

# INFORMATION of PLASMIDS pJFE1 & pJFE2 & pJFE3

Constructor: 彭炳银

ADVISED USING OF RESTRICTION ENZYME SITES					
Vector	Integrating between	Vector digested by	Expressed gene		
			Before ATG	After TAA	
pJFE1	TEF1 promoter and PGK1 terminator	BamHI/XbaI	BamHI	XbaI	
			BclI	SpeI	
			BglII	BlnI	
		BamHI/PstI		NheI	
			BamHI	PstI	
			BclI	SbfI	
pJFE3	TEF1 promoter and PGK1 terminator	BamHI/XbaI	BglII	EcoT22I	
			BamHI	XbaI	
			BclI	SpeI	
		BamHI/PstI	BglII	NheI	
			BamHI	PstI	
			BclI	SbfI	
pJFE2	TEF1 promoter and PGK1 terminator	BamHI/PstI	BglII	EcoT22I	
			BamHI	PstI	
			BclI	SbfI	
	TDH3 promoter and CYC1 terminator	XbaI/SphI	BglII	EcoT22I	
			XbaI	SphI	
			SpeI		
XbaI/XhoI	XbaI/XhoI	NheI			
		BlnI			
		XbaI	XhoI		
			SpeI	Sall	
			NheI		
			BlnI		

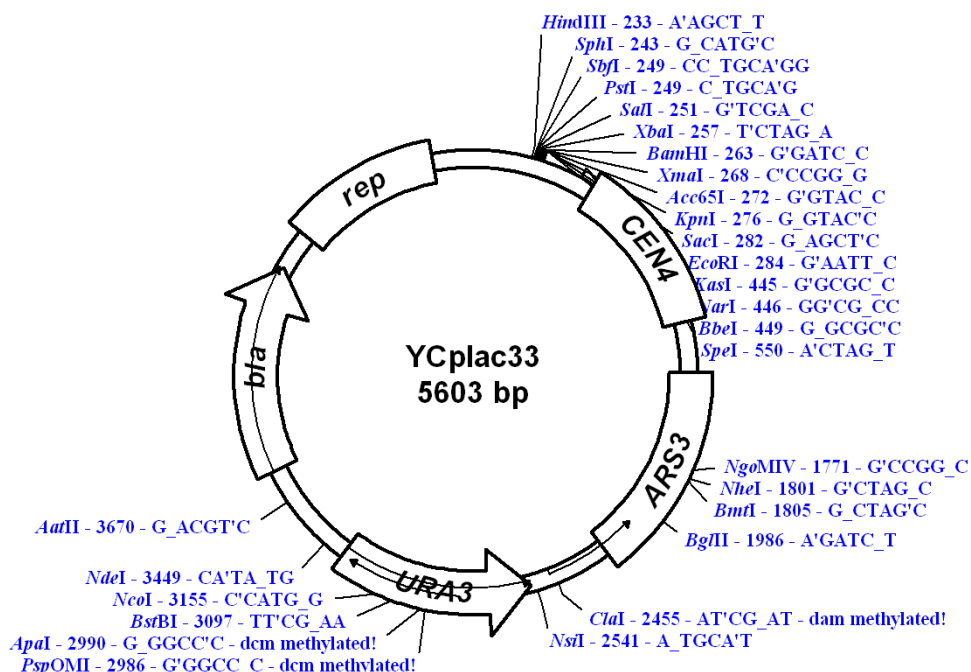
**Constructing information:** To construct pJFE1 and pJFE3, PGK1 terminator was cloned into HindIII/PstI sites of YCplac33 and YEplac195. The TEF1 promoter was cloned into KpnI/BamHI sites. To construct pJFE2, the XbaI site of pJFE1 was deleting. Then CYC1 terminator was cloned into KpnI-EcoRI sites. Then TDH3 terminator was

cloned into SphI-EcoRI sites.

