<110> CJ CheilJedang Corporation

<120> GLUTAMATE-CYSTEINE LIGASE VARIANT AND METHOD FOR PRODUCING GLUTATHIONE USING SAME

<130> OPA21295

<150> KR10-2021-0000361
<151> 2021-01-04

<160> 15

<170> KoPatentIn 3.0

<210> 1
<211> 678
<212> PRT
<213> Saccharomyces cerevisiae

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Thr Tyr Asn Glu His Ile Arg Asp Glu Gly Ile Glu Gln Leu Leu Tyr
             20   25   30

Ile Phe Gln Ala Ala Gly Lys Arg Asp Asn Asp Pro Leu Phe Trp Gly
         35   40   45

Asp Glu Leu Glu Tyr Met Val Val Asp Phe Asp Asp Lys Glu Arg Asn
     50   55   60

Ser Met Leu Asp Val Cys His Asp Lys Ile Leu Thr Glu Leu Asn Met
 65   70   75   80

Glu Asp Ser Ser Leu Cys Glu Ala Asn Asp Val Ser Phe His Pro Glu
                 85   90   95

Tyr Gly Arg Tyr Met Leu Glu Ala Thr Pro Ala Ser Pro Tyr Leu Asn
            100   105   110

Tyr Val Gly Ser Tyr Val Glu Val Asn Met Gln Lys Arg Arg Ala Ile
        115   120   125

Ala Glu Tyr Lys Leu Ser Glu Tyr Ala Arg Gln Asp Ser Lys Asn Asn
    130   135   140

Leu His Val Gly Ser Arg Ser Val Pro Leu Thr Leu Thr Val Phe Pro
145   150   155   160

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Arg Met Gly Cys Pro Asp Phe Ile Asn Ile Lys Asp Pro Trp Asn His
                165                 170                 175

Lys Asn Ala Ala Ser Arg Ser Leu Phe Leu Pro Asp Glu Val Ile Asn
            180                 185                 190

Arg His Val Arg Phe Pro Asn Leu Thr Ala Ser Ile Arg Thr Arg Arg
        195                 200                 205

Gly Glu Lys Val Cys Met Asn Val Pro Met Tyr Lys Asp Ile Ala Thr
    210                 215                 220

Pro Glu Thr Asp Asp Ser Ile Tyr Asp Arg Asp Trp Phe Leu Pro Glu
225                 230                 235                 240

Asp Lys Glu Ala Lys Leu Ala Ser Lys Pro Gly Phe Ile Tyr Met Asp
                245                 250                 255

Ser Met Gly Phe Gly Met Gly Cys Ser Cys Leu Gln Val Thr Phe Gln
            260                 265                 270

Ala Pro Asn Ile Asn Lys Ala Arg Tyr Leu Tyr Asp Ala Leu Val Asn
        275                 280                 285

Phe Ala Pro Ile Met Leu Ala Phe Ser Ala Ala Ala Pro Ala Phe Lys
    290                 295                 300

Gly Trp Leu Ala Asp Gln Asp Val Arg Trp Asn Val Ile Ser Gly Ala
305                 310                 315                 320

Val Asp Asp Arg Thr Pro Lys Glu Arg Gly Val Ala Pro Leu Leu Pro
                325                 330                 335

Lys Tyr Asn Lys Asn Gly Phe Gly Gly Ile Ala Lys Asp Val Gln Asp
            340                 345                 350

Lys Val Leu Glu Ile Pro Lys Ser Arg Tyr Ser Ser Val Asp Leu Phe
        355                 360                 365

Leu Gly Gly Ser Lys Phe Phe Asn Arg Thr Tyr Asn Asp Thr Asn Val
    370                 375                 380

Pro Ile Asn Glu Lys Val Leu Gly Arg Leu Leu Glu Asn Asp Lys Ala
385                 390                 395                 400

Pro Leu Asp Tyr Asp Leu Ala Lys His Phe Ala His Leu Tyr Ile Arg
                405                 410                 415

Asp Pro Val Ser Thr Phe Glu Glu Leu Leu Asn Gln Asp Asn Lys Thr
            420                 425                 430
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Ser Ser Asn His Phe Glu Asn Ile Gln Ser Thr Asn Trp Gln Thr Leu
        435                 440                 445

Arg Phe Lys Pro Pro Thr Gln Gln Ala Thr Pro Asp Lys Lys Asp Ser
    450                 455                 460

