# PULSED FIELD GEL

# PFG – SOLUTIONS FOR DNA PREP IN AGAROSE PLUGS

### Lysis Solution for Plugs

- 1.5 ml 3 M Tris.HCl pH 8.8 (~20 mM)
  250 ml 0.5 M EDTA pH 8 (~500 mM)
  2.5 g N-lauroylsarcosine (1%)
  **Total ~250 ml**
  No need to sterilise (already sterile)
- If required, add immediately before:
  Add RNase (30 mg/ml) to 10 µg/ml
  Add proteinase K (powder) to 1 mg/ml

### Wash Solution for Plugs

- 6.25 ml 1 M Tris.HCl pH 7.5 (25 mM)
  50 ml 0.5 M EDTA pH 8 (100 mM)
  **$dH_2O$ to 250 ml**
  Autoclave
- If required:
  Add 1 mM PMSF (100 mM in ethanol)

### Wash Solution + Glycerol for Plug Storage

- 2.5 ml 1 M Tris.HCl pH 7.5 (25 mM)
  20 ml 0.5 M EDTA pH 8 (100 mM)
  50 ml glycerol (50%)
  Autoclave
  **$dH_2O$ to 100 ml**

# DAY 1

- Set up overnight culture in 5 ml Hv-YPC

Hitch et al (2025); Ausiannikava et al (2018)

# DAY 2

- Prepare 1% low-melt agarose (SeaPlaque GTG) in 18% SW:
  - Add 0.1 g low-melt agarose to 4 ml $dH_2O$, boil to melt
  - Add 6 ml hot (>80°C) 30% SW (only required for H. volcanii – use $dH_2O$ for e.g. E. coli)
  - Mix thoroughly, boil once more and leave at 42°C (for 30 minutes) to cool
- Pellet 1 ml cells at 6000 rpm for 10 minutes, use 2 ml round-bottom tube
  Use 2 ml for slow-growing strains (e.g. Δ*radA*)
- Resuspend cell pellet gently in 100 µl of 18% SW
  - Transfer 80 µl of cells to fresh tube, leave at 42°C for 5 minutes
- Add 320 µl of 1% low-melt agarose at 42°C, and mix gently by pipetting.
  Immediately pipette 80 µl into each plug mould, avoiding air bubbles
  - Leave in fridge for 10 minutes, normally get 3-4 plugs per sample
- Transfer agarose plug to 2 ml tube containing 2 ml lysis solution +proteinase K (1 mg/ml)
  - Incubate shaking at 45°C for 3–4 hours (low speed), can put 2 plugs in each tube
  - Clean plug moulds: rinse in hot water to remove agarose, rinse in IMS, wash in $dH_2O$, leave to air dry
- Remove lysis solution, add 2 ml fresh lysis solution +proteinase K (1 mg/ml) +RNase (10 µg/ml)
  - Incubate shaking at 45°C overnight

Hitch et al (2025); Ausiannikava et al (2018)

# DAY 3 – OPTION 1 – GAMMA RADIATION

- Remove lysis solution with pipette, replace with 2 ml wash solution. Incubate shaking at 37°C for 30 minutes
- Transfer plugs into fresh 2 ml tubes containing 0.5 ml wash solution
- Gamma irradiate plugs for required exposure time (optional)

  - Beverley (1989) estimates rate of $7x10^{-6}$ DSB / kb / Gy
  - $^{137}Cs$ source, 30.17 yr half-life: 9.2333 Gy/min (Jan 1990) = 4.13175 Gy/min (Jan 2025)
  - For H. volcanii, normally ~5-10 mins for chromosome, ~10-20 mins for pHV3 or pHV1
- Transfer plugs to fresh 2 ml tubes with 2 ml wash solution +PMSF (1 mM). Incubate with shaking at 37°C for 1 hour.
  - Repeat wash twice more, once with PMSF and once without
  - 100 mM stock solution of PMSF in 100% ethanol (8.7 mg in 500µl), needed to inactivate proteinase K

