Discovery and Functional Evaluation of Heat Tolerance Genes in the Nonconventional Yeast *Yarrowia lipolytica*

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* Correspondence: chrqrq@sjtu.edu.cn

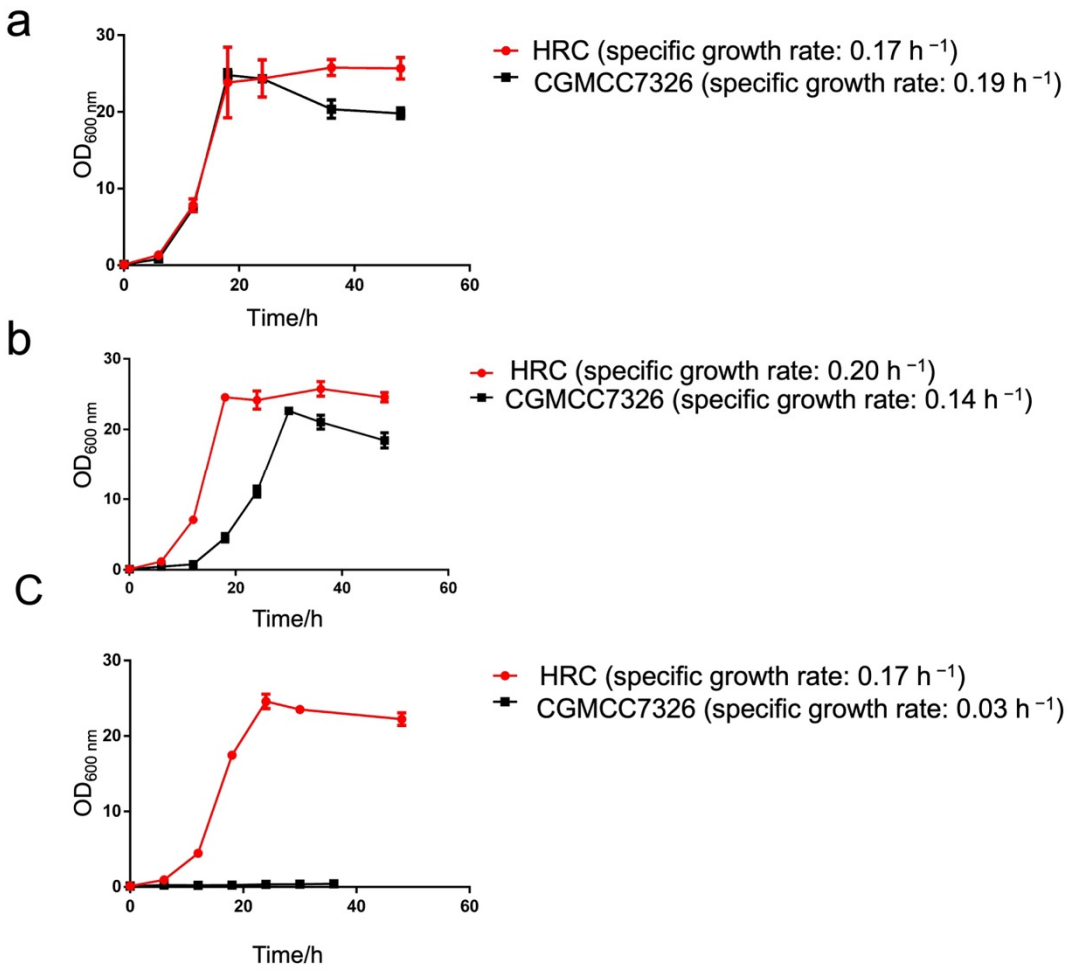

Figure S1 Growth curve of HRC and *Y. lipolytica* CGMCC7326 at different temperatures. a. Growth curve of HRC and *Y. lipolytica* CGMCC7326 at 30°C. b. Growth curve of HRC and *Y. lipolytica* CGMCC7326 at 33°C. c. Growth curve of HRC and *Y. lipolytica* CGMCC7326 at 35°C.

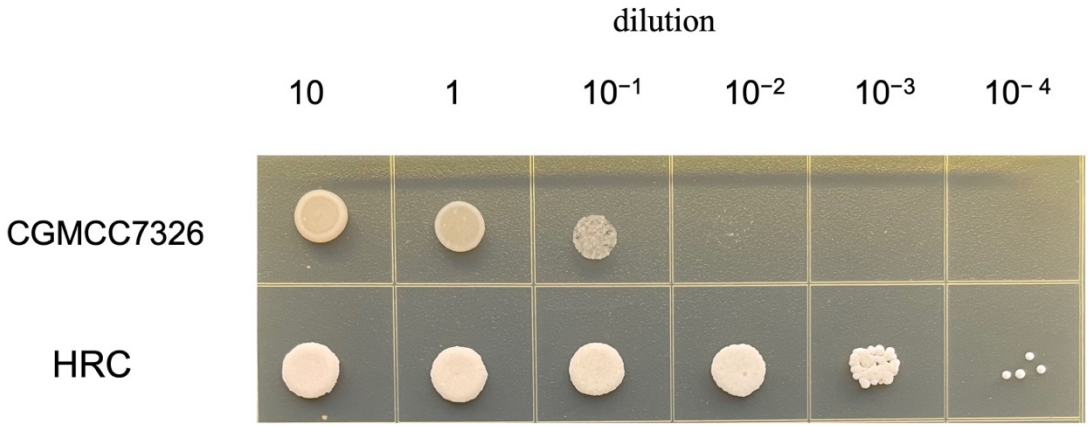

Figure S2 Spot assay of *Y. lipolytica* CGMCC7326 and HRC

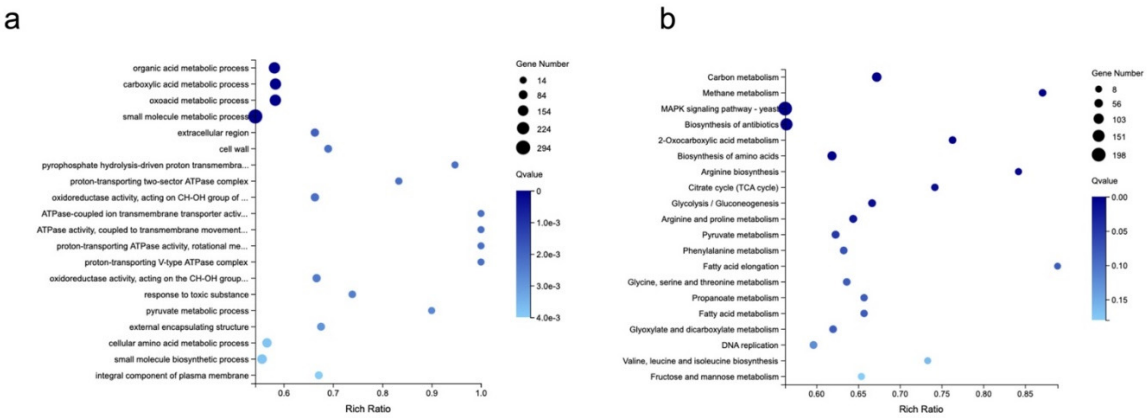

Figure S3 Differentially expressed genes (DEGs) analysis of D group (HRC at 35 ℃) vs C Group (CGMCC7326 at 30 ℃). a. Go enrichment analysis of DEGs of D group (HRC at 35 ℃) vs C Group (CGMCC7326 at 30 ℃). b. KEGG enrichment analysis of DEGs of D group (HRC at 35 ℃) vs C Group (CGMCC7326 at 30 ℃).

