DryGel bidirect and IEFGel bidirect
large and normal, nativ and denaturing

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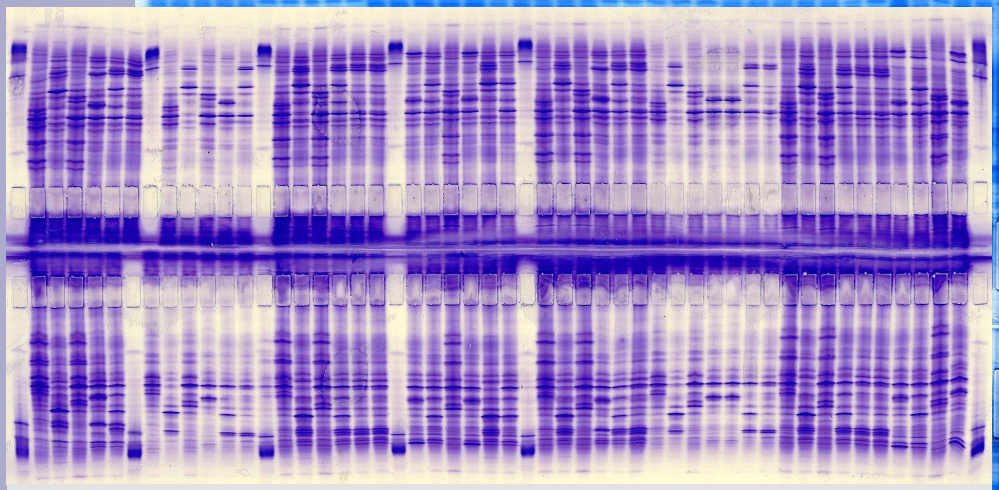


Fig1a: Wheat varieties on a IEFGel bidirect 104S EQ-type

Fig.1b: Four different potato varieties on DryGel IEF large bidirect. 104 samp-

General*:DryGels IEF bidirect large (250 mm x 180 mm) and normal (250 mm x 110 mm) are 0.5 mm ultrathin polyacrylamide gel with a specially designed matrix for isoelectric focusing (IEF). These gels are washed and dried onto their support-films.*
Due to the bidirect running technique up to 104 samples can be applied in the same time. Running time is ~ 1.5 hours.
The 104S gels are multi-channel pipette compatible.*
Quick running time: ~90 min. Staining time with Coomassie Violet is ~1 hour.
The gels have been polymerized under special conditions to obtain an optimal matrix suitable for native and denaturing focusing (1-7 M urea).
The dry gels must be stored at -20°C in order to preserve their reconstitution properties. Before use, they are reconstituted in a flat tray (rehydration pool) in the appropriate carrier ampholyte solution with or without additives (e.g. urea, nonionic detergents and reducing agents).
Two Coomassie procedures and one silver-staining are described.

DryGels (package à 4 gels)

DryGel IEF bidirect normal 104S, 2 x 52 slots* à 10 µl	(edc-1117)
DryGel IEF bidirect large no slots	(edc-1118)
DryGel IEF bidirect large 60S, 2 x 30 slots à 12 µl	(edc-1119)
DryGel IEF bidirect large 80S, 2 x 40 slots à 8.5 µl	(edc-1113)
DryGel IEF bidirect large 104S, 2 x 52 slots* à 15 µl	(edc-1116)

IEFGel cereal

IEFGel cereal bidirect small 104 slots à 8µl	(edc-1022)

Additionally required

RehydrationPool Large Tray for rehydration of dry gels (large size)	(*edc-me-l*)
Flatbed electrode, 8 mm wide (for small bidirect gels)	(edc-elec2843)

Ampholytes (for DryGels):
SepaLyte 7-10 EXTENDED, Sepalyte 3-10, (ProTec, Heidelberg 68008), BioLyte 3/10 (BioRad 163-1113), Triton X100, Ethylenglycol, Tetramethyl-urea (TMU), 2-Chlorethanol, Urea, Kerosene, Sample Application Strips (54 à 10 µl) (Biostep BS146.668).

Rehydration of the DryGel

Place the DryPool Combi (for normal sized gels) res. Rehydration Pool Large (for large sized gels) on a horizontal table. Clean it with distilled water and tissue paper.
Rehydration volumes: 28 ml for a large-sized and 18 ml for a normal sized gel.
Carefully cast this volume into the RehydrationPool Large.
Place the edge of the gel film - with the dry gel surface facing downward - into the rehydration solution (Fig. 2a, b and c) and slowly lower the film. At the same time move the gel film to and fro in order to achieve an even distribution of the liquid and to avoid trapping air bubbles. Lift the film at the edges with tweezers, and slowly lower them down, in order to maintain an even distribution of the liquid (Fig. 3) and to remove air bubbles.

