

INFORMATION of PLASMIDS pJPPP3&PJPPP4

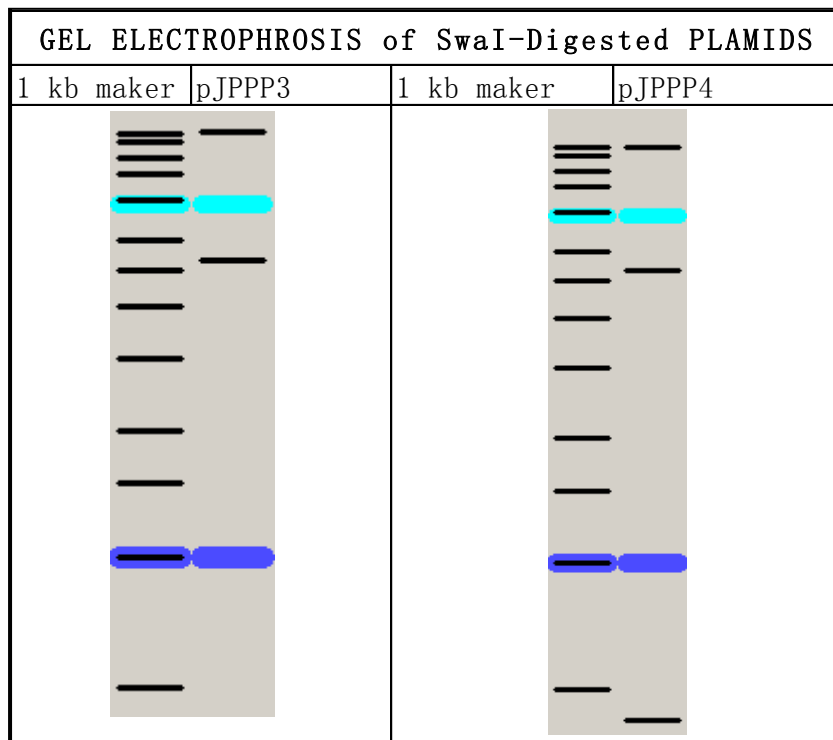
Constructor: 彭炳银

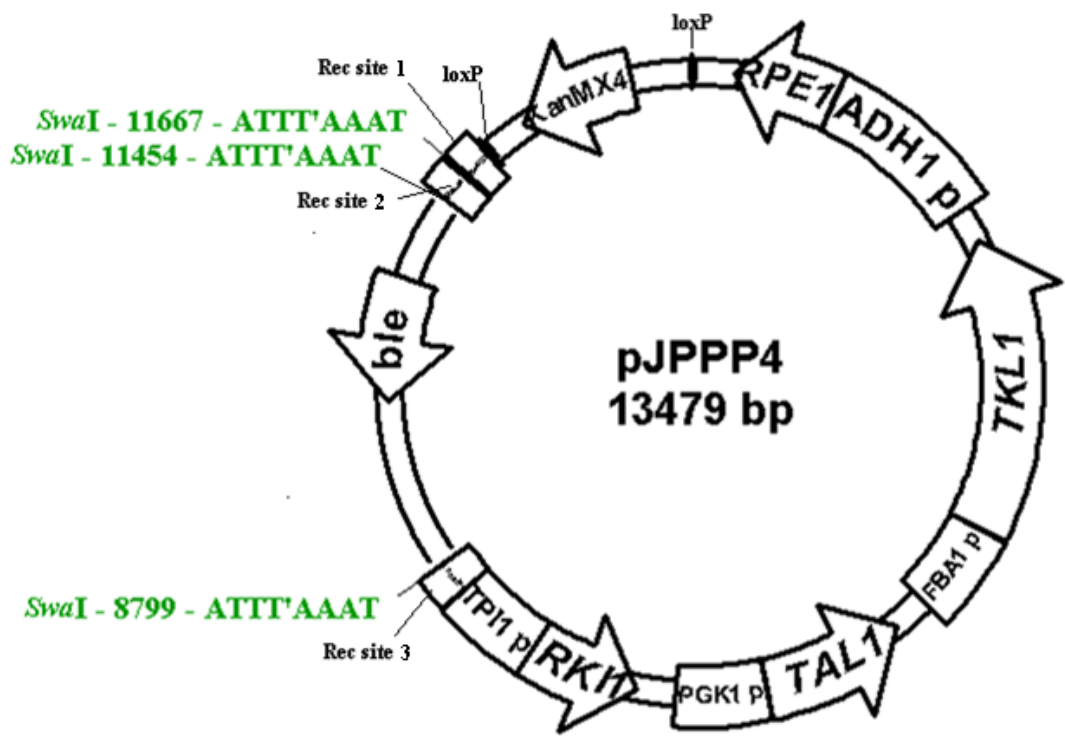
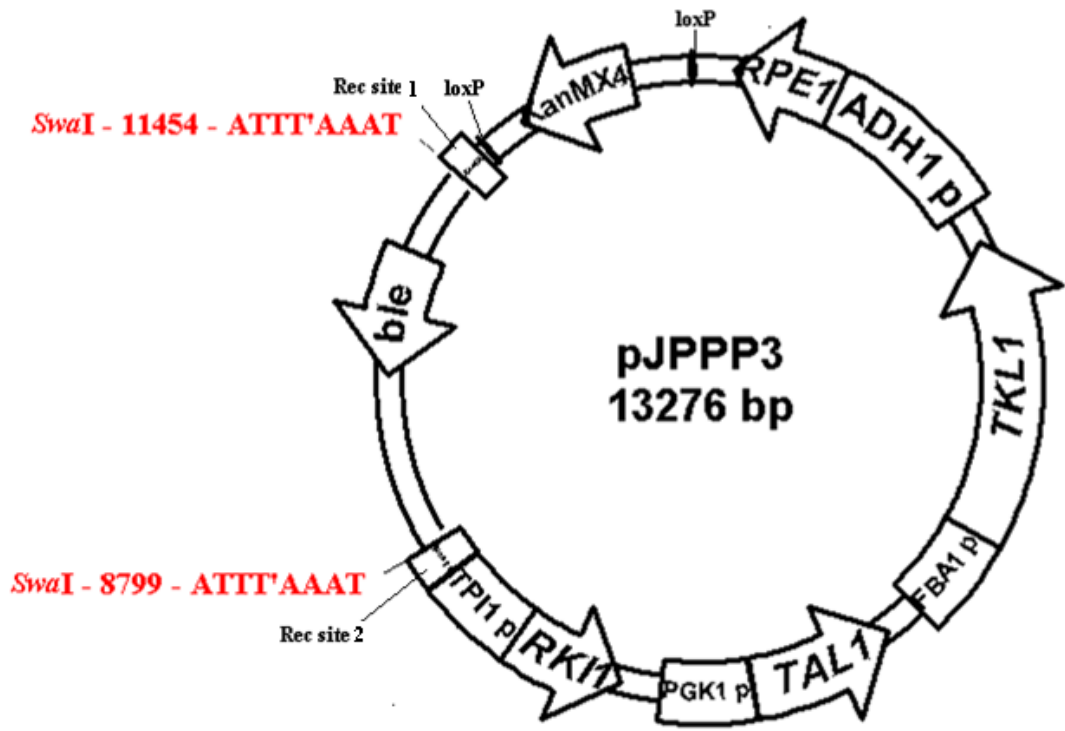
ELEMENTS of the PLASMIDS							
Plamsid	Element	From	To	Plamsid	Element	From	To
pJPPP3	bla	9989	10847	pJPPP4	bla	9989	10847
	M13F	11427	11445		M13F	11427	11445
	M13R	8827	8844		M13R	8827	8844
	Recsit1	8575	8799		Recsit1	8575	8799
	Recsit2	11451	11655		Recsit2	11451	11655
	RKI1	7180	7954		RKI1	7180	7954
	TPI1 p	7955	8568		TPI1 p	7955	8568
	TAL1	5274	6279		TAL1	5274	6279
	PGK1 p	6280	6907		PGK1 p	6280	6907
	TKL1	2354	4394		TKL1	2354	4394
	FBA1 p	4395	5024		FBA1 p	4395	5024
	RPE1	234	948		RPE1	234	948
	ADH1 p	949	2113		ADH1 p	949	2113
	KanMX4	11996	12803		KanMX4	12199	13006
	loxP	11695	11719		RecSit3	11676	11873
	loxP	13202	13236		loxP	11898	11922
				loxP	13405	13439	