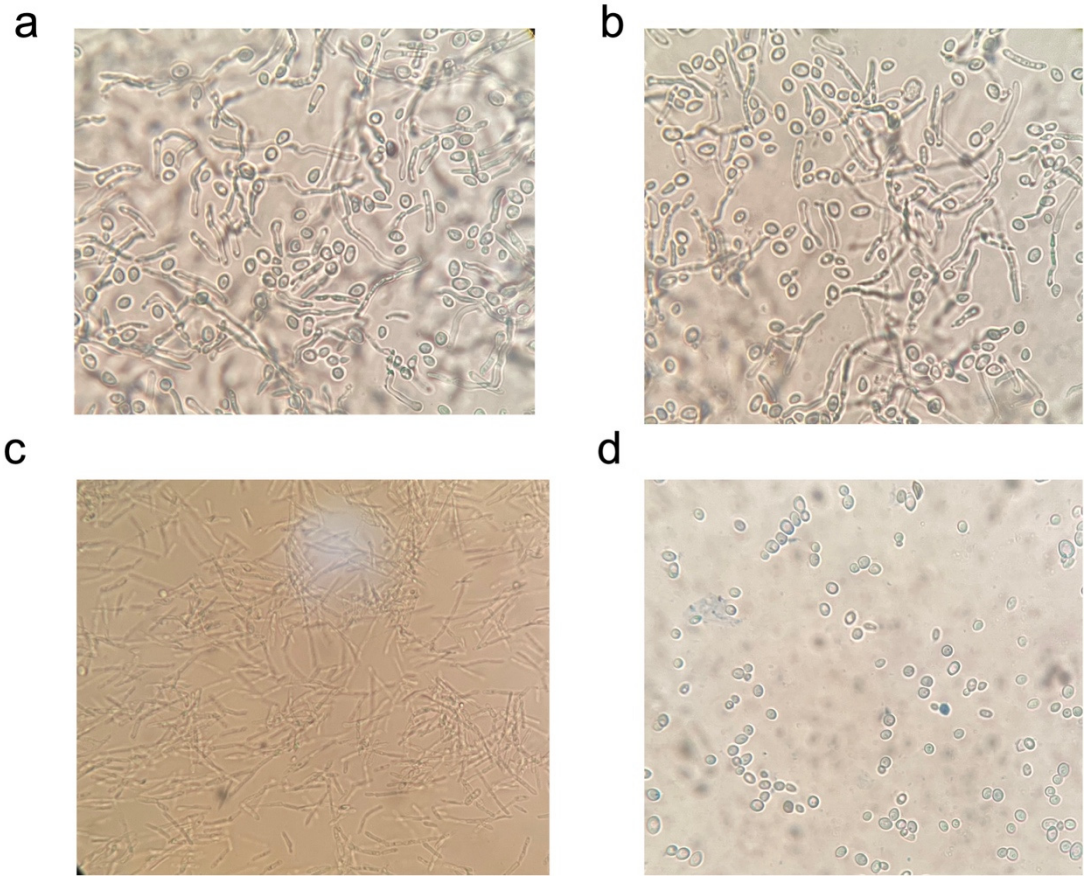

Figure S4 Morphological characteristics of HRC2 and *Y. lipolytica* CGMCC7326. a. b. Filamentous growth of HRC2 on solid YPD plate. c. Diploid pseudohyphal formation of HRC2 during fermentation. d. *Yarrowia lipolytica* cells morphology.

Sequence 1: Control cassette

1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1165-1170: KpnI

1171-1176: PstI

1177-1434: terminator

1435-1440: Afl II

1441-1446: BamH I

1447-1452: Bmt I

1453-1486: loxP

1487-2018: hp4d

2019-2024: SacI

2025-3491: guab

3492-3497: Xho I

3498-3730: cyc1 terminator

3731-3764: loxP

3765-3770: Nar I

3771-4172: rDNA

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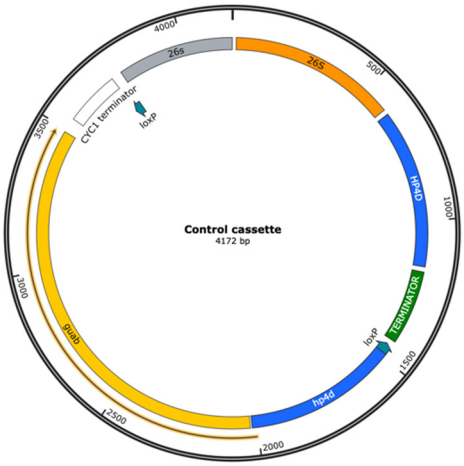

Sequence 2: YALI0_F01452g overexpression cassette

1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

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604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: Kpn I

1171-2457: YALI0_F01452g

2458-2463: KpnI

2464-2469: PstI

2470-2727: terminator

27128-2733: Afl II

2734-2739: BamH I

2740-2745: Bmt I

27467-2779: loxP

2780-3311: hp4d

3312-3317: SacI

3318-4784: guab

4785-4790: Xho I

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5024-5057: loxP

5058-5063: Nar I

5064-5465: rDNA

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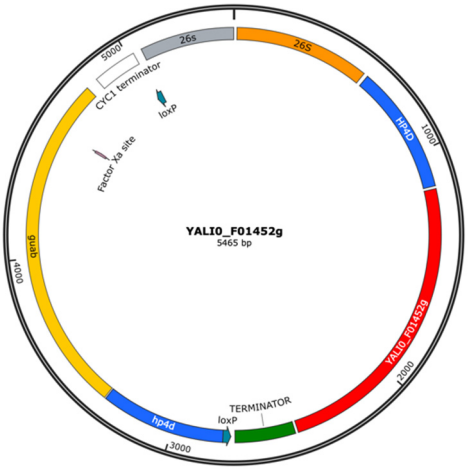
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Sequence 3: YALI0_C04279g overexpression cassette

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9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: KpnI

1171-2319: YALI0_C04279g

2320-2325: Kpn I

2326-2331: Pst I

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2602-2607: BmtI

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3174-3179: SacI

3180-4646: guab

4647-4652: XhoI

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1261 GAGATCGCTG CCGAGCTTTT CAAGCGACAA GCAGGTGTTT TCCACAGCAC CCAAACGAAG
1321 GGAGACATCT TTTACGAGGC CGAGATCGAG ATTGGAACCC CTCCCCAGAA GGTCTCCGTC
1381 TGCTTCGACA CCGGATCCCC CCTTCTGTGG GTGCCCGGAT CTAACTCCAC CCAATGCAAG
1441 GATAGCTGTA AGGGACGAAC CTACGATGTC AGCAAGTCTT CCTCCTGGCA GTACCAGAAG
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5161 TACAAACCAT GAAAGTGTGG CCTATCGATC CTTTAGTTGT TCGGAGTTTG AACCTAGAGG
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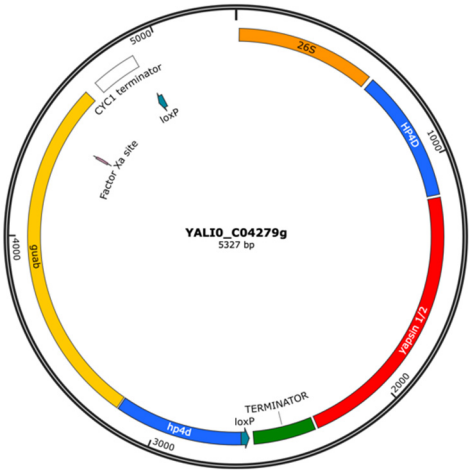
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hp4d
Yblsn LTZ
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Factor Xa site
loxP
CYC1 terminator
1000
2000
3000
4000
5000