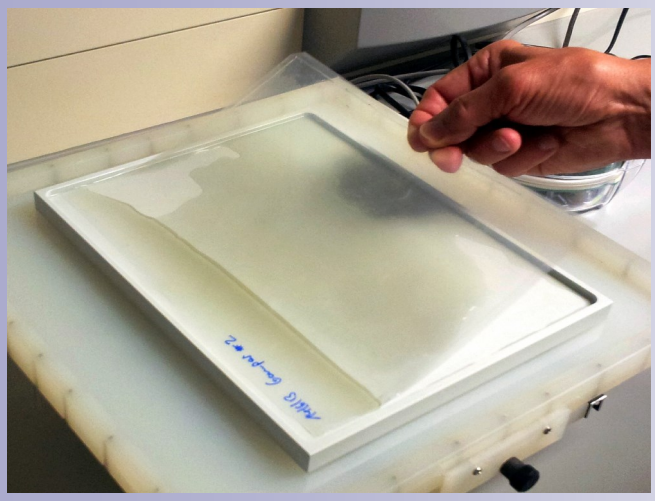


Fig.2: Lowering the gel onto the rehydration liquid

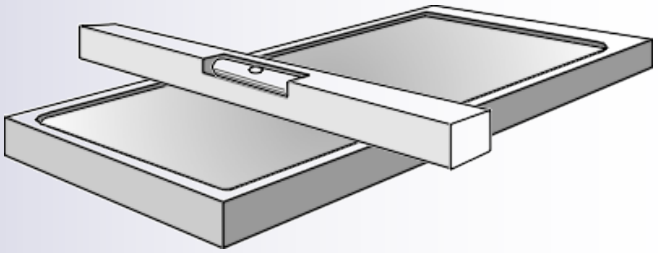


Fig.2a: Adjusting the rehydration pool horizontally

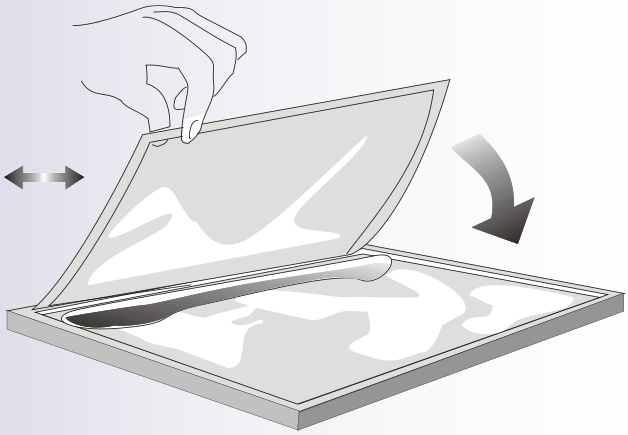


Fig.2b: Placing the DryGel IEF large into the Rehydration Pool Large: 28 ml

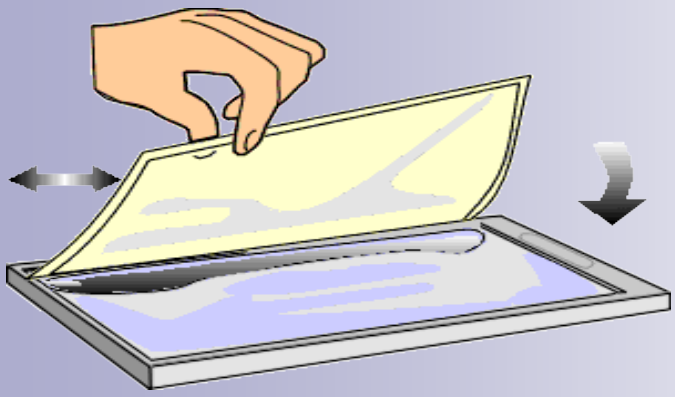


Fig.2c: DryPool Combi (normal sized gels): 18 ml

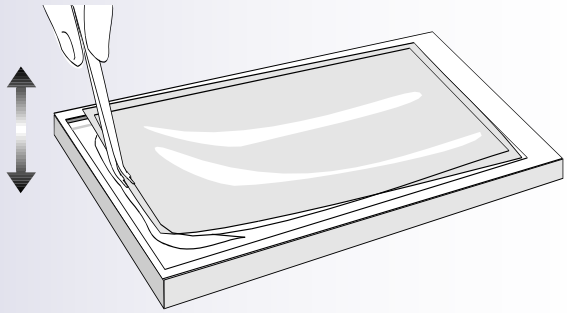


Fig.3: Lifting the edges for an even distribution of the liquid under the gel

Rehydration time is ~2 hours 20 min.
During this time the gel have to be lifted several times with a forceps:

1. Directly after setting the gel onto the liquid
2. Then 2 x after every 5 min and then after 10 min.
3. Finally 6-7 x after every 20 min.

