

METHOD FOR SILENCING GENES

Field of the Invention

The present invention relates to improved methods and products for silencing expression of target genes in eukaryotic cells.

5 **Background of the Invention**

The ability to silence the expression of specific genes provides great opportunities in a range of different fields, including research tools, therapeutics and agriculture. In relation to agriculture, improved crops are required so that the global food supply can keep up with continuing population growth. There is a limit to the traits that can be generated using
10 traditional breeding, and this is particularly true for certain crops, so silencing genes using genetic manipulation presents a promising approach.

Silencing of genes in plants and other eukaryotes is routinely achieved using transgenic RNAi. Generation of an RNAi silencing construct requires designing a sequence that encodes a silencing RNA, such as a double stranded hairpin construct. Specificity for the
15 target gene of interest is achieved by using a sequence with complementarity to the target gene of interest. The sequence encoding the silencing RNA is then combined with an independent promoter and a terminator that drive strong expression. In order to reduce exogenous sequence, a promoter derived from the target organism may be used and fused to the sequencing construct (e.g. Miroshnichenko *et al.*, 2020, *PCTOC*, 140:691–705).

20 WO 2019/058255 and WO 2019/058253 describe methods for silencing gene expression in eukaryotic cells that utilise a DNA editing agent and involve modification of an endogenous locus *in situ* and *in vivo*.

Improved methods for silencing expression of target genes are required.

Summary

25 The inventors have developed an improved system for silencing the expression of target genes in eukaryotes. The system of the disclosure is efficient, utilises minimal genetic modifications, and achieves a range of silencing effects. In particular, in the system of the disclosure an endogenous silencing sequence is identified that has a desired expression pattern and that can be modified and redirected to silence the target gene with minimal nucleotide
30 changes. The endogenous silencing sequence, including its promoter and terminator, is used to generate a silencing insertion that includes minimal nucleotide changes in order to provide specificity for the target gene. The silencing insertion is inserted into the genome of the

eukaryotic cell to provide specific silencing of the target gene in desired tissues and conditions.

The silencing insertion is generated from an endogenous silencing sequence, including its promoter and terminator, and so it is highly similar to the endogenous sequence.

5 Therefore, advantageously, the silencing insertion is processed efficiently by the host because the sequences of the insertion and their arrangement are endogenous to the host. Also, the silencing insertion is a cisgenic modification that introduces endogenous sequence in its endogenous arrangement with no juxtaposition of promoters and silencing constructs, which is attractive to consumers and presents greatly reduced potential issues for regulators.

10 The silencing insertion is inserted randomly into the genome, which is highly efficient. Inclusion of a promoter and terminator in the silencing insertion allows it to be expressed effectively when inserted randomly and can avoid the need for targeting sequences. Insertion of a sequence also allows for selection of successful transformants, if the insertion comprises a selectable marker.

15 The silencing insertion of the disclosure benefits from introducing an endogenous sequence in its endogenous arrangement with minimal nucleotide changes and also benefits from specific silencing of target genes in desired tissues and conditions. This is achieved, in part, because the insertion maintains the link between the endogenous sequence encoding a silencing RNA and its cognate promoter and terminator, and also other sequences that control
20 expression such as 5'UTR and 3'UTR sequences, whilst also including modification to the targeting sequence in order to redirect silencing to the target gene of interest.

Accordingly, the disclosure provides a method of reducing the expression of a first target gene comprising inserting a silencing insertion into the genome of a eukaryotic cell,

25 wherein the silencing insertion comprises a promoter or a part thereof, a sequence encoding a silencing RNA that silences the expression of the first target gene, and a terminator or a part thereof,

wherein the silencing insertion has over 95% sequence identity across its length to an endogenous silencing sequence in the eukaryotic cell that comprises a promoter, a sequence encoding a silencing RNA and a terminator, and

30 wherein the silencing RNA encoded by the endogenous silencing sequence is not active or silences the expression of a second target gene that is different from the first target gene.

The disclosure also provides a eukaryotic cell comprising in its genome:

(a) a silencing insertion comprising a promoter or a part thereof, a sequence encoding a silencing RNA that silences the expression of a first target gene, and a terminator or a part thereof,

(b) an endogenous genome sequence that has been separated by the insertion, and

(c) an endogenous silencing sequence comprising a promoter, a sequence encoding a silencing RNA and a terminator,

wherein the silencing insertion and the endogenous silencing sequence have over 95% sequence identity across the length of the insertion, and

wherein the silencing RNA encoded by the endogenous silencing sequence is not active or silences the expression of a second target gene that is different from the first target gene.