Sequence 4: YALI0_B21582g overexpression cassette

1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: KpnI

1171-2028: *HRC1*

2329-2334: Kpn I

2335-2340: Pst I

2041-2298: terminator

2299-2304: Afl II

2305-2310: Nhe I

2311-2316: BmtI

2317-2350: loxP

2351-2882: hp4d

2883--2888: SacI

2889-4355: guab

4356-4361: XhoI

4362-4594: terminator

4595-4628: loxP

4629-4634: Nar I

4635-5035: rDNA

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1       GCGGCCGCGA ATTCCAGATC TTGGTGGTAG TAGCAAATAT TCAAATGAGA ACTTTGAAGA
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481 CGGCAAAATA GATCCGTAAC TTCGGGATAA GGATTGGCTC TGGGGGTTGG TGGATGGAAG
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901 GTACATTATC GAGACCGTTG TTCCCGCCCA CCTCGATCCG GCTGAGGTGT CTCACAAGTG
961 CCGTGCAGTC CCGCCCCCAC TTGCTTCTCT TTGTGTGTAG TGTACGTACA TTATCGAGAC
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1081 TGCCAGCCAC AGATTTTCAC TCCACACACC ACATCACACA TACAACCACA CACATCCACA
1141 ATGGAACCCG AAACTAAGCA TATGGGTACC ATGGACCTCG AATTGGAAAT TCCCGTCTTG
1201 CATTCCATGG ACTCGCACCA CCAGGTGGTG GACTCCCACA GACTGGCACA GCAACAGTTC
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2161 TGTAGGTGGC AAAATCCCGT CTTTGTTCGT CGGTTCCCTC TGTGACTGCT CGTCGTCCCT
2221 TTGTGTTCGA CTGTCGTGTT TTGTTTTCCG TGCGTGCGCA AGTGAGATGC CCGTGTTCGA
2281 ATACGGTAGT CGCACGGACT TAAGGGATCC GCTAGCATAA CTTCGTATAA TGTATGCTAT
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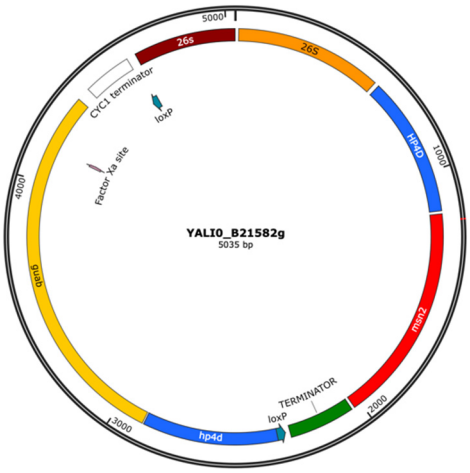
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TERMINATOR
loxP
2000
3000
hp4d

Sequence 5: YALI0_C13750g overexpression cassette

1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: KpnI

1171-2370: *HRC2*

2371-2376: Kpn I

2377-2382: Pst I

2383-2640: terminator

2641-2646: Afl II

2647-2652: Nhe I

2653-2658: BmtI

2659-2692: loxP

2693-3224: hp4d

3225-3230: SacI

3231-4697: guab

4698-4703: XhoI

4704-4936: terminator

4937-4970: loxP

4971-4976: Nar I

4977-5377: rDNA

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301 GTCTAGAGAT AGGGTATCGT TCCGGAAGAG GGGGGCAGCT TTGTCCCCTC CGATGCACTT
361 GTGACGCCCC TTGAAAACCC GCAGGAAGGA ATAGTTTTCA CGCCAAGTCG TACTGATAAC
421 CGCAGCAGGT CTCCAAGGTG AACAGCCTCT AGTTGATAGA ATAATGTAGA TAAGGGAAGT
481 CGGCAAAATA GATCCGTAAC TTCGGGATAA GGATTGGCTC TGGGGGTTGG TGGATGGAAG
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4381 CTTACCGAGG TATGGGTTCC CTGGGTGCCA TGTCCAAAGG TTCCTCTGAC CGATATTTCC
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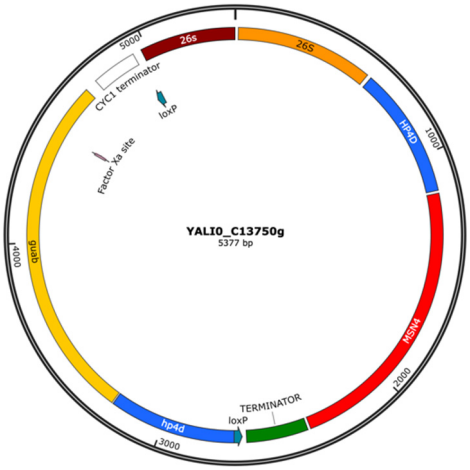
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26S
hp4D
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TERMINATOR
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hp4d
gene
Factor Xa site
loxP
CYC1 terminator
1000
2000
3000
4000
5000

Sequence 6: YALI0_B10626g overexpression cassette

1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: KpnI

1171-3105: *HRC3*

3106-3111: Kpn I

3112-3117: Pst I

3118-3375: terminator

3376-3381: Afl II

3382-3387: Nhe I

3388-3393: BmtI

3394-3427: loxP

3428-3959: hp4d

3960-3965: SacI

3966-5432: guab

5433-5438: XhoI

5439-5671: terminator

5672-5705: loxP

5706-5711: Nar I

5712-6113: rDNA

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1       GCGGCCGCGA ATTCCAGATC TTGGTGGTAG TAGCAAATAT TCAAATGAGA ACTTTGAAGA
61      CTGAAGTGGG GAAAGGTTCC GTGTGAACAG CAGTTGGACA CGGGTAAGTC GATCCTAAGG
121     GGTGGCATAA CTGTCGCGTA CGGCCCGATA AGGGCCTTCT CCAAAAGGGA AGCCGGTTGA
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241 ACAAGTCTTG GAAGGAGTTA TCTTTTCTTT TTAACGGAGT CAACACCCTG GAATTAGTTT
301 GTCTAGAGAT AGGGTATCGT TCCGGAAGAG GGGGGCAGCT TTGTCCCCTC CGATGCACTT
361 GTGACGCCCC TTGAAAACCC GCAGGAAGGA ATAGTTTTCA CGCCAAGTCG TACTGATAAC
421 CGCAGCAGGT CTCCAAGGTG AACAGCCTCT AGTTGATAGA ATAATGTAGA TAAGGGAAGT
481 CGGCAAAATA GATCCGTAAC TTCGGGATAA GGATTGGCTC TGGGGGTTGG TGGATGGAAG
541 CGTGGGAGAC CCCAAGGGAC TGGCGGCTGG GCAACTGGCA GCCGGACCCG CAAGCTTGTC
601 GACACTAGTG CCGGACCCGC AAGCATCTGA GGTGTCTCAC AAGTGCCGTG CAGTCCCGCC
661 CCCACTTGCT TCTCTTTGTG TGTAGTGTAC GTACATTATC GAGACCGTTG TTCCCGCCCA
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781 TTGTGTGTAG TGTACGTACA TTATCGAGAC CGTTGTTCCC GCCCACCTCG ATCCGGCTGA
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1441 TGGATCAGCA GTGTTATTGC TGCCCTCGAA AAATATGAGC AAAGCCCCGA CTTCACCTCT
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2041 AAGCCCGTTA CTACTGTCAA GTCTGAAGAT CCCGTTGCAT CTGGACTTGC CTCCGAAGAA
2101 CCTATTACTG CCGAGTCCCC CCTGTCTAGT GCCTCTGGTC TGGCTTCCGG GGAGGCTTCT
2161 GTATCTTCTG ACACACCTGT CTCTGCAAGC GCTAACACTG ACGATACTAG CCCTCAGACC
2221 TCTGGTGTCT CCTCTTCCGG TACTCCGGAG ACTGTCGCTC CCACCTCTGC ACCTTCTTCA
2281 GCGTCCATCT CTAGTGCTGC TGGTGTTACG AACGGCACTG TTTTTGGGTC CTCTGAGCCG
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2701 AAAACCGGGA AGACAGAGGA GACAGAAGAG GCTGAGAAGG CCGATAAGAC CCATGAGTCT
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2821 ACTGAGAAAA CTGACAAGAC CAAGAAAACT GACAAGACTG ACAACTCTGA AACCCCTAAG
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2941 AACGACTCAT CCAACAAGCC CACCAACACC GGCGCTGCTG ATAAGCCTTC AAACGGAGGA
3001 TCTGCTGAGC CCAAGCCAGT TGCCCAGGCC AACTCAGGCT CAAAGTTGGT CATCTCGGTC
3061 GGCGCACTAC TTGCTGGAAT TCTGGCTCTC GTTGCTTTAT CGTAGGGTAC CCTGCAGGCA
3121 ATTAACAGAT AGTTTGCCGG TGATAATTCT CTTAACCTCC CACACTCCTT TGACATAACG
3181 ATTTATGTAA CGAAACTGAA ATTTGACCAG ATATTGTTGT AAATAGAAAA TCTGGCTTGT
3241 AGGTGGCAAA ATCCCGTCTT TGTTCGTCGG TTCCCTCTGT GACTGCTCGT CGTCCCTTTG
3301 TGTTCGACTG TCGTGTTTTG TTTTCCGTGC GTGCGCAAGT GAGATGCCCG TGTTCGAATA
3361 CGGTAGTCGC ACGGACTTAA GGGATCCGCT AGCATAACTT CGTATAATGT ATGCTATACG
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3541 TCTCACAAGT GCCGTGCAGT CCCGCCCCCA CTTGCTTCTC TTTGTGTGTA GTGTACGTAC
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4081 GACTGAATAT CCCTATGCTC TCCGCTGCTA TGGATACCGT TACCGAAGCC CGACTGGCTA
4141 TTGCTCTGGC TCAGGAAGGC GGTATCGGCT TTATCCACAA AAACATGTCC ATTGAACGAC
4201 AGGCTGAAGA AGTTCGACGA GTGAAAAAAC ACGAATCTGG TGTGGTGACT GATCCCCAGA
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5341 TTGTTCGAAT CTCCGGTGCC GGCATTCAGG AATCCCACGT TCACGACGTG ACCATTACTA
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5521 TGAAGTCTAG GTCCCTATTT ATTTTTTTAT AGTTATGTTA GTATTAAGAA CGTTATTTAT

