TRF analysis with PFGE

06/2015

Kits: Gentra Puregene Kit (Qiagen)
TeloTAGGG Telomere Length Assay Kit (Roche)

Day 1

1. Genomic DNA isolation

- DNA isolation of at least one T75 flask with Gentra Puregene Kit (Qiagen)
- Resuspend DNA in as little hydration solution as possible to increase concentration
- Centrifuge DNA solution 5 min at maximum speed and use only supernatant in digestion reaction

2. Digestion

- Digest 5 µg DNA with RsaI/HinfI
- Add 5 µg DNA to tube and fill up to 25 µl with H2O
- Prepare a mastermix containing for each 5 µg DNA sample:
  1.3 µl HinfI (10 U/µl, Roche)
  1.3 µl RsaI (10 U/µl, Roche)
  3 µl 10X digestion buffer SURE/Cut A (if total volume is 30 µl)
- Add 5 µl mastermix to each tube
- Incubate O/N @37°C

Day 2

- Add DNA loading dye to stop digestion reaction
- Prepare marker by mixing two markers:
  2 µl GeneRuler High Range DNA ladder + 7 µl Dig High molecular weight marker from Roche kit + loading dye + fill up with H2O to same volume as samples

3. PFGE

- Prepare 450 ml 0.6% agarose with Biozym gold agarose in 1X TAE (heat while stirring until agarose is dissolved completely, then cool down to 60-70 °C)
- Meanwhile: Prepare the casting chamber by putting the black plastic cover in a big chamber (25 cm long) and the comb on top of sticks on both sides (so that it doesn't touch the black cover)
- Fill the CHEF-DRII chamber with ca. 3 l cooled 0.5X TAE
- Turn the pump to a flow of ca. 50, remove all air from the tubes, then cover the tube with ice in a box
- When the gel has solidified, cut the gel to the size of the black cover with a scalpel
- While the pump is OFF, put the black cover with the gel on top inside the frame attached to the bottom of the CHEF-DRII chamber
- Load the HinfI/RsaI digested DNA and the molecular weight marker
- Run the PFGE gel with the following conditions:
  Pump: 70-80
  Volt: 4 V/cm
  b1: Initial switch time: 1 s
  Final switch time: 6 s
  Run time: 13 h

Day 3

- Incubate gel 30 min in SYBR Safe (1:10000 in 1X TAE)
- Make an image

4. Depurination/Neutralization/Denaturation of gel

- To facilitate DNA transfer, partially depurinate the DNA by placing the gel in 0.25 M HCl solution for 20 min under gentle agitation (bromphenol should turn yellow)
- Rinse gel twice with H_2O
- Denature the gel in denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 2x15 min with gentle shaking
- Rinse gel twice with H_2O
- Submerge gel in neutralization solution (0.5 M Tris-HCl, 3 M NaCl, pH 7.5) for 30 min with gentle shaking

5. Southern Blot

- cut a positive charged nylon transfer membrane and 3 Whatman papers to the size of the gel and soak in 10X SSC
- fold paper towels to a stack of about 10 cm height and place about 3 cm paper soaked in 10X SSC onto the stack
- place 4 pre-cut Whatman papers soaked in 20X SSC onto the stack
- Place the membrane onto the stack and mark the upper right corner of the membrane
- remove any trapped air-bubbles by rolling a pipette over the surface of the membrane
- carefully place the neutralized gel onto the membrane
- cover the stack with saran wrap and put the whole blot in a basket
- put the big gel chamber on top and a 2x250 g weight (after some time more and more weight)
- the transfer of DNA to the membrane will take ca. 12 hours

Day 4

- After transfer: label the membrane at slots with a pencil
- remove the membrane from the stack and air-dry it
- bake membrane at 120°C for 20 min
- Wash membrane twice in 2X SSC

6. Hybridization and Detection (according to telomere length assay from Roche)

- Prewarm DIG Easy Hyb Granules (bottle 7) to 42 °C
- Prehybridization: Cover the membrane with prewarmed DIG Easy Hyb Granules in a tupper box and incubate for at least 1 h at 42 °C with gentle agitation
- Prepare hybridization solution (3 µl telomere probe in 15 ml DIG Easy Hyb Granules)
- Discard prehybridization solution and immediately add hybridization solution to the membrane
- Incubate at 42 °C O/N with gentle agitation

Day 5

- Discard hybridization solution
- Wash membrane twice for 5 min at RT with stringent wash buffer I (2X SSC, 0.1% SDS)
- Wash membrane twice for 15-20 min at 50 °C with prewarmed stringent wash buffer II (0.2X SSC, 0.1% SDS) with gentle agitation
- Rinse membrane in at least 50 ml 1X washing buffer for 5 min at RT with gentle agitation
- Prepare ~100 ml 1X blocking solution: Dilute maleic acid buffer (bottle 12) 1:10 with H_2O to get a 1X solution. Then, dilute 10X blocking buffer (bottle 11) 1:10 with1X maleic acid buffer.
- Incubate membrane in 50 ml freshly prepared 1X blocking solution for 30 min at RT with gentle agitation

- Prepare Anti-DIG-AP working solution: Spin vial for 5 min at 13,000 rpm before use. Dilute Anti-DIG-AP (bottle 13) with 1X blocking solution to a final concentration of 75 mU/ml (1:10,000)
- Incubate the membrane in 50 ml Anti-DIG-AP working solution for 30 min at RT with gentle agitation
- Wash membrane 2x 15 min at RT with 1X washing buffer (bottle 10; 50 ml each time) with gentle agitation
- Prepare 1X detection buffer: Dilute 10X detection buffer (bottle 14) 1:10 with H_2O
- Incubate membrane in 50 ml of 1X detection buffer for 2-5 min at RT with gentle agitation
- Shortly dip membrane on whatman paper then place the wet membrane, DNA side facing up, on an open transparent sheet and very quickly apply ca. 3-4 ml substrate solution (bottle 15) to the membrane
- Immediately cover the membrane with the transparent sheet and spread the substrate solution without trapping air bubbles
- Incubate the membrane for >30 min at RT
- Squeeze out excess substrate solution and expose the membrane in a Bio-Rad Chemi-Doc MP System (Program “Chemi High Resolution”)