

## Recombinant microorganisms and process

### Field of the invention

The present invention relates to processes for producing hydrogen, nucleic acid constructs and recombinant microorganisms for producing hydrogen.

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### Related application

This application claims priority from Australian provisional application AU 2020900990, the entire contents of which are hereby incorporated by reference.

### Background of the invention

There has been an increasing interest in alternative fuels due to rising petroleum  
10 costs, escalating diplomatic tensions with oil producing countries, and the rising levels  
of greenhouse gases in the atmosphere. Hydrogen has enormous potential to serve as  
a non-polluting fuel, thereby alleviating the environmental and political concerns  
associated with fossil energy utilization. Thus, efforts to identify a candidate for  
replacing or supplementing fossil fuels as a source of clean energy have focused on the  
15 production of molecular hydrogen.

Key to a hydrogen economy is finding an efficient, inexpensive, and renewable  
process for the production of hydrogen while also achieving the equally important goal  
of economically converting hydrogen into usable energy.

One approach to the production of hydrogen on a commercial scale is the  
20 exploitation of photobiological production of hydrogen by eukaryotic organisms. For  
example, green algae respond to anaerobic stress by switching the oxidative pathway to  
a fermentative metabolism.

The ability of green algae, such as *Chlamydomonas reinhardtii*, to produce  
hydrogen from water has long been recognized. This reaction is catalyzed by a  
25 reversible hydrogenase, an enzyme that is induced in the cells after exposure to a short  
period of anaerobiosis. Thus, the use of algal bioreactors has been one approach to  
producing hydrogen. However, the activity of the hydrogenase is rapidly lost when cells

are illuminated because of the immediate inactivation of the reversible hydrogenase by photosynthetically generated O<sub>2</sub>.

Other approaches for the production of hydrogen include the generation of recombinant microorganisms, and the fermentation of a carbohydrate feedstock by those microorganisms. In some examples, hydrogenases from bacteria, archaea and algae have been expressed in *E. coli*, although expression of the exogenous enzymes in *E. coli* has been complicated by low expression rates and protein instability, despite codon optimisation.

Various hydrogenases have been proposed as candidates for use in such fermentation approaches. For example, the [NiFe] hydrogenase from the purple bacterium *Allochromatium vinosum* is a remarkably active electrocatalyst. Though [NiFe]-hydrogenases exhibit promise, there remain problems associated with use of these and other hydrogenase enzymes. The stability of hydrogenases has been one of the major disadvantages in their use in enzyme fuel cells. Furthermore, though the enzymes demonstrate less susceptibility to CO poisoning than does platinum, commercial use requires further improvement in terms of both the sensitivity to CO as well as to oxygen. In addition, the lack of hydrogenase availability in large quantities limits their potential application in enzyme fuel cells. Therefore, production of stable hydrogenase in large quantities and with desired catalytic properties will greatly enhance the application of this interesting bioelectrocatalyst for hydrogen fuel.

Algal bioreactors are expensive to scale up due to a number of light capture and hydrogen capture technical barriers. The rate of hydrogen production is also an important consideration as hydrogen is difficult to contain and collect at low volumes and concentrations. Algal systems produce hydrogen at very low rates and require nutrient limitation to start production. To date, both the production rates and the yields of hydrogen produced by either engineered microalgae or via fermentation of carbohydrate feedstock have been too slow and low, respectively, to be commercially viable.

There is therefore a need for improved processes for the generation of hydrogen.

Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any

jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

### Summary of the invention

5       The invention relates generally to expression vectors, microorganisms, methods and reactor systems to produce hydrogen and active hydrogenase enzymes for energy and electricity-generating applications. The expression vectors and microorganisms can be used in culture methods to produce the products of interest. Both the hydrogen and active hydrogenase products can be incorporated into a system such as, for example, a  
10   fuel cell system for producing electricity from hydrogen.

In a first aspect, the present invention provides a recombinant microorganism for producing hydrogen gas, wherein the microorganism comprises:

exogenous nucleic acid sequences encoding one or more proteins for enabling the microorganism to produce hydrogen,

- 15       - wherein the one or more proteins comprise an Fe-Fe-dependent hydrogenase and optionally at least one assembly protein for enabling maturation and activation of the hydrogenase;
- wherein the nucleic acid sequences are operably linked to one or more promoters for enabling expression of the nucleic acid sequences in the  
20   microorganism, and
- wherein the exogenous nucleic acid sequences are codon optimised to provide for optimised expression of the hydrogenases in the microorganism.

Preferably, the Fe-Fe hydrogenase is a member of the A1 class of Fe-Fe hydrogenases.

25       Preferably, the Fe-Fe-dependent hydrogenase is HydA (Hyd1) or a functionally equivalent homolog or derivative thereof.

In preferred embodiments, the Fe-Fe-dependent hydrogenase comprises the amino acid sequence of the HydA protein selected from the group consisting of: *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*,

*Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*,  
*Veillonella parvula*, *Veillonella atypica*, *Peptoclostridium bifermentans* *Clostridium*  
*arbusti*, *Pseudoflavonifractor capillosus*, *Lachnoclostridium citroniae*, *Lachnoclostridium*  
*clostridioforme*, *Pelosinus fermentans*, *Thermodesulfovibrio islandicus*, *Sutterella*  
5 *wadsworthensis*, *Clostridium beijerinckii*, *Fusobacterium ulcerans*, *Clostridium*  
*tyrobutyricum*, *Clostridium perfringens*, *Cetobacterium somerae*, *Clostridium beijerinckii*,  
*Clostridium colicanis*, *Clostridium intestinale*, *Clostridium chauvoei*, *Cellulomonas fimi*,  
*Ruminiclostridium thermocellum*, *Naegleria gruberi*, *Chlorella variabilis*,  
*Fervidobacterium nodosum*, *Thermotoga petrophila*, *Thermotoga lettingae*,  
10 *Thiomicrospira pelophila*, *Caldatibacterium californiense*, *Fusobacterium necrophorum*,  
*Omnitrophus fodinae*, *Syntrophothermus lipocalidus*, *Ammonifex degensii*,  
*Desulfotomaculum hydrothermale*, *Fusobacterium mortiferum*, *Desulfotomaculum*  
*kuznetsovii*, and *Lachnoclostridium phytofermentans* or functionally equivalent  
homologs or derivatives thereof. Preferably, the HydA protein is selected from the group  
15 consisting of: *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba*  
*nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*,  
*Veillonella parvula*, *Veillonella atypica*, and *Peptoclostridium bifermentans*, and  
functionally equivalent homologs thereof. More preferably, the HydA protein is from the  
*Chlamydomonas reinhardtii* or a functionally equivalent homolog or derivatives thereof.

20 In certain embodiments, the microorganism may be provided, during culturing of  
the microorganism, with one or more factors for enabling maturation and activation of  
the hydrogenase. Preferably the one or more factors is in the form of a small molecule.  
Examples of factors for enabling maturation and activation of the hydrogenase are  
[2Fe]-subsite mimetics containing an azadithiolate bridge. Such factors are described,  
25 for example in Esselborn et al., (2013) *Nat Chem Biol* 9 (10):607-609, and Berggren et  
al., (2013) *Nature*, 499: 66-69 the contents of which are incorporated herein by  
reference.

Preferably, the exogenous nucleic acid sequences encode at least one assembly  
protein for enabling maturation and activation of the hydrogenase, wherein the at least  
30 one protein is selected from the group consisting of: HydEF and/or HydG. More  
preferably, the exogenous nucleic acid sequences comprise sequences encoding both  
assembly proteins HydEF and HydG. In a particularly preferred embodiment, the HydEF

and HydG proteins comprise the amino acid sequence of the HydEF and HydG proteins from *Chlamydomonas reinhardtii* or functionally equivalent homologs or derivatives thereof.

Accordingly, in a preferred embodiment, the present invention provides a  
5 recombinant microorganism for producing hydrogen gas, wherein the microorganism comprises:

exogenous nucleic acid sequences encoding one or more proteins for enabling the microorganism to produce hydrogen,

- 10 - wherein the one or more proteins comprise an Fe-Fe-dependent hydrogenase HydA, or a functionally equivalent homolog or derivative thereof, and the assembly proteins HydEF and HydG from *Chlamydomonas reinhardtii*, or functionally equivalent homologs or derivatives thereof;
- wherein the nucleic acid sequences are operably linked to one or more promoters for enabling expression of the nucleic acid sequences in the  
15 microorganism, and
- wherein the exogenous nucleic acid sequences are codon optimised to provide for optimised expression of the hydrogenases in the microorganism.

In any embodiment, the microorganism further comprises nucleic acid sequences encoding the proteins Ferredoxin NADP reductase (FNR) and ferredoxin (encoded by  
20 petF), or functionally equivalent homologs or derivatives thereof.

Preferably the source of the FNR is a Flavin containing ferredoxin reductase that utilises NADPH as the reducing agent to reduce Ferredoxin. More preferably, the ferredoxin protein is from *Chlamydomonas reinhardtii* and the FNR is any FNR capable of reducing the Ferredoxin from *Chlamydomonas reinhardtii*. In a particularly preferred  
25 embodiment, the FNR and Ferredoxin proteins comprise the amino acid sequences from *Chlamydomonas reinhardtii* or functionally equivalent homologs or derivatives thereof.

The recombinant microorganism may be any microorganism suitable for use of expression of recombinant proteins. In certain embodiments, the recombinant  
30 microorganism is selected from the group consisting of: *Escherichia coli*, *Bacillus*

*subtilis*, *Lactobacillus* sp., or a *Streptococcus* sp., In preferred embodiments, the microorganism is a strain of *Escherichia coli* (*E. coli*).

In certain embodiments, the recombinant microorganism is partially or completely inactivated and/or non-viable.

5 In any embodiment, the exogenous nucleic acid sequences are provided in one or more polynucleotide constructs. In a preferred embodiment, the exogenous nucleic acid sequences encoding HydEF, HydG, HydA, and optionally Ferredoxin and FNR are provided in a single polynucleotide construct. In alternative embodiments, the nucleic acid sequences encoding the proteins are provided in separate polynucleotide  
10 constructs.

In a preferred embodiment, the present invention provides an *E. coli* cell comprising a recombinant construct encoding a cluster of protein that enable the cell to produce hydrogen, wherein the cluster of proteins comprises, consists or consists essentially of the polypeptides HydEF, HydG, HydA, ferredoxin and FNR from  
15 *Chlamydomonas reinhardtii*. In a preferred embodiment, the recombinant construct comprises, consists or consists essentially of the sequence set forth in SEQ ID NO: 10.

In further embodiments, the microorganism comprises one or more genetic modifications for redirecting carbon utilisation into the pentose phosphate pathway. The modification may result in the reduction or inhibition of activity of a protein that directs  
20 carbon towards the glycolytic pathway, thereby redirecting carbon utilisation towards the pentose phosphate pathway.

For example, the microorganism may be further modified to reduce or inhibit the activity or levels of one or more endogenous proteins selected from the group consisting of: phosphofructokinase, pyruvate kinase, glycerate mutase, glyceraldehyde-3-  
25 phosphate dehydrogenase, 6-phosphogluconate dehydratase, and 2-keto-3-deoxy-6-phosphogluconate aldolase. These proteins are encoded by the genes *pfkA*, *pps*, *gpmA/gpmM*, *gapA*, *edd* and *eda*, respectively.

Preferably, the microorganism is genetically modified to delete or reduce expression of one or more of the genes *pfkA*, *pps*, *gpmA/gpmM*, *gapA*, *edd* and *eda*,  
30 encoding phosphofructokinase, pyruvate kinase, glycerate mutase, glyceraldehyde-3-

phosphate dehydrogenase, 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase respectively. The modification may be any modification that partially or completely reduces expression of the gene. Where there is a partial reduction in expression, the expression may be reduced by at least 5%, at least 10%, at  
5 least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more, compared to the expression in the wild-type microorganism of the same strain.

The genetic modification may be made using a CRISPR-Cas9 system or other genome modification system (such as lambda red recombinase) to partially or  
10 completely inhibit expression of the one or more genes. The genetic modification may result in the introduction of a complete or partial loss-of-function mutation in the gene, preferably a complete loss-of-function mutation. The modification may be the complete or partial excision of the gene sequence.

In certain embodiments, only one of *pfkA*, *pps*, *gpmA/gpmM*, *gapA*, *edd* and *eda*  
15 genes are deleted or knocked-down. Preferably *pfkA* or *gpmA* is deleted or knocked-down. In further embodiments, the microorganism is genetically modified to delete or reduce expression of two, three, four, five or all of the genes *pfkA*, *pps*, *gpmA/gpmM*, *gapA*, *edd* and *eda*. In certain embodiments, the genetic modification results in deletion or reduction in expression of: *pfkA* and *gpmA*; or *edd* and *eda*; or *gpmM*, *edd* and *eda*;  
20 or *gpmA*, *edd* and *eda*; or *gpmM*, *edd*, *eda* and *pfkA*; or *gpmA*, *edd*, *eda* and *pfkA* or all of *pfkA*, *pps*, *gpmA/gpmM*, *edd* and *eda*.

In still further embodiments, the microorganism is genetically modified to increase the level or activity of one or more proteins of the pentose phosphate pathway.

Preferably the one or more proteins is selected from the group consisting of:  
25 phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, 6-phosphogluconate dehydrogenase, transketolase and transaldolase. These proteins are encoded by the genes *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB*, respectively. In a particularly preferred embodiment, the protein is glucose-6-phosphate dehydrogenase.

In further embodiments, the level or activity of endogenous NAD kinase (NADK, encoded by *yjfB*) and/or soluble pyridine nucleotide transhydrogenase (UdhA, encoded by *sthA*) is increased.

Preferably the increased level, or activity, of the proteins of the microorganism is accomplished by increasing expression of nucleic acid sequences encoding the one or more proteins of the pentose phosphate pathway, such that the level of the protein produced by the microorganism is increased relative to a wild-type microorganism of the same strain. In alternative embodiments, the increased level or activity of the proteins is accomplished by the introduction of one or more point mutations which result in increased activity of the protein.

In a preferred embodiment, the gene encoding glucose-6-phosphate dehydrogenase, *zwf* is overexpressed. In further embodiments, the gene encoding 6-phosphogluconate dehydrogenase, *gnd* is overexpressed. In other embodiments, the gene encoding 6-phosphogluconolactonase, *pgl*, is overexpressed.

Overexpression and increased levels or activity of phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, 6-glucophosphonate dehydrogenase, transketolase and transaldolase can optionally be accomplished by modification of the promoter sequences of one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB*. In certain embodiments, the endogenous promoters for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with an exogenous promoter for increasing expression of the gene. In certain embodiments, the endogenous promoter for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with an endogenous promoter which regulates expression of a different gene in the microorganism. In alternative embodiments, the endogenous promoter for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with an exogenous promoter. The exogenous promoter may regulate expression of a homologous gene in a non-cognate microorganism or may regulate expression of a non-homologous protein in a non-cognate microorganism.

In particularly preferred embodiments, the endogenous promoter for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with a promoter selected

from the group consisting of: the *osmY* promoter, the *gapA* promoter, the *nirB* promoter and the *nar* promoter.

Still further, the host cell may be transformed with a recombinant construct which encodes a heterologous protein from another microbial species, for example for the purpose of increasing the level or activity of the relevant enzyme of the pentose phosphate pathway.

The recombinant construct may enable expression of the exogenous gene concomitantly with expression of the endogenous gene. Alternatively, the recombinant construct may be stably introduced into the microorganism genome, such that the endogenous gene sequence is replaced with the exogenous gene sequence.

Overexpression and increased levels or activity of phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, 6-glucophosphonate dehydrogenase, transketolase and transaldolase can be accomplished by supplementation or replacement of the endogenous gene encoding said protein, with an exogenous gene encoding a homologous protein. In certain examples, the endogenous *zwf* gene is replaced with the *zwf* gene from *Zymomonas mobilis*. Preferably, the microorganism is *E. coli* and the *E. coli zwf* gene is replaced with the *zwf* gene from *Zymomonas mobilis*. In still further examples, the *gnd* gene is replaced with the *gnd* gene from *Corynebacterium glutamicum*. Preferably the microorganism is *E. coli* the gene encoding transketolase and transaldolase is supplemented or replaced with the homologous gene from *Saccharomyces cerevisiae*. Preferably, the microorganism is *E. coli* and the *E. coli gnd* gene is replaced with the *gnd* gene from *Corynebacterium glutamicum*. Further still, the endogenous *gapA* gene (encoding glyceraldehyde-3-phosphate dehydrogenase) is replaced with the *gapC* gene from *Clostridium acetobutylicum*. Preferably, the microorganism is *E. coli* and the *E. coli gapA* gene is replaced with the *gapC* gene from *Clostridium acetobutylicum*.

Preferably expression of the one or more genes encoding phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, and 6-glucophosphonate dehydrogenase is increased by at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 5-fold or more.

In still further embodiments, the recombinant microorganism is modified so as to metabolise sucrose for energy consumption. In embodiments where the microorganism is *E. coli*, the microorganism is preferably genetically modified to express *cscA* and *cscB* genes, encoding sucrose hydrolase, and sucrose permease respectively from strains of *E. coli* that metabolise sucrose. Further still, the *E. coli* microorganism may be genetically modified to increase the levels or activity of endogenous *E. coli* phosphoglucomutase (*pgm*) or xylose isomerase (*xylA*). The genetic modification may be to increase expression of the endogenous gene (for example, by modification of the promoter region) or by introduction and expression of an exogenous nucleic acid encoding the gene.

In further embodiments, the microorganism is modified to express sucrose phosphorylase from *Leuconostoc mesenteroides*

In any embodiment described herein requiring expression of an exogenous gene, the gene may be codon optimised for expression in the microorganism.

Where the microorganism is a recombinant *E. coli* microorganism, the microorganism may be any strain of *E. coli* capable of expressing an exogenous nucleic acid sequence. In certain preferred embodiments, the *E. coli* strain is selected from any K12 derived or W derived strain. In certain embodiments, the *E. coli* strain is selected from the group consisting of: DH5 $\alpha$  (DH5alpha).

In a further aspect, the present invention provides a method for producing hydrogen gas, the method comprising:

- providing a host cell comprising one or more recombinant polynucleotides comprising nucleic acid sequences encoding an Fe-Fe-dependent hydrogenase, wherein the nucleic acid sequences are operably linked to one or more promoters for enabling expression of the nucleic acid sequences in the microorganism,

- contacting the host cell with an exogenous factor for enabling maturation and activation of the hydrogenase;

- culturing the host cell under suitable conditions for enabling production of hydrogen therefrom.

Preferably the one or more factors is in the form of a small molecule. Examples of factors for enabling maturation and activation of the hydrogenase are [2Fe]-subsite mimetics containing an azadithiolate bridge. Such factors are described, for example in Esselborn et al., (2013) *Nat Chem Biol* 9 (10):607-609, and Berggren et al., (2013) *Nature*, 499: 66-69 the contents of which are incorporated herein by reference.

Further, the present invention provides a method for producing hydrogen gas, the method comprising:

- providing a host cell comprising one or more recombinant polynucleotides comprising nucleic acid sequences encoding an Fe-Fe-dependent hydrogenase and at least one assembly protein for enabling maturation and activation of the hydrogenase;
  - o wherein the nucleic acid sequences are operably linked to one or more promoters for enabling expression of the nucleic acid sequences in the microorganism, and
  - o wherein the exogenous nucleic acid sequences are codon optimised to provide for optimised expression of the hydrogenases in the microorganism.

In another aspect, the present invention provides a method for producing hydrogen gas, the method comprising:

- providing one or more polynucleotides comprising nucleic acid sequences encoding an Fe-Fe-dependent hydrogenase and optionally at least one assembly protein for enabling maturation and activation of the hydrogenase, wherein the nucleic acid sequences are operably linked to a promoter for enabling expression of the nucleic acid sequences and wherein the nucleic acid sequences are codon optimised for expression in a heterologous host cell;
- providing a heterologous host cell;
- transforming or transfecting the host cell with the polynucleotide(s);
- providing cell culture media; and
- culturing the transformed or transfected host cell in the cell culture media under conditions sufficient for expression of the polynucleotide.

In still a further aspect, the present invention provides a method for maximising the expression of a hydrogen-generating Fe-Fe hydrogenase, preferably A1 Fe-Fe hydrogenase in a heterologous host cell, the method comprising:

- 5       - providing a polynucleotide comprising nucleic acid sequences encoding an Fe-Fe-dependent hydrogenase and at least one assembly protein for enabling maturation and activation of the hydrogenase, wherein the nucleic acid sequences are operably linked to a promoter and are codon optimised for enabling expression of the nucleic acid sequences in a heterologous host cell;
- 10       - providing a heterologous host cell;
- transforming or transfecting the host cell with the polynucleotide;
- providing cell culture media; and
- culturing the transformed or transfected host cell in the cell culture media under conditions sufficient for expression of the polynucleotide.
- 15       Preferably the Fe-Fe-dependent hydrogenase is a class A1 Fe-Fe hydrogenase.

Preferably, the Fe-Fe-dependent hydrogenase is HydA (Hyd1) or a functionally equivalent homolog or derivative thereof.

- In preferred embodiments, the Fe-Fe-dependent hydrogenase comprises the amino acid sequence of the HydA protein selected from the group consisting of:
- 20   *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*, *Veillonella parvula*, *Veillonella atypica*, *Peptoclostridium bifermentans*, *Clostridium arbusti*, *Pseudoflavonifractor capillosus*, *Lachnoclostridium citroniae*, *Lachnoclostridium clostridioforme*, *Pelosinus fermentans*, *Thermodesulfobacterium islandicus*, *Sutterella wadsworthensis*,
  - 25   *Clostridium beijerinckii*, *Fusobacterium ulcerans*, *Clostridium tyrobutyricum*, *Clostridium perfringens*, *Cetobacterium somerae*, *Clostridium beijerinckii*, *Clostridium colicanis*, *Clostridium intestinale*, *Clostridium chauvoei*, *Cellulomonas fimi*, *Ruminiclostridium thermocellum*, *Naegleria gruberi*, *Chlorella variabilis*, *Fervidobacterium nodosum*, *Thermotoga petrophila*, *Thermotoga lettingae*,
  - 30   *Thiomicrospira pelophila*, *Caldatibacterium californiense*, *Fusobacterium necrophorum*, *Omnitrophus fodinae*, *Syntrophothermus lipocalidus*, *Ammonifex degensii*,

*Desulfotomaculum hydrothermale*, *Fusobacterium mortiferum*, *Desulfotomaculum kuznetsovii*, and *Lachnoclostridium phytofermentans* or functionally equivalent homologs or derivatives thereof. Preferably, the HydA protein is selected from the group consisting of: *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba*  
 5 *nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*, *Veillonella parvula*, *Veillonella atypica*, and *Peptoclostridium bifermentans*, and functionally equivalent homologs thereof. More preferably, the HydA protein is from the *Chlamydomonas reinhardtii* or a functionally equivalent homolog or derivatives thereof.

Preferably, the at least one assembly protein comprises a protein from the group  
 10 consisting of: HydEF and/or HydG. More preferably, the exogenous nucleic acid sequences comprise sequences encoding both assembly proteins HydEF and HydG. In a particularly preferred embodiment, the HydEF and HydG proteins comprise the amino acid sequence of the HydEF and HydG proteins from *Chlamydomonas reinhardtii* or functionally equivalent homologs or derivatives thereof.

15 Accordingly, in preferred embodiments, the present invention provides a method for producing hydrogen gas, the method comprising:

- providing a host cell comprising one or more recombinant polynucleotides comprising nucleic acid sequences encoding the *Chlamydomonas reinhardtii* polypeptides HydEF, HydG and HydA,  
 20     o wherein the nucleic acid sequences are operably linked to a promoter for enabling expression of the nucleic acid sequences,  
       o wherein the nucleic acid sequences are codon optimised for expression in a heterologous host; and
- culturing said host cell in a suitable culture medium under conditions to effect  
 25 expression of the polynucleotides.

In another aspect, the present invention provides a method for producing hydrogen gas, the method comprising:

- providing one or more polynucleotides comprising nucleic acid sequences encoding the *Chlamydomonas reinhardtii* polypeptides HydEF, HydG and  
 30 HydA, wherein the nucleic acid sequences are operably linked to a promoter

for enabling expression of the nucleic acid sequences and wherein the nucleic acid sequences are codon optimised for expression in a heterologous host cell;

- providing a host cell;
- 5    - transforming or transfecting the host cell with the polynucleotide(s);
- providing cell culture media; and
- culturing the transformed or transfected host cell in the cell culture media under conditions sufficient for expression of the polynucleotide.

In still a further aspect, the present invention provides a method for maximising  
10   the expression of a hydrogen-generating Fe-Fe hydrogenase from *Chlamydomonas reinhardtii* in a heterologous host cell, the method comprising:

- providing a polynucleotide comprising nucleic acid sequences encoding the *Chlamydomonas reinhardtii* polypeptides HydEF, HydG and HydA, wherein the nucleic acid sequences are operably linked to a promoter and are codon  
15    optimised for enabling expression of the nucleic acid sequences in a heterologous host cell;
- providing a heterologous host cell;
- transforming or transfecting the host cell with the polynucleotide;
- providing cell culture media; and
- 20    - culturing the transformed or transfected host cell in the cell culture media under conditions sufficient for expression of the polynucleotide.

Preferably the heterologous host cell is an *E. coli* cell and the nucleic acid sequences are codon optimised for expression in *E. coli*. Preferably the promoters in the polynucleotide are for expression of the polynucleotides in *E. coli*.

25    In a preferred embodiment of any of the above aspects, the recombinant polynucleotide(s) comprise nucleic acid sequences encoding Ferredoxin NADP reductase and Ferredoxin, or functionally equivalent homologs or derivatives thereof.

Preferably the source of the FNR is a Flavin containing ferredoxin reductase that utilises NADPH as the reducing agent to reduce Ferredoxin. More preferably, the  
30   ferredoxin protein is from *Chlamydomonas reinhardtii* and the FNR is any FNR capable

of reducing the Ferredoxin from *Chlamydomonas reinhardtii*. In a particularly preferred embodiment, the FNR and Ferredoxin proteins comprise the amino acid sequences from *Chlamydomonas reinhardtii* or functionally equivalent homologs or derivatives thereof.

- 5           The host cell may be any microorganism suitable for use of expression of recombinant proteins. In certain embodiments, the host cell is selected from the group consisting of: *Escherichia coli*, *Bacillus subtilis*, *Lactobacillus* sp., or a *Streptococcus* sp., In preferred embodiments, the microorganism is a strain of *Escherichia coli* (*E coli*).

          In certain embodiments, the host cell is partially or completely inactivated and/or  
10   non-viable.

          As used herein, the combination of nucleic acid sequences encoding HydEF, HydG, HydA, Ferredoxin NADP reductase and Ferredoxin may also be referred to as the hydrogen producing gene cluster (HPGC).

          In any embodiment, the above methods may further comprise utilising a  
15   genetically modified host cell, or modifying the microorganism or host cell, or contacting the microorganism or host cell with an agent to reduce or inhibit the activity or levels of one or more endogenous host cell proteins selected from the group consisting of: phosphofructokinase, pyruvate kinase, glycerate mutase, 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase.

20           The agent for reducing or inhibiting the activity or levels of one or more of phosphofructokinase, pyruvate kinase, glycerate mutase, 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase may be selected from: a small molecule, a peptide, an antibody, an interfering RNA, for example an antisense RNA, microRNA, shRNA, siRNA, that can reduce the activity or levels of one or more of  
25   the proteins.

          In preferred embodiments, the methods comprise contacting or having contexted the the microorganism or host cell with an agent which genetically modifies the microorganism or host cell such that the levels or activity of one or more of the *pfkA*, *pps*, *gpmA/gpmM*, *gapA*, *edd* and *eda* (encoding phosphofructokinase, pyruvate kinase,  
30   glycerate mutase, glyceraldehyde-3-phosphoate dehydrogenase, 6-phosphogluconate

dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase, respectively) are partially or completely reduced. For example, the agent may comprise a gRNA molecule for use in combination with a CRISPR-Cas9 or other genome-editing system (such as lambda red recombinase) for deleting part or all of the gene.

5 In still further embodiments, the methods further comprise genetically modifying or having modified the microorganism or host cell to increase the level or activity of one or more proteins of the pentose phosphate pathway. Preferably the one or more proteins is selected from the group consisting of: phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, 6-phosphogluconate  
10 dehydrogenase, transketolase and transaldolase. These genes are encoded by the genes *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and, *talA* or *talB*, respectively. In a particularly preferred embodiment, the protein is glucose-6-phosphate dehydrogenase.

In further embodiments, the level or activity of endogenous NAD kinase (NADK, encoded by *yfjB*) and/or soluble pyridine nucleotide transhydrogenase (UdhA, encoded  
15 by *sthA*) is increased.

Preferably the methods comprise modifying the microorganism or host cell to comprise nucleic acid sequences for overexpressing the genes encoding the one or more proteins of the pentose phosphate pathway, such that the level of the protein produced by the microorganism is increased relative to a wild-type microorganism of the  
20 same strain. In alternative embodiments, the increased level or activity of the proteins is accomplished by the introduction or one or more point mutations which result in increased activity of the protein.

In a preferred embodiment, the gene encoding glucose-6-phosphate dehydrogenase, *zwf* is overexpressed. In further embodiments, the gene encoding 6-phosphogluconate dehydrogenase, *gnd* is overexpressed. In other embodiments, the  
25 gene encoding 6-phosphogluconolactonase, *pgl*, is overexpressed. In other embodiments, the gene encoding glyceraldehyde-3-phosphate dehydrogenase, *gapA*, is overexpressed. Overexpression can optionally be accomplished by modification of the promoter sequences of one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and, *talA* or  
30 *talB*.

In certain embodiments, the endogenous promoters for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with an exogenous promoter for increasing expression of the gene. In certain embodiments, the endogenous promoter for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with an  
5 endogenous promoter which regulates expression of a different gene in the microorganism. In alternative embodiments, the endogenous promoter for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with an exogenous promoter. The exogenous promoter may regulate expression of a homologous gene in a non-cognate microorganism or may regulate expression of a non-homologous protein in  
10 a non-cognate microorganism.

In particularly preferred embodiments, the endogenous promoter for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with a promoter selected from the group consisting of: the *osmY* promoter, the *gapA* promoter, the *nirB* promoter and the *nar* promoter.

15 Overexpression and increased levels or activity of phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, 6-glucophosphonate dehydrogenase, transketolase and transaldolase may also be accomplished by replacement of the endogenous gene encoding said protein, with an exogenous gene encoding a homologous protein.

20 Preferably expression of the one or more genes encoding phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, and 6-glucophosphonate dehydrogenase is increased by at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 5-fold or more.

In still further embodiments, the recombinant microorganism or host cell is  
25 modified so as to metabolise sucrose for energy consumption. In embodiments where the microorganism is *E. coli*, the microorganism is preferably genetically modified to express *cscA* and *cscB* genes, encoding sucrose hydrolase, and sucrose permease respectively from strains of *E. coli* that metabolise sucrose. Further still, the *E. coli* microorganism may be genetically modified to increase the levels or activity of  
30 endogenous *E. coli* phosphoglucomutase (*pgm*) or xylose isomerase (*xylA*). The genetic modification may be to increase expression of the endogenous gene (for example, by

modification of the promoter region) or by introduction and expression of an exogenous nucleic acid encoding the gene.

In further embodiments, the microorganism is modified to express sucrose phosphorylase from *Leuconostoc mesenteroides*

- 5           In embodiments where the host cell is *E. coli*, the *E. coli* microorganism may be any strain of *E. coli* capable of expressing an exogenous nucleic acid sequence. In certain preferred embodiments, the *E. coli* strain is selected from any K12 derived or W derived strain. In certain embodiments, the *E. coli* strain is selected from the group consisting of: DH5 $\alpha$  (DH5alpha).
- 10           In further embodiments, the methods described herein further comprise culturing the microorganism or host cell in conditions which are optimised for enabling expression of the hydrogen producing gene cluster (HPGC) described herein, and thereby increasing the production of hydrogen by the microorganism. In one example, the methods comprise culturing the host cell under anaerobic conditions. The skilled person
- 15           will be familiar with methods for culturing cells under anaerobic conditions, including by the addition of a neutral gas as a reductant.

Further still, the culture conditions may include addition of ferric (iron III) or ferrous (iron II) to the culture medium. In preferred embodiments, the ferrous iron (Fe II) is added to the culture medium at a concentration of at least about 20  $\mu$ M or greater,

20           preferably no more than about 50  $\mu$ M.

The culturing conditions are preferably performed at no more than 37 °C, more preferably at less than about 35 °C, less than about 32 °C, most preferably at less than about 30 °C.

The present invention also provides various nucleic acid constructs or

25           polynucleotides for use in a system for generating molecular hydrogen.

In one embodiment, the invention provides a nucleic acid construct or polynucleotide comprising nucleotide sequence encoding the polypeptides HydEF, HydG and HydA, wherein the nucleic acid sequences are operably linked to a promoter for enabling expression of the nucleic acid sequences and wherein the nucleic acid

sequences are codon optimised for expression in a heterologous host. Preferably, the nucleic acid sequences are codon optimised for expression in *E. coli*. Preferably, the HydEF and HydG polypeptides are from *Chlamydomonas reinhardtii*.

In preferred embodiments, the nucleic acid encoding the HydA protein in the  
5 nucleic acid constructs of the invention, encodes the amino acid sequence of the HydA protein from an organism selected from the group consisting of: *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*, *Veillonella parvula*, *Veillonella atypica*, *Peptoclostridium bifermentans*, *Clostridium arbusti*, *Pseudoflavonifractor*  
10 *capillosus*, *Lachnoclostridium citroniae*, *Lachnoclostridium clostridioforme*, *Pelosinus fermentans*, *Thermodesulfovibrio islandicus*, *Sutterella wadsworthensis*, *Clostridium beijerinckii*, *Fusobacterium ulcerans*, *Clostridium tyrobutyricum*, *Clostridium perfringens*, *Cetobacterium somerae*, *Clostridium beijerinckii*, *Clostridium colicanis*, *Clostridium intestinale*, *Clostridium chauvoei*, *Cellulomonas fimi*, *Ruminiclostridium thermocellum*,  
15 *Naegleria gruberi*, *Chlorella variabilis*, *Fervidobacterium nodosum*, *Thermotoga petrophila*, *Thermotoga lettingae*, *Thiomicrospira pelophila*, *Caldatribacterium californiense*, *Fusobacterium necrophorum*, *Omnitrophus fodinae*, *Syntrophothermus lipocalidus*, *Ammonifex degensii*, *Desulfotomaculum hydrothermale*, *Fusobacterium mortiferum*, *Desulfotomaculum kuznetsovii*, and *Lachnoclostridium phytofermentans* or  
20 functionally equivalent homologs or derivatives thereof. Preferably, the HydA protein is selected from the group consisting of: *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*, *Veillonella parvula*, *Veillonella atypica*, and *Peptoclostridium bifermentans*, and functionally equivalent homologs thereof. More  
25 preferably, the HydA protein is from the *Chlamydomonas reinhardtii* or a functionally equivalent homolog or derivatives thereof

The present invention also provides a polynucleotide comprising nucleic acid sequences encoding the hydrogen producing gene cluster (HPGC), wherein the HPGC comprises genes encoding HydEF, HydG, HydA, ferredoxin NADP reductase and  
30 ferredoxin. Preferably the nucleic acid encodes an FNR that is a Flavin containing ferredoxin reductase that utilises NADPH as the reducing agent to reduce Ferredoxin. More preferably, the nucleic acid encodes a ferredoxin protein from *Chlamydomonas*

*reinhardtii* and encodes an FNR that is any FNR capable of reducing the Ferredoxin from *Chlamydomonas reinhardtii*. In a particularly preferred embodiment, the polynucleotide comprises nucleic acids encoding FNR and Ferredoxin proteins from *Chlamydomonas reinhardtii* or functionally equivalent homologs or derivatives thereof.

- 5           In a particularly preferred embodiment, the sequence of the polynucleotide comprising the HPGC comprises, consists, or consists essentially of the nucleotide sequence set forth in SEQ ID NOs: 10 or 30 to 40.

          The present invention also provides a microorganism as described herein, when used, or for use in a system for producing hydrogen. Accordingly, the present invention  
10       provides a system for producing hydrogen, wherein the system comprises:

- a culture or population of recombinant microorganisms as herein described;
- a feedstock for use by the recombinant microorganisms to induce expression of one or more proteins for enabling production of hydrogen by the microorganisms.

- 15           Optionally, the system also comprises means for storing or transferring the hydrogen produced by the recombinant microorganisms.

          Preferably, the feedstock is a carbohydrate-based feedstock, such as glucose or sucrose or any other carbohydrate source.

          The present invention also provides a bioreactor for producing hydrogen,  
20       comprising: a vessel which comprises a hydrogen producing system as described herein, said system comprising a suspension of hydrogen generating microorganisms of the invention, a feedstock for providing a source of carbon for use by the recombinant microorganisms and means for separating or extracting hydrogen gas from said suspension.

- 25           The present invention also provides a microorganism as described herein, when used, or for use in a system for producing electricity. Accordingly, the present invention provides a system or device for producing electricity from hydrogen, wherein the system or device comprises:

- a culture or population of recombinant microorganisms as herein described;

- a feedstock for use by the recombinant microorganisms to induce expression of one or more proteins for enabling production of hydrogen by the microorganisms;
- a hydrogen fuel cell;
- means for transferring the hydrogen produced by the recombinant microorganisms to the hydrogen fuel cell.

The present invention also provides a method for producing electricity, the method comprising operating a system or device comprising a recombinant microorganism as described herein, or utilising hydrogen produced according to a method described herein.

The present invention also provides for use of a recombinant microorganism as herein described, in a system or device for producing electricity from hydrogen.

As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and 15 "comprised", are not intended to exclude further additives, components, integers or steps.

Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

20 **Brief description of the drawings**

**Figure 1: Hydrogen production in wild-type and mutant strains expressing the hydrogen producing gene cluster (HPGC).** H<sub>2</sub> gas production after addition of 20 mM glucose to 50 mL *Escherichia coli* culture. DH5α without the hydrogen producing gene cluster (HPGC) makes no hydrogen under these conditions. The four strains DH5α with plasmid pHPGC; DH5α  $\Delta pfk$  with pHPGC; DH5α  $\Delta gpmA$  with pHPGC; and DH5α with plasmid pH1-HEFG (pHPGC without petF-FNR), rapidly start to accumulate hydrogen after the addition of glucose. Hydrogen concentration in gas phase measured by gas chromatography.

**Figure 2: Accumulation of total organic acid fermentation products (succinate plus pyruvate plus lactate) in wild-type and mutant strains expressing the hydrogen producing gene cluster.** Organic acid accumulation after addition of glucose for hydrogen production using HPGC.

5 **Figure 3: Schematic of exemplary device comprising microorganisms of the invention.**

**Figure 4: Hydrogen production rates of various recombinant microorganisms containing pHPGC, relative to wild-type *E. coli* DH5 $\alpha$  with pHPGC (control).** Rate of hydrogen production (L/h) by *E. coli* genetically modified to  
10 reduce the flow of carbon from glucose through the lower section of the glycolytic pathway by deleting *gpmM*,  $\Delta$ *gpmA*. Rate of hydrogen production increased with deletion of *gpmA* and *gpmM*. Increased expression of *gnd* and *zwf* also improved the rate of hydrogen production.

**Figure 5: Ratio of hydrogen to carbon dioxide produced by various recombinant microorganisms containing pHPGC, relative to wild-type *E. coli* DH5 $\alpha$  with pHPGC (control).** Increasing expression of *zwf*, *Gp::zwf* or reducing *pfk* activity,  $\Delta$ *pfk*, increases the ratio of hydrogen to CO<sub>2</sub> and hence flux through the  
15 pentose phosphate pathway. As *gnd* encodes a protein that is downstream of the protein encoded by *zwf*, the increase in activity of *gnd*, *Gp::gnd*, in this mutant has no  
20 significant effect on the ratio H<sub>2</sub> to CO<sub>2</sub> ratio compared to the wt DH5.

**Figure 6: Utilisation of Sucrose in making hydrogen by modified DH5 $\alpha$  cscAB.** DH5 $\alpha$  cscAB with HPGC strain is able to utilise sucrose to make hydrogen like the positive control W strain with HPGC. Wild type DH5 $\alpha$  with HPGC is unable to utilise sucrose and no hydrogen is detectably made when sucrose is supplied to this strain.

25 **Detailed description of the embodiments**

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

Reference will now be made in detail to certain embodiments of the invention. While the invention will be described in conjunction with the embodiments, it will be understood that the intention is not to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and  
5 equivalents, which may be included within the scope of the present invention as defined by the claims.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials  
10 described. It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

All of the patents and publications referred to herein are incorporated by  
15 reference in their entirety.

For purposes of interpreting this specification, terms used in the singular will also include the plural and vice versa.

Microorganisms are able to synthesise molecular hydrogen using classes of enzymes known generally as hydrogenases. Seeking to harness the capacity of these  
20 enzymes to generate hydrogen, researchers have endeavoured to express hydrogenases from various microorganisms and algae in heterologous expression systems. Typically, this approach has involved screening various hydrogenases from algal and 'extremophile microorganisms'. However, efforts to generate sufficient amounts of hydrogen using this type approach have been hampered by the instability of  
25 these hydrogenases and their cognate maturation proteins, and the resulting Fe-Fe hydrogenase enzyme complex, when expressed in heterologous organisms.