The disclosure also provides an isolated silencing construct comprising a silencing insertion that comprises a promoter or a part thereof, a sequence encoding a silencing RNA that silences the expression of a first target gene in a eukaryotic cell, and a terminator or a part thereof,

wherein the silencing insertion has over 95% sequence identity across its length to an endogenous silencing sequence in the eukaryotic cell that comprises a promoter, a sequence encoding a silencing RNA and a terminator, and

wherein the silencing RNA encoded by the endogenous silencing sequence is not active or silences the expression of a second target gene that is different from the first target gene.

In preferred examples, the silencing insertion is inserted by particle bombardment (biolistic bombardment), protoplast transfection, electroporation or nanoparticle-mediated transfection. Such techniques do not require transgenic DNA, and so they introduce only the minimal genetic changes that are provided by the system of the disclosure.

In preferred examples, the eukaryotic cell is a plant cell. Methods of silencing target genes whilst making minimal genetic changes are particularly useful in plants because they allow the generation of crops and plant produce that do not comprise exogenous DNA or endogenous DNA sequences that are not in their endogenous arrangement, which is preferred by consumers and regulators. It is also possible to breed out transgenic DNA in plants.

In preferred examples, the eukaryotic cell is a banana plant cell or a coffee plant cell or a rice plant cell. The methods and products of the disclosure are particularly useful for silencing genes in banana plants, because they are asexual and it is impossible to breed bananas to achieve improved traits or phenotypes. Similarly, the methods and products of the

disclosure are particularly useful for silencing genes in coffee plants, because breeding coffee plants takes 15-25 years and is uneconomical. Similarly, the methods and products of the disclosure are particularly useful for silencing genes in rice plants, because breeding rice plants is particularly time consuming.

5 In alternative examples, the eukaryotic cell is an animal cell, preferably a livestock animal cell. A livestock animal may be any breed or population of animal kept by humans for a useful, commercial purpose. Examples of livestock animals for use in the disclosure include horse, donkey, cattle or any other ruminants, zebu, yak, buffalo, sheep, goat, reindeer, camel, llama, alpaca, pig, rabbit or any other rodent, dog, cat, poultry such as chicken, turkey, goose
10 or duck, fish, crustaceans and molluscs. A livestock animal is not a human. Methods of silencing target genes whilst making minimal genetic changes are particularly useful in livestock animals because they allow the generation of animals and animal produce that do not comprise exogenous DNA or endogenous DNA sequences that are not in their endogenous arrangement, which is preferred by consumers and regulators. It is also possible
15 to breed out transgenic DNA in livestock animals. The disclosure also provides methods of treating or preventing disease in livestock animals comprising administering a silencing insertion or animal cell of the disclosure, and provides silencing insertions and animal cells of the disclosure for use in treating or preventing disease.

In alternative examples, the eukaryotic cell is a human cell. Methods of silencing
20 target genes whilst making minimal genetic changes are particularly useful in humans because they allow therapeutic sequences to be introduced with minimal other changes that might be deleterious. The disclosure also provides methods of treating or preventing disease in humans comprising administering a silencing insertion or human cell of the disclosure, and provides silencing insertions and human cells of the disclosure for use in treating or
25 preventing disease. In examples using human cells, the cells are isolated and handled *in vitro*, or the methods are therapeutic. According to some examples, the eukaryotic cell is a human cell isolated from the human body, wherein the silencing insertion of the disclosure is introduced *in vitro*, optionally wherein the human cell with the silencing insertion of the disclosure is re-introduced into a human. According to some examples, the disclosure
30 provides methods of treating or preventing disease in humans comprising: (1) isolating a cell from a human body; (2) introducing the silencing insertion of the disclosure into the cell *in vitro*, resulting in a modified cell; and (3) administering the modified cell into a human subject, optionally the same human subject from which the non-modified cell originated. According to some examples, in such a method the silencing insertion of the disclosure

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introduces silencing specificity towards a target gene associated with the disease that the method is treating or preventing.

