Pulsed Field Gel Electrophoresis
Adapted from Sanderson Lab protocol (U. Calgary)

Preparation of peptide-treated cultures
-Inoculate O/N culture: LT2 (or mutant, or…) in MH broth
-Next day, subculture 1:100 into 1ml MH broth for each treatment
-2 hours at 37C, with shaking
-Add peptides, control compounds
-3 hours at 37C, with shaking
-Dilute 100ul of each sample to 1000ul (1:10 dilution) and take OD_{600}
-Spin down remaining 900ul at 13K rpm, 3 minutes
-Resuspend Pellet in Resuspension Solution to make OD_{600}=1.2-1.4
[(actual OD600)/ 1.3 (desired)] x 0.9 = ml resuspension buffer to use
(the factor of 0.9 is to correct because 900ul is left, instead of 1000)

Preparation of bacteria-imbedded agarose plugs
-Prepare 1.5% PFGE agarose (Bio-Rad) in Sterile nH20
-Combine equal of volumes molten, cooled agarose and bacterial suspension
(Final agarose concentration in plugs = 0.75%)
-Pipet into plug molds, allow to harden at 4C for 10-20 minutes
-Typically yields 10 plugs/treatment (treatments typically yield less)
-Remove plugs to 15ml conical tubes (10 plugs of one treatment/tube)

Lysis and protein inactivation
- Add 4ml of Cell Lysis Solution to each 15ml conical containing plugs
- Incubate in waterbath at 65-70^{0}C for 1 hour with periodic shaking*
- Decant Cell Lysis Solution (I decant, so I don't lose plugs)
- Add 4ml Proteinase K solution (1mg/ml in Proteinase K buffer)
- Incubate in waterbath at 42^{0}C for 18-72 hours (usually at least 48 hours**) with periodic shaking*

**I have found that 48 and 72 both work, so they can be left over the weekend or pulled early depending on your schedule.

- Discard the Proteinase K buffer by decanting
- Wash the plugs in 4ml wash solution (10 minutes @ room temperature) with shaking.*
- Decant wash buffer
- Add 3ml of 1mM PMSF solution to the plugs (to destroy the Proteinase K) for 1 hour at room temperature with gentle shaking*
- Wash the plugs in 4ml wash solution (10 minutes @ room temperature) with shaking.*
- Decant wash buffer
- Wash the plugs in 4ml wash solution (10 minutes @ room temperature) with shaking.*
- Decant wash buffer
- Wash the plugs in 4ml storage solution (10 minutes @ room temperature) with shaking.*
- Decant wash buffer
- Wash the plugs in 4ml storage solution (10 minutes @ room temperature) with shaking.*
- Decant wash buffer
- Store plugs at 4C in 4ml storage solution.

*Sanderson Lab Protocol calls for continuous gentle shaking, but we only have stagnant water baths, so I try to remember to jostle them from time to time to facilitate diffusion, but find it works without shaking, so no worries.

Plug digest
- Pull one plug per condition (unless smaller than 5mm x 10mm)
- Cut plug in half (~5mm square, use 2 different plugs if necessary) move each half to separate eppi-tube (one for cutting, the other for uncut)
- Add 1ml sterile nH20 to each plug
- Keep “uncut” plugs in water at RT while performing the digest
- Place “cut” plugs in 37 water bath for 20 min
- Aspirate water from “cut” plugs
- To each, add 500ul 2x buffer #4 (#4 is for ICeuI digestion), 10min at room temp
- Aspirate buffer 4
- Add 500ul 1x buffer #4 + BSA + 10U ICeuI to each “cut” plug
- Incubate 37C waterbath, 1 hour
- Aspirate reaction buffer from “cut” plugs
- Add 1ml sterile nH20 to “cut” plugs (10min, room temp)
- Aspirate water from ALL plugs
- Add 500ul running buffer (0.5xTBE) to ALL plugs

Casting and Running the Gel
- Prepare 1% PFGE Agarose (Bio-rad) in 0.5x TBE
- Melt and set aside to cool
- Place each plug (5x5mm) on a tooth of the comb (we currently have 15 and 20 combs)
- Place comb/plugs in casting tray, with 1mm space underneath plugs
- Pour molten, cooled agarose to a level 1mm above plugs (yields a 7mm thick gel, perfect!)
- Allow to cool and harden 10-15min at RT (shouldn’t take long, or you melted your plugs)
- Place gel/platform in recess in PFGE rig
- Add 2200ml of 0.5x TBE
- Start pump, clear air bubbles from the lines
- Start cooling module,
- Allow buffer to cool
- Start run

For looking at ICeuI fragments, I use the following:

> 14C, 22 hours, 6.0V/cm, 120 degree included angle, 50-90 second switch time, with a linear ramp.

Reagents

Cell Suspension solution
10 mM Tris HCl (pH 7.2), 20 mM NaCl, 10 mM EDTA

Cell Lysis Solution
10 mM Tris HCl (pH 7.2), 50 mM NaCl, 100 mM EDTA, 0.2% Sodium dodecyl sulfate, 0.5% N-Lauryl Sarcosine

Wash Solution
20mM tris Hcl (pH 8), 50 mM EDTA

Storage solution
(10X diluted wash solution)

PMSF (Phenyl Methyl Sulfonyl Fluoride) Solution
Make a working stock of 1mM PMSF solution
(Eg: For 12 tubes, add 0.36 ml PMSF (1mM) to 36 ml wash solution and add 3ml to each tube)

Proteinase K buffer
100 mM EDTA, 0.2% Sodium dodecyl sulfate, 1% N-Lauryl sarcosine