In order to address such limitations, others in the field have endeavoured to co-express maturation proteins from non-cognate organisms when expressing hydrogenases in heterologous organisms. However, this approach has generally failed

to overcome the difficulties in obtaining sufficient levels of hydrogen for use in a commercial system.

Others have sought to focus on the type of hydrogenase being expressed, for example, seeking to express Ni-Fe hydrogenases rather than Fe-Fe hydrogenases.

5 Such systems have been demonstrated to be functional in vitro and efficient at producing hydrogen utilising only the NADPH dependent Ni-Fe hydrogenase and a mixture of commercially available enzymes from the pentose phosphate pathway enzymes. However, such systems have not proved commercially viable due to the cost of providing the additional enzymes. Further, the rate of hydrogen production was too  
10 slow for commercial production.

The present inventors have identified a new approach for maximising production of molecular hydrogen from algal genes expressed in heterologous host cells. The approach adopted by the inventors allows for the stable production of an Fe-Fe hydrogenase complex from various microorganisms. Moreover, the inventors believe  
15 that their approach provides for increased rates of hydrogen production over time, and increased yield (production per input). The approach of the inventors represents a major advance over previous non-optimised approaches for generation of biological hydrogen.

#### *Hydrogen Producing Gene Cluster*

The invention includes providing a microorganism, as described herein, with  
20 various nucleic acid sequences encoding components of the molecular machinery required to produce hydrogen in that microorganism. In addition, the invention provides genetically modified microorganisms comprising those nucleic acid sequences.

In particular, the present invention includes the provision of a host cell with nucleic acid sequences (including recombinant polynucleotides) encoding the HydEF,  
25 HydG and HydA proteins. In preferred embodiments, the host cell is also provided with nucleic acids encoding Ferredoxin NADP reductase (FNR) and Ferredoxin (petF). It will be appreciated that hydrogen may be produced by a microorganism, as herein described, where the microorganism is modified to express a nucleic acid sequence encoding HydA, and wherein the microorganism comprises endogenous ferredoxin.

Moreover, maturation of the hydrogenase may be accomplished using small molecules such as [2Fe]-subsite mimetics containing an azadithiolate bridge. Such factors are described, for example in Esselborn et al., (2013) *Nat Chem Biol* 9 (10):607-609, and Berggren et al., (2013) *Nature*, 499: 66-69 the contents of which are  
5 incorporated herein by reference.

However, in preferred embodiments, the microorganism is preferably modified to express the components of the HPGC as herein defined.

As used herein, the hydrogen producing gene cluster (HPGC) preferably comprises nucleic acid sequences encoding HydA, HydEF, HydG, ferredoxin NADP  
10 reductase and ferredoxin, wherein HydA refers to a Fe-Fe hydrogenase (preferably A1 subclass), and HydEF and HydG refer to the maturation and assembly complex of proteins required for formation of an active FeFe-hydrogenase.

As used herein HydA refers to any Fe-Fe-hydrogenase protein HydA, also referred to as iron hydrogenase, or iron hydrogenase HydA1 or Hyd1. This protein is  
15 encoded by the gene *hyd1*.

The skilled person will be familiar with methods for classification of different hydrogenases, including methods for determining whether a given hydrogenase is a Fe-Fe hydrogenase (including A1 class), as distinct from an Ni-Fe hydrogenase or an Fe-hydrogenase. Such methods are described for example in S ndergaard et al., (2016)  
20 Scientific Reports, 6:34212.

The HydA protein may be the HydA protein from a microorganism selected from the group consisting of: *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*, *Veillonella parvula*, *Veillonella atypica*, *Peptoclostridium bifermentans*  
25 *Clostridium arbusti*, *Pseudoflavonifractor capillosus*, *Lachnoclostridium citroniae*, *Lachnoclostridium clostridioforme*, *Pelosinus fermentans*, *Thermodesulfovibrio islandicus*, *Sutterella wadsworthensis*, *Clostridium beijerinckii*, *Fusobacterium ulcerans*, *Clostridium tyrobutyricum*, *Clostridium perfringens*, *Cetobacterium somerae*, *Clostridium beijerinckii*, *Clostridium colicanis*, *Clostridium intestinale*, *Clostridium chauvoei*,  
30 *Cellulomonas fimi*, *Ruminiclostridium thermocellum*, *Naegleria gruberi*, *Chlorella*

*variabilis*, *Fervidobacterium nodosum*, *Thermotoga petrophila*, *Thermotoga lettingae*, *Thiomicrospira pelophila*, *Caldatibacterium californiense*, *Fusobacterium necrophorum*, *Omnitrophus fodinae*, *Syntrophothermus lipocalidus*, *Ammonifex degensii*, *Desulfotomaculum hydrothermale*, *Fusobacterium mortiferum*, *Desulfotomaculum*  
 5 *kuznetsovii*, and *Lachnoclostridium phytofermentans* or functionally equivalent homologs or derivatives thereof.

Preferably, the HydA protein is selected from the group consisting of: *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*,  
 10 *Veillonella parvula*, *Veillonella atypica*, and *Peptoclostridium bifermentans*, and functionally equivalent homologs thereof. More preferably, the HydA protein is from *Chlamydomonas reinhardtii* or a functionally equivalent homolog or derivatives thereof.

Exemplary sequences of the *Chlamydomonas reinhardtii* protein sequence for HydA are provided under UniProt accession number Q9FYU1 and exemplary nucleic  
 15 acid sequences encoding said protein may be found under accession number AJ308413, CAC83731.1 (EBI) and XP\_001693376.1.

An exemplary nucleic acid sequence encoding HydA is provided in SEQ ID NO:  
 6. An exemplary promoter for enabling expression of *hydA* is provided in SEQ ID NO: 5.

Exemplary accession numbers providing sequence information for HydA from  
 20 *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*, *Veillonella parvula*, *Veillonella atypica*, and *Peptoclostridium bifermentans* include XP002956049, XP001709915, XP008860420, WP013388849 (and XP002948483), XP001330775, WP006942403, WP004697562, WP005375825 and WP021432477, respectively.

25 Exemplary codon optimised nucleic acid sequences (including restriction sites) encoding HydA from *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*, *Veillonella parvula*, *Veillonella atypica*, and *Peptoclostridium bifermentans* are provided in SEQ ID NOs: 18 to 27.

As used herein, HydEF preferably refers to the *Chlamydomonas reinhardtii* Fe-hydrogenase assembly protein HydEF, also referred to as iron hydrogenase assembly protein HydEF. This protein is encoded by the gene *hydEF*. Exemplary sequences of the *Chlamydomonas reinhardtii* protein sequence for HydEF are provided under UniProt  
5 accession number Q6PSL5 and exemplary nucleic acid sequences encoding said protein may be found under accession numbers DS496119, EDP05198.1 (EBI) and XP\_001691465.1.

An exemplary nucleic acid sequence encoding HydEF is provided in SEQ ID NO:  
2. An exemplary promoter for enabling expression of *hydEF* is provided in SEQ ID NO:  
10 1.

As used herein HydG refers to the *Chlamydomonas reinhardtii* Fe-hydrogenase assembly protein HydG, also referred to as iron hydrogenase assembly protein HydG. This protein is encoded by the gene *hydG*. Exemplary sequences of the *Chlamydomonas reinhardtii* protein sequence for HydG are provided under UniProt  
15 accession number Q6PSL4 and exemplary nucleic acid sequences encoding said protein may be found under accession number DS496119, EDP05052.1 (EBI) and XP\_001691319.1.

An exemplary nucleic acid sequence encoding HydG is provided in SEQ ID NO:  
4. An exemplary promoter for enabling expression of *hydG* is provided in SEQ ID NO: 3.

20 As used herein ferredoxin refers to the *Chlamydomonas reinhardtii* ferredoxin protein encoded by the *petF* gene. Exemplary sequences of the *Chlamydomonas reinhardtii* protein sequence for ferredoxin are provided under UniProt accession number A8IV40 and exemplary nucleic acid sequences encoding said protein may be found under accession number DS496124, EDP03827.1 (EBI) and XP\_001692808.1.

25 An exemplary nucleic acid sequence encoding ferredoxin is provided in SEQ ID NO: 8. An exemplary promoter for enabling expression of *petF* is provided in SEQ ID NO: 7.

As used herein, *Chlamydomonas reinhardtii* ferredoxin NADP reductase (FNR) refers to EC:1.18.1.2. The protein is encoded by the gene *petH* or *fnr1*. Exemplary  
30 sequences of the *Chlamydomonas reinhardtii* protein sequence for FNR are provided

under UniProt accession number A8J6Y8 and P53991 and exemplary nucleic acid sequences encoding said protein may be found under accession number DS496140, EDP00292.1 (EBI) and XP\_001697352.1.

An exemplary nucleic acid sequence encoding FNR is provided in SEQ ID NO: 9.

- 5 In preferred embodiments, the nucleic acid sequences encoding the HydEF, HydG, HydA, Ferredoxin and FNR proteins are provided in a single polynucleotide construct. In one example, the polynucleotide has the nucleic acid sequence as set forth in SEQ ID NO: 10. *Redirection oxidation of glucose towards pentose phosphate pathway*

- The inventors have found that isolation of the pentose phosphate pathway from  
10 the glycolytic pathway allows for optimal conversion of carbohydrate to hydrogen. Accordingly, in preferred embodiments, the microorganisms of the present invention are further modified to reduce or delete the expression of one or more of endogenous genes encoding phosphofructokinase, pyruvate kinase, glycerate mutase, 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase.  
15 These proteins are encoded by the genes *pfkA*, *pps*, *gpmA*, *gpmM*, *gapA*, *edd* and *eda*, respectively,

- Further, the methods of the invention include contacting the microorganism with one or more agents for inhibiting the activity or levels of one or more of the proteins phosphofructokinase, pyruvate kinase, glycerate mutase, 6-phosphogluconate  
20 dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase.

As used herein, phosphofructokinase, (E.C. 2.7.1.11 and E.C. 2.7.1.105), also known as PFK is a kinase enzyme that phosphorylates fructose 6-phosphate in glycolysis. Phosphofructokinase catalyses the phosphorylation of fructose-6-phosphate to fructose-1,6- diphosphate, a key regulatory step in the glycolytic pathway.

- 25 As used herein pyruvate kinase, (E.C. 2.7.1.40) encoded by the *pps* gene, is an enzyme that catalyses the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), yielding one molecule of pyruvate and one molecule of ATP.

As used herein glycerate mutase, may refer to either the 2,3-bisphosphoglycerate-dependent (dPGM, GpmA) or the cofactor-independent (iPGM, GpmM) phosphoglycerate mutase. 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase is encoded by the gpmA gene and catalyses the reaction 2-phospho-D-glycerate  $\leftrightarrow$  3-phospho-D-glycerate. 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (also known as gpmC; pgml; gpml or yibO) is encoded by the gpmM gene and catalyses the same reaction. The iPGM enzyme has significantly lower specific activity. Therefore, preferably pgmA is targeted for deletion or inhibition in accordance with the methods of the present invention.

As used herein 6-phosphogluconate dehydratase (E.C. 4.2.1.12) is an enzyme that catalyses the reaction 6-phospho-D-gluconate  $\leftrightarrow$  2-dehydro-3-deoxy-6-phospho-D-gluconate + H<sub>2</sub>O. Other names in common use include 6-phosphogluconate dehydratase, 6-phosphogluconic dehydrase, gluconate-6-phosphate dehydratase, gluconate 6-phosphate dehydratase, 6-phosphogluconate dehydrase, and 6-phospho-D-gluconate hydro-lyase.

As used herein 2-keto-3-deoxy-6-phosphogluconate aldolase (E.C. 4.1.2.14), commonly known as KDPG aldolase is an enzyme that catalyses the reaction 2-dehydro-3-deoxy-D-gluconate 6-phosphate  $\leftrightarrow$  pyruvate + D-glyceraldehyde 3-phosphate.

It will be appreciated that any one or more of phosphofructokinase, pyruvate kinase, glycerate mutase, 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase may be inhibited through contacting the microorganism or host cell of the invention with any agent which reduces or inhibits the levels or activity of the proteins. The inhibition may be direct or indirect. The inhibition may be partial or complete.

The inhibitor is preferably selected from: a small molecule, a peptide, an antibody, an interfering RNA, for example an antisense RNA, microRNA, shRNA, siRNA, that can reduce the activity or levels of one or more of the proteins.

In preferred embodiments, the microorganism or host cell is genetically modified so as to completely delete or partially reduce the expression of one or more of the genes *pfkA*, *pps*, *gpmA*, *gpmM*, *gapA*, *edd* and *eda*.

The skilled person will be familiar with various techniques for deleting or  
5 modifying gene sequences so as to partially or complete reduce gene expression. In certain embodiments, the genetic modification is by use of a CRISPR-Cas9 system. Other genome editing techniques that can be employed include the lambda red recombinase system, random mutagenesis and selection and Multiplex Automated Genome Engineering (MAGE). In one example, a combination of CRISPR-Cas9 and  
10 lambda red recombinase may be used, such as outlined in Reisch CR and Prather KL, (2015) *The no-SCAR (Scarless Cas9 Assisted Recombineering) system for genome editing in Escherichia coli*, Sci Rep. 14(5):15096.

The reduced expression of any one or more of the *pfkA*, *pps*, *gpmA*, *gpmM*, *gapA*, *edd* and *eda* genes may be a reduced expression of at least 20%, at least 30%,  
15 at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or 100% reduced expression.

The gene which is deleted or has reduced expression is preferably *pfk* or *gpmA*. In other embodiments, both *pfk* and *gpmA* or *pfk* and *gpmM* are deleted. Further still, both *edd* and *eda* may be deleted. In alternative embodiments, *gpmM* and/or *gpmA* in  
20 combination with *edd* and *eda* are deleted. In still further embodiments, *pfk*, *edd-eda* and *gpmA* or *gpmM* are deleted.

To further direct oxidation of glucose toward the pentose phosphate pathway, and to maximise the rate of production and yield of hydrogen, the invention also contemplates the increased expression or activity of various endogenous genes (or  
25 inhibition of the proteins they encode).

Accordingly, in preferred embodiments, the microorganisms of the present invention are further modified to increase the levels or activity of one or more of the genes encoding phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, 6-glucophosphonate dehydrogenase NAD kinase and  
30 soluble pyridine nucleotide transhydrogenase. These proteins are encoded by the

genes *pgm*, *zwf*, *pgl*, *gnd*, *yfjB* and *sthA* respectively. Moreover, the methods of the invention include increasing the expression of one or more of the proteins phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, and 6-phosphogluconate dehydrogenase.

5 As used herein phosphoglucomutase, (PGM) (E.C. 5.3.1.9), also known as glucose-phosphate isomerase, phosphoglucose isomerase/phosphoglucoisomerase (PGI) or phosphohexose isomerase (PHI), is an enzyme that functions as a glycolytic enzyme (glucose-6-phosphate isomerase) that interconverts glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P). Since the reaction is reversible, its direction is  
10 determined by G6P and F6P concentrations.

In certain embodiments, level or activity of PGM is increased by increasing the expression of the endogenous *pgm* gene, for example, by introducing a promoter that enables increased expression of the gene.

In preferred embodiments, the promoter of the *pgm* gene in *E. coli* is replaced  
15 with the *gapA* promoter from *E. coli*. An exemplary *gapA* promoter (*gapAp*) 5'-3' is set forth in SEQ ID NO: 13.

As used herein glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), also known as G6PD is an enzyme that catalyses the chemical reaction D-glucose 6-phosphate + NADP+  $\rightleftharpoons$  6-phospho-D-glucono-1,5-lactone + NADPH + H+. G6PD converts G6P into  
20 6-phosphoglucono- $\delta$ -lactone and is the rate-limiting enzyme of the pentose phosphate pathway. Thus, regulation of G6PD has downstream consequences for the activity of the rest of the pentose phosphate pathway. Glucose-6-phosphate dehydrogenase is stimulated by its substrate G6P. In *E. coli*, the *zwf* gene encodes glucose-6-phosphate 1-dehydrogenase. An exemplary amino acid sequence of the *E. coli* G6PD protein can  
25 be found under Uniprot accession POAC53, and exemplary nucleic acid sequences under accession numbers M55005, NP\_416366.1, and NC\_000913.3. The cognate protein from *Zymomonas mobilis* is encoded by *zwf*, an exemplary nucleic acid sequence of which is provided in SEQ ID NO: 16, herein.

In certain embodiments, level or activity of G6PD is increased by increasing the  
30 expression of the endogenous *zwf* gene, for example, by introducing a promoter that

enables increased expression of the gene. In certain embodiments, the *E. coli zwf* promoter is replaced with the *osmY* promoter (*osmYp*). An exemplary *osmY* promoter (*osmYp*) sequence is set forth in SEQ ID NO: 12. In alternative embodiments, the *E. coli zwf* promoter is replaced with the *E. coli gapA* promoter as set forth in SEQ ID NO: 13.

5           In preferred embodiments, level or activity of G6PD is increased by replacing or supplementing the *zwf* gene of *E. coli* with the *zwf* gene from *Zymomonas mobilis*. In further embodiments, the *zwf* gene of *E. coli* is replaced or supplemented with the *zwf* gene from any gram negative facultative bacterium.

As used herein 6-phosphogluconolactonase, (E.C. 3.1.1.31), also known as  
10 6PGL or PGLS, is an enzyme that catalyzes the hydrolysis of 6-phosphogluconolactone to 6-phosphogluconic acid (or 6-phospho-D-gluconate + H<sup>+</sup>) in the oxidative phase of the pentose phosphate pathway. 6-phosphogluconolactonase catalyzes the conversion of 6-phosphogluconolactone to 6-phosphogluconic acid, both intermediates in the oxidative phase of the pentose phosphate pathway, in which glucose is converted into  
15 ribulose 5-phosphate. The oxidative phase of the pentose phosphate pathway releases CO<sub>2</sub> and results in the generation of two equivalents of NADPH from NADP<sup>+</sup>. The final product, ribulose 5-phosphate, is further processed by the organism during the non-oxidative phase of the pentose phosphate pathway to synthesize biomolecules including nucleotides, ATP, and Coenzyme A. The enzyme that precedes 6PGL in the pentose  
20 phosphate pathway, glucose-6-phosphate dehydrogenase, exclusively forms the  $\delta$ -isomer of 6-phosphogluconolacton. An exemplary *E. coli* 6PGL sequence can be found under Uniprot accession P52697 and exemplary nucleic acid sequence can be found under accession numbers U27192, NP\_415288.1 and NC\_000913.3.

In certain embodiments, level or activity of PGL is increased by increasing the  
25 expression of the endogenous *pgl* gene, for example, by introducing a promoter that enables increased expression of the gene.

In preferred embodiments, promoter of the *pgl* gene in *E. coli* is replaced with the *gapA* promoter from *E. coli*. An exemplary *gapA* promoter (*gapAp*) 5'-3' is set forth in SEQ ID NO: 13.

As used herein glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12), also known as GAPDH, and less commonly as G3PDH, is encoded by the *gapA* gene in *E. coli*. The protein catalyses the oxidative phosphorylation of glyceraldehyde 3-phosphate (G3P) to 1,3-bisphosphoglycerate (BPG) using the cofactor NAD. The first reaction step  
5 involves the formation of a hemiacetal intermediate between G3P and a cysteine residue, and this hemiacetal intermediate is then oxidized to a thioester, with concomitant reduction of NAD to NADH. The reduced NADH is then exchanged with the second NAD, and the thioester is attacked by a nucleophilic inorganic phosphate to produce BPG. An exemplary amino acid sequence of the *E. coli* GapA protein can be  
10 found under Uniprot accession POA9B2, and exemplary nucleic acid sequences under accession numbers X02662, NP\_416293.1 and NC\_000913.3. The cognate protein from *Clostridium acetobutylicum* is encoded by *gapC*, an exemplary nucleic acid sequence of which is provided in SEQ ID NO: 15, herein.

In certain embodiments, level or activity of GAPDH is decreased by decreasing  
15 or eliminating the expression of the endogenous *gapA* gene, by deleting the gene or changing the promoter to reduce the expression of the gene.

In preferred embodiments, the *gapA* gene in *E. coli* is replaced with the *gapC* gene from *Clostridium acetobutylicum*.

As used herein 6-glucophosphonate dehydrogenase, also referred to as 6-phosphogluconate dehydrogenase, decarboxylating (E.C. 1.1.1.44), is an enzyme that  
20 catalyses the oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate and CO<sub>2</sub>, with concomitant reduction of NADP to NADPH. In *E. coli*, 6-glucophosphonate dehydrogenase is encoded by the *gnd* gene. An exemplary amino acid sequence of the *E. coli* 6-glucophosphonate dehydrogenase can be found under  
25 UniProt accession P00350, and nucleic acid sequences can be found under accession numbers K02072, NP\_416533.1 and NC\_000913.3. The cognate protein from *Corynebacterium glutamicum* is encoded by *gnd*, an exemplary nucleic acid sequence of which is provided in SEQ ID NO: 14, herein.

In certain embodiments, level or activity of 6-glucophosphonate dehydrogenase  
30 is increased by increasing the expression of the endogenous *gnd* gene, for example, by introducing a promoter that enables increased expression of the gene. In certain

embodiments, wherein the microorganism is *E. coli*, the promoter of the *gnd* gene in *E. coli* is replaced with the *gapA* promoter from *E. coli*. An exemplary *gapA* promoter (*gapAp*) 5'-3' is set forth in SEQ ID NO: 13. In alternative embodiments, the *E. coli gnd* promoter is replaced with the *osmY* promoter (*osmYp*). An exemplary *osmY* promoter  
 5 (*osmYp*) sequence is set forth in SEQ ID NO: 12.

In particularly preferred embodiments, the endogenous promoter for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with a promoter selected from the group consisting of: the *osmY* promoter, the *gapA* promoter, the *nirB* promoter and the *nar* promoter.

10 In preferred embodiments, the *gnd* gene in from the microorganism (.e.g, *E. coli*) is replaced with the *gnd* gene from *Corynebacterium glutamicum*.

In particularly preferred embodiments, the endogenous promoter for one or more of *pgm*, *zwf*, *pgl*, *gnd*, *tktB* or *tktA*, and *talA* or *talB* is replaced with a promoter selected from the group consisting of: the *osmY* promoter, the *gapA* promoter, the *nirB* promoter  
 15 and the *nar* promoter. Preferably the *osmY*, *gapA*, *nirB* and/or *nar* promoters are the endogenous promoters of the organism. More preferably, wherein the microorganism is *E. coli*, the *osmY*, *gapA*, *nirB* and/or *nar* promoters are from *E. coli*.

#### *Sucrose metabolising genes*

Since most *E. coli* strains are unable to utilise sucrose as a source of carbon, the  
 20 microorganisms and methods of the present invention also include modification of the host microorganisms to enable metabolism of sucrose. In certain embodiments, this can be accomplished by modifying the microorganism to express a gene cluster, *cscRAKB* which have been identified in those strains of *E. coli* which are able to metabolise sucrose.

25 Thus, in preferred embodiments, the methods of the invention further comprise providing the host microorganism with a recombinant polynucleotide for enabling expression of nucleic acid sequences encoding sucrose hydrolase (encoded by the *cscA* gene) and sucrose permease (encoded by the *cscB* gene). In further embodiments, the methods also comprise providing the microorganism with a

recombinant polynucleotide encoding the regulatory proteins CscR and CscK (encoded by the genes *cscR* and *cscK*, respectively).

As used herein, sucrose hydrolase refers to the enzyme sucrose-6-phosphate hydrolase, also referred to as sucrose or invertase (E.C. 3.2.1.26) encoded by the *cscA* gene. An exemplary amino acid sequence of sucrose hydrolase is provided under UniProt accession P40714 and exemplary nucleotide sequences are provided under accession number X81461.

As used herein, sucrose permease refers to the protein encoded by the *cscB* gene. Sucrose permease is also known as sucrose transport protein and an exemplary amino acid sequence can be found under Uniprot accession number P3000. An exemplary nucleotide sequence encoding sucrose permease can be found under accession X63740 or X81461.

Further still, the *E. coli* microorganism may be genetically modified to increase the levels or activity of endogenous *E. coli* phosphoglucomutase (*pgm*) or xylose isomerase (*xylA*). The genetic modification may be to increase expression of the endogenous gene (for example, by modification of the promoter region) or by introduction and expression of an exogenous nucleic acid encoding the gene.

In further embodiments, the microorganism is modified to express sucrose phosphorylase from *Leuconostoc mesenteroides*

## **Nucleic acids**

An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide encoding nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes nucleic acid molecules contained in cells that ordinarily express the nucleic acid where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The terms “nucleic acid molecule” and “polynucleotide” are used interchangeably herein and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogues thereof. Non-limiting examples of polynucleotides include a gene, a gene fragment, messenger RNA (mRNA), cDNA, 5 recombinant polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A nucleic acid sequence which “encodes” a selected polypeptide is a nucleic acid molecule which is transcribed (in the case of DNA) and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are 10 determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A transcription termination sequence may be located 3' to the coding sequence.

Polynucleotides of the invention can be synthesised according to methods well known in the art, as described by way of example in Sambrook et al (1989, Molecular 15 Cloning—a laboratory manual; Cold Spring Harbor Press).

As used herein, “codon optimised” refers to optimisation of the DNA sequence to resemble the codon usage of genes in host microorganism. In preferred embodiments, the codon usage in the sequence is optimised to resemble that of highly expressed *E. coli* genes.

20 The polynucleotide molecules of the present invention may be provided in the form of an expression cassette which includes control sequences operably linked to the inserted sequence, thus allowing for expression of the polypeptide. These expression cassettes, in turn, are typically provided within vectors (e.g., plasmids or recombinant vectors). A suitable vector may be any vector which is capable of carrying a sufficient 25 amount of genetic information, and allowing expression of a polypeptide of the invention.

The present invention thus includes expression vectors that comprise such polynucleotide sequences. Expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate 30 initiators, promoters, enhancers and other elements which may be necessary, and which are positioned in the correct orientation, in order to allow for expression of a

desired polypeptide. Other suitable vectors would be apparent to persons skilled in the art. By way of further example in this regard we refer to Sambrook et al.

Thus, a polypeptide of the invention may be provided by delivering such a vector to a cell and allowing transcription from the vector to occur. The skilled person will be  
5 familiar with standard techniques for delivery such expression vectors to a cell, including transformation techniques and the like.

The vector may be a plasmid. In certain embodiments, the plasmid is a high copy number plasmid or a low copy number plasmid. Vectors are well known in the art and may include cloning vectors, expression vectors, etc. A cloning vector is a recombinant  
10 nucleic acid construct which is able to replicate autonomously or integrated in the genome in a host cell, and which is further characterized by one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the new recombinant vector retains its ability to replicate in the host cell. In the case of plasmids, replication of the desired  
15 sequence may occur many times as the plasmid increases in copy number within the host bacterium or just a single time per host before the host reproduces by mitosis. In the case of phage, replication may occur actively during a lytic phase or passively during a lysogenic phase. An expression vector is a recombinant nucleic acid construct into which a desired DNA sequence may be inserted by restriction and ligation such that  
20 it is operably joined to regulatory sequences and may be expressed as an RNA transcript. Vectors may further contain one or more marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which  
25 encode polypeptides or enzymes whose activities are detectable by standard assays known in the art (e.g.,  $\beta$ -galactosidase, luciferase or alkaline phosphatase), and genes which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques (e.g., fluorescent proteins such as green fluorescent protein). Preferred vectors are those capable of autonomous replication and expression of the structural gene  
30 products present in the DNA segments to which they are operably joined.

As used herein, a coding sequence and regulatory sequences are said to be "operably" joined or linked when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory sequences. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined or linked if induction of a promoter in the 5' regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined or linked to a coding sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript can be translated into the desired protein or polypeptide.

The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribed and 5' non-translated sequences involved with the initiation of transcription and translation respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. In particular, such 5' non-transcribed regulatory sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined gene.

Regulatory sequences may also include enhancer sequences or upstream activator sequences as desired. The vectors of the invention may optionally include 5' leader or signal sequences. The choice and design of an appropriate vector is within the ability and discretion of one of ordinary skill in the art.

A "promoter" is a nucleotide sequence which initiates and regulates transcription of a polypeptide-encoding polynucleotide. Promoters can include inducible promoters (where expression of a polynucleotide sequence operably linked to the promoter is induced by an analyte, cofactor, regulatory protein, etc.), repressible promoters (where expression of a polynucleotide sequence operably linked to the promoter is repressed by an analyte, cofactor, regulatory protein, etc.), and constitutive promoters. It is intended that the term "promoter" or "control element" includes full-length promoter

regions and functional (e.g., controls transcription or translation) segments of these regions.

The nucleic acids of the present invention are preferably operably linked to promoters such that the subject enzymes are expressed in the cell when cultured under  
5 suitable conditions for enabling production of hydrogen, as described herein. The promoters may be specific for individual bacterial cell species. The promoter may be a heterologous promoter which increases the expression of the gene above the typical expression level observed in the cell. The promoter may be an inducible promoter.

A polynucleotide, expression cassette or vector according to the present  
10 invention may additionally comprise a signal peptide sequence. The signal peptide sequence is generally inserted in operable linkage with the promoter such that the signal peptide is expressed and facilitates secretion of a polypeptide encoded by coding sequence also in operable linkage with the promoter. It may further be understood that in any embodiment, any of the exemplary expression cassettes, vectors or sequences  
15 described herein may be further modified so as to not include a signal peptide sequence.

Any appropriate expression vector (e.g., as described in Pouwels et al., Cloning Vectors: A Laboratory Manual (Elsevier, N.Y.: 1985)) and corresponding suitable host can be employed for production of recombinant polypeptides. Expression hosts include,  
20 but are not limited to, bacterial species within the genera *Escherichia*, *Bacillus*, *Pseudomonas*, *Salmonella*, host cell systems and the like. The skilled person is aware that the choice of expression host has ramifications for the type of polypeptide produced.

In some embodiments, the cell is engineered or selected (e.g., as described  
25 herein) to produce or have altered, optionally increased, production of a molecule of interest. In some embodiments, the cell comprises a deletion or mutation of one or more genes (e.g., one or more regulatory or competing metabolic genes as described herein). In other examples, the one or more genes that are deleted or mutated are in a competing pathway. Mutations can be single or multiple point mutations, additions,  
30 partial internal deletions, N-terminal or C-terminal deletions (truncations), or complete deletions, all of which can affect amino acid sequence encoded the gene(s).

Deletions or mutations can be made using standard methods in the art. Mutations can be non-random, partially random or random, or a combination of these mutations. For example, for a partially random mutation, the mutation(s) may be confined to a certain portion of the nucleic acid molecule encoding a polypeptide in which mutation(s)

5 are to be made.

#### *Culturing and modification of microorganisms*

In particularly preferred embodiments, culturing of the microorganisms or host cells, as described herein, is performed under aerobic conditions initially to produce biomass then transferred to anaerobic conditions to induce anaerobiosis during

10 expression of the HPGC. The skilled person will be familiar with techniques for creating anaerobiosis, including with the addition of a neutral gas (such as N<sub>2</sub>) or a reductant. However, it will also be appreciated that anaerobiosis can be accomplished simply by culturing the microorganisms in a sealed container in the presence of an oxidisable carbon source.

15 Further still, culturing of the microorganisms or host cells is preferably performed by inclusion of ferric (iron III) or ferrous (iron II) salts in the culture media. Preferably the ferric (iron III) or ferrous (iron II) salts are provided at a final concentration in the media of at least about 5 µM, at least about 10 µM, at least about 20 µM or at least about 30 µM or more. Preferably the final concentration of ferric (iron III) or ferrous (iron II) salts

20 provided in the culture media is equal to or greater than about 20 µM.

The skilled person will appreciate that culturing of recombinant host cells for production of recombinant proteins will be carried out at a temperature that is optimal for the growth and expression of proteins in the organism. For example, the optimum temperature for growth of *E. coli* and related bacterial organisms is about 37 °C and the

25 temperature for growth of yeasts for producing recombinant proteins is about 30-32°C. However, the present inventors have found that expression of functional hydrogenase can be further enhanced when using a bacterial expression system, such as *E. coli*, when the culturing temperature is reduced. Accordingly, in preferred embodiments, where the microorganism or host cell is *E. coli*, the culturing temperature is no more

30 than about 30°C. The temperature may be between about 10°C to about 30°C, preferably at least about 15 °C. In certain embodiments, the temperature is about 20°C

to about 30°C. In particularly preferred embodiments, the temperature is about 20°C (for example, 18°C, 19°C, 20°C, 21°C, 22°C). .

“Genetically engineered” or “genetically modified” refers to any cell modified by any recombinant DNA or RNA technology. In other words, the cell has been transfected, 5 transformed, or transduced with a recombinant polynucleotide molecule, and thereby been altered so as to cause the cell to alter expression of a desired protein. Methods and vectors for genetically engineering host cells are well known in the art; for example, various techniques are illustrated in Current Protocols in Molecular Biology, Ausubel et al., eds. (Wiley & Sons, New York, 1988, and quarterly updates). Genetic engineering 10 techniques include but are not limited to expression vectors, targeted homologous recombination, and gene activation (see, for example, U.S. Pat. No. 5,272,071), and trans-activation by engineered transcription factors (see, for example, Segal et al., 1999, Proc Natl Acad Sci USA 96(6):2758-63).

In certain embodiments, the genetic modifications described herein result in an 15 increase in gene expression or function and can be referred to as amplification, overproduction, overexpression, activation, enhancement, addition, or up-regulation of a gene. More specifically, reference to increasing the action (or activity) of enzymes or other proteins discussed herein generally refers to any genetic modification in the microorganism in question that results in increased expression and/or functionality 20 (biological activity) of the enzymes or proteins and includes higher activity of the enzymes (e.g., specific activity or in vivo enzymatic activity), reduced inhibition or degradation of the enzymes, and overexpression of the enzymes. For example, gene copy number can be increased, expression levels can be increased by use of a promoter that gives higher levels of expression than that of the native promoter, or a 25 gene can be altered by genetic engineering or classical mutagenesis to increase the biological activity of an enzyme. Combinations of some of these modifications are also possible.

As used herein, the term "exogenous polynucleotides" is intended to mean polynucleotides that are not derived from naturally occurring polynucleotides in a given 30 organism. Exogenous polynucleotides may be derived from polynucleotides present in a different organism. In accordance with the present invention, an *E. coli* cell may be

genetically modified with a nucleic acid construct which contains one or more exogenous polynucleotides, encoding one or more enzymes which enable the cell to produce hydrogen.

5 The exogenous polynucleotides may be heterologous or homologous. The term "heterologous" refers to a molecule or activity derived from a source other than the referenced species whereas "homologous" refers to a molecule or activity derived from the host microbial organism. Accordingly, exogenous expression of a nucleic acid molecule of the invention can be through the use of either or both a heterologous or homologous nucleic acid molecule.

10 The exogenous polynucleotides may be provided in one or more expression constructs (plasmid vectors).

Methods of transforming microorganisms are well known in the art, and can include such non-limiting examples as electroporation, calcium chloride-, or lithium acetate-based methods.

15 The skilled person will be familiar with methods for confirming successful transformation of relevant constructs, as well as methods for determining whether the transformants possess the relevant enzyme activity provided by the encoded protein. For example, phosphofructokinase activity (and therefore inferring correct protein folding of the encoded protein) can be inferred using a commercially available enzyme  
20 assay kit.

Similarly, the skilled person will be familiar with standard techniques to confirm inhibition or deletion of the level of activity of a relevant protein or level of expression of the relevant gene. Successful gene modification, deletion or replacement can be confirmed using standard sequencing techniques. Successful inhibition of protein  
25 activity following contacting the cell with an inhibitor can be assessed by assessing for the activity of the relevant protein, for example using a commercially available enzyme assay kit.

The skilled person will also be familiar with general culturing techniques required to induce expression of the polynucleotides in the recombinant microorganism, and  
30 thereby induce production of the proteins of the HPGC to produce hydrogen, when

required. In some examples, a liquid culture of the recombinant microorganism is grown under anaerobic conditions, supplemented with glucose.

Successful transformation can also be determined by the inclusion of selection marker genes in the plasmid of vector to be transformed into the cell. As used herein, 5 the term "selection marker genes" refer to genetic material that encodes a protein necessary for the survival and/or growth of a host cell grown in a selective culture medium. Typical selection marker genes for use in microorganisms, including in *E. coli* are well known to the skilled person.

Measurement of hydrogen production can be by any suitable method including 10 as outlined in the Examples. In one simple example, hydrogen production can be gauged simply by observing for the production of bubbles of gas in the culture. In other examples, the production and quantification of hydrogen production is by sampling the gas bubbles and analysing the gas composition by gas chromatography with detection by thermal conductivity or mass spectrometry. In other examples, a Clark-type electrode 15 known to the skilled person may be used, or any other suitable method for detecting hydrogen production.

In any embodiment of the invention, the microorganism, preferably an *E. coli* microorganism, may be stored for a period of time prior to inducing the production of hydrogen. For example, in certain embodiments, the microorganism of the invention or 20 methods described herein may involve transformation of the microorganism with the required polynucleotides in order to generate a recombinant microorganism capable of generating hydrogen. The microorganism may then be harvested and stored under conditions suitable for storage of the microorganism (for example, at 4°C, -20°C or -80°C in a suitable buffer) until required for hydrogen production. It will also be 25 appreciated that the microorganism may be lyophilised until required for further use. Further, it will be understood that the microorganism can be grown under conditions to enable expression of the HPGC and then harvested, where necessary stored, and then resuspended in appropriate solutions supplemented with glucose to initiate bacterial production of hydrogen.

In some examples, the cultured bacteria that have been produced, and that have expressed the HPGC are harvested and fed glucose under isoosmotic conditions to produce hydrogen.

5 In certain embodiments, the bacteria are encapsulated, for example in calcium alginate beads using standard techniques and are fed glucose in an isosmotic media to produce hydrogen. The skilled person will be familiar with standard manual and mechanism techniques and equipment for bio-encapsulation, including by using a device such as the Inotech Encapsulator IE-50R (EncapBioSystems Inc), or Encapsulator B-390/B-395 pro (Buchi), or related systems. Other methods are  
10 described, for example in: Heidebach, et al., (2012) Critical Reviews in Food Science and Nutrition, 52: 291-311; Martín et al., (2015) Innovative Food Science & Emerging Technologies 27:15-25, the entire contents of which are hereby incorporated by reference.

In other examples, the recombinant microorganism does not need to be viable  
15 (i.e., capable of reproducing, "growing" or increasing in cell numbers) in order to be able to produce hydrogen in accordance with the present invention. For example, in any embodiment, the methods involve providing or generating a recombinant microorganism as herein described, culturing the microorganism under conditions and for a sufficient time to induce expression of the proteins required for producing hydrogen (e.g., the  
20 proteins encoded by the HPGC) and then inactivating the microorganism. Preferably, the inactivated microorganisms remain intact, although it will be understood that this is not an essential requirement.

Inactivated recombinant microorganisms of the invention can be then be used to generate hydrogen, for example as described herein in the Examples.

25 The skilled person will be familiar with methods for inactivating microorganisms so that the cells remain intact, but can still be utilised to produce hydrogen (i.e., from the HPGC and other proteins that have been expressed by the cells). Inactivation may be by gamma irradiation or by treatment with an antibiotic (such as mitomycin or similar).

In any embodiment, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%  
30 or 100% of the microorganisms are inactivated.

*Systems and devices*

The present invention also provides systems and devices comprising the microorganisms of the invention, or reactor systems which include methods described herein for producing hydrogen.

5 In preferred embodiments, the invention further comprises a hydrogen gas collection system for collecting hydrogen gas produced by the microorganisms. A hydrogen gas collection system can be included in the reactor system such that the hydrogen gas generated is collected and is optionally stored for use. Alternatively, the generated hydrogen gas can be directed to a point of use, such as, for example, to a  
10 hydrogen fuel powered device.

In some embodiments, a hydrogen gas collection unit includes one or more hydrogen gas conduits for directing a flow of hydrogen gas produced in the reactor system to a storage container or directly to a point of use. In other embodiments, a hydrogen gas conduit is optionally connected to a source of a sweep gas, wherein the  
15 hydrogen gas is collected using the sweep gas. An exemplary sweep gas is nitrogen. For example, as hydrogen gas is initially produced, a sweep gas can be introduced into a hydrogen gas conduit, flowing in the direction of a storage container or point of hydrogen gas use. In further embodiments, a hydrogen collection system can include a container for collection of hydrogen from the reactor system. In still other embodiments,  
20 a collection system can further include a conduit for passage of hydrogen. The conduit and/or container can be in gas flow communication with a channel provided for outflow of hydrogen gas from the reaction chamber

Fuel cells are electrochemical devices that convert the energy of a fuel directly into electrochemical and thermal energy. Typically, a fuel cell consists of an anode and  
25 a cathode, which are electrically connected via an electrolyte. A fuel such as, for example, hydrogen, is fed to the anode where it is oxidized with the help of an electrocatalyst. At the cathode, the reduction of an oxidant such as oxygen (or air) takes place. The electrochemical reactions which occur at the electrodes produce a current and thereby electrical energy. Commonly, thermal energy is also produced which may  
30 be harnessed to provide additional electricity or for other purposes. Currently, the most common electrochemical reaction for use in a fuel cell is that between hydrogen and

oxygen to produce water. Molecular hydrogen itself can be fed to the anode where it is oxidized, and the electrons produced are passed through an external circuit to the cathode where oxidant is reduced. Ion flow through an intermediate electrolyte maintains charge neutrality.