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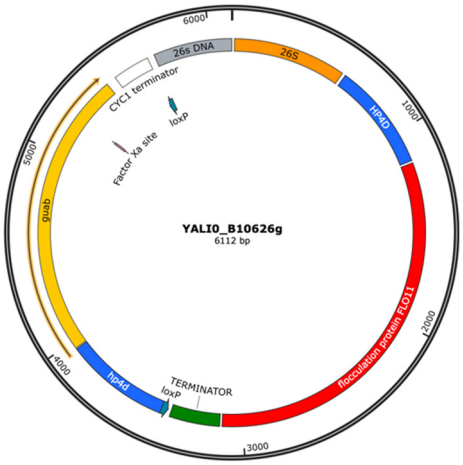

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1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: KpnI

1171-2754: YALI0_B20768g

2755-2760: Kpn I

2761-2766: Pst I

2767-3024: terminator

3025-3030: Afl II

3031-3036: Nhe I

3037-3042: BmtI

3043-3076: loxP

3077-3608: hp4d

3609-3614: SacI

3615-5081: guab

5082-5087: XhoI

5088-5320: terminator

5321-5354: loxP

5355-5360: Nar I

5361-5762: rDNA

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1621 GGAGCAAGTA ACCCTCTTGC TCCCGGCGAA CAAAACACAA TGGACTCCCT GCTGCAAAAC
1681 ATTGACAACT TTGACTTCCT CCTGCACCCG GGAGACCTGG CCTATGCGGA TTACTGGCTC
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5221 GTTATTTATA TTTCAAATTT TTCTTTTTTT TCTGTACAGA CGCGTGTACG CATGTAACAT
5281 TATACTGAAA ACCTTGCTTG AGAAGGTTTT GGGACGCTCG ATAACTTCGT ATAATGTATG
5341 CTATACGAAG TTATGGCGCC GGTGAAATAC CACTACCCTT ATCGTTTCTT TACTTATTTA

5401 GTAAGTGGAA GTGGTTTAAC AACCATTTTC TAGCATTCCT TTCCAGGCTG AAGACATTGT

5461 CAGGTGGGGA GTTTGGCTGG GGCGGCACAT CTGTTAAAAG ATAACGCAGA TGTCCTAAGG

5521 GGGACTCAAT GAGAACAGAA ATCTCATGTA GAACAAAAGG GTAAAAGTCC CCTTGATTTT

5581 GATTTTCAGT GTGAATACAA ACCATGAAAG TGTGGCCTAT CGATCCTTTA GTTGTTCGGA

5641 GTTTGAACCT AGAGGTGCCA GAAAAGTTAC CACAGGGATA ACTGGCTTGT GGCAGTCAAG

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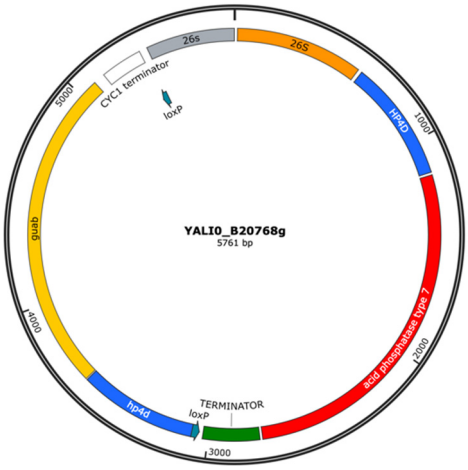
5761 AG