Pro Gly Trp Arg Val Glu Phe Arg Pro Phe Glu Val Gln Leu Leu Asp
465                 470                 475                 480

Phe Glu Asn Ala Ala Tyr Ser Val Leu Ile Tyr Leu Ile Val Asp Ser
                485                 490                 495

Ile Leu Thr Phe Ser Asp Asn Ile Asn Ala Tyr Ile His Met Ser Lys
            500                 505                 510

Val Trp Glu Asn Met Lys Ile Ala His His Arg Asp Ala Ile Leu Phe
        515                 520                 525

Glu Lys Phe His Trp Lys Lys Ser Phe Arg Asn Asp Thr Asp Val Glu
    530                 535                 540

Thr Glu Asp Tyr Ser Ile Ser Glu Ile Phe His Asn Pro Glu Asn Gly
545                 550                 555                 560

Ile Phe Pro Gln Phe Val Thr Pro Ile Leu Cys Gln Lys Gly Phe Val
                565                 570                 575

Thr Lys Asp Trp Lys Glu Leu Lys His Ser Ser Lys His Glu Arg Leu
            580                 585                 590

Tyr Tyr Tyr Leu Lys Leu Ile Ser Asp Arg Ala Ser Gly Glu Leu Pro
        595                 600                 605

Thr Thr Ala Lys Phe Phe Arg Asn Phe Val Leu Gln His Pro Asp Tyr
    610                 615                 620

Lys His Asp Ser Lys Ile Ser Lys Ser Ile Asn Tyr Asp Leu Leu Ser
625                 630                 635                 640

Thr Cys Asp Arg Leu Thr His Leu Asp Asp Ser Lys Gly Glu Leu Thr
                645                 650                 655

Ser Phe Leu Gly Ala Glu Ile Ala Glu Tyr Val Lys Lys Asn Lys Pro
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Ser Ile Glu Ser Lys Cys
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gacaatgacc ctctttttg gggagacgag cttgagtaca tggttgtaga ttttgatgat 180

aaggagagaa attctatgct cgacgtttgc catgacaaga tactcactga gcttaatatg 240

gaggattcgt ccctttgtga ggctaacgat gtgagttttc accctgagta tggccggtat 300

atgttagagg caacaccagc ttctccatat ttgaattacg tgggtagtta cgttgaggtt 360

aacatgcaaa aaagacgtgc cattgcagaa tataagctat ctgaatatgc gagacaagat 420

agtaaaaata acttgcatgt gggctccagg tctgtccctt tgacgctgac tgtcttcccg 480

aggatgggat gccccgactt tattaacatt aaggatccgt ggaatcataa aaatgccgct 540

tccaggtctc tgtttttacc cgatgaagtc attaacagac atgtcaggtt tcctaacttg 600

acagcatcca tcaggaccag gcgtggtgaa aaagtttgca tgaatgttcc catgtataaa 660

gatatagcta ctccagaaac ggatgactcc atctacgatc gagattggtt tttaccagaa 720

gacaaagagg cgaaactggc ttccaaaccg ggtttcattt atatggattc catgggtttt 780

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cctgctttta aaggttggct agccgaccaa gatgttcgtt ggaatgtgat atctggtgcg 960

gtggacgacc gtactccgaa ggaaagaggt gttgcgccat tactacccaa atacaacaag 1020

aacggatttg gaggcattgc caaagacgta caagataaag tccttgaaat accaaagtca 1080

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acattcgaag aactgttgaa tcaggacaac aaaacgtctt caaatcactt tgaaaacatc 1320

caaagtacaa attggcagac attacgtttt aaacccccca cacaacaagc aaccccggac 1380

aaaaaggatt ctcctggttg gagagtggaa ttcagaccat ttgaagtgca actattagat 1440

tttgagaacg ctgcgtattc cgtgctcata tacttgattg tcgatagcat tttgaccttt 1500

tccgataata ttaacgcata tattcatatg tccaaagtat gggaaaatat gaagatagcc 1560

catcacagag atgctatcct atttgaaaaa tttcattgga aaaaatcatt tcgcaacgac 1620

accgatgtgg aaactgaaga ttattctata agcgagattt tccataatcc agagaatggt 1680

atatttcctc aatttgttac gccaatccta tgccaaaaag ggtttgtaac caaagattgg 1740

aaagaattaa agcattcttc caaacacgag agactatact attatttaaa gctaatttct 1800

gatagagcaa gcggtgaatt gccaacaaca gcaaaattct ttagaaattt tgtactacaa 1860

catccagatt acaaacatga ttcaaaaatt tcaaagtcga tcaattatga tttgctttct 1920

acgtgtgata gacttaccca tttagacgat tcaaaaggtg aattgacatc ctttttagga 1980

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Thr Tyr Asn Glu His Ile Arg Asp Glu Gly Ile Glu Gln Leu Leu Tyr
20 25 30