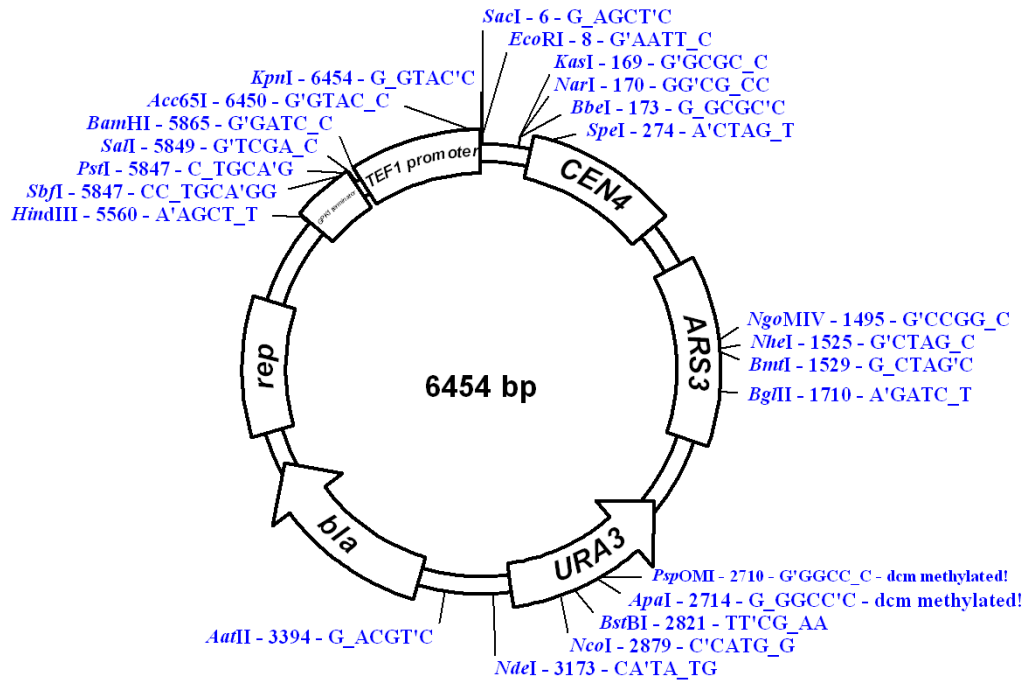
The primers used to amplify the above fragments were listed below.

PRIMERS USED IN PCR			
No.	Amplified Fragment	Primer	Sequence
1	PGK1 terminator	PGK1ts	ATCCTGCAGGATTGAATTGAATTGAAATCGATAGA
		PGK1tal	CTCGAAAGCTTTAACGAACGCAGAATTTTCGAGT
2	TEF1 promoter	TEF1ps	TATGGTACCACAATGCATACTTTGTACGTTCAAAAT
		TEF1pal	TGTGGATCCTTTGTAATTA AAACTTAGATTAGATTG
3	CYC1 terminator	CYC1ts	AAAGAATTCAAAGCATGCACCTCGAGTCATGTAATTAGT
		CYC1ta	TTTGAATTCGAGCTCGGTACCGGCCGCAAATTAAG
4	TDH3 promoter	TDH3ps	AAAGAATTCGAGCTCGGTACCATAAAAAACACGCTTTTTCAGTTC
		TDH3pa	TTTGAATTCCTTGCATGCTTTCTAGATTGTTTGTATGTGTATTTCG

