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 ddH_2O

∑ = 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 ddH_2O to 1l total)

· Denaturing solution: (1 l)

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

· Neutralizing solution: (1 l)

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 cm^2) 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	ddH_2O

· 20x SSC: (1 l)

175.3	g	NaCl
88.2	g	Na-citrate

Add ddH_2O and adjust pH to 7.0.
Add ddH_2O to 1l total.

· Prehybridization buffer: *(20 ml are needed)* (200ml)

5x	SSC	(20x SSC -> 50 ml)
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 10ml prehybridization buffer.
The hybridzation 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)

0.1	M	maleic acid	(116.1g / mol -> 11.61 g)
0.15	M	NaCl	(8.78 g)

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:

325 ml maleic acid buffer
975 µl 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).

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