

Monday 8/3/09

Analyzed primers for Pu promoter

Prabhjyot's colony PCR for the Pu promoter did not work. The primers were double checked using DNA Star's megalign program and the PCR conditions were reevaluated.

Results

Primer Analysis

EcoRI-XbaI- Seq downstream Pu

Primer Sequence

GCG CGA ATT CAA ATC TAG AAA CGG GGC TCG GAC TAC TTC TTC

EcoRI

XbaI

pWW0 sequence from NCBI

Alignment with DNA Star MegAlign between plasmid (74987-77725 bp region) and primer

Martinez/Needleman-Wunsch DNA Alignment

Minimum Match: 9; Gap Penalty: 1.10; Gap Length Penalty: 0.33

Seq1(1>2739)	Seq2(1>42)	Similarity	Gap	Gap	
Consensus					
pWW0 74987 to 7772! 5	EcoRI-XbaI-Seq downstream Pu	Index	Number	Length	
Length					
(656>928)	(42<-1)	12.3	3	231	273
(656>928)	(42<1)	12.3	3	231	273
v660	v670	v680	v690	v700	v710
GAAGAAGTAGTCCGAGCCCCG (pWW0 plasmid)					
GAAGAAGTAGTCCGAGCCCCG (alignment)					
GAAGAAGTAGTCCGAGCCCCG(primer)					
^40 ^30					

PstI-SpeI-Seq upstream Pu

Primer Sequence

AAACTGCAGAAAACTAGTAAAGCACGCACGCCAATGACCT

PstI

SpeI

pWW0 sequence from NCBI

Alignment with DNA Star MegAlign between plasmid (74987-77725 bp region) and primer

Martinez/Needleman-Wunsch DNA Alignment

Minimum Match: 9; Gap Penalty: 1.10; Gap Length Penalty: 0.33

Seq1(1>2739)	Seq2(1>40)	Similarity	Gap	Gap	
Consensus					
pWW0 74987 to 77725	Pu upstream primer pstI-speI-sequence	Index	Number	Length	
Length					
(194>233)	(1>40)	63.0 !	3	6	43
(194>233)	(1>40)	63.0	3	6	43
v200	v210	v220	v230		
GCACGCACGCCAATGACCT (pWW0 plasmid)					
GCACGCACGCCAATGACCT(alignment)					
GCACGCACGCCAATGACCT(primer)					
^10	^20	^30	^40		

PCR Conditions for Probyjot's PCR run

- 1) 95C for 6 min
- 2) 98C for 10 sec
- 3) 52C to 69C for 30 sec
- 4) ext4) 72C for 5 min
- 5) Goto step 2 four times
- 6) 98C for 10 sec
- 7) 69C for 30 sec
- 8) 72C for 5 min
- 9) Goto step 6 twenty nine times
- 10) 4C forever
- 11) End

Suggestions from Jeremy

- Use phusion polymerase NOT hot-start (antibody will not anneal due to low temperatures)
 - The reaction must be started on ice
- Modifications to PCR conditions
 - Shorten extension time to 15 seconds at 72C
- Check Tm of only the region homologous to the pWW0 plasmid using oligocalc online
 - Upper: 55C
 - Lower: 58C