Constructing information: To over-express non-oxidative phosphate pathway genes, *RPE1*, *TKI1*, *TAL1*, and *TKL1*, two plasmids pJPPP3 and pJPPP4 were constructed. pJPPP3 was designed for manipulating on the chromosome of laboratory strain and the first homogenous chromosome of diploid industrial strain. pJPPP4 was designed to integrate into the second homogenous chromosome of diploid industrial strain. The sequences of *RPE1*, *TKI1*, *TAL1*, and *TKL1* with native terminators, the fragments of *TPI1*, *ADH1*, *PGK1*, *FBA1* promoters and the recombinant arms 1/2/3 targeting GRE3 locus were amplified from CEN.PK113-5D genome. And KanMX4-loxP selective marker was amplified from plasmid

pUG6. And over-lap PCR was done to fuse fragments of recombinant arm A with recombinant arm B, *RPE1* with *TPI1* promoter, *TAL1* with *PGK1* promoter, *TKL1* with *FBA1* promoter, and selective marker KanMX4-loxP with recombinant arm C. The primers (listed below) were designed with relative restriction endonuclease sites. The construction procedure was referred to the below Figure.

How to Use: Before transforming yeasts, digest the plasmids with *SmaI* (*SwaI*). The plasmids have the recombinant sites targeting to *GRE3* loci. The locus for pJPPP3 is ***gre3(-241, +338)***, and for pJPPP4 is ***gre3(-241, +113)***.





pJPPP3 Sequence ..

