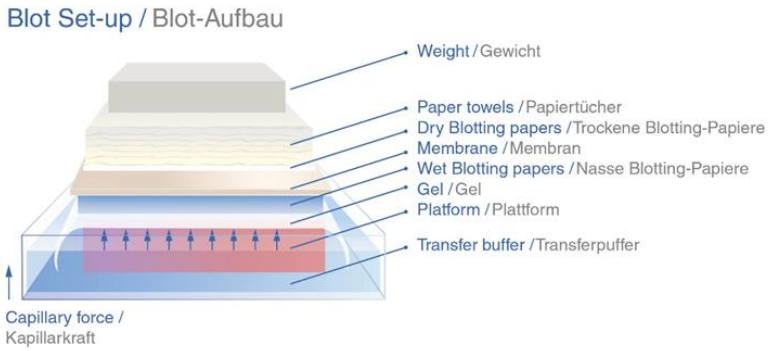
Technical Info

Well advised with Roth.

Southern & Northern Blot

General information



Southern and Northern blotting are molecular biology techniques for the transfer of nucleic acids onto membranes, with a subsequent hybridisation procedure that allows specific nucleic acid sequences to be detected. Southern blots are used to identify specific DNA sequences, e.g. to find out how many copies of a certain gene are present in an organism, while northern blotting is used to compare mRNA pools between different organisms. Since RNAseq, microarrays and RT-PCR are now common and sometimes more sensitive methods for analysing interspecies mRNA pools, Northern blotting is nowadays less commonly used. Southern blot, on the other hand, is still a very popular method because, in contrast to PCR, it can also be used to identify orthologous or paralogous genes, partial insertions of foreign genes, or the number of copies of a specific gene within a genome, since only the basic sequence of the gene, but no specific primer binding site, needs to be known. As the Northern blot is rarely performed nowadays, the main focus of this information brochure will be on the Southern blot.

General procedure

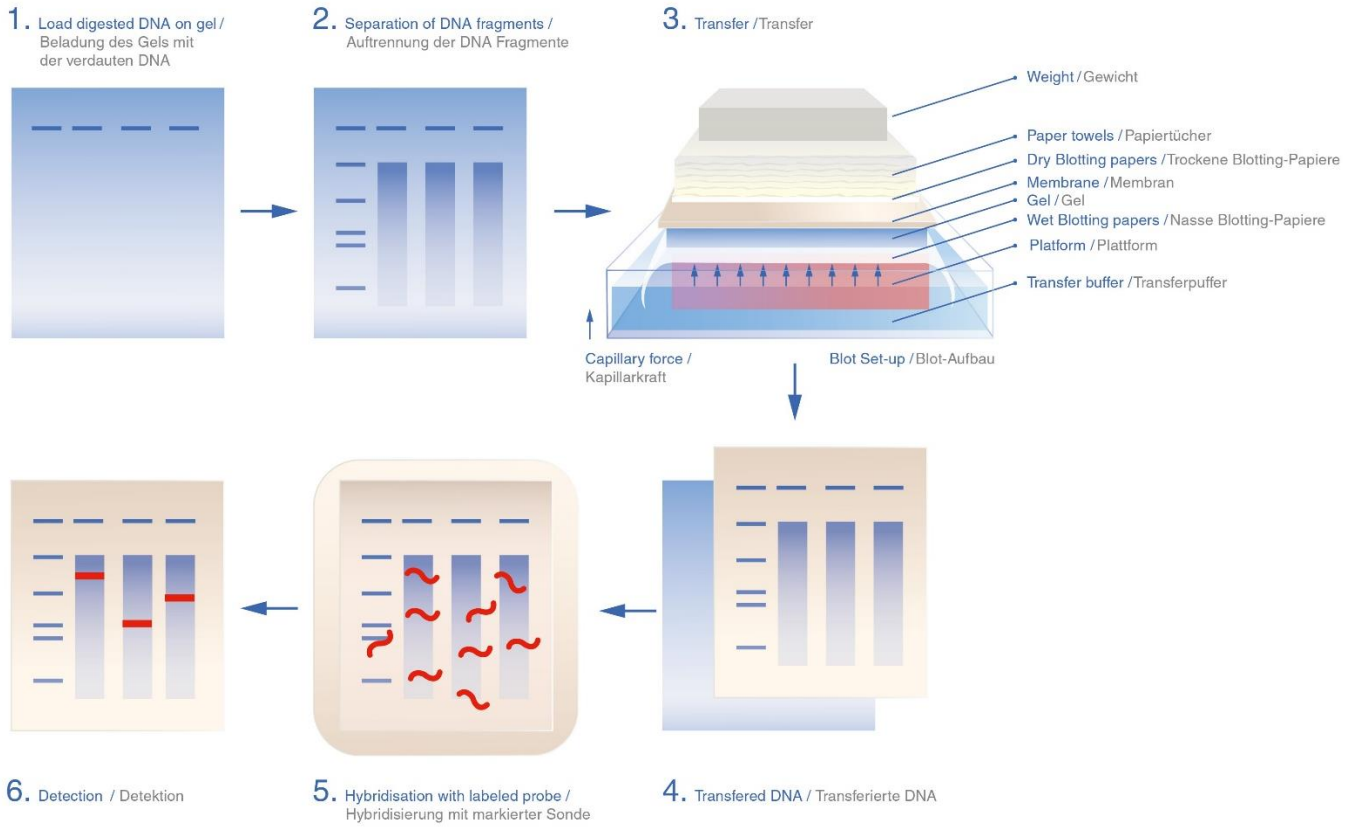
The first step required to perform a Southern blot is to **isolate gDNA** from an organism or from specific cells. The obtained gDNA must then be fragmented in the next step. This is usually done by **digestion** with selected restriction enzymes, which ideally should not cut within the sequence of interest. To separate the resulting DNA fragments, an agarose **gel electrophoresis** is performed afterwards, with a subsequent quality check by gel documentation. If the gel shows the expected DNA band pattern, the procedure can be continued. If large fragments are to be transferred, the gel can be placed in 0.25 M HCl solution, as this **depurinates** the DNA, causing it to break into smaller pieces.