Ile Phe Gln Ala Ala Gly Lys Arg Asp Asn Asp Pro Leu Phe Trp Gly
35 40 45

Asp Glu Leu Glu Tyr Met Val Val Asp Phe Asp Asp Lys Glu Arg Asn
50 55 60

Ser Met Leu Asp Val Cys His Asp Lys Ile Leu Thr Glu Leu Asn Met
65 70 75 80

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Glu Asp Ser Ser Leu Cys Glu Ala Asn Asp Val Ser Phe His Pro Glu
                 85                  90                  95

Tyr Gly Arg Tyr Met Leu Glu Ala Thr Pro Ala Ser Pro Tyr Leu Asn
            100                 105                 110

Tyr Val Gly Ser Tyr Val Glu Val Asn Met Gln Lys Arg Arg Ala Ile
        115                 120                 125

Ala Glu Tyr Lys Leu Ser Glu Tyr Ala Arg Gln Asp Ser Lys Asn Asn
    130                 135                 140

Leu His Val Gly Ser Arg Ser Val Pro Leu Thr Leu Thr Val Phe Pro
145                 150                 155                 160

Arg Met Gly Cys Pro Asp Phe Ile Asn Ile Lys Asp Pro Trp Asn His
                165                 170                 175

Lys Asn Ala Ala Ser Arg Ser Leu Phe Leu Pro Asp Glu Val Ile Asn
            180                 185                 190

Arg His Val Arg Phe Pro Asn Leu Thr Ala Ser Ile Arg Thr Arg Arg
        195                 200                 205

Gly Glu Lys Val Cys Met Asn Val Pro Met Tyr Lys Asp Ile Ala Thr
    210                 215                 220

Pro Glu Thr Asp Asp Ser Ile Tyr Asp Arg Asp Trp Phe Leu Pro Glu
225                 230                 235                 240

Asp Lys Glu Ala Lys Leu Ala Ser Lys Pro Gly Phe Ile Tyr Met Asp
                245                 250                 255

Ser Met Gly Phe Gly Met Gly Cys Ser Cys Leu Gln Val Thr Phe Gln
            260                 265                 270

Ala Pro Asn Ile Asn Lys Ala Arg Tyr Leu Tyr Asp Ala Leu Val Asn
        275                 280                 285

Phe Ala Pro Ile Met Leu Ala Phe Ser Ala Ala Ala Pro Ala Phe Lys
    290                 295                 300

Gly Trp Leu Ala Asp Gln Asp Val Arg Trp Asn Val Ile Ser Gly Ala
305                 310                 315                 320

Val Asp Asp Arg Thr Pro Lys Glu Arg Gly Val Ala Pro Leu Leu Pro
                325                 330                 335

Lys Tyr Asn Lys Asn Gly Phe Gly Gly Ile Ala Lys Asp Val Gln Asp
            340                 345                 350
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Lys Val Leu Glu Ile Pro Lys Ser Arg Tyr Ser Ser Val Asp Leu Phe
        355                 360                 365

Leu Gly Gly Ser Lys Phe Phe Asn Arg Thr Tyr Asn Asp Thr Asn Val
    370                 375                 380

Pro Ile Asn Glu Lys Val Leu Gly Arg Leu Leu Glu Asn Asp Lys Ala
385                 390                 395                 400

Pro Leu Asp Tyr Asp Leu Ala Lys His Phe Ala His Leu Tyr Ile Arg
                405                 410                 415

Asp Pro Val Ser Thr Phe Glu Glu Leu Leu Asn Gln Asp Asn Lys Thr
            420                 425                 430

Ser Ser Asn His Phe Glu Asn Ile Gln Ser Thr Asn Trp Gln Thr Leu
        435                 440                 445

Arg Phe Lys Pro Pro Thr Gln Gln Ala Thr Pro Asp Lys Lys Asp Ser
    450                 455                 460

Pro Gly Trp Arg Val Glu Phe Arg Pro Phe Glu Val Gln Leu Leu Asp
465                 470                 475                 480

