DETECTION OF POINT MUTATIONS BY SSCP-ELECTROPHORESIS

EDC V2-03/13

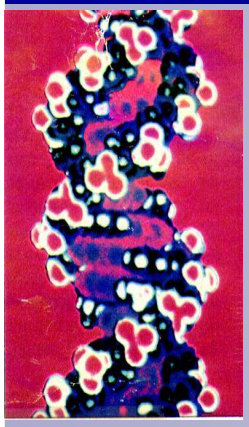


Fig.A: *Double stranded DNA*

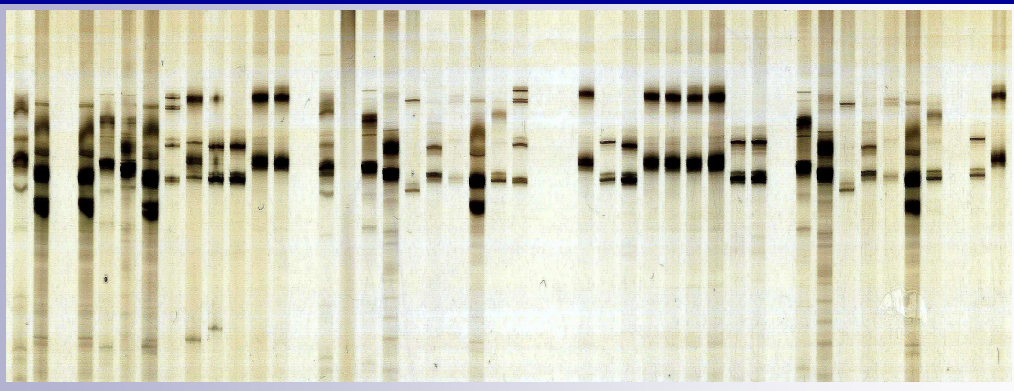


Fig.1: Patterns of single-stranded DNA (upper part of the gel) of various fish species obtained from a 464 bp amplicon of the cytochrome b-gene. (Dr. H. Rehbein, Ute Schröder, MRI, Hamburg)

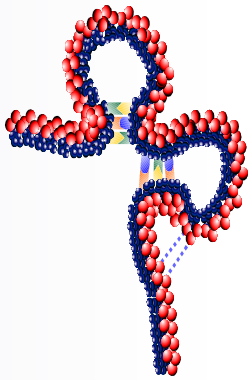


Fig.B: *Single Stranded DNA*

General: The 4 different bases in the DNA are orientated in the center of the molecule, see right picture. So the electrophoretic migration speed of double strands with different sequences is rather identical. Point mutations normally cannot be detected when double-stranded DNA is used as electrophoretic sample.
When a single stranded DNA-fragment is produced by heating up the sample, then a sequence-specific conformation is set up automatically, see picture 1.
This leads to sequence-depending electrophoretic mobilities.
Major advantage of this method: 52 samples can be indentificated by a simple silver-stain.

Denaturating sample solution
25 ml Formamide + 100 µl Xylenecyanol (1%)

Sample treatment
Dilute the samples at least 1+1 with the sample solution**.** Dilute as much as possible to reach the upper picogramm region, this gives best results. To compare the sample concentrations: Take Promega´s 100 BP ladder and dilute 30 µl+720 µl with the gel buffer (add colour) and run at least one lane per gel. During the developing step of the silver staining procedure the sample-lanes should appear synchronuously with this standard lane.After gel rehydration and assembling of the horizontal electrophoresis: Heat the samples 3 - 5 minutes (depends of the sample volume) to 95°C, then chill them in an ice/water-bath.

Strategy of SSCP-Electrophoresis
Single-stranded DNA migrate rather slowly in the gel-matrix relative to their double-strands. Normally you will not loose any information at the anodal side when running the samples till the dye „Xylencyanol" (mixed to the sample's denaturating buffer) has reached the anodal strip.