In preferred examples, the silencing insertion comprises 1-40, such as 5-40, 5-30, 5-20, 3-40, 3-30, 3-20, 5-15, 3-10, 10-30, or 10-20, nucleotide additions, deletions and substitutions relative to the endogenous silencing sequence. Such alterations are suitable for redirecting the endogenous silencing sequence to target the target gene of interest, whilst presenting only minimal changes to the endogenous sequence. Therefore, preferably these changes are in the sequence encoding the silencing RNA, and preferably the promoter and terminator, and any other sequences that control expression such as 5'UTR and 3'UTR sequences, in the silencing insertion are identical to the corresponding sequences in the endogenous silencing sequence.

In preferred examples, the silencing insertion is adjacent to a selectable marker and the method of the disclosure comprises inserting the silencing insertion with the adjacent selectable marker into the genome of a eukaryotic cell. Use of a selectable marker allows the selection of successful transformants, which makes their generation much more efficient. Preferably, the selectable marker is highly similar to an endogenous gene of the eukaryotic cell, such as a mutant version of a gene endogenous to the eukaryotic cell, to minimise the exogenous genetic material that is introduced. The selectable marker can be a mutant version of an endogenous gene of the eukaryotic cell, optionally a gene which does not function as a marker until the mutation (preferably a dominant mutation) is introduced and enables it to function as a selectable marker. A selectable marker which is a mutant version of an endogenous gene is preferably used with the promoter and terminator of the endogenous gene. Preferably the mutant version of the marker includes a mutation which naturally exists in another eukaryotic cell of the same genus or the same species. A non-limiting example of a selectable marker is a mutated version of an ALS gene that confers chlorsulfuron herbicide resistance. The system of the disclosure allows multiple silencing insertions to be inserted together, providing silencing of multiple genes and "stacking" of traits. This is particularly advantageous for use with asexual crops, such as banana, which cannot be crossed to combine traits.

The disclosure also provides plants, plant parts, seeds, fruit and plant products obtained from the methods of the disclosure and comprising cells with silencing insertions of the disclosure. Such products will exhibit the improved traits provided by specific gene silencing, whilst being cisgenic and not containing any exogenous DNA or endogenous DNA in a non-endogenous arrangement.

The disclosure also provides animals and animal products obtained from the methods of the disclosure and comprising cells with silencing insertions of the disclosure. Such animals and products will exhibit the improved traits provided by specific gene silencing, whilst being cisgenic and not containing any transgenic DNA or endogenous DNA in a non-endogenous arrangement.

In one aspect, the present disclosure relates to a method of reducing the expression of a first target gene comprising inserting a silencing insertion into the genome of a eukaryotic cell, (a) wherein the silencing insertion comprises a promoter or a part thereof, a sequence encoding a silencing RNA that silences the expression of the first target gene, and a terminator or a part thereof, (b) wherein the silencing insertion has over 95% sequence identity across its length to an endogenous silencing sequence in the eukaryotic cell that comprises a promoter, a sequence encoding a silencing RNA and a terminator, and (c) wherein the silencing RNA encoded by the endogenous silencing sequence is not active or silences the expression of a second target gene that is different from the first target gene.

In another aspect, the present disclosure relates to a eukaryotic cell comprising in its genome: (a) a silencing insertion comprising a promoter or a part thereof, a sequence encoding a silencing RNA that silences the expression of a first target gene, and a terminator or a part thereof, (b) an endogenous genome sequence that has been separated by the insertion, and (c) an endogenous silencing sequence comprising a promoter, a sequence encoding a silencing RNA and a terminator, wherein the silencing insertion and the endogenous silencing sequence have over 95% sequence identity across the length of the insertion, and wherein the silencing RNA encoded by the endogenous silencing sequence is not active or silences the expression of a second target gene that is different from the first target gene.

In another aspect, the present disclosure relates to an isolated silencing construct comprising a silencing insertion that comprises a promoter or a part thereof, a sequence encoding a silencing RNA that silences the expression of a first target gene in a eukaryotic cell, and a terminator or a part thereof, (a) wherein the silencing insertion has over 95% sequence identity across its length to an endogenous silencing sequence in the eukaryotic cell that comprises a promoter, a sequence encoding a silencing RNA and a terminator, and (b) wherein the silencing RNA encoded by the endogenous silencing sequence is not active or silences the expression of a second target gene that is different from the first target gene.

In another aspect, the present disclosure relates to a plant or a part of plant comprising a eukaryotic cell as disclosed herein. In another aspect, the present disclosure relates to a plant produced by the method as disclosed herein. In another aspect, the present disclosure relates to a seed of the plant as disclosed herein.

