

Fig.1: DNA-fragments on DryGel Elpho 12.5% with the DNA-Buffer. Kit: Silver staining



Fig.2: Fluorescence detection with GelStar

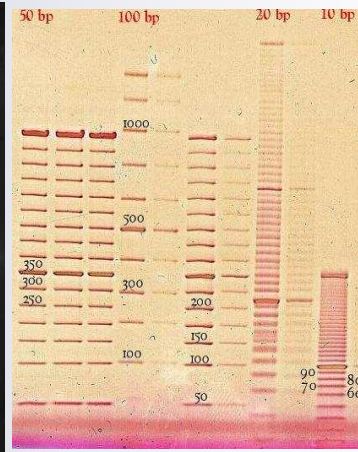


Fig.3: Direct staining using "Stains All"

General: Normally DNA-fragments are separated in Agarose gels through a submarine electrophoresis followed by a fluorescence visualization with Ethidium Bromide. The reasons for this are: 1.) Semipreparative technique. 2.) Separation optimum is between 2 kB and 20 kB.

PCR-reaction products are DNA-fragments below 1000 bases. If an analytical electrophoresis with high resolution is needed, the DNA-fragments should run on Polyacrylamide gels because of their separation optimum below 1 kB.

The DNA Buffer Kit contains a buffer system, which produces extremely sharp bands, resulting in a very high resolution even within short separation distances of 8 cm.

For highest sensitivity (~100 pg) and highest resolution the silver-staining as visualization is recommended, see figure 1.

For fluorescence-staining a specific dye must be used: The GelStar stain is excitable with normal light, the backing material of the DryGels will not interfere under these condition (~1 ng). See figure 2.

Equipment:

flatbed professional: horizontal chamber (edc-ief-2836)

Power Supply minimum 1000 V, 150 mA, 100 W; thermostatic circulator (lab supply)

Steel Staining Tray: (stainless steel tray with elevated stainless steel grid) (edc-wm-n1)

optional: Multi 6 Tray: for staining 6 gels simultaneous (edc-wm-m6)

Accessories:

Dry Pool Combi: tray for rehydration dry gels and soaking the electrpde strips (edc-me-d)

DryGel Elpho:

DryGel Elpho 12.5% 24S:, 4 gels, for 24 samples à 12 µl (edc-4112)

DryGel Elpho 12.5% 52S:, 4 gels, for 52 samples à 5 µl (edc-4113)

Buffer:

DNA-Buffer Kit Amphoter for 4 DryGels Elpho, rehydr., anod. and cathod. Buffer (edc-5005)

Staining: dsDNA: GelStar (Cambrex 50535), ssDNA/RNA: Sybr Green II Molecular Probes S7564, Dark Reader: (Clare Chemicals DR45M), "Stains All" Sigma Aldrich # E9379-1G

Markers: Promega: 100 bp (G210A), Invitrogen: 50 bp (10416-014), 10 bp (10821-015)

Sample preparation

Sample buffer: 25 ml rehydration buffer + 80 µl Orange G solution (1%) + 60 µl Bromophenol Blue solution (1%) + 40 µl Xylencyanol solution (1%) + 250 µl 0.2 mol/l EDTA.

Fluorescence pre-staining: Prepare the tagging mix: 200 µl sample buffer + 2 µl 10% dye solution (dilute with DMSO). Tagging: 2 µl marker + ~16 µl sample buffer + 2 µl tagging-mix. Depending of the PCR-reaction further dilutions can be necessary. Single-stranded samples should be used double concentrated.

Fluorescence staining: Dilute as much as the sensitivity of the used chromogen makes it possible.

Direct staining: Dilute the markers 10 + ~20 µl sample buffer.

Silver-staining: Dilute as much as possible, to reach the upper picogramm region, this gives best results. To control the sample concentration: Take Promega's 100 BP-ladder and dilute 30 µl + 720 µl sample buffer and run at least one lane per gel. During the development step of the staining procedure, the samples should appear synchronously with this standard lane.