Hitch et al (2025); Ausiannikava et al (2018)

# DAY 3 – OPTION 2 – RESTRICTION DIGEST

- Remove lysis solution with pipette and replace with 2 ml wash solution.
  Incubate shaking at 37°C for 30 minutes
- Transfer agarose plugs to fresh 2 ml tubes containing 2 ml wash solution + PMSF (1 mM).
  Incubate shaking at 37°C for 1 hour
  - *100 mM stock solution of PMSF in 100% ethanol (8.7 mg in 500µl), to inactivate proteinase K*
- Repeat wash step one more time with PMSF and one without PMSF, then:
  - Wash plugs 2X in 2 ml wash solution diluted 1/10 for 30 minutes at 37°C
  - Wash plugs 1X in 2 ml wash solution diluted 1/100 for 30 minutes at 37°C
  - Wash plugs 1X in 2 ml wash solution diluted 1/1000 for 30 minutes at 37°C

- Transfer plugs to fresh 1.5 ml tubes containing 1 ml 1X restriction buffer
  Incubate shaking at 37°C for 1 hour
- Remove restriction buffer and replace with 250 µl fresh 1X restriction buffer
- Add 5 µl restriction enzyme (50 U, adjust accordingly), incubate shaking at 37°C overnight

Hitch et al (2025); Ausiannikava et al (2018)

# DAY 3 – POURING AND LOADING GEL

- For temporary storage of plugs, transfer into 1 ml fresh wash solution, leave at 4°C
  - For long term storage, equilibrate plugs in 1 ml wash solution +50% glycerol, then store at –20°C
- Prepare 1.2% PFG gel, (SeaKem Gold agarose) ★
  - For small gel melt 1.26 g PFG agarose in 105 ml 0.5X TBE. Pour 100 ml gel
  - For large gel melt 1.98 g PFG agarose in 165 ml 0.5X TBE. Pour 160 ml gel
  - Allow to set for >1 hour (keep ~5 ml molten agarose for later)
- Wash plugs twice in 2 ml 0.5X TBE buffer at 37°C for 30 mins before loading
- Set up PFG apparatus (BioRad CHEF Mapper) with 2.2 litres of 0.5X TBE
  - Leave circulating at 14°C, for 30 minutes to cool
- Insert plug into each well (between 1/3 and whole plug, depending on well size)
  - Seal plugs in place with molten agarose (from earlier) – use as little as possible
  - Use thin slice of lambda ladder as marker – also seal in place with molten agarose

★*Alternatively, arrange plugs on teeth of PFG comb (horizontal), seal in place with molten agarose along top edge, place comb in PFG mould, and pour agarose gel around comb*

- *Remove comb carefully (watch plugs), insert slice of lambda ladder in well, fill empty wells with agarose*

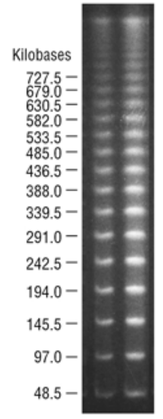
Hitch et al (2025); Ausiannikava et al (2018)

# DAY 3 – POURING AND LOADING GEL – CONT…

- Place gel into frame in PFG apparatus, leave to equilibrate for ~30 minutes at 14°C with circulating buffer
  - Watch out for air bubbles in circulation tubing
- Run 1.2% PFG at 14°C with following conditions:
  - Running time of 20 hrs 46 mins (vary as appropriate)
  - Two state, 120° included angle
  - Gradient 6 V/cm (0 ramping)
  - Initial switch time 0.64 sec
  - Final switch time 1 min 13.22 sec

# DAY 4/5

- Stain gel with ethidium bromide and visualise
- Proceed to Southern blot protocol if required
  - Acid nick, denature and blot for 1.5x longer than usual (due to higher agarose%)




Hitch et al (2025); Ausiannikava et al (2018)