**Southern**

**Restriction digest:**

1 µg DNA  
2 U restriction enzyme  
1 µl 10x restriction enzyme buffer  
(0.1 µl 100x BSA 100µg/ml, depending on enzyme)  
x µl ddH₂O  

∑ = 10 µl

Incubate digest 1h at 37°C (temperature depending on the enzyme). Stop digest by adding 1 µl of 10x loading buffer.

**Gel:**

1% agarose gel, big (20 slots), 1x TBE-buffer (e.g.)

2 g agarose  
200 ml1x TBE-buffer (from 10x TBE-stock solution)

Heat in microwave 2.5 min.  
+ 10 µlEthidiumbromide (10 µg/µl)

Let cool for 10 min.  
Pour gel (big gel ca. 150 ml, small gel ca. 80 ml).

**Loading:**

Load 50 - 100 ng of digested plasmid DNA or all digested chromosomal DNA.

Run gel at 25 V overnight or for a shorter time at up to 110 V.

Take a picture of the gel (with a ruler beside it).

**Treatment of Gel:**

- gently shake gel for 15 min in 0.25 M HCl (depurinization)  
- gently shake gel for 2x 15 min in denaturing solution  
- gently shake gel for 2x 15 min in neutralizing solution
material:

- 0.25 M HCl  (20.8 ml  37% HCl, add ddH2O to 1l total)

- **Denaturing solution:**
  
  0.5 M NaOH (40 g/mol -> 20g)
  
  1.5 M NaCl  (58.5g/mol -> 87.5g)

- **Neutralizing solution:**
  
  1 M Tris-HCl, pH 7.5  (1mol Tris-base 121.14g/mol -> 121.14g)
  
  1.5 M NaCl  (87.75g)
  
  (adjust pH to 7.5 with concentrated HCl)

**Southern Blot:**

- turn the gel over and put on clean table (gloves!)
- wet a piece of nylon membrane of the size of the gel in neutralizing solution and lay it onto the gel (no airbubbles!)
- wet 3 pieces of 3mm Whatman paper (same size as gel!) in neutralizing solution and lay onto gel, one after each other (no airbubbles!)
- then ca. 7cm of a stack of paper towels (about the same size as gel) is put on top
- a tray (or something with a flat surface) should be put on top with a weight of ca. 250g on it
- blot for 4h (or longer if gel is thick)
- remove paper and label membrane

**Covalent Binding of DNA to Nylon Membrane:**

- use the “UV-Stratalink”
- push “Autocrosslink” once to burn out UV-lamps
- put in the nylon membrane (on a piece of household wrap), the DNA facing up
- push “Autocrosslink” again
- the DNA is now covalently bound to the membrane
- the membrane can be stored in wrap at 4˚C
**Hybridization:**

- **Prehybridization:**
  - place membrane in a hybridization tube by rolling it; the edge of the membrane should face the direction of rotation in the Hybridization Incubator (FISHER Biotech)
  - incubate membrane in 10ml (-20ml) Prehybridization buffer at 60°C for at least 1h

- **Hybridization:**
  - denature probe DNA in microcentrifuge tubes by heating them to 95°C for 10 min
  - incubate membrane in 10ml (2.5 ml/ 100 cm²) Hybridization buffer at 63°C overnight

- **Stringent Washing:**
  - wash 2x for 15 min in 50 ml of 2x SSC, 0.1 % SDS at room temperature
  - wash 2x for 15 min in 50 ml of 0.5x SSC, 0.1 % SDS at 55°C (prewarmed)
  - or, for higher stringency:
    - wash 2x for 15 min in 50 ml of 0.1x SSC, 0.1 % SDS at 60°C (prewarmed) (1l: 10ml 10% SDS, 5ml 20x SSC makes 0.1x SSC, 0.1 % SDS)

**Material:**

- **10 % SDS:** (100 ml)
  - 10% (10g) (w/v) sodium dodecyl sulfate
  - 100ml ddH₂O

- **20x SSC:** (1 l)
  - 175.3 g NaCl
  - 88.2 g Na-citrate

  Add ddH₂O and adjust pH to 7.0.
  Add ddH₂O to 1l total.

