DNA Electrophoresis using DNA-Buffer Disk Kit

EDC V3-08/14

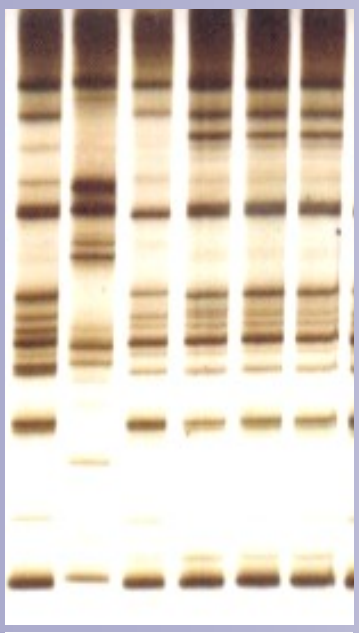


Fig.1: *DNA-fragments run on a DryGel Elpho 12.5% with the DNA Buffer Disk Kit: silver staining*

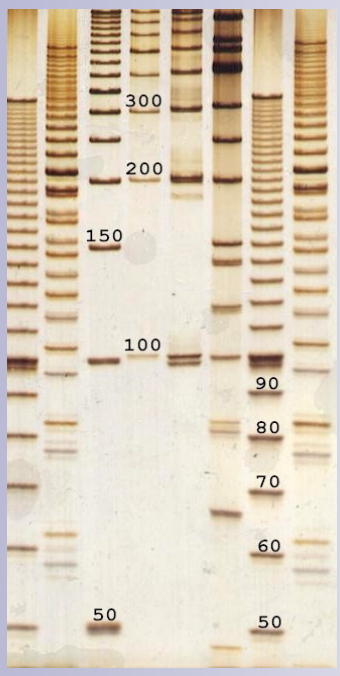


Fig.2: *A DryGel 10%, denaturated run, silver-stain*

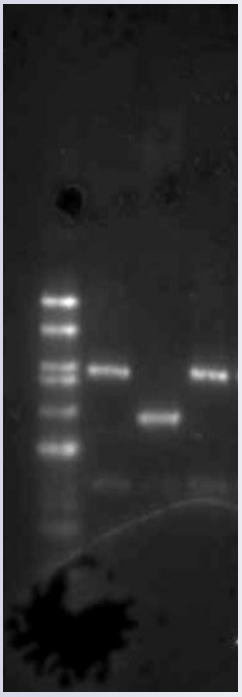


Fig.3: *Fluorescence stai-*

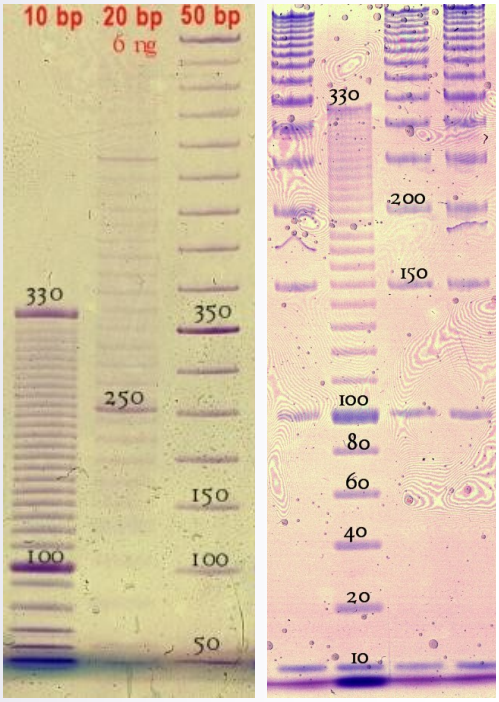


Fig.4: *Direct staining using "Stains All". Native (left) and denatured*

***General**: Normally DNA-fragments are separated in Agarose gels through a submarine electrophoresis followed by a fluorescence visualization with Ethidium Bromide. The reasons 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 hight resolution is needed, the DNA-fragments should run on Polyacrylamide gels because of their separation optimum below 1 kB.*
The DNA Buffer Disk Buffer Kit pH 8.4 contains a buffer system, which produces sharp bands in native (figure 1) and under denaturing conditions (fig. 2, and fig. 4 right side).
For highest sensitivity (~100 pg) and highest resolution the silver-staining as visualization is recommended, see figure 1.
An easy direct staining method is described, figure 4.