In another aspect, the present disclosure relates to an animal or an animal product comprising a eukaryotic cell as disclosed herein. In yet another aspect, the present disclosure relates to an animal produced by the method as disclosed herein.

In another aspect, the present disclosure relates to a human or livestock animal cell as disclosed herein or a silencing insertion as disclosed herein for use in therapy.

Brief Description of the Figures

Figure 1 - Promoter identification pipeline: Process Overview.

Figure 2 - Terminator identification pipeline: Process Overview.

Figure 3. WT (A) and GEiGSTM-Insertion (B) sequences and stem-loop

5 **structures (C) for a miRNA example (ath-miR173).** Promoter, ncRNA (non-coding RNA) and terminator regions are indicated in underlined, italics and italics + underlined, respectively. Changes from the WT sequence are indicated in bold. 5' to 3' orientation is indicated.

Figure 4. WT (A) and GEiGSTM-Insertion (B) sequences for a tasiRNA example

10 **(ath-Tas3a).** Promoter, ncRNA (non-coding RNA) and terminator regions are indicated in underlined, italics and italics + underlined, respectively. Changes from the WT sequence are indicated in bold. 5' to 3' orientation is indicated.

Figure 5. Overlapping PCR strategy to introduce GEiGSTM-Insertion

15 **modifications.** Two separate PCR reactions with primers A+B (PCR 1) and C+D (PCR 2), respectively, are carried out using genomic DNA as template. Primers B and C contain the modified GEiGSTM sequence at their 5' (dotted lines). A third PCR reaction (PCR 3) is carried out annealing the modified region of products from PCR 1 and 2 and using primers A+D allowing to amplify the whole GEiGSTM-Insertion cassette.

Figure 6. GEiGSTM-Insertion with selection diagrams. The GEiGSTM-Insertion

20 cassette can be designed in such a way that the GEiGSTM ncRNA and mutated P197S expression cassettes face the opposite directions (A) or the same direction (B). Orientation A is preferred to prevent read-through transcription. ALS cassette is natively expressed from the complementary strand. ALS endogenous promoter (including 5'UTR) and terminator (including 3'UTR) regions are shown. ncRNA endogenous promoter and terminator are
25 shown. GEiGSTM ncRNA contains minimum mutations, which do not affect its secondary structure, to redirect the silencing activity and specificity of the sRNA from that of the silencing gene on which the insertion is based. ALS P197S: single point ALS mutant in

CLAIMS

1. A method of reducing the expression of a first target gene comprising inserting a silencing insertion into the genome of a eukaryotic cell,
 - 5 wherein the silencing insertion comprises a promoter or a part thereof, a sequence encoding a silencing RNA that silences the expression of the first target gene, and a terminator or a part thereof,
 - wherein the silencing insertion has over 95% sequence identity across its length to an endogenous silencing sequence in the eukaryotic cell that comprises a promoter, a
 - 10 sequence encoding a silencing RNA and a terminator, and
 - wherein the silencing RNA encoded by the endogenous silencing sequence is not active or silences the expression of a second target gene that is different from the first target gene.
- 15 2. The method of claim 1, wherein the silencing insertion is inserted by particle bombardment, protoplast transfection, electroporation, nanoparticle-mediated transfection or Agrobacterium-mediated transformation.
3. A eukaryotic cell comprising in its genome:
 - 20 (a) a silencing insertion comprising a promoter or a part thereof, a sequence encoding a silencing RNA that silences the expression of a first target gene, and a terminator or a part thereof,
 - (b) an endogenous genome sequence that has been separated by the insertion, and
 - 25 (c) an endogenous silencing sequence comprising a promoter, a sequence encoding a silencing RNA and a terminator,
 - wherein the silencing insertion and the endogenous silencing sequence have over 95% sequence identity across the length of the insertion, and
 - wherein the silencing RNA encoded by the endogenous silencing sequence is not
 - 30 active or silences the expression of a second target gene that is different from the first target gene.
4. The eukaryotic cell of claim 3, wherein an endogenous genome sequence has been deleted from between the endogenous genome sequence that has been separated.

