Typing Mice by Southern Blot in 2 Days

**Tail DNA** (The protocol of the awesome Luis)

**Digestion Buffer (DB)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td>TRIS</td>
<td>10 mM</td>
</tr>
<tr>
<td>EDTA</td>
<td>5 mM</td>
</tr>
<tr>
<td>SDS</td>
<td>1%</td>
</tr>
<tr>
<td>NaAc</td>
<td>0.3 M</td>
</tr>
</tbody>
</table>

autoclave or filter, then add

200 µg/ml Proteinase K

**TE**

<table>
<thead>
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1 Cut [tails and] toes. Process immediately or store @ -20 C.

2 Add 250-400 µl of DB. Digest 2-4 h or o/n @ 45 C until free hairs and bones are visible.


4 Spin 10’ @ 13,000 rpm, RT.

5 With a wide bore tip, take off the sup (rather leaving junk behind) and transfer to a new vial with 1 ml of 100 % ethanol. Invert until the DNA is visible. Transfer with a yellow tip to a new vial with 100 µl of TE.

6 Resuspend 10 mins. @ 37-65 C. DNA is ready for use. Store @ 4 C.

**The Gel**

1 Digest around 10 µg DNA (equals around 10-15 µl) 4 h or overnight.

2 Run the digest in a 0.8 % agarose gel @ 75 V. (The medium size BioRad gel trays with two combs work nicely and fit the size of the Hybond-N\(^+\) membrane exactly (20 cm). Running takes around 4 h.)

3 Take a picture with a ruler next to it.
The Probe

3 x RP Buffer
120 mM TRIS-HCl, pH 8.0
12 mM MgCl2
400 mM HEPES, pH 6.5
24 mM 2-ME
48 μM dATP, dGTP, dTTP
0.5 mg/ml BSA
5 ng/ml hexamer

1 Dilute 25-50 ng purified DNA fragment in H2O, 15 μl total.
2 5 mins. @ 95 C, chill on ice, e.g., in a PCR machine.
3 Add 10 μl 3 x RP buffer
   1 μl Klenow (5 U)
   4 μl 32P-dCTP (3000 Ci/mmol), 3 μl is fine in times of shortage.
4 1 h @ 37 C.
5 Prepare G50 column: fill 1 ml syringe without needle & plunger over cotton wool from 10 ml pipette with 0.8 ml G50, spin in a 15 ml tube for 1 min. @ 1000 rpm. Discard flow through, put in an Eppi without lid.
6 Fill up labeling reaction with H2O to 200 μl, put on column, spin 1 min. @ 1000 rpm.
7 Transfer the flowthrough to a new Eppi. Count 2 μl without scintillation in γ-counter: 10^8 cpm/μg or more is good (i.e., 50,000 cpm for 50 ng DNA is the lower limit).
8 Essential: Denaturation of the probe!
   In case you use Stratagene QuikHybe solution for hybing, just boil the probe, mix with 0.5 ml QuikHybe and hybe.
   Add 100 μl of 10 mg/ml salmon or herring sperm DNA (blocking non-specific binding sites on the membrane) and denature by adding 0.2 N NaOH, that is 12 μl of 5 N NaOH. Incubate 5 mins. @ 37 C.
   Alternative: Add 100 μl of 10 mg/ml salmon or herring sperm DNA (blocking non-specific binding sites on the membrane) and denature by boiling at 95 C for 5 mins. (Danger! prick the lid with a needle, otherwise it will pop, messy!).
The Blot (Chomczynski, Anal. Biochem. 201: 134 (1992), modified)

Transfer Solution (TS)
3 M NaCl
0.4 M NaOH

1 Soak the gel 30 mins in TS.

2 Transfer DNA 1 h downwards with TS:
   weight (gel tray is sufficient)
   cover everything with Saran
   3 x 3MM paper bridge (prewetted in TS)
   3 x 3MM paper (prewetted in TS)
   gel
   membrane (prewetted in H$_2$O, then TS)
   3 3MM paper (prewetted in TS)
   2 thick Whatmans
   paper towels

   In the meantime, warm the Stratagene QuikHyb solution to 50-65 C as well as the tube (this avoids precipitation of dextran from the QuikHyb solution after adding it into the cold tube) and have a coffee.

3 Before taking everything apart, mark the pockets with a ballpoint pen on the membrane. Wash the membrane briefly in 2 x SSC or 0.2 M TRIS, pH 7.0.

4 To check the transfer, restain the gel with ethidium bromide and take a picture.

5 Prehybe the membrane 15 mins. (no more!) in 8 ml (small) or 12 ml (large hybridization tubes) QuikHyb in rotating oven @ 68 C.

6 Mix the probe with 1 ml of warm prehybe solution, vortex, and add to hybridization tube. Hybridize for no more than 1 h @ 68 C.

7 Pour out probe, rinse with 2 x SSC, 0.1 % SDS, wash two times with 2 x SSC, 0.1 % SDS, and with 0.2 x SSC, 0.1 % SDS if required.

8 Expose overnight, develop, have a happy life ever after...