Rehydration of the dry gel

Lay the DryPool Combi onto a horizontal table so that the rehydration chamber shows upwards; pipette rehydration solution into the chamber, for a complete DryGel 50 ml. Lay the edge of the gel-film - with the gel surface facing down - into the rehydration buffer (fig. 4) and slowly lower it, avoiding air bubbles. Lift the film at the edges up to the middle, using forceps and lower them again without catching air bubbles, in order to achieve an even distribution of the liquid (fig. 5 A). Repeat this procedure during the first 15 min. Very even rehydration is also obtained when performing it on a shaker at a slow rotation rate (fig. 5 B). 90 min later the gel has rehydrated completely and can be removed from the GelPool. Remove excess buffer, dry the sample wells and wipe off exceed buffer volume from the gel surface with the edge of a drying cardboard (fig. 6) until you can hear a "squeaking".

Important, the gel surface must be total dry!

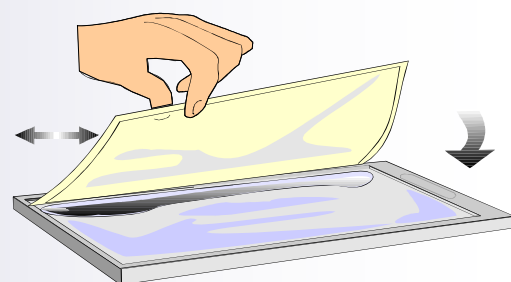


Fig.4: Placing the DryGel into the DryPool

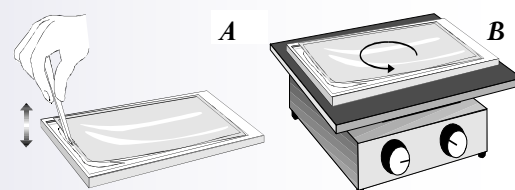


Fig5: A Lifting the edges for an even distribution of the liquid. B Rehydration on a rocking platform

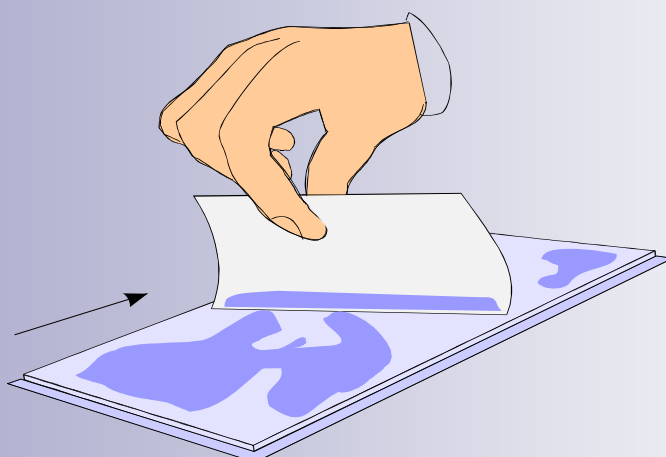


Fig.6: Removing the excess buffer from the gel surface using the edges of a drying cardboard

Application of the gel and the electrode wicks

Switch on the thermostatic circulator, adjusted to 15 °C. Apply a very thin layer of kerosene (ca. 1.5 ml) onto the cooling plate with a pipette, in order to ensure good cooling contact. Place the gel (surface up) on to the center of the cooling plate: The side containing the wells must be orientated towards the cathode (Fig. 8).

Lay two of the electrode wicks into the 2 compartments of the DryPool Combi's reverse side. Apply 25 ml of the respective electrode buffer to each wick using a pipette (fig. 7). Place the cathode strip onto the cathodal edge of the gel, overlapping the gel edge 1-2 mm. Place the anode strip over the anodal edge in the same way. Smooth out air bubbles by sliding bent tip forceps along the edges of the wicks laying in contact with the gel.

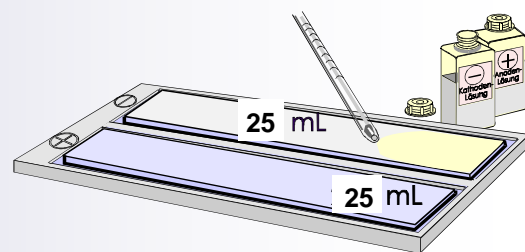


Fig.7: Soaking the electrode wicks with the electrode solution in the DryPool Combi's backside

Sample application and electrophoresis

Apply 6 µL of each sample to the sample wells using a micropipette (or use appropriate multipipette). Don't leave sample slots unfilled. Clean platinum electrode wires before (and after) each electrophoresis run with a wet tissue paper. Place the security lid onto its 4 stands of the basic unit. Move the electrodes so that they will rest on the outer edges of the electrode wicks (fig.9). Move the sliders forward and lower the safety lid until the platinum wires stand on the paper-electrodes (fig.10).