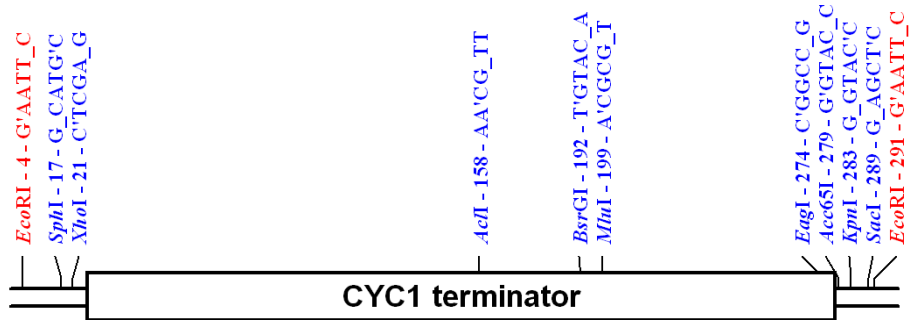
1. 在 CEN.PK113-5D 基因组中扩增 PGK1 终止子, TEF1 启动子和 TDH3 启动子。在质粒 pUG30 中扩增 CYC1 终止子。
2. 将 PGK1 终止子克隆到 Ycplac33 的 HindIII 和 PstI 位点, 将 TEF1 克隆到 KpnI 和 BamHI 位点, 但到 pJFE1。



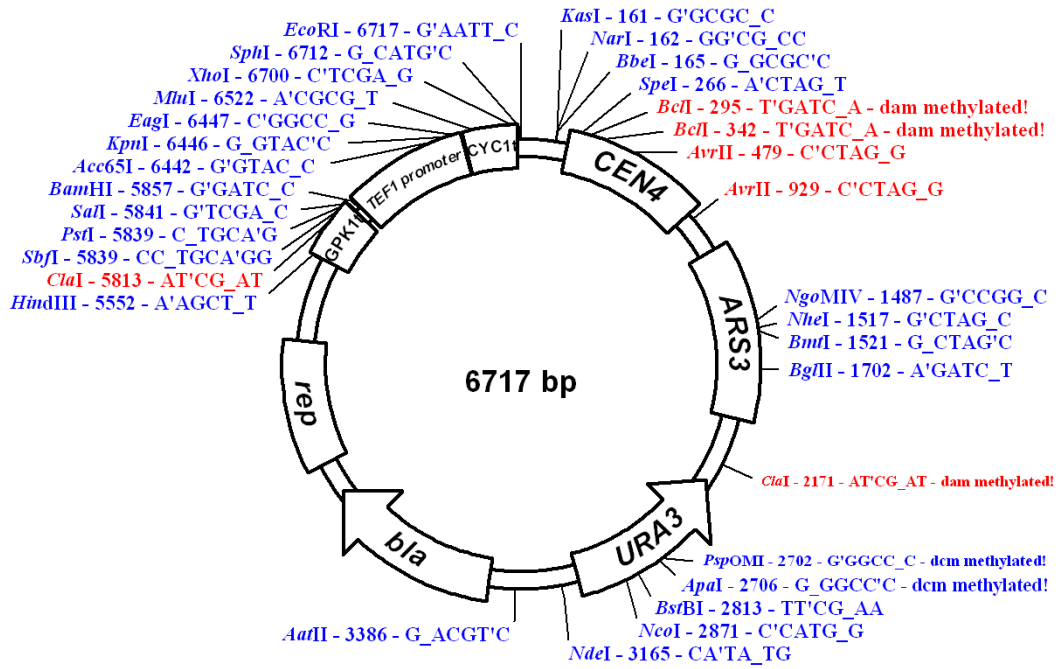
3. 用 XbaI 切 pJFE1，添平末端，连接。去除 pJFE1 中的 XbaI 位点，得到下面质粒。



