

Fingerprint Analysis of Large-Insert BAC Clones

The following protocol describes a high throughput scheme to yield precise and robust BAC clone restriction fragments which are used to identify other clones that share a large proportion of fragments with the same relative mobilities. By utilizing FPC and I.m.a.g.e software developed at the Sanger Centre, we infer the overlap of clones and can construct a contig where the relative positions of the clones reflect the extent to which they overlap.

MATERIALS:

- | | |
|-----------------------------|--|
| 1) BAC clone DNA: | Autogen prepped |
| 2) Cycleplate: | Robbins Scientific (#1038-00-0) |
| 3) HindIII (40U/ul): | B/M #798983 |
| 4) EcoRI (40U/ul): | B/M#200310 |
| 5) Foil tape: | Scotch #425 (7126-2-5) |
| 6) Ficoll: | Sigma |
| 7) Bromophenol blue: | Bio-Rad |
| 8) Xylene Cyanol FF: | Bio-Rad |
| 9) Seakem LE Agarose: | FMC #50004 |
| 10) SYBR Green 1: | FMC #50512 |
| 11) EDTA: | Baker |
| 12) Glacial acetic acid | |
| 13) TRIS-base: | Sigma |
| 14) Gel Apparatus: | Owl Scientific Gator A3-1 |
| 15) Power Supply: | Bio-Rad, Model 300 |
| 16) 51-well combs: | 2mm wide x 1mm thick x 3mm deep
(Wash U #CMB-47) |
| 121-well combs: | 1mm wide x 0.8mm thick x 3mm deep
(Wash U #CMB-59A) |
| 17) Recirculating chiller: | VWR Model 1170 (#11025-012) |
| 18) DNA Markers: | Promega marker:#DG1931
B/M DNA marker II: #236250
B/M DNA marker V: #821705
B/M DNA marker III: #528552
Life Tech. 1kb ladder: # 15615-024 |
| 19) Tygon tubing | Tygon LFL #6429-17 |
| 20) Nalgene tank (16 Liter) | |
| 21) RNaseA: | SIGMA |
| 22) Imager: | Molecular Dynamics Typhoon |

Solutions:

1) 0.5 EDTA, pH 8.0

93 g EDTA / 500ml H₂O
pH to 8.0 with NaOH

2) 50X TAE: filter and dilute to 1x before use (500ml to 25 L filtered ddH₂O)

TRIS-Base	242 g
Glacial Acetic Acid	57.1 ml
0.5 M EDTA, pH 8.0	100 ml
QS to 1 liter with ddH ₂ O	
Store at room temperature	

3) 6X Loading Buffer:

7.5 g Ficoll
125 mg bromophenol blue
125 mg xylene cyanol FF
to 50 ml ddH₂O
Store at room temperature for six months

4) Marker DNA :

FP Marker 1: 15.6 ul (0.2 ug/ul) Promega (Analytical marker DNA, Wide Range)
2 ul (250 ng/ul) B/M Marker V
42 ul 6x gel loading buffer
189.6 ul 10:0.1 TE, pH 8.0
Store at -20 C

FP Marker 2: 0.83 ul (1ug/ul) Life Tech. 1 kb ladder
3.33 ul (250 ng/ul) B/M Marker II
3.33 ul (250 ng/ul) B/M Marker III
92.5 ul TE (10: 0.1), pH 8.0
25 ul 6x loading dye
store at -20C

Before use, mix: 20ul Marker 2 stock, 17ul TE, 3ul 6x loading dye and incubate at 60C for 5 min.

5) SYBR Green 1: (1:10000 dilution)

40 ul SYBR Green 1 in 500 ml 1 x TAE
Store in foil-covered rubbermaid recyclable #5 container at 4C
Use for 2-3 days, Protect from light.

6) RNase A: Stock Solution: 10 mg/ml

Resuspend 100mg RNase A in 10 ml of 10 mM Tris-HCL, pH 7.5, 15mM NaCl.
Boil for 10 min.
Store as 1 ml aliquots at -20C

RNase A Working Solution: 50 ng/3.75 ul

1.5 ul of stock RNase (10mg/ml) in 998 ul H₂O
prepare before use.

