

pctgb2016051438-seq1
SEQUENCE LISTING

<110> Lucite International UK Limited

<120> Process for the Biological Production of Methacrylic Acid

<130> P31059W0

<150> GB1508582.2

<151> 2015-05-19

<150> GB1517545.8

<151> 2015-10-05

<160> 24

<170> PatentIn version 3.5

<210> 1

<211> 471

<212> DNA

<213> Artificial Sequence

<220>

<223> optimised 4-hydroxybenzoyl-coA thioesterase (4HBT) from
Arthrobacter sp. strain SU

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<223> HHT.F forward primer for cloning of 4-HBT

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36

<210> 3

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> HHT.R reverse primer for cloning of 4HBT

<400> 3

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24

<210> 4

<211> 469

<212> DNA

<213> Artificial Sequence

<220>

<223> Product of the PCR performed to modify the 4HBT gene for sub cloning into the pET20b(+) plasmid to form the pET20b(+)::CtHis 4HBT plasmid.

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120

cgacgaaatg accccggaac gtgcgaccgc gtctgttgaa gttaccgaca ccctgcgtca

180

gcgttgggggt ctggttcacg gtggtgctta ctgcgcactg gcggagatgc tggcgaccga

240

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300

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360

gggttctacc acctggttct gggacgtttc cctgcgcgat gatgcgggtc gcctgtgtgc

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469

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<210> 5
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<220>
 <223> carboxy-terminal hexahistidine tagged 4HBT in the
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> optimised acyl-coA oxidase 4 (ACX4) from Arabidopsis thaliana

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<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> OE.A.F forward primer for overlap extension polymerase chain reaction A

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27

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<210> 8
 <211> 39
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> OE.A.R reverse primer for overlap extension polymerisation chain reaction B

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 <210> 9
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 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> OE.B.F forward primer for overlap extension polymerase chain reaction B

 <400> 9
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 <210> 10
 <211> 40
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> OE.B.R reverse primer for overlap polymerase chain reaction B

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 <210> 11
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 <212> DNA
 <213> Artificial Sequence

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 <223> product of the overlap polymerase chain reaction to concatenate 4HBT and ACX4 into one polynucleotide

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tcgag	1805

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 <212> DNA
 <213> Artificial Sequence

<220>
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 chlororaphis B23

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<210> 13
 <211> 3447
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> The sequence between, and inclusive of, the NdeI and XhoI restriction sites in pET20b(+):4HBT ACX4 AcsA.

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<210> 14
 <211> 4996
 <212> DNA
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<220>
 <223> ppBCKD polynucleotide containing the four genes encoding the subunits of the Pseudomonas putida KT2440 branched chain keto acid dehydrogenase delivered in the pBSK::ppBCKD plasmid

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