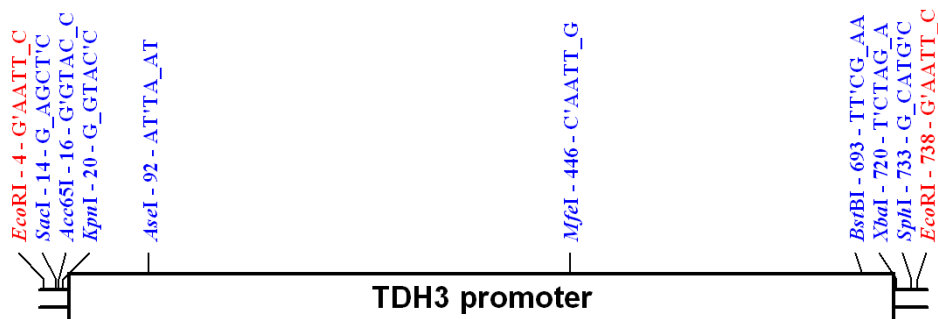
4. 将 *CYC1* 终止子克隆到上述质粒中的 *KpnI* 和 *EcoRI* 位点。



得到下面质粒

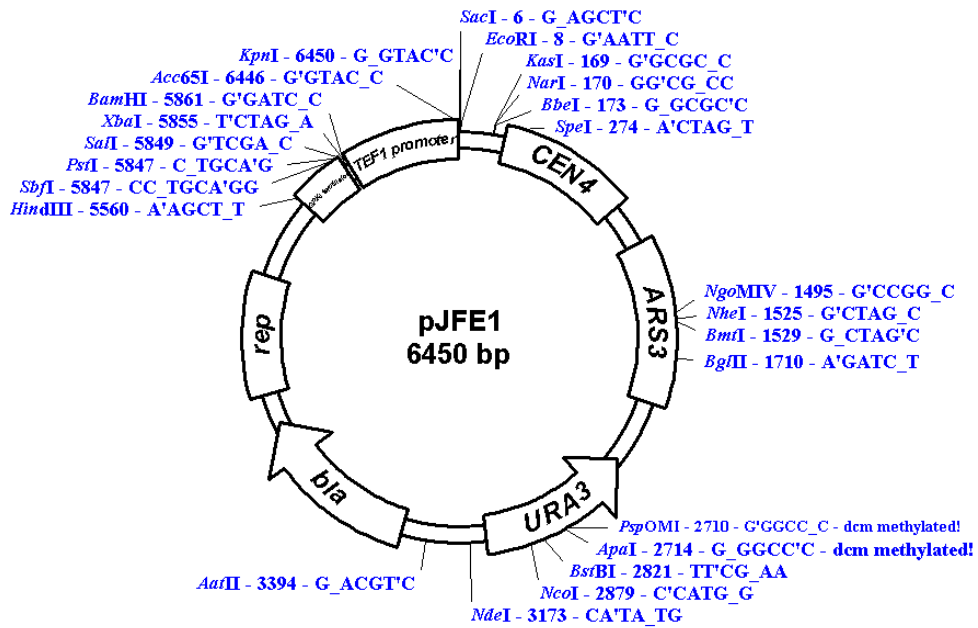


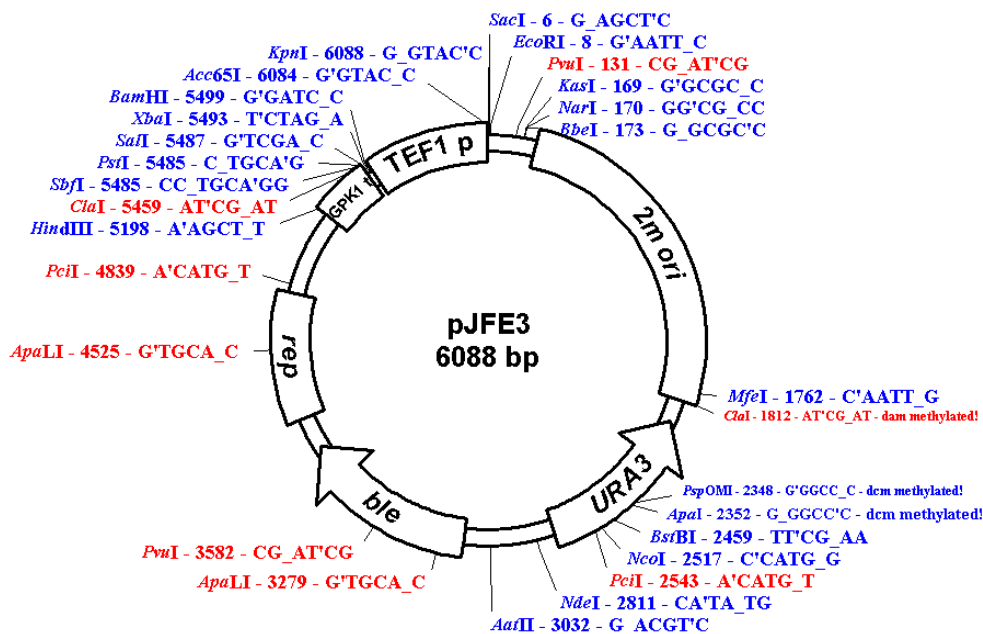
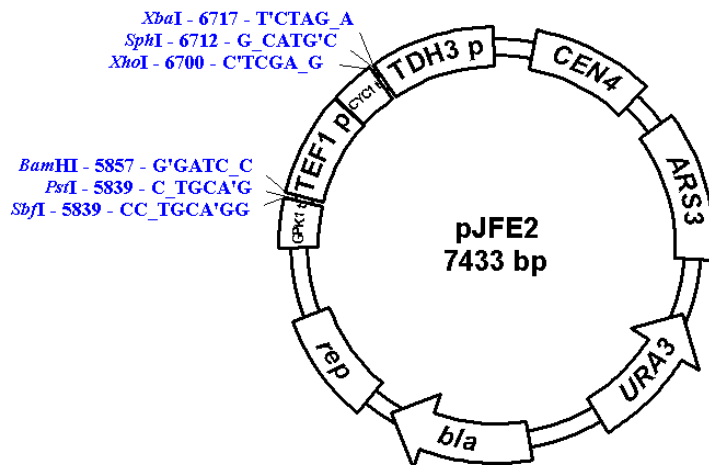
5. 再将 TDH3 启动子克隆到所得质粒的 SphI 和 EcoRI 位点，最终得到质粒 pJFE2.



## ELEMENTS of the PLASMIDS

Plasmid	Element	From	To	Plasmid	Element	From	To
pJFE1	CEN4	227	904	pJFE3	URA3	1924	2725
	ARS3	1113	1951		ble	3164	4022
	URA3	2285	3089		rep	4183	4798
	bla	3525	4386		2m ori	230	1813
	rep	4545	5160		GPK1 t	5205	5479
	GPK1 t	5567	5841		TEF1 p	5506	6084
	TEF1 p	5868	6446				
pJFE2	CEN4	219	896				
	ARS3	1105	1943				
	URA3	2277	3081				
	bla	3517	4378				
	rep	4537	5152				
	GPK1 t	5559	5833				
	TEF1 p	5864	6442				
	CYC1 t	6448	6700				
TDH3 p	6723	7417					





## pJFE1 Sequence ..

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1 CGAGCTCGAA TTCACTGGCC GTCGTTTTAC AACGTCGTGA CTGGGAAAAC
51 CCTGGCGTTA CCCAACTTAA TCGCCTTGCA GCACATCCCC CTTTCGCCAG
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151 GCAGCCTGAA TGGCGAATGG CGCCTGATGC GGTATTTTCT CCTTACGCAT
201 CTGTGCGGTA TTTCACACCG CATATATCGC TGGGCCATTC TCATGAAGAA