Sequence 8: YALI0_D07898g overexpression cassette

1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: KpnI

1171-1872: YALI0_D07898g

1873-1878: Kpn I

1879-1884: Pst I

1885-2142: terminator

2143-2148: Afl II

2149-2154: Nhe I

2155-2160: BmtI

2161-2194: loxP

2195-2726: hp4d

2727-2732: SacI

2733-4199: guab

4200-4205: XhoI

4206-4438: terminator

4439-4472: loxP

4473-4478: Nar I

4479-4880: rDNA

```
1       GCGGCCGCGA ATTCCAGATC TTGGTGGTAG TAGCAAATAT TCAAATGAGA ACTTTGAAGA
61      CTGAAGTGGG GAAAGGTTCC GTGTGAACAG CAGTTGGACA CGGGTAAGTC GATCCTAAGG
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121 GGTGGCATAA CTGTCGCGTA CGGCCCGATA AGGGCCTTCT CCAAAAGGGA AGCCGGTTGA
181 AATTCCGGCA CTTGGATGTG GATTCTCCAC GGCAACGTAA CTGAATGTGG GGACGGTGGC
241 ACAAGTCTTG GAAGGAGTTA TCTTTTCTTT TTAACGGAGT CAACACCCTG GAATTAGTTT
301 GTCTAGAGAT AGGGTATCGT TCCGGAAGAG GGGGGCAGCT TTGTCCCCTC CGATGCACTT
361 GTGACGCCCC TTGAAAACCC GCAGGAAGGA ATAGTTTTCA CGCCAAGTCG TACTGATAAC
421 CGCAGCAGGT CTCCAAGGTG AACAGCCTCT AGTTGATAGA ATAATGTAGA TAAGGGAAGT
481 CGGCAAAATA GATCCGTAAC TTCGGGATAA GGATTGGCTC TGGGGGTTGG TGGATGGAAG
541 CGTGGGAGAC CCCAAGGGAC TGGCGGCTGG GCAACTGGCA GCCGGACCCG CAAGCTTGTC
601 GACACTAGTG CCGGACCCGC AAGCATCTGA GGTGTCTCAC AAGTGCCGTG CAGTCCCGCC
661 CCCACTTGCT TCTCTTTGTG TGTAGTGTAC GTACATTATC GAGACCGTTG TTCCCGCCCA
721 CCTCGATCCG GCTGAGGTGT CTCACAAGTG CCGTGCAGTC CCGCCCCCAC TTGCTTCTCT
781 TTGTGTGTAG TGTACGTACA TTATCGAGAC CGTTGTTCCC GCCCACCTCG ATCCGGCTGA
841 GGTGTCTCAC AAGTGCCGTG CAGTCCCGCC CCCACTTGCT TCTCTTTGTG TGTAGTGTAC
901 GTACATTATC GAGACCGTTG TTCCCGCCCA CCTCGATCCG GCTGAGGTGT CTCACAAGTG
961 CCGTGCAGTC CCGCCCCCAC TTGCTTCTCT TTGTGTGTAG TGTACGTACA TTATCGAGAC
1021 CGTTGTTCCC GCCCACCTCG ATCCGGCACG GGCAAAAGTG CGTATATATA CAAGAGCGTT
1081 TGCCAGCCAC AGATTTTCAC TCCACACACC ACATCACACA TACAACCACA CACATCCACA
1141 ATGGAACCCG AAACTAAGCA TATGGGTACC ATGCTTCTTT CTAATATTTT CACCCACATG
1201 CTTCTGGCCT CTGTGGTCTC CTCCGCCGCC ATTCCCGTGG CTGACGCCGA ATCTGTTCAG
1261 CTGGCTGAGC GAAAAGACGC CAAGGAGGTC TTCCACGAGA TTGGAAACTT CTTCAAGACT
1321 GGTGACATTC ACGACGATGG AAAGCACAAC GACAAGCGAG AGCTTGAGGA GCGAAAGAAC
1381 GCCAAGGAGG TCTTTGGTGA GATTGGCAAC TTCTTCAAGA CCGGTGACAT TCACGACGAC
1441 GGCAAGCACC ACGACAAGCG AGAGCTTGAG GAGCGAAAGA ACGCCAAGGA GGTCTTTGGT
1501 GAGATTGGCA ACTTCTTCAA GACCGGTGAT ATTCACGACG ACGGCAAGCA CCACGACAAG
1561 CGAGAGCTTG AGGAGCGAAA GGACGCCAAG GAGGTCTTTA GCGAGATCGG CAACTTCTTC
1621 AAGACCGGTG ACATTCACGA CGACGGCAAG CACCACGACA AGCGAGAGCT TGAGGAGCGA
1681 AAGGACGCCA AGGAGGTCTT TGGTGAGATT GGCAACTTCT TCAAGACCGG AGATATCCAC
1741 GACGACGGCA AGCACCACGA CAAGCGAGAG CTTGAGGAGC GAAAGGACGC CAAGGAGGTC
1801 TTTGGTGAGA TTGGCAACTT CTTCAAGACC GGAGATATCC ACGACGACGG CAAGCACCAC
1861 AAGGACGACT AAGGTACCCT GCAGGCAATT AACAGATAGT TTGCCGGTGA TAATTCTCTT
1921 AACCTCCCAC ACTCCTTTGA CATAACGATT TATGTAACGA AACTGAAATT TGACCAGATA
1981 TTGTTGTAAA TAGAAAATCT GGCTTGTAGG TGGCAAAATC CCGTCTTTGT TCGTCGGTTC
2041 CCTCTGTGAC TGCTCGTCGT CCCTTTGTGT TCGACTGTCG TGTTTTGTTT TCCGTGCGTG
2101 CGCAAGTGAG ATGCCCGTGT TCGAATACGG TAGTCGCACG GACTTAAGGG ATCCGCTAGC
2161 ATAACTTCGT ATAATGTATG CTATACGAAG TTATCTGAGG TGTCTCACAA GTGCCGTGCA
2221 GTCCCGCCCC CACTTGCTTC TCTTTGTGTG TAGTGTACGT ACATTATCGA GACCGTTGTT
2281 CCCGCCCACC TCGATCCGGC TGAGGTGTCT CACAAGTGCC GTGCAGTCCC GCCCCCACTT
2341 GCTTCTCTTT GTGTGTAGTG TACGTACATT ATCGAGACCG TTGTTCCCGC CCACCTCGAT
2401 CCGGCTGAGG TGTCTCACAA GTGCCGTGCA GTCCCGCCCC CACTTGCTTC TCTTTGTGTG
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2581 ATCGAGACCG TTGTTCCCGC CCACCTCGAT CCGGCACGGG CAAAAGTGCG TATATATACA
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2701 CATCCACAAT GGAACCCGAA ACTAAGGAGC TCATGCTCCG AATCGCTAAA GAAGCTCTGA

2761 CCTTTGACGA CGTTCTCCTC GTTCCTGCTC ACTCTACCGT TCTGCCCAAT ACTGCTGACC
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2881 ATACCGTTAC CGAAGCCCGA CTGGCTATTG CTCTGGCTCA GGAAGGCGGT ATCGGCTTTA
2941 TCCACAAAAA CATGTCCATT GAACGACAGG CTGAAGAAGT TCGACGAGTG AAAAAACACG
3001 AATCTGGTGT GGTGACTGAT CCCCAGACTG TTCTGCCTAC CACCACCCTG CGAGAAGTGA
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3301 TCCACCTGAT CGGCATGATC ACCGTGAAAG ACTTCCAGAA AGCCGAACGA AAACCCAACG
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3481 CCCACGGTCA CTCTGAAGGT GTTCTGCAAC GAATCCGAGA AACCCGAGCT AAATATCCCG
3541 ATCTGCAAAT TATCGGCGGC AACGTGGCTA CTGCTGCTGG TGCCCGAGCC CTGGCTGAAG
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3661 TGACTGGCGT CGGTGTTCCC CAGATTACCG CTGTTGCTGA CGCTGTTGAA GCCCTGGAAG
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3901 CCCTGGGTGC CATGTCCAAA GGTTCCTCTG ACCGATATTT CCAGTCCGAT AACGCTGCCG
3961 ACAAACTGGT GCCCGAAGGT ATCGAAGGTC GAGTTGCCTA TAAAGGACGA CTGAAAGAGA
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4081 TCGACGAACT GCGAACTAAA GCCGAGTTTG TTCGAATCTC CGGTGCCGGC ATTCAGGAAT
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4381 CGTGTACGCA TGTAACATTA TACTGAAAAC CTTGCTTGAG AAGGTTTTGG GACGCTCGAT
