

Single Strand DNA Sequencing Analysis

Objective

Use primer walking method to full-length sequence a 3.7kb insert of a plasmid in single strand

Starting Materials

1. 5µg of plasmid DNA.
2. The predicted sequence of the 3.7 kb region.

Methods

1. Design and synthesis of 5 internal sequencing primers every 500 bases.
2. Sequencing the plasmid with the designed primers and two universal primers.
3. Assembly of the sequencing results by Sequencher 4.2.
4. Determination of consensus sequences in the overlapping region.

Results

1. All 5 designed primers and two universal primers generated good sequencing data.
2. After assembly by Sequeuncher and proofreading of the overlapping region, full length sequences had been generated.
3. All primers information, sequencing data and consensus sequence are available at our secure website.

Primer Information

Primer Name	Primer Sequences
M13F	5' (GTA AAA CGA CGG CCA GT) 3'
M13R	5' (GGA AAC AGC TAT GAC CAT G) 3'
Primer 1	5' (ACG GGA CGT CGG TGA CAT CA) 3'
Primer 3	5' (GGA CGT CGG TTA TGA TGA CGT) 3'
Primer 5	5' (ACG GGA ACT CGG GCG GGA AT) 3'
Primer 7	5' (TTG CAT CGT CGG TGA CAT TTG) 3'
Primer 9	5' (ACT CGT CGT CCT CGT GAA CT) 3'