Restriction Digest of BAC Clone DNA:

Individual digests:

5.0 ul BAC DNA
3.75 ul RNase A working solution (50 ng/3.75 ul)
1.0 ul of 10 x buffer
0.25 ul of Hind III or EcoR1 (40 U/ul), 10 units

110ul reactions digestion cocktail (96-well plate):

412.5 ul RNase A (50ng/3.75ul)
110 ul of 10x buffer
27.5 ul of HindIII or EcoR1 (mix well on ice)

- 1) 5ul of each BAC clone DNA is added to a 96-well thin wall cycle plate followed by 5ul of digestion cocktail.
- 2) Plates are covered with foil tape, spun briefly, and incubated at 37C for 4.5 hours in a water bath.
- 3) After digestion, spin briefly again, and add 2ul of 6x loading dye to each well.
- 4) Plates are sealed with foil tape and, if necessary, stored at 4C overnight before electrophoresis

Electrophoresis of BAC Clone DNA Digests:

OWL SCIENTIFIC EP TANKS: 121-well combs

- 1) 400 ml (1%) Seakem LE agarose gels are prepared in 1x TAE. Molten agarose is cooled to 55C in a water bath with occasional stirring and then poured into 23x40-cm UVT trays resting on a **level** surface. Tip UVT tray to evenly distribute molten agarose. Two, 121-well combs are then inserted. After the gel is solidified, 4.0 liters of 1x TAE buffer is added, the combs are removed, and the buffer re-circulated at 14C (speed 5).

1% Seakem LE:

4.0g agarose in 400ml 1x TAE (3mm gel height).

Boil agarose in 500ml storage bottles, with caps on loosely, in microwave 3 x 60 seconds, swirling between.

Heat additional 10 seconds to ensure boiling

Cool to 55C in water bath

- 2) Allow gel to equilibrate 10 min. in circulating buffer before loading.
Turn off recirculator.
- 3) In the first well and every fifth well thereafter, load 0.8ul of FP marker 1 (or 1.5ul of FP marker 2) for a total of 25 times. See gel loading sheets for well-sample identity.
- 4) 1.5ul of restriction digestion/loading dye mixture is loaded to remaining wells using a Hamilton 8-channel syringe. 48 samples are loaded in duplicate (colony a & b, 96 total) in each comb. Each gel can accommodate 192 samples.
- 5) Samples are electrophoresed at 140V for 15 minutes after which time the recirculation of the electrophoresis buffer commences, maintaining the buffer at 16C.
- 6) Total electrophoresis time is eight hours. Set timer on power supplies to assure identical EP run times.
- 7) Alternatively, samples can be separated at 105V for 12 hours, or 70V for 16 hours (960 minutes).
- 8) After electrophoresis, gels are cut in half and removed to Rubbermaid trays containing 500ml of 1:10000 SYBR green in 1x TAE and agitated in the dark for 30-45 minutes.
- 9) SYBR green can be reused for 6-8 gels if stored at 4C in foil-covered contained.

10) After staining, gel images are captured sideways using a Molecular Dynamics Typhoon imager (see Typhoon – FP protocol).

Additional Notes:

- 1) EP tanks, combs, trays and dams must be cleaned and rinsed with dH₂O after each use.
- 2) Drain cooling lines of circulating buffer before disconnecting EP tanks.
- 3) Check water level in cooling tanks and change water monthly. Add 10ml of 10% Roccal per liter of water to prevent growth of algae and fungi.
- 4) Check the level of EP tanks before casting gels.
- 5) Turn off the circulating pumps and refrigerated recirculator ASAP after running gels to minimize wear.
- 6) If FP image demonstrates bubbles, frowning or smiling bands, rerun the same digest on a new FP gel.

Marker sizes and bands

FP Marker 1 sizes:		FP Marker 2 sizes:	
1	1000000	1	1000000
	29950		23130
	24510		21226
	20560		12216
5	17053	5	11198
	15258		10180
	13822		9416
	12188		9162
	10171		8144
10	8453	10	7126
	7242		6557
	6369		6108
	5687		5148
	5220		5090
15	4822	15	4973
	4324		4361
	3976		4268
	3675		4072
	3194		3530
20	2804	20	3054
	2582		2322
	2323		2036/2027
	2121		1904
	1929		1636
25	1747	25	1584
	1560		1375
	1503		1018
	1371		947
	1264		831
30	1079	30	564
	963		517/506
	702		396
	587	33	344
	540		298
35	504		
	458		34 bands
	434		
	267		
	234		
40	213		
	192		
	184		

Vector band = 6382

42 bands