The rehydrated gel must have a dry surface!

Special native procedure for potatoes:
Rehydrating solution large size: for 1 gel with 3.8% Ampholytes (28 ml)
2.25 ml Sepalyte 7-10 EXTENDED, 460 µl Biolyte 3/10, 1.4 ml Ethylene glycol
30 µl Triton 10% (v/v). Fill up to 28 ml (+24 ml H_2O).

Rehydrating solution normal size: for 1 gel with 3.8% Ampholytes (18 ml)
1.44 ml Sepalyte 7-10 EXTENDED, 295 µl Biolyte 3/10, 900 µl Ethylene glycol
20 µl Triton 10% (v/v). Fill up to 18 ml (+15.4 ml H_2O).

Protein Extraction Mix Potatoe: for 10 ml nativ extraction solution
130 µl Sepalyte 3-10, 80 µl Orange G (1%), 10 µl Bromophenol Blue, 15 µl Bromophenol-red, spatula-tip of Dithiothreitol

Protein extraction potatoe: Press potatoe cubes with a squeezer (garlic-press) and collect the sap in a eppendorf centrifuge.
Dilute with the extraction solution according to the visualization procedure: e.g. Coomassie=1+3. Sample volume: 8 - 15 µl. Centrifuge for 5 min at 5000 rpm.

Fig.4: garlic press for sqeezing the potatoe cubes

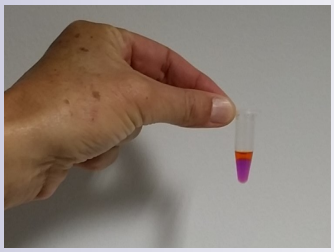


Fig.5: pulverizing single grains of maize (above) and overlaying with kerosene for defatting (left)

Special denaturing procedure for maize:
Rehydration solution large size: for 1 gel with 4M Urea and 4.4% Ampholytes (28 ml)
6.8 g Urea, 2.18 ml Sepalyte 3-10, 360 µl Biolyte 3/10, 80 µl Serva 9-11, 60 µl Triton 10% (v/v), Fill up to 28 ml (+20 ml H_2O). Stir until the urea has dissolved completely.

Rehydration solution normal size: : for 1 gel with 4M Urea and 4.4% Ampholytes (18 ml)
4.4 g Urea, 1.4 ml Sepalyte 3-10, 230 µl Biolyte 3/10, 50 µl Serva 9-11, 40 µl Triton 10% (v/v), Fill up to 18 ml (+13 ml H_2O). Stir until the urea has dissolved completely.

Protein Extraction Mix Maize: for 10 ml denaturing extraction solution
0,5 ml Tetramethyl urea (TMU), 0,5 ml 2-Chloroethanol, 100 µl Sepalyte 3-10, 50 µl Triton 10%, 65 µl Pyronin Y, 15 µl Methylene Blue, spatula-tip Dithiothreitol.
Fill up to 10ml (+9 ml H_2O)

Protein Extraction Procedure Maize:
Pulverice one grain with a shredder, or pulverize it in a plastic bag with a hammer (fig. 5).
Extracting the proteins out of one grain maize with the following volumes:
Hybrid: 1000 µl — male: 650 µl — female: — 800 µl
of the extraction solution.

Apply 15 min ultrasonic treatment.

Optional: defatting by overlaying 200 µl kerosene (+ spatula tip of Sudan-red). Shake the cups well and leave for 10 min. Then take the lower layer, see fig 5.

Centrifuge the Eppendorfs 5 min 5000 rpm.

Isoelectric Focusing and Sample Application

Gel application: At the end of rehydration (~140 min), the gel is ready for removing from the Rehydration Pool. Normally the gel's surface should be dry, otherwise the gel starts to sweat during isoelectric focusing and the application strips will not be leakage-free!
Spread 2.5 ml kerosene onto the cooling plate of your horizontal chamber in order to ensure good cooling contact. Take the gel with the film down and bend it a little like in figure 6, 7.

