

HAVEY'S LAB BAC-END SEQUENCING PROTOCOL

Material:

- 1) BAC DNA (it is recommended to use 1-2 ug of BAC DNA per sequencing reaction, if you are using lab's miniprep just use 5 ul, it will work well for majority of the samples)
- 2) M13REV and M13FOR primers, 10 uM solution (several »versions« of these primers are available, I used M13REV-BIOTECH GAAACAGCTATGACCATG and M13FOR CGCCAGGGTTTTCCCAGTCACGA, TIGR is using TIGR universal FORWARD 18 bp TGTA AACGACGGCCAGT and TIGR universal REVERSE 18 bp CAGGAAACAGCTATGACC, probably other are suitable too, check the pBeloBac11 sequence, Accession Number is U51113)
- 3) BIG DYE 5x Buffer and Enzyme solution

Procedure (20 ul volume):

- 1) Put 5 ul of BAC DNA into PCR tube, keep on ice.
- 2) Add 3 ul of 5x BIG DYE Buffer.
- 3) Add 2 ul (=20 pmol per reaction) of M13REV or M13FOR primers.
- 4) Add 8 ul of dH₂O.
- 5) Add 2 ul of BIG DYE Enzyme mix.

For larger quantities you can prepare Master mix of 2-5 components and add 15 ul to each sample.

Cycling procedure (worked well for majority of samples):

95°C 5 min, then 50 cycles of 95°C 30 sec 50°C 20 sec 60°C 4 min.

Clean up procedure (Biotech recommendations, use 8 channel pipette):

Add 10 ul CleanSeq beads (AGENCOURT).

Add 80 ul 80% EtOH, mix by pipetting.

Put on magnetic plate 2-3 min.

Withdraw liquid.

Add 200 ul 80% EtOH, withdraw as much liquid as possible.

Remove from magnetic plate (further drying not required).

Add back 50 ul water, when beads have fully released from the side, transfer 10 ul to plate for capillary sequencing, reseal with foil, take over to Biotech (it is good idea to upload the Excel sheet with the names of the samples, especially if you have full plate).

NOTES:

- 1) Reading lengths are usually slightly shorter than compared to PCR or plasmid samples, everything above 500 bp is good.

2) If sample is bad, resequencing will often not improve anything (can be type of DNA which is hard to sequence).