Protein electrophoresis troubleshooting

By

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Troubleshooting of protein isolation

Troubleshooting

1- Chemical expiry

2- Temperature, PH

3-Lysis of sample

4- Protenase enzyme

5- Contamination (bacteria, fungi, etc.)

To Be Right You Should Measure Total Protein Using Spectrophotometer

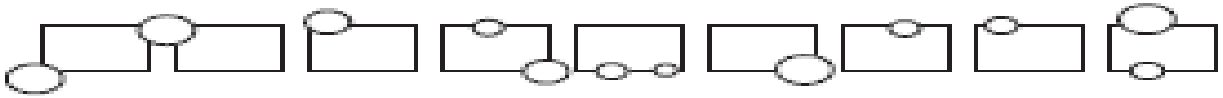
Troubleshooting of Electrophoresis

Trouble shooting: SDS-electrophoresis

1- Gel casting.

Symptom	*Cause*	*Remedy*
Incomplete polymerization.	Poor quality chemicals.	Only use analytical grade quality reagents.
	The acrylamide and/or APS solutions were kept too long.	Always store the stock solutions in the refrigerator in the dark; the 40% APS solution can be stored for one week, solutions of lower concentration should be freshly prepared every day; in case of doubt make new stock solutions.
	The water is of poor quality.	Always use double-distilled water.
The gel sticks to the glass plate.	The glass plate is too hydrophilic.	Wash the glass plate and coat it with Repel Silane.
Leakage from the gradient mixer .	The rubber gasket is dry.	Open the gradient mixer, coat the gasket with a thin layer of CelloSeal®.
Gradient gels: the gel solution already polymerizes in the gradient mixer.	Too much APS was used.	Reduce the amount of APS. Open the gradient mixer and clean it.

Symptom	*Cause*	*Remedy*
Air bubbles are trapped in the cassette.	This cannot always be prevented.	Carefully pull them out with a strip of film.
Gradient gels: one half of the gel is not or is incompletely polymerized.	The APS solution has not mixed with the gel solution (it has stayed on the sides or on the dense solution).	Pipette carefully, stir vigorously for a short time so that the APS solution is drawn into the dense solution.
The gel separates from the support film.	Wrong support film was used.	Only use GelBond PAG film for polyacrylamide gels not GelBond film (for agarose).
	The wrong side of the support film was used.	Cast the gel on the hydrophilic side; test with a drop of water.
	The support film was wrongly stored or too old.	Always store the GelBond PAG film in a cool, dry and dark place.

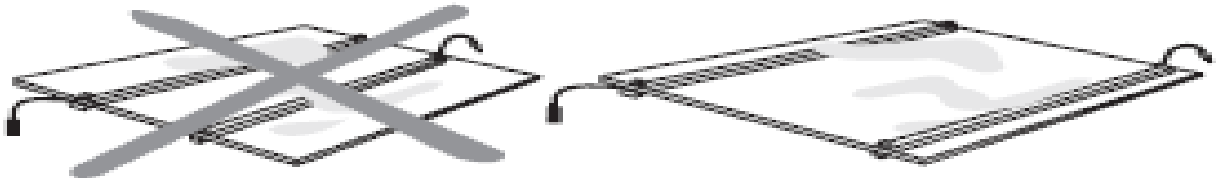
Symptom	Cause	Remedy
There is a liquid film on the surface.	The polyacrylamide matrix has hydrolysed because the buffer is too alkaline (pH 8.8).	Alkaline polyacrylamide gels should not be stored longer than 10 days in the refrigerator.
Holes in the sample wells. 	Air bubbles were incorporated during casting.	Cut out the slot former with a sharp scalpel. Press the cut edge down with a pair of curved tweezers; use "crystal clear" Tesafilm or Dymo tape with a smooth surface; otherwise small air bubbles which inhibit polymerization in their vicinity can form.
Holes in the gel ("Swiss cheese effect").	Many very small bubbles in the gel solution.	Degas the gel solution; when casting gradient gels do not stir too fast, because SDS solutions foams.
The edges of the gel have not polymerized enough.	The gel solution was not overlayed.	Overlay the gel solution.
	Oxygen from the air has diffused through the seal.	Polymerize the gel at a higher temperature (37 to 50 °C, ca. 30 min).
	Polymerization is too slow.	Degas the gel solution; add a little more TEMED and APS.