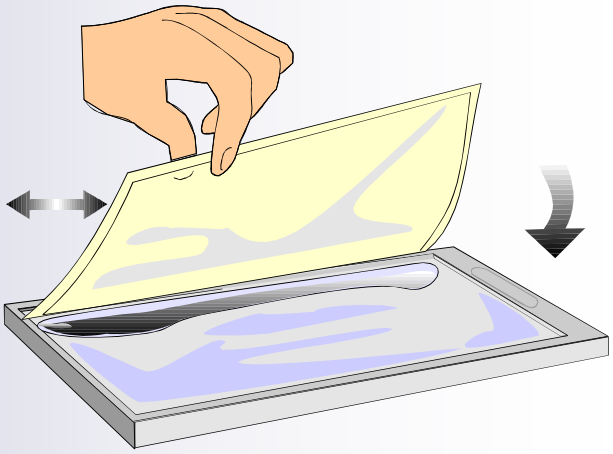


Fig.2: *Placing the dry gel into the Dry-Pool Combi for rehydration*

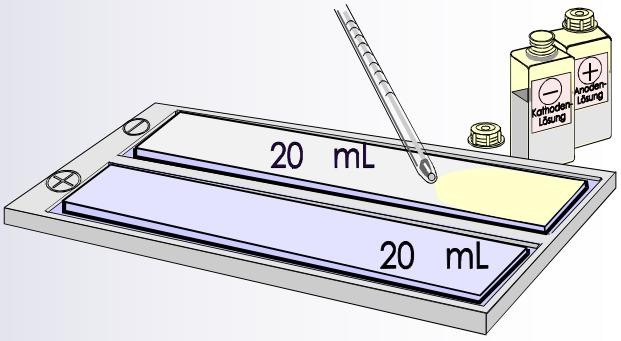
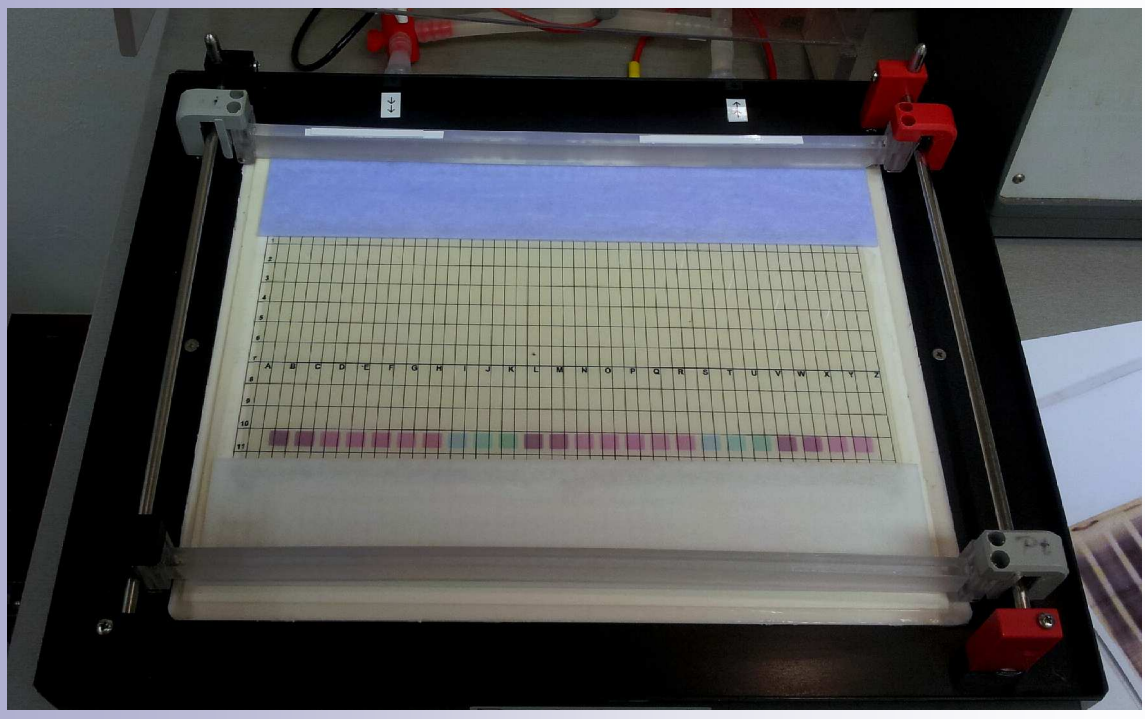


Fig.3: *Soaking the electrode strips with the electrode solution in the DryPool Combi*



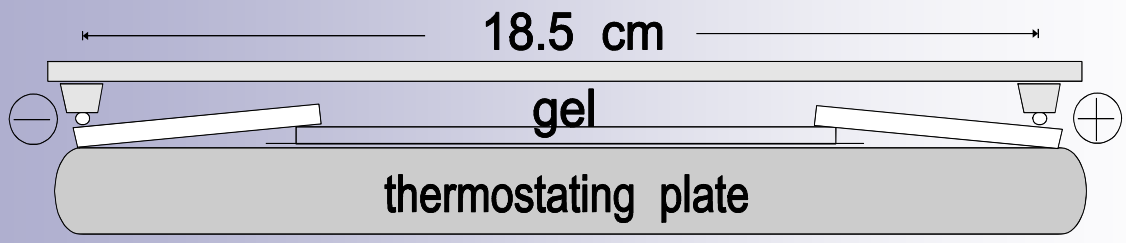


Fig.4: *Arrangement of gel, buffer wicks and electrodes in a horizontal electrophoresis chamber: flatbed professional*

Results

SSCP can be recommended as a rapid, sensitive, cost-effective (50 samples per gel!) screening method for controlling the declaration of fish and seafood species.

Silver Staining

Fixing: 40 min (0.6% Benzenesulfonic Acid, 24% Ethanol)
Washing: 3 x 10 min (0.07% Benzenesulfonic Acid)
Silvering: 40 min (0.2% $AgNO_3$, 0.07% Benzenesulfonic Acid, 0.05% Formaldehyde)
Water: 2 min
Developing: 5-6 min (2.5% Na_2CO_3, 0.05% Formaldehyde, 0.002% Sodiumthiosulfate)
Stopping & preserving: 3 x 10 min (10% Acetic Acid, 10% Glycerin)