Phe Glu Asn Ala Ala Tyr Ser Val Leu Ile Tyr Leu Ile Val Asp Ser
                485                 490                 495

Ile Leu Thr Phe Ser Asp Asn Ile Asn Ala Tyr Ile His Met Ser Lys
            500                 505                 510

Val Trp Glu Asn Met Lys Ile Ala His His Arg Asp Ala Ile Leu Phe
        515                 520                 525

Glu Lys Phe His Trp Lys Lys Ser Phe Arg Asn Asp Thr Asp Val Glu
    530                 535                 540

Thr Glu Asp Tyr Ser Ile Ser Glu Ile Phe His Asn Pro Glu Asn Gly
545                 550                 555                 560

Ile Phe Pro Gln Phe Val Thr Pro Ile Leu Cys Gln Lys Gly Phe Val
                565                 570                 575

Thr Lys Asp Trp Lys Glu Leu Lys His Ser Ser Lys His Glu Arg Leu
            580                 585                 590

Tyr Tyr Tyr Leu Lys Leu Ile Ser Asp Arg Ala Ser Gly Glu Leu Pro
        595                 600                 605

Thr Thr Ala Lys Phe Phe Arg Asn Phe Val Leu Gln His Pro Asp Tyr
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    610                 615                 620

Lys His Asp Ser Lys Ile Ser Lys Ser Ile Asn Tyr Asp Leu Leu Ser
625                 630                 635                 640

Thr Cys Asp Arg Leu Thr His Leu Asp Asp Ser Lys Met Glu Leu Thr
                645                 650                 655

Ser Phe Leu Gly Ala Glu Ile Ala Glu Tyr Val Lys Lys Asn Lys Pro
            660                 665                 670

Ser Ile Glu Ser Lys Cys
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<210> 5
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<212> DNA
<213> Artificial Sequence

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<223> R_GSH1_G653M

<400> 5
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<400> 6
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<210> 7
<211> 35
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<223> R_XhoI_GSH1

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<211> 33
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<223> F_SpeI_GSH1_DW

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<210> 9
<211> 35
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<223> R_NcoI_GSH1_DW

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<210> 10
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> R_AL killer

<400> 10
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<210> 11
<211> 24
<212> DNA
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<220>
<223> F_BR killer

<400> 11
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<210> 12
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<223> S. cerevisiae CJ-5 promoter

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cagcatggcg cgcacgtgac tacaactgtg gctggaaacc ttttcgtcct ccccggtttt 180

tcagtgagcc gactctacta caatgctttt tcatttttca ctcagaaaaa cctgcaattt 240

tccaaattag tcatgctctg tgcctccctt gacaaaggac atcttccctg tttataaacg 300

gcggcttacc aaaagttgaa gcttgttctt gcttcttatg agtggagcaa tcgattatat 360

tgaatcgttg tgctggagta gttggatctt tccacgtggt ctcgagtcac ttgtagaagc 420

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<211> 678

<212> PRT
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             20   25   30

Ile Phe Gln Ala Ala Gly Lys Arg Asp Asn Asp Pro Leu Phe Trp Gly
         35   40   45

Asp Glu Leu Glu Tyr Met Val Val Asp Phe Asp Asp Lys Glu Arg Asn
     50   55   60

Ser Met Leu Asp Val Cys His Asp Lys Ile Leu Thr Glu Leu Asn Met
 65   70   75   80

Glu Asp Ser Ser Leu Arg Glu Ala Asn Asp Val Ser Phe His Pro Glu
                 85   90   95

Tyr Gly Arg Tyr Met Leu Glu Ala Thr Pro Ala Ser Pro Tyr Leu Asn
            100   105   110

Tyr Val Gly Ser Tyr Val Glu Val Asn Met Gln Lys Arg Arg Ala Ile
        115   120   125

Ala Glu Tyr Lys Leu Ser Glu Tyr Ala Arg Gln Asp Ser Lys Asn Asn
    130   135   140

Leu His Val Gly Ser Arg Ser Val Pro Leu Thr Leu Thr Val Phe Pro
145   150   155   160

Arg Met Gly Cys Pro Asp Phe Ile Asn Ile Lys Asp Pro Trp Asn His
                165   170   175

Lys Asn Ala Ala Ser Arg Ser Leu Phe Leu Pro Asp Glu Val Ile Asn
            180   185   190