To prepare blotting and hybridisation, the agarose gel is placed in alkaline solution to denature double stranded DNA into single stranded DNA. This method is named **alkaline transfer** and has the advantage in improving binding of the negatively charged DNA to the positively charged membrane. Afterwards the gel is washed in a neutralisation buffer and then incubated in transfer buffer. For the transfer of nucleic acids from the gel to a membrane, usually capillary blotting is used. To build up capillary forces, a buffer reservoir is filled into a tray and the blot is built up on a platform in the tray. First, a couple of blotting papers are placed on the platform with their ends protruding into the buffer reservoir. The gel is placed on top of the blotting papers and a membrane made of nitrocellulose or nylon is placed directly above it. A layer of dry blotting papers is placed on top of the membrane, followed by a layer of dry paper towels. Finally, the blot is weighted down with a glass plate and a weight on top to support the capillary forces required for transfer. After the transfer, the DNA can be **covalently fixed** on the membrane by UV crosslinking or alternatively, by baking it for 15 min at 80 °C.

In preparation for hybridisation and to avoid false positive signals, the free binding sites of the membrane need to be **blocked** in the next step. For this purpose, the membrane is incubated in a prehybridisation solution consisting of salmon sperm DNA, BSA, surfactants and polymers. The salmon sperm DNA binds to the unspecific

binding sites of the transferred DNA, whereas the surfactants, proteins and polymers saturate the membrane and as consequence preventing the non-specific binding of the labeled probe DNA to the membrane. Afterwards the **hybridisation step** can be performed by probing the memrane with labeled ssDNA complementary to your sequence of interest. For Southern Blotting, DNA is either labeled radioactively, with Biotin, Digoxygenin (DIG) or a fluorescent dye. It is possible to order the labeled ssDNA or to label it by yourself, e.g. by using a DIG-labeling Kit.

In the final step the sequence of interest is visualized either by autoradiogram or in case of a labeled antibody/Streptavidin against DIG or Biotin, in a documentation system or without any further devices.



Recommended Products

Blotting Step	Product	Product name	Art. Nr.
Electrophoresis	**Agarose**	Agarose HR-Plus	HP30.2
		Agarose Broad Range	T846.2
		Agarose GTQ	6352.2
	Running Buffer	ROTIPHORESE®10x TBE Buffer	3061.1
	Loading dye	ROTI®Load DNA tricolor	HP06.1
	DNA ladder	DNA-Ladder combi	CL22.1
	DNA stain	ROTI®GelStain Red	0984.1
		ROTI®Methylene blue staining concentrate	0648.1
Transfer	**SSC buffer**	ROTI®Fair 20x SSC	1232.1
		ROTI®Stock 20x SSC	1054.1
	SSPE buffer	ROTI®Fair SSPE	1233.1
	Nylon membrane	Transfer membrane ROTI®Nylon plus	K058.1
		Transfer membrane ROTI®Nylon 0.2	AE50.1
	NC membrane	Transfer membrane ROTI®NC 0.2	9302.1
		Transfer membrane ROTI®NC 0.45	9200.1
	Blotting papers	Blotting papers ROTILABO®, 0.75 mm	0943.1
		Gel Blotting Papers Whatman®, 1.5 mm	A125.1
	Blotting devices	Semi dry blotter ROTIPHORESE® PROfessional MAXI	KK59.1
Crosslinking	**UV crosslinker**	UV Crosslinker, 254 nm	1782.1
Blocking	**Denhardt's solution**	Denhardt's solution, lyophilised	HP33.1
		Salmon Sperm DNA	5434.1
	SDS	ROTI®Stock 20 % SDS	1057.1
	Incubation tray	Blot incubation trays	4811.1
Detection	**Autoradiography**	Autoradiography Pen	CL10.1
		X-ray-cassettes ROTILABO®,	X265.1
	Documentation systems	Gel documentation system gelLITE	1Y4N.1
		Documentation system chemiLITE	1YE6.1