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 simultanous (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% 25S:, 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 Disk Kit 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 Bromo-phenol Blue solution (1%) + 40 µ 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 synchronuously 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 foreceps 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!

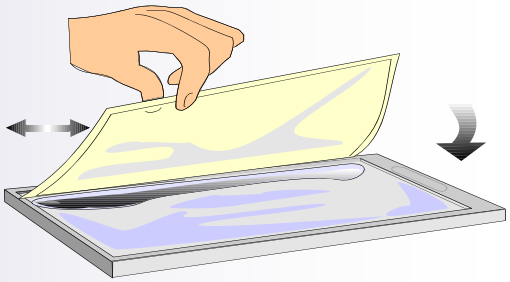


Fig.4: *Placing the DryGel into the DryPool*

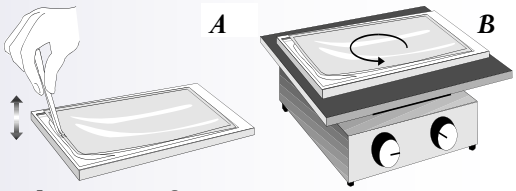


Fig5: *A Lifting the edges for an even distribution of the liquid. B Rehydration on a rocking platform*

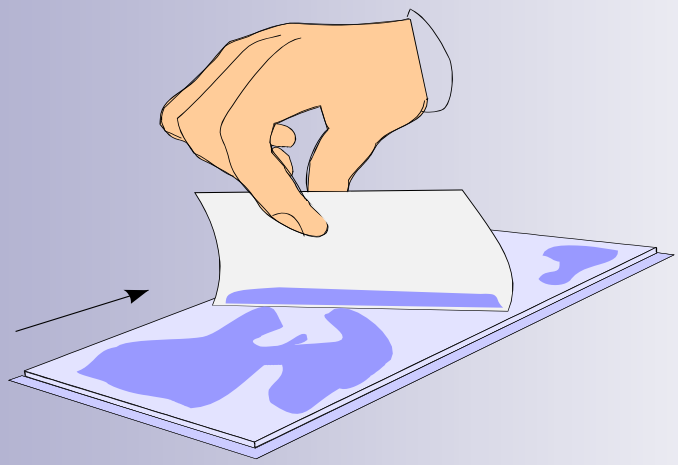


Fig.6: *Removing the excess buffer from the gel surface using the edges of a drying cardboard*

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.

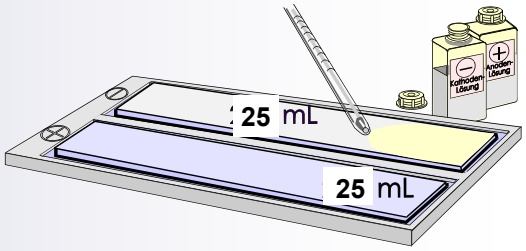


Fig.7: *Soaking the electrode wicks with the electrode solution in the DryPool Combi‘s backside*

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).