Arg His Val Arg Phe Pro Asn Leu Thr Ala Ser Ile Arg Thr Arg Arg
        195   200   205

Gly Glu Lys Val Cys Met Asn Val Pro Met Tyr Lys Asp Ile Ala Thr
    210   215   220

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Pro Glu Thr Asp Asp Ser Ile Tyr Asp Arg Asp Trp Phe Leu Pro Glu
225                 230                 235                 240

Asp Lys Glu Ala Lys Leu Ala Ser Lys Pro Gly Phe Ile Tyr Met Asp
                245                 250                 255

Ser Met Gly Phe Gly Met Gly Cys Ser Cys Leu Gln Val Thr Phe Gln
            260                 265                 270

Ala Pro Asn Ile Asn Lys Ala Arg Tyr Leu Tyr Asp Ala Leu Val Asn
        275                 280                 285

Phe Ala Pro Ile Met Leu Ala Phe Ser Ala Ala Ala Pro Ala Phe Lys
    290                 295                 300

Gly Trp Leu Ala Asp Gln Asp Val Arg Trp Asn Val Ile Ser Gly Ala
305                 310                 315                 320

Val Asp Asp Arg Thr Pro Lys Glu Arg Gly Val Ala Pro Leu Leu Pro
                325                 330                 335

Lys Tyr Asn Lys Asn Gly Phe Gly Gly Ile Ala Lys Asp Val Gln Asp
            340                 345                 350

Lys Val Leu Glu Ile Pro Lys Ser Arg Tyr Ser Ser Val Asp Leu Phe
        355                 360                 365

Leu Gly Gly Ser Lys Phe Phe Asn Arg Thr Tyr Asn Asp Thr Asn Val
    370                 375                 380

Pro Ile Asn Glu Lys Val Leu Gly Arg Leu Leu Glu Asn Asp Lys Ala
385                 390                 395                 400

Pro Leu Asp Tyr Asp Leu Ala Lys His Phe Ala His Leu Tyr Ile Arg
                405                 410                 415

Asp Pro Val Ser Thr Phe Glu Glu Leu Leu Asn Gln Asp Asn Lys Thr
            420                 425                 430

Ser Ser Asn His Phe Glu Asn Ile Gln Ser Thr Asn Trp Gln Thr Leu
        435                 440                 445

Arg Phe Lys Pro Pro Thr Gln Gln Ala Thr Pro Asp Lys Lys Asp Ser
    450                 455                 460

Pro Gly Trp Arg Val Glu Phe Arg Pro Phe Glu Val Gln Leu Leu Asp
465                 470                 475                 480

Phe Glu Asn Ala Ala Tyr Ser Val Leu Ile Tyr Leu Ile Val Asp Ser
                485                 490                 495
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Ile Leu Thr Phe Ser Asp Asn Ile Asn Ala Tyr Ile His Met Ser Lys
            500                 505                 510

Val Trp Glu Asn Met Lys Ile Ala His His Arg Asp Ala Ile Leu Phe
        515                 520                 525

Glu Lys Phe His Trp Lys Lys Ser Phe Arg Asn Asp Thr Asp Val Glu
    530                 535                 540

Thr Glu Asp Tyr Ser Ile Ser Glu Ile Phe His Asn Pro Glu Asn Gly
545                 550                 555                 560

Ile Phe Pro Gln Phe Val Thr Pro Ile Leu Cys Gln Lys Gly Phe Val
                565                 570                 575

Thr Lys Asp Trp Lys Glu Leu Lys His Ser Ser Lys His Glu Arg Leu
            580                 585                 590

Tyr Tyr Tyr Leu Lys Leu Ile Ser Asp Arg Ala Ser Gly Glu Leu Pro
        595                 600                 605

Thr Thr Ala Lys Phe Phe Arg Asn Phe Val Leu Gln His Pro Asp Tyr
    610                 615                 620

Lys His Asp Ser Lys Ile Ser Lys Ser Ile Asn Tyr Asp Leu Leu Ser
625                 630                 635                 640

Thr Cys Asp Arg Leu Thr His Leu Asp Asp Ser Lys Met Glu Leu Thr
                645                 650                 655

Ser Phe Leu Gly Ala Glu Ile Ala Glu Tyr Val Lys Lys Asn Lys Pro
            660                 665                 670

Ser Ile Glu Ser Lys Cys
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<210> 15
<211> 25
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<223> F_GSH1_C86R

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