- 5           The fuel cells of the present subject matter utilize hydrogen as a fuel wherein the source of hydrogen is from the recombinant microorganisms of the present subject matter.

Typically, hydrogen is present in the fuel source in an amount of at least about 2% by volume, preferably at least about 5% and more preferably at least about 10% by  
10 volume, for example about 25%, 50%, 75% or 90% by volume. Where an inert gas is used to form part of the fuel gas, the inert gas is typically present in an amount of at least about 10%, such as at least about 25%, 50 % or 75% by volume, most preferably at least about 80% by volume.

Generally, the fuel source is supplied from an optionally pressurized container of  
15 the fuel source in gaseous or liquid form. The fuel source is supplied to the electrode via an inlet, which can optionally comprise a valve. An outlet is also provided which enables used or waste fuel source to leave the fuel cell.

The oxidant typically includes oxygen, although any other suitable oxidant can be used. The oxidant source typically provides the oxidant to the cathode in the form of a  
20 gas which includes the oxidant, in some embodiments, the oxidant can be provided in liquid form. Generally, the oxidant source also includes an inert gas, although the oxidant in its pure form can also be used. For example, a mixture of oxygen with one or more gases such as nitrogen, helium, neon or argon can be used. The oxidant source can optionally comprise further components, for example alternative oxidants or other  
25 additives. An example of a suitable oxidant source is air.

Typically, oxygen is present in the oxidant source in an amount of at least about 2% by volume, preferably at least about 5% and more preferably at least about 10% by volume.

Generally, the oxidant source is supplied from an optionally pressurized container  
30 of the oxidant source in gaseous or liquid form. The oxidant source is supplied to the

electrode via an inlet, which optionally comprises a valve. An outlet is also provided which enables used or waste oxidant source to leave the fuel cell.

The anode can be made of any conducting material for example stainless steel, brass or carbon, which can be graphite. The surface of the anode can, at least in part,  
5 be coated with a different material which facilitates adsorption of the catalyst. The surface onto which the catalyst is adsorbed is of a material which does not cause the hydrogenase to denature. Suitable surface materials include graphite, such as, for example, a polished graphite surface or a material having a high surface area such as carbon cloth or carbon sponge. Materials with a rough surface and/or with a high  
10 surface area are generally preferred.

The cathode can be made of any suitable conducting material which will enable an oxidant to be reduced at its surface. For example materials used to form the cathode in conventional fuel cells can be used. An electrocatalyst can, if desired, be present at the cathode. This electrocatalyst can, for example, be coated or adsorbed on the  
15 cathode itself, or it can be present in a solution surrounding the cathode. Suitable electrocatalysts include those used in conventional fuel cells such as platinum. Biological catalysts can also be used for this purpose, and in particular, the combination of enzymes and accessory proteins described herein.

The fuel cell of the present subject matter is typically operated at a temperature  
20 of at least about 25°C, more preferably at least about 30°C. It is preferred that the fuel cell is operated at a temperature of from about 35 °C to about 65°C, such as from about 40°C to about 50°C. A higher temperature increases the rate of reaction and leads to a higher oxidation current.

A fuel cell, as described above, can be operated under the conditions described  
25 above, to produce a current in an electrical circuit. The fuel cell is operated by supplying hydrogen to the anode and supplying an oxidant to the cathode. The fuel cell of the invention is capable of producing current densities of at least about 0.5 mA, typically at least about 0.8 mA, 1 mA or 1.5 mA per cm<sup>2</sup> of surface area of the positive electrode. For example, the fuel cell of the invention can produce a current of at least about 2 mA,  
30 such as at least about 3 mA per cm<sup>2</sup> of surface area of the positive electrode.

## Examples

### Example 1

#### Materials and methods

##### *Bacterial strains and plasmids*

5 Wild-type (DH5 $\alpha$ ) *Escherichia coli* NEB 5- $\alpha$  (CP017100.1, (Anton and Raleigh, 2016)), an immediate *fhuA2* derivative of DH5 $\alpha$  and derivative of K-12, was purchased from New England Biolabs and maintained on plates containing Luria-Bertani (LB) medium and 1.5% agar. Antibiotics chloramphenicol (Cam) 30 mg L<sup>-1</sup> and kanamycin sulfate (Kan) 50 mg L<sup>-1</sup> were included as required. Mutant strains of DH5 $\alpha$   
10 were constructed using CRISPR/Cas9 methods (Reisch and Prather, 2015). Plasmid pHPGC (Cam<sup>R</sup>) was constructed using standard Biobrick assembly methods, restriction digest and ligation. The resulting plasmid was transformed into WT and mutant strains by standard procedures. Strains and plasmids used for hydrogen production are listed in Table 2. The sequences of the various components of the HPGC and heterologous  
15 promoter and gene sequences are given in Table 1. The genome sequences of wild type and mutant strains used for hydrogen production were confirmed using Nanopore Sequencing technology.

##### *Fermentation*

Pre-cultures in 100 mL Super Optimal broth with Catabolite repression (SOC)  
20 media containing Cam were inoculated with single colonies of *E. coli* DH5 $\alpha$ , DH5 $\alpha$ -HPGC,  $\Delta$ pfkA-HPGC or  $\Delta$ gpmA-HPGC (using the HPGC constructs specified in SEQ ID NO: 10 and 30-40). The pre-cultures were incubated overnight at 37°C to approximately OD<sub>600</sub> 2 (1.6x10<sup>9</sup> cells) and inoculated into 2 L of SOC media (pH 7) with the addition of sterile filtered (0.2  $\mu$ m) 20 mM D-glucose, 1 mM iron sulfate and Cam (30 mg L<sup>-1</sup>).

25 Cells were grown anaerobically to OD<sub>600</sub> 0.6 (4.8x10<sup>8</sup> cells) at 100 rpm agitation speed with temperature being controlled at 37°C and pH maintained at 7 by titrating 1 M sodium hydroxide with the aid of a fermenter (Eppendorf, BioFlow 120 and BioFlo®/CelliGen® 115 Fermenter/Bioreactor). When cell growth reached OD<sub>600</sub> 0.6 (4.8x10<sup>8</sup> cells), the culture temperature was reduced to 18°C prior to induction. The

cells were induced with sterile filtered Isopropyl-b-D-thiogalactopyranoside (IPTG, 1 mM) and 1 mM iron sulfate. Fermentation parameters during induction were the same as above and cells were grown overnight to approximately OD<sub>600</sub> 2 (1.6x10<sup>9</sup> cells). Following, cells were harvested by centrifugation, 4650 rcf for 15 minutes at 18°C. The  
5 cell pellets were washed three times in 1x phosphate-buffered saline (PBS) pH 7.4 (10 mM) or 1x PBS (approximately 50 mM) pH 8.0 containing 1 mM sodium dithionite and stored at 4°C.

#### *Biohydrogen reactor*

Cell pellets of DH5 $\alpha$ , DH5 $\alpha$ -HGPC,  $\Delta$ pfkA-HGPC or  $\Delta$ gpmA-HGPC obtained  
10 through fermentation were resuspended in 1x PBS (10 mM) pH 7.4 or 1x PBS (approximately 50 mM) pH 8.0 at 20 OD<sub>600</sub> with optionally 1 mM dithionite (final volume 50 mL) and placed into a 100 mL side-arm conical flask to test and measure hydrogen production. The flask was sealed with a rubber stopper with a pH probe protruding into the suspended cells. The side-arm of the flask was connected to a custom-made  
15 apparatus designed to measure gas volume. A Teflon coated magnetic bar was placed inside the conical flask and the flask was placed on a magnetic stirrer plate. The headspace of the conical flask was purged with three volumes of 100% nitrogen gas prior to the addition of D-glucose (final concentration of 20 mM) to initiate bacterial hydrogen formation. The experiment was performed at room temperature of  
20 approximately 22°C.

#### *Hydrogen, carbon dioxide and pH measurements*

Headspace gas samples (5  $\mu$ L) and pH measurements were taken and recorded, respectively, from the biohydrogen reactor at the start (immediately following D-glucose addition) and approximately every 15-20 minutes until gas production has almost  
25 ceased. The gas samples were analysed using Shimadzu Nexis, GC-2030 with column (Restek, ShinCarbon ST Micropacked GC Column, Cat. # 19808) and GC method: SPL1 temperature 100°C, column flow 6 mL.min<sup>-1</sup>, DTCD temperature 180°C, oven temperature 40°C held for 3 minutes, then to 170°C at 15°C.min<sup>-1</sup>, hold for 2 minutes at 170°C. Carrier gas was Argon. Column specifications, ShinCarbon ST, 100/120 mesh, 2  
30 m, 1/16 in. OD, 1.0 mm).

Gas standards (20% hydrogen, 20% nitrogen, 20% carbon monoxide and 20% carbon dioxide [product number: PGS402470D]; 10% hydrogen, 10% nitrogen, 10% carbon monoxide and 10% carbon dioxide with argon balance gas [product number: PGS402469D]; and 50% oxygen with argon balance gas [product number: PGS402471D2]) were used to determine the % concentration of hydrogen and carbon dioxide. Oxygen and nitrogen gas were also measured to monitor air leaks into the conical side-arm flask during the experiment. Gas standards were supplied by BOC Australia.

#### *NMR analysis*

For each sample, 700  $\mu$ L of cell culture was pelleted by centrifugation at 20,018 rcf for 2 minutes. The supernatant was collected (600  $\mu$ L) into 15-mL Falcon tubes and then frozen at  $-80^{\circ}\text{C}$ . The samples were then freeze-dried and resuspended in deuterium oxide (800  $\mu$ L). Resuspension was placed into NMR tubes (Norell Sample Vault Series, standard wall, closed cap, parameter 700 MHz frequency, diam. x L 5 mm x 178 mm, mfr no. Norell, SVCP-5-178-96PK). All NMR spectra were recorded at 298K on a Bruker AVIIIHD 400 MHz NMR Spectrometer equipped with a 5mm BBFO SmartProbe. Spectra were processed and analysed using Topspin 3.5.  $^1\text{H}$  spectra were recorded with a spectral width of 8013 Hz (20.0 ppm) over 64K data points.

#### Results

The DH5 $\alpha$ -HPGC,  $\Delta pfkA$ -HPGC,  $\Delta gpmA$ -HPGC or DH5 $\alpha$ -H1-HEGF (which is HPGC lacking *petF* and *FNR*) strains of *E. coli* produce significant quantities of gas within a 2-hour period after the addition of glucose. The cessation of hydrogen production correlates with the complete consumption of glucose.  $\Delta gpmA$ -HGPC (e.g., SEQ ID NO: 10) produced 0.95 moles of hydrogen per mole of glucose;  $\Delta pfkA$ -HGPC produced 0.85 moles of hydrogen per mole of glucose; DH5 $\alpha$ -HGPC produced 0.45 moles of hydrogen per mole of glucose, DH5 $\alpha$ -H1-HEFG produced 0.45 moles of hydrogen per mole of glucose.

Maximum rates of hydrogen gas production are similar for the HPGC containing strains at  $22^{\circ}\text{C}$ ; being  $3.6 \pm 0.06$  L of hydrogen gas per L cells at 200 OD<sub>600</sub> per hour. Rates were lower for those strains lacking HPGC; with no detectable hydrogen under

these conditions for DH5 $\alpha$  and DH5 $\alpha$ -H1-HEFG giving ~ 1.2 L of hydrogen gas per L cells at 200 OD600 per hour, which lacked the petF-FNR.

If not buffered sufficiently the hydrogen production ceases when the pH falls below 5. The drop in pH is due to production of organic acids lactate, succinate, pyruvate and acetate, and the  $\Delta pfkA$  and  $\Delta gpmA$  mutants have reduced production of organic acids (Fig. 2).

**Example 2: rate of hydrogen production by targeting lower section of glycolytic pathway**

Evidence that reducing the flow of carbon from glucose through the lower section of the glycolytic pathway, is shown in Fig 4. Deletion of the genes *gpmM* or *gpmA* improves the rate of hydrogen production.

Increasing expression of *gnd* and *zwf* also unexpectedly improved the rate of hydrogen production as shown in Fig 4.

Figure 5 also provides evidence that increasing the flux of carbon from glucose through the Pentose Phosphate pathway (PPP) increases the ratio of H<sub>2</sub> to CO<sub>2</sub>. The theoretical maximum ratio under anaerobic conditions is 2:1 if all the carbons in glucose are metabolised to CO<sub>2</sub> via the pentose phosphate pathway and the reductants produced are used to make H<sub>2</sub>. If the glucose goes through glycolysis then the ratio is 1:1 under anaerobic conditions. Increasing the flux through the PPP will improve the overall yield of H<sub>2</sub> made from glucose.

An increase in metabolism through the PPP can be achieved by increasing activity and/or expression of *zwf* and/or *gnd*. Alternatively this can also be achieved by reducing the flux from the section of glycolysis with intermediates having 6 carbons to the section of glycolysis with intermediates having 3 carbons by reducing activity of *pfk* (as for  $\Delta pfk$ ). Data shown in Fig 5 shows increasing expression of *zwf* or reducing *pfk* activity increases the ratio of hydrogen to CO<sub>2</sub> and hence flux through the pentose phosphate pathway. As *gnd* encodes an enzyme that is downstream in the metabolic pathway compared to the enzyme encoded by *zwf*, the increase in activity of *gnd* in this mutant has no significant effect on the ratio H<sub>2</sub> to CO<sub>2</sub> ratio compared to the wt DH5 $\alpha$ .

Table 1: Sequence information

| SEQ ID NO: | Description                                          | Nucleic acid or amino acid sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|------------|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1          | Promoter for <i>hydEF</i> gene                       | CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br>GTGGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 2          | <i>hydEF</i> nucleic acid<br>sequence encoding HydEF | AAGAAGGAGATATACATATGGCTCATAGTTTAAGCGCACAT<br>TCCCGTCAGGCCGGAGATCGCAAACCTGGGCGCAGGTGCG<br>GCATCTAGCCGCCCATCATGTCCTTCTCGCCGCATTGTCC<br>GCGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACTCCCG<br>ATGTACCTGTTGACGATCTTCTCCTGCGCATGCCCGTGCA<br>GCAGTAGCGGCCGCTAACCCTCGTGCGCGCGCTATGGCA<br>TCAGCGGAGGCCGCGGCAGAGACCCTGGGCGATTTCTG<br>GGCTTAGGGAAGGGGGGGCTTTCGCCGGGCGCAACCGCC<br>AACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGAGGCGG<br>TCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGCGTTGTA<br>CAGCCATGCCAATGCGGTGACTAACAAATACTGTGGTGGG<br>GGGGTCTACTATCGCGGCCTTGTGGAGTTCAGTAATATTTG<br>CCAAAACGATTGCTCATATTGTGGGATTGCAACAATCAAA<br>AAGAAGTTTGGCGCTATACTATGCCGGTTGAAGAGGTGGT<br>CGAGGTAGCTAAGTGGGCGCTTGAACCGGCATTGCAAC<br>ATTATGTTGCAAGGGGGTGAATTAAGACAGAGCAACGCTT<br>AGCCTACTTGGAGGCATGCGTCCGTGCGATTGCGGAGGAG<br>ACAACGCAGTTAGACCTTGAATGCGCGCACGTGCCGCGA<br>GTACGACAACAGCGGAAGCGGCAGCCAGTGACAGGCAG<br>ACGCCGAAGCTAAACGCGCGAGCCAGAATTGGGTGTGGT<br>CGTATCCTTAAGCGTCGGAGAACTTCTATGGAACAGTATG<br>AGCGTTTGTTCGCGCCGCGCGCCGTCGCTATCTGATCCG<br>CATTGAAACCTCGAATCCCGACCTGTACGCGGCACCTTAC<br>CCGGAGCCGATGTCTGGCACGCGCGTGTAGAATGCCTG<br>CGCAACCTGAAGAAAGCTGGGTATATGTTGGGCACAGGAG<br>TGATGGTCGGCTTGCCGGGACAAACCTTACACGACCTGGC<br>TGGGGATGTCATGTTCTTTCGCGACATTAAAGCGGACATGA<br>TCGGTATGGGCCCCCTTACATTACGCAGCCTGGGACGCCTGC<br>AACTGATAAGTGGACCGCGCTGTATCCGAACGCCAATAAG<br>AACAGCCACATGAAATCTATGTTGATCTGACCACTGCTAT<br>GAATGCACTTGACGTATTACGATGGGGAACGTAAATATCA<br>GTGCTACGACTGCATTACAAGCGATTATCCCCACTGGACGT<br>GAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCATGCCTA<br>TTTTAACGCCCTACTCAGTATCGCGAGTCTATCAGTTATATG<br>AGGGTAAGCCCTGCATTACGGATACTGCTGTTCACTGTGCGT<br>CGTTGCCTGGACATGCGTTTCACTCCGTTGGCAAAACGT<br>CTGCGGCCGGAGTTTGGGGCATCCTGCTTCGTTCTTGCA<br>TCCCATCGTTGGCGTCCAGTCCCGCACGACTTGTATCA |

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|--|--|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  |  | <p> CCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTCACGAGG<br/> TTGGCGCAGGTCCCTGGAACCCCATCCGACTAGAGCGTCT<br/> GGTTGAAGTGCCGGACCGTTACCCTGACCCCGATAACCCAC<br/> GGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGCAAGGCG<br/> CATGACTCTCACGACGATGGCGATCACGACGACCACCACC<br/> ACCACCACGGAGCTGCCCCGCGGGTGACGCGGCTGGAA<br/> AGGGTACCGGTGCAGCTGCAATTGGTGGCGGAGCGGGGG<br/> CTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCGCCTCTG<br/> CTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCCGTGTTGT<br/> CGCGTCGCCATTGCGCCCAGCTGCCGCTTGCCGTGGTGT<br/> GGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGAAGACGC<br/> TGGTGCTGGTACGTCTGGCGTAGGTTCAAATATTGTTACGT<br/> CTCCCGGAATCGCTAGCACTACGGCACATGGTGTTCTCG<br/> TATCAATATTGGGGTGTTCCGCGTGATGAATGCAGGAAAGT<br/> CTACACTTGTGAATGCTCTGGCGCAACAGGAAGCATGCATT<br/> GTTGACTCAACCCCCGGCACGACCGCAGATGTAAAAACAG<br/> TTTTGCTTGAGCTTCATGCCCTTGGACCAGCCAAGTTGCTG<br/> GACACAGCCGGATTAGACGAAGTCGGTGGACTTGGGGATA<br/> AAAAGCGCCGTAAAGCCCTGAATACGCTGAAGGAGTGC GA<br/> TGTTGCTGTGCTGGTTGTTGACACTGATACGGCAGCCGCC<br/> GCAATCAAATCGGGACGCCCTTGCTGAAGCCCTGGAATGGG<br/> AATCGAAAGTAATGGAACAGGCACACAAGTATAATGTCAGT<br/> CCTGTACTGCTTCTGAATGTAAATCACGCGGGCTGCCTGA<br/> AGCGCAGGCAGCTAGCATGCTTGAGGCGGTGCTGGTATG<br/> CTTGACCCATCCAAGCAAATCCCTCGTATGTCGCTGGATTT<br/> AGCGTCCACCCCCCTGCACGAGCGTAGTACGATTACGTCT<br/> GCATTCGTCAAGGAAGGAGCAGTGCGCAGTTCACGCTATG<br/> GGGCTCCTCTGCCGGGGTGTTTGCCCGTTGGTCTCTTGG<br/> ACGCAACGCCCCTTACTGATGGTAATCCCGATGGATGCC<br/> GAAACCCAGGTGGACGTCTTCTTCGTCTCAAGCGCAAG<br/> TTATGGAGGAAGCAATCCGCCATTGGGCTACCGTGCTTTC<br/> CGTACGCTGGATTTAGACGCCGCACGTGGCAAATGGGG<br/> CCCGAGGCCTGTGAGATGGAGCGTCAGCGTTTCGACGGG<br/> GTAATTGCTATGATGGAACGTAATGACGGACCCACCCTGGT<br/> GGTGACAGATTCTCAAGCGATCGATGTTGTACACCCTTGGA<br/> CCCTGGACCGCTCATCAGGACGTCCGTTGGTTCCAATTACT<br/> ACCTTTAGCATCGCTATGGCGTACCAACAAAACGGCGGAC<br/> GTTTGGACCCGTTTGTGGAAGGATTGGAAGCATTGGAGAC<br/> GTTACAAGATGGGGATCGCGTTCTTATTCGGAAGCGTGTA<br/> ATCATAACCGTATCACCTCCGCTTGCAACGACATTGGAATG<br/> GTGCAGATCCCTAACAGCTGGAGGCAGCACTTGGCGGGA<br/> AAAAGTTACAAATCGAACATGCGTTTGGTCGCGAGTTTCCC<br/> GAGCTTGAGAGTGGGGGTATGGATGGATTGAAGTTAGCGA<br/> TCCATTGTGAGGGTGTATGATCGACGCTCAGAAAATGCA </p> |
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|---|----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|   |                                        | GCAGCGTATGAAAGACTTGCATGAGGCTGGGGTGCCGGTC<br>ACTAATTATGGGGTGTCTTTCTCTTGGGCAGCTTGCCCCGA<br>CGCCCTTCGTCGCGCATTGGAACCGTGGGGAGTGAGCC<br>ACCGGTAGGTACTCCTGCTACCCCGCAGCCGCGCCTGCT<br>ACGGCAGCGTCCGGGGTATAATACTA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 3 | Promoter for <i>hydG</i> gene          | CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br>GTGGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 4 | Nucleic acid sequence<br>encoding HydG | AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br>GCAGGCCGTCTTTTGGCGGGCCAGCGCCCTGCGGCGTC<br>CGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br>CACACGGCAAAGCATCTGCCACGCGCAATACGCTGGTGA<br>CTTTCTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br>AGCGTGAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br>AAGGCTGATGCTCAGGACGCAGGACGCGTGCGTGAGATT<br>TGCGGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br>GCCTGTAAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br>TTACCTTGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br>TGCTATCAAAGAACGTATCTACGGGAACGCGTCGTGCTG<br>TTGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br>CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br>GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br>CGCCTTGCAACGTCAAGGTCATCGCCGCAATTTAGCCCTTA<br>CTGGAGAACATCCTAAATACACTTTCGACAATTTCTTGATG<br>CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br>GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br>TCTCAGACATGCGCCGCTTAAGAATACAGATTCAGTTGGC<br>ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br>CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br>TTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT<br>GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br>ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br>TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br>TCCCCGTATGCGCCCCGCGACGGAAGCGAGCTTAGTATC<br>GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br>GTTGCGGTCCCTTCGTATTGCTGTACCATACACAGGTATGAT<br>CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCGCTT<br>CTGAAGTGTGGGATGAGTCAAATGAGTGCCGCTCGCGCA<br>CTGATGTAGGAGCCTATCACAAAGATCACACGTTAAGTACA<br>GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br>GGATGAACGTCCAACATAATGAGATTGTTAAGTGGCTGATGG<br>AGGAGGGCTATGTCCCGTCTTGGTGACACAGCTTGTTACCG |

|   |                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|---|-----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|   |                                               | TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br>GCTGGTGACATTCACGATTTCTGTCATCCCAATAGTCTGCT<br>TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br>TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG<br>ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br>CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br>ACGATGTATATCTTTAACCAGGCATCAAATAAACGAAAGG<br>CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTG<br>TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br>GGGTGGGCCTTCTGCGTTTATA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 5 | Promoter for <i>hydA</i>                      | CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br>GTGGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 6 | Nucleic acid sequence<br>encoding HydA (Hyd1) | AAGAAGGAGATATACATATGTCCGCATTAGTACTTAAGCCC<br>TGTGCGGCAGTATCCATCCGTGGCTCCTCGTGTCGCGCAC<br>GCCAAGTAGCCCCACGCGCTCCGCTTGACAGTAGCACGGT<br>TCGTGTCGCTCTTGCAACCTTGAGGACACGCGCTCGT<br>TTAGGAAACGTCGCCTGTGCCGACGCGCACCGGCCGCA<br>GAGGCACCCCTGTCTCACGTCCAGCAAGCACTGGCCGAAC<br>TGGCAAAGCCCAAAGATGACCCACGCGTAAGCACGTTTG<br>CGTTCAAGTCGCTCCCGCAGTGCGTGTGCTATTGCTGAA<br>ACCTTAGGGCTTGCGCCGGGCGCTACGACACCGAAACAAT<br>TAGCAGAAGGCCTGCGTCGCTTGGGCTTTGACGAGTTTT<br>CGATACGCTGTTGGGGCCGACTTGACGATCATGGAAGAG<br>GGCTCAGAACTTTTGACCCGTCTGACGGAGCACTTGGAAG<br>CACACCCGCACTCTGATGAGCCGCTGCCTATGTTTACCAG<br>CTGCTGTCCTGGTTGGATCGCGATGCTGGAGAAATCATAC<br>CCAGACCTTATCCCTTATGTAAGTTCTTGCAAATCCCTCAA<br>ATGATGCTGGCTGCTATGGTCAAATCGTATCTGGCGGAAAA<br>AAAGGGGATTGCACCTAAAGATATGGTTATGGTAAGCATT<br>TGCCATGCACACGCAAGCAGAGTGAGGCGGATCGTGATTG<br>GTTTTGTGTCGACGCGGACCCAACACTTCGCCAATTGGAC<br>CATGTGATCACGACCGTAGAGCTGGGAATATTTCAAAGA<br>GCGTGGGATCAACCTTGCGGAATTACCCGAGGGGGAGTG<br>GGACAACCAATGGGGTAGGCTCCGGTGCTGGAGTACTT<br>TTTGGCACCACTGGAGGGGTAATGGAGGCGGCGCTGCGT<br>ACTGCATACGAATTATTTACTGGAACCCCTTACCCGCCT<br>TTCGCTTTCGGAGGTGCGCGCATGGATGGCATTAAAGAG<br>ACTAACATCACGATGGTACCGGCCCTGGCAGCAAGTTTG<br>AAGAACTGTTAAAGCACCGCGCGGAGCGCGTGCCGAGG<br>CTGCTGCACACGGAACACCTGGTCCCTTGGCCTGGGACGG<br>CGGCGCAGGGTTCACCTCGGAGGACGGGCGTGGGGGTAT<br>CACTCTTCGTGTGGCGGTAGCTAATGGCTTGGGAAACGCC<br>AAAAAGTTGATCACAAGATGCAGGCGGGAGAGGCGAAGT |

|   |                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
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|   |                                           | ATGACTTTGTAGAAATCATGGCGTGTCCAGCGGGATGCGTT<br>GGGGGCGGCGGGCAGCCACGTTCCACCGATAAAGCAATT<br>ACCCAGAAGCGCCAGGCTGCACTTTACAATCTGGACGAGA<br>AGTCGACTCTGCGCCGCTCCCATGAAAACCGTCTATCCG<br>TGAGTTATACGACACTTATTTGGGTGAGCCCTTAGGGCACA<br>AAGCACACGAACTTTTACATACTCACTATGTAGCTGGCGGG<br>GTCGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAAAT<br>AAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCTGTTTTA<br>TCTGTTGTTTGTGCGGTGAACGCTCTCTACTAGAGTCACACT<br>GGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| 7 | Promoter for Ferredoxin-FNR               | CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 8 | Nucleic acid sequence encoding Ferredoxin | AAGAAGGAGATATACATATGGCGATGCGTTCCACATTTGCT<br>GCGCGTGTGCGGTGCCAAACCGGCAGTGCGTGGGGCGCGT<br>CCCGCGTCGCGTATGTCTTGATGGCCTATAAGGTTACACT<br>GAAAACGCCATCAGGCGATAAAACAATCGAATGTCCGGCG<br>GACACCTATATCCTGGATGCTGCGGAGGAAGCCGGGCTGG<br>ACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTTCCTCTGT<br>GCTGGCAAAGTGGCAGCTGGAACGGTAGATCAATCTGATC<br>AGTCCTTTCTGGATGACGCTCAGATGGGGAACGGATTCTGT<br>CCTGACATGTGTGCGGTATCCTACAAGCGACTGCACTATCC<br>AAACCTACCAAGAGGAGGCTTTATATTAA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 9 | Nucleic acid sequence encoding FNR        | AAGAAGGAGATATACATATGCAAACGTTCGCGCTCCAGCA<br>GCTTCAGGTGTTGCCACACGTGTGCGAGTCTCGTATGT<br>GTCGTCCGGTTGCGGCGACGAAGGCTTCACGGCTGTTAC<br>CACAGACATGTGGAAGCGCACTGTTCCAACCAAGTTAGAG<br>GAAGGTGAAATGCCGTTGAACACGTACAGTAATAAAGCTCC<br>GTTCAAGGCAAAGGTTGCTTCCGTGGAAAAATCACAGGA<br>CCAAAAGCCACAGGTGAGACGTGCCACATCATTATTGAAAC<br>CGAGGGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGT<br>GTAATTCCGCCGGGACCAAGATCAACTCTAAGGGAAAAG<br>AAGTGCCTCATGGTACTCGTCTTTATTCTGATTGCTTCTAGTC<br>GTTACGGAGATGACTTCGATGGTCAAACGGCATCGCTGTG<br>TGTTGCGCGCGCGGTATACGTGATCCAGAGACTGGAAAG<br>GAGGACCCCGCGAAAAAAGGACTGTGTAGTAACCTTTTGTG<br>TGATGCCACACCAGGCACGAAATTTCCATGACAGGGCCC<br>ACAGGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGC<br>CATTAATCTGTGTGCAACGGGGACTGGAATCGCGCCTTTT<br>CGCTCATTCTGGCGCCGTTGCTTCATCGAGAATGTCCAA<br>GTTATAAGTTCACTGGCCTTTTCTGGTTGTTATGGGTGTC<br>GCTAACTCTGATGCTAAATTGTACGATGAGGAACTGCAAGC<br>TATCGCAAAGCGTATCCGGGACAATCCGCCTGGACTAT<br>GCCTTGCTCGTGAACAAAATAATCGCAAGGGCGGGAAAA |

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|----|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                                                                                                  | <p>TGTACATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATT<br/> TTCGATTATTGGATAATGGGGCGCACATGTACTTCTGCGG<br/> GTTAAAGGGGATGATGCCAGGCATCCAAGATATGTTAGAAC<br/> GCGTTGCAAAAGAAAAGGGGCTGAACTACGAAGAGTGGGT<br/> CGAGGGGTTAAAGCACAGAATCAATGGCATGTTGAAGTCT<br/> ACTAACCAGGCATCAATAAAACGAAAGGCTCAGTCGAAAG<br/> ACTGGGCCTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCT<br/> CTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTT<br/> CTGCGTTTATATA</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| 10 | Exemplary HPGC (hydrogen producing gene cluster) with HydA from <i>Chlamydomonas reinhardtii</i> | <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/> GTGGAAAGAAGGAGATATACATATGGCGATGCGTTCCACAT<br/> TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGC GTGGGG<br/> CGCGTCCCGCGTGC GTATGTCTTG CATGGCCTATAAGGT<br/> TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br/> CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br/> GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br/> ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br/> TCTGATCAGTCCTTTCTGGATGACGCTCAGATGGGGAACG<br/> GATTCGTCCTGACATGTGTCGCGTATCCTACAAGCGACTGC<br/> ACTATCCAACTCACCAAGAGGAGGCTTTATATTAAGAA<br/> GGAGATATACATATGCAAACGTTCGCGCTCCAGCAGCTTC<br/> AGGTGTTGCCACACGTGTCGCAGGTGCTCGTATGTGTCGT<br/> CCGGTTGCGGCGACGAAGGCTTCCACGGCTGTTACCACAG<br/> ACATGTCGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br/> TGAAATGCCGTTGAACACGTACAGTAATAAGCTCCGTTCA<br/> AGGCAAAGGTTGTTCCGTGGAAAAATCACAGGACCAAA<br/> AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br/> GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br/> TTCCGCGGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br/> GCCTCATGGTACTCGTCTTTATTCGATTGCTTCTAGTCGTTA<br/> CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/> CGCCGCGCGGTATACGTCGATCCAGAGACTGGAAAGGAG<br/> GACCCCGCAAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br/> TGCCACACCAGGCACGGAAATTTCCATGACAGGGCCCACA<br/> GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/> TAATCTGTGTCGAACGGGGACTGGAATCGCGCCTTTTCG<br/> CTCATTCTGGCGCGTTGCTTCATCGAGAATGTCCCAAGTT<br/> ATAAGTTCACTGGCCTTTTCTGTTGTTTATGGGTGTCGCT<br/> AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br/> CGCAAAAGCGTATCCGGGACAATTCCGCCTGGACTATGCC<br/> TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAAAATGTA<br/> CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/> ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA</p> |

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|  |  | AAAGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br>TTGCAAAAGAAAAGGGGCTGAACTACGAAAGTGGGTGCA<br>GGGGTTAAAGCACAAGAATCAATGGCATGTTGAAGTCTACT<br>AACCAGGCATCAAATAAACGAAAGGCTCAGTCGAAAGACT<br>GGGCCTTTCGTTTTATCTGTTGTTTGTGGTGAACGCTCTC<br>TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br>GCGTTTATATACTAGAGGGCTTTACACTTTATGCTTCCGGC<br>TCGTATGTTGTGTGGAAGAAGGAGATATACATATGTCCGC<br>ATTAGTACTTAAGCCCTGTGCGGCAGTATCCATCCGTGGCT<br>CCTCGTGTGCGGCACGCCAAGTAGCCCCACGCGCTCCGCT<br>TGCAGCTAGCACGGTTCGTGTGCTCTTGCAACCCTGGAG<br>GCACCAGCGCGTCGTTTAGGAAACGTGCGCTGTGCCGCGAG<br>CGGCACCGGCCGCGAGAGGCACCCCTTGCTCACGTCCAGC<br>AAGCACTGGCCGAACTGGCAAAGCCCAAAGATGACCCAC<br>GCGTAAGCACGTTTGCGTTCAAGTCGCTCCCGCAGTGCGT<br>GTCGCTATTGCTGAAACCTTAGGGCTTGCGCCGGGCGCTA<br>CGACACCGAAACAATTAGCAGAAGGCCTGCGTCGCTTGGG<br>CTTTGACGAGGTTTTGATACGCTGTTGCGGGCCGACTTG<br>ACGATCATGGAAGAGGGCTCAGAACTTTGCACCGTCTGA<br>CGGAGCACTTGAAGCACACCCGCACTCTGATGAGCCGCT<br>GCCTATGTTTACCAGCTGCTGTCCTGGTTGGATCGCGATG<br>CTGGAGAAATCATACCCAGACCTTATCCCTTATGTAAGTTC<br>TTGCAAATCCCTCAAATGATGCTGGCTGCTATGGTCAAAT<br>CGTATCTGGCGGAAAAAAGGGGATTGCACCTAAAGATAT<br>GGTTATGGTAAGCATTATGCCATGCACACGCAAGCAGAGT<br>GAGGCGGATCGTGATTGTTTTGTGTCGACGCGGACCCAA<br>CACTTCGCCAATTGGACCATGTGATCACGACCGTAGAGCT<br>GGGGAATATTTTCAAAGAGCGTGGGATCAACCTTGCGGAA<br>TTACCCGAGGGGAGTGGGACAACCCAAATGGGGTAGGC<br>TCCGGTGCTGGAGTACTTTTTGGCACCCTGGAGGGGTAA<br>TGGAGGCGGCGCTGCGTACTGCATACGAATTATTACTGG<br>AACCCCTTACCCGCCTTTTCGCTTTTCGGAGGTGCGCGGC<br>ATGGATGGCATTAAAGAGACTAACATCACGATGGTACCGG<br>CCCCTGGCAGCAAGTTTGAAGAACTGTTAAAGCACCGCGC<br>GGCAGCGCGTGCCGAGGCTGCTGCACACGGAACACCTGG<br>TCCCTTGGCCTGGGACGGCGGCGCAGGGTTCACCTCGGA<br>GGACGGGCGTGGGGGTATCACTCTTCGTGTGGCGGTAGC<br>TAATGGCTTGGGAAACGCCAAAAGTTGATCACAAAGATGC<br>AGGCGGGAGAGGCGAAGTATGACTTTGTAGAAATCATGGC<br>GTGTCCAGCGGGATGCGTTGGGGGCGGCGGGCAGCCACG<br>TTCCACCGATAAAGCAATTACCCAGAAGCGCCAGGCTGCA<br>CTTTACAATCTGGACGAGAAGTCGACTCTGCGCCGCTCCC<br>ATGAAAACCGCTCTATCCGTGAGTTATACGACACTTATTTG<br>GGTGAGCCCTTAGGGCACAAAGCACACGAACTTTTACATAC |
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|  |  | <p>TCAGTATGTAGCTGGCGGGGTCGAGGAGAAGGATGAGAAA<br/>AAGTGACCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAA<br/>GACTGGGCCTTTCGTTTTATCTGTTGTTTGTGGTGAACGC<br/>TCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTT<br/>TCTGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>CACATTCCCGTCAGGCCGAGATCGCAAACCTGGGCGCAG<br/>GTGCGGCATCTAGCCGCCCATCATGCTTCTCGCCGCAT<br/>TGTCCGCGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCC<br/>GTGCAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGGAGAGACCTGGGCGATT<br/>TCTGGGCTTAGGGAAGGGGGGCTTTCGCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>GTGGGGGGTCTACTATCGCGCCTTGTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTGCAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAAAACGGCATT<br/>GCAACATTATGTTGCAAGGGGGTGAATTAAAGACAGAGCAA<br/>CGCTTAGCCTACTTGGAGGCATGCGTCCGTGCGATTGCGG<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC<br/>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGACACA<br/>GGCAGACGCCGAAGCTAAACGCGGCGAGCCAGAATTGGG<br/>TGTGGTTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>AGTATGAGCGTTTGTTCGCGCCGGCGCCCGTCGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>CTTCACCCGGAGCCGATGTCCTGGCACGCGCGTGTAGAAT<br/>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>CTGGCTGGGGATGTCATGTTCTTCGCGACATTAAAGCGG<br/>ACATGATCGGTATGGGCCCTTCATTACGCAGCCTGGGAC<br/>GCCTGCAACTGATAAGTGGACCGCGCTGTATCCGAACGCC<br/>AATAAGAACAGCCACATGAAATCTATGTTGATCTGACCAC<br/>TGCTATGAATGCACTTGTACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA<br/>TGCTATTTTAAACGCTACTCAGTATCGCGAGTCCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA<br/>GTGTCGTGCTGCTGACATGCGTTTGCACTCCGTTGGC<br/>AAAACGTCTGCGGCCGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGATCCCATCGTTGGCGTCCAGTCCCGCACGACTT</p> |
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|  |  | <p>GTGATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGTTGAAGTGCCGGACCGTTACCCTGACCCCGAT<br/>AACCACGGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCACCACCGAGCTGCCCCCGGGGTGCAGCGG<br/>CTGGAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTGTGCTGGTACGTCTGGCGTAGGTTCAAATATT<br/>GTTACGTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCCCTCGTATCAATATTGGGGTGTTGCGCGTGATGAATGCA<br/>GGAAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCGGCACACCGCAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGACCAGCCA<br/>AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCCGCAATCAAATCGGGACGCCTTGCTGAAGCCCT<br/>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTCTGTCAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGTGTTTGCCCCGTT<br/>GGTCTCTTGACGCAACGCCCGCTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCAGGTGGACGTCTTCTTCGTCT<br/>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>CCGTGCTTTCCGTACGCCTGGATTTAGACGCCGCACGTGG<br/>CAAATTGGGGCCCGAGGCCTGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>CACCCCTGGACCCTGGACCGCTCATCAGGACGTCCGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGACCCGTTTGTTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTG<br/>GAAGCGTGTAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTCG<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC</p> |
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|  |  | <p>AGAAAATGCAGCAGCGTATGAAAGACTTGCATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTTCCTCTTGGGCAG<br/>CTTGCCCCGACGCCCTTCGTCGCGCATTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCCGACG<br/>CGCGCCTGCTACGGCAGCGTCCGGGGTATAATACTA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGA</p> <p>AAGAAGGAGATATACATATGTCGGTCCCCCTACAG<br/>TGCAATGCAGGCCGTCTTTTGGCGGGCCAGCGCCCTGC<br/>GGCGTCCGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAG<br/>TGACCGCACACGGCAAAGCATCTGCCACGCGCGAATACGC<br/>TGGTGACTTTCTCCAGGGACCACTATTAGTCATGCGTGGT<br/>CCGTGGAGCGTGAAACTCATCACCGTTATCGTAATCCGGC<br/>CGAGTGGATTAACGAGGCTGCCATCCACAAGGCGCTTGAA<br/>ACGTCAAAGGCTGATGCTCAGGACGCAGGACGCGTGCGT<br/>GAGATTTTGGCGAAGGCTAAGGAAAAGGCTTTTGTTACTGA<br/>ACATGCGCCTGTAAATGCGGAAAGTAAATCCGAATTTGTAC<br/>AAGGGCTTACCTTGGAGGAGTGCGCCACCTTAATTAACGTT<br/>GATTCTAATAATGTCGAGTTGATGAATGAAATCTTCGACACT<br/>GCGCTTGCTATCAAAGAACGTATCTACGGGAACCGCGTCG<br/>TGCTGTTGCGGCCCTTGCTACATTGCTAATCATTGTATGAAC<br/>ACTTGCACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCAT<br/>GGAACGCTCTATTCTGACAGACGATGACCTGCGTGAAGAA<br/>GTTGCCGCCCTTGCAACGTCAAGGTCATCGCCGCATTTTAG<br/>CCCTTACTGGAGAACATCCTAAATACACTTTCGACAATTTCT<br/>TGCATGCAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCT<br/>GAAGGGTCCATTGCGCGTATCAATGTCGAAATCCCACCACT<br/>TAGTGTCTCAGACATGCGCCGCCCTTAAGAATACAGATTCAG<br/>TTGGCACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGAC<br/>ACATTC AAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTT<br/>TGATTTTTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCA<br/>GGCTTGAGACGATGTTGGTATTGGTGCTTTATTCCGGTTATA<br/>TGATTACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCG<br/>AGCATTTGGAACGTGAGTACAACGCAGGACCACATACTATC<br/>TCTGTTCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTA<br/>GTATCGCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGA<br/>AATTAGTTGCGGTCCTTCGTATTGCTGTACCATACACAGGT<br/>ATGATCCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGC<br/>GCTTCTGAAGTGTGGGATGAGTCAAATGAGTGCCGGCTCG<br/>CGCACTGATGTAGGAGCCTATCACAAAGATCACACGTTAAG<br/>TACAGAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCT<br/>TGCAGGATGAACGTCCAATAATGAGATTGTTAAGTGGCTG<br/>ATGGAGGAGGGCTATGTCCCGTCTTGGTGCACAGCTTGT</p> |
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|    |                | ACCGTCAAGGTCGTACGGGCGAAGACTTCATGAACATCTG<br>TAAGGCTGGTGACATTCACGATTTCTGTCATCCCAATAGTC<br>TGCTTACGCTTCAAGAGTACCTTATGGATTATGCAGATCCA<br>GACCTTCGTAAGAAAGCGAGCAAGTAATTGCCCGTGAGA<br>TGGGACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCC<br>GTAAGCGCCTTGAACGCAAGATGAAACAAGTATTGGAGGG<br>GGAGCACGATGTATATCTTTAACCAGGCATCAAATAAACG<br>AAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTT<br>GTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCA<br>CCTTCGGGTGGGCCTTCTGCGTTTATA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| 11 | pSB1C3 plasmid | TACTAGTAGCGGCCGCTGCAGTCCGGCAAAAAAGGGCAAG<br>GTGTACCCACCCCTGCCCTTTTTCTTTAAACCGAAAAAGATTA<br>CTTGCGTTATGCAGGCTTCCTCGCTCACTGACTCGCTGC<br>GCTCGGTCGTTGCGCTGCGCGAGCGGTATCAGCTCACTC<br>AAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC<br>GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCA<br>GGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCACAG<br>GCTCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCA<br>AGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACC<br>AGGCGTTTTCCCCCTGGAAGCTCCCTCGTGGCTCTCCTGT<br>TCCGACCCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTC<br>CCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTA<br>GGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGG<br>CTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCC<br>TTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACA<br>CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATT<br>AGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGA<br>AGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTT<br>GGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAA<br>GAGTTGGTAGCTCTTGATCCGGCAACAAACACCGCTGG<br>TAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCA<br>GAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACG<br>GGGTCTGACGCTCAGTGAACGAAACTCACGTTAAGGGA<br>TTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCC<br>TTTTAAATAAAAATGAAGTTTTAAATCAATCTAAAGTATATA<br>TGAGTAAACTTGGTCTGACAGCTCGAGGCTTGGATTCTCAC<br>CAATAAAAAACGCCCGCGGCAACCGAGCGTTCTGAACAA<br>ATCCAGATGGAGTTCTGAGGTCACTTGGATCTATCAACA<br>GGAGTCCAAGCGAGCTCGATATCAAATTACGCCCGGCCCT<br>GCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTG<br>CCGACATGGAAGCCATCACAAACGGCATGATGAACCTGAA<br>TCGCCAGCGGCATCAGCACCTTGTGCGCTTGCCTATAATAT<br>TTGCCCATGGTGAAAACGGGGCGAAGAAGTTGTCATAT |