1 GATCCGAAGC CTTATGGAGT GTCATTGACG ATACTGCATC CCAGGGATTG

51 ATCATCGAAA ACGGACAAGG CACCACCCTG GGCGGCCCAT TTTCCCATG
101 AGTTAGGCAC TTACGTATCT TGTATAGTAG GAATGGCTCG GTTTATGTAT
151 ATTAGGAGAT CAAAACGAGA AAAAAATACC ATATCGTATA GTATAGAGAG
201 TATAAATATA AGAAATGCCG CATATGTACA ACTAATCTAG CAAATCTCTA
251 GAACGCAATT CCTTCGAGAC TTCTTCTTTC ATGAAGGAGA TAACATCGTG
301 CGGGTCAGCT GCAGTGAAAA CACTGGTACC AGCGACAATA ACGTTGGCAC
351 CGGCTTTGGC GGCTTTCGGG ATGGTCTCCT TGCCCAAACC ACCATCGACT
401 TGGATATTCA AATGGGGGAA CTTGGCTCTC AAAGTTTCCA CTTTTGGCAT
451 CATGTCTTCC ATGAATTTTT GGCCTCCAAA CCCAGGTTC ACAGTCATAA
501 CAAGAGCCAT ATCCAAATGA GGAGCTAGTT CAAATAAAAC GTCAACAGAA
551 GTACCAGGTT TGATGGCGCA TGCAGCTTTG ATGCCCTTAG ACTTAATCAA
601 CTTAACTAAA TGCAAAGGGT CTTGTGTGGC CTCGTAGTGG AACGTAAATT
651 GGTCAGCACC ACATTTAGCA AAATCGTCGA CCCATTTTTC AGGATTTTCA
701 ACCATCATGT GACAATCGAA GAACGCAGTG GGCTTCTTTT CTGTGTTGCT
751 AGCATCGCCA GGGCGTGGCA CAGAACGACG TAGGGAGGTA ACAATTGGTT
801 GGCCCAGAGT AATGTTTGGG ACAAATGGC CGTCCATGAC ATCGATATGT
851 AACCAATCTG CGCCGGCGTT GATGACCTTA TGACATTCGC AACCCAAGTT
901 GGCGAAGTCA GAAGCAAGGA TACTGGGAGC TATAATTGGT TTGACCATTG
951 TATATGAGAT AGTTGATTGT ATGCTTGGTA TAGCTTGAAA TATTGTGCAG
1001 AAAAAGAAAC AAGGAAGAAA GGGAACGAGA ACAATGACGA GGAAACAAAA
1051 GATTAATAAT TGCAGGTCTA TTTATACTTG ATAGCAAGAC AGCAAACCTT
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1151 ATTAGAGAGT AGTGTGCGTG AATGAAGGAA GGAAAAAGTT TCGTGTGCTT
1201 CGAGATACCC CTCATCAGCT CTGGAACAAC GACATCTGTT GGTGCTGTCT
1251 TTGTCGTAA TTTTTCCTT TAGTGTCTTC CATCATTTTT TTGTCATTGC
1301 GGATATGGTG AGACAACAAC GGGGGAGAGA GAAAAGAAAA AAAAAGAAAA
1351 GAAGTTGCAT GCGCCTATTA TTA CT TCAAT AGATGGCAA TGGAAAAAGG
1401 GTAGTGAAAC TTCGATATGA TGATGGCTAT CAAGTCTAGG GCTACAGTAT
1451 TAGTTCGTAA TGTACCACCA TCAATGAGGC AGTGTAATTG GTGTAGTCTT
1501 GTTTAGCCCA TTATGTCTTG TCTGGTATCT GTTCTATTGT ATATCTCCCC
1551 TCCGCCACCT ACATGTTAGG GAGACCAACG AAGGTATTAT AGGAATCCCG
1601 ATGTATGGGT TTGGTTGCCA GAAAAGAGGA AGTCCATATT GTACACCCGG
1651 AAACAACAAA AGGATATCCG AAATATTCCA CGGTTTAGAA AAAAATCGGA
1701 AAAGAGCGCG GAGGGGTGTT ACCCCCCTTC TCTACTAGCA TTGGACTTTA
1751 ATTAATATAT GTGCATAGGA GAAGTGTAAG GTTCCCTTCC ATATTGTAAC
1801 ATAATAAAGT GCACACCCAA ATGAATTGAA AGCGTACTCA AACAGACAAC
1851 CATTTCCAGT GTTGTATGTA CCTGTCTATT TATACTGGTA GCAACCCTAT
1901 TGCTGTTTCC TCTTCAAAGT ACTCTAGCGG TTATGCGCGT CTCACCTTCA
1951 AGGTCATGGT CGCTCTATTG TTCGCACCAC CGGCAA ACTC GCGTCTCGCA
2001 AGTCTTGGCT CATTCTTCTA GTATACTCAT GTTGCAAATG CACTCAGGTT
2051 CTTTCGGCAA CT TAAATAAT GACACCAGTT GTCGTGGTCG TCATCATCGC
2101 AACCCCAACC GGCAGATCCA TGTGCTGGTA CAAGGAAAAA GAAAGAAAAA
2151 GTAATCATTG AAATTTTTCT ATAATACGTA CACCTATAAT ATAAGAAGGA
2201 AAAGGACTGA GTGTTTGAA TATTGT TAAA AGTTTGAAAC TATTTTTTTG

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2301 CATTTTTTCA CAAATAATAT CATATCAAAT CTGATGATCT ACGATCAGAA
2351 TTTAGAAAGC TTTTTTCAAA GGAGAAATTA GCTTGTCACC CTTATAGAAT
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3251 CGGCAGAACC ACCAATCAAC TCTGGCAATT GATTGTAAAC ATCCTCAAGA
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13201 TTTGCTTCGC GCCGTGCGGC CATCAAAATG TATGGATGCA AATGATTATA
13251 CATGGGGATG TATGGGCTAA ATGTACGGGC GACAGTCACA TCATGCCCCT
13301 GAGCTGCGCA CGTCAAGACT GTCAAGGAGG GTATTCTGGG CCTCCATGTC
13351 GCTGGCCGGG TGACCCGGCG GGGACGAGGC AAGCTAAACA GATCTCTAGA
13401 CCTAATAACT TCGTATAGCA TACATTATAC GAAGTTATAT TAAGGGTTGT
13451 CGACCTGCAG CGTACGAAGC TTCAGCTGG