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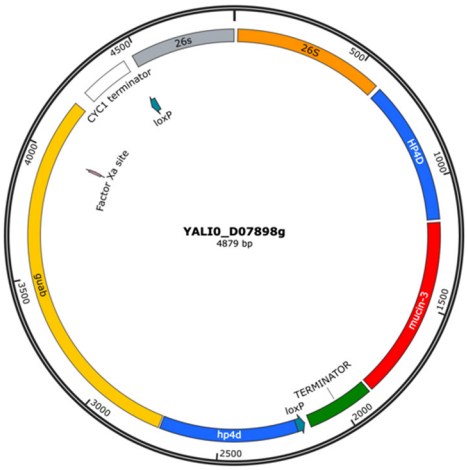
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HP4D
1000
muGFP-3
1500
TERMINATOR
2000
hp4d
2500
loxP
3000
gUAP
3500
Factor Xa site
4000
CYC1 terminator
loxP
4500
26s

Sequence 9: YALI0_B19866g overexpression cassette

1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: KpnI

1171-2223: YALI0_B19866g

2224-2229: Kpn I

2230-2235: Pst I

2236-2493: terminator

2494-2499: Afl II

2500-2505: Nhe I

2506-2511: BmtI

2512-2545: loxP

2546-3077: hp4d

3078-3083: SacI

3084-4550: guab

4551-4556: XhoI

4557-4789: terminator

4790-4823: loxP

4824-4829: Nar I

4830-5231: rDNA

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1       GCGGCCGCGA ATTCCAGATC TTGGTGGTAG TAGCAAATAT TCAAATGAGA ACTTTGAAGA
61      CTGAAGTGGG GAAAGGTTCC GTGTGAACAG CAGTTGGACA CGGGTAAGTC GATCCTAAGG
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241 ACAAGTCTTG GAAGGAGTTA TCTTTTCTTT TTAACGGAGT CAACACCCTG GAATTAGTTT
301 GTCTAGAGAT AGGGTATCGT TCCGGAAGAG GGGGGCAGCT TTGTCCCCTC CGATGCACTT
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421 CGCAGCAGGT CTCCAAGGTG AACAGCCTCT AGTTGATAGA ATAATGTAGA TAAGGGAAGT
481 CGGCAAAATA GATCCGTAAC TTCGGGATAA GGATTGGCTC TGGGGGTTGG TGGATGGAAG
541 CGTGGGAGAC CCCAAGGGAC TGGCGGCTGG GCAACTGGCA GCCGGACCCG CAAGCTTGTC
601 GACACTAGTG CCGGACCCGC AAGCATCTGA GGTGTCTCAC AAGTGCCGTG CAGTCCCGCC
661 CCCACTTGCT TCTCTTTGTG TGTAGTGTAC GTACATTATC GAGACCGTTG TTCCCGCCCA
721 CCTCGATCCG GCTGAGGTGT CTCACAAGTG CCGTGCAGTC CCGCCCCCAC TTGCTTCTCT
781 TTGTGTGTAG TGTACGTACA TTATCGAGAC CGTTGTTCCC GCCCACCTCG ATCCGGCTGA
841 GGTGTCTCAC AAGTGCCGTG CAGTCCCGCC CCCACTTGCT TCTCTTTGTG TGTAGTGTAC
901 GTACATTATC GAGACCGTTG TTCCCGCCCA CCTCGATCCG GCTGAGGTGT CTCACAAGTG
961 CCGTGCAGTC CCGCCCCCAC TTGCTTCTCT TTGTGTGTAG TGTACGTACA TTATCGAGAC
1021 CGTTGTTCCC GCCCACCTCG ATCCGGCACG GGCAAAAGTG CGTATATATA CAAGAGCGTT
1081 TGCCAGCCAC AGATTTTCAC TCCACACACC ACATCACACA TACAACCACA CACATCCACA
1141 ATGGAACCCG AAACTAAGCA TATGGGTACC ATGACTTCTT CTACTACCAA CAGCCCCAGA
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1441 CCTTCCAGAT TCAGCTTCAC TGGTCCGGTG CTGTCTATTA TTCAGGAAGT TGCAGACGGC
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2041 TCAGAACTGG CAGGATATAA GCAGTTAGTC CCAGCCAGCT CAGAGCTCGA AACGTACCTT
2101 CCCCAAGTCA ACTCTCTCAG TGAACAGATC CACACCTTGG CCAATGCAAT GTTCGAGTTT
2161 GAACAGGAGG TTTCTGAATC AGAGAAGCTT TTGAAGCATG GAAGTGAGTG GAGTCACCAC
2221 TAGGGTACCC TGCAGGCAAT TAACAGATAG TTTGCCGGTG ATAATTCTCT TAACCTCCCA
2281 CACTCCTTTG ACATAACGAT TTATGTAACG AAACTGAAAT TTGACCAGAT ATTGTTGTAA
2341 ATAGAAAATC TGGCTTGTAG GTGGCAAAAT CCCGTCTTTG TTCGTCGGTT CCCTCTGTGA
2401 CTGCTCGTCG TCCCTTTGTG TTCGACTGTC GTGTTTTGTT TTCCGTGCGT GCGCAAGTGA
2461 GATGCCCGTG TTCGAATACG GTAGTCGCAC GGACTTAAGG GATCCGCTAG CATAACTTCG
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2581 CCACTTGCTT CTCTTTGTGT GTAGTGTACG TACATTATCG AGACCGTTGT TCCCGCCCAC
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2881 CGTGCAGTCC CGCCCCCACT TGCTTCTCTT TGTGTGTAGT GTACGTACAT TATCGAGACC
2941 GTTGTTCCCG CCCACCTCGA TCCGGCACGG GCAAAAGTGC GTATATATAC AAGAGCGTTT
3001 GCCAGCCACA GATTTTCACT CCACACACCA CATCACACAT ACAACCACAC ACATCCACAA
3061 TGGAACCCGA AACTAAGGAG CTCATGCTCC GAATCGCTAA AGAAGCTCTG ACCTTTGACG
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3181 AGCTGACCAA AACTATTCGA CTGAATATCC CTATGCTCTC CGCTGCTATG GATACCGTTA
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3301 ACATGTCCAT TGAACGACAG GCTGAAGAAG TTCGACGAGT GAAAAAACAC GAATCTGGTG
3361 TGGTGACTGA TCCCCAGACT GTTCTGCCTA CCACCACCCT GCGAGAAGTG AAAGAACTGA
3421 CCGAGCGAAA CGGTTTTGCC GGCTATCCCG TCGTTACCGA AGAAAACGAA CTGGTGGGTA
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3781 GAGTTGACGC CCTGGTTGCC GCTGGCGTTG ACGTTCTGCT GATCGACTCC TCCCACGGTC
3841 ACTCTGAAGG TGTTCTGCAA CGAATCCGAG AAACCCGAGC TAAATATCCC GATCTGCAAA
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4081 TTCCCGTTAT CGCTGATGGC GGTATTCGAT TCTCCGGCGA CATCGCCAAA GCTATCGCCG
4141 CTGGCGCTTC CGCCGTGATG GTTGGTTCCA TGCTGGCCGG TACTGAAGAA TCTCCCGGTG
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4321 TGCCCGAAGG TATCGAAGGT CGAGTTGCCT ATAAAGGACG ACTGAAAGAG ATCATTCACC
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5101 TTGTTCGGAG TTTGAACCTA GAGGTGCCAG AAAAGTTACC ACAGGGATAA CTGGCTTGTG
5161 GCAGTCAAGC GTTCATAGCG ACATTGCTTT TTGATCCTTC GATGTCGGCT CTTCCTATCA
5221 TACCGAAGCA G

YALI0_B19866g
5230 bp
26S
26S
hmD
structural maintenance of chromosome
TERMINATOR
loxP
hp4d
gmb
CYC1 terminator
1000
2000
3000
4000
5000

Sequence 10: YALI0_F26543g overexpression cassette

1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: KpnI

1171-2016: *YALI0_F26543g*

2017-2022: Kpn I

2023-2028: Pst I

2029-2286: terminator

2287-2292: Afl II