5. An isolated silencing construct comprising a silencing insertion that comprises a promoter or a part thereof, a sequence encoding a silencing RNA that silences the expression of a first target gene in a eukaryotic cell, and a terminator or a part thereof, wherein the silencing insertion has over 95% sequence identity across its length to an endogenous silencing sequence in the eukaryotic cell that comprises a promoter, a sequence encoding a silencing RNA and a terminator, and wherein the silencing RNA encoded by the endogenous silencing sequence is not active or silences the expression of a second target gene that is different from the first target gene.
6. The method, eukaryotic cell or isolated silencing construct of any preceding claim, wherein:
- (a) said eukaryotic cell is:
- (i) a plant cell, such as a banana plant cell, a coffee plant cell or a rice plant cell, optionally wherein the eukaryotic cell is a plant protoplast; or
- (ii) a human cell or an animal cell, preferably a livestock animal cell, such as a horse, donkey, cattle or any other ruminant, zebu, yak, buffalo, sheep, goat, reindeer, camel, llama, alpaca, pig, rabbit or any other rodent, dog, cat, poultry such as chicken, turkey, goose or duck, fish, crustacean or mollusc cell; and/or
- (b) the silencing RNA encoded by the silencing insertion is a small interfering RNA (siRNA), a microRNA (miRNA), a piwi-interacting RNA (piRNA), a phased small interfering RNA (phasiRNA), a trans-acting siRNA (tasiRNA), a short-hairpin RNA (shRNA), inverted repeat RNA forming double stranded RNA, a small nuclear RNA (snRNA or U-RNA), a small nucleolar RNA (snoRNA), a Small Cajal body RNA (scaRNA), a transfer RNA (tRNA), a ribosomal RNA (rRNA), a repeat-derived RNA, an autonomous or non-autonomous transposable or retro-transposable element-derived RNA, an autonomous or non-autonomous transposable or retro-transposable element RNA, a transfer RNA fragment (tRF), or a long non-coding RNA (lncRNA); and/or
- (c) the silencing insertion comprises 1-40, such as 5-40, 5-30, 5-20, 3-40, 3-30, 3-20, 5-15, 3-10, 10-30, or 10-20 nucleotide substitutions, additions and/or deletions relative to the endogenous silencing sequence, optionally wherein:
- (i) substitutions are not contiguous; and/or

- (ii) the nucleotide substitutions are all present in:
- (A) the sequence encoding the silencing RNA; or
 - (B) the transcribed sequence; and/or
- (d) the silencing RNA encoded by the silencing insertion and the silencing RNA encoded by the endogenous silencing sequence form the same secondary structure; and/or
- (e) the promoter and terminator in the silencing insertion are identical or partly identical to the promoter and terminator in the endogenous silencing sequence; and/or
- (f) the silencing insertion and the endogenous silencing sequence have over 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity across the length of the insertion; and/or
- (g) the silencing insertion is inserted with an adjacent selectable marker, or the eukaryotic cell or isolated silencing construct of any preceding claim, wherein the silencing insertion is adjacent to a selectable marker, optionally wherein the selectable marker is endogenous to the eukaryotic cell, such as wherein the selectable marker is a mutant version of a marker endogenous to the eukaryotic cell or wherein the selectable marker is a mutant version of a gene endogenous to the eukaryotic cell which does not function as a selectable marker; optionally wherein:
- (i) the mutation in the mutant version is the same as a naturally occurring mutation in another eukaryotic cell of the same genus, optionally of the same species; and/or
 - (ii) the selectable marker is a mutated version of an ALS gene that confers herbicide resistance, such as chlorsulfuron herbicide resistance.
7. The method of any of claims 1, 2 and 6, wherein:
- (a) the silencing insertion is inserted into the genome of the eukaryotic cell in a construct that additionally comprises sequence encoding an RNA-guided DNA endonuclease and sequence encoding a guide RNA targeted to the endogenous silencing sequence, optionally wherein:
- (i) the RNA-guided DNA endonuclease and guide RNA introduce a modification into the endogenous silencing sequence, such as an insertion, deletion or insertion and deletion, which reduces or abolishes expression or activity of the endogenous silencing sequence; or

(ii) the RNA-guided DNA endonuclease and guide RNA mediate substitution of the endogenous silencing sequence with the silencing insertion in the eukaryotic cell, optionally wherein the eukaryotic cell is a plant cell or a livestock animal cell and wherein the method additionally comprises generating a plant or a livestock animal and breeding out the construct that was inserted into the genome, thereby generating a plant or livestock animal that comprises the silencing insertion substituted at the location of the endogenous silencing sequence and that does not comprise the construct that was inserted into the genome of the eukaryotic cell; or