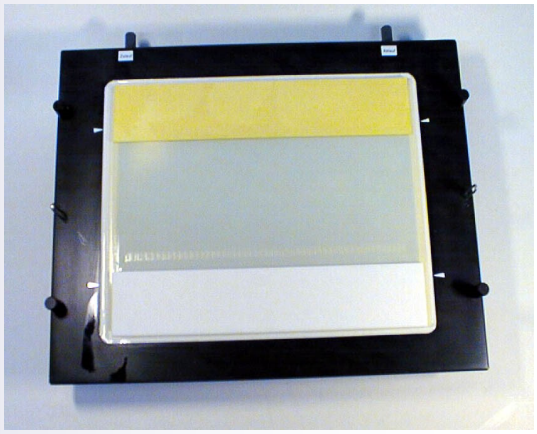


Fig.8: *Arrangement of gel and buffer wicks*

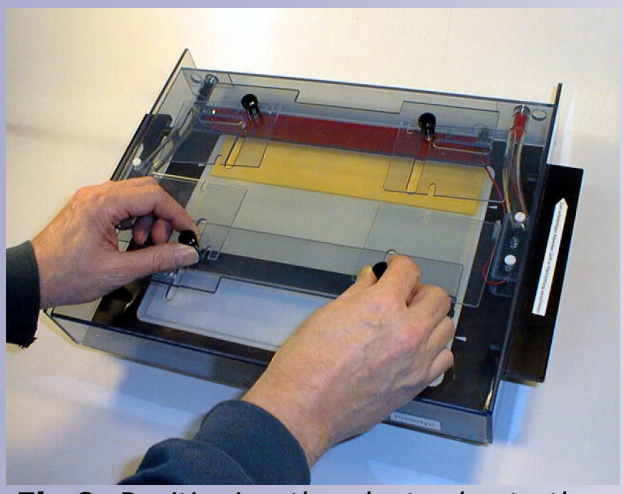


Fig.9: *Positioning the electrodes to the outer edges of the paper wicks*

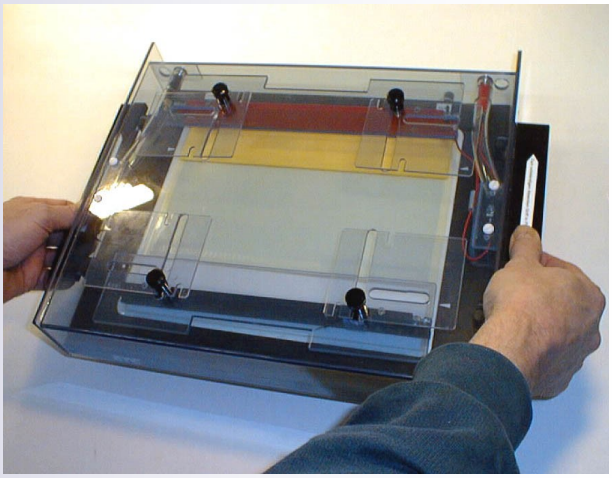
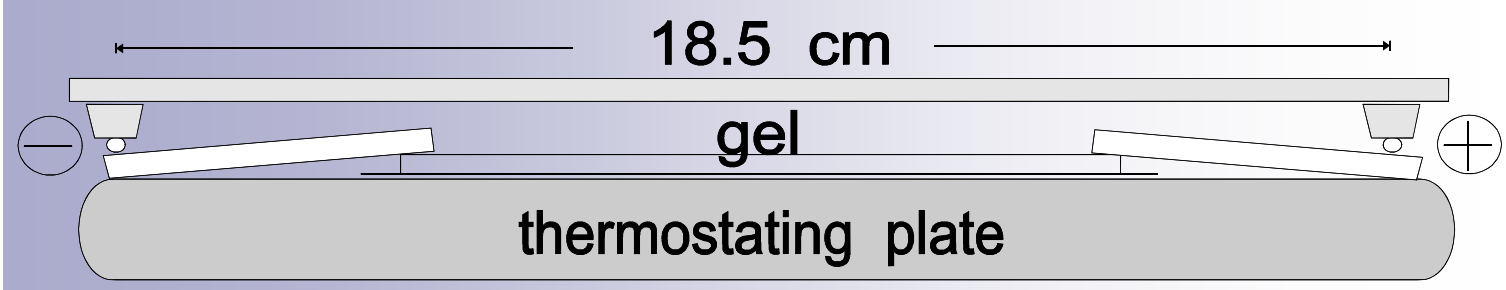


Fig.10: *Lowering the security lid to the contact position*



Running conditions whole gel, 24S, 52S: (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

Native run

1 Gel:	Set V	SET mA	Set W	Time	Temp	Comment
phase 1 (sample entrance 1)	120 V	20 mA	10 W	20 min	15°	buffer discontinuity through the slot
phase 2 (main run)	600 V	37 mA	15 W	70 min	15°	buffer discontinuity in the resolving gel

Stopping the electrophoresis:

Denatured run

1 Gel:	Set V	SET mA	Set W	Time	Temp	Comment
phase 1 (sample entrance 1)	240 V	30 mA	5 W	10 min	40°	buffer discontinuity through the slot
phase 2 (main run)	600 V	40 mA	20 W	50 min	50°	**Cover the surface!** discontinuity in the resolving gel

Stopping the electrophoresis:
Control with Xylencyanol band (XC): 12.5 % gel mR= 55 bases

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 $AgNO_3$ + 1.75 g Benzene sulfonic acid. Fill up to 500 ml with dist water.
Developing Concentrate (5x): 62.5 g Na_2CO_3. 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 Conc. + 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% $AgNO_3$, 0.06% Benzene sulfonic acid, 0.05%Formaldehyde, 0.002% Na-Thiosulfate),
Water: 2 min
Developing: 5-6 min (2.5% Na_2CO_3, 0.05% Formaldehyde, 0.002% Sodiumthiosulfate)
Stopping & preserving: 3 x 10 min (12.5% Acetic Acid, 12.5% 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 H_20 + 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 H_2O 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