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|    |                                                                                                                                                          | <p>TGGCCACGTTTAAATCAAACTGGTGAACTCACCCAGGGA<br/> TTGGCTGAGACGAAAAACATATTCTCAATAAACCCTTTAGG<br/> GAAATAGGCCAGGTTTTTCACCGTAACACGCCACATCTTGCG<br/> AATATATGTGTAGAACTGCCGGAATCGTCGTGGTATTCA<br/> CTCCAGAGCGATGAAACGTTTCAGTTTGCTCATGGAAAC<br/> GGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCAC<br/> CGTCTTTTCATTGCCATACGAAATTCGGGATGAGCATTTCATC<br/> AGGCGGGCAAGAATGTGAATAAAGGCCGGATAAACTTGT<br/> GCTTATTTTCTTTACGGTCTTTAAAGGCCGTAATATCCA<br/> GCTGAACGGTCTGGTTATAGGTACATTGAGCACTGACTGA<br/> AATGCCTCAAAATGTTCTTTACGATGCCATTGGGATATATCA<br/> ACGGTGGTATATCCAGTGATTTTTTCTCCATTTTAGCTTCC<br/> TTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGG<br/> TAGTGATCTTATTTTCATTATGGTGAAAGTTGGAACCTCTTAC<br/> GTGCCCGATCAACTCGAGTGCCACCTGACGTCTAAGAAAC<br/> CATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCAC<br/> GAGGCAGAATTCAGATAAAAAAATCCTTAGCTTTTCGCTA<br/> AGGATGATTTCTGGAATTCGCGGCCGCTTCTAGAG</p> |
| 12 | osmYp, 5' – 3' promoter                                                                                                                                  | <p>TTGTTAAATATAGATCACAATTTTGAAACCGCTCGGGATATC<br/> AGCGAAAACATAAGCAAAAGTGAATGTTTTAAGAACATTCC<br/> GTAA</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| 13 | gapAp, 5' – 3'<br>(gapA promoter)                                                                                                                        | <p>ATATTCCACCAGCTATTTGTTAGTGAATAAAAGGTTGCCTGT<br/> AAAATTACAAAAACCTTACGCAGCGTCAAGCGGAATCGTGT<br/> CAATCATTGCGACAAATCAATCCTGTGCCTAAGCATTACGC<br/> GACTGACTCGCCTCACTCTTCCTTTGGGCTTGAGACCACAT<br/> GGGGTCGGCGCCCCGAATTTTAAAGGGCAATTACGATAAA<br/> AATGTGATTT</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 14 | Nucleic acid sequence<br>encoding 6-<br>glucophosphonate<br>dehydrogenase from<br><i>Corynebacterium<br/>glutamicum</i> , 5' – 3'<br>(gnd gene sequence) | <p>AGCCTCTACCTCGCTGCGGTGCGCCAGACCACTCTGTATGG<br/> AACGAACCGTCTTTGTGCGATGCGTTTGTATGTGTGGCACC<br/> AAAAAATCGCGCTGCCCTTGGAATACGCTGCTGGAAGG<br/> CGCTCGGCGCGTAATGAGTCGTAATAACTAAGACTACTAGC<br/> AAAGACGGGAATCGGCAACCCAGTTGGGTGGCGGTACACA<br/> ATGACGCGGCGCCAGCTATCGATTAAGTCCCCAAGCTCGG<br/> ATTTAAAGTAAGGATCTAACAGCAACGACTCTAACTCTGCG<br/> TTCGCGTCATACGCTTCAACGATGCGATTTAAAAATTTAGC<br/> GCGGATGATACAACCGCCGCGCCAAATAGTCGCTAAATCA<br/> CGGGGGTCTACATCCCAGTTGTTCTCATCAGAACCGGCTTT<br/> AATTTTCGTCGAAACCTTGTGCGTAGGCTACCAACTTAGATG<br/> CGTAAAGCGCACGACGGACATCCTCGACAAATTGAGCCTT<br/> ATCTACGCCAAGCGCTTCAAGGTCTGTGAGTACCCAGCG<br/> GGAAGATTGCCCTGGGCGGCAGCACGTTGTGAAGTTGCAC<br/> CGGACAGGGCACGGGCAAATACGGCCTCGCCAATTCGGG<br/> TGTTTGCAATTCCTCAATCCAGAGCCGCTTTTACTGTCCAG</p>                                                                                               |

|    |                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                                                                                                                                                             | CGTCCTGTACCTTTTGGCCCGCCGCATCCACGATAACATC<br>AATCAACGGCTTGCCCTGTCTCTGCGTCGACCTGAGATAAAA<br>CCTCAGCCGTAATTTGATCAAGTAGGAGTCAAGATCACCA<br>GCATTCCACTCCTTAAATACCTCGGCAATTTGGCCGGCTG<br>CATTCCAGCCGCATAGCGCAGCAGGTGATACGCTTCCCA<br>ATTACTTGCATATCGGCGTACTCAATGCCGTTATGTACCAT<br>CTTGACGAAGTGCTCTGCTCCGCTGGCCCGATATGCGTA<br>ACACAAGGGGTACCATCCACGTTGGCAGCAATTGACTCAA<br>GCAAAGGGCCCAAGCTTTCATAACTCTTCGCAGGACCTCC<br>CGGCATAATAGAAGGTCCGTTCAAGGCCCTTCTTCTCCG<br>CCGGAATGCCCCGACCCACAAAGTGCAGTCCACGGGCAC<br>TGATCTCTTTTTCGCGGCGGATGGTATCCGTATACAGAGCG<br>TTGCCACCATCGATAATGATGTCGCCCTTCGTCCATTGCGTC<br>TGCTAATTGATTGATGACGGCATCTGTAGCGTTGCCGGCCT<br>GGACCATGATGATGGCGCGACGGGGCTTCTCTAAACTCGC<br>TACAACTCCTCGACCGTTGCGGAGGGAATAAAATTTCCCT<br>CGCTGCCATGATCCGCGATCAACTTATCCGTTTTGTGCGTA<br>CTGCGGTTATAGACTGCAACGGTGTGCGTTACGGGCAA<br>AATTGCGGGCAAGGTTTGAAGCCATAACCGCAAGCCCAAC<br>AACGCCAATTTGAGCCAGATTATCACCGTTTGTCT                                                                                                                                            |
| 15 | Nucleic acid sequence<br>encoding glyceraldehyde-3-<br>phosphate from <i>Clostridium</i><br><i>acetobutylicum</i> , 5' – 3'<br>( <i>gapC</i> gene sequence) | ATGGCGAAGATTGCAATCAATGGCTTTGGGCGTATTGGCC<br>GCCTTGCGCTTCGTGCGATTCTGGAAGTACGGGGTCTGGA<br>AGTTGTGGCGATTAATGATCTGACCGATGCAAAGATGCTGG<br>CACATTTGTTTAAATACGATTCTTCGCAAGGACGCTTCAAC<br>GGGGAGATTGAGGTGAAAGAAGGCGCGTTTGTGCTCAACG<br>GCAAAGAAGTCAAGGTATTCGCCGAGGCCGATCCAGAAAA<br>GTTGCCGTGGGGAGACCTGGGCATTGATGTGGTCCCTTGAG<br>TGACGGGCTTTTTCACTAAGAAAGAGAAGGCCGAGGCAC<br>ACGTCCGTGCAGGGGCGAAGAAGGTCTGTATCTCTGCTCC<br>AGCTGGGAATGATCTTAAACCATTTGATTCAACGTCAACA<br>ATGAAGATCTGGACGGGACGGAACGGTCATCTCGGGTGC<br>CAGCTGCACGACGAATTGTCTGGCTCCAATGGCAAAAGTC<br>TTAAATGACAAGTTTGGCATTGAGAAAGGATTGATGACAAC<br>TATTCATGCGTTTACCAACGACGAGAATACGTTAGATGGGC<br>CGCACCGTAAAGGAGACCTGCGTCGTGCACGTGCCGCGG<br>CCGTTTCCATCATCCCAAATTAACAGGTGCCGCAAAAGCT<br>ATCTCGCAAGTTATCCCGGATCTGGCTGGTAAATTAGATGG<br>AAATGCACAACGTGTACCGGTGCCTACCGGTTCCATCACT<br>GAGCTGGTCTCAGTCCTGAAGAAAAAGGTAACGGTAGAAG<br>AGATTAATGCAGCGATGAAAGAAGCAGCCGATGAGTCTTTT<br>GGGTACACCGAAGATCCCATTTGATCCGCGGACGTCTGGG<br>GAATTAACACGTTCTTTGTTTACGCAACTCTGACTAAAA<br>TTGTAGACGTGAATGGGTCTCAATTGGTTAAGACCGCTGCA |

|    |                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|----|--------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                                                                                                                                                        | TGGTATGATAATGAGATGAGTTACACTTCTCAGCTGGTACG<br>CACACTTGCATATTTGCGCAAGATTGCTAAATAG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 16 | Nucleic acid sequence<br>encoding glucose-6-<br>phosphate dehydrogenase<br>from <i>Zymomonas mobilis</i> , 5'<br>– 3'<br>( <i>zwf</i> gene sequence)   | GTCATACCAGGTCACCTCCGTACGTTCTGCCAATGCGATAG<br>CGGTAGACGGTCCCCAGGTTCCCGATACGTAAGTCTTAGG<br>TTTCATAGAGTTAGCTTTCCACCCCTCACGAATTCGGTCAAT<br>CCAGACCCACTGGGCCTCAACTTCGTACGGCGTACAAAC<br>AGAGTGGCGTCTCCCTCAATCAGGTCAAGCATCAGACGCT<br>CATAGGCGATACGGCGTTTGGGTCTTAAAAACATCAGTC<br>AACGACAAGTCCAGCCACACCTCGCGCATGTGGGCTCCGT<br>TACGGTCCAATCCGGGCTCTTTCACCATCATCGAGATTGGA<br>ATAGTCTCGTCCGGTTGTAAGACGATGCGTAACCTATTAGG<br>TTGCAAGATGCCGCCACTCGAAGTGAAGATAGAGTGCGGT<br>ACAGGTTTGAAGTGTACCACGATTTTCGAGCGACGGGCCG<br>GCAAGCGTTTACCGTACGGATGTAAATGGAACACCCCTG<br>CCAACGCCAATTGTCCACATGGGCTTTAATTGCTACGAAAG<br>TTTCAGTATCACTTGTTGCCCTAACTCGTCGATGTATCCT<br>GCAACTTCCTTTCCGCCAGAGACCCCGCCCGTACTGCC<br>CGGTTACGGTGTGGGTAAACACCGTATCATTGTTGATAGG<br>GCGCAAAGCGCGGAATACTTTAACCTTTCATCAGGCACAG<br>CATTGGCCTCCATGTGTGCGGGGGGCTCCATTGCGACCAA<br>CGCTACAAGCTGAAGAATATGACTCTGAACCATGTCACGCA<br>GGGAGCCACTACCGTCGAAGTAACCAATGCGCCCTTCAAG<br>TCCGACTGTTTCCGCGACCGAAATCTGTACGTGGTCAATCC<br>CCTTGCTATTCCAAAGGGGCTCGAACAATGCGTTCCCAAAA<br>CGCAAGGTCAAAAGATTCTGCACCGTTTCTTTGCCCAAATA<br>GTGGTCGATGCGGTAAACTTGCTTCTCTGAGAATACCTTCA<br>GTACAGCATCGTTAATATGGTCGCTGGAGGCAAGGTCTTGT<br>CCCAGGGGTTTTTCCAATGCCAAACGTGAGGTTGGCCCCG<br>CTAATCCCGCCTGCTTTAATCCAGCAATGGCGCCTTCAAAA<br>AGTGAAGGCGCCGTACTTAAGTAAATTGCGATGCCTTTTTTC<br>TACGGGACCACACAGATCCGCTAATTTGCCGAAGTGGGTG<br>GGGTGCGTGATGTCCACGGTAGCGTAAAAAGCTTATTTAA<br>AAACTTCGCTTTAGCATCGTCATTCAAACGGTCAGAGGCCA<br>CGAAACGGTCCAGCGCTTTCTCCGCAAAGTCACGGAATCC<br>GTCAGTATCATACTCGGAACGCGAGGTGCATACGATACGC<br>AAGTCGTCCGCCAGTAAGCCGTCTGCATCAAGCCCATAAA<br>GGCTAGGCAGCAACATGCGTTGGCTTAAATCCCGGTGCT<br>ACCGAACAGAATCATTGTTGACACAGTATTGGTCAT |
| 17 | Codon optimised nucleic<br>acid sequence encoding<br>HydA from <i>Chlamydomonas<br/>reinhardtii</i> , 5' – 3' (including<br>restriction sites shown in | CCCGGGATGTCCGCATTAGTTCTGAAGCCATGTGCGGCGG<br>TCAGTATCCGCGGAAGCAGCTGCCGCGCCCGTCAAGTCGC<br>GCCGCGCGCGCCGTTAGCAGCGTCTACAGTTCTGTGTTGCA<br>TTAGCAACACTTGAAGCTCCTGCGCGTCCCTGGGCAACG<br>TCGCTTGTGACGACGCGCTCCGGCGGCGGAGGCCCTT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

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|    | bold)                                                                                                                                       | <p>             TGAGCCACGTCCAACAAGCCTTAGCTGAATTGGCCAAACC<br/>             GAAAGATGACCCACACGCAAGCATGTTTGTGTGCAAGTC<br/>             GCCCCAGCGGTCCGCGTCGCTATTGCCGAAACACTTGGAC<br/>             TTGCTCCCGGTGCCACGACCCGAAGCAATTGGCGGAGG<br/>             GCTTACGCCGTCTGGGTTTCGACGAAGTATTCGACACGCTT<br/>             TTCGGGGCAGATTTGACAATTATGGAAGAAGGGTCAGAGTT<br/>             GTTGCAATCGTTTAAACGGAACATTTGGAGGCTCACCCCTACT<br/>             CTGACGAACCGTTGCCCATGTTTACCTCGTGTTGTCCGGGT<br/>             TGGATTGCGATGCTTGAGAAATCGTACCCGGATTTAATTCC<br/>             TTATGTGTCCTCGTGCAAATCTCCTCAAATGATGTTAGCCG<br/>             CGATGGTGAAGTCATATCTTGCCGAAAAGAAGGGCATCGC<br/>             ACCTAAAGACATGGTGATGGTGTCAATCATGCCTTGACCCC<br/>             GTAAGCAGTCCGAGGCCGATCGCGACTGGTTTTGTGTGGA<br/>             TGCTGACCCCTACATTACGCCAGTTAGATCATGTTATTACAA<br/>             CCGTAGAGTTGGGTAACATCTTTAAGGAGCGTGATCAAT<br/>             TTGGCTGAGTTACCGGAAGGTGAATGGGACAACCCGATGG<br/>             GGGTGGGTTCCGGAGCCGAGTATTATTTGGGACCACTGG<br/>             CGGCGTAATGGAGGCAGCATTACGTACGGCCTACGAAGT<br/>             TTCACAGGTACCCCGCTGCCTCGCCTGAGTTTGAGCGAGG<br/>             TCGTGGTATGGACGGTATTAAGGAAACGAACATCACAATG<br/>             GTTCCTGCTCCCGTTCAAAATTCGAGGAACTGTTGAAGCA<br/>             TCGCGCTGCCGCACGTGCAGAAGCAGCCGCACACGGTAC<br/>             TCCTGGTCCACTTGCTGGGACGGCGGGGCCGATTACT<br/>             TCAGAAGACGGTCGTGGTGAATTACTTTACGTGTCGCTGT<br/>             TGCGAATGGGCTGGGTAACGCTAAAAAGTTAATTACAAAAA<br/>             TGCAGGCCGGAGAGGCCAAAATATGATTTCTAGAGATTATG<br/>             GCCTGTCCCGCTGTTGCGTAGGAGGTGGGGGCCAGCCC<br/>             CGTTCAACTGACAAAGCTATCACGCAGAAACGTCAAGCGG<br/>             CATTATACAATCTGGATGAAAAATCAACGCTTCGCCGTTCA<br/>             CATGAGAATCCATCAATCCGTGAATTGTACGATACTTATTTA<br/>             GGTGAGCCTCTGGGGCATAAGGCACACGAACCTCTGCATA<br/>             CTCATTACGTCGCGGGTGGAGTTGAGGAGAAGGATGAAAA<br/>             GAAGTAGGGATCC           </p> |
| 18 | Codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Volvox carteri</i> , 5' – 3' (including restriction sites shown in bold) | <p> <b>CCCGGG</b>ATGTACGTTTGGCTCGCACAAAGTTACGTTACATGA<br/>             TTGCTTAGCATGCTCAGGGTGCATCACATCTGCCGAAACG<br/>             GTTTTGCTTCAGCAACAGTCCGGGGATGAGTTCTTATCCCG<br/>             CTTGGCAGACCCGCACACTACTGTCGTAGTTACCGTCTCG<br/>             CCCCAATCGCGCACAGCTTTGGCGGCTTACTATGGATTATG<br/>             CCCGTCTCAGGCTCTTGCCCGCCTTGTTGGGCTGGTTGAAG<br/>             TGGCTTGGGGTTCGCGCCGTATGGGATCTGACAACAGCCC<br/>             GCGATCTTGTATTGTTGGAAGAGGCAGCTGAGTTTATGAAC<br/>             CGTTGGGTCTGCATGTATGTTAGCGCCGGGCCTCTTCCGG<br/>             TCATGGGCAGTTCATGTCCTGGCACAGGCTGGGTTTGTAT           </p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

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|    |                                                                                                                                             | <p>GCTGAAAAGACGCACGGGACGCGTGATTACCCATTGTA<br/> GCACCACCCGTTCACCCCAAGGAGCAATGGGTGGCTTGGT<br/> AAAGTCCCTGGTGGCAGCAGCTTGGGGCGTTACTCCAGGG<br/> TCTTTGTACCACGTCAACAATTATGCCGTGTTATGACAAGAA<br/> ATTGGAAGCATCTCGCGATGAATTAACCACAACGGCGACAA<br/> CTACTACCGCTGCGGGGACAGACGCGGCTGGAGCAGGTG<br/> CCGCCGTAGGCGGGCCTCTGCCTGAAGTGATGGTCCGTCA<br/> TGCAACCGCAGCGCCTGATCCGTTGCTTCCGGGCGTGTA<br/> CCAGCCGACGACCAGCTGTACTCCCTTCCACATGGCTCCA<br/> GCTCGGGTGGATACGCGGACTTCGTTTTCCGCACTGCCGC<br/> GCGCGAGTTGTGGGGGGTAGAAGTCCGCCAGGACCTTTA<br/> CCATGGCGTACTCGTCGTAACGCCGACTTACAGCCTCGTG<br/> TTGGTGCCCCAGGCCAATCTCTGGTCGTTGCGCGTGTA<br/> TGGATTCCGCAATATTCAGACACTTTTACAGCAGCTTAAGC<br/> GTGGACGCTGCCCTTATCATTATGTGGAAGTCATGGCTTGT<br/> CCTAGCGGGTGTGTTGAACGGAGGAGGACAAATCAAACCGG<br/> GGCCAGGGGTAAACGCCGCAACAATTAATTGAACAATTGGA<br/> GCTTTTGTATGACGTTGCGGCACGTTCTCCAGCAGACAACC<br/> CGGCGGTAGCAGCTTTATATGGCTCTTGGTTAGGCGGTCG<br/> TCCAGGGGCACCCCCGCTCGTCAACTGTTACATACGACA<br/> TTTCGCGAGCGCGAAAAGACTGTAAGTCCGCAACCGTCA<br/> CGAATTGGTAGGGATCC</p> |
| 19 | Codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Giardia lamblia</i> 5' – 3' ((including restriction sites shown in bold) | <p><b>CCCGGGAT</b>GCCTCCAAAACCCCAACGATGTAACAGGCG<br/> TGGACTCCAATAACGCGATCATGATTGATTACGCAAAGTGC<br/> ATTGGCTGCAACATGTGCATCAAAGCATGCGACGTGCAAG<br/> GTATTGGTGTGTTACAAGCAGAACGAAAAACCGAAGTACCCG<br/> CCTATCGTGAAGCTGAGCACCCCTGTTTAAAGCGATTGTAT<br/> CGGCTGCGGTCAATGTGCAACGATTTGTCCGGTGGACGCG<br/> ATTGCTCCAAAGAACAACTCTGGAATTTACAAAGGCGAATC<br/> TGCTTCAAAAAAGTTCTGTGTGGCGTTGATCGCGCCAAGC<br/> ACTCGCGTGGCGTTTGGAGATGTCTTCGGGCTGCCGATCG<br/> GTACAAATACCATTTACTCCCTGATTGCGATGCTGAAGCAA<br/> TATCTGGGTTTCGATTATGTGTTGACGTGAATTTCCGGCGC<br/> AGATGAAACCACCGTCATCGACACCCAAGAGCTGTTGCAC<br/> TTCAAACACGAGGGCCGTGGTCCGGTGTTCACCAGCTGCT<br/> GCCCGGCGTGGGTAAACCTCTGTGAAATGAAGTACCCGGA<br/> GCTGCTGCCCCAGGTTAGCACCGCTAAATCTTGTTGCAA<br/> TGGTAGCGACCCTTGTA AAAAGACGTTGGGTTCAAGAACAT<br/> TTAATCCCGAAGGGTATTGTAGACAGCGTTGACGATGTCTA<br/> CGTGGCTGATATTATGCCGTGTACCGGAAAAAGGATGAA<br/> AGCATGCGTCCACAAGTGAACCGCGATGTGGACATCTGCC<br/> TGACGGTGCCTGAAGTTGCGGAGCACCTGTATTTCTGCA<br/> CGGCGCGCGCTTGACGCTGGAGGAAGTCGAGGCGGATGC</p>            |

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|    |                                                                                                                                                                                | <p>GTTGGTTTTGCGTCCGGGCCGTAGCACGCAGAAAAAGTGG<br/> GACTTTGACGCTCCGTTTAAACACCGTGCTGGTGGCTCCCA<br/> TATCTTTGGTAAGACCGGTGGCGTTGCCGAGACGTGCTC<br/> CGTTTCATCAGCTATATGAAAAAGTCGCCGATAGAAACGT<br/> TAAGGAGGAATTGCTGAAAGAGTTCAAGACGCCGGGTGAG<br/> CTGGTGCAAACCGTTAAGCTGGTCAGCTGCGAGATCGCCG<br/> GTGAGACTTATCGTGCCCTGATTGCGCATGGCGGTTGAGC<br/> AATCAATGCCGCCGCGCGTATGGTGCTCAATAAAGAGGTG<br/> GAGTGCGACGTTGTTGAGCAGATGGCCTGTCCGGGTGGCT<br/> GCCAGAACGGTGGAGGCATGCCGAAGATCAAGGGTAAAAA<br/> AGAGGCAGTTTTGACGCGTGCGTCTACCTTGACATCCTG<br/> GACGGTAAAGAGCGCTTTGCGAGCGCGGGCGAGAACAAA<br/> ACTCTGTGGGGTTTCAACGGCTGCTTAACCGAACACGAAG<br/> CGCACGAGTTGCTTCACCCCATTATCAGCATCGCCCGGT<br/> GGAATCGCTGCTGCCGCAGTAG<b>GGATCC</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| 20 | <p>Codon optimised nucleic<br/> acid sequence encoding<br/> HydA (Hyd1) from<br/> <i>Entamoeba nuttalli</i>, 5' – 3'<br/> (including restriction sites<br/> shown in bold)</p> | <p><b>CCCGGGAT</b>GCCTCCAAAACCTCACACACTGTAACAGGCC<br/> ACGACCACAACCACAGCATCCAGTTTGACTGGAGCAAGTG<br/> TATGGGCTGCGGTATGTGTGCAACCAAATGCACCTTCGGC<br/> GTATTGGTTAAGCAGCCGCCGAAGATCCCGCCATTTGTCC<br/> AGCCGAACCGTGAAAACTGAGCCAAGAGAACACCGATAA<br/> AACGCGTGTCCTGATCGATGAAAGCGAGTGCAACCGGCTGC<br/> GGTCAATGCTCTCTGGTGTGCAACTTTGGATCTATCACCCC<br/> GATCGACCACCTCGTTGACACCTTCAAAGCAAAGGAGGCG<br/> GGCAAAAACTCGTGGCCATGATTGCCCGAGCACTCGCC<br/> TTGGTGTCGCTGAGGCTATGGGTATGCCGATTGGCTCCAC<br/> CGCTATGGCGCAGCTGGTGCATTGTCTGCGTCTGATTGGT<br/> TTTGACTACGTTTTTGACGTGGACGCGGGTGCGGATAAGA<br/> CGACCATGGACGATTACGCGGAAGTTATCGAAATGAAAAA<br/> GGAGGGTAAGGGTCCGGCGATCACCAGCTGCTGCCCGGC<br/> GTGGATTGAGCTGGTGGAGAAAGAATATCCGATTTGATC<br/> CCGAACGTGTCCACTGCGCGTAGCCCGATCGGCTGCTTGG<br/> CCGGTTGCATCAAACGTGGTTGGGCAAAGATGTTGGCAT<br/> CGCGGTAGAGGATTTGTACACCGTGGGTATCATGCCTTGT<br/> ATTGCGAAGAAGACGGAATCCCAACGTGAGCAAATTCATCA<br/> AGATTACGACGCAAGCTGTACGTCTAACGAGATTGCGGCG<br/> TACTTCAAGAAGCACCTGCCACCGGAAGAGTGCAAATTCAC<br/> CCAGGAGCGCGAGGAAGCTCTGGCAAAGACTGAGGACGG<br/> CCAATGTGATCTGCCGTTCCGTCGCATTAGCGGTGGCTCC<br/> AACATTTTTGGTAAGACCGGCGGGGTGTGTGAAACCGTGT<br/> TGCGGGTGATTGCGCGTAATGCGGGTGTGACTGGAATAC<br/> GTGCACCGTTAAACAAGAGGAAACATTCAAACACGCCGCTA<br/> GCGGTTTCGACCATGACCAATCTGAGTGTTGACATCGGCGG<br/> AACTATAATCACCGGGGCAGTTTGCCATGGTGGTTATGCAA</p> |

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|    |                                                                                                                                                          | <p>TCCGTCATGCCTGCGAACTGATTGCGAAAGGTGAACTGAAA<br/> GTCGACGTTGTTGAGATGATGGCCTGTGTTGGCGGCTGCC<br/> TGGGCGGTGCTGGCCAGCCTAAGATCCCGCCGCGGAAGA<br/> AGCTGGAATGGATAAGCGCAGAGTGATGTTAGATATTCTG<br/> GACCAGCAAACGGACATTCGTGCAGCGAATGAAAACACCG<br/> ACGTGCTAGGCTGGATTGATAAACACTTCGACCACCAAGG<br/> TGCGCATCAACATCTGCACACCTATTTACCCCGCGTTATC<br/> AGAATTAGGGATCC</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 21 | <p>Codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Ilyobacter polytrophus</i> 5' – 3' (including restriction sites shown in bold)</p> | <p><b>CCCGGGATGAAGAACAAAACAGTATCAAATGATAAAATAGA</b><br/> CAATAAACTGAACTGCTCCGAAAACCTGGAAAACCGTACCA<br/> TCGACAAAAGCTTCTACACCTTTTCAGTGATACCAGCAAG<br/> TGCATTAAAGTGCTACAAGTGCCTGAAAGTTTGAAGGACAC<br/> TCAGGGCATTTCGTTTTTCAGGTTGAGGAGACGGCACG<br/> GTGGGCATCAAAGAGGAGAATATGCGGCCACCTTGTGTA<br/> TCAGCTGTGGTCAATGTATTAAAGTGTGCACCGCAGGTGC<br/> GCTGAAGGAGAAATCCAACATCTCTCTGTTGAAGGAGCAGT<br/> TAAATAACCCGAATAAGCACGTTGTCGCGCAGCTGTCTCCA<br/> AGCTTCAAACACACCATTGGTGATGGCTTCGGCATCAGCTC<br/> GGGTACCGATACTTCCCGAAAATCATCAGCGCTCTGAAA<br/> GAAATTGGTTTCTCTAAGGTTTTTCAGTACCGGTTTCGCCAG<br/> CGACGTGAATATCGTTGAGACCAGCGCGGATCTGAAAAA<br/> CGCCTAGATGAAAACGGTCCGTTCCCGGTGTTACCTCCA<br/> CGTGACGGGTTGGATTAATATGCGGAAAAGTTTTGTCCG<br/> GAGTTCCTGGGGCTGCTCTCTCCGTGCAAAAGCCCGCAAC<br/> AGATCCTGGGTAGCCTGAGCAAGTCTACTACGAAGAGAG<br/> CATTGACATCAGCAGAGAAAATATCTTTAGCGTGGCGCTGA<br/> TGCTTGCATCGCCAAAAGGATGAAGCTAATCGTTTTGAC<br/> ATGAAGGACGAATATGGTAACAAAGATGTGGACCTGGTCTCT<br/> GACCGTGAATGAAGTTGCTAGCCTTTTGAACAAAAGGGCA<br/> TTGATTTAAACAATTACTCTAAGTTCGGTACTTTTGATAAGC<br/> CGATGAAATCCGACACTGGTTCTTCGCGTATTAAGGCGGTT<br/> ACGGGTGGCCTGGCAGAGGCTATCCTGCGCAACACCGCA<br/> CACATGATCGGTGAAGATCCGTTTTCTGTAGACCTGAAGAA<br/> GCTGCGTGGTATGGATGGTATTAAGCTCACGAGCGTGGTT<br/> CTGGGCGGGAAAAAATTAAACATTGCGGTCGTGAACGGCA<br/> TCAAGAACGTGCCGGTATTCTGGACATGATTAAGATGGC<br/> ATTACCGAGTTCCACTTGGTCGAGTTATGGCCTGTCCGG<br/> GTGGCTGCGTCGGCGGCGGTGGTATCCCGTTGTGAGAAG<br/> ACCCGGACATTATCCAAAACGCGCAGAGAAAATCTACTCG<br/> TACGACGCGAGCAGCGAAATTCGTTGTAGCTGGGAAAACC<br/> CAGATGTTAAAACCTGTATAGCGAGTACCTGAAGGAGCC<br/> GCTGGGAGAGGAGTCTCAACGTTTGTTCATTTTCATTATA<br/> AAAACCGCCGTACCAAACGTATCTTCTAGGGATCC</p> |

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| 22 | Codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Volvox carteri</i> , 5' – 3' (including restriction sites shown in bold) | <p> <b>CCCGGGATGAGTGCTATGCTATCTTCATTAACAAATTCTCG</b><br/> <b>CTTGGGCGCTTACGTGCCGCAAGTGCGGTAAGCCGTTAT</b><br/> <b>GGTTCCAAGCCGGTCTACGTCCCGTGCGTGCCGTTTCGTA</b><br/> <b>AGTGACCTCGCGGGTGCCAGTGCCGGTTAAGACGGTGC</b><br/> <b>AAGAAGTAATTCGGTCAGACTTATGCCAGCGGCGGCGGC</b><br/> <b>TCCGGCGGGTGCGGCAGCGGACCCACATTGGAACAAGC</b><br/> <b>ATTTGCAGAGCTTGATAAACCAGAAAGCCGAACGTAAAGTTA</b><br/> <b>TGATCGCACAGGTTGCTCCGGCGGTGCGCGTTGCCATCTC</b><br/> <b>CGAGAGCTTTGGTCTGGCACCGGGGGCTACCACTCCGGG</b><br/> <b>TCAGCTGGCCGAGTCCCTGCGTTGTCTGGGTTTTGATATG</b><br/> <b>GTGTTGACACCTTTGACGCGCGGACCTGACCATTATGG</b><br/> <b>AAGAGGGCACTGAGTTGCTGTCCCGTCTGCAAGCGCAATT</b><br/> <b>GGAAGCCACCCGCATAGCGAAGAACCCTGCCGATGTTT</b><br/> <b>ACCAGCTGCTGTCCGGGCTGGATCGCCCTGTTGGAGAAGA</b><br/> <b>GCTATCCGGAATTAATTCCGTATGTGTCTAGCTGCAAGTCT</b><br/> <b>CCGCAGATGATGTTAGGCGCTATGGTGAAGACCTATCTGG</b><br/> <b>CAGAGAAGAAAGGCATCTCTCCGAGCGACATCTCCATGGT</b><br/> <b>TTCGATTATGCCGTGTGTGAGGAAGCAAGGTGAAGCGGAC</b><br/> <b>CGTGATTGGTTTTGCAGCGGCGGCCCGCGCTTCGTGATG</b><br/> <b>TGGACCACGTTATTACCACGGCGGAGCTAGGCAACATCCT</b><br/> <b>GAAAGAACGTAATATCAACCTCCCCGAAGTCCGGAAGGC</b><br/> <b>GGTTGGGATGAGCCGCTGGGTTGGGTAGCGGTGCGGGC</b><br/> <b>GTCGTTTTCGGTACAACCGGTGGTGTATGGAAGCGCGC</b><br/> <b>TGCGCACCGGTACGAGTTGGTTACCCAGCAACCGCTGCC</b><br/> <b>TCGTTTGAATCTGAGTGAGGTTGCGGTATGGACGGCATT</b><br/> <b>AAGAAACCGAAATCAAGATGGTTCCGCCTCCGGGTAGCAA</b><br/> <b>ATTCGCCGAGCTTGTGCGCGACGTGCAGCGGCGAAGGCT</b><br/> <b>ATGGATGAAGCGGCCGCGAGCGCAGGCGCGATTAAAGTGG</b><br/> <b>GATGGTGGCAGCAACTTACCCGCGACGACGGTGCTAAG</b><br/> <b>GGCATCACCTGCGCGTGGCGGTGGCTAATGGTCTGGGAA</b><br/> <b>ACGCGAAAAAGCTGCTGACGAAAAATGCAGACCGGTGAGTG</b><br/> <b>CAAATACGACTTCGTGGAAATTATGGCATGTCCGTCTGGGT</b><br/> <b>GCGTTGGTGGTGGTGCTAGCCGCGCAGCACCGATAAACA</b><br/> <b>GGTGGCGGTCAAACGTACGAGGCACTGTACGACCTTGAC</b><br/> <b>GAGCGCGCAACGATCCGTCTAGCCACGAGAACCCGGCT</b><br/> <b>GTCCAAAAGCGTACGAAGATTTCTGGGCGAGCCGAACA</b><br/> <b>GCCACAAAGCGCACGATCTGTTGCACACCCATTATGTTCCG</b><br/> <b>GGTGGCGTTGACACGGAGTAGGGATCC</b> </p> |
| 23 | Codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Trichomonas vaginalis</i> , 5' – 3' (including restriction sites         | <p> <b>CCCGGGATGCTAGCGTCAAGTGCAACAGCTATGAAAGGCT</b><br/> <b>TCGCCAATAGCCTCCGTATGAAAGATTACAGCAGCACCGG</b><br/> <b>TATTAACCTTCGACATGACTAAGTGATTAAGTCCAAAGCT</b><br/> <b>GCGTTCGCGCCTGCACGAACATCGCTGGCCAAAACGTCTT</b><br/> <b>GAAGAGCTTGACGGTGAACGGCAAGTCCGTTGTGACAGC</b> </p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |

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|    | shown in bold)                                                                                                                                           | <p>GTTACCGGTAAACCGCTGGCGGAGACCAACTGCATCAGCT<br/> GCGGTCAATGTACCCTTGGCTGCCCGAAGTTCACCATCTTC<br/> GAGGCGGACGCCATCAATCCAGTTAAAGAGGTCTTGACCA<br/> AGAAAAACGGTCGTATTGCCGTGTGTGAGATTGCTCCGGC<br/> GATTCGTATCAACATGGCTGAAGCTCTGGGCGTTCCGGCT<br/> GGTACGATCTCCTTGGGTAAGGTGGTGACCGCGCTGAAAC<br/> GTCTGGGTTTTGACTACGTTTTCGATACCAATTCGCTGCC<br/> GATATGACCATCGTGGAGGAAGCGACCGAACTGGTTCAAC<br/> GTTTGTCTGACAAAAACGCAGTGCTGCCTATGTTTACTTCA<br/> TGCTGTCCAGCGTGGGTTAATTACGTAGAGAAATCCGATCC<br/> GAGCTTGATCCCGCATCTGAGCTCTTGCTGAGCCCGATG<br/> AGCATGCTGAGCAGCGTTATCAAGAACGCTTTCCGAAAAA<br/> GATTGGTACGACCGCAGACAAAATCTACAATGTGGCGATCA<br/> TGCCGTGCACCGCGAAGAAGGACGAAATTCAGCGCAGCCA<br/> ATTTACCATGAAGGATGGTAAACAAGAGACAGGTGCGGTG<br/> CTGACTTCTCGTGAACGCGGAAAATGATTAAGGAGGCGAA<br/> GATCAACTTCAAAGAGCTGCCGGATACGCCGTGTGATAACT<br/> TTTATTCTGAAGCATCGGGCGCGGTGCGATCTTTGTGCA<br/> ACCGGCGGCGTGATGGAAGCCGCGGTGCGCAGCGCGTAT<br/> AAGTTCCTGACGAAGAAAGAGCTGGCACCATTGACCTGC<br/> AAGACGTTGCGCGGTGTTGCGAGCGCGTTAACTGGCGGA<br/> GGTGGATATTGCTGGTACCAAAGTAAAGTGGCGGTTGCG<br/> CACGGCATTAAAGACGCCATGACCCTGATTAAGAAGATCAA<br/> ATCGGGTGAAGAGCAGTTTAAAGACGTTAAGTTCGTGGAA<br/> GTGATGGCATGTCCGGGTGGCTGCGTGTTGGTGGTGGC<br/> AGCCCGAAGGCTAAGACCAAGAAAGCTGTCCAGGCACGAT<br/> TGAATGCAACGTAATCCATTGACAAAAGCTCCAAACACAGA<br/> ACTTCTCAGGACAACCCGAGTTGTTACAGCTGTATAAAGA<br/> ATCGTTTGAAGGTAAATTCGGTGGTCATGTCGCGCACCACC<br/> TGCTGCACACCCATTATAAGAATCGTAAAGTAAATCCGTAG<br/> <b>GGATCC</b></p> |
| 24 | Codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Megasphaera micronuciformis</i> , 5' – 3' (including restriction sites shown in bold) | <p><b>CCCGGGAT</b>GAAAGCTGTAGAGGGATTGAATCAAAATATAA<br/> ATTTTACGACAAGCGCGTGCCGATTGCGGACGACAACCCA<br/> GCTGTGCACTTTGACGAAACGAAATGCAAAAATTGTACCCT<br/> TTGCCGTGCGTGCGTGTGAAACTACGCAAACCGTGCTGGAC<br/> TATTACAGCCTGGAACGCACCGGCGATGTTCCGGTTTGTG<br/> TTCCTGCGGTCAATGTGCAATGCCTGCCCGTTTGGTGC<br/> CATGATGGAAGTTGATGATACGAACCTAGTCAAAGCTGCGA<br/> TCGCGGACCCGGATAAGGTGGTGGTTTTCCAAACGGCACC<br/> GGCGGTACGTGTGGCCATTGCGGAAGAGTTCGGTGCGGA<br/> GGCGGTACATTGCGACAGGGTAAAATGATTTAGCGTTG<br/> AGAGCCCTGGGTGGCGACTACGTGTTGACACCAACTTGTG<br/> GCGCTGACATGACCATCATGGAAGAACTCCGAGCTGGT</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

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|    |                                                                                                                                                  | <p>GCGTCGTATTACCACTGGTAACTTCGCAATGCCGCAGTTTACAGCTGCTGCCCGCGTGGGTTGAGTTTGCTGAGACCTTCTACGCAGAATACATCCCGCATCTGTCCAGCGCAAGAGCCGATTCTGATGCAAAACACCACGGAAAAATCTGGTTTGC CGAGAAGGCGGGCATCGATCCGAAGAAGATGGTGACGGTTTGCGTTACCCCGTGACCGCTAAAAAGCCGAGATCAAGCGCAAAGAATTGAATGCTGCGGCTGAGTACTGGCATATTGATGGCTTAAAGGACTCCGACATTTGCATCACGACCCGTGAACGGCACGTTGGCTGAAAGCGGAGAACATTGACTTCAATACCCTGGATGATGGTATTTTTGATAGCCACCTGGGCGAGGCGAGCGGTGGTGATCATCTTCGGCAGCACCGGTGGTGTCATGGAGTCCGCACTGCGCAGCGCGTATTACTTCTATACCGGTAAACCGATGCCAGCGGAGTATATACCGTATGAACCGGTGCGTGCGCTTGACCGCGTTAAAGAGGCGACCATCGACTTCAGCGGTATCTCTCTGCATGTGCGAGTCGTGAGCGGTCTCGGCAATGCGCGTCGTTTTCTGGATAAAATTATGGCGGACGGCACCTTCAAAGATTACACCTTTATCGAGTTCATGGCGTGCCAGGTGGCTGCATCAACGGTGGTGGCCAACCGAAGGTTAAGATGCCTCTGGTTCAGAAAACCAACCAGGCTCGCATGAATAGCTTGTAACAACGTGATTGCGAGGTTTCGATTAAGGCCGCCTGGAAAACCCCGAAATCCAGGAACCTACAGCGACTTCTACGGCCAGCCGCTGTCTGAGCGCTCTGAGAAGTATATTCACACCTTCTTTGAGGATAAGTCCGGCAACCTGGGTGAAGGTGGTGCTGTGACCCCGCAAACGAACCCATTGTCTCCGAAGTATAAGCCGATTGAATAGGGATCC</p> |
| 25 | Codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Veillonella parvula</i> , 5' – 3' (including restriction sites shown in bold) | <p><b>CCCGGGATGTCAAATATCAATTTT</b>AGATAGAAGGGTCCC GATTGAGGACGGTAACATCGCTCTGGTTCAGGATTTGACTA AGTGCAAAAATTGTAGCCTGTGTCGTAAGCTTGCGCGGTG GATATGGGCGTCTTTGACTACTATGATCTGACCACGAATGG TGACCACCCGATTTGCATCCACTGCGGTCAATGTGCGTCC ATCTGCCCATTCGATTCTATTAATGAACGCAGCGAGATCGA TGAAGTTAAGGCGCGATTGCGGACCCGAACAAAATCGTCA TCTTCCAGACCGCACCGGCTGTGCGTGTGGGTCTTGCGG AGGAGTTCGGCTTGAGGCGGGTACCTTTGTGAAGGCAA GATGGTTGCAGCCCTGAGAAAGCTGGGCGGTGACTACATT CTGGACACCAACTTTGGTGCGGACATGACCATCATGGAAG AGGCGTCCGAGTTGCTGGAACGTGTTATCAACTCGGATGCG TGTTCGCCGCAATTTACCAGCTGTTGTCCGGCGTGGGTTA AGTTCGCGGAGACATTCTACCCGGAGTTCCTGCCTAACCT GAGCACCGCGAAGTCCCCGATTGCCATGCAGGCACCGAC CCAGAAAACCTATTTCCGCCGAAAAATGGGTCTGGACGCG AAGCAGATTGTGGCGGTGGCCGTAACCTCCGTGTACTGCCA AGAAATTTGAGATTGCGCGTGACGAAATGAACAGCAGCGC</p>                                                                                                                                                                                                                                         |

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|    |                                                                                                                                                  | AGAGTATTGGAATACCCAGAAATGCGTGATACCGATTACT<br>GCATCACCACGCGTGAGCTGGCAAAGTGGCTGCGCGCAG<br>AAGAAATCAACTTTGACGACCTCGAGGACAGCGCATTTGAT<br>CCGCTGATGGGTGAGGCTAGCGGTGGCGGTATTATTTTGG<br>GCAACACCGGCGCGGTTATGGAAGCTGCCATGCGCGCGG<br>CGTATAAAATGGCAACCGGTGAAGATGCCCCCAAACCT<br>TATCCCATTGAGGCCATCAGAGGCATGGATGGTGCGCGC<br>GAAGCTGATGTTGTGATCGGTGACAAGACCCTGCACGTTG<br>CGGCGGTGCATGGTACGGGCAATTTACGTAAATTCATTGA<br>GCGCATGCGTGCGGAGAACATCCACTACGACTTCATCGAG<br>GTGATGGCATGCCGTGGTGGGTGCATCGGCGGCGGTGGC<br>CAACCGCGTGTTAAATTGCCGATGGCCGACAAAGCGCGCG<br>AAGCTCGTATCGCGTCTCTATACACCCGTGACGCAGAAAT<br>GACTGTAAAGGCCGCGTGCGATAATCCGGATATTCAGAAAT<br>TATATGCAGAGTTTTTCGACGGCAAACCGATGAGCCATAAA<br>GCACATCACATGCTGCATACCAGTTCGTGAATCGTAGCGA<br>AGATTTGGGTCCGAACGGTGCGTGACCCCGGCGACGTG<br>CCCGACCAAGTTCGGAACCTGAAAAAGGCTGCTGAAGCG<br>GCGAAGGCGGCGGCTGAAGTTAACTCTTAGGGATCC                                                                                                                                                                                                                                                 |
| 26 | Codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Veillonella atypica</i> , 5' - 3' (including restriction sites shown in bold) | <b>CCCGGG</b> ATGTCACAATTTGAATTTATAGATAAAAGGGTCCC<br>TATTGCGCTCGACAACCCGAGCATCTATCAGATATCTCCA<br>AATGCAAAAACCTGCACCTTATGCCGTGCGTGCGCCGA<br>CGTGATGAGCGTTCTGGACTACTACGATCTGGAAGCGACC<br>GGTGACGTGCCAGTTTGTATTATTGTGGACAATGTGCAGC<br>GGCGTGCCCGTTTGACTCGATGCATGCAAAAAGCGAATTG<br>GATAAGGTGAAGGTGGCTATTGCCGATCCGGATAAAATCG<br>TCGTTATTCAGACCGCTCCGGCTGTACGCGTGTCGATTGG<br>CGAGGGTTTCGGCTTTGAACCGGGTACGTTTCTCGAGGGC<br>AAAATGGTTAGCGCGCTGCGTAAACTGGGTGCCGACTACG<br>TGGTCGACACGAACCTCGGCGCGGATCTGACCATTATGGA<br>GGAGGCGTCTGAATTGGTTGATCGTCTGAAGAACGGTGGC<br>ACGATCCCGCAATTCACCAAGTTGCTGCCCGGCTTGGGTTT<br>GTTTTGCGGAAATCTACTTCCCGGAGTTGATCCCAAATCTG<br>TCTAGCACCCGTAGCTGCATCGCAATGGAGGCCGCTATGA<br>TTAAAACCTATTTGCTGAGAAAAAGGGTATTAACCCGGCG<br>AATATCGTGTCCGTCTCCGTTAATCCGTGTACTGCGAAGAA<br>GGCTGAGACAAAGCGCGTGGAAGAAATGCTGCCGCGCG<br>TTATTACGACGACGAATCCCTTGGCATGGATACCGACATCA<br>GCATTACCACCAGAGAATTTATCCGTTGGCTGAACGACGAA<br>GGCGTGGACTTCGAGTCCCTGGAGGACAGCCAGTTTGATG<br>ATCTTATCGGTATGGAACTGGCGCGTCTATCATTTTTGGT<br>AATACCGGTGGCGTTATGGAGGCTGCTATGAGAACGGCGT<br>ACAAACTGATTACGGATAAGGAGCCGCCACCGTATGCACT |

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|    |                                                                                                                                                                                                       | <p>GACCCACCTGGAGGATGTGCGCGGTATGAACGGTGTGAAA<br/> GAGGCGACGGTTCAGCTGGGTGATGACGTGACTCTGAGC<br/> GTTGCGGTGGTTCACGCGGTAAGAACACCCGTGATTTTTT<br/> GAATGCGCTGAAGGAGAACGGCAAGCACTATGACTTCATC<br/> GAAGTCATGGCATGTCCGGGTGGGTGCATTGGTGGCGGT<br/> GGCCAACCGCTACCAAACTGCCGCAGGCGGTTAAGACCA<br/> AAGAGGCGCGTATCGGCGGTCTGTACAAGGCGGACGAAG<br/> AATATAAGTACGTTGCCAGCTATGAATCACCAGAAATCCAA<br/> GAGTTGTACAAGAACTTCTTGGGAGAACCTCTGGGTCACAA<br/> AGCACATGAATTGCTGCATACCCACTTCACCGATCGCAGC<br/> GCACAGTTAGGCGACCGCAAAGATGTTGTCCCGGAGACCT<br/> GTCCGACCAGCCCGAAATACAAGGGTTAGGGATCC</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 27 | <p>Codon optimised nucleic<br/> acid sequence encoding<br/> HydA (Hyd1) from<br/> <i>Peptoclostridium</i><br/> <i>bifermentans</i>, 5' - 3'<br/> (including restriction sites<br/> shown in bold)</p> | <p><b>CCCGGG</b>ATGAAGCACCTATTTACAGAAAAAGTAGTTCGGAT<br/> CGAGCTGGATAAACCCTCCATTGAGTTGACTTTGATAAGT<br/> GCATCAAGTGCGGTCTGTGTAACGCGTTTGTGAAAACGA<br/> GATTGGTGTTAATGGTTATTTGACCTGGAGAAGACCGGTG<br/> ACATCGCCATCTGCATCAACTGCGGCCAATGTGTTCAAGC<br/> ATGTCCAAAAAGGCGATCACCCAGGTTATCGATGTGGATC<br/> GCGTGAAGGAAGCGATCAACGATCCGGAAAAGATTGTTAT<br/> CTTCAGCACAGCGCCAGCTGTGCGTGTGCACTGGGCGAA<br/> GAATTTAACCTGGAAGAAGGCGCGTATGTTGAGGACAAAAT<br/> GGTGGACGCCCTGCGTAACTGGGTGGAGATTACGTTTTT<br/> GACGTTACCTTTGGCGCCGATATGACCATCATGGAAGAGG<br/> CGAACGAACCTGTTTCTCGTATCAAAAACGGCAAAGGCAAA<br/> ACCCCGCAATTTACCAGCTGCTGCCCGTCTGGGTTAAGTT<br/> CGCGGAAACGTTCTATCCGGAGTTGATTCCGAATCTGTCTA<br/> CCACGAAATCTCCGATTGGCATTGAGGTGCTGTATCAAG<br/> ACCTATTTGCGACAGAAAGCAAATATCGACCCGGAGAAAAT<br/> CGTGAACGTAACCATTACTCCGTGCACCGCTAAGAAGTAC<br/> GAAATTGACCGTCCGGAGATGAATGCGAGCGCAAAATACA<br/> ACAAAAGCGAAAATATGAGAGATAATGACATCATCCTCACC<br/> ACTAAGGAGCTGGCGCAGTGGCTCCGTGACGAAGAGATCG<br/> ACTTCAACGCTTTGGAGGGCTCGAAATTTGACAACATCTTG<br/> GGCTTGGGTAGCGGTGCGGGCATTATCTTCGGTAATAGCG<br/> GTGGTGTGATGGAAGCCGCGGTGCGCACGGTCTACAATAT<br/> CCTCACCCATGAGAACCACATAAAGAACTGCTGCACTTTA<br/> ATCCGGTTCGTGGTCTGGAAGACGTGAAGGAGGCTACCCCT<br/> TACCATTGGCGATACCACCCTGCGCCTGGCAGCGGTGCAA<br/> GGCACGGCGAACGTGCGTACGTTGATCGAAAAGCTGAAAT<br/> CCGGTGAGGTGGAGTACGACTTCATAGAGGTAATGACTTG<br/> CAAAGGTGGTTGATTGGTGGCGCTGGTCAACCGAAGATG<br/> AAAGCGCGTATTAGCAATGAGATGCGTCTCAAGCGCATTGA<br/> GGGTCTGTACGATAAGGACAAGCACATTGCGGTCAAATGC</p> |

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|    |                                                                                                                                                                                                                   | AGCTATGAAAACCCGGATGTCATCAACGTGTACAAGGAGTT<br>CTTCAAACAACCGCTGAGCCATTTATCCCACGAGTTGCTGC<br>ACACCACCTTTGAAAGCAAGCACGATATGTTGGGTCTGAAG<br>GATGACAACAACGTTTCGGATATTGGCTAGGGATCC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| 28 | nirB promoter                                                                                                                                                                                                     | GGTAAATTTGATGTACATCAAATGGATCCCCTTGCTGAATC<br>GTTAAGGTAGGCGGTAATAGAAAAGAAATCGAGGCAAAAAT<br>G                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| 29 | nar promoter                                                                                                                                                                                                      | CTCTTGATCGTTATCAAATCCCAATACTGTGTTAGAGAGTAT<br>AATGCCCTTA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| 30 | Exemplary HPGC (hydrogen producing gene cluster) with alternative codon optimised nucleic acid sequence encoding HydA from <i>Chlamydomonas reinhardtii</i> , 5' – 3' (including restriction sites shown in bold) | CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br>GTGGAAGAAGGAGATATACATATGGCGATGCGTTCCACAT<br>TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGCCTGGGG<br>CGCGTCCCGCGTGCCTATGCTTGCATGGCCTATAAGGT<br>TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br>CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br>GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br>ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br>TCTGATCAGTCCTTTCTGGATGACGCTCAGATGGGGAACG<br>GATTCGTCTGACATGTGTCGCGTATCCTACAAGCGACTGC<br>ACTATCCAACTCACCAAGAGGAGGCTTTATATTAAGAA<br>GGAGATATACATATGCAAAGTTCGCGCTCCAGCAGCTTC<br>AGGTGTTGCCACACGTGTCGAGGTCGTGATGTGTCGT<br>CCGGTTGCGGCGACGAAGGCTTCCACGGCTGTTACACAG<br>ACATGTCGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br>TGAAATGCCGTTGAACACGTACAGTAATAAGCTCCGTTCA<br>AGGCAAAGGTTTCGTTCCGTGGAAAAATCACAGGACCAAA<br>AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br>GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br>TTCCGCGGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br>GCCTCATGGTACTCGTCTTTATTCGATTGCTTCTAGTCGTTA<br>CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br>CGCCGCGCGGTATACGTGATCCAGAGACTGGAAGGAG<br>GACCCCGCGAAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br>TGCCACACCAGGCACGGAATTTCCATGACAGGGCCCA<br>GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br>TAATCTGTGTCGAACGGGGACTGGAATCGCGCCTTTTCG<br>CTCATTCTGGCGCGTTGCTTCATCGAGAATGTCCCAAGTT<br>ATAAGTTCACTGGCCTTTTCTGGTTGTTTATGGGTGTCGCT<br>AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br>CGCAAAAGCGTATCCGGGACAATCCGCCTGGACTATGCC<br>TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAATGTA<br>CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br>ATTTATTGGATAATGGGGCGCACATGTAATCTGCGGGTTA |

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|  | <p>AAAGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/>TTGCAAAAGAAAAGGGGCTGAACTACGAAAGTGGGTCGA<br/>GGGGTTAAAGCACAGAATCAATGGCATGTTGAAGTCTACT<br/>AACCAGGCATCAAATAAACGAAAGGCTCAGTCGAAAGACT<br/>GGGCCTTTCGTTTTATCTGTTGTTTGTGGTGAACGCTCTC<br/>TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br/>GCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/>TGTTGTGTGAAAGAAGGAGATATACAT</p> <p>ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>ATGTCCGC<br/>ATTAGTTCTGAAGCCATGTGCGCGGTCAAGTATCCGCGGA<br/>AGCAGCTGCCGCGCCGTCAAGTCGCGCCGCGCGCGCCG<br/>TTAGCAGCGTCTACAGTTCGTGTTGCATTAGCAACACTTGA<br/>AGCTCCTGCGCGTGCCTGGGCAACGTCGCTTGTGCAGCA<br/>GCGGCTCCGCGGCGGAGGCCCTTTGAGCCACGTCCAA<br/>CAAGCCTTAGCTGAATTGGCCAAACGAAAGATGACCCCA<br/>CACGCAAGCATGTTTGTGTGCAAGTCGCCCCAGCGGTCCG<br/>CGTCGCTATTGCCGAAACACTTGGAATTGCTCCCGGTGCC<br/>ACGACCCCGAAGCAATTGGCGGAGGGCTTACGCCGTCTG<br/>GGTTTCGACGAAGTATTCGACACGCTTTTCGGGGCAGATT<br/>GACAATTATGGAAGAAGGGTCAGAGTTGTTGCATCGTTAA<br/>CGGAACATTTGGAGGCTCACCTCACTCTGACGAACCGTT<br/>GCCCATGTTTACCTCGTGTGTCGGGTTGGATTGCGATG<br/>CTTGAGAAATCGTACCCGATTAAATTCCTTATGTGCTCTC<br/>GTGCAAATCTCTCAAATGATGTTAGCCGCGATGGTGAAGT<br/>CATATCTTGCCGAAAAGAAGGGCATCGCACCTAAAGACATG<br/>GTGATGGTGTCAATCATGCCTTGACCCGTAAGCAGTCCG<br/>AGGCCGATCGCGACTGGTTTTGTGTGGATGCTGACCCTAC<br/>ATTACGCCAGTTAGATCATGTTATTACAACCGTAGAGTTGG<br/>GTAACATCTTTAAGGAGCGTGGTATCAATTTGGCTGAGTTA<br/>CCGGAAGGTGAATGGGACAACCCGATGGGGGTGGGTTTCG<br/>GGAGCCGGAGTATTATTTGGGACCACTGGCGGCGTAATGG<br/>AGGCAGCATTACGTACGGCTACGAACTGTTACAGGTAC<br/>CCCGCTGCCTCGCCTGAGTTTGAGCGAGGTGCGTGGTATG<br/>GACGGTATTAAGGAAACGAACATCACAATGGTTCCTGCTCC<br/>CGGTTCAAAATTCGAGGAACTGTTGAAGCATCGCGCTGCC<br/>GCACGTGCAGAAGCAGCCGACACGGTACTCCTGGTCCAC<br/>TTGCCTGGGACGGCGGGGCCGATTACTTCAGAAGACGG<br/>TCGTGGTGGAATTACTTTACGTGTCGCTGTTGCGAATGGGC<br/>TGGGTAACGCTAAAAAGTTAATTACAAAAATGCAGGCCGGA<br/>GAGGCAAAATATGATTTCTGAGAGATTATGGCCTGTCCCGC<br/>TGTTGCGTAGGAGGTGGGGGCCAGCCCCGTTCAACTGA<br/>CAAAGCTATCACGCAGAAACGTCAAGCGGCATTATACAATC</p> |
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|  |  | <p>TGGATGAAAAATCAACGCTTCGCCGTTACATGAGAATCCA<br/>TCAATCCGTGAATTGTACGATACTTATTTAGGTGAGCCTCT<br/>GGGGCATAAGGCACACGAACTTCTGCATACTCATTACGTC<br/>GCGGGTGGAGTTGAGGAGAAGGATGAAAAGAAGTAGGGA<br/>TCC</p> <p>GGGTCGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>ATAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCTGTTT<br/>TATCTGTTGTTTGTCTGGTGAACGCTCTCTACTAGAGTCACA<br/>CTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCGGGCTCGTATGTTGT<br/>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>CACATTCCCGTCAGGCCGGAGATCGCAAACCTGGGCGCAG<br/>GTGCGGCATCTAGCCGCCCATCATGTCTTCTCGCCGCAT<br/>TGTCGCGGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCC<br/>GTGCAAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGGAGAGACCTGGGCGATT<br/>TCTTGGGCTTAGGGAAGGGGGGGCTTTGCGCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>GTGGGGGGTCTACTATCGCGGCCTTGTTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTGCAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAAAACGGCATT<br/>GCAACATTATGTTGCAAGGGGGTGAATTAAAGACAGAGCAA<br/>CGCTTAGCCTACTTGAGGCATGCGTCCGTGCGATTGCGG<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC<br/>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGACACA<br/>GGCAGACGCCGAAGCTAAACGCGGCGAGCCAGAATTGGG<br/>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>AGTATGAGCGTTTGTTCGCGCCGGCGCCCGTCGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>CTTCACCCGGAGCCGATGTCTGGCACGCGCGTGTAGAAT<br/>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>CTGGCTGGGGATGTCATGTTCTTTCGCGACATTAAAGCGG<br/>ACATGATCGGTATGGGCCCCCTTACATCGCAGCCTGGGAC<br/>GCCGCAACTGATAAGTGGACCGCGCTGTATCCGAACGCC<br/>AATAAGAACAGCCACATGAAATCTATGTTGATCTGACCAC<br/>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA</p> |
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|  |  | <p>TGCCTATTTTAACGCCTACTCAGTATCGCGAGTCCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA<br/>GTGTCGTCGTTGCCTGGACATGCGTTTGCCTCCGTTGGC<br/>AAAACGTCTGCGGCCGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGATCCCATCGTTGGCGTCCCAGTCCCGCACGACTT<br/>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGGTTGAAGTGCCGGACCGTTACCTGACCCCGAT<br/>AACCACGGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCAACCACCGAGCTGCCCCCGGGGTGCAGCGG<br/>CTGGAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTGTGGTACGTCTGGCGTAGGTTCAAATATT<br/>GTTACGTCTCCCGAATCGCTAGCACTACGGCACATGGTG<br/>TTCCTCGTATCAATATTGGGGTGTTCGGCGTGATGAATGCA<br/>GGAAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCGGCACGACCGCAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGGAACAGCCA<br/>AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCGATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCGCAATCAAATCGGGACGCCTTGCTGAAGCCCT<br/>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTGCTCAAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGGTGTTCGCCCGTT<br/>GGTCTCTTGACGCAACGCCCGCTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCAGGTGGACGCTTCTTCGTCCT<br/>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>CCGTGCTTTCCGTACGCTGGATTTAGACGCCGCACGTGG<br/>CAAATTGGGGCCCGAGGCCGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>CACCCCTGGACCCTGGACCGCTCATCAGGACGTCCGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTTCG</p> |
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|  | <p>GAAGCGTGTAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGCGGGGAAAAAGTTACAAATCGAACATGCGTTTGGTCG<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAATGCAGCAGCGTATGAAAGACTTGCAATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTCTTTCTCTTGGGCAG<br/>CTTGCCCCGACGCCCCTCGTCGCGCATTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCCGACG<br/>CGCGCCTGCTACGGCAGCGTCCGGGGTATAATACTA<br/><br/>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGA<br/><br/>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/>GCAGGCCGTCTTTTGGCGGGCCAGCGCCCCCTGCGGCGTC<br/>CGCGCGGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/>CACACGGCAAAGCATCTGCCACGCGCGAATACGCTGGTGA<br/>CTTTCTTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/>AGCGTGAAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/>AAGGCTGATGCTCAGGACGCAGGACGCGTGCCTGAGATT<br/>TGGCGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/>GCCTGTAAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/>TTACCTTGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/>TGCTATCAAAGAACGTATCTACGGGAACCGCGTCGTGCTG<br/>TTCGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br/>CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/>GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/>CGCCTTGCAACGTCAAGGTCATCGCCGCATTTTAGCCCTTA<br/>CTGGAGAACATCCTAAATACACTTTTCGACAATTTCTTGATG<br/>CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/>GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br/>TCTCAGACATGCGCCGCCCTTAAGAATACAGATTCAGTTGGC<br/>ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br/>CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br/>TTCGCGTCTTGACCCAGGACCGGCCATGCGCGCAGGCTT<br/>GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br/>ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br/>TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br/>TCCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br/>GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br/>GTTGCGGTCCCTTCGATTGCTGTACCATACACAGGTATGAT<br/>CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCCTT</p> |
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|    |                                                                                                                                                                                                   | <p>CTGAAGTGTGGGATGAGTCAAATGAGTGCCGCTCGCGCA<br/> CTGATGTAGGAGCCTATCACAAAGATCACACGTTAAGTACA<br/> GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br/> GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br/> AGGAGGGCTATGTCCCGTCTTGGTGACAGCTTGTTACCG<br/> TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br/> GCTGGTGACATTACGATTTCTGTCATCCCAATAGTCTGCT<br/> TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br/> TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG<br/> ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br/> CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br/> ACGATGTATATCTTTAACCAGGCATCAAATAAACGAAAGG<br/> CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTG<br/> TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br/> GGGTGGGCCTTCTGCGTTTATA</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 31 | Exemplary HPGC (hydrogen producing gene cluster) with codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Volvox carteri</i> , 5' – 3' (including restriction sites shown in bold) | <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/> GTGAAAGAAGGAGATATACATATGGCGATGCGTTCCACAT<br/> TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGCGTGGGG<br/> CGCGTCCCGCGTGTGCGTATGCTTTCATGGCCTATAAGGT<br/> TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br/> CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br/> GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br/> ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br/> TCTGATCAGTCCTTCTGGATGACGCTCAGATGGGGAACG<br/> GATTCGTCCTGACATGTGTCGCGTATCCTACAAGCGACTGC<br/> ACTATCCAACTCACCAAGAGGAGGCTTTATATTAAGAA<br/> GGAGATATACATATGCAAAGTTCGCGCTCCAGCAGCTTC<br/> AGGTGTTGCCACACGTGTGCGAGGTGCTCGTATGTGTCGT<br/> CCGGTTGCGGCGACGAAGGCTTCCACGCTGTTACACAG<br/> ACATGTGCAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br/> TGAAATGCCGTTGAACACGTACAGTAATAAGCTCCGTTCA<br/> AGGCAAAGGTTGTTCCGTGGAAAAATCACAGGACCAAA<br/> AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br/> GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br/> TTCCGCCGGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br/> GCCTCATGGTACTCGTCTTTATTGATTGCTTCTAGTCGTTA<br/> CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/> CGCCGCGCGGTATACGTGATCCAGAGACTGGAAGGAG<br/> GACCCGCGAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br/> TGCCACACCAGGCACGGAATTTCCATGACAGGGCCCA<br/> GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/> TAATCTGTGTCGCAACGGGGACTGGAATCGCGCCTTTTCG<br/> CTCATTCTGGCGCCGTTGCTTCATCGAGAATGTCCCAAGTT</p> |

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|  | <p>ATAAGTTCACCTGGCCTTTTCTGGTTGTTTATGGGTGTCGCT<br/>AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br/>CGCAAAAGCGTATCCGGGACAATTCCGCCTGGACTATGCC<br/>TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAAAATGTA<br/>CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/>ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA<br/>AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/>TTGCAAAAGAAAAGGGGCTGAACTACGAAGAGTGGGTCGA<br/>GGGGTTAAAGCACAGAATCAATGGCATGTTGAAGTCTACT<br/>AACCAGGCATCAAATAAACGAAAGGCTCAGTCGAAAGACT<br/>GGGCCTTTCGTTTTATCTGTTGTTGTCGGTGAACGCTCTC<br/>TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br/>GCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/>TGTTGTGTGGAAAGAAGGAGATATACAT</p> <p>ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>ATGTACGT<br/>TTGCGTCGCACAAGTTACGTTACATGATTGCTTAGCATGCT<br/>CAGGGTGTCATCACATCTGCGGAAACGGTTTTGCTTCAGCA<br/>ACAGTCCGGGGATGAGTTCCTTATCCCGCTTGGCAGACCCG<br/>CACACTACTGTCGTAGTTACCGTCTCGCCCCAATCGCGCA<br/>CAGCTTTGGCGGCTTACTATGGATTATGCCCGTCTCAGGCT<br/>CTTGCCCGCCTTGTTGGGCTGGTTGAAGTGGCTTGGGGTTC<br/>GCGCCGTATGGGATCTGACAACAGCCCGCATCTTGATT<br/>GTTGGAAGAGGCAGCTGAGTTCATGAACCGTTGGGTCTGC<br/>ATGTATGTTAGCGCCGGGCCTCTTCGGTCATGGGCAGTT<br/>CATGTCCTGGCACAGGCTGGGTTTGTTATGCTGAAAAGAC<br/>GCACGGGACGCGTGATTACCCTATTTGAGCACCACCCGT<br/>TCACCCCAAGGAGCAATGGGTGGCTTGGTAAAGTCCCTGG<br/>TGGCAGCAGCTTGGGGCGTTACTCCAGGGTCTTTGTACCA<br/>CGTCACAATTATGCCGTGTTATGACAAGAAATTGGAAGCAT<br/>CTCGCGATGAATTAACCACAACGGCGACAACACTACTACCGT<br/>GCGGGGACAGACGCGGCTGGAGCAGGTGCCGCCGTAGG<br/>CGGGCCTCTGCCTGAAGTGATGGTCCGTCATGCAACCGCA<br/>GCGCCTGATCCGTTGCTTCCGGGCGTGGTACCAGCCGAC<br/>GACCAGCTGTACTCCCTTCCACATGGCTCCAGCTCGGGTG<br/>GATACGCGGACTTCGTTTTCCGCACTGCCGCGCGCAGTT<br/>GTGGGGGGTAGAAGTTCGCCAGGACCTTACCATGGCGT<br/>ACTCGTCGTAACGCCGACTTACAGCCTCGTGTTGGTGCCC<br/>CAGGCCAATCTCTGGTCGTTGCGCGTGTGTATGGATTCCG<br/>CAATATTCAGACACTTTTACAGCAGCTTAAGCGTGGACGCT<br/>GCCCTTATCATTATGTGGAAGTCATGGCTTGCTAGCGGG<br/>TGTTTGAACGGAGGAGGACAAATCAAACCGGGGCCAGGG<br/>GTAACGCCGCAACAATTAATTGAACAATTGGAGCTTTTGTGA</p> |
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|  |  | <p>             TGACGTTGCGGCACGTTCTCCAGCAGACAACCCGGCGGTA<br/>             GCAGCTTTATATGGCTCTTGTTAGGCGGTCGTCCAGGGG<br/>             CACCCCCCGCTCGTCAACTGTTACATACGACATTTGCGGAG<br/>             CGCGAAAAGACTGTAAGTGCCGCAACCGTCACGAATTGGT<br/> <b>AGGGATCC</b><br/>             GGGTCGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>             ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTGTTTT<br/>             TATCTGTTGTTTGTGCGGTGAACGCTCTCTACTAGAGTCACA<br/>             CTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATA<br/>             CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>             GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>             CACATTCCCGTCAGGCCGAGATCGCAAAGTGGGCGCAG<br/>             GTGCGGCATCTAGCCGCCCATCATGTCTTCTCGCCGCAT<br/>             TGTCCGCGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>             CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCC<br/>             GTGCAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA<br/>             TGGCATCAGCGGAGGCCGCGGAGAGACCTGGGCGATT<br/>             TCCTGGGCTTAGGGAAGGGGGGGCTTTGCGCGGGCGCAA<br/>             CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>             GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>             GTTGACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>             GTGGGGGGTCTACTATCGCGGCCTTGTTGGAGTTCAGTAA<br/>             TATTTGCCAAAACGATTGCTCATATTGTGGGATTCGCAACA<br/>             ATCAAAAAGAAGTTTGGCGCTATACTATGCCGGTTGAAGAG<br/>             GTGGTCGAGGTAGCTAAGTGGGCGCTTGAACGCGCATTC<br/>             GCAACATTATGTTGCAAGGGGGTGAATTAAAGACAGAGCAA<br/>             CGCTTAGCCTACTTGAGGCGATGCGTCCGTGCGATTGCGG<br/>             AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC<br/>             CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGACACA<br/>             GGCAGACGCCGAAGCTAAACGCGGCGAGCCAGAATTGGG<br/>             TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>             AGTATGAGCGTTTGTTCGCGCCGGCGCCCGTCGCTATCT<br/>             GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>             CTTACCCGGAGCCGATGTCTGGCACGCGCGTGTAGAAT<br/>             GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>             AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>             CTGGCTGGGGATGTCATGTTCTTTCGCGACATTAAAGCGG<br/>             ACATGATCGGTATGGGCCCCCTTACACGAGCCTGGGAC<br/>             GCCTGCAACTGATAAGTGGACCGCGCTGTATCCGAACGCC<br/>             AATAAGAACAGCCACATGAAATCTATGTTTCGATCTGACCAC<br/>             TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>             ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>             GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA           </p> |
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|  |  | <p>             TGCCTATTTTAACGCCTACTCAGTATCGCGAGTCCTATCAG<br/>             TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA<br/>             GTGTCGTCGTTGCCTGGACATGCGTTTGCCTCCGTTGGC<br/>             AAAACGTCTGCGGCCGAGTTTGGGGCGATCCTGCTTCGT<br/>             TCTTGATCCCATCGTTGGCGTCCCAGTCCCGCACGACTT<br/>             GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>             ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>             GCGTCTGGTTGAAGTGCCGGACCGTTACCTGACCCCGAT<br/>             AACCACGGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>             AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>             ACCACCACCACCGAGCTGCCCCCGGGGTGCAGCGG<br/>             CTGGAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>             CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>             CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>             GTGTTGTCGCGTCGCCATTGCGCCCAGCTGCCGCTTGCCG<br/>             TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA<br/>             AGACGCTGGTGTGGTACGTCTGGCGTAGGTTCAAATATT<br/>             GTTACGTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>             TTCCTCGTATCAATATTGGGGTGTTCGGCGTGATGAATGCA<br/>             GGAAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>             CATGCATTGTTGACTCAACCCCGGCACGACCGCAGATGT<br/>             AAAACAGTTTTGCTTGAGCTTCATGCCCTTGGAACAGCCA<br/>             AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>             TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>             GAGTGCGATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>             CAGCCGCGCAATCAAATCGGGACGCCTTGCTGAAGCCCT<br/>             GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>             AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>             GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>             CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>             CGCTGGATTTAGCGTCCACCCCTGCACGAGCGTAGTAC<br/>             GATTACGTCTGCATTGCTCAAGGAAGGAGCAGTGCGCAGT<br/>             TCACGCTATGGGGCTCCTCTGCCGGGGTGTTCGCCCGTT<br/>             GGTCTCTTGACGCAACGCCCGCTTACTGATGGTAATCCC<br/>             GATGGATGCCGAAACCCAGGTGGACGCTTCTTCGTCTCT<br/>             CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>             CCGTGCTTTCCGTACGCTGGATTTAGACGCCGCACGTGG<br/>             CAAATTGGGGCCCGAGGCCGTGAGATGGAGCGTCAGCG<br/>             TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>             CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>             CACCCTTGACCCTGGACCGCTCATCAGGACGTCCGTTGG<br/>             TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>             AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/>             CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTTCG           </p> |
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|  |  | <p>GAAGCGTGTAATCATAACCGGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAAGCTGGAGGCAGCA<br/>CTTGCGGGGAAAAAGTTACAAATCGAACATGCGTTTGGTCG<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAATGCAGCAGCGTATGAAAGACTTGCATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTCTTTCTCTTGGGCAG<br/>CTTGCCCCGACGCCCCTCGTGCGGCATTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCCGACG<br/>CGCGCCTGCTACGGCAGCGTCCGGGGTATAATACTA<br/><br/>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGA</p> <p>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/>GCAGGCCGTCTTTTGGCGGGCCAGCGCCCCCTGCGGCGTC<br/>CGCGCGGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/>CACACGGCAAAGCATCTGCCACGCGCGAATACGCTGGTGA<br/>CTTTCTTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/>AGCGTGAAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/>AAGGCTGATGCTCAGGACGCAGGACGCGTGCCTGAGATT<br/>TGGCGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/>GCCTGTAAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/>TTACCTTGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/>TGCTATCAAAGAACGTATCTACGGGAACCGCGTCTGCTG<br/>TTCGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br/>CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/>GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/>CGCCTTGCAACGTCAAGGTATCGCCGCATTTTAGCCCTTA<br/>CTGGAGAACATCCTAAATACACTTTTCGACAATTTCTTGCATG<br/>CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/>GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br/>TCTCAGACATGCGCCGCCCTTAAGAATACAGATTCAGTTGGC<br/>ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br/>CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br/>TTCGCGTCTTGACCCAGGACCGGCCATGCGCGCAGGCTT<br/>GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br/>ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br/>TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br/>TCCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br/>GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br/>GTTGCGGTCCCTTCGTATTGCTGTACCATACACAGGTATGAT<br/>CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCCTT</p> |
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|    |                                                                                                                                                                                                    | <p>CTGAAGTGTGGGATGAGTCAAATGAGTGCCGCTCGCGCA<br/> CTGATGTAGGAGCCTATCACAAAGATCACACGTTAAGTACA<br/> GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br/> GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br/> AGGAGGGCTATGTCCCGTCTTGGTGACACAGCTTGTTACCG<br/> TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br/> GCTGGTGACATTACAGATTTCTGTCATCCCAATAGTCTGCT<br/> TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br/> TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG<br/> ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br/> CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br/> ACGATGTATATCTTTAACCAGGCATCAAATAAACGAAAGG<br/> CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTG<br/> TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br/> GGGTGGGCCTTCTGCGTTTATA</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 32 | Exemplary HPGC (hydrogen producing gene cluster) with codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Giardia lamblia</i> 5' – 3' ((including restriction sites shown in bold)) | <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/> GTGGAAGAAGGAGATATACATATGGCGATGCGTTCACAT<br/> TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGCGTGCGG<br/> CGCGTCCCGCGTGTGCGTATGCTTTCATGGCCTATAAGGT<br/> TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br/> CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br/> GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br/> ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br/> TCTGATCAGTCCTTCTGGATGACGCTCAGATGGGGAACG<br/> GATTCGTCCTGACATGTGTCGCGTATCCTACAAGCGACTGC<br/> ACTATCCAACTCACCAAGAGGAGGCTTTATATTAAGAA<br/> GGAGATATACATATGCAAAGTTCGCGCTCCAGCAGCTTC<br/> AGGTGTTGCCACACGTGTGCGAGGTGCGTATGTGTCGT<br/> CCGGTTGCGGCGACGAAGGCTTCCACGCTGTTACACAG<br/> ACATGTGGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br/> TGAAATGCCGTTGAACACGTACAGTAATAAGCTCCGTTCA<br/> AGGCAAAGGTTGTTCCGTGGAAAAATCACAGGACCAAA<br/> AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br/> GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br/> TTCCGCCGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br/> GCCTCATGGTACTCGTCTTTATTGATTGCTTCTAGTCGTTA<br/> CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/> CGCCGCGCGGTATACGTGATCCAGAGACTGGAAGGAG<br/> GACCCGCGAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br/> TGCCACACCAGGCACGGAATTTCCATGACAGGGCCCA<br/> GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/> TAATCTGTGTCGCAACGGGGACTGGAATCGCGCCTTTTCG<br/> CTCATTCTGGCGCCGTTGCTTCATCGAGAATGTCCCAAGTT</p> |