(iii) cell or a livestock animal cell, and wherein the method additionally comprises generating a plant or a livestock animal and breeding the plant or livestock animal for at least one generation until the RNA-guided DNA endonuclease and guide RNA mediate substitution of the endogenous silencing sequence with the silencing insertion, optionally wherein the eukaryotic cell is a plant cell or a livestock animal cell and wherein the method additionally comprises generating a plant or a livestock animal and breeding out the construct that was inserted into the genome, thereby generating a plant or livestock animal that comprises the silencing insertion substituted at the location of the endogenous silencing sequence and that does not comprise the construct that was inserted into the genome of the eukaryotic cell; or

(b) the eukaryotic cell comprises in its genome a construct that comprises sequence encoding an RNA-guided DNA endonuclease, sequence encoding a guide RNA targeted to the endogenous silencing sequence, and a silencing insertion as defined in any preceding claim, optionally wherein the endogenous silencing sequence comprises a modification introduced by the RNA-guided DNA endonuclease and guide RNA, such as an insertion, deletion or insertion and deletion, which reduces or abolishes expression or activity of the endogenous silencing sequence.

8. The silencing construct of claims 5 or 6, additionally comprising sequence encoding an RNA-guided DNA endonuclease and sequence encoding a guide RNA targeted to the endogenous silencing sequence.

9. The method of any of claims 1, 6 and 7(a) wherein the silencing insertion is introduced into the eukaryotic cell in a T-DNA cassette using Agrobacterium-mediated transformation, or the eukaryotic cell of any of claims 3, 4, 6 and 7(b) wherein the silencing insertion is within an inserted T-DNA cassette sequence, or the isolated silencing construct of any of claims 5, 6 and 8, which is a T-DNA cassette, optionally the T-DNA cassette comprises a selectable marker and/or sequence encoding an RNA-guided DNA endonuclease and sequence encoding a guide RNA targeted to the endogenous silencing sequence.
10. The method, eukaryotic cell or isolated silencing construct of any preceding claim, wherein:
- (a) the method comprises inserting multiple silencing insertions, wherein the eukaryotic cell comprises multiple silencing insertions, or wherein the isolated silencing construct comprises multiple silencing insertions, optionally, wherein the silencing RNA encoded by each silencing insertion targets a different gene; and/or
 - (b) the target gene is endogenous to the eukaryotic cell, optionally wherein the silencing of the target gene provides an improved trait or phenotype.
11. The method, eukaryotic cell or isolated silencing construct of any of claims 1-10(a), wherein the target gene is exogenous to the eukaryotic cell, such as a pest gene, optionally wherein:
- (a) silencing of the target gene provides pest resistance, disease tolerance or resistance; or
 - (b) the target gene is exogenous to the eukaryotic cell but is present within the eukaryotic cell; or
 - (c) the target gene is exogenous to the eukaryotic cell and is not present within the eukaryotic cell.
12. The method, eukaryotic cell or isolated silencing construct of any preceding claim, wherein:
- (a) the first target gene is a homeolog of the second target gene; and/or

(b) the promoter, terminator and/or other regulatory sequence in the silencing insertion is tissue-specific, biotic-condition specific, abiotic-condition specific, inducible, developmentally regulated or constitutive.

- 5 13. A plant or a part of plant comprising a eukaryotic cell of any one of claims 3, 4 or 6-12.
14. The method of any of claims 1, 2, 6-12 wherein the eukaryotic cell is a plant cell, and wherein the method further comprises growing a plant from the cell and propagating
10 the plant, optionally wherein the method further comprises harvesting fruit or other plant part or product.
15. A plant produced by the method of claim 14.
- 15 16. A seed of the plant of claim 13 or claim 14.
17. An animal or an animal product comprising a eukaryotic cell of any one of claims 3, 4 or 6-12.
- 20 18. The method of any of claims 1, 2, 6-12, wherein the eukaryotic cell is an animal cell, and wherein the method further comprises developing an animal from the cell and breeding the animal, optionally wherein the method further comprises producing an animal
product.
- 25 19. An animal produced by the method of claim 18.
20. A human or livestock animal cell of any of claims 3, 4 or 6-12 or a silencing insertion of any of claims 5-12 for use in therapy.