Fig.6: Lowering the bended gel –film down– on the cooling-plate's Kerosene

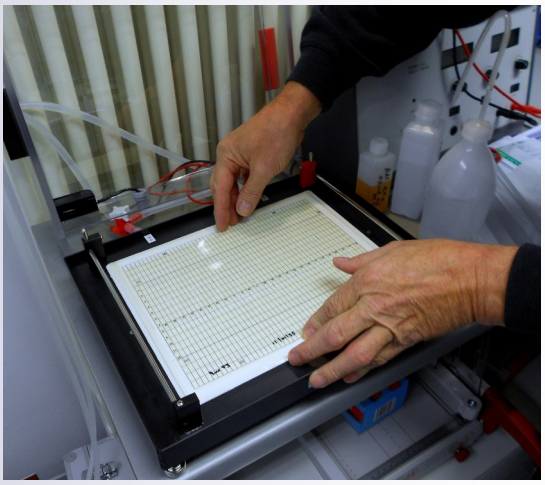


Fig.7: Set the gel down and avoid trapping air-bubble

Setting up the chamber
Before (and after) IEF, thoroughly clean the platinum wires with wet tissue paper.
Insert the platinum electrodes to their positions in the middle and over the edges of the gel. Normally the central electrode is the Anode (red colour) and the peripheral electrodes are the two cathodes (black colour). Figure 8
Be sure that the cathodes hit the gel-edges and the central electrode's plastic bar is not overlapping the sample slots. Figure 9 and 10. Please note: the central electrode for small bidirect gels should be the 8 mm wide electrode (see page 1). Otherwise the electrode will overlap the slots!

Sample application: Apply the appropriate sample volumes into the preformed sample slots See figure 7-10. DryGel bidirect large: 15 µl, DryGel bidirect: 10 µl

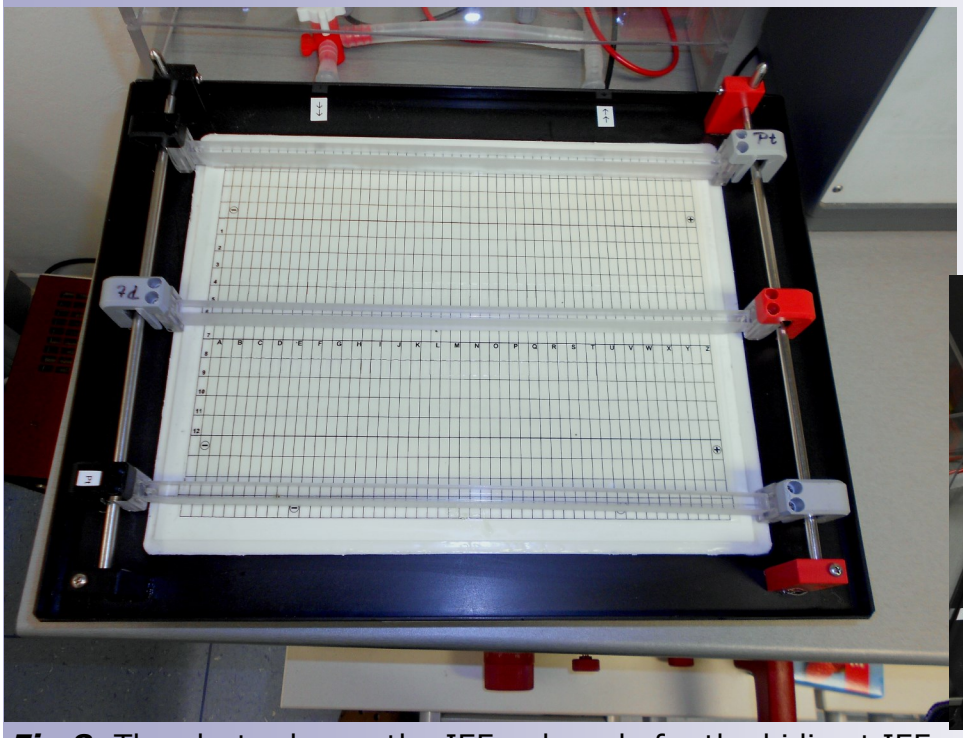


Fig.8: The electrodes on the IEF-gel ready for the bidirect IEF

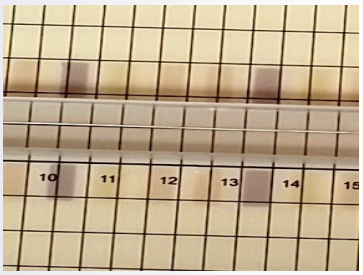


Fig.9: Electrode is exactly between the sample

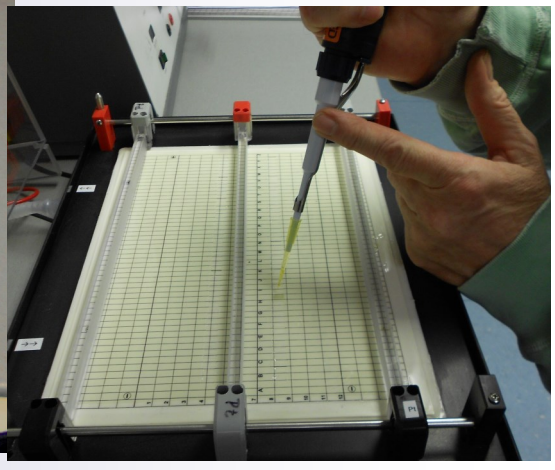


Fig.10: Pipetting the samples

Temperature: Isoelectric focusing has to be performed at a defined constant temperature because the pH gradient and the isoelectric points are dependent on the temperature. Switch on the thermostatic circulator, set to 10°C.

