RAGE

Rotating Field Gel Electrophoresis (RAGE) is a variation on Pulsed Field Gel Electrophoresis (PFGE) and gives similar results. We use equipment that was only briefly marketed by Stratagene, at far lower cost than BioRad PFGE equipment.

Plugs for RAGE/PFGE

Starting material for BF: 100 ml (50 ml is also fine) of 1.5–2x10^6 cells/ml.

1. Prepare 1.6% LMP agarose (Sigma agarose type VII: low gelling, Cat. No. A-4018) in L buffer and leave it at 50°C.
2. Centrifuge cells and wash once in TDB.
3. Resuspend cells up-and-down in L buffer in order to obtain 5x10^8 cells/ml
4. Incubate at 42-50°C for 10 min.
5. Add 1 volume of LMP to 1 volume of cells, (i.e. 2.5x10^8 cells/ml)
6. Mix well BUT QUICKLY and pour ~85 µl into disposable molds with a 200 µl pipette.
7. After 5–10 min, plugs are solid. Transfer plugs into a 5ml tube containing 2ml of L buffer+1% Sarkosyl.
8. Add 100µL of Proteinase K 20 mg/ml (Final concentration 1 mg/ml)
9. Incubate at 50°C for 48 h.
10. Discard Proteinase K digest, wash 2x with L buffer and repeat Proteinase K treatment for another 48 h.
11. Wash again as above.
12. Store plugs at 4°C in L buffer.
13. Use 1/2 plug / lane, i.e. ~ 1 x 10^7 cells / lane (2.5x10^8 cells/ml x 1/2 x 85 µl = 1x10^7 cells).

L buffer (per 500 ml)

mix	vol	final concentration
1 M Tris-HCl pH 7.6	5 ml	0.01 M
5 M NaCl	2 ml	0.02 M
0.5 M EDTA pH 8.0	10 0ml	0.1 M

Pre-run and post-run steps

Prepare agarose gel

1. Prepare 2–3 l of 0.5x TBE and place them in gel box to refrigerate.
2. Prepare 300 ml of a 0.8% (or more!) agarose gel in 0.5x TBE.

   To make sure the volume is 300 ml, I usually weigh the flask containing the agarose + TBE before and after microwaving.
3. Cool agarose to 50-60°C on a stirring plate. Turn on heating if necessary.
4. Melt 1.6% LMP agarose and cool it. Leave it over a heating block at 50-70°C
5. Set up gel tray (drawer underneath RAGE apparatus): 4 pieces. Make sure that the rotating circular platform indicates the correct angle: 106 or 120°.
6. Seal with the 0.8% agarose.

Load plug samples into comb

1. Decide the order of the samples. Do not forget size marker(s) (BioRad).
2. Prepare a working area by placing some parafilm over the bench and providing a clean scalpel and spatula.
3. Make sure you have the comb nearby.
4. Place 1 small drop of 0.5x TBE on each well of the comb, where a plug will be placed.
5. Take 1 plug out of the vial to the parafilm with the help of the spatula.
6. Cut 1 plug into two halves (0.5 x 0.5 cm) with the scalpel.
7. Replace one half in the vial. Place the other half on the well.
8. After 2–3 half-plugs were placed on the comb, seal them with 1 drop of the previously melted 1.6% LMP agarose.

Pour gel and begin run

1. When all plugs are loaded onto comb, place comb on the gel tray over blue line.
2. Pour 0.8% agarose in gel tray and let it cool (~30 min).
3. Carefully remove comb from gel (don't worry, the plugs will stick to the gel).

4. Fill each well with 0.5x TBE. Add 1.6% LMP agarose to those same wells. Avoid bubbles. Allow agarose to cool (10 min)
5. Start run: conditions for different size separations follow on next pages.

Staining and destaining

1. 1 h in 0.5x TBE containing 1 μg/ml EtBr .
2. Discard EtBr in appropriate container (in the hood), for later disposal.
3. Destain for 1 h in 0.5x TBE.
4. Take picture of gel. "Analytical" UV mode can be used, since DNA fragments are going to be depurinated anyway. Do not forget to have the ruler on the side of the gel!

Depurination

1. Depurinate for 30-40 min in HCl 0.25 M (~500 ml).
2. Neutralize for 30 min in NaOH 0.4 M (~500 ml).

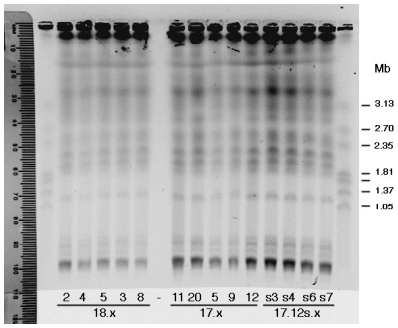
DNA transfer and Southern blotting (as described in Protocol 8 of Maniatis)

1. Cut Hybond N+ and 2 pieces of Whatman paper to the same size as the gel.
2. Use 0.4 M NaOH as transfer buffer (~1,000 ml).
3. Transfer for at least O/N: 3 days (Friday-Monday) is fine, but paper towels need to be changed when the stack is soaked and more 0.4N NaOH added to the bottom container.
4. Pre-hybridization: choose between QuickHyb or Church solution.
5. Label probe with ^{32}P-dCTP (fridge; hot room).
6. Hybridization: 2 h or O/N.
7. Washing: 1–2 h in 2x SSC, 0.1% SDS and 0.1x SSC, 0.1% SDS.
8. Expose to a film or PhosphorImager, depending whether the signal is strong or weak, respectively, or if quantification is needed.

