Friday 10/2/09

Protocol for Primer Design using DNA star

1. Compose desired sequence with restriction sites
    a. Save as a .txt file
    b. Add extra base pairs on the end of the cut sites otherwise the restriction enzyme will not cut
2. Log into Linad (see Ann for password)
3. Create sequence file using SEQ Builder
    a. Application
        i. DNA Star
            1. Lasergene
                a. SEQ builder
                    i. File
                        1. Open .txt file from step 1
                            a. Save as .sbd
4. Design primers using primer select
    a. Application
        i. DNA Star
            1. Lasergene
                a. Primer Select
                    i. File
                        1. Enter sequence
                            a. Choose .sbd from step 3
                        2. Conditions
                            a. Location
                                i. Select entire sequence to be amplified
                            b. Characteristics
                                i. Tm 50-70C
                                ii. Length 20-40 bp
                        3. Locate
                            a. PCR primer probes
                            b. PCR primer pairs
                        4. Report
                            a. Self dimmers
                            b. Pair dimmers
                            c. Hair pins
                            d. Amplification Summary

Primer Design for PrXylR part from P. putida *mt-2*

In the past two days primers were design for the PrXylR region of the pWW0 TOL plasmid. To get acceptable primers, the DNA sequence directly before and after PrXylR was changed to try and eliminate primer pair dimmers, primer self dimmers and hairpins.

Results

Primer Design for PrXylR part from P. putida *mt-2*

Pr XylR Primers From sequence 12 on linlab computer icestorm (it took a few tries to manipulate the sequence correctly)

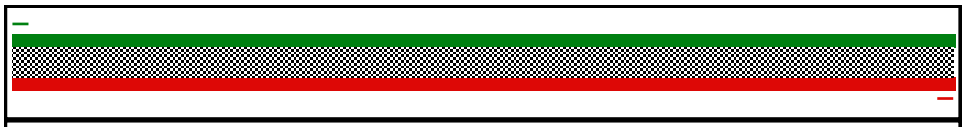
Upper Primer

5’ TATACTAGTCTGGGGCGAGAGGCGACGAC 3’

Lower Primer

5’ GGGGAATTCTAGAATGTGGGCTGCTTGGTG 3’

Amplification Summary from Primer Select



Upper Primer: 29-mer 5' TATACTAGTCTGGGGCGAGAGGCGACGAC 3'
Lower Primer: 30-mer 5' GGGGAATTCTAGAATGTGGGCTGCTTGGTG 3'

DNA 250 pM, Salt 50 mM	Upper Primer	Lower Primer
Primer Tm	66.7 °C	68.9 °C
Primer Overall Stability	-56.7 kc/m	-59.7 kc/m
Primer Location	1..29	2338..2309
Product Tm - Primer Tm	16.0 °C	
Primers Tm Difference	2.2 °C	
Optimal Annealing Temperature	63.0 °C	
Product Length	2338 bp	
Product Tm (%GC Method)	82.7 °C	
Product GC Content	56.3%	
Product Tm at 6xSSC	104.3 °C	

Product Melting Temperature (%GC Method)

Salt			Formamide			
mM	xSSC	xSSPE	0%	10%	20%	50%
1	0.005	0.006	54.5	48.0	41.5	22.0
10	0.051	0.062	71.1	64.6	58.1	38.6
50	0.256	0.312	82.7	76.2	69.7	50.2
165	0.846	1.031	91.3	84.8	78.3	58.8
330	1.692	2.062	96.3	89.8	83.3	63.8
500	2.564	3.125	99.3	92.8	86.3	66.8
1000	5.128	6.250	104.3	97.8	91.3	71.8

195	1.000	1.219	+ 0.0	%formamide = Tm 92.5 °C

Tm of Pr Xyl R Analogous regions only
Upper primer: 62 C
Lower primer: 49 C
*this large difference may cause problems with the PCR cycles, but these were the only good primers that could be designed without primer self dimmers, primer pair dimmers or hairpin formations

From NCBI sequence
*27 bp from the end of xylU
*19 bp from the start of xylS