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251 TATCTTGAAT TTATTGTCAT ATTACTAGTT GGTGTGGAAG TCCATATATC  
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401 ATTGACTTTA TAACTTATTT AGGTGGTAAC ATTCTTATAA AAAAGAAAAA  
451 AATTACTGCA AAACAGTACT AGCTTTTAAAC TTGTATCCTA GGTATCTAT  
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751 AATTGCAAAA TTTAATTGCT TGCAAAAGGT CACATGCTTA TAATCAACTT  
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1101 ATGAGAATTA ATTCCACATG TAAAATAGT GAAGGAGCAT GTTCGGCACA  
1151 CAGTGGACCG AACGTGGGGT AAGTGCACTA GGGTCCGGTT AAACGGATCT  
1201 CGCATTGATG AGGCAACGCT AATTATCAAC ATATAGATTG TTATCTATCT  
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1351 CGTAGAAGAT AATAGTAAAA GCGGTTAATA AGTGTATTTG AGATAAGTGT  
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6451

## **pJFE2 Sequence ..**

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3901 TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG  
3951 AGCTAACCGC TTTTTTGAC AACATGGGGG ATCATGTAAC TCGCCTTGAT  
4001 CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC  
4051 CACGATGCCT GTAGCAATGG CAACAACGTT GCGCAAATA TTACTIONGGCG  
4101 AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG  
4151 GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT  
4201 TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG  
4251 CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG  
4301 ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT  
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4401 ATATACTTTA GATTGATTTA AACTIONCATT TTTAATTTAA AAGGATCTAG  
4451 GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT  
4501 TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT  
4551 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAACCA  
4601 CCGTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT



4651 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC  
4701 TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT  
4751 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA  
4801 TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG  
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4901 CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC TATGAGAAAG  
4951 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA  
5001 GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG  
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5751 ATAAAATAAA CTATTATTTT AGCGTAAAGG ATGGGGAAAAG AGAAAAGAAA  
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6051 AACGTGATAA AAATTTTAT TGCCTTTTTC GACGAAGAAA AAGAAACGAG  
6101 GCGGTCTCTT TTTTCTTTTC CAAACCTTTA GTACGGGTAA TTAACGACAC  
6151 CCTAGAGGAA GAAAGAGGGG AAATTTAGTA TGCTGTGCTT GGGTGTTTTG  
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6251 GGAGTAGAAA CATTTTGAAG CTATGGTGTG TGGGGGATCA CTTGTGGGGG  
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6351 CGCCTGTTGC TTCCTATGTG ATATGTATTA TATGTAATAT GCATAAATAT  
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6451 CGCAAATTAA AGCCTTCGAG CGTCCCAAAA CCTTCTCAAG CAAGGTTTTC  
6501 AGTATAATGT TACATGCGTA CACGCGTCTG TACAGAAAAA AAAGAAAAAT  
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6601 TAGGGACCTA GACTTCAGGT TGTCTAACTC CTCCTTTTC GGTTAGAGCG  
6651 GATGTGGGGG GAGGGCGTGA ATGTAAGCGT GACATAACTA ATTACATGAC  
6701 TCGAGGTGCA TGCTTTTCTA GATTTGTTTG TTTATGTGTG TTTATTCGAA  
6751 ACTAAGTTCT TGGTGTTTTA AACTAAAAA AAAGACTAAC TATAAAAGTA  
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6851 CCGTCTTTAT ATACTTATTA GTCAAGTAGG GGAATAATTT CAGGGAAGTG  
6901 GTTTC AACCT TTTTTTTCAG CTTTTTCCAA ATCAGAGAGA GCAGAAGGTA  
6951 ATAGAAGGTG TAAGAAAATG AGATAGATAC ATGCGTGGGT CAATTGCCTT  
7001 GTGTCATCAT TTA CTCCAGG CAGGTTGCAT CACTCCATTG AGGTTGTGCC  
7051 CGTTTTTTGC CTGTTTGTGC CCCTGTTCTC TG TAGTTGCG CTAAGAGAAT  
7101 GGACCTATGA ACTGATGGTT GGTGAAGAAA ACAATATTTT GGTGCTGGGA  
7151 TTCTTTTTTT TTCTGGATGC CAGCTTAAAA AGCGGGCTCC ATTATATTTA  
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7251 TCTGTGTAAC CCGCCCCCTA TTTTGGGCAT GTACGGGTTA CAGCAGAATT  
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7351 TTATTTACGT ATTCTTTGAA ATGGCAGTAT TGATAATGAT AACTCGAAC  
7401 TGAAAAGCG TGTTTTTTAT GGTACCGAGC TCG