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|  |  | <p>ATAAGTTCACCTGGCCTTTTCTGGTTGTTTATGGGTGTCGCT<br/> AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br/> CGCAAAAGCGTATCCGGGACAATTCCGCCTGGACTATGCC<br/> TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAAAATGTA<br/> CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/> ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA<br/> AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/> TTGCAAAAGAAAAGGGGCTGAACTACGAAGAGTGGGTCTGA<br/> GGGGTTAAAGCACAGAATCAATGGCATGTTGAAGTCTACT<br/> AACCAGGCATCAAATAAACGAAAGGCTCAGTCGAAAGACT<br/> GGGCCTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTC<br/> TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br/> GCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/> TGTGTGTGGAAAGAAGGAGATATACAT</p> <p>ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>ATGCCTCC<br/> AAAACCCCAACACGATGTAACAGGCGTGGACTCCAATAAC<br/> GCGATCATGATTGATTACGCAAAGTGCAATTGGCTGCAACAT<br/> GTGCATCAAAGCATGCGACGTGCAAGGTATTGGTGTTTACA<br/> AGCAGAACGAAAAACCGAAGTACCCGCCTATCGTGAAGCT<br/> GAGCACCTGTTTAATAGCGATTGTATCGGCTGCGGTCAAT<br/> GTGCAACGATTTGTCCGGTGGACGCGATTGCTCCAAAGAA<br/> CAATCTGGAAATTTACAAAGGCGAATCTGCTTCAAAAAAG<br/> TTCGTGTGGCGTTGATCGCGCCAAGCACTCGCGTGGCGTT<br/> TGGAGATGTCTTCGGGCTGCCGATCGGTACAAATACCATTT<br/> ACTCCCTGATTTCGCATGCTGAAGCAATATCTGGGTTTCGAT<br/> TATGTGTTGACGTGAATTCGGCGCAGATGAAACCAACCGT<br/> CATCGACACCCAAGAGCTGTTGCACTTCAAACACGAGGGC<br/> CGTGGTCCGGTGTTCACCAGCTGCTGCCCAGCGTGGGTTA<br/> ACCTCTGTGAAATGAAGTACCCGGAGCTGCTGCCCCAGGT<br/> TAGCACCGCTAAATCTTGTGTTGCAATGGTAGCGACCTTG<br/> TAAAAAGACGTTGGGTTCAAGAACATTTAATCCCGAAGGGT<br/> ATTGTAGACAGCGTTGACGATGTCTACGTGGCTGATATTAT<br/> GCCGTGTACCGCGAAAAAGGATGAAAGCATGCGTCCACAA<br/> CTGAACCGCGATGTGGACATCTGCCTGACGGTGCGTGAAG<br/> TTGCGGAGCACCTGTATTTTCTGCACGGCGCGCGCTTGAC<br/> GCTGGAGGAAGTCGAGGCGGATGCGTTGGTTTTGCGTCCG<br/> GGCCGTAGCACGCAGAAAAAGTGGGACTTTGACGCTCCGT<br/> TTAACACCGTGTCTGGTGGCTCCCATATCTTTGGTAAGACC<br/> GGTGGCGTTGCCGAGACGTGTCTCCGTTTCATCAGCTATAT<br/> GAAAAAGTCGCCGATAGAAAACGTTAAGGAGGAATTGCTG<br/> AAAGAGTTCAAGACGCCGGGTGAGCTGGTGCAAACCGTTA<br/> AGCTGGTCAGCTGCGAGATCGCCGGTGAGACTTATCGTGC</p> |
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|  | <p>CCTGATTGCGCATGGCGGTTTCAGCAATCAATGCCGCCGCG<br/>CGTATGGTGCTCAATAAAGAGGTGGAGTGCGACGTTGTTG<br/>AGCAGATGGCCTGTCCGGGTGGCTGCCAGAACGGTGGAG<br/>GCATGCCGAAGATCAAGGGTAAAAAGAGGCAGTTTTGAC<br/>GCGTGCGTCTACCTTGACATCCTGGACGGTAAAGAGCGC<br/>TTTGCGAGCGGGGCGAGAACAACTCTGTGGGTTTCA<br/>ACGGCTGCTTAACCGAACACGAAGCGCACGAGTTGCTTCA<br/>CACCCATTATCAGCATCGCCCGGTGGAATCGCTGCTGCCG<br/>CAGTAGGGATCC</p> <p>GGGTCGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT<br/>TATCTGTTGTTTGTGGTGAACGCTCTCTACTAGAGTCACA<br/>CTGGCTCACCTTCGGGTGGGCCTTCTGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>CACATTCCCGTCAGGCCGGAGATCGCAAACGGGCGCAG<br/>GTGCGGCATCTAGCCGCCCATCATGTCTTCTCGCCGCAT<br/>TGTCGCGGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCC<br/>GTGCAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGGAGAGACCCTGGGCGATT<br/>TCCTGGGCTTAGGGAAGGGGGGCTTTGCGCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>GTGGGGGGGTCTACTATCGCGGCCTTGTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTCGCAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAACCGGCATT<br/>GCAACATTATGTTGCAAGGGGGTGAATTAAAGACAGAGCAA<br/>CGCTTAGCCTACTTGAGGCATGCGTCCGTGCGATTGCGG<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC<br/>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGACACA<br/>GGCAGACGCCGAAGCTAACCGCGGCGAGCCAGAATTGGG<br/>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCTATGGAAC<br/>AGTATGAGCGTTTGTTCGCGCCGCGCGCCGTCGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>CTTCACCCGGAGCCGATGTCTGGCACGCGCGTGTAGAAT<br/>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>CTGGCTGGGGATGTCATGTTCTTTCGCGACATTAAAGCGG<br/>ACATGATCGGTATGGGCCCTTCATTACGCAGCCTGGGAC<br/>GCCTGCAACTGATAAGTGGAACGCGCTGTATCCGAACGCC</p> |
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|  |  | <p>AATAAGAACAGCCACATGAAATCTATGTTGATCTGACCAC<br/>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA<br/>TGCCTATTTTAACGCCTACTCAGTATCGCGAGTCCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGATACTGCTGTTCA<br/>GTGTCGTCGTTGCCTGGACATGCGTTTGCACTCCGTTGGC<br/>AAAACGTCTGCGGCCGGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGCATCCCATCGTTGGCGTCCCAGTCCCGCACGACTT<br/>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGTTGAAGTGCCGGACCGTTACCCTGACCCCGAT<br/>AACCACGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCACCACCACGGAGCTGCCCCCGGGGTGCAGCGG<br/>CTGAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTGTGCTGGTACGCTGGCGTAGGTTCAAATATT<br/>GTTACGTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCTCGTATCAATATTGGGGTGTTGCGCGTGATGAATGCA<br/>GGAAGTCTACACTTGTAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCCGGCACGACCGCAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGACCAGCCA<br/>AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCGATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCCGCAATCAAATCGGGACGCCCTTGCTGAAGCCCT<br/>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTGCTCAAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGGTGTTTGCCCCGTT<br/>GGTCTCTTGGACGCAACGCCCGCTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCCAGGTGGACGTCTTCTTCGTCCCT<br/>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>CCGTGCTTTCCGTACGCTGGATTTAGACGCCGCACGTGG<br/>CAAATTGGGGCCCGAGGCCTGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA</p> |
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|  | <p>CACCCCTTGGACCCTGGACCGCTCATCAGGACGTCCGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTG<br/>GAAGCGTGTAAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTGC<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAATGCAGCAGCGTATGAAAGACTTGCATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTCTTCTTGGGCAG<br/>CTTGGCCCGACGCCCTTCGTGCGCATTTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCCGACG<br/>CGCGCTGCTACGGCAGCGTCCGGGGTATAATACTA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGA</p> <p>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/>GCAGGCCGTCTTTTGGCGGGCCAGCGCCCTGCGGCGTC<br/>CGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/>CACACGGCAAAGCATCTGCCACGCGGAATACGCTGGTGA<br/>CTTTCTTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/>AGCGTGAAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/>AAGGCTGATGCTCAGGACGCAGGACGCGTGCGTGAGATTT<br/>TGCGGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/>GCCTGTAAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/>TTACCTTGGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/>TGCTATCAAAGAACGTATCTACGGGAACCGCGTCGTGCTG<br/>TTCGCGCCCTTGATATTGCTAATCATTGTATGAACACTTG<br/>CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/>GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/>CGCCTTGCAACGTCAAGGTCATCGCCGATTTTAGCCCTTA<br/>CTGGAGAACATCCTAAATACACTTTTCGACAATTTCTTGCATG<br/>CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/>GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br/>TCTCAGACATGCGCCGCTTAAGAATACAGATTCAAGTTGGC<br/>ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br/>CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br/>TTGCGCTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT<br/>GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br/>ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br/>TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT</p> |
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|    |                                                                                                                                                                                                       | <p>TCCCCGTATGCGCCCCGCGACGGAAGCGAGCTTAGTATC<br/> GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br/> GTTGCGGTCCTTCGTATTGCTGTACCATACACAGGTATGAT<br/> CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCGCTT<br/> CTGAAGTGTGGGATGAGTCAAATGAGTGCCGCTCGCGCA<br/> CTGATGTAGGAGCCTATCACAAGATCACACGTTAAGTACA<br/> GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br/> GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br/> AGGAGGGCTATGTCCCGTCTTGGTGCACAGCTTGTTACCG<br/> TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br/> GCTGGTGACATTCACGATTTCTGTCTATCCCAATAGTCTGCT<br/> TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br/> TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG<br/> ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br/> CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br/> ACGATGTATATCTTTAACCAGGCATCAAATAAACGAAAGG<br/> CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTG<br/> TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br/> GGGTGGGCCTTCTGCGTTTATA</p>                                                                                                                                                                                                                                          |
| 33 | Exemplary HPGC (hydrogen producing gene cluster) with codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Entamoeba nuttalli</i> , 5' – 3' (including restriction sites shown in bold) | <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/> GTGGAAAGAAGGAGATATACATATGGCGATGCGTTCCACAT<br/> TTGCTGCGCGTGTGGTGCCAAACCGGCAGTGCGTGGGG<br/> CGCGTCCCGCGTCGCGTATGTCCTTGATGGCCTATAAGGT<br/> TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br/> CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br/> GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br/> ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br/> TCTGATCAGTCCTTCTGGATGACGCTCAGATGGGGAACG<br/> GATTCTGCTCCTGACATGTGTCGCGTATCTACAAGCGACTGC<br/> ACTATCCAAACTACCAAGAGGAGGCTTTATATTAAGAA<br/> GGAGATATACATATGCAAACGTTCGCGCTCCAGCAGCTTC<br/> AGGTGTTGCCACACGTGTGCGAGGTCGTGATGTGTCGT<br/> CCGGTTGCGGCGACGAAGGCTTCACGGCTGTTACCACAG<br/> ACATGTGGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br/> TGAAATGCCGTTGAACACGTACAGTAATAAAGCTCCGTTCA<br/> AGGCAAAGGTTGTTCCGTGGAAAAATCACAGGACCAAA<br/> AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br/> GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br/> TTCCGCGGGGACCAAGATCAACTCTAAGGGAAAAAGAAGT<br/> GCCTCATGGTACTCGTCTTTATTGATTGCTTCTAGTCGTTA<br/> CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/> CGCCGCGCGGTATACGTGATCCAGAGACTGGAAGGAG<br/> GACCCGCGAAAAAAGGACTGTGTAGTAACTTTTGTGTGA</p> |

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|  |  | <p>TGCCACACCAGGCACGGAAATTTCCATGACAGGGCCCCACA<br/>GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/>TAATCTGTGTCGCAACGGGGACTGGAATCGCGCCTTTTCG<br/>CTCATTCTGGCGCCGTTGCTTCATCGAGAATGTCCCAAGTT<br/>ATAAGTTCACTGGCCTTTTCTGGTTGTTTATGGGTGTCGCT<br/>AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br/>CGCAAAAGCGTATCCGGGACAATTCGCGCTGGACTATGCC<br/>TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAAAATGTA<br/>CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/>ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA<br/>AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/>TTGCAAAAGAAAAGGGGCTGAACTACGAAGAGTGGGTGCA<br/>GGGGTTAAAGCACAAGAATCAATGGCATGTTGAAGTCTACT<br/>AACCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT<br/>GGGCCTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTC<br/>TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTCT<br/>GCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCGGGCTCGTA<br/>TGTTGTGTGGAAGAAGGAGATATACAT</p> <p>ATGTCCGCATTAGTACTTAAG<b>CCCCGGG</b>ATGCCTCC<br/>AAAACCTCACACACTGTAACAGGCCACGACCACAACCAC<br/>AGCATCCAGTTTGACTGGAGCAAGTGATGGGCTGCGGTA<br/>TGTGTGCAACCAATGCACCTTCGGCGTATTGGTTAAGCAG<br/>CCGCCGAAGATCCCGCCATTTGTCCAGCCGAACCGTGAAA<br/>AACTGAGCCAAGAGAACACCGATAAAACGCGTGCTCTGAT<br/>CGATGAAAGCGAGTGACCCGGCTGCGGTCAATGCTCTCTG<br/>GTGTGCAACTTTGGATCTATCACCCCGATCGACCACCTCGT<br/>TGACACCTTCAAAGCAAAGGAGGCGGGCAAAAACCTCGTG<br/>GCCATGATTGCCCGAGCACTCGCCTTGGTGTGCTGAGG<br/>CTATGGGTATGCCGATTGGCTCCACCGCTATGGCGAGCT<br/>GGTGCATTGTCTGCGTCTGATTGGTTTTGACTACGTTTTTG<br/>ACGTGGACGCGGGTGCGGATAAGACGACCATGGACGATTA<br/>CGCGGAAGTTATCGAAATGAAAAGGAGGGTAAGGGTCCG<br/>GCGATCACCAAGCTGCTGCCCGGCGTGGATTGAGCTGGTG<br/>GAGAAAGAATATCCGGATTTGATCCCGAACGTGTCCACTGC<br/>GCGTAGCCCGATCGGCTGCTTGCCGGTTGCATCAAACGT<br/>GGTTGGGCAAAAGATGTTGGCATCGCGGTAGAGGATTTGT<br/>ACACCGTGGGTATCATGCCTTGATTGCGAAGAAGACGGA<br/>ATCCCAACGTCAGCAAATTCATCAAGATTACGACGCAAGCT<br/>GTACGTCTAACGAGATTGCGGCGTACTTCAAGAAGCACCT<br/>GCCACCGGAAGAGTGCAAATTCACCCAGGAGCGCGAGGA<br/>AGCTCTGGCAAAGACTGAGGACGGCCAATGTGATCTGCCG<br/>TTCCGTGCGATTAGCGGTGGCTCCAACATTTTTGGTAAGAC</p> |
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|  |  | <p>CGGCGGGGTGTGTGAAACCGTGTTCGGGTGATTGCGCG<br/>TAATGCGGGTGTGACTGGAATACGTGCACCGTTAACAAAG<br/>AGGAAACATTCAAACACGCCGCTAGCGGTTGACCATGAC<br/>CAATCTGAGTGTGACATCGGCGGAAGTATAATCACCGGG<br/>GCAGTTTGCCATGGTGGTTATGCAATCCGTCATGCCTGCG<br/>AACTGATTGCAAAGGTGAAGTGAAGTGCACGTTGTTGAG<br/>ATGATGGCCTGTGTTGGCGGCTGCCTGGGCGGTGCTGGC<br/>CAGCCTAAGATCCCGCCGCGCAAGAAGCTGGAATGGATA<br/>AGCGCAGAGTGATGTTAGATATTCTGGACCAGCAAACGGA<br/>CATTCTGTCAGCGAATGAAAACACCGACGTGCTAGGCTGG<br/>ATTGATAAACACTTCGACCACAGGGTGCGCATCAACATCT<br/>GCACACCTATTTACCCCGCGTTATCAGAATTAGGGATCC</p> <p>GGGTCGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT<br/>TATCTGTTGTTTGTGCGGTGAACGCTCTCTACTAGAGTCACA<br/>CTGGCTCACCTTCGGGTGGGCCTTCTGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>CACATTCCCGTCAGGCCGAGATCGAAACTGGGCGCAG<br/>GTGCGGCATCTAGCCGCCATCATGTCCTTCTCGCCGCAT<br/>TGTCGCGGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCCC<br/>GTGCAGCAGTAGCGGCCGCTAACCCTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGCAGAGACCTGGGCGATT<br/>TCCTGGGCTTAGGGAAGGGGGGGCTTTCGCCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>GTGGGGGGTCTACTATCGCGGCCTTGTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTCGCAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAACGCGCATTC<br/>GCAACATTATGTTGCAAGGGGGTGAATTAAGACAGAGCAA<br/>CGCTTAGCCTACTTGAGGCATGCGTCCGTGCGATTGCGC<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC<br/>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGACACA<br/>GGCAGACGCCGAAGCTAACGCGGCGAGCCAGAATTGGG<br/>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>AGTATGAGCGTTTGTTCGCGCCGGCGCCGCTCGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>CTTCACCCGGAGCCGATGTCCTGGCACGCGCGTGTAGAAT<br/>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC</p> |
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|  |  | <p>CTGGCTGGGGATGTCATGTTCTTTGCGGACATTAAAGCGG<br/>ACATGATCGGTATGGGCCCCCTTATTACGCAGCCTGGGAC<br/>GCCTGCAACTGATAAGTGGACCGCGCTGTATCCGAACGCC<br/>AATAAGAACAGCCACATGAAATCTATGTTGATCTGACCAC<br/>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA<br/>TGCCTATTTTAACGCCTACTCAGTATCGCGAGTCCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA<br/>GTGTCGTCGTTGCCTGGACATGCGTTTGCACTCCGTTGGC<br/>AAAACGTCTGCGGCCGGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGATCCCATCGTTGGCGTCCCAGTCCCGCACGACTT<br/>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGGTTGAAGTGCCGGACCGTTACCCTGACCCCGAT<br/>AACCACGGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCAGCAGATGGCGATCAGCAGACC<br/>ACCACCACCACCGAGCTGCCCCCGGGTGCAGCGG<br/>CTGAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTCTGGTACGCTCTGGCGTAGGTTCAAATATT<br/>GTTACGTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCTCGTATCAATATTGGGGTGTTGCGCGTGATGAATGCA<br/>GGAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCCGGCACGACCGCAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGACCAGCCA<br/>AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCCGCAATCAAATCGGGACGCCTTGCTGAAGCCCT<br/>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTGCTCAAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGGTGTTTGCCCCGTT<br/>GGTCTCTTGACGCAACGCCCGCTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCAGGTGGACGTCTTCTTCGTCT<br/>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>CCGTGCTTCCGTACGCCTGGATTAGACGCCGCACGTGG</p> |
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|  |  | <p>CAAATTGGGGCCCGAGGCCTGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>CACCCCTGGACCCTGGACCGCTCATCAGGACGTCGGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTGCG<br/>GAAGCGTGTAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTGC<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAATGCAGCAGCGTATGAAAGACTTGCAATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTTTTCTCTTGGGCAG<br/>CTTGGCCCGACGCCCTTCGTCGCGCATTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCGCAGC<br/>CGCGCTGCTACGGCAGCGTCCGGGGTATAATACTA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGA</p> <p>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/>GCAGGCCGTCTTTTGGCGGGCCAGCGCCCTGCGGCGTC<br/>CGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/>CACACGGCAAAGCATCTGCCACGCGCAATACGCTGGTGA<br/>CTTTCTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/>AGCGTGAAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/>AAGGCTGATGCTCAGGACGCAGGACGCGTGCGTGAGATT<br/>TGCGGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/>GCCTGTAAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/>TTACCTTGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/>TGCTATCAAAGAACGTATCTACGGAACCGCGTCGTGCTG<br/>TTCGCGCCCTGTACATTGCTAATCATTGTATGAACACTTG<br/>CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/>GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/>CGCCTTGCAACGTCAAGGTCATCGCCGCATTTTAGCCCTTA<br/>CTGGAGAACATCCTAAATACACTTTGACAATTTCTTGATG<br/>CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/>GTCCATTGCGCGTATCAATGTGAAATCCCACCACTTAGTG<br/>TCTCAGACATGCGCCGCTTAAGAATACAGATTCAGTTGGC<br/>ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br/>CAAGGTAATGCATCCGTGAGGTCCAAAGAGCGACTTTGATT<br/>TTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT</p> |
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|    |                                                                                                                                                                                                                              | GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br>ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br>TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br>TCCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br>GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br>GTTGCGGTCCTTCGTATTGCTGTACCATACACAGGTATGAT<br>CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCGCTT<br>CTGAAGTGTGGGATGAGTCAAATGAGTGCCGGCTCGCGCA<br>CTGATGTAGGAGCCTATCACAAAGATCACACGTTAAGTACA<br>GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br>GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br>AGGAGGGCTATGTCCCGTCTTGGTGACACAGCTTGTACCG<br>TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br>GCTGGTGACATTCACGATTTCTGTCTATCCCAATAGTCTGCT<br>TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br>TTCGTAAGAAAGGCGAGCAAGTAATTGCCGTGAGATGGG<br>ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br>CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br>ACGATGTATATCTTTAACCAGGCATCAAATAAACGAAAGG<br>CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTG<br>TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br>GGGTGGGCCTTCTGCGTTTATA |
| 34 | Exemplary HPGC (hydrogen<br>producing gene cluster) with<br>codon optimised nucleic acid<br>sequence encoding HydA<br>(Hyd1) from<br><i>Ilyobacter polytrophus</i> 5' – 3'<br>(including restriction sites<br>shown in bold) | CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br>GTGGAAAGAAGGAGATATACATATGGCGATGCGTTCCACAT<br>TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGCGTGGGG<br>CGCGTCCCGCGTCGCGTATGTCTTGATGGCCTATAAGGT<br>TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br>CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br>GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br>ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br>TCTGATCAGTCCTTCTGGATGACGCTCAGATGGGGAACG<br>GATTCGTCCTGACATGTGTGCGGTATCCTACAAGCGACTGC<br>ACTATCCAACTCACCAAGAGGAGGCTTTATATTTAAAGAA<br>GGAGATATACATATGCAAACGTTCGCGCTCCAGCAGCTTC<br>AGGTGTTGCCACACGTGTGCGAGGTGTCGTATGTGTCGT<br>CCGGTTGCGGCGACGAAGGCTTCCACGGCTGTTACCACAG<br>ACATGTCGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br>TGAAATGCCGTTGAACACGTACAGTAATAAAGCTCCGTTCA<br>AGGCAAAGGTTGTTCCGTGGAAAAATCACAGGACCAAA<br>AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br>GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br>TTCCGCGGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br>GCCTCATGGTACTCGTCTTTATTCGATTGCTTCTAGTCGTTA                                        |

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|  |  | <p>CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/>CGCCGCGCGGTATACGTGATCCAGAGACTGGAAGGAG<br/>GACCCGCGGAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br/>TGCCACACCAGGCACGGAAATTTCCATGACAGGGCCCACA<br/>GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/>TAATCTGTGTCGCAACGGGGACTGGAATCGCGCCTTTTCG<br/>CTCATTCTGGCGCCGTTGCTTCATCGAGAATGTCCCAAGTT<br/>ATAAGTTCACTGGCCTTTTCTGGTTGTTTATGGGTGTCGCT<br/>AACTCTGATGCTAAATTGTACGATGAGGAAGTGAAGCTAT<br/>CGCAAAAGCGTATCCGGGACAATCCGCCTGGACTATGCC<br/>TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAAAATGTA<br/>CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/>ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA<br/>AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/>TTGCAAAAGAAAAGGGGCTGAAGTACGAAGAGTGGGTGGA<br/>GGGGTTAAAGCACAAGAATCAATGGCATGTTGAAGTCTACT<br/>AACCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT<br/>GGGCCTTTCGTTTTATCTGTTGTTTGTGGTGAACGCTCTC<br/>TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br/>GCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/>TGTTGTGTGGAAGAAGGAGATATACAT</p> <p>ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>GATGAAGAACAAAAC<br/>AGTATCAAATGATAAAATAGACAATAAACTGAACTGCTCCG<br/>AAAACCTGGAAAACCGTACCATCGACAAAAGCTTCTACACC<br/>TTTTACGTGATACCAGCAAGTGCATTAAGTGTACAAGTG<br/>CGTGAAAGTTTGCAAGGACACTCAGGGCATTTCGTTTTTC<br/>AGGTTGAGGAGGACGGCACGGTGGGCATCAAAGAGGAGA<br/>ATATGGCGGCCACCTTGTGTATCAGCTGTGGTCAATGTATT<br/>AAAGTGTGCACCGCAGGTGCGCTGAAGGAGAAATCCAACA<br/>TCTCTCTGTTGAAGGAGCAGTTAAATAACCCGAATAAGCAC<br/>GTTGTCGCGCAGCTGTCTCCAAGCTTCAAACACACCATTGG<br/>TGATGGCTTCGGCATCAGCTCGGGTACCGATACTTCCCCG<br/>AAAATCATCAGCGCTCTGAAAGAAATTGTTTTCTCTAAGGT<br/>TTTCAGTACCGGTTTCGCCAGCGAGTGAATATCGTTGAGA<br/>CCAGCGCGGATCTGAAAAACGCCTAGATGAAAACGGTCC<br/>GTTCCCGGTGTTACCTCCACGTGCACGGGTTGGATTAAAC<br/>TATGCGGAAAAGTTTTGTCCGGAGTTCCTGGGGCTGCTCT<br/>CTCCGTGCAAAAGCCCGCAACAGATCCTGGGTAGCCTGAG<br/>CAAGTCCTACTACGAAGAGAGCATTGACATCAGCAGAGAAA<br/>ATATCTTTAGCGTGGCGCTGATGCCTTGATCGCCAAAAG<br/>GATGAAGCTAATCGTTTTGACATGAAGGACGAATATGGTAA<br/>CAAAGATGTGGACCTGGTCCTGACCGTGAATGAAGTTGCT</p> |
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|  |  | <p>AGCCTTTTGAACAAAAAGGGCATTGATTTAAACAATTACTCT<br/>AAGTTCGGTACTTTTGATAAGCCGATGAAATCCGACACTGG<br/>TTCTTCGCGTATTAAGGCGGTTACGGGTGGCCTGGCAGAG<br/>GCTATCCTGCGCAACACCGCACACATGATCGGTGAAGATC<br/>CGTTTTCTGTAGACCTGAAGAAGCTGCGTGGTATGGATGGT<br/>ATTAAGCTCACGAGCGTGGTTCTGGGCGGGAAAAAATTA<br/>CATTGCGGTGCGTAACGGCATCAAGAACGTGCCGTTATT<br/>CTGGACATGATTAAAGATGGCATTACCGAGTTCCACTTGGT<br/>CGAGGTTATGGCCTGTCCGGGTGGCTGCGTCGGCGGCGG<br/>TGGTATCCCGTTGTCAGAAGACCCGGACATTATCCAAAAAC<br/>GCGCAGAGAAAACTACTCGTACGACGCGAGCAGCGAAAT<br/>TCGTTGTAGCTGGGAAAACCCAGATGTTAAAACCTGTATA<br/>GCGAGTACCTGAAGGAGCCGCTGGGAGAGGAGTCTCAAC<br/>GTTTGTTCATTTTCATTATAAAAAACCGCCGTACCAAACGTA<br/>TCTTCTAGGGATCCGGGTGCGAGGAGAAGGATGAGAAAAAG<br/>TGACCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGA<br/>CTGGGCCCTTCGTTTTATCTGTTGTTGTCGGTGAACGCTC<br/>TCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTC<br/>TGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>CACATTCCCGTCAGGCCGGAGATCGAAACTGGGCGCAG<br/>GTGCGGCATCTAGCCGCCCATCATGTCTTCTCGCCGCAT<br/>TGTCGCGGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCC<br/>GTGCGAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGGAGAGACCTGGGCGATT<br/>TCCTGGGCTTAGGGAAGGGGGGGCTTTCGCCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>GTGGGGGGGTCTACTATCGCGGCCTTGTTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTGCAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAAAACGGCATT<br/>GCAACATTATGTTGCAAGGGGGTGAATTAAAGACAGAGCAA<br/>CGCTTAGCCTACTTGGAGGCATGCGTCCGTGCGATTGCGG<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC<br/>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGACACA<br/>GGCAGACGCCGAAGCTAAACGCGGCGAGCCAGAATTGGG<br/>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>AGTATGAGCGTTTGTTCGCGCCGGCGCCCGTCGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>CTTCACCCGAGCCGATGTCCTGGCACGCGCGTGTAGAAT</p> |
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|  |  | <p>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>CTGGCTGGGGATGTCATGTTCTTTCGCGACATTAAGCGG<br/>ACATGATCGGTATGGGCCCCTTACATTACGCAGCCTGGGAC<br/>GCCTGCAACTGATAAGTGGACCGCGCTGTATCCGAACGCC<br/>AATAAGAACAGCCACATGAAATCTATGTTGATCTGACCAC<br/>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA<br/>TGCTATTTTAACGCCTACTCAGTATCGCGAGTCCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA<br/>GTGTCGTCGTTGCCTGGACATGCGTTTGCACTCCGTTGGC<br/>AAAACGTCTGCGGCCGGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGCACTCCCATCGTTGGCGTCCCAGTCCCGCACGACTT<br/>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGGTTGAAGTGCCGGACCGTTACCCTGACCCCGAT<br/>AACCACGGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCACCACCGAGCTGCCCCCGGGGTGCAGCGG<br/>CTGGAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTGCTGGTACGCTTGCGGTAGGTTCAAATATT<br/>GTTACGTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCTCGTATCAATATTGGGGTGTTGCGCGTGATGAATGCA<br/>GGAAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCGGCACGACCGCAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGACACGCCA<br/>AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCGATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCCGCAATCAAATCGGGACGCCTTGCTGAAGCCCT<br/>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCACTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTGCTCAAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGGTGTTCGCCCGTT<br/>GGTCTCTTGACGCAACGCCCGCTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCAGGTGGACGCTTCTTCGTCCT</p> |
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|  |  | <p>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGGCTA<br/>CCGTGCTTTCCGTACGCCTGGATTTAGACGCCGCACGTGG<br/>CAAATTGGGGCCCGAGGCCTGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>CACCCCTGGACCCTGGACCCTCATCAGGACGTCCGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTTCG<br/>GAAGCGTGTAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTGC<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAATGCAGCAGCGTATGAAAGACTTGCATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTCTTCTTTCTTGGGCAG<br/>CTTGGCCCGACGCCCTTCGTGCGCATTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCGCAGC<br/>CGCGCTGCTACGGCAGCGTCCGGGGTATAATACTA<br/><br/>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGA<br/><br/>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/>GCAGCCCGTCTTTTGGCGGGCCAGCGCCCTGCGGCGTC<br/>CGCGCGCTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/>CACACGGCAAAGCATCTGCCACGCGCGAATACGCTGGTGA<br/>CTTTCTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/>AGCGTGAAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/>AAGGCTGATGCTCAGGACGCAGGACGCGTGCGTGAGATTT<br/>TGCGGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/>GCCTGTAAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/>TTACCTTGGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/>TGCTATCAAAGAACGTATCTACGGGAACCGCGTCGTGCTG<br/>TTCGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br/>CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/>GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/>CGCCTTGCAACGTCAAGGTCATCGCCGCATTTTAGCCCTTA<br/>CTGGAGAACATCCTAAATACACTTTTCGACAATTTCTTGCA<br/>CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/>GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br/>TCTCAGACATGCGCCGCTTAAGAATACAGATTGAGTTGGC<br/>ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT</p> |
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|    |                                                                                                                                                                                                   | CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br>TTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT<br>GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br>ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br>TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br>TCCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br>GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br>GTTGCGGTCCTTCGTATTGCTGTACCATACACAGGTATGAT<br>CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCGCTT<br>CTGAAGTGTGGGATGAGTCAAATGAGTGCCGCTCGCGCA<br>CTGATGTAGGAGCCTATCACAAAGATCACACGTTAAGTACA<br>GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br>GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br>AGGAGGGCTATGTCCCGTCTTGGTGCACAGCTTGTTACCG<br>TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br>GCTGGTGACATTCACGATTTCTGTCTATCCCAATAGTCTGCT<br>TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br>TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG<br>ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br>CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br>ACGATGTATATCTTTAACCAGGCATCAAATAAAACGAAAGG<br>CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTG<br>TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br>GGGTGGGCCTTCTGCGTTTATA |
| 35 | Exemplary HPGC (hydrogen producing gene cluster) with codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Volvox carteri</i> , 5' – 3' (including restriction sites shown in bold) | CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br>GTGGAAAGAAGGAGATATACATATGGCGATGCGTTCACAT<br>TTGCTGCGCGTGTCGGTGCCAAACCGGCAGTGCGTGGGG<br>CGCGTCCCGCGTCGCGTATGTCTTGATGGCCTATAAGGT<br>TAACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br>CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br>GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br>ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br>TCTGATCAGTCCTTCTGGATGACGCTCAGATGGGAACG<br>GATTCGTCCTGACATGTGTCGCGTATCCTACAAGCGACTGC<br>ACTATCCAACTCACCAAGAGGAGGCTTTATATTTAAAGAA<br>GGAGATATACATATGCAAAGTTCGCGCTCCAGCAGCTTC<br>AGGTGTTGCCACACGTGTCGAGGTGTCGTATGTGTCGT<br>CCGGTTGCGGCGACGAAGGCTTCACGGCTGTTACCACAG<br>ACATGTCGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br>TGAAATGCCGTTGAACACGTACAGTAATAAAGCTCCGTTCA<br>AGGCAAAGGTTTCGTTCCGTGGAAAAAATCACAGGACCAAA<br>AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br>GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA                                                                                                                                                                                                                               |

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|  | <p>TTCCGCCGGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br/>GCCTCATGGTACTCGTCTTTATTGATTGCTTCTAGTCGTTA<br/>CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/>CGCCGCGCGGTATACGTCGATCCAGAGACTGGAAAGGAG<br/>GACCCGCGGAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br/>TGCCACACCAGGCACGGAAATTTCCATGACAGGGCCCACA<br/>GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/>TAATCTGTGTCGCAACGGGGACTGGAATCGCGCCTTTTCG<br/>CTCATTCTGGCGCGTGTGCTTCATCGAGAATGTCCCAAGTT<br/>ATAAGTTCACTGGCCTTTTCTGGTTGTTTATGGGTGTCGCT<br/>AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br/>CGCAAAAGCGTATCCGGGACAATCCGCCTGGACTATGCC<br/>TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAAAATGTA<br/>CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/>ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA<br/>AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/>TTGCAAAAGAAAAGGGGCTGAACACGAAGAGTGGGTGCA<br/>GGGGTTAAAGCACAAGAATCAATGGCATGTTGAAGTCTACT<br/>AACCAGGCATCAAATAAACGAAAGGCTCAGTCGAAAGACT<br/>GGGCCTTTCGTTTTATCTGTTGTTTGTGCGTGAACGCTCTC<br/>TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br/>GCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/>TGTTGTGTGGAAAGAAGGAGATATACAT</p> <p>ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>ATGAGTGC<br/>TATGCTATCTTCATTAACAAATTCGCTTGGGCGCTTACGT<br/>GCCGCAAGTGGCGGTAAGCCGTTATGGTTCCAAGCCGGTC<br/>TACGTCCCCTGCGTGCCGTTTCGTAAGTGACCTCGGCGG<br/>TGCCAGTGCCGTTAAGACGGTGCAAGAAGGTAATTCGGT<br/>CAGACTTATGCCAGCGGCGGCGGCTCCGGCGGGTGCGGC<br/>AGCGGACCCACATTGGAACAAGCATTTCAGAGCTTGATA<br/>AACCGAAAGCCGAACGTAAAGTTATGATCGCACAGGTTGCT<br/>CCGGCGGTGCGCGTTGCCATCTCCGAGAGCTTTGGTCTGG<br/>CACCGGGGGCTACCACTCCGGGTGAGCTGGCCGAGTCCC<br/>TGCGTTGTCTGGGTTTTGATATGGTGTTCGACACCTGTAC<br/>GGCGCGGACCTGACCATTATGGAAGAGGGCACTGAGTTGC<br/>TGTCCTGCTGCAAGCGCAATTGGAAGCCACCCGCATAG<br/>CGAAGAACCCTGCCGATGTTTACCAGCTGCTGTCCGGGC<br/>TGGATCGCCCTGTTGGAGAAGAGCTATCCGGAATTAATTCC<br/>GTATGTGTCTAGCTGCAAGTCTCCGCAGATGATGTTAGGC<br/>GCTATGGTGAAGACCTATCTGGCAGAGAAGAAAGGCATCT<br/>CTCCGAGCGACATCTCCATGGTTTCGATTATGCCGTGTGTG<br/>AGGAAGCAAGGTGAAGCGGACCGTGATTGGTTTGCAGCG</p> |
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|  | <p>GCGGCGCCGGCGTTCTGTGATGTGGACCACGTTATTACCAC<br/>GGCGGAGCTAGGCAACATCCTGAAAGAACGTAATATCAAC<br/>CTCCCCGAACTGCCGGAAGCGGTTGGGATGAGCCGCTG<br/>GGTTCGGGTAGCGGTGCGGGCGTCGTTTTCGGTACAACCG<br/>GTGGTGTATGGAAGCGCGCTGCGCACCGCGTACGAGTT<br/>GGTTACCCAGCAACCGCTGCCTCGTTTGAATCTGAGTGAG<br/>GTTGCGGTATGGACGGCATTAAAGAAACCGAAATCAAGAT<br/>GGTTCGCTCCGGGTAGCAAATTCGCCGAGCTTGTGGCC<br/>GCACGTGCAGCGGCGAAGGCTATGGATGAAGCGGCCGCG<br/>AGCGCAGGCGCGATTAAAGTGGGATGGTGGCAGCAACTTCA<br/>CCGCGGACGACGGTGCTAAGGGCATCACCTGCGCGTGG<br/>CCGTGGCTAATGGTCTGGGAAACGCGAAAAAGCTGCTGAC<br/>GAAAAATGCAGACCGGTGAGTGCAAATACGACTTCGTGGAA<br/>ATTATGGCATGTCCGTCTGGGTGCGTTGGTGGTGGTGGTC<br/>AGCCGCGCAGCACCGATAAACAGGTGGCGGTCAAACGTCA<br/>GCAGGCACTGTACGACCTTGACGAGCGCGCAACGATCCGT<br/>CGTAGCCACGAGAACCCGGCTGTCCAAAAGCGTACGAAG<br/>ATTTCTGGGCGAGCCGAACAGCCACAAAGCGCACGATCT<br/>GTTGCACACCCATTATGTTCCGGGTGGCGTTGACACGGAG<br/><b>TAGGGATCC</b></p> <p>GGGTGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT<br/>TATCTGTTGTTTGTGCGGTGAACGCTCTCTACTAGAGTCACA<br/>CTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>CACATTCCCGTCAGGCCGGAGATCGCAAAGTGGGCGCAG<br/>GTGCGGCATCTAGCCGCCCATCATGTCTTCTCGCCGCAT<br/>TGTCGCGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCC<br/>GTGCAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGCAGAGACCTGGGCGATT<br/>TCCTGGGCTTAGGGAAGGGGGGCTTTGCGCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>GTGGGGGGGTCTACTATCGCGGCCCTTGTTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTCGCAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAAAACGGCATTC<br/>GCAACATTATGTTGCAAGGGGGTGAATTAAAGACAGAGCAA<br/>CGCTTAGCCTACTTGGAGGCATGCGTCCGTGCGATTGCGG<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC</p> |
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|  |  | <p>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGCACA<br/>GGCAGACGCCGAAGCTAAACGCGGCGAGCCAGAATTGGG<br/>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>AGTATGAGCGTTTGTTCGCGCCGCGCCCGTCGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>CTTCACCCGGAGCCGATGTCCTGGCAGCGCGTGTAGAAT<br/>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>CTGGCTGGGGATGTCATGTTCTTTCGCGACATTAAGCGG<br/>ACATGATCGGTATGGGCCCCTTACATTACGCAGCCTGGGAC<br/>GCCTGCAACTGATAAGTGGACCGCGCTGTATCCGAACGCC<br/>AATAAGAACAGCCACATGAAATCTATGTTTCGATCTGACCAC<br/>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA<br/>TGCCTATTTTAACGCCTACTCAGTATCGCGAGTCCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA<br/>GTGTCGTCGTTGCTGGACATGCGTTTGCACTCCGTTGGC<br/>AAAACGTCTGCGGCCGGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGATCCCATCGTTGGCGTCCAGTCCCGCACGACTT<br/>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGGTTGAAGTGCCGGACCGTTACCCTGACCCCGAT<br/>AACCACGGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCACCACCACGGAGCTGCCCCCGCGGGTGCACGCG<br/>CTGGAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGACGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTGCTGGTACGCTTGCGGTAGGTTCAAATATT<br/>GTTACGCTCTCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCCTCGTATCAATATTGGGGTGTTCGGCGTGATGAATGCA<br/>GGAAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCGGCACGACCGCAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGGAACAGCCA<br/>AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCGATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCCGCAATCAAATCGGGACGCCTTGCTGAAGCCCT<br/>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT</p> |
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|  |  | <p>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTGCTCAAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGGTGTTCGCCCGTT<br/>GGTCTCTTGACGCAACGCCCGCTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCAGGTGGACGCTTCTTCGTCTCT<br/>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>CCGTGCTTTCGTACGCCTGGATTAGACGCCGCACGTGG<br/>CAAATTGGGGCCCGAGGCCTGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>CACCCCTGGACCCTGGACCCTCATCAGGACGTCCGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTTCG<br/>GAAGCGTGAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTCG<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAATGCAGCAGCGTATGAAAGACTTGCATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTTCCTCTTGGGCAG<br/>CTTGGCCCGACGCCCTTCGTGCGCATTTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTAATCCTGCTACCCCGCAGC<br/>CGCGCTGCTACGGCAGCGTCCGGGGTATAATACTA<br/><br/>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGA<br/><br/>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/>GCAGGCCGTCTTTTGGCGGGCCAGCGCCCTGCGGCGTC<br/>CGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/>CACACGGCAAAGCATCTGCCACGCGCGAATACGCTGGTGA<br/>CTTCTTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/>AGCGTGAAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/>AAGGCTGATGCTCAGGACGCAGGACGCGTGCCTGAGATTT<br/>TGGCGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/>GCCTGTAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/>TTACCTTGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/>TGCTATCAAAGAACGTATCTACGGGAACCGCGTCGTGCTG<br/>TTCGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br/>CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/>GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC</p> |
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|    |                                                                                                                                                                                                          | <p>CGCCTTGCAACGTCAAGGTCATCGCCGCATTTTAGCCCTTA<br/> CTGGAGAACATCCTAAATACACTTTGACAATTTCTTGCATG<br/> CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/> GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br/> TCTCAGACATGCGCCGCCTTAAGAATACAGATTCAGTTGGC<br/> ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br/> CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br/> TTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT<br/> GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br/> ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br/> TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br/> TCCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br/> GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br/> GTTGCGGTCCCTTCGTATTGCTGTACCATACACAGGTATGAT<br/> CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCGCTT<br/> CTGAAGTGTGGGATGAGTCAAATGAGTGCCGGCTCGCGCA<br/> CTGATGTAGGAGCCTATCACAAGATCACACGTTAAGTACA<br/> GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br/> GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br/> AGGAGGGCTATGTCCCGTCTTGGTGACAGCTTGTTACCG<br/> TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br/> GCTGGTGACATTACGATTTCTGTATCCCAATAGTCTGCT<br/> TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br/> TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG<br/> ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br/> CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br/> ACGATGTATATCTTTAACCAGGCATCAAATAAACGAAAGG<br/> CTCAGTCGAAAGACTGGGCCCTTCGTTTTATCTGTTGTTG<br/> TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br/> GGGTGGGCCTTTCTGCGTTTATA</p> |
| 36 | Exemplary HPGC (hydrogen producing gene cluster) with codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Trichomonas vaginalis</i> , 5' – 3' (including restriction sites shown in bold) | <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/> GTGGAAGAAGGAGATATACATATGGCGATGCGTTCCACAT<br/> TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGCGTGGGG<br/> CGCGTCCCGCGTCGCGTATGTCTTGCAATGGCCTATAAGGT<br/> TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br/> CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br/> GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br/> ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br/> TCTGATCAGTCCTTTCTGGATGACGCTCAGATGGGGAACG<br/> GATTCGTCCTGACATGTGTCGCGTATCCTACAAGCGACTGC<br/> ACTATCCAAACTCACCAAGAGGAGGCTTTATATTAAGAA<br/> GGAGATATACATATGCAAACGTTCGCGCTCCAGCAGCTTC<br/> AGGTGTTGCCACACGTGTCGCGAGGTCGTCGTATGTGTCGT</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