Nativ Running Conditions (25°C, 15°C)
Close the safety lid and begin the focusing procedure (Table 1 large- and 2 normal sized).
Running conditions: During isoelectric focusing the electric resistance of the gel changes dramatically, and small current or power values ultimately determine the voltage values of the run. The commonly used method is to limit the voltage via the mA and watts achieved in the gel during the run (see Table 1 and 2 for large sized gels ; table 3 and 4 for normal sized gels.).

Table 1 Run of a native IEFGel bidirect large; total time: 2h

Temp: first step 25°C, then15°C

Table 1	**SET V**	***Start Value***	**SET mA**	**SET W**	Time	Temp	Process
Step 1:	400 V	*(160 V)*	35 mA*	15 W	30 min	**25°***	sample entrance *
Step 2:	1000 V	*(600 V)*	35 mA*	30 W	80 min	15°C	main focusing (switch to cooling)
Step 3:	1400 V	*(1000 V)*	40 mA*	40 W	10 min	15°C	band sharpening

Table 2 Run of a native IEFGel bidirect normal; total time: 1h 55 min

Temp: first step in 25°C, then 15°C

Table 1	**SET V**	***Start Value***	**SET mA**	**SET W**	Time	Temp	Process
Step 1:	100 V	*(40 V)*	12 mA*	5 W	20 min	**25°***	sample entrance *
Step 2:	200 V	*(110 V*	20 mA*	5 W	15 min	15°C	sample entrance *
Step 3:	500 V	*(260 V)*	30 mA*	10 W	10 min	15°C	focusing (switch to 15°)
Step 4:	750 V	*(490 V)*	30 mA*	20 W	60 min	15°C	main focusing
Step 5:	900 V	*(860 V)*	25 mA	20 W	10 min	15°C	band sharpening

* *Note 1: If other rehydration mixes are applied (pH-gradients, ampholyte-concentrations) adjust the starting Volt-values with the mA set-value.*
* *25°: then in step 3: do not forget to set the kryostat down to 15°C.*
After the run: Immediately take the application pieces away with a pair of forceps and start the staining procedure
Prefocusing: Seed samples are normally running without prefocusing.
Other samples could be applied after a short prefocusing step (Step 1).
Exception: Salty samples, e.g. serum or cerebrospinal fluid, are applied at the beginning of the IEF.
In case of prefocusing, the IEF is stopped after the first phase to apply the samples. Modern power supplies are set to "Autohold" after the first step. Focusing is then continued with Step 1.

Denaturing Running Conditions (10°C)
Close the safety lid and begin the focusing procedure (Table 1 large- and 2 normal sized).
Running conditions: During isoelectric focusing the electric resistance of the gel changes dramatically, and small current or power values ultimately determine the voltage values of the run. The commonly used method is to limit the voltage via the mA and watts achieved in the gel during the run (see Table 1 and 2 for large sized gels ; table 3 and 4 for normal sized gels.).

Table 2 Bidirect run of a denaturing large sized gel; total time: 1h 30 min

Temp: 10°C

Table 1	**SET V**	***Start Value***	**SET mA**	**SET W**	Time	Process
Step 1:	350 V	*(120 V)*	30 mA*	10 W	25 min	sample entrance *
Step 2:	1500 V	*(410 V)*	40 mA*	45 W	65 min	main focusing

* *Note 1: If other rehydration mixes are applied (pH-gradients, ampholyte-concentrations) adjust the starting Volt-values with the mA set-value.*
After the run: Immediately take the application pieces away with a pair of forceps and start the staining procedure
Prefocusing: Seed samples are normally running without prefocusing.
Other samples could be applied after a short prefocusing step (Step 1).
Exception: Salty samples, e.g. serum or cerebrospinal fluid, are applied at the beginning of the IEF.
In case of prefocusing, the IEF is stopped after the first phase to apply the samples. Modern power supplies are set to "Autohold" after the first step. Focusing is then continued with Step 1.

Table 2 Bidirect run of a denaturing normal sized gel; total time: 1h

Temp: 10°C

Table 1	**SET V**	***Start Value***	**SET mA**	**SET W**	Time	Process
Step 1:	200 V	*(90 V)*	30 mA*	10 W	15 min	sample entrance *
Step 2:	1000 V	*(250 V)*	45 mA*	40 W	45 min	main focusing

* *Note 1: If other rehydration mixes are applied (pH-gradients, ampholyte-concentrations) adjust the starting Volt-values with the mA set-value.*
After the run: Immediately take the application pieces away with a pair of forceps and start the staining procedure
Prefocusing: Seed samples are normally running without prefocusing.
Other samples could be applied after a short prefocusing step (Step 1).
Exception: Salty samples, e.g. serum or cerebrospinal fluid, are applied at the beginning of the IEF.
In case of prefocusing, the IEF is stopped after the first phase to apply the samples. Modern power supplies are set to "Autohold" after the first step. Focusing is then continued with Step 1.