Trouble shooting: SDS-electrophoresis

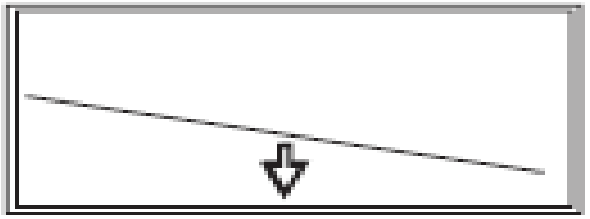
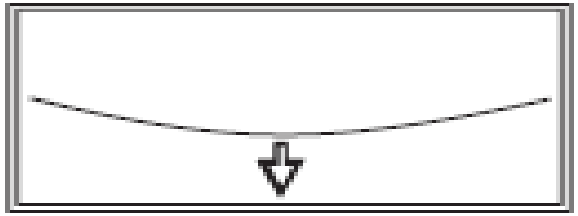
2- Effects during electrophoresis.

Symptom	Cause	Remedy
Droplets on the surface of the gel.	Buffer dripped on the surface of the gel when the electrode wicks soaked in buffer were placed on the surface of the gel.	Make sure that the electrode strips are never held over the surface of the gel; if this happens, carefully remove the drops with a bit of filter paper.
	High ambient temperature and high humidity, water condensation on the surface of the cooled gel. (summer time !).	Apply gel and samples on the cooling plate before the cooling waterbath is connected to the cooling plate. Do not connect to the cooling system before electrodes and safety lid cover the gel.
Sample applicator strip: the samples merge together.	The sample applicator strip is not applied well enough.	Press the sample applicator down properly, do not touch it anymore, do not touch the strip with the tip of the pipette when applying the sample; use sample application pieces.
Sample wells: the samples leave the wells and spread over the gel surface.	Glycerol oder sucrose in the sample, osmotic distribution.	Prepare samples without glycerol or sucrose; those are only necessary for vertical PAGE.

Symptom	Cause	Remedy
	High protein content, sample contains proteins with low surface tension.	Add 8 mol/L urea to each sample (**after** heating!), the urea does not influence the electrophoresis pattern.
No current.	The electrode cable is not plugged in.	Check whether all the cables are properly connected.
The power supply switches itself off and exhibits ground leakage.	Electricity is leaking from the chamber.	Make sure that the laboratory bench is dry; if the ambient temperature and humidity are high, regularly wipe off the condensation water from the tubings: ideally use a foam rubber tubing to cover the cooling tubing.
The current decreases quickly, the voltage increases quickly.	The system runs out of buffer ions, because the electrodes are placed too close together.	Place electrodes as far as possible to the outer edges of the buffer strips, in order to include the complete buffer between the electrodes.



Symptom	*Cause*	*Remedy*
The front migrates into the wrong direction.	The plugs are inverted: the gel is wrongly oriented.	Check whether the cable is plugged in properly; place the gel so that the sample application point lies near the cathode.
The front migrates too slowly; the separation takes too long.	The current flows under the support film.	Use kerosene as contact fluid, not water.
The electrophoresis takes too long.	There are chloride ions in the cathode buffer.	The cathode buffer (Tris glycine) must not be titrated with HCl even if the pH value given in the recipe (usually pH 8.3) is not reached with glycine; the pH usually sets itself at 8.9 which is all right.
Condensation.	The power is too high.	Check the setting of the power supply: at most 2.5 W/mL of gel.
	The cooling is insufficient.	Check the cooling temperature (10 to 15 °C are recommended); check the flow of the cooling fluid (bend in the tubing?); add contact fluid (kerosene) between the cooling plate and the support film.

Symptom	*Cause*	*Remedy*
The front is crooked. 	Irregular electrical contact.	See above.
	The buffer concentration in the electrode strips is irregular because they were not held straight when they were soaked or placed on the gel.	Make sure that the electrode wicks are always held straight.
	The casting mold was not levelled when the disk or gradient gels were cast.	Set the mold level with a spirit-level.
The front is curved. 	The gel polymerized before the density gradient had settled or before the density discontinuity was finished.	Delay the onset of the polymerization: either by reducing the quantity of APS and/or by cooling the casting cassette in the refrigerator.
The front is uneven or wavy.	The gel surface has dried out in places under the holes in the lid.	Only for Multiphor I: Place a glass plate or electrode holder over the electrode wicks and the gel.