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|  |  | <p>CCGGTTGCGGCGACGAAGGCTTCCACGGCTGTTACCACAG<br/> ACATGTGCGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br/> TGAAATGCCGTTGAACACGTACAGTAATAAAGCTCCGTTCA<br/> AGGCAAAGGTTGTTCCGTGGAAAAATCACAGGACCAAA<br/> AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br/> GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br/> TTCCGCCGGGGACCAAGATCAACTCTAAGGGAAAAGAGT<br/> GCCTCATGGTACTCGTCTTTATTGATTGCTTCTAGTCGTTA<br/> CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/> CGCCGCGCGGTATACGTGATCCAGAGACTGGAAGGAG<br/> GACCCCGCGAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br/> TGCCACACCAGGCACGGAATTTCCATGACAGGGCCCA<br/> GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/> TAATCTGTGTCGCAACGGGGACTGGAATCGCGCCTTTTCG<br/> CTCATTCTGGCGCCGTTGCTTCATCGAGAATGTCCCAAGTT<br/> ATAAGTTCACTGGCCTTTTCTGGTTGTTTATGGGTGTCGCT<br/> AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br/> CGCAAAAGCGTATCCGGGACAATCCGCCTGGACTATGCC<br/> TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAAAATGTA<br/> CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/> ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA<br/> AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/> TTGCAAAAGAAAAGGGGCTGAACTACGAAGAGTGGGTCGA<br/> GGGGTTAAAGCACAGAATCAATGGCATGTTGAAGTCTACT<br/> AACCAGGCATCAAATAAACGAAAGGCTCAGTCGAAAGACT<br/> GGGCCTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTC<br/> TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br/> GCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/> TGTTGTGTGGAAGAAGGAGATATACAT</p> <p>ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>ATGCTAGC<br/> GTCAAGTGCAACAGCTATGAAAGGCTTCGCCAATAGCCTC<br/> CGTATGAAAGATTACAGCAGCACCGGTATTAACCTTCGACAT<br/> GACTAAGTGCAATTAAGTCCAAAGCTGCGTTTCGCGCCTGC<br/> ACGAACATCGCTGGCCAAAACGTCTTGAAGAGCTTGACGG<br/> TGAACGGCAAGTCCGTTGTGACAGACCGTTACCGGTAAACC<br/> GCTGGCGGAGACCAACTGCATCAGCTGCGGTCAATGTACC<br/> CTTGGCTGCCCGAAGTTCACCATCTTCGAGGGCGGACGCCA<br/> TCAATCCAGTTAAAGAGGTCTTGACCAAGAAAAACGGTCGT<br/> ATTGCCGTGTGTCAGATTGCTCCGGCGATTCTGATCAACAT<br/> GGCTGAAGCTCTGGGCGTTCCGGCTGGTACGATCTCCTTG<br/> GGTAAGGTGGTGACCGCGCTGAAACGCTGGGTTTTGACT<br/> ACGTTTTCGATACCAATTTTCGCTGCCGATATGACCATCGTG</p> |
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|  | <p>GAGGAAGCGACCGAACTGGTTCAACGTTTGTCTGACAAAA<br/>ACGCAGTGCTGCCTATGTTTACTTCATGCTGTCCAGCGTGG<br/>GTTAATTACGTAGAGAAATCCGATCCGAGCTTGATCCCGCA<br/>TCTGAGCTCTTGTCTAGCCCGATGAGCATGCTGAGCAGC<br/>GTTATCAAGAACGCTTTCCGAAAAAGATTGGTACGACCGC<br/>AGACAAAATCTACAATGTGGCGATCATGCCGTGCACCGCG<br/>AAGAAGGACGAAATTCAGCGCAGCCAATTTACCATGAAGG<br/>ATGGTAACAAGAGACAGGTGCGGTGCTGACTTCTCGTGA<br/>ACTCGCGAAAATGATTAAGGAGGCGAAGATCAACTTCAAAG<br/>AGCTGCCGGATACGCCGTGTGATAACTTTTATTCTGAAGCA<br/>TCGGGCGGCGGTGCGATCTTTGTGCAACCGGCGGCGTG<br/>ATGGAAGCCGCGGTGCGCAGCGCGTATAAGTTCCTGACGA<br/>AGAAAGAGCTGGCACCGATTGACCTGCAAGACGTTCCGCG<br/>TGTTGCGAGCGGCGTTAAACTGGCGGAGGTGGATATTGCT<br/>GGTACCAAAGTAAAAGTGGCGGTTGCGCACGGCATTAAAG<br/>ACGCCATGACCCTGATTAAGAAGATCAAATCGGGTGAAGA<br/>GCAGTTTAAAGACGTTAAGTTCGTGGAAGTGATGGCATGTC<br/>CGGGTGGCTGCGTGTTGGTGGTGGCAGCCGAAGGCTA<br/>AGACCAAGAAAGCTGTCCAGGCACGATTGAATGCAACGTA<br/>CTCCATTGACAAAAGCTCCAAACACAGAACTTCTCAGGACA<br/>ACCCGCAGTTGTTACAGCTGTATAAGAATCGTTTGAAGGT<br/>AAATTCGGTGGTCATGTGCGCACCCACCTGCTGCACACCC<br/>ATTATAAGAATCGTAAAGTAAATCCGTAGGGATCC</p> <p>GGGTCGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT<br/>TATCTGTTGTTTGTGCGGTGAACGCTCTCTACTAGAGTCACA<br/>CTGGCTCACCTTCGGGTGGGCCTTCTGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>CACATTCCCGTCAGGCCGGAGATCGAAACTGGGCGCAG<br/>GTGCGGCATCTAGCCGCCCATCATGCTTCTCGCCGCAT<br/>TGTCGCGGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCCC<br/>GTGCAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGGCAGAGACCCTGGGCGATT<br/>TCCTGGGCTTAGGGAAGGGGGGGCTTTCGCCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>GTGGGGGGGTCTACTATCGCGGCCCTTGTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTCGCAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAAAACGGCATTTC</p> |
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|  |  | <p>GCAACATTATGTTGCAAGGGGGTGAATTAAGACAGAGCAA<br/>CGCTTAGCCTACTTGAGGCATGCGTCCGTGCGATTGCGG<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGGCGACGTGC<br/>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGCACA<br/>GGCAGACGCCGAAGCTAAACGCGGCGAGCCAGAATTGGG<br/>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>AGTATGAGCGTTTGTCCGCGCCGCGGCCGCTGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>CTTCACCCGGAGCCGATGTCCTGGCACGCGCGTGTAGAAT<br/>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>CTGGCTGGGGATGTCATGTTCTTTGCGGACATTAAGCGG<br/>ACATGATCGGTATGGGCCCCCTTATTACGCAGCCTGGGAC<br/>GCCTGCAACTGATAAGTGGAACCGCGCTGTATCCGAACGCC<br/>AATAAGAACAGCCACATGAAATCTATGTTTCGATCTGACCAC<br/>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA<br/>TGCCTATTTTAACGCCTACTCAGTATCGCGAGTCCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA<br/>GTGTCGTCGTTGCCTGGACATGCGTTTGCACTCCGTTGGC<br/>AAAACGTCTGCGGCCGGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGATCCCATCGTTGGCGTCCCAGTCCCGCACGACTT<br/>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGTTGAAGTGCCGGACCGTTACCCTGACCCCGAT<br/>AACCACGGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCACCACCACGAGCTGCCCCCGGGGTGCAGCGG<br/>CTGAAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGACGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTGCTGGTACGCTTGGCGTAGGTTCAAATATT<br/>GTTACGCTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCCTCGTATCAATATTGGGGTGTTCGGCGTGATGAATGCA<br/>GGAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCCGGCACGACCGCAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGACCAGCCA<br/>AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCGATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCCGAATCAAATCGGGACGCCTTGCTGAAGCCCT</p> |
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|  |  | <p>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTCTGCAAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGGTGTTTGCCCCGTT<br/>GGTCTCTTGGACGCAACGCCCGCTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCAGGTGGACGCTTCTTCGTCTCT<br/>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>CCGTGCTTTCCGTACGCCTGGATTTAGACGCCGCACGTGG<br/>CAAATTGGGGCCCGAGGCCGTGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>CACCCCTTGACCCCTGGACCGCTCATCAGGACGTCCGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTTCG<br/>GAAGCGTGTAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTGC<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAATGCAGCAGCGTATGAAAGACTTGCATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTCTTTCTTTGGGCAG<br/>CTTGGCCCGACGCCCTTCGTGCGCATTTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCCGCAGC<br/>CGCGCCTGCTACGGCAGCGTCCGGGGTATAATACTA<br/><br/>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGA<br/><br/>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/>GCAGGCCGTCTTTTGGCGGGCCAGCGCCCCCTGCGGCGTC<br/>CGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/>CACACGGCAAAGCATCTGCCACGCGCGAATACGCTGGTGA<br/>CTTTCTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/>AGCGTGAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/>AAGGCTGATGCTCAGGACGCAGGACGCGTGCGTGAGATTT<br/>TGGCGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/>GCCTGTAAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/>TTACCTTGGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/>TGCTATCAAAGAACGTATCTACGGGAACCGCGTCGTGCTG</p> |
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|    |                                                                                                                                                                                                                                                                                                                                                                     | <p>             TTCGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br/>             CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/>             GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/>             CGCCTTGCAACGTCAAGGTCATCGCCGATTTTAGCCCTTA<br/>             CTGGAGAACATCCTAAATACACTTTCGACAATTTCTTGCATG<br/>             CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/>             GTCCATTGCGCGTATCAATGTCGAAATCCCACCACCTTAGTG<br/>             TCTCAGACATGCGCCGCCTTAAGAATACAGATTCAAGTTGGC<br/>             ACGTTCGTTCTGTTTCAAGAGACATATCACCAGCAGACATT<br/>             CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br/>             TTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT<br/>             GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br/>             ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br/>             TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br/>             TCCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br/>             GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br/>             GTTGCGGTCCTTCGTATTGCTGTACCATACACAGGTATGAT<br/>             CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCGCTT<br/>             CTGAAGTGTGGGATGAGTCAAATGAGTGCCGGCTCGCGCA<br/>             CTGATGTAGGAGCCTATCACAAGATCACACGTTAAGTACA<br/>             GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br/>             GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br/>             AGGAGGGCTATGTCCCGTCTTGGTGACAGCTTGTACCG<br/>             TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br/>             GCTGGTGACATTCACGATTTCTGTATCCCAATAGTCTGCT<br/>             TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br/>             TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG<br/>             ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br/>             CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br/>             ACGATGTATATCTTTAACCAGGCATCAAATAAACGAAAGG<br/>             CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTG<br/>             TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br/>             GGGTGGGCCTTTCTGCGTTTATA           </p> |
| 37 | <p>             Exemplary HPGC (hydrogen<br/>             producing gene cluster) with<br/>             codon optimised nucleic acid<br/>             sequence encoding HydA<br/>             (Hyd1) from <i>Megasphaera<br/>             micronuciformis</i>, 5' – 3'<br/>             (including restriction sites<br/>             shown in bold)           </p> | <p>             CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>             GTGGAAAGAAGGAGATATACATATGGCGATGCGTTCCACAT<br/>             TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGCGTGGGG<br/>             CGCGTCCCGCGTCGCGTATGCTTGCATGGCCTATAAGGT<br/>             TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br/>             CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br/>             GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br/>             ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br/>             TCTGATCAGTCCTTCTGGATGACGCTCAGATGGGGAACG<br/>             GATTCGTCCTGACATGTGTCGCGTATCCTACAAGCGACTGC           </p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

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|  | <p>             ACTATCCAACTCACCAAGAGGAGGCTTTATATTAAGAA<br/>             GGAGATATACATATGCAAAGTTCGCGCTCCAGCAGCTTC<br/>             AGGTGTTGCCACACGTGTCGAGGTCGTATGTGTCGT<br/>             CCGGTTGCGGCGACGAAGGCTTCCACGGCTGTTACCACAG<br/>             ACATGTCGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br/>             TGAAATGCCGTTGAACACGTACAGTAATAAGCTCCGTTCA<br/>             AGGCAAAGGTTGTTCCGTGAAAAAATCACAGGACCAAA<br/>             AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br/>             GGGAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br/>             TTCCGCGGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br/>             GCCTCATGGTACTCGTCTTTATTCGATTGCTTCTAGTCGTTA<br/>             CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/>             CGCCGCGCGGTATACGTGATCCAGAGACTGGAAGGAG<br/>             GACCCCGCGAAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br/>             TGCCACACCAGGCACGGAATTTCCATGACAGGGCCCCACA<br/>             GGAAGATATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/>             TAATCTGTGTCGCAACGGGGACTGGAATCGCGCCTTTTCG<br/>             CTCATTCTGGCGCGTTGCTTCATCGAGAATGTCCCAAGTT<br/>             ATAAGTTCACTGGCCTTTTCTGGTTGTTTATGGGTGTCGT<br/>             AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br/>             CGCAAAGCGTATCCGGGACAATCCGCCTGGACTATGCC<br/>             TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAATGTA<br/>             CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/>             ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA<br/>             AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/>             TTGCAAAAGAAAAGGGGCTGAACTACGAAGAGTGGGTCGA<br/>             GGGGTAAAGCACAGAATCAATGGCATGTTGAAGTCTACT<br/>             AACCAGGCATCAAATAAACGAAAGGCTCAGTCGAAAGACT<br/>             GGGCCTTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTC<br/>             TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br/>             GCGTTTATATA<br/> <br/>             CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/>             TGTGTGTGGAAGAAGGAGATATACAT<br/> <br/>             ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>ATGAAGGC<br/>             TGTAGAGGGATTGAATCAAAATATAAATTTACGACAAGC<br/>             GCGTGCCGATTGCGGACGACAACCCAGCTGTGCACTTTGA<br/>             CGAAACGAAATGCAAAAATTGTACCCTTTGCCGTCGTGCGT<br/>             GTGAACTACGCAAACCGTGCTGGACTATTACAGCCTGGA<br/>             ACGCACCGGCGATGTTCCGGTTTGTGTTCACTGCGGTCAA<br/>             TGTGCAAATGCCTGCCCGTTTGGTGCCATGATGGAAGTTG<br/>             ATGATACGAACTTAGTCAAAGCTGCGATCGCGGACCCGGA<br/>             TAAGGTGGTGGTTTTCCAAACGGCACCGGCGGTACGTGTG<br/>             GCCATTGCGGAAGAGTTCGGTGCGGAGGCGGGTACATTC           </p> |
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|  |  | <p>GCACAGGGTAAAATGATTTACGCGTTGAGAGCCCTGGGTG<br/>GCGACTACGTGTTTCGACACCAACTTTGGCGCTGACATGAC<br/>CATCATGGAAGAACTTCCGAGCTGGTGCGTCGTATTACCA<br/>CTGGTAACCTCGCAATGCCGAGTTTACCAGCTGCTGCCC<br/>GGCGTGGGTTGAGTTTGCTGAGACCTTCTACGCAGAATAC<br/>ATCCCGCATCTGTCCAGCGCGAAGAGCCCGATTCTGATGC<br/>AAAACACCACGGAAAAAATCTGGTTTGGCGAGAAGGCGGG<br/>CATCGATCCGAAGAAGATGGTGACGGTTTGCGTTACCCCG<br/>TGTACCGCTAAAAAGCCGAGATCAAGCGCAAAGAATTGAA<br/>TGCTGCGGCTGAGTACTGGCATATTGATGGCTTAAAGGACT<br/>CCGACATTTGCATCACGACCCGTGAACTGGCACGTTGGCT<br/>GAAAGCGGAGAACATTGACTTCAATACCCTGGATGATGGTA<br/>TTTTTGATAGCCACCTGGGCGAGGCGAGCGGTGGTGGCAT<br/>CATCTTCGGCAGCACCGGTGGTGTGTCATGGAGTCCGCACTG<br/>CGCAGCGCGTATTACTTCTATACCGGTAAACCGATGCCAG<br/>CGGAGTATATACCGTATGAACCGGTGCGTGGCTTGACGG<br/>CGTTAAAGAGGCGACCATCGACTTCAGCGGTATCTCTCTG<br/>CATGTCGCAGTCGTGAGCGGTCTCGGCAATGCGCGTCGTT<br/>TTCTGGATAAAATTATGGCGGACGGCACCTTCAAAGATTAC<br/>ACCTTTATCGAGTTCATGGCGTGCCAGGGTGGCTGCATCA<br/>ACGGTGGTGGCCAACCGAAGGTTAAGATGCCTCTGGTTCA<br/>GAAAACCAACCAGGCTCGCATGAATAGCTTGACAAACGTG<br/>ATTCGAGGTTTCGATTAAGGCCGCTGGGAAAACCCCGA<br/>AATCCAGGAACTCTACAGCGACTTCTACGGCCAGCCGCTG<br/>TCTGAGCGCTCTGAGAAGTATATTACACCTTCTTTGAGGA<br/>TAAGTCCGGCAACCTGGGTGAAGGTGGTGTGTGACCCCCG<br/>CAAACGAACCCATTGTCTCCGAAGTATAAGCCGATTGAATA<br/><b>GGGATCC</b></p> <p>GGGTCGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT<br/>TATCTGTTGTTTGTGCGGTGAACGCTCTCTACTAGAGTCACA<br/>CTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>CACATTCCCGTCAGGCCGAGATCGCAAACCTGGGCGCAG<br/>GTGCGGCATCTAGCCGCCCATCATGTCCTTCTCGCCGCAT<br/>TGTCGCGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCC<br/>GTGCAGCAGTAGCGGCCGCTAACCCTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGGCAGAGACCTGGGCGATT<br/>TCCTGGGCTTAGGGAAGGGGGGCTTTCGCCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTAATCTGGAGCGTGC</p> |
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|  |  | <p>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>GTGGGGGGTCTACTATCGCGCCTTGTTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTTCGAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAAAACGGCATTTC<br/>GCAACATTATGTTGCAAGGGGGTGAATTAAGACAGAGCAA<br/>CGCTTAGCCTACTTGGAGGCATGCGTCCGTGCGATTTCGCG<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC<br/>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGCACA<br/>GGCAGACGCCGAAGCTAAACGCGCGAGCCAGAATTGGG<br/>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>AGTATGAGCGTTTGTTCGCGCCGGCGCCCGTCGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>CTTCACCCGGAGCCGATGTCCTGGCACGCGCGTGTAGAAT<br/>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>CTGGCTGGGGATGTCATGTTCTTCGCGACATTAAGCGG<br/>ACATGATCGGTATGGGCCCTTCATTACGCAGCCTGGGAC<br/>GCCTGCAACTGATAAGTGGACCGCGCTGTATCCGAACGCC<br/>AATAAGAACAGCCACATGAAATCTATGTTGATCTGACCAC<br/>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGCGCAAATGTTGTCA<br/>TGCTATTTTAAACGCCTACTCAGTATCGCGAGTCCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA<br/>GTGTCGTCGTTGCCTGGACATGCGTTTGCACTCCGTTGGC<br/>AAAACGTCTGCGGCCGGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGCAATCCCATCGTTGGCGTCCAGTCCCGCACGACTT<br/>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGGTTGAAGTGCCGGACCGTTACCTGACCCCGAT<br/>AACCACGGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCAACCAACGGAGCTGCCCCCGGGGTGCAGCGG<br/>CTGGAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTGTGGTACGTCTGGCGTAGGTTCAAATATT<br/>GTTACGTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCCTCGTATCAATATTGGGGTGTTCGGCGTGATGAATGCA<br/>GGAAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCGGCACGACCGCAGATGT</p> |
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|  |  | <p> AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGGACCAGCCA<br/> AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/> TGGGATAAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/> GAGTGCATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/> CAGCCGCCGCAATCAAATCGGGACGCCCTTGCTGAAGCCCT<br/> GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/> AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/> GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/> CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/> CGCTGGATTTAGCGTCCACCCCTGCACGAGCGTAGTAC<br/> GATTACGTCTGCATTCTGTCGAAGGAAGGAGCAGTGCGCAGT<br/> TCACGCTATGGGGCTCCTCTGCCGGGGTGTTCGCCCGTT<br/> GGTCTCTTGGACGCAACGCCCGCTTACTGATGGTAATCCC<br/> GATGGATGCCGAAACCCAGGTGGACGTCTTCTTCGTCCCT<br/> CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/> CCGTGCTTTCCGTACGCCTGGATTTAGACGCCGCACGTGG<br/> CAAATTGGGGCCCGAGGCCTGTGAGATGGAGCGTCAGCG<br/> TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/> CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/> CACCCTTGGACCCTGGACCGCTCATCAGGACGTCCGTTGG<br/> TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/> AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/> CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTCCG<br/> GAAGCGTGTAATCATAACCGTATCACCTCCGCTTGCAACGA<br/> CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/> CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTGC<br/> CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/> AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/> AGAAAATGCAGCAGCGTATGAAAGACTTGATGAGGCTGG<br/> GGTGCCGGTCACTAATTATGGGGTGTTCCTTCTTGGGCAG<br/> CTTGGCCCGACGCCCTTCGTGCGCATTGGAACCGTGGG<br/> GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCGCAGC<br/> CGCGCCTGCTACGGCAGCGTCCGGGGTATAATACTA<br/> <br/> CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/> GTGGA<br/> <br/> AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/> GCAGGCCGTCTTTTGGCGGGCCAGCGCCCTGCGGCGTC<br/> CGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/> CACACGGCAAAGCATCTGCCACGCGGAATACGCTGGTGA<br/> CTTCTTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/> AGCGTGAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/> GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/> AAGGCTGATGCTCAGGACGCAGGACGCGTGCCTGAGATTT </p> |
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|    |                                                                                                                                          | <p>TGGCGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/> GCCTGTAAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/> TTACCTTGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/> AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/> TGCTATCAAAGAACGTATCTACGGGAACCGCGTCGTGCTG<br/> TTCGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br/> CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/> GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/> CGCCTTGCAACGTCAAGGTCATCGCCGCATTTTAGCCCTTA<br/> CTGGAGAACATCCTAAATACACTTTTCGACAATTTCTTGCA<br/> CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/> GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br/> TCTCAGACATGCGCCGCTTAAGAATACAGATTCAGTTGGC<br/> ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br/> CAAGGTAATGCATCCGTGAGGTCCAAAGAGCGACTTTGATT<br/> TTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT<br/> GGACGATGTTGGTATTGGTGCTTTATTGCGGTTATATGATT<br/> ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br/> TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br/> TCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br/> GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br/> GTTGCGGTCTTCGTATTGCTGTACCATACACAGGTATGAT<br/> CCTTAGCACTCGTGAATCACCAAGATGCGCTCGGCGCTT<br/> CTGAAGTGTGGGATGAGTCAAATGAGTGCCGCTCGCGCA<br/> CTGATGTAGGAGCCTATCACAAAGATCACACGTTAAGTACA<br/> GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br/> GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br/> AGGAGGGCTATGTCCCGCTTGGTGACACAGCTTGTACCG<br/> TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br/> GCTGGTGACATTCACGATTTCTGTCATCCCAATAGTCTGCT<br/> TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br/> TTCGTAAGAAAGGCGAGCAAGTAATTGCCCCTGAGATGGG<br/> ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br/> CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br/> ACGATGTATATCTTTAACCAGGCATCAAATAAACGAAAGG<br/> CTCAGTCGAAAGACTGGGCCTTTCTGTTTTATCTGTTGTTG<br/> TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br/> GGGTGGGCCTTTCTGCGTTTATA</p> |
| 38 | Exemplary HPGC (hydrogen producing gene cluster) with codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Veillonella</i> | <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/> GTGGAAAGAAGGAGATATACATATGGCGATGCGTTCCACAT<br/> TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGCGTGGGG<br/> CGCGTCCCGCGTCGCGTATGTCTTGCAATGGCCTATAAGGT<br/> TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

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|  | <p><i>parvula</i>, 5' – 3' (including restriction sites shown in bold)</p> | <p>CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br/> GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTC<br/> ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br/> TCTGATCAGTCCTTTCTGGATGACGCTCAGATGGGGAACG<br/> GATTCGTCCTGACATGTGTCGCGTATCCTACAAGCGACTGC<br/> ACTATCCAAACTCACCAAGAGGAGGCTTTATATTTAAAGAA<br/> GGAGATATACATATGCAAACGTTCGCGCTCCAGCAGCTTC<br/> AGGTGTTGCCACACGTGTCGCAGGTGCTCGTATGTGTCGT<br/> CCGGTTGCGGCGACGAAGGCTTCCACGGCTGTTACCACAG<br/> ACATGTCGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br/> TGAATGCCGTTGAACACGTACAGTAATAAAGCTCCGTTCA<br/> AGGCAAAGGTTTCGTTCCGTGGAAAAATCACAGGACCAAA<br/> AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br/> GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br/> TTCCGCGGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br/> GCCTCATGGTACTCGTCTTTATTTCGATTGCTTCTAGTCGTTA<br/> CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/> CGCCGCGCGGTATACGTCGATCCAGAGACTGGAAAGGAG<br/> GACCCCGCGAAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br/> TGCCACACCAGGCACGGAATTTCCATGACAGGGCCCACA<br/> GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/> TAATCTGTGTCGCAACGGGACTGGAATCGCGCCTTTTCG<br/> CTCATTCTGGCGCGGTTGCTTCATCGAGAATGTCCCAAGTT<br/> ATAAGTTCACTGGCCTTTTCTGGTTGTTTATGGGTGTCGCT<br/> AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br/> CGCAAAAGCGTATCCGGGACAATTCCGCCTGGACTATGCC<br/> TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAATGTA<br/> CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/> ATTTATTGGATAATGGGGCGCACATGTACTTCTCGGGTTA<br/> AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/> TTGCAAAAGAAAAGGGGCTGAACACGAAGAGTGGGTCGA<br/> GGGGTTAAAGCACAGAATCAATGGCATGTTGAAGTCTACT<br/> AACCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT<br/> GGGCCTTTCGTTTTATCTGTTGTTGTCGGTGAACGCTCTC<br/> TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br/> GCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/> TGTTGTGTGGAAAGAAGGAGATATACAT</p> <p>ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>GATGTCAAA<br/> ATATCAATTTTATAGATAGAAGGGTCCCGATTGAGGACGGTA<br/> ACATCGCTCTGGTTCAGGATTTGACTAAGTGCAAAAATTGT<br/> AGCCTGTGTCGTAAAGCTTGCGCGGTCGATATGGGCGTCT<br/> TTGACTACTATGATCTGACCACGAATGGTGACCACCCGATT</p> |
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|  |  | <p>TGCATCCACTGCGGTCAATGTGCGTCCATCTGCCCATTCGA<br/>TTCTATTAATGAACGCAGCGAGATCGATGAAGTTAAGGCGG<br/>CGATTGCGGACCCGAACAAAATCGTCATCTTCCAGACCGC<br/>ACCGGCTGTGCGTGTGGGTCTTGGCGAGGAGTTGCGCTTG<br/>GAGGCGGGTACCTTTGTGAAGGCAAGATGGTTGCAGCCC<br/>TGAGAAAGCTGGGCGGTGACTACATTCTGGACACCAACTTT<br/>GGTGCGGACATGACCATCATGGAAGAGGCGTCCGAGTTGC<br/>TGGAACGTGTTATCAACTCGGATGCTGTTCTGCCGCAATTT<br/>ACCAGCTGTTGTCCGGCGTGGGTTAAGTTGCGGAGACAT<br/>TCTACCCGGAGTTCCCTGCCTAACCTGAGCACCGCGAAGTC<br/>CCCGATTGCCATGCAGGCACCGACCCAGAAAACCTATTTTC<br/>GCCGAAAAAATGGGTCTGGACGCGAAGCAGATTGTGGCGG<br/>TGCCCGTAACCTCCGTGTAAGTCCGCAAGAAATTTGAGATTGCG<br/>CGTGACGAAATGAACAGCAGCGCAGAGTATTGGAATACCC<br/>CAGAAATGCGTGATACCGATTACTGCATCACCACGCGTGA<br/>GCTGGCAAAGTGGCTGCGCGCAGAAGAAATCAACTTTGAC<br/>GACCTCGAGGACAGCGCATTTGATCCGCTGATGGGTGAGG<br/>CTAGCGGTGGCGGTATTATTTTGGCAACACCGGCGGCGT<br/>TATGGAAGCTGCCATGCGCGCGGCGTATAAAATGGCAACC<br/>GGTGAAGATGCCCCCAAACCTTATCCCATTCGAGGCCA<br/>TCAGAGGCATGGATGGTGCGCGCGAAGCTGATGTTGTGAT<br/>CGGTGACAAGACCTGCACGTTGCGGCGGTGCATGGTAC<br/>GGGCAATTTACGTAAATTCATTGAGCGCATGCGTGCGGAG<br/>AACATCCACTACGACTTCATCGAGGTGATGGCATGCCGTG<br/>GTGGGTGCATCGGCGGCGGTGGCCAACCGCGTGTAAATT<br/>GCCGATGGCCGACAAAGCGCGCGAAGCTCGTATCGCGTCT<br/>CTATACACCCGTGACGCGAGAAGTGAAGTGTAAAGGCCGCGT<br/>GCGATAATCCGGATATTCAGAAATTATATGCAGAGTTTTTC<br/>GACGGCAAACCGATGAGCCATAAAGCACATCACATGCTGC<br/>ATACCACGTTCTGTGAATCGTAGCGAAGATTTGGGTCCGAAC<br/>GGTGCGTGACCCCGGCGACGTGCCCCGACCAAGTGTTCG<br/>AACCTGAAAAAGGCTGCTGAAGCGGCGAAGGCGGCGGCT<br/>GAAGTTAACTCTTAGGGATCC</p> <p>GGGTCGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTTCGTTT<br/>TATCTGTTGTTTGTGCGGTGAACGCTCTCTACTAGAGTCACA<br/>CTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG<br/>CACATTCCCGTCAGGCCGAGATCGCAAACCTGGGCGCAG<br/>GTGCGGCATCTAGCCGCCCATCATGTCCTTCTCGCCGCAT<br/>TGTCGCGGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCTGCGCATGCC</p> |
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|  |  | <p>GTGCAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGGCAGAGACCTGGGCGATT<br/>TCCTGGGCTTAGGGAAGGGGGGGCTTTCGCCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG<br/>GTGGGGGGGTCTACTATCGCGGCCCTGTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTCGCAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAAAACGGCATT<br/>GCAACATTATGTTGCAAGGGGGTGAAATTAAGACAGAGCAA<br/>CGCTTAGCCTACTTGGAGGCATGCGTCCGTGCGATTTCGG<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC<br/>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGCACA<br/>GGCAGACGCCGAAGCTAAACGCGGCGAGCCAGAATTGGG<br/>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>AGTATGAGCGTTTGTTCGCGCCGGCGCCCGTCTGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGCA<br/>CTTACCCCGAGCCGATGTCCTGGCACGCGCGTGTAGAAT<br/>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>CTGGCTGGGGATGTCATGTTCTTTCGCGACATTAAAGCGG<br/>ACATGATCGGTATGGGCCCCCTTACATCGCAGCCTGGGAC<br/>GCCTGCAACTGATAAGTGGAACGCGCTGTATCCGAACGCC<br/>AATAAGAACAGCCACATGAAATCTATGTTGATCTGACCAC<br/>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA<br/>TGCTATTTTAAACGCCTACTCAGTATCGCGAGTCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGATACTGCTGTTCA<br/>GTGTCGTCGTTGCCTGGACATGCGTTTGCACTCCGTTGGC<br/>AAAACGTCTGCGGCCGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGATCCCATCGTTGGCGTCCCAGTCCCGCACGACTT<br/>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGGTTGAAGTGCCGGACCGTTACCCTGACCCCGAT<br/>AACCACGGACGAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCACCACCGAGCTGCCCCCGCGGGTGCAGCGG<br/>CTGGAAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA</p> |
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|  |  | <p>AGACGCTGGTGCTGGTACGCTGGCGTAGGTTCAAATATT<br/>GTTACGCTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCCTCGTATCAATATTGGGGTGTTCGGCGTGATGAATGCA<br/>GGAAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCGGCACGACCGCAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGACCAGCCA<br/>AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCGATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCCGCAATCAAATCGGGACGCCTTGCTGAAGCCCT<br/>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTGCTCAAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGTGTTTGCCCCGTT<br/>GGTCTCTTGACGCAACGCCCGTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCAGGTGGACGTCCTTCTCGTCT<br/>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>CCGTGCTTTCCGTACGCCTGGATTTAGACGCCGCACGTGG<br/>CAAATTGGGGCCCGAGGCCTGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>CACCCCTGGACCCTGGACCGCTCATCAGGACGTCCGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCCTATTTCG<br/>GAAGCGTGTAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTCG<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAAATGCAGCAGCGTATGAAAGACTTGATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTTCCTTGGGCAG<br/>CTTGGCCCGACGCCCTTCGTCGCGCATTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCGCAGC<br/>CGCGCCTGCTACGGCAGCGTCCGGGGTATAATACTA<br/><br/>CTAGAGGGCTTTACACTTTATGCTTCGGCTCGTATGTTGT<br/>GTGGA<br/><br/>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/>GCAGGCCGTCTTTTGGCGGGCCAGCGCCCTGCGGCGTC<br/>CGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG</p> |
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|  |  | <p>CACACGGCAAAGCATCTGCCACGCGCGAATACGCTGGTGA<br/>CTTTCTTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/>AGCGTGAAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/>AAGGCTGATGCTCAGGACGCAGGACGCGTGCGTGAGATT<br/>TGGCGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/>GCCTGTAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/>TTACCTTGGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/>TGCTATCAAAGAACGTATCTACGGGAACCGCGTCGTGCTG<br/>TTCGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br/>CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/>GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/>CGCCTTGCAACGTCAAGGTCATCGCCGCATTTTAGCCCTTA<br/>CTGGAGAACATCCTAAATACACTTTTCGACAATTTCTTGCATG<br/>CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/>GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br/>TCTCAGACATGCGCCGCTTAAGAATACAGATTCAAGTTGGC<br/>ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br/>CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br/>TTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT<br/>GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br/>ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br/>TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br/>TCCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br/>GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br/>GTTGCGGTCTTCGTATTGCTGTACCATACACAGGTATGAT<br/>CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCGCTT<br/>CTGAAGTGTGGGATGAGTCAAATGAGTGCCGCTCGCGCA<br/>CTGATGTAGGAGCCTATCACAAGATCACACGTTAAGTACA<br/>GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br/>GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br/>AGGAGGGCTATGTCCCGTCTTGGTGACAGCTTGTTACCG<br/>TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br/>GCTGGTGACATTACGATTTCTGTCATCCCAATAGTCTGCT<br/>TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br/>TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG<br/>ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br/>CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br/>ACGATGTATATCTTTAACCAGGCATCAAATAAAACGAAAGG<br/>CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTG<br/>TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br/>GGGTGGGCCTTCTGCGTTTATA</p> |
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| 39 | Exemplary HPGC (hydrogen producing gene cluster) with codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Veillonella atypica</i> , 5' – 3' (including restriction sites shown in bold) | <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/> GTGGAAGAAGGAGATATACATATGGCGATGCGTTCCACAT<br/> TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGCCTGCGG<br/> CGCGTCCCGCGTGTGCGTATGTCTTGCATGGCCTATAAGGT<br/> TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br/> CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br/> GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br/> ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br/> TCTGATCAGTCCTTTCTGGATGACGCTCAGATGGGGAACG<br/> GATTCGTCTGACATGTGTCGCGTATCCTACAAGCGACTGC<br/> ACTATCCAACTCACCAAGAGGAGGCTTTATATTAAGAA<br/> GGAGATATACATATGCAAACGTTCGCGCTCCAGCAGCTTC<br/> AGGTGTTGCCACACGTGTGCGAGGTGCGTATGTGTCGT<br/> CCGGTTGCGGCGACGAAGGCTTCCACGGCTGTTACCACAG<br/> ACATGTGGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br/> TGAAATGCCGTTGAACACGTACAGTAATAAGCTCCGTTCA<br/> AGGCAAAGGTTGTTCCGTGGAATAATCACAGGACCAAA<br/> AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br/> GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br/> TTCCGCCGGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br/> GCCTCATGGTACTCGTCTTTATTGATTGCTTCTAGTCGTTA<br/> CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br/> CGCCGCGCGGTATACGTGATCCAGAGACTGGAAGGAG<br/> GACCCGCGAAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br/> TGCCACACCAGGCACGAAATTTCCATGACAGGGCCCA<br/> GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br/> TAATCTGTGTCGCAACGGGGACTGGAATCGCGCCTTTTCG<br/> CTCATTCTGGCGCCGTTGCTTCATCGAGAATGTCCCAAGTT<br/> ATAAGTTCACTGGCCTTTTCTGTTGTTTATGGGTGTCGCT<br/> AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br/> CGCAAAAGCGTATCCGGGACAATTCGCCCTGGACTATGCC<br/> TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAAAATGTA<br/> CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br/> ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA<br/> AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br/> TTGCAAAAGAAAAGGGGCTGAACTACGAAGAGTGGGTCGA<br/> GGGGTTAAAGCACAAGAATCAATGGCATGTTGAAGTCTACT<br/> AACCAGGCATCAAATAAACGAAAGGCTCAGTCGAAAGACT<br/> GGGCCTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTC<br/> TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCT<br/> GCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/> TGTTGTGTGGAAGAAGGAGATATACAT</p> |
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|  |  | <p>ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>GATGTCACA<br/>ATTTGAATTTATAGATAAAAGGGTCCCTATTGCGCTCGACA<br/>ACCCGAGCATCTATCAGATATCTCCAAATGCAAAAAGTGC<br/>ACCTTATGCCGTCGTGCGTGCGCCGACGTGATGAGCGTTC<br/>TGGACTACTACGATCTGGAAGCGACCGGTGACGTGCCAGT<br/>TTGTATTCAATTGTGGACAATGTGCAGCGGCGTGCCCGTTTG<br/>ACTCGATGCATGCAAAAAGCGAATTGGATAAGGTGAAGGT<br/>GGCTATTGCCGATCCGGATAAAATCGTCGTTATTCAGACCG<br/>CTCCGGCTGTACGCGTGTGATTGGCGAGGGTTTCGGCTT<br/>TGAACCGGTACGTTTCTCGAGGGCAAAATGGTTAGCGCG<br/>CTGCGTAACTGGGTGCCGACTACGTGGTCGACACGAAGT<br/>TCGGCGCGGATCTGACCATTATGGAGGAGGCGTCTGAATT<br/>GGTTGATCGTCTGAAGAACGGTGGCACGATCCCGCAATTC<br/>ACCAAGTTGCTGCCCGGCTTGGGTTTCGTTTTCGCGAAATCTA<br/>CTTCCCGGAGTTGATCCCAAATCTGTCTAGCACCCGTAGCT<br/>GCATCGCAATGGAGGCCGCTATGATTAACCTATTTTCGCT<br/>GAGAAAAAGGGTATTAACCCGGCGAATATCGTGTCCGTCT<br/>CCGTTAATCCGTGTACTGCGAAGAAGGCTGAGACAAAGCG<br/>CGTGGAAGAAAATGCTGCCGCGCGTTATTACGACGACGAA<br/>TCCCTTGGCATGGATACCGACATCAGCATTACCACCAGAGA<br/>ATTTATCCGTTGGCTGAACGACGAAGGCGTGGACTTCGAG<br/>TCCCTGGAGGACAGCCAGTTTGATGATCTTATCGGTATGGA<br/>AACTGGCGCGTCTATCATTTTTGGTAATACCGGTGGCGTTA<br/>TGGAGGCTGCTATGAGAACGGCGTACAACTGATTACGGA<br/>TAAGGAGCCGCCACCGTATGCACTGACCCACCTGGAGGAT<br/>GTGCGCGGTATGAACGGTGTGAAAGAGGCGACGGTTCAG<br/>CTGGGTGATGACGTGACTCTGAGCGTTGCGGTGGTTCACG<br/>GCGGTAAAGAACCCCGTGATTTTTTGAATGCGCTGAAGGA<br/>GAACGGCAAGCACTATGACTTCATCGAAGTCATGGCATGTC<br/>CGGGTGGGTGCATTGGTGGCGGTGGCCAACCGCGTACCA<br/>AACTGCCGCAGGCGGTTAAGACCAAAGAGGCGCGTATCGG<br/>CGGTCTGTACAAGGCGGACGAAGAATATAAGTACGTTGCC<br/>AGCTATGAATCACCGGAAATCCAAGAGTTGTACAAGAACTT<br/>CTTGGGAGAACCTCTGGGTCACAAAGCACATGAATTGCTG<br/>CATACCCACTTCACCGATCGCAGCGCACAGTTAGGCGACC<br/>GCAAAGATGTTGTCCCGGAGACCTGTCCGACCAGCCCGAA<br/>ATACAAGGGTTAG<b>GGATCC</b></p> <p>GGGTCGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT<br/>TATCTGTTGTTTGTGCGGTGAACGCTCTCTACTAGAGTCACA<br/>CTGGCTCACCTTCGGGTGGGCCTTTCGCGTTTATATA<br/>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG</p> |
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|  |  | <p>CACATTCCCGTCAGGCCGGAGATCGCAAACCTGGGCGCAG<br/>GTGCGGCATCTAGCCGCCCATCATGTCCTTCTCGCCGCAT<br/>TGTCGCGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT<br/>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCCC<br/>GTGCAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA<br/>TGGCATCAGCGGAGGCCGCGGCAGAGACCCTGGGCGATT<br/>TCCTGGGCTTAGGGAAGGGGGGGCTTTCGCCGGGCGCAA<br/>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA<br/>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC<br/>GTTGTACAGCCATGCCAATGCGGTGACTAACAAACTGTG<br/>GTGGGGGGTCTACTATCGCGGCCCTTGTTGGAGTTCAGTAA<br/>TATTTGCCAAAACGATTGCTCATATTGTGGGATTCGCAACA<br/>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGGTTGAAGAG<br/>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAAAACGGCATT<br/>GCAACATTATGTTGCAAGGGGGTGAATTAAGACAGAGCAA<br/>CGCTTAGCCTACTTGAGGCATGCGTCCGTGCGATTGCGG<br/>AGGAGACAACGCAGTTAGACCTTGAAATGCGCGCACGTGC<br/>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGCACA<br/>GGCAGACGCCGAAGCTAAACGCGGCGAGCCAGAATTGGG<br/>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC<br/>AGTATGAGCGTTTGTTCGCGCCGGCGCCCGTCGCTATCT<br/>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA<br/>CTTCACCCGGAGCCGATGTCCTGGCACGCGCGTGTAGAAT<br/>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC<br/>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC<br/>CTGGCTGGGGATGTCATGTTCTTTCGCGACATTAAAGCGG<br/>ACATGATCGGTATGGGCCCTTCATTACGCAGCCTGGGAC<br/>GCCTGCAACTGATAAGTGGACCGCGCTGTATCCGAACGCC<br/>AATAAGAACAGCCACATGAAATCTATGTTGATCTGACCAC<br/>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA<br/>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT<br/>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA<br/>TGCTATTTTAAACGCCTACTCAGTATCGCGAGTCCTATCAG<br/>TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA<br/>GTGTCGTCGTTGCCTGGACATGCGTTTGCACTCCGTTGGC<br/>AAAACGTCTGCGGCCGGAGTTTGGGGCGATCCTGCTTCGT<br/>TCTTGATCCCATCGTTGGCGTCCCAGTCCCGCACGACTT<br/>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC<br/>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA<br/>GCGTCTGGTTGAAGTGCCGGACCGTTACCCTGACCCCGAT<br/>AACCACGGACGAAAAAGGCAGGAGCCGGGAAAGGAGGC<br/>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCACCACCGAGCTGCCCCGCGGGTGCAGCGG<br/>CTGGAAGGGTACCGGTGCAGCTGCAATTGGTGGCGGAG</p> |
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|  |  | <p>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTGCTGGTACGCTGCGGTAGGTTCAAATATT<br/>GTTACGTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCCTCGTATCAATATTGGGGTGTTGCGCGTGATGAATGCA<br/>GGAAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCCGGCACGCCGAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGGAACAGCCA<br/>AGTTGCTGGACACAGCCGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCGATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCGCAATCAAATCGGGACGCCTTGCTGAAGCCCT<br/>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTGCTCAAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGGTGTTTGCCCCGTT<br/>GGTCTCTTGACGCAACGCCGCTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCCAGGTGGACGCTCTTCTCGTCCT<br/>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>CCGTGCTTTCCGTACGCCTGGATTTAGACGCCGCACGTGG<br/>CAAATTGGGGCCCGAGGCCTGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>CACCCCTGGACCCTGGACCGCTCATCAGGACGTCCGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGGACCCGTTTGTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTTCG<br/>GAAGCGTGTAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTCG<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAAATGCAGCAGCGTATGAAAGACTTGCAATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTTTTTCTCTTGGGCAG<br/>CTTGGCCCCGACGCCCTTCGTCGCGCATTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTAATCCTGCTACCCCCGACG<br/>CGCGCTGCTACGGCAGCGTCCGGGGTATAATACTA<br/>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT</p> |
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|  |  | <p>GTGGA</p> <p>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/> GCAGGCCGTCTTTTGGCGGGCCAGCGCCCTGCGGCGTC<br/> CGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/> CACACGGCAAAGCATCTGCCACGCGCGAATACGCTGGTGA<br/> CTTCTTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/> AGCGTGAAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/> GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/> AAGGCTGATGCTCAGGACGACGACGCGTGCGTGAGATT<br/> TGGCGAAGGCTAAGGAAAAGGCTTTTGTACTGAACATGC<br/> GCCTGTAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/> TTACCTTGGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/> AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/> TGCTATCAAAGAACGTATCTACGGGAACCGCGTCGTGCTG<br/> TTCGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br/> CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/> GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/> CGCCTTGCAACGTCAAGGTCATCGCCGATTTTAGCCCTTA<br/> CTGGAGAACATCCTAAATACACTTTTCGACAATTTCTTGCATG<br/> CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/> GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br/> TCTCAGACATGCGCCGCCTTAAGAATACAGATTCAAGTTGGC<br/> ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br/> CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br/> TTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT<br/> GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br/> ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br/> TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br/> TCCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br/> GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br/> GTTGCGGTCTTCGTATTGCTGTACCATACACAGGTATGAT<br/> CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCGCTT<br/> CTGAAGTGTGGGATGAGTCAAATGAGTGCCGCTCGCGCA<br/> CTGATGTAGGAGCCTATCACAAGATCACACGTTAAGTACA<br/> GAGGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br/> GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br/> AGGAGGGCTATGTCCCGTCTTGGTGACACAGCTTGTTACCG<br/> TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br/> GCTGGTGACATTACGATTTCTGTATCCCAATAGTCTGCT<br/> TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br/> TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG<br/> ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br/> CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br/> ACGATGTATATCTTTAACCAGGCATCAAATAAACGAAAGG</p> |
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|    |                                                                                                                                                                                                                  | CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTG<br>TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br>GGGTGGGCCTTCTGCGTTTATA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| 40 | Exemplary HPGC (hydrogen producing gene cluster) with codon optimised nucleic acid sequence encoding HydA (Hyd1) from <i>Peptoclostridium bifermentans</i> , 5' – 3' (including restriction sites shown in bold) | CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br>GTGGAAAGAAGGAGATATACATATGGCGATGCGTTCACAT<br>TTGCTGCGCGTGTGCGTGCCAAACCGGCAGTGCGTGGGG<br>CGCGTCCCGCGTGTGCGTATGTCTTGATGGCCTATAAGGT<br>TACACTGAAAACGCCATCAGGCGATAAAACAATCGAATGTC<br>CGGCGGACACCTATATCCTGGATGCTGCGGAGGAAGCCG<br>GGCTGGACCTTCCCTATAGTTGCCGTGCGGGCGCCTGTTT<br>ATCCTGTGCTGGCAAAGTGGCAGCTGGAACGGTAGATCAA<br>TCTGATCAGTCCTTCTGGATGACGCTCAGATGGGGAACG<br>GATTCGTCCTGACATGTGTCGCGTATCCTACAAGCGACTGC<br>ACTATCCAAACTCACCAAGAGGAGGCTTTATATTAAGAA<br>GGAGATATACATATGCAAACGTTCGCGCTCCAGCAGCTTC<br>AGGTGTTGCCACACGTGTGCGAGGTCGTGATGTGTCGT<br>CCGGTTGCGGCGACGAAGGCTTCCACGGCTGTTACCACAG<br>ACATGTGGAAGCGCACTGTTCCAACCAAGTTAGAGGAAGG<br>TGAAATGCCGTTGAACACGTACAGTAATAAGCTCCGTTCA<br>AGGCAAAGGTTGTTCCGTGGAAAAATCACAGGACCAAAA<br>AGCCACAGGTGAGACGTGCCACATCATTATTGAAACCGAG<br>GGGAAGATCCCGTTTTGGGAGGGACAATCGTACGGTGTA<br>TTCCGCGGGGACCAAGATCAACTCTAAGGGAAAAGAAGT<br>GCCTCATGGTACTCGTCTTTATTCGATTGCTTCTAGTCGTTA<br>CGGAGATGACTTCGATGGTCAAACGGCATCGCTGTGTGTT<br>CGCCGCGCGGTATACGTGATCCAGAGACTGGAAAGGAG<br>GACCCCGCGAAAAAAGGACTGTGTAGTAACTTTTGTGTGA<br>TGCCACACCAGGCACGGAATTTCCATGACAGGGCCCCACA<br>GGAAAAGTATTGCTTCTGCCAGCAGACGCGAACGCGCCAT<br>TAATCTGTGTCGCAACGGGGACTGGAATCGCGCCTTTTCG<br>CTCATTCTGGCGCCGTTGCTTCATCGAGAATGTCCCAAGTT<br>ATAAGTTCACTGGCCTTTTCTGGTTGTTTATGGGTGTCGCT<br>AACTCTGATGCTAAATTGTACGATGAGGAAGTCAAGCTAT<br>CGCAAAAGCGTATCCGGGACAATTCCGCCTGGACTATGCC<br>TTGTCTCGTGAACAAAATAATCGCAAGGGCGGGAAAATGTA<br>CATCCAAGATAAGGTTGAAGAGTATGCCGACGAAATTTTCG<br>ATTTATTGGATAATGGGGCGCACATGTACTTCTGCGGGTTA<br>AAGGGGATGATGCCAGGCATCCAAGATATGTTAGAACGCG<br>TTGCAAAAGAAAAGGGGCTGAACTACGAAGAGTGGGTGCA<br>GGGGTTAAAGCACAGAATCAATGGCATGTTGAAGTCTACT<br>AACCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT<br>GGGCCTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTC<br>TACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTCT |

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|  |  | <p>GC GTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTA<br/>TGTTGTGTGGAAAGAAGGAGATATACAT</p> <p>ATGTCCGCATTAGTACTTAAG<b>CCCGGG</b>GATGAAGCA<br/>CCTATTTACAGAAAAAGTAGTTCCGATCGAGCTGGATAACC<br/>CGTCCATTGAGATTGACTTTGATAAGTGCATCAAGTGCGGT<br/>CTGTGTAAACGCGTTTGTGAAAACGAGATTGGTGTTAATGG<br/>TTATTTGACCTGGAGAAGACCGGTGACATCGCCATCTGCA<br/>TCAACTGCGGCCAATGTGTTGAGCATGTCCAAAAAGGC<br/>GATCACCCAGGTTATCGATGTGGATCGCGTGAAGGAAGCG<br/>ATCAACGATCCGAAAAAGATTGTTATCTTCAGCACAGCGCC<br/>AGCTGTGCGTGTGCGACTGGGCGAAGAATTTAACCTGGAA<br/>GAAGGCGCGTATGTTGAGGACAAAATGGTGGACGCCCTGC<br/>GTAACTGGGTGGAGATTACGTTTTTGACGTTACCTTTGGC<br/>GCCGATATGACCATCATGGAAGAGGCGAACGAACCTGTTT<br/>CTCGTATCAAAAACGGCAAAGGCAAACCCCGCAATTTACC<br/>AGCTGCTGCCCGTCTGGGTTAAGTTCGCGGAAACGTTCT<br/>ATCCGGAGTTGATTCCGAATCTGTCTACCACGAAATCTCCG<br/>ATTGGCATTGAGGTGCTGTCTCAAGACCTATTTGCGACA<br/>GAAAGCAAATATCGACCCGGAGAAAATCGTGAACGTAACC<br/>ATTACTCCGTGCACCGCTAAGAAGTACGAAATTGACCGTCC<br/>GGAGATGAATGCGAGCGCAAAATACAACAAAAGCGAAAT<br/>ATGAGAGATAATGACATCATCTCACCCTAAGGAGCTGGC<br/>GCAGTGGCTCCGTGACGAAGAGATCGACTTCAACGCTTTG<br/>GAGGGCTCGAAATTTGACAACATCTTGGGCTTGGTAGCG<br/>GTGCGGGCATTATCTTCGGTAATAGCGGTGGTGTGATGGA<br/>AGCCGCGGTGCGCACGGTCTACAATATCTCACCCATGAG<br/>AACCACATAAAGAACTGCTGCACTTTAATCCGGTTCGTGG<br/>TCTGGAAGACGTGAAGGAGGCTACCCTTACCATTGGCGAT<br/>ACCACCTGCGCCTGGCAGCGGTGCAAGGCACGGCGAAC<br/>GTGCGTACGTTGATCGAAAAGCTGAAATCCGGTGAGGTGG<br/>AGTACGACTTCATAGAGGTAATGACTTGCAAAGGTGTTGT<br/>ATTGGTGGCGCTGGTCAACCGAAGATGAAAGCGCGTATTA<br/>GCAATGAGATGCGTCTCAAGCGCATTGAGGGTCTGTACGA<br/>TAAGGACAAGCACATTGCGGTCAAATGCAGCTATGAAAACC<br/>CGGATGTCATCAACGTGTACAAGGAGTTCTTCAAACAACCG<br/>CTGAGCCATTTATCCACGAGTTGCTGCACACCACCTTTGA<br/>AAGCAAGCACGATATGTTGGGTCTGAAGGATGACAACAAC<br/>GTTTCGGATATTGGCTAG<b>GGATCC</b></p> <p>GGGTGAGGAGAAGGATGAGAAAAAGTGACCAGGCATCAA<br/>ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTT<br/>TATCTGTTGTTGTGCGGTGAACGCTCTCTACTAGAGTCACA</p> |
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|  |  | <p>CTGGCTCACCTTCGGGTGGGCCTTCTGCGTTTATATA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT</p> <p>GTGGAAAGAAGGAGATATACATATGGCTCATAGTTTAAGCG</p> <p>CACATTCCCCTCAGGCCGGAGATCGCAAACCTGGGCGCAG</p> <p>GTGCGGCATCTAGCCGCCCATCATGTCCTTCTCGCCGCAT</p> <p>TGTCCGCGTGGCGGCCCATGCTTCTGCGTCCAAGGCGACT</p> <p>CCCGATGTACCTGTTGACGATCTTCTCCTGCGCATGCCC</p> <p>GTGCAGCAGTAGCGGCCGCTAACCGTCGTGCGCGCGCTA</p> <p>TGGCATCAGCGGAGGCCGCGGCAGAGACCTGGGCGATT</p> <p>TCCTGGGCTTAGGGAAGGGGGGGCTTTCGCCGGGCGCAA</p> <p>CCGCCAACCTTGATCGTGAGCAAGTATTAGGTGTGTTGGA</p> <p>GGCGGTCTGGCGTCGTGGAGACCTTAATCTGGAGCGTGC</p> <p>GTTGTACAGCCATGCCAATGCGGTGACTAACAAATACTGTG</p> <p>GTGGGGGGTCTACTATCGCGGCCCTTGTTGAGTTCAGTAA</p> <p>TATTTGCCAAAACGATTGCTCATATTGTGGGATTCGCAACA</p> <p>ATCAAAAAGAAGTTTGGCGCTATACTATGCCGGTTGAAGAG</p> <p>GTGGTCGAGGTAGCTAAGTGGGCGCTTGAAAACGGCATT</p> <p>GCAACATTATGTTGCAAGGGGGTGAATTAAGACAGAGCAA</p> <p>CGCTTAGCCTACTTGAGGCATGCGTCCGTGCGATTGCGG</p> <p>AGGAGACAACGCAGTTAGACCTTGAATGCGCGCACGTGC</p> <p>CGCGAGTACGACAACAGCGGAAGCGGCAGCCAGTGACACA</p> <p>GGCAGACGCCGAAGCTAACGCGGCGAGCCAGAATTGGG</p> <p>TGTGGTCGTATCCTTAAGCGTCGGAGAACTTCCTATGGAAC</p> <p>AGTATGAGCGTTTGTTCGCGCCGGCGCCCGTCGCTATCT</p> <p>GATCCGCATTGAAACCTCGAATCCCGACCTGTACGCGGCA</p> <p>CTTCACCCGGAGCCGATGTCCTGGCACGCGCGTGTAGAAT</p> <p>GCCTGCGCAACCTGAAGAAAGCTGGGTATATGTTGGGCAC</p> <p>AGGAGTGATGGTCGGCTTGCCGGGACAAACCTTACACGAC</p> <p>CTGGCTGGGGATGTCATGTTCTTTCGCGACATTAAGCGGG</p> <p>ACATGATCGGTATGGGCCCCCTTATTACGCAGCCTGGGAC</p> <p>GCCTGCAACTGATAAGTGGACCGCGCTGTATCCGAACGCC</p> <p>AATAAGAACAGCCACATGAAATCTATGTTGATCTGACCAC</p> <p>TGCTATGAATGCACTTGACGTATTACGATGGGGAACGTAA</p> <p>ATATCAGTGCTACGACTGCATTACAAGCGATTATCCCCACT</p> <p>GGACGTGAAATTGCGCTTGAGCGCGGCGCAAATGTTGTCA</p> <p>TGCCTATTTTAACGCCTACTCAGTATCGCGAGTCCTATCAG</p> <p>TTATATGAGGGTAAGCCCTGCATTACGGATACTGCTGTTCA</p> <p>GTGTCGTCGTTGCCTGGACATGCGTTTGCACTCCGTTGGC</p> <p>AAAACGTCTGCGGCCGGAGTTTGGGGCGATCCTGCTTCGT</p> <p>TCTTGATCCCATCGTTGGCGTCCAGTCCCGCACGACTT</p> <p>GTCATCACCTGCTTTGGCCGCTGCTGCAAGTGCGGATTTTC</p> <p>ACGAGGTTGGCGCAGGTCCCTGGAACCCCATCCGACTAGA</p> <p>GCGTCTGTTGAAGTGCCGGACCGTTACCCTGACCCCGAT</p> <p>AACCACGGACGCAAAAAGGCAGGAGCCGGGAAAGGAGGC</p> |
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|  |  | <p>AAGGCGCATGACTCTCACGACGATGGCGATCACGACGACC<br/>ACCACCACCACCACGGAGCTGCCCCCGCGGGTGCAGCGG<br/>CTGAAAGGGTACCGGTGCAGCTGCAATTGGTGCGGAG<br/>CGGGGGCTAGCCGTCAACGCGTAGCAGGGGCTGCTGCCG<br/>CCTCTGCTCGTCTGTGTGCGGGAGCTCGCCGCGCTGGCC<br/>GTGTTGTCGCGTCGCCATTGCGCCAGCTGCCGCTTGCCG<br/>TGGTGTGGCCGTCAAGGCCGAGCTGCTGCTGCGGGTGA<br/>AGACGCTGGTGTGGTACGCTCTGGCGTAGGTTCAAATATT<br/>GTTACGTCTCCCGGAATCGCTAGCACTACGGCACATGGTG<br/>TTCCTCGTATCAATATTGGGGTGTTCGGCGTGATGAATGCA<br/>GGAAAGTCTACACTTGTGAATGCTCTGGCGCAACAGGAAG<br/>CATGCATTGTTGACTCAACCCCCGGCAGCAGCCGAGATGT<br/>AAAAACAGTTTTGCTTGAGCTTCATGCCCTTGACCAGCCA<br/>AGTTGCTGGACACAGCCGGATTAGACGAAGTCGGTGGACT<br/>TGGGGATAAAAAGCGCCGTAAAGCCCTGAATACGCTGAAG<br/>GAGTGCATGTTGCTGTGCTGGTTGTTGACACTGATACGG<br/>CAGCCGCCGCAATCAAATCGGGACGCCTTGCTGAAGCCCT<br/>GGAATGGGAATCGAAAGTAATGGAACAGGCACACAAGTAT<br/>AATGTCAGTCCTGTACTGCTTCTGAATGTAAATCACGCGG<br/>GCTGCCTGAAGCGCAGGCAGCTAGCATGCTTGAGGCGGT<br/>CGCTGGTATGCTTGACCCATCCAAGCAAATCCCTCGTATGT<br/>CGCTGGATTTAGCGTCCACCCCCCTGCACGAGCGTAGTAC<br/>GATTACGTCTGCATTGCTCAAGGAAGGAGCAGTGCGCAGT<br/>TCACGCTATGGGGCTCCTCTGCCGGGGTGTTCGCCCGTT<br/>GGTCTCTTGACGCAACGCCCGCTTACTGATGGTAATCCC<br/>GATGGATGCCGAAACCCCAGGTGGACGTCTTCTTCGTCT<br/>CAAGCGCAAGTTATGGAGGAAGCAATCCGCCATTGGGCTA<br/>CCGTGCTTTCCGTACGCCTGGATTTAGACGCCGCACGTGG<br/>CAAATTGGGGCCCGAGGCCTGTGAGATGGAGCGTCAGCG<br/>TTTCGACGGGGTAATTGCTATGATGGAACGTAATGACGGAC<br/>CCACCCTGGTGGTGACAGATTCTCAAGCGATCGATGTTGTA<br/>CACCCCTGGACCCCTGGACCGCTCATCAGGACGTCCGTTGG<br/>TTCCAATTACTACCTTTAGCATCGCTATGGCGTACCAACAA<br/>AACGGCGGACGTTTGACCCGTTTGTGGAAGGATTGGAAG<br/>CATTGGAGACGTTACAAGATGGGGATCGCGTTCTTATTTCCG<br/>GAAGCGTGTAATCATAACCGTATCACCTCCGCTTGCAACGA<br/>CATTGGAATGGTGCAGATCCCTAACAAGCTGGAGGCAGCA<br/>CTTGGCGGGAAAAAGTTACAAATCGAACATGCGTTTGGTGC<br/>CGAGTTTCCCGAGCTTGAGAGTGGGGGTATGGATGGATTG<br/>AAGTTAGCGATCCATTGTGGAGGGTGTATGATCGACGCTC<br/>AGAAAATGCAGCAGCGTATGAAAGACTTGATGAGGCTGG<br/>GGTGCCGGTCACTAATTATGGGGTGTTCCTTCTTGGGCAG<br/>CTTGGCCCGACGCCCTTCGTGCGCATTGGAACCGTGGG<br/>GAGTGGAGCCACCGGTAGGTACTCCTGCTACCCCCGACG</p> |
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|  |  | <p>CGCGCCTGCTACGGCAGCGTCCGGGGTATAATACTA</p> <p>CTAGAGGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT<br/>GTGGA</p> <p>AAGAAGGAGATATACATATGTCGGTCCCCCTACAGTGCAAT<br/>GCAGGCCGTCTTTTGGCGGGCCAGCGCCCTGCGGCGTC<br/>CGCGCGCGTCTGAACCGCCGTGTATGTGTTCCAGTGACCG<br/>CACACGGCAAAGCATCTGCCACGCGGAATACGCTGGTGA<br/>CTTTCTTCCAGGGACCACTATTAGTCATGCGTGGTCCGTGG<br/>AGCGTGAAACTCATCACCGTTATCGTAATCCGGCCGAGTG<br/>GATTAACGAGGCTGCCATCCACAAGGCGCTTGAAACGTCA<br/>AAGGCTGATGCTCAGGACGACGACGCGTGCGTGAGATTT<br/>TGCGGAAGGCTAAGGAAAAGGCTTTTGTTACTGAACATGC<br/>GCCTGTAAATGCGGAAAGTAAATCCGAATTTGTACAAGGGC<br/>TTACCTTGGAGGAGTGCGCCACCTTAATTAACGTTGATTCT<br/>AATAATGTCGAGTTGATGAATGAAATCTTCGACACTGCGCT<br/>TGCTATCAAAGAACGTATCTACGGGAACCGCGTCGTGCTG<br/>TTCGCGCCCTTGACATTGCTAATCATTGTATGAACACTTG<br/>CACTTATTGTGCGTTTCGCTCAGCCAATAAGGGCATGGAAC<br/>GCTCTATTCTGACAGACGATGACCTGCGTGAAGAAGTTGC<br/>CGCCTTGCAACGTCAAGGTCATCGCCGCATTTTAGCCCTTA<br/>CTGGAGAACATCCTAAATACACTTTTCGACAATTTCTTGATG<br/>CAGTCAATGTAATCGCTTCCGTGAAGACAGAGCCTGAAGG<br/>GTCCATTGCGCGTATCAATGTCGAAATCCCACCACTTAGTG<br/>TCTCAGACATGCGCCGCTTAAGAATACAGATTCAAGTTGGC<br/>ACGTTCTGTTCTGTTTCAAGAGACATATCACCGCGACACATT<br/>CAAGGTAATGCATCCGTCAGGTCCAAAGAGCGACTTTGATT<br/>TTCGCGTCTTGACCCAGGACCGCGCCATGCGCGCAGGCTT<br/>GGACGATGTTGGTATTGGTGCTTTATTCGGGTTATATGATT<br/>ACCGTTATGAAGTTTGCGCCATGTTAATGCATAGCGAGCAT<br/>TTGGAACGTGAGTACAACGCAGGACCACATACTATCTCTGT<br/>TCCCCGTATGCGCCCCGCGGACGGAAGCGAGCTTAGTATC<br/>GCGCCTCCTTATCCTGTTAATGATGCTGATTTTATGAAATTA<br/>GTTGCGGTCCTTCGTATTGCTGTACCATACACAGGTATGAT<br/>CCTTAGCACTCGTGAATCACCAGAAATGCGCTCGGCGCTT<br/>CTGAAGTGTGGGATGAGTCAAATGAGTGCCGCTCGCGCA<br/>CTGATGTAGGAGCCTATCACAAGATCACACGTTAAGTACA<br/>GAGCGAATTTGAGTAAGTTAGCTGGGCAGTTTACCTTGCA<br/>GGATGAACGTCCAATAATGAGATTGTTAAGTGGCTGATGG<br/>AGGAGGGCTATGTCCCGTCTTGGTGACAGCTTGTTACCG<br/>TCAAGGTCGTACGGGCGAAGACTTCATGAACATCTGTAAG<br/>GCTGGTGACATTCACGATTTCTGTCATCCCAATAGTCTGCT<br/>TACGCTTCAAGAGTACCTTATGGATTATGCAGATCCAGACC<br/>TTCGTAAGAAAGGCGAGCAAGTAATTGCCCGTGAGATGGG</p> |
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|  |  | ACCGGACGCCTCTGAGCCGTTATCGGCGCAAAGCCGTAAG<br>CGCCTTGAACGCAAGATGAAACAAGTATTGGAGGGGGAGC<br>ACGATGTATATCTTTAACAGGCATCAAATAAACGAAAGG<br>CTCAGTCGAAAGACTGGGCCTTTCTGTTTTATCTGTTGTTG<br>TCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC<br>GGGTGGGCCTTTCTGCGTTTATA |
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Table 2: Strains and plasmids

| Strain          | Relevant characteristics                                                                     | Genotype                                                                                                             |
|-----------------|----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| DH5α            | Wildtype                                                                                     | fluA2::IS2 Δ(mmuP-mhpD)169 ΔphoA8 glnX44<br>φ80d[ΔlacZ58(M15)] rfbD1 gyrA96 luxS11 recA1<br>endA1 rphWT thiE1 hsdR17 |
| DH5α-HPGC       | Wildtype with HPGC                                                                           | DH5α with pHPGC (Cam <sup>R</sup> )                                                                                  |
| ΔpfkA-HPGC      | Deletion of <i>pfkA</i> , with HPGC                                                          | DH5α Δ <i>pfkA</i> :: <i>KanR</i> with pHPGC (Cam <sup>R</sup> )                                                     |
| ΔgpmA-HPGC      | Deletion of <i>gpmA</i> , with HPGC                                                          | DH5α Δ <i>gpmA</i> :: <i>KanR</i> with pHPGC (Cam <sup>R</sup> )                                                     |
| <b>Plasmids</b> |                                                                                              |                                                                                                                      |
| pHPGC           | pSB1C3 derivative with <i>hydA</i> , <i>petF</i> , <i>fnr</i> , <i>hydEF</i> and <i>hydg</i> |                                                                                                                      |

| Strain                    | Relevant characteristics                                                                                | Genotype                                                                                         |
|---------------------------|---------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| ΔgpmM-HPGC                | Deletion of <i>gpmM</i> , with HPGC                                                                     | DH5α Δ <i>gpmM</i> :: <i>KanR</i> , pHPGC (Cam <sup>R</sup> )                                    |
| Δedd-eda-HPGC             | Deletion of <i>edd-eda</i> , with HPGC                                                                  | DH5α Δ <i>edd-eda</i> :: <i>KanR</i> , pHPGC (Cam <sup>R</sup> )                                 |
| Δpps-HPGC                 | Deletion of <i>pps</i> , with HPGC                                                                      | DH5α Δ <i>pps</i> :: <i>KanR</i> , pHPGC (Cam <sup>R</sup> )                                     |
| ΔgpmM-ΔpfkA-HPGC          | Deletion of <i>gpmM</i> , <i>pfk</i> , with HPGC                                                        | DH5α Δ <i>gpmM</i> , Δ <i>pfkA</i> :: <i>KanR</i> , pHPGC (Cam <sup>R</sup> )                    |
| ΔgpmA-ΔpfkA-HPGC          | Deletion of <i>gpmA</i> , <i>pfk</i> , with HPGC                                                        | DH5α Δ <i>gpmA</i> , Δ <i>pfkA</i> :: <i>KanR</i> , pHPGC (Cam <sup>R</sup> )                    |
| ΔgpmM-Δedd-eda-HPGC       | Deletion of <i>gpmM</i> , <i>edd-eda</i> , with HPGC                                                    | DH5α Δ <i>gpmM</i> , Δ <i>edd-eda</i> :: <i>KanR</i> , pHPGC (Cam <sup>R</sup> )                 |
| ΔgpmA-Δedd-eda-HPGC       | Deletion of <i>gpmA</i> , <i>edd-eda</i> , with HPGC                                                    | DH5α Δ <i>gpmA</i> , Δ <i>edd-eda</i> :: <i>KanR</i> , pHPGC (Cam <sup>R</sup> )                 |
| ΔgpmM-Δedd-eda-ΔpfkA-HPGC | Deletion of <i>gpmM</i> , <i>edd-eda</i> , <i>pfk</i> , with HPGC                                       | DH5α Δ <i>gpmM</i> , Δ <i>pfkA</i> , Δ <i>edd-eda</i> :: <i>KanR</i> , pHPGC (Cam <sup>R</sup> ) |
| ΔgpmA-Δedd-eda-ΔpfkA-HPGC | Deletion of <i>gpmA</i> , <i>edd-eda</i> , <i>pfk</i> , with HPGC                                       | DH5α Δ <i>gpmA</i> , Δ <i>pfkA</i> , Δ <i>edd-eda</i> :: <i>KanR</i> , pHPGC (Cam <sup>R</sup> ) |
| Δzwf::zwfZm-HPGC          | <i>E. coli</i> <i>zwf</i> replaced with <i>zwf</i> from <i>Zymomonas mobilis</i> , with HPGC            | DH5α Δ <i>zwf</i> :: <i>zwfZm</i> , pHPGC (Cam <sup>R</sup> )                                    |
| Δgnd::gndCg-HPGC          | <i>E. coli</i> <i>gnd</i> replaced with <i>gnd</i> from <i>Corynebacterium glutamicum</i> , with HPGC   | DH5α Δ <i>gnd</i> :: <i>gndCg</i> , pHPGC (Cam <sup>R</sup> )                                    |
| ΔgapA::gapCCa-HPGC        | <i>E. coli</i> <i>gapA</i> replaced with <i>gapC</i> from <i>Clostridium acetobutylicum</i> , with HPGC | DH5α Δ <i>gapA</i> :: <i>gapCCa</i> , pHPGC (Cam <sup>R</sup> )                                  |
| zwf::osmYp-HPGC           | <i>zwf</i> promoter replaced with <i>osmY</i> promoter, with HPGC                                       | DH5α <i>zwf</i> :: <i>osmYp</i> , pHPGC (Cam <sup>R</sup> )                                      |
| zwfp::gapAp-HPGC          | <i>zwf</i> promoter replaced with <i>gapA</i>                                                           | DH5α <i>zwf</i> :: <i>gapAp</i> , pHPGC (Cam <sup>R</sup> )                                      |

|                         |                                                                      |                                        |
|-------------------------|----------------------------------------------------------------------|----------------------------------------|
| <b>gndp::osmYp-HGPC</b> | promoter, with HPGC<br><i>gnd</i> promoter replaced with <i>osmY</i> | DH5α <i>gnd::osmYp</i> , pHPGC (CamR)  |
| <b>gndp::gapAp-HGPC</b> | promoter, with HPGC<br><i>gnd</i> promoter replaced with <i>gapA</i> | DH5α <i>gnd::gapAp</i> , pHPGC (CamR)  |
| <b>pgip::gapAp</b>      | promoter, with HPGC<br><i>Pgi</i> promoter replaced with <i>gapA</i> | DH5α <i>pgip::gapAp</i> , pHPGC (CamR) |
| <b>pglp::gapAp</b>      | promoter, with HGPC<br><i>Pgl</i> promoter replaced with <i>gapA</i> | DH5α <i>pglp::gapAp</i> , pHPGC (CamR) |

**Table 3: Nucleic acids and corresponding proteins referred to in description, exemplary sequences**

| Gene                                                                      | Protein encoded by gene                                                    | Exemplary nucleic acid sequence accession | Exemplary amino acid sequence accession |
|---------------------------------------------------------------------------|----------------------------------------------------------------------------|-------------------------------------------|-----------------------------------------|
| <i>Heterologous sequences for expression in recombinant microorganism</i> |                                                                            |                                           |                                         |
| <i>hydA</i>                                                               | HydA (Fe-Fe hydrogenase A1 class)                                          | AJ308413, CAC83731.1<br>XP_001693376.1.   | Q9FYU1                                  |
| <i>hydG</i>                                                               | HydG (assembly protein) preferably from <i>Chlamydomonas reinhardtii</i>   | DS496119, EDP05052.1<br>XP_001691319.1.   | Q6PSL4                                  |
| <i>hydEF</i>                                                              | HydEF (assembly protein) preferably from <i>Chlamydomonas reinhardtii</i>  | DS496119, EDP05198.1<br>XP_001691465.1.   | Q6PSL5                                  |
| <i>petF</i>                                                               | Ferredoxin preferably from <i>Chlamydomonas reinhardtii</i>                | DS496124, EDP03827.1<br>XP_001692808.1.   | A8IV40                                  |
| <i>FNR</i>                                                                | Ferredoxin NADP reductase preferably from <i>Chlamydomonas reinhardtii</i> | DS496140, EDP00292.1<br>XP_001697352.1.   | A8J6Y8 and P53991                       |
| <i>gtfA</i>                                                               | Sucrose phosphorylase (e.g., from <i>Leuconostoc mesenteroides</i> )       | NC_008531.1<br>(336262..337740)           | WP_011679246                            |
| <i>cscB</i>                                                               | Sucrose permease (e.g., from another <i>E. coli</i> strain)                | X63740 or X81461                          | P3000                                   |
| <i>cscA</i>                                                               | Sucrose hydrolase (e.g., from another <i>E. coli</i> strain)               | X81461                                    | P40714                                  |
| <i>Targets for reduced or deleted expression</i>                          |                                                                            |                                           |                                         |
| <i>pfkA</i>                                                               | phosphofructokinase                                                        | NC_000913,<br>EcoGene:EG10699             | P0A796                                  |
| <i>pps</i>                                                                | pyruvate kinase                                                            | EcoGene:EG10759                           | P23538                                  |
| <i>gpmA</i>                                                               | glycerate mutase                                                           | EcoGene:EG11699                           | P62707                                  |
| <i>gpmM</i>                                                               | glycerate mutase                                                           | EcoGene:EG12296                           | P37689                                  |
| <i>gapA</i>                                                               | glyceraldehyde-3-phosphate dehydrogenase                                   | EcoGene:EG10367                           | P0A9B2                                  |
| <i>edd</i>                                                                | 6-phosphogluconate dehydratase                                             | EcoGene:EG10257                           | P0ADF6                                  |
| <i>eda</i>                                                                | 2-keto-3-deoxy-6-phosphogluconate aldolase                                 | EcoGene:EG10256                           | P0A955                                  |
| <i>Endogenous targets for increased expression/activity</i>               |                                                                            |                                           |                                         |
| <i>pgm</i>                                                                | phosphoglucomutase                                                         | EcoGene:EG12144                           | P36938                                  |
| <i>zwf</i>                                                                | glucose-6-phosphate dehydrogenase                                          | M55005, NP_416366.1,<br>NC_000913.        | POAC53                                  |
| <i>pgl</i>                                                                | 6-phosphogluconolactonase                                                  | U27192, NP_415288.1<br>NC_000913.3.       | P52697                                  |
| <i>gnd</i>                                                                | 6-glucophosphonate dehydrogenase                                           | K02072, NP_416533.1<br>NC_000913.3.       | P00350                                  |
| <i>yfiB</i>                                                               | NAD kinase                                                                 | EcoGene:EG12192                           | P0A7B3                                  |
| <i>sthA</i>                                                               | soluble pyridine nucleotide transhydrogenase (UdhA)                        | EcoGene:EG11428                           | P27306                                  |
| <i>tktA</i> or <i>tktB</i>                                                | Transketolase                                                              | EcoGene:EG11427<br>EcoGene:EG12100        | P27302 P33570                           |
| <i>talA</i> or <i>talB</i>                                                | transaldolase                                                              | EcoGene:EG11797<br>EcoGene:EG11556        | P0A867 P0A870                           |
| <i>pgm</i>                                                                | phosphoglucomutase                                                         | EcoGene:EG12144                           | P36938                                  |

|      |                  |                 |        |
|------|------------------|-----------------|--------|
| xylA | Xylose isomerase | EcoGene:EG11074 | P00944 |
|------|------------------|-----------------|--------|

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations  
5 constitute various alternative aspects of the invention.