Basic Protocol

1. Digestion with appropriate restrictrion enzyme → Use 5-10x more restriction enyme than recommended, digest at 37 °C for 2 h (plasmid DNA) or overnight (genomic DNA).
2. Concentrate DNA to a volume of 25 µl, e.g. with Ethanol precipiation and resuspension in ddH_2O or with DNA purification coloumns
3. Separate DNA-fragments in agarose gel electrophoresis (0.7-2% agarose gel) for 1-16h depending on the range of fragments to be separated. To separate gDNA it is recommended to run at low voltage (5-20 V) for Plasmid DNA up to 100 V can be used.

4. Visualize separated DNA on a transilluminator or in a gel documentation system. Plasmid DNA should appear as distinct bands, whereas gDNA should appear as a smear, with brighter bands that represent repetitive DNA elements. Place a ruler alongside the gel to take a picture, as this facilitates matching the hybridisized sequence of interest to the position in the gel
5. If large fragments are to be transferred the DNA can be depurinated by placing the gel in 0.25 M HCl for 10 min.
6. To prepare the transfer, denature the DNA by placing the gel in alkaline solution with 1.5 M NaCl and 0.5 M NaOH for 15-30 min, afterwards wash the gel in ddH_2O
7. Wash the gel twice for 15 min with neutralization buffer consisting of 3 M NaCl and 0.5 M Tris HCl (pH 7)
8. Equilibrate the gel by placing it for 30 min in 20x SSC buffer (or 20x SSPE buffer).
9. Cut a piece of membrane (either nylon or nitrocellulose (NC)) to the size of your gel and incubate the membrane for 5 min in 0.5x SSC buffer, than for 1 min in 20x SSC (alternatively in 10x SSPE for 5 min)
10. Prepare the blotting papers and paper towels. These should be slightly larger than the membrane. One half of the blotting papers should be immersed in 20x SSC buffer (alternatively 10x SSPE) until saturated, the other half should remain dry.

11. Set up the blot as follows:
    a. Fill a tray with 20x SSC buffer (alternatively 10x SSPE)
    b. Lay a stable platform, e.g. a glassplate in the tray
    c. Place some SSC soaked blotting papers on the platfrom. Make sure that the corners of the blotting papers protrude into the SSC-buffer reservoir in the tray.
    d. Place the gel (top side down) on the wet blotting papers.
    e. Place the membrane on the gel. Take care to eliminate air bubbles between the gel and the membrane, e.g. by gently rolling a glass stick over the membrane. Do not move the membrane once it has been placed, as the transfer may have already started.
    f. Place 3 layers of dry blotting papers on the membrane.
    g. Stack some dry paper towels on the blot to increase the capillary force.
    h. Weigh down the blot set up with a glass plate and a weight on top.

12. Let the Blot set-up stand to transfer the DNA. The transfer takes at least 3-16 h, depending on the size of the DNA fragments.
13. Fix the DNA covalently on the membrane, either by UV-crosslinking (254 nm, 1 min → nylon membrane) or by baking it for 15 min at 80 °C(NC-membrane).
14. Block the membrane by incubating it for 1 h at 68 °C in Denhardt's solution containing 100 µg/ml salmon sperm DNA and 1 % SDS dissolved in 6x SSC buffer.
15. Prehybridize the mebrane for at least 1 h at 68 °C in the blocking solution
16. Denature you labeled probe for 10 min at 100 °C
17. Dilute the labelled probe DNA with 20 ml blocking buffer (6x SSPE, 1% SDS, alternatively: 2x SSC, 2% SDS)
18. Perform the hybridisation step by incubatig the membrane overnight in blocking buffer
19. Wash the membrane twice for 5 min at room temperature in 6x washing buffer (6x SSPE, 0.2% SDS, alternatively 2x SSC, 0.2% SDS)

20. Wash the membrane twice for 15 min at 68 °C in 1x washing buffer (1x SSPE, 0.2% SDS, alternatively: 0.5x SSC, 0.2% SDS)
21. Incubate the membrane for 5 min at room temperature in washing buffer
22. For the following blocking steps and the detection of the probe, please proceed according to the instructions of the probe manufacturer.

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