### **pJFE3 Sequence ..**

1 CGAGCTCGAA TTC ACTGGCC GTCGTTTTAC AACGTCGTGA CTGGGAAAAC  
51 CCTGGCGTTA CCCAACTTAA TCGCCTTGCA GCACATCCCC CTTTCGCCAG  
101 CTGGCGTAAT AGCGAAGAGG CCCGCACCGA TCGCCCTTCC CAACAGTTGC  
151 GCAGCCTGAA TGGCGAATGG CGCCTGATGC GGTATTTTCT CCTTACGCAT  
201 CTGTGCGGTA TTTCACACCG CATATATCGG ATCGTACTTG TTACCCATCA  
251 TTGAATTTTG AACATCCGAA CCTGGGAGTT TTCCCTGAAA CAGATAGTAT  
301 ATTTGAACCT GTATAATAAT ATATAGTCTA GCGCTTTACG GAAGACAATG  
351 TATGTATTTT GGTTCCTGGA GAAACTATTG CATCTATTGC ATAGGTAATC

401 TTGCACGTCG CATCCCCGGT TCATTTTCTG CGTTTCCATC TTGCACTTCA  
451 ATAGCATATC TTTGTTAACG AAGCATCTGT GCTTCATTTT GTAGAACAAA  
501 AATGCAACGC GAGAGCGCTA ATTTTTCAAA CAAAGAATCT GAGCTGCATT  
551 TTTACAGAAC AGAAATGCAA CGCGAAAGCG CTATTTTACC AACGAAGAAT  
601 CTGTGCTTCA TTTTGTAAA ACAAAAATGC AACGCGAGAG CGCTAATTTT  
651 TCAAACAAAG AATCTGAGCT GCATTTTTAC AGAACAGAAA TGCAACGCGA  
701 GAGCGCTATT TTACCAACAA AGAATCTATA CTTCTTTTTT GTTCTACAAA  
751 AATGCATCCC GAGAGCGCTA TTTTCTAAC AAAGCATCTT AGATTACTTT  
801 TTTTCTCCTT TGTGCGCTCT ATAATGCAGT CTCTTGATAA CTTTTTGCAC  
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901 TCCATAAAAA AAGCCTGACT CCACTTCCCG CGTTTACTGA TTACTAGCGA  
951 AGCTGCGGGT GCATTTTTTC AAGATAAAGG CATCCCCGAT TATATTCTAT  
1001 ACCGATGTGG ATTGCGCATA CTTTGTGAAC AGAAAGTGAT AGCGTTGATG  
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1301 AAGAGATACT TTTGAGCAAT GTTTGTGGAA GCGGTATTCG CAATATTTTA  
1351 GTAGCTCGTT ACAGTCCGGT GCGTTTTTGG TTTTTTGAAA GTGCGTCTTC  
1401 AGAGCGCTTT TGGTTTTCAA AAGCGCTCTG AAGTTCCTAT ACTTTCTAGC  
1451 TAGAGAATAG GAACTTCGGA ATAGGAACTT CAAAGCGTTT CCGAAAACGA

1501 GCGCTTCCGA AAATGCAACG CGAGCTGCGC ACATACAGCT CACTGTTCAC  
1551 GTCGCACCTA TATCTGCGTG TTGCCTGTAT ATATATATAC ATGAGAAGAA  
1601 CGGCATAGTG CGTGTTTATG CTTAAATGCG TACTTATATG CGTCTATTTA  
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2501 GGCTTAACTG TGCCCTCCAT GGAAAAATCA GTCAAGATAT CCACATGTGT  
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