Programs for chromosome separation by RAGE

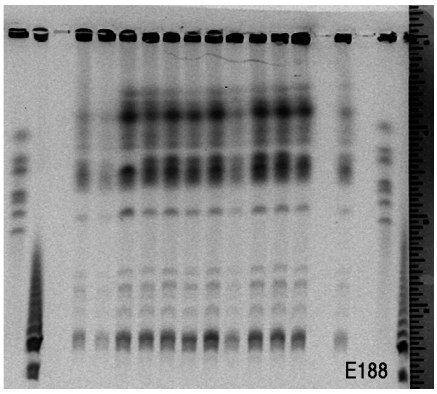
CLASSICAL PROGRAM: MEGABASE CHROMOSOMES ARE SEPARATED FROM MINI+INTERMEDIATE CHROMOSOMES

Gel:	0.8% in TBE 0.5x	
Temperature:	12C	
Window 1		
	Linear pulse time:	100-300 sec
	Constant voltage:	120 V
	Time:	10 hr
	Angle:	106 Degrees
	Range of resolved fragments:	0.600-2.0 Mb
Window 2		
	Linear pulse time:	1000-2500 sec
	Constant voltage:	50 V
	Time:	84 hr
	Angle:	106 Degrees
	Range of resolved fragments:	1.7-3.8 Mb
Totals		
	Effective Time:	4 days
	Effective Distance:	10 cm



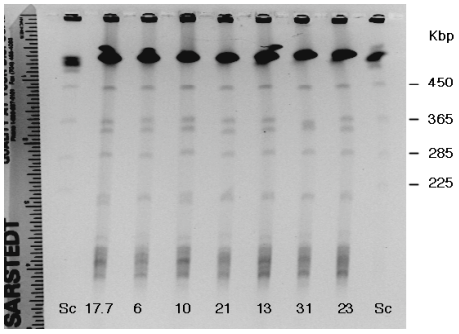
MBC+IC PROGRAM: MEGABASE AND INTERMEDIATE CHROMOSOMES ARE SEPARATED FROM MINI CHROMOSOMES

Gel:	0.8% in TBE 0.5x	
Temperature:	12C	
Window 1		
	Linear pulse time:	10-40 sec
	Constant voltage:	150 V
	Time:	8 hr
	Angle:	106 Degrees
	Range of resolved fragments:	100-455 kb
Window 2		
	Linear pulse time:	100-300 sec
	Constant voltage:	120 V
	Time:	2 hr
	Angle:	106 Degrees
	Range of resolved fragments:	0.600-2.0 Mb
Window 3		
	Pulse:	1200-2500 sec
	Voltage:	50 V
	Time:	85 hr
	Angle:	106 Degrees
	Range of resolved fragments:	2.0-5.0 Mb
Totals		
	Effective Time:	4 days
	Effective Distance:	9 cm



IC PROGRAM: INTERMEDIATE CHROMOSOMES ARE SEPARATED; MINI CHROMOSOMES ARE ONLY PARTIALLY RESOLVED

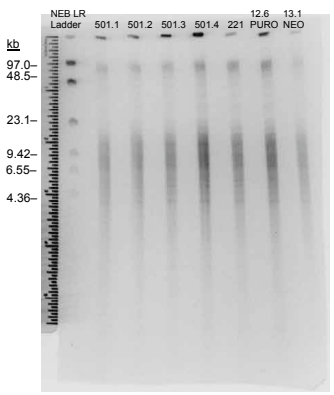
Gel:	1% in TBE 0.5x	
Temperature:	12C	
Window 1		
	Linear pulse time:	20-60 sec
	Constant voltage:	110 V
	Time:	60 hr
	Angle:	120 Degrees
	Range of resolved fragments:	130-460 Mb
Totals		
	Effective Time:	3 days, 5hours
	Effective Distance:	11 cm



Low Range PFGE *(adapted from Navarro and Cross, MCB 1996) (example shown is with XmnI digestion and probing with Es promote: Grace Teng, 040816)*

Low Range PROGRAM: for fragments between 5-100kb.

Gel:	1% in TBE 0.5x	
Temperature:	12C	
Window 1		
	Linear pulse time:	1-2 sec
	Constant voltage:	150 V
	Time:	18 hr
	Angle:	120 Degrees
	Range of resolved fragments:	5-100 Kb
Totals		
	Effective Time:	69 hr
	Effective Distance:	11 cm



Empirical Equation for fragment resolution and velocity using Pulse-Field Gels:

Maximum well-resolved size (Kb) = $0.034 \times (T+40) \times V^{1.1} \times (3-A)^{0.6} \times t^{0.875}$

Minimum well-resolved size (Kb) = 0.75 x Maximum Size

Velocity of 10kb fragment (cm/hr) = $[0.0012 \times (T+25) \times V^{1.6} \times \cos(\theta/2)] / A$

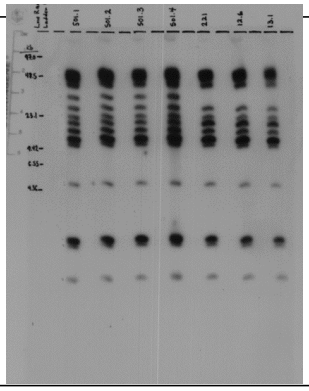
T, temperature (°C)

V, field strength (Volts/cm)

A, % agarose gel (multiply by 0.8 for pulse field grade agarose)

T, pulse time (secs)

θ, reorientation angle



In-agarose XmnI restriction digest

1. Incubate 5 mm x 5 mm agarose plugs in TE with rotation for 30 min.
2. Incubate plugs in 1x NEB buffer 2 + 1x BSA at RT for 15 min.
3. Discard buffer, replace with fresh 400 μl 1x NEB buffer 2 + 1x BSA
4. Add 40 U XmnI and incubate at 37 ºC O/N.
5. Run under Low Range conditions (above) to separate ES promoter fragments, etc