2293-2298: Nhe I

2299-2304: BmtI

2305-2338: loxP

2339-2870: hp4d

2871-2876: SacI

2877-4343: guab

4344-4349: XhoI

4350-4582: terminator

4583-4616: loxP

4617-4622: Nar I

4623-5024: rDNA

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301 GTCTAGAGAT AGGGTATCGT TCCGGAAGAG GGGGGCAGCT TTGTCCCCTC CGATGCACTT
361 GTGACGCCCC TTGAAAACCC GCAGGAAGGA ATAGTTTTCA CGCCAAGTCG TACTGATAAC
421 CGCAGCAGGT CTCCAAGGTG AACAGCCTCT AGTTGATAGA ATAATGTAGA TAAGGGAAGT
481 CGGCAAAATA GATCCGTAAC TTCGGGATAA GGATTGGCTC TGGGGGTTGG TGGATGGAAG
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1321 GCCCAAGCCG ACAATGGACC CTACTATGCT GTCAGAGTTG GCCATAAGCC TGGTATTTAT
1381 ACCAGCTACA ATGCAGCACT ACACGCCGCC GAAAATTATC CATGCTCACA GCCCAAGAAG
1441 TTCGACACTC GAGCCAAGGC AGAGGACTAC ATGGAAGAGT CCTTCACTTG CGCACCCGAG
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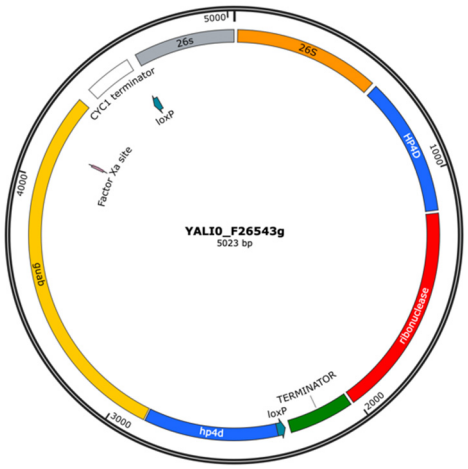
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26S
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loxP
Factor Xa site
HP4D
1000
YALI0_F26543g
5023 bp
4000
gluab
fibronuclease
TERMINATOR
loxP
2000
3000
hp4d

Sequence 11: YALI0_D20482g overexpression cassette

1-8: NotI

9-14: EcoRI

15-591: rDNA

592-597: Hind III

598-603: Sal I

604-609: SpeI

610-1158: hp4d

1159-1164: NdeI

1164-1170: KpnI

1171-1887: *YALI0_D20482g*

1888-1893: Kpn I

1894-1899: Pst I

1900-2157: terminator

2158-2163: Afl II

2164-2169: Nhe I

2170-2175: BmtI

2176-2209: loxP

2210-2741: hp4d

2742-2747: SacI

2748-4214: guab

4215-4220: XhoI

4221-4453: terminator

4454-4487: loxP

4488-4493: Nar I

4494-4895: rDNA

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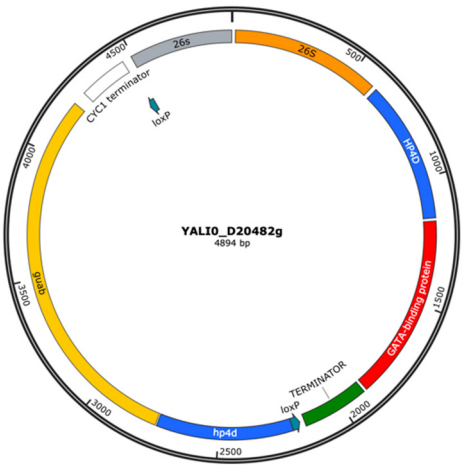
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TERMINATOR
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1500
2000
2500
3000
3500
4000
4500

Table S1. Strains, gene cassettes, and primers used in this study.

Strains/plasmids/primers	Properties (5′→3′)	Source
DH5α	*F⁻, endA1, hsdR17, recA1, supE44, thi-1, ϕ80lacZΔM15*	Purchasing from Promega Crop. (Madison, WI)
Y. lipolytica CGMCC7326	Suc2⁻, Lac⁻, Mal⁻	Cheng et al., 2018 [1]
HRC	Adaptive evolution in laboratory from CGMCC7326	This study
HRC1	Overexpression of YALI0_B21582g in CGMCC7326	This study
HRC2	Overexpression of YALI0_C13750g in CGMCC7326	This study
HRC3	Overexpression of YALI0_B10626gin CGMCC7326	This study
Primers	**Sequences (5′→3′)**	
P (B21582)-F	GAACCCGAAACTAAGCATATGGGTACCATGGACCTCGAATTGGAAAT	
P (B21582)-R	CTATCTGTTAATTGCCTGCAGGGTACCCTAGTCCCGAGGATGCGTACG	
P (B21582)-VF	CAGCAGATCCACATGCTGCAGCAG	
P (B21582)-VR	CGTGCGACTACCGTATTCGAACAC	
P (C13750)-F	GAACCCGAAACTAAGCATATGGGTACCATGACCACCTACGATCTCA	
P (C13750)-R	CTATCTGTTAATTGCCTGCAGGGTACCTTAACATCGCCGCTTCTGTC	
P (C13750)-VF	CGTTTGTTGGCATGAAGCTCAACTT	
P (C13750)-VR	CGTGCGACTACCGTATTCGAACAC	
P (B10626)-F	GAACCCGAAACTAAGCATATGGGTACCATGAAGCTATCATCAATTGCC	
P (B10626)-R	CTATCTGTTAATTGCCTGCAGGGTACCCTACGATAAAGCAACGAGAGC	
P (B10626)-VF	GATCTGTACGCTCCAGCTTAC	
P (B10626)-VR	CGTGCGACTACCGTATTCGAA	
P (B20768)-F	GAACCCGAAACTAAGCATATGGGTACCATGAAGTTCAGCGGTCTCGTT	
P (B20768)-R	CTATCTGTTAATTGCCTGCAGGGTACCCTAGAAGAAGAAGGCCAAGAA	
P (B20768)-VF	CTGCTAAGAAGAAGCACAGATGTC	
P (B20768)-VR	CGTGCGACTACCGTATTCGAACAC	
P (D07898)-F	GAACCCGAAACTAAGCATATGGGTACCATGCTTCTTTCTAATATTTTC	
P (D07898)-R	CTATCTGTTAATTGCCTGCAGGGTACCTTAGTCGTCCTTGTGGTGCTT	
P (D07898)-VF	ATGCTTCTTTCTAATATTTTCACC	
P (D07898)-VR	CGTGCGACTACCGTATTCGAACA	
P (B19866)-F	GAACCCGAAACTAAGCATATGGGTACCATGACTTCTTCTACTACCAAC	
P (B19866)-R	CTATCTGTTAATTGCCTGCAGGGTACCCTAGTGGTGACTCCACTCACT	
P (B19866)-VF	AAGGCCAAAAAGCTCGGCTTTG	
P (B19866)-VR	CGTGCGACTACCGTATTCGAACA	
P (F26543)-F	GAACCCGAAACTAAGCATATGGGTACCATGTGTGGGGAACTCGTGTGT	
P (F26543)-R	CTATCTGTTAATTGCCTGCAGGGTACCCTAAGGCTTCCAAACTGACTC	
P (F26543)-VF	ACGTTCAGACCAACCAGAGAGCTG	
P (F26543)-VR	CGTGCGACTACCGTATTCGAACAC	
P (D20482)-F	GAACCCGAAACTAAGCATATGGGTACCATGAACCAGAATCTGACGGTG	

P (D20482)-R	CTATCTGTTAATTGCCTGCAGGGTACCCTATAAAGCCATGCTCAGCCA
P (D20482)-VF	CTGAGGGTGAGCCGTTGTGCAACG
P (D20482)-VR	CGTGCGACTACCGTATTCGAACAC
P (C04279)-F	GAACCCGAAACTAAGCATATGGGTACCATGATCTTTAGCAACATTCTC
P (C04279)-R	CTATCTGTTAATTGCCTGCAGGGTACCCTAAGGCTTGAAGGGGACGGC
P (C04279)-VF	TAAGGGACGAACCTACGATGTCA
P (C04279)-VR	CGTGCGACTACCGTATTCGAACAC
P (F01452)-F	GAACCCGAAACTAAGCATATGGGTACCATGAACCAGAACATGCAACGG
P (F01452)-R	CTATCTGTTAATTGCCTGCAGGGTACCCTACCACGCTCCAACCATGCT
P (F01452)-VF	GGGGTATTCCCGAGGACGAGGTGC
P (F01452)-VR	CGTGCGACTACCGTATTCGAACAC

Table S2 Ten selected genes which are overexpressed in *Y. lipolytica* CGMCC7326

gene	A	B	C	D	E
XP501434(yapsin 1/2) YALI0_C04279g	1.38 ±0.05	6.29 ±0.23	23.40 ±0.24	1.00	1.25 ±0.06
XP504861(chitinase) YALI0_F01452g	5.48 ±0.22	17.31 ±1.07	18.11 ±0.81	1.00	1.52 ±0.13
XP501114(structural maintenance of chromosome) YALI0_B19866g	3.51 ±0.11	5.80 ±0.24	42.46 ±1.57	1.00	0.61 ±0.03