Symptom	*Cause*	*Remedy*
Formation of white precipitates and an irregular gel surface.	There is dirt in the electrode wicks which forms a precipitate with SDS.	Use electrode paper of the best quality; only handle the paper with rubber gloves.
The gel dries along one edge of the paper and burns through there.	This is an electroendosmotic effect due to poor quality of the chemicals and/or an old acrylamide solution.	Only use chemicals of analytical grade quality; store the acrylamide stock solution in the refrigerator in the dark for a short time (at most 2 weeks for SDS gels).
The slots dry out and burn through after a while; at the same time water collects at the cathodic side of the slots.	The samples were wrongly prepared; free SH groups lead to the formation of disulfide bridges between the various polypeptides; these aggregates are too large for the gel pores and highly negatively charged (SDS), → electroosmotic flow of water towards the cathode.	Protect DTT from oxidation with EDTA; after reducing, heating and recooling the samples, add the same quantity of DTT as for the reduction; or else alkylate with iodoacetamide.

Trouble shooting: SDS-electrophoresis

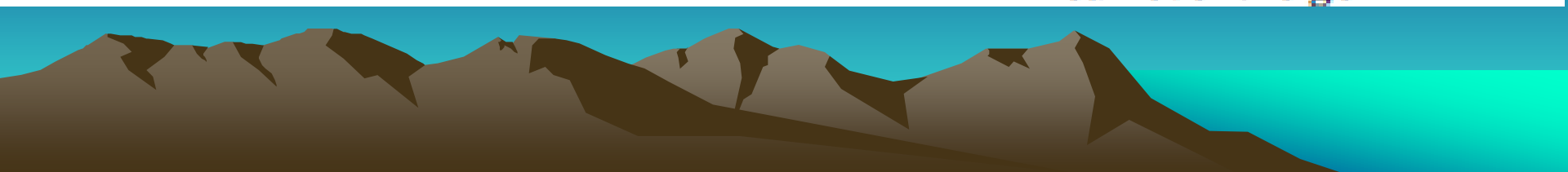
3- Separation results.

Symptom	*Cause*	*Remedy*
The bands are not straight but curved.	Part of the gradient was shifted by heat convection during polymerization.	Add a little more APS to the light solution than to the dense one so that the polymerization starts at the top and proceeds towards the bottom.
The bands are close together at the buffer front.	The concentration of the resolving gel is too low.	Increase the concentration of the resolving gel.
	Low molecular weight peptides are poorly resolved.	Use gradient gels (if necessary concave exponential pore gradients); or use the buffer system of Schägger and von Jagow (1987).
The low molecular weight proteins are missing.	The post-polymerization is not finished.	Polymerize the gel at least one day ahead.
The bands are "fuzzy" and diffuse.	The post-polymerization is not finished. The resolving gel is not properly polymerized.	Polymerize the resolving gel at least one day ahead. Always prepare the resolving gel at least one day before use, because a slow post-polymerization takes place in the gel matrix.

Symptom	*Cause*	*Remedy*
The bands are diffuse.	The proteins were applied too close to the cathode.	Apply the samples at least 1 cm from the edge of the cathode wick or the buffer strips.
	The samples are old.	Prepare fresh samples; it is helpful to boil the samples again (adding reducing agent before and after); or else alkylate them.
	A homogeneous gel system was used or else the buffer system was not optimal for the samples.	Try disc PAGE and/or gradient gel PAGE or another buffer system.
	Insufficient gel polymerization.	See above.
Artifactual double bands.	Partial refolding of the molecules because the SH groups are not sufficiently protected.	Alkylate the samples after reduction.
The sample concentration is irregularly distributed in the bands.	Very small sample volumes were applied so the filling of the sample wells was irregular.	Dilute the samples with buffer and apply a correspondingly larger volume or apply the sample directly on the surface of the gel (for < 5 µL).

Symptom	*Cause*	*Remedy*
Crooked bands.	There is too much salt in the samples which leads to marked conductivity differences at the beginning.	Desalