附件

附表 1 重组反应体系

Supplementary Tab.1 Recombination System

组分 Component	加入量
2×Seamless cloning Master Mix	10 μL
线性化载体	50-100 ng
插入片段	X ng
ddH_2O	to 20 μL
PCR 仪上 50 ℃反应 20 min。反应结束后，立即将离心管置于冰上冷却 2 min，待转化	

附表 2 醋酸锂转化体系配比

Supplementary Tab.2 Lithium acetate conversion system ratio

试剂	体积
PEG3350（50%（W/V 过滤除菌））	240ul
LiAc 1.0M（过滤除菌）	36 ul
SSDNA（（鲑鱼精 DNA）10mg/mL）	10 ul
DNA 片段和水（质粒 2.5ul）	74 ul
Total	360ul

注：进行多片段共转化时，按每个片段加入 400ng 混合混匀即可

Note : When performing multi-fragment co-transformation, 400 ng of each fragment is added to mix well.

附表 3 底盘片段克隆引物序列表

Supplementary Tab.3 Chassis Fragment Clone Primer Sequence List

基因 Gene	上游引物 Upstream primer（5F'→3'）	下游引物 Downstream primer（5F'→3'）
ADH1	ACTAGTTAGTAGATGATAGTTGATTTCTATTCCAACA	GTTTGAAAGATGGGTCCGTCACCTGCATTA
	GCATGCCGGTAGAGGTGTGGTC	AATCCTAAAGTTATAAAAAAAATAAGTGT
tHMG1	ACTTTAAAATTTGTATACACTTATTTTTTTTATAACTTT	CACAAAAACAAAAAGTTTTTTTAATTTTAA
	AGGATTTAATGCAGGTGACG	TCAAAAAATGGCTGCAGACCAATTGGTGA
HXT7	GGTGACTTCAGTTTTCACCAATTGGTCTGCAGCCATT	TACGCTTGACATCTACTATATGTAAGTATAC
	TTTTGATTAAAATTAAAAAAAC	GGCCCCCTTCTCGTAGGAACAATTTCG
TEF2	GAACACGCAGGGGCCCGAAATTGTTCCTACGAGAA	ATCGAATGGAGAAACCTTAACTGAAGAGG
	GGGGGCCGTATACTTACATATAG	ACTGCATGTTTAGTTAATTATAGTTCGTTG
VvCPR	AATATACGGTCAACGAACTATAATTAACTAAACATGC	AAATCATTAAAGTAACTTAAGGAGTTAAAT
	AGTCCTCTTCAGTTAAGGTTTC	TTATCAGACATCCCTCAAGTATCTACCAG
TDH2	GGTAGATACTTGAGGGATGTCTGATAAATTTAACTCC	CAGTGATATGCATATGGGAGATGGAGATGA
	TTAAGTTACTTTAATGATTTAG	TACCTGCGAAAAGCCAATTAGTGTGATAC
ENO2	TAGTATCACACTAATTGGCTTTTCGCAGGTATCATCTC	ATACAGAGTTATTCCAGGCATCTACTGAGT
	CATCTCCCATATGCATATCAC	GCTTTTAACTAAGAATTATTAGTCTTTTC
StDWF5	GCAGAAAAGACTAATAATTCTTAGTTAAAAGCACTC	AATCTATAACTACAAAAAACACATACATAA
	AGTAGATGCCTGGAATAACTCTG	ACTAAAAATGGCCGAATCTCAATTGGTTC
TPI1	GATGAACCAATTGAGATTCGGCCATTTTTAGTTTATG	CTGCTCACAAATCTTAAAGTCATACATTGC
	TATGTGTTTTTTGTAGTTATAG	ACGACTATATATCTAGGAACCCATCAGGT
GPM1	AATCTTCCACCAACCTGATGGGTTCCTAGATATATAG	CAAACCTGCACCTAAAGACCAAACAGCTG
	TCGTGCAATGTATGACTTTAAG	ACATTTATTGTAATATGTGTGTTTGTTTGG

GgDHCR24	TCTTAATAATCCAAACAAACACACATATTACAATAAA	TTGTCTAACTCCTTCCTTTTCGGTTAGAGC
	TGTCAGCTGTTTGGTCTTTAGG	GGATTTAATGTCTTGCAGCCTTACAGATC
CYC1	GTTTACGATAAGATCTGTAAGGCTGCAAGACATTAAA	TGCAAATGCCTATTATGCAGATGTTATAATA
	TCCGCTCTAACCGAAAAGGAAG	TCTGTGCGTAGTCGAGCGTCCCAAAACC
PGK1p	CTGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCG	CGGATGCAATGCCAATTGTAATAGCTTTCC
	ACTACGCACAGATATTATAACATC	CATTGTTTTATATTTGTTGTAAAAAGTAG
ERG9	GGAAGTAATTATCTACTTTTTACAACAAATATAAAAC	ACTCATTAAAAAACTATATCAATTAATTTG
	AATGGGAAAGCTATTACAATTGG	AATTAACTTCACGCTCTGTGTAAAGTGTAT
FBAI	TTGGGTTTTATTATATATACACTTTACACAGAGCGTGA	ACTTCTTGTTGTTGACGCTAACATTCAACG
	AGTTAATTCAAATTAATTGATA	CTAGTATAGTAAGCTACTATGAAAGACTT
TDH3	TTCGAGTTCTTTGTAAAGTCTTTCATAGTAGCTTACTA	CGATTGTGTCAGAATAAAATTCTGTCATTT
	TACTAGCGTTGAATGTTAGCG	TTGTTTGTTTATGTGTGTTTATTCGAAAC
ERG7	GTTTCGAATAAACACACATAAACAAACAAAAATGAC	GTTCTTTAGGTATATATTTAAGAGCGATTTG
	AGAATTTTATTCTGACACAATCG	TTTTAAAGCGTATGTGTTTCATATGCCC
GPT	GTACAGCAGGGCATATGAAACACATACGCTTTAAAA	GCGTATTTTAAGTTTAATAACTCGAAAATT
	CAAATCGCTCTTAAATATATACC	CTGCGTTGGTATACTGGAGGCTTCATGAG
T*PGK1t*	GAAGGACATAACTCATGAAGCCTCCAGTATACCAAC	AAGAGATTGCCAATTAAGGTTAGACCATT
	GCAGAATTTTCGAGTTATTAAAC	GGGTGACGATCTCCCATGTCTCTACTGGTG
VcCYP90B27	GAAGCACCACCACCAGTAGAGACATGGGAGATCGT	ATAGCAATCTAATCTAAGTTTTAATTACAA
	CACCCAATGGTCTAACCTTAATTG	AATGGCTATGGAATTGTTGTTATTGATTC
VnigCYP063	GAAGCACCACCACCAGTAGAGACATGGGAGATCTC	AGAAAGCATAGCAATCTAATCTAAGTTTTA
	AGTCCCCGAGTTTTTCGA	ATTACAAAATGGCGATGGAGCTCCTCTTG
TEF1	GCTGGAATCAATAACAACAATTCCATAGCCATTTTGT	ctcttgcatcttacgatacctgagtattcccacagttAGTGATCC
	AATTAAAACTTAGATTAGATTG	CCCACACACCATAG
LEU2	TAGAAACATTTTGAAGCTATGGTGTGTGGGGGATCA	TAACTCTCAACAGACAACAACACCTGCTT
	CTaactgtgggaatactcaggta	CATttaagcaaggattttcttaacttcttc
Down	tgctgtcgccgaagaagttaagaaaatccttgcttaaATGAAGCAGGTGT	AGGAGAACTTCTAGTATATTCTGTATACC
	TGTTGTCTG	
TDH2	GGTAGATACTTGAGGGATGTCTGATAAATTTAACTCC	CAGTGATATGCATATGGGAGATGGAGATGA
	TTAAGTTACTTTAATGATTTAG	TACCTGCGAAAAGCCAATTAGTGTGATAC
StDWF5	GCAGAAAAGACTAATAATTCTTAGTTAAAAGCACTC	AATCTATAACTACAAAAAACACATACATAA
	AGTAGATGCCTGGAATAACTCTG	ACTAAAAATGGCCGAATCTCAATTGGTTC

注：小写字母表示基因同源臂序列
Note : Lowercase letters represent gene homologous arm sequences

附表 4 底盘片段融合引物序列表
Supplementary Tab.4 Chassis fragment fusion primer sequence table

模块表达盒 Module expression box	上游引物 Upstream primer（5F'→3'）	下游引物 Downstream primer（5F'→3'）
F1:UP-ADH1-HMG1-XHT7	GGATATAGGAATCCTCAAAATG	TACGCTTGACATCTACTATATGTAAGTAT ACGGCCCCCTTCTCGTAGGAACAATTTC G
F2 :TEF2-VnCPR1-TDH2 TEF2-VgCPR3-TDH2	GAACACGCAGGGGCCCGAAATTGTTC CTACGAGAAGGGGGCCGTATACTTACA TATAG	CAGTGATATGCATATGGGAGATGGAGAT GATACCTGCGAAAAGCCAATTAGTGTGA TAC

F3:ENO2-StDWF5-TPI-GPM1	TAGTATCACACTAATTGGCTTTTCGCA GGTATCATCTCCATCTCCCATATGCATA TCAC	CAAACCTGCACCTAAAGACCAAACAGC TGACATTTATTGTAATATGTGTGTTTGTT TGG
F4:GgDHCR24-tCYC1-PGK1p	TCTTAATAATCCAAACAAACACACATA TTACAATAAATGTCAGCTGTTTGGTCT TTAGG	CGGATGCAATGCCAATTGTAATAGCTTT CCCATTGTTTTATATTTGTTGTAAAAAGT AG
F5:ERG9-FBA1-TDH3	GGAAGTAATTATCTACTTTTTACAACA AATATAAAACAATGGGAAAGCTATTAC AATTGG	CGATTGTGTCAGAATAAAATTCTGTCAT TTTTGTTTGTTTATGTGTGTTTATTCGAA AC
22-F6:ERG7	GTTTCGAATAAACACACATAAACAAA CAAAAATGACAGAATTTTATTCTGACA CAATCG	ctcttgcatcttacgatacctgagtattcccacagttGGTATA CTGGAGGCTTCATGAG
F7:PGT-tPGK1-VcCYP90B27 PGT-tPGK1-VnCYP90B27-1	GTACAGCAGGGCATATGAAACACATA CGCTTTAAAACAAATCGCTCTTAAATA TATACC	ATAACTACAAAAAACACATACATAAACT AAAAATGACTCCATTGGTCGTGTTATTC TTC
F8:TEF1-LEU2-Down	GCTGGAATCAATAACAACAATTCCATA GCCATTTTGTAATTAAAACTTAGATTA GATTG	AGAAAGCATAGCAATCTAATCTAAGTTT TAATTACAAAATGGCGATGGAGCTCCTC TTG

注：小写字母表示基因同源臂序列
Note : Lowercase letters represent gene homologous arm sequences

附表 5 模块表达盒信息表

Supplementary Tab.5 Module expression box information table

名称 Name	组成 Composition	长度 Length（bp）
Fragment 1（F1）	UP-ADH1-HMG1-XHT7	2374
Fragment 2（F2）	TEF2-VvCPR-TDH2	3085
	TEF2-VnCPR1-TDH2	3085
	TEF2-VgCPR3-TDH2	3088
	TEF2-ATR-TDH2	3085
Fragment 3（F3）	ENO2-StDWF5-TPI-GPM1	2886
Fragment 4（F4）	GgDHCR24-tCYC1-PGK1p	2493
Fragment 5（F5）	ERG9-FBA1-TDH3	2538
Fragment 6（F6）	ERG7	2196
Fragment 7（F7）	PGT-tPGK1-VcCYP90B27	2118
	PGT-tPGK1-VnCYP90B27-1	
Fragment 8（F8）	TEF1-LEU2-Down	2175

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CAAACGGGACGTCGACCAAACCGCTGAAACTCCCGCCGGGCCAAATGGGTTGGCCTTTCATCGGCTACACC
ATCCCCTTCATGCAGCCCCACTCCTCCGCGTCCCTCGGCCCCTACATCGACCTCAACACCGCTAGGTATGGGA
CCATCTTTCGGATGAACTTGTTGGCGAAACCGACGATTGTGTCGGCGGATCCGGAGTTCAACCGGTACATAC
TGCAGAACGAGGGCCGGCTTTTCGAGAACAGCTGCCCGACGAGCATCGCGGAGATCATGGGCCGTTGGTC
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TCTAAAGCATCGCCCAGTGAAATCACTAGGACCTTTCATGGAACAACACATATCAAGGTACGGGAAGATCTT
CTCATCGAACCTGTTAGGGAAGCAGGTAGTAATCACGGCCGACCCGGAGCTGAACCGATTCATACTGAAGA
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CTCATACTGAACTATGAGTGGGAGTCCGTAGAGCCTGACCATCCTGTGAGCATACCGGTCATCAAGTTCCCTA
AAGGACTACAAATTAGGGTCAGGGCCCTGCCAAATGAGATGGAGATGATGTAG