XP501151(acid phosphatase type 7) YALI0_B20768g	6.28 ±0.17	15.83 ±0.69	36.09 ±1.82	1.00	2.02 ±0.09
XP503074(GATA-binding protein 5) YALI0_D20482g	0.41 ±0.02	30.77 ±1.60	32.68 ±1.39	1.00	2.66 ±0.14
XP502553(mucin-3/17; K10955//mucin-2; K01216) YALI0_D07898g	1.54 ±0.07	19.84 ±0.81	29.78 ±0.93	1.00	2.20 ±0.16
XP505912(ribonuclease HI) YALI0_F26543g	5.04 ±0.14	50.86 ±1.02	16.26 ±0.60	1.00	0.68 ±0.01
XP500729(flocculation protein FLO11+ko04011//MAPK signaling pathway - yeast) YALI0_B10626g	3.63 ±0.04	3.78 ±0.12	19.83 ±0.43	1.00	1.13 ±0.04
XP501188（Msn2 like protein）YALI0_B21582g	0.28 ±0.01	2.24 ±0.11	16.55 ±0.72	1.00	1.65 ±0.09
XP501804(transcription factor STE12) YALI0_C13750g	3.86 ±0.09	5.12 ±0.29	5.02 ±0.31	1.00	1.42 ±0.09

Relative expression level of 10 genes

Table S3 relative expression level of 22 genes

gene	A	B	C	D	E
1. XP502012(heat shock protein 90kDa beta+ko04141) YALI0_C19404g	3.03 ±0.17	3.35 ±0.16	3.08 ±0.25	1.00	1.42 ±0.06
2. XP501399(HSP20 family protein+ko04141) YALI0_C03465g	1.47 ±0.10	2.84 ±0.04	2.49 ±0.13	1.00	0.44 ±0.02
3.XP504554（heat shock 70kDa protein ）YALI0_E29491g	2.39 ±0.04	3.96 ±0.06	6.84 ±0.28	1.00	1.13 ±0.05
4. XP503922（heat shock transcription factor）YALI0_E13948g	0.90 ±0.05	2.38 ±0.04	1.88 ±0.06	1.00	1.76 ±0.08
*5. XP505273（hsp70-interacting protein+ko04141）YALI0_F11121g	1.99 ±0.04	1.64 ±0.06	1.41 ±0.06	1.00	0.88 ±0.02
*6. XP504564(proton-dependent transporter) YALI0_E29711g	2.12 ±0.02	2.43 ±0.04	8.56 ±0.23	1.00	1.21 ±0.06
*7. XP500382(MFS transporter, SP family, sugar:H+ symporter+ko04113//Meiosis -) YALI0_B01342g	3.42 ±0.06	4.17 ±0.14	5.67 ±0.46	1.00	0.83 ±0.05
*8. XP502309(solute carrier family 66 (lysosomal lysine-arginine transporter)) YALI0_D02046g	3.05 ±0.05	7.33 ±0.23	5.29 ±0.06	1.00	1.86 ±0.09
9. XP_504809 agglutinin (YALI0_F00220g)	0.31 ±0.01	2.74 ±0.11	4.35 ±0.17	1.00	1.09 ±0.05
10. XP501434(yapsin 1/2) YALI0_C04279g	1.38 ±0.05	6.29 ±0.23	23.40 ±0.24	1.00	1.25 ±0.06
11. XP504861(chitinase) YALI0_F01452g	5.48 ±0.22	17.31 ±1.07	18.11 ±0.81	1.00	1.52 ±0.13
12. XP501114(structural maintenance of chromosome) YALI0_B19866g	3.51 ±0.11	5.80 ±0.24	42.46 ±1.57	1.00	0.61 ±0.03
13. XP501151(acid phosphatase type 7) YALI0_B20768g	6.28 ±0.17	15.83 ±0.69	36.09 ±1.82	1.00	2.02 ±0.09
14. XP503074(GATA-binding protein 5) YALI0_D20482g	0.41 ±0.02	30.77 ±1.60	32.68 ±1.39	1.00	2.66 ±0.14
15. XP502553(mucin-3/17; K10955//mucin-2; K01216) YALI0_D07898g	1.54 ±0.07	19.84 ±0.81	29.78 ±0.93	1.00	2.20 ±0.16
16. XP505912(ribonuclease HI) YALI0_F26543g	5.04 ±0.14	50.86 ±1.02	16.26 ±0.60	1.00	0.68 ±0.01
17. XP506120(glucose transport transcription regulator RGT1) YALI0_F32065g	4.52 ±0.04	11.65 ±0.55	5.17 ±0.23	1.00	2.16 ±0.08
18. XP499684(phosphatidylinositol phospholipase C) YALI0_A02288g	3.42 ±0.18	4.71 ±0.24	5.61 ±0.32	1.00	1.52 ±0.11
19. XP500595(glycerol 2-dehydrogenase)) YALI0_B07117g	1.33 ±0.07	12.97 ±0.71	12.63 ±1.13	1.00	2.25 ±0.14
20.XP500729(flocculation protein FLO11+ko04011//MAPK signaling pathway - yeast) YALI0_B10626g	3.63 ±0.04	3.78 ±0.12	19.83 ±0.43	1.00	1.13 ±0.04
21. XP501188（Msn2 like protein）YALI0_B21582g	0.28 ±0.01	2.24 ±0.11	16.55 ±0.72	1.00	1.65 ±0.09
22. XP501804(transcription factor STE12) YALI0_C13750g	3.86 ±0.09	5.12 ±0.29	5.02 ±0.31	1.00	1.42 0.09

* Membrane proteins

Relative expression level of 22 genes

Table S4 13 genes were significant upregulated in HRC strains at 30°C compared with the *Y. lipolytica* CGMCC7326

gene	A	D
XP502012(heat shock protein 90kDa beta+ko04141) YALI0_C19404g	3.03 ±0.17	1.00
XP504554（heat shock 70kDa protein ）YALI0_E29491g	2.39 ±0.04	1.00
XP504564(proton-dependent transporter) YALI0_E29711g	2.12 ±0.02	1.00
XP500382(MFS transporter, SP family, sugar:H+ symporter+ko04113//Meiosis -) YALI0_B01342g	3.42 ±0.06	1.00
*XP502309(solute carrier family 66 (lysosomal lysine-arginine transporter)) YALI0_D02046g	3.05 ±0.05	1.00
XP504861(chitinase) YALI0_F01452g	5.48 ±0.22	1.00
XP501114(structural maintenance of chromosome) YALI0_B19866g	3.51 ±0.11	1.00
XP501151(acid phosphatase type 7) YALI0_B20768g	6.28 ±0.17	1.00
XP505912(ribonuclease HI) YALI0_F26543g	5.04 ±0.14	1.00
XP506120(glucose transport transcription regulator RGT1) YALI0_F32065g	4.52 ±0.04	1.00
XP499684(phosphatidylinositol phospholipase C) YALI0_A02288g	3.42 ±0.18	1.00
XP500729(flocculation protein FLO11+ko04011//MAPK signaling pathway - yeast) YALI0_B10626g	3.63 ±0.04	1.00
XP501804(transcription factor STE12) YALI0_C13750g	3.86 ±0.09	1.00

* Membrane proteins
Relative expression level of 13 genes

1. Cheng, H., et al., *Identification, characterization of two NADPH-dependent erythrose reductases in the yeast Yarrowia lipolytica and improvement of erythritol productivity using metabolic engineering*. Microb Cell Fact, 2018. **17**(1): p. 133.