1. Staining native IEF with Coomassie-Violet

Stock solution
Dye-Concentrate (10 x conc): 0,1% Coom.Violet = 1 g in 1000 ml H_2O dist

Staining solution (Mix just before using!)
approx. 170 ml H_2O dist. + 20 ml dye-conc. + 6 ml HAc + 8 ml H_3PO_4

Staining programme

Fix:	15 min in 200 ml 20 % TCA (at room temperature)
Wash:	1 min in 200 ml H_2O dist.
Stain:	30 min 0.01% Coomassie-Violet in 3,4% (w/v) phosphoric acid + 3% (v/v) HAc. (*Optimal staining: leave staining solution overnight)*
Destain:	20 min in H_2O dist
Impregnate:	5 min in 200 ml 5 % (v/v) glycerol
Dry:	air-dry (leave at room temperature)

Abbreviations: TCA = trichloroacetic acid

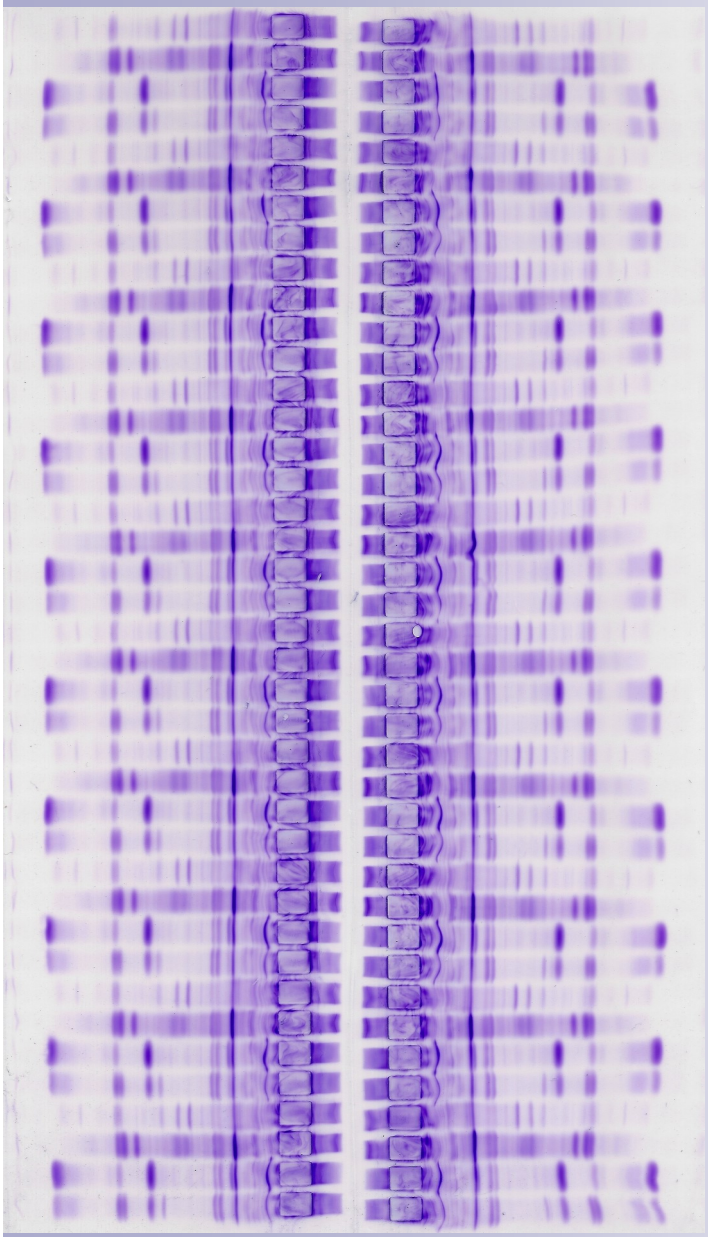
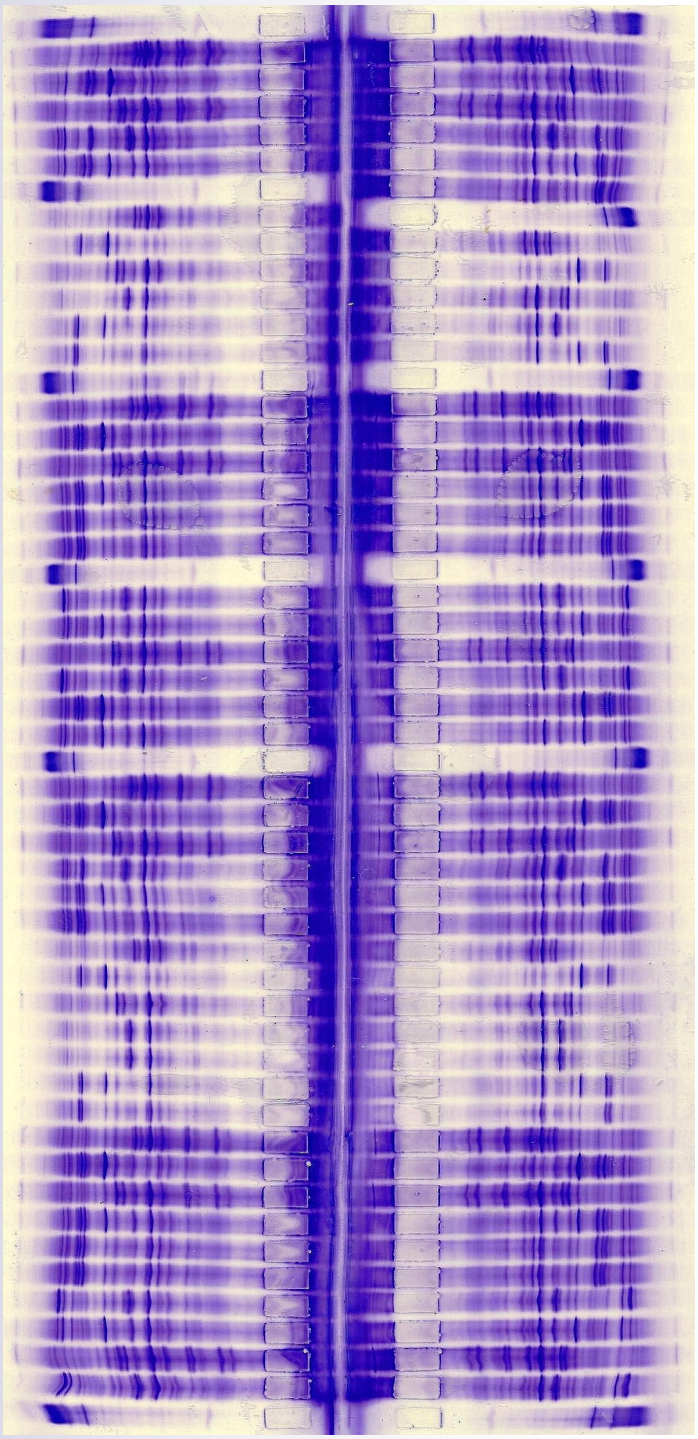


Fig. 10: Diverse potato-varieties on a DryGel IEF large bidirect 80S. 2 x 40 samples à 8.5

Wheat varieties on a IEFGel bidirect 104S



2. Staining denaturing IEF with Blakesley / Roti Blue

Based on „Blakesley-Staining": ANALYTICAL BIOCHEMISTRY 82, 580-582 (1977)

A five fold concentrate staining solution can be purchased from Roth (Karlsruhe):
1 liter **Roti-Blue** (#A152.1). Sensitivity is 3-5 fold higher than other Coomassie-stainings.

Recipe and procedure for urea-containing IEF-gels:
(double volumes for large sized gels)

Abbreviations: TCA = Trichloroacetic acid, MeOH = Methanol

fixing	100 ml H_2O	+ 12.5g TCA	30 min
washing	70 ml H_2O + 30 ml MeOH	+ 1 ml Phosphoric acid	15 min
staining	mix: 60 ml H_2O + 40 ml MeOH	stir in: 20 ml Roti-Blue	3h-overnight
washing	75 ml H_2O + 25 ml MeOH	- - -	5 min
preserving	3% Glycerol	- - -	20 min

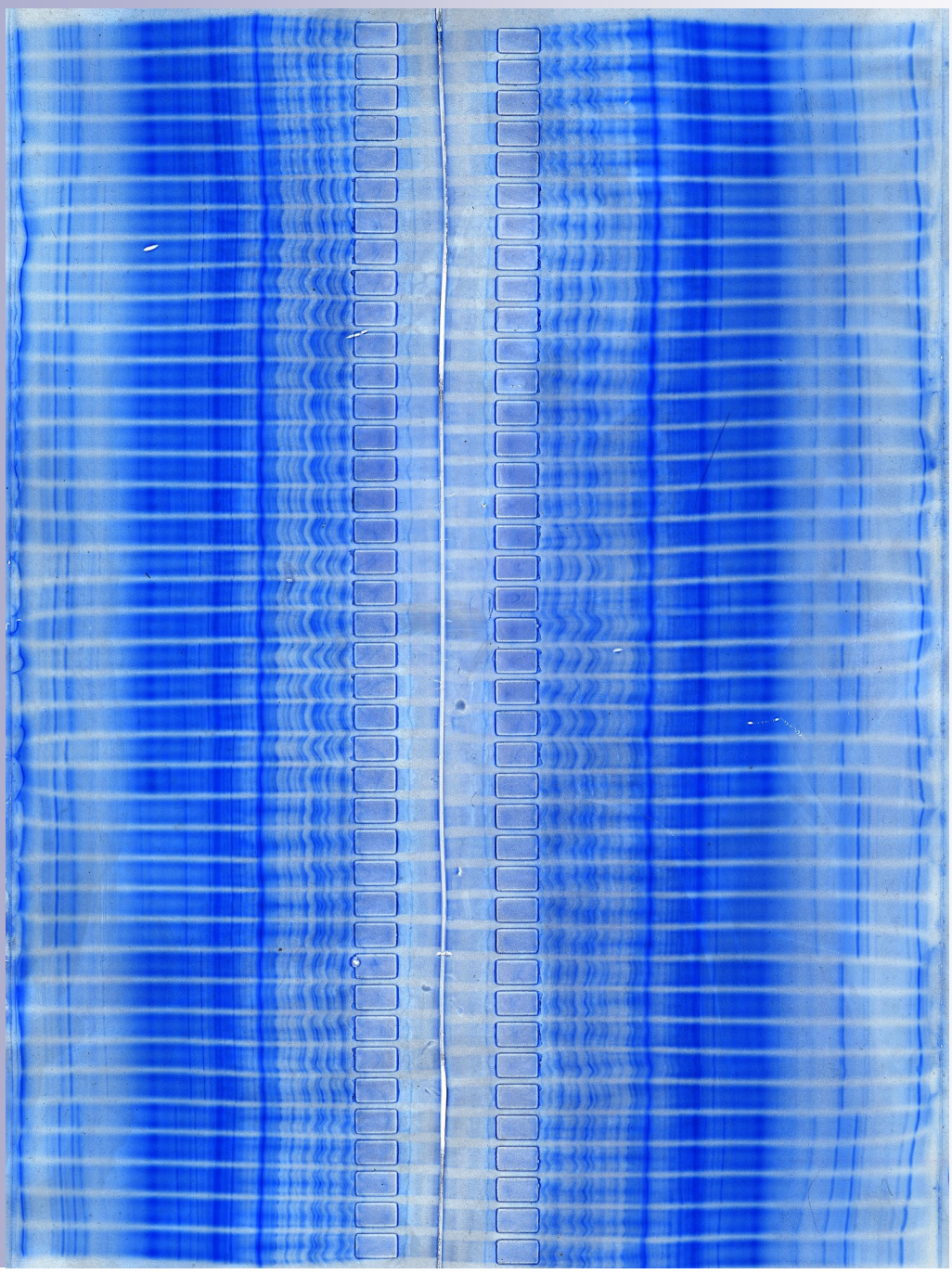


Fig.11: Maize family; male-female-hybrid on DryGel bidirect latge 80S

2. Silver Staining

Automated silver-staining is performed **at ambient temperature** with the Hoefer Automated Gel Stainer (GE 80-6395-02).
Adapted staining programme [1]

Staining solutions and programme:

step	reagent	volume	time
1 Fixing	20% trichloroacetic acid (w/v)	200 ml	20 min
2+3 Rinsing	20 % ethanol/ 8% acetic acid (v/v)	2 x 200 ml	2 x 10 min
4 Incubation	0.1 % sodium thiosulphate; 0.4 mol/l sodium acetate/acetic acid pH 6.5; 0.125 % glutardialdehyde	200 ml	15 min
5 Rinsing	20 % ethanol / 8% acetic acid	200 ml	10 min
6-8 Washing	H_2O dist (place gel into a glass tray, with the gel surface side up)	3x 200 ml	3x10 min
9 Silvering	0.1% $AgNO_3$ /0.004% formaldehyde (w/v) 20µl HCHO (37% w/v) per 200 ml 0.1% $AgNO_3$	200 ml	30 min
10 Developing	2.5 % Na_2CO_3 / 0.004% formaldehyde 40µl HCHO (37% w/v) per 400 ml 2.5% Na_2CO_3	2x 200 ml observe to stop	0.5 min 2-3 min
11 Stopping/ Preserving	10% HAc, 5% glycerol	200 ml	20 min
Drying	air dry , on the support-film		16 h

Ammoniacal Silver Staining (spezial staining recipe for basic proteins):
http://www.*electrophoresis-development-consulting.de*/download/csffocus.pdf

References:

[1] A.Jonitz[1], D.Miranda[2], VDLUFA Kongressband 2008
[1] Landwirtschaftliches Technologiezentrum Augustenberg, Karlsruhe, Germany
[2] Universidade de Pelotas, Pelotas, Brazil