- **Prehybridization buffer:** (20 ml are needed) (200ml)
  - 5x SSC
  - 2% (w/v) blocking reagent (from 10% sterile stock solution) (40 ml)
  - 0.1% (w/v) N-laurylsarcosine (0.2 g)
  - 0.02% (w/v) SDS (10% SDS -> 0.4ml)

Storage at room temperature, dark.
• **Hybridization buffer:** (10 ml are needed)
  
  50 ng probe DNA should be added to 10 ml prehybridization buffer.
  
  The hybridization buffer can be used several times. If used, it should be stored in microcentrifuge tubes at -20˚C. For reusage, the whole hybridization buffer with the probe DNA in it needs to be denatured.
  
  Storage at room temperature, dark.

• **Blocking reagent stock solution:** (10 %)
  
  10% (w/v) blocking reagent (from Boehringer Mannheim)
  
  soluted in maleic acid buffer (buffer 1).
  
  Solve blocking reagent by constantly stirring it at 65˚C or by heating it in the microwave.
  
  Autoclave and store at 4˚C. (Will look milky.)

• **Maleic acid buffer (buffer 1):** (ca. 450 ml are needed)
  
  (1 l)
  
<table>
<thead>
<tr>
<th>Molarity</th>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M</td>
<td>maleic acid</td>
<td>(116.1 g/mol -&gt; 11.61 g)</td>
</tr>
<tr>
<td>0.15 M</td>
<td>NaCl</td>
<td>(8.78 g)</td>
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</tbody>
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  Adjust pH to 7.5 (20˚C) with solid (or concentrated) NaOH.
  
  Autoclave and store at room temperature.

**Detection:** (for DIG-labeled probes)

For a detailed protocol see “CDP-Star” from “Boehringer Mannheim”.

The detection is completed at room temperature, beginning inside of the “Hybridization Incubator” (FISHER Biotech) at room temperature.

- wash membrane for 1-5 min in (25 ml) wash buffer
- block membrane by incubating it in 100 ml buffer 2 for 30 min
- dilute “Anti-DIG-AP Conjugate” 1:30,000 (0.666 µl / 20 ml) (1:20,000 - 1:50,000 are recommended) in buffer 2
- incubate membrane in 20 ml Anti-DIG-AP dilution for 30 min
- wash membrane 3x (or 2x) for 15 min in 100 ml wash buffer
- equilibrate 2-5 min in 20 ml of buffer 3
- take out the membrane, still wet, and place in a plain bowl
- dilute CDP-Star (25 mM) 1:1000 (10µl / 10 ml) (1:100 - 1:1500 are recommended) in buffer 3
- incubate membrane for 5 min, gently shaking, with 10 ml of CDP-Star dilution
- lift the membrane up and let the liquid drop off
- place membrane in a hybridisation bag and seal, still slightly wet
- expose membrane to x-ray film inside a cassette (expose film to redlight only!) for 15 s - 5 min and develop film
material:

- **Wash buffer:** *(325 ml are needed)*
  0.3% (w/v) Tween 20 (detergent)
  in maleic acid buffer.
  
  e.g. 325 ml:
  
  \[
  \begin{array}{c}
  325 \text{ ml} \\
  975 \mu l
  \end{array}
  \]
  maleic acid buffer
  Tween 20 (very viscous, cut tip before pipeting!)

- **Buffer 2 (blocking solution):** *(120 ml are needed)*
  5% (w/v) milk powder (not like original protocol!)
  in maleic acid buffer.
  Cannot be autoclaved, because milk powder will denature!
  Storage at 4°C.

- **Buffer 3 (detection buffer):** *(30 ml are needed)*
  0.1 M Tris-HCl
  0.1 M NaCl
  50 mM MgCl\(_2\)
  
  Adjust pH to 9.5 (20°C).

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**Stripping:**

The probe is being removed, the membrane can be reused for hybridization with another probe.

- incubate membrane for 30 min with 100 ml of 0.4 M NaOH at 45°C
- wash membrane 1x for 15 min with 50 ml 1x SSC, 0.1% SDS at room temperature