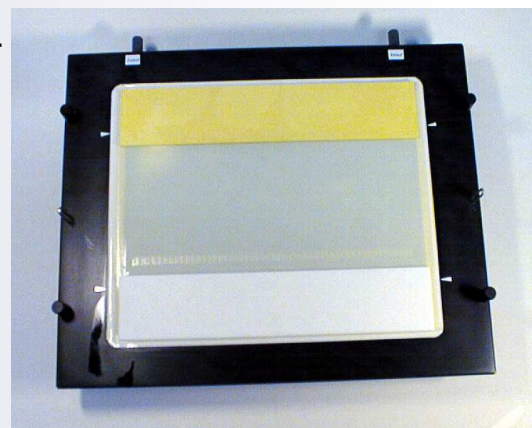


Fig.8: Arrangement of gel and buffer wicks

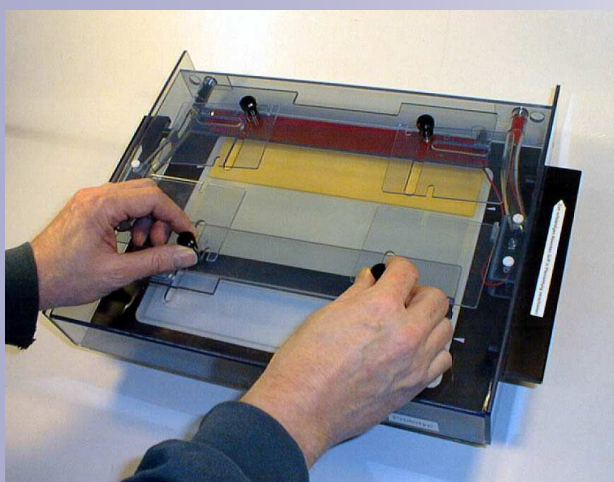


Fig.9: Positioning the electrodes to the outer edges of the paper wicks

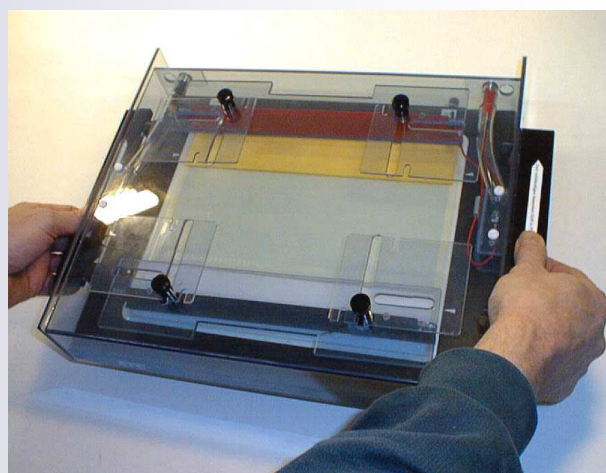
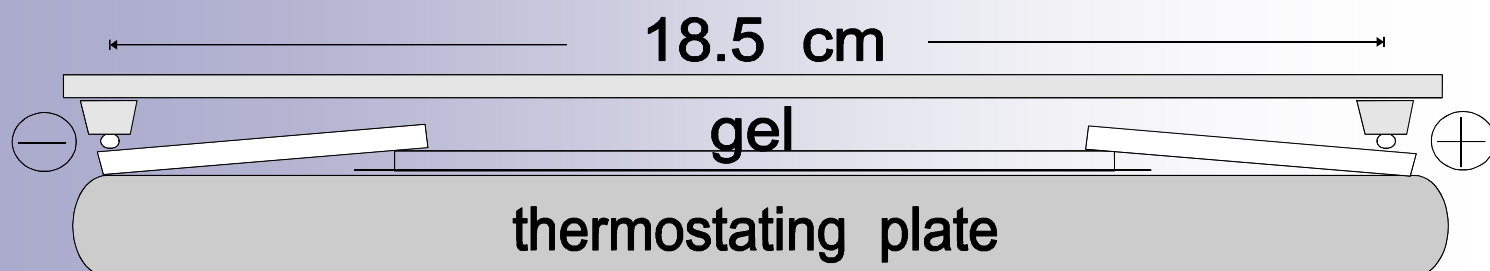


Fig.10: Lowering the security lid to the contact position



Running conditions (15°C) whole gel: (half gel: keep Volts and halve mA and Watt)

Bio-Rad Power Pac HV: type in this parameter as constant ---> n mA
(Select „BASIC“ mode) type in the other settings as limits ---> n V

n mA
n V

1 Gel:	Set V	Start Value	SET mA	Set W	Time	Comment
phase 1 (sample entrance 1)	200 V	(200 V)	20 mA	10 W	20 min	buffer discontinuity through the slot
phase 2 (sample concentration)	375 V	(375 V)	30 mA	20 W	50 min	buffer discontinuity in the resolving gel
phase 3 (main electrophoresis)	450 V	(450 V)	30 mA	20 W	40 min	buffer discontinuity in the anode

Stopping the electrophoresis:

Control with Xylencyanol band (XC): 12.5 % gel mR= 150 bp

Fast and sensitive silver staining [4]

We use this staining protocol because it will fix also smaller DNA-fragments below 150 bp! This staining procedure gives also best results with denaturing, 7 M Urea containing gels! We recommend to use the Autostainer from GE, see fig.7.

Our Stock Solutions: (These solutions can be ordered ready to use: Amersham "DNA-Silver Staining Kit")

Fixing Concentrate (5x): 15 g Benzene sulfonic acid + 120 ml ethanol. Fill up to 500 ml with dist. water.

Fixing diluter: 120 ml ethanol. Fill up to 500 ml with dist. water.

Washing Concentrate (6x): 1.75 g Benzene sulfonic acid. Fill up to 500 ml with dist. water.

Silvering Concentrate (5x): 5 g AgNO₃ + 1.75 g Benzene sulfonic acid. Fill up to 500 ml with dist. water.

Developing Concentrate (5x): 62.5 g Na₂CO₃. Fill up to 500 ml with dist. water.

Na-Thiosulfate (2%): 0.2 g Na-Thiosulfate fill up to 10 ml.

The Final Solutions:

Fixing solution: 40 ml Fixing Concentrate + 160 ml Fixing Diluter

Washing solution: 100 ml Washing Concentrate + 500 ml water

Silvering solution: 40 ml Silvering Concentrate + 160 ml dist. water + 260 µl Formaldehyde.

Developing solution: 40 ml Developing Concentrate + 160 ml dist. water + 260 µl Formaldehyde + 200 µl Na-Thiosulfate solution

Stopping and preserving: 75 ml Acetic Acid + 75 ml Glycerol + 600 ml dist. water

The Protocol:

Fixing: 40 min (0.6% Benzene sulfonic acid, 24% Ethanol)

Washing: 3 x 10 min (0.07% Benzene sulfonic acid)

Silvering: 40 min (0.2% AgNO₃, 0.07% Benzene sulfonic acid, 0.05% Formaldehyde), 0.002% Na-Thiosulfate

Water: 2 min

Developing: 5-6 min (2.5% Na₂CO₃, 0.05% Formaldehyde, 0.002% Sodiumthiosulfate)

Stopping & preserving: 3 x 10 min (10% Acetic Acid, 10% Glycerol)

Air Drying: Overnight

Fluorescence staining on DryGels Elpho

The chromogen must not be activated in the UV-region. If the samples were not tagged with the fluorescence dye: Equilibrate the gel in a solution of 50 ml H₂O + 1.5 µl GelStar dye solution for several minutes. Excitation 440-500 nm, emission light: orange.

Fluorescence staining on DryGels Elpho NF

Please follow the chromogen manufacturer's manual.

Direct staining

Staining solution: 7 mg "Stains All" + 50 ml o-Methylpyrrolidon + 50 ml H₂O dist. (final volume 100 ml).

Staining time: 30 min. Destain for 45 min in 10% Ethanol. Scan the result. Presevation: 20 min 10% Glycerol. Then air-dry overnight.

References:

- [1] Barros, F., Carracedo, A., Victoria M.L., Rodriguez-Calvo, M.S.: *Electrophoresis* 12 (1991) 1041-1045
- [2] Seymore, C., Gronau, S.; Pharmacia Eurolab Application Bulletin 17-9211-09E
- [3] Bassam, B.J., Caetano-Annollés, G., Gresshoff, P.M.: *Anal. Biochem.* 80, (1991) 81-84
- [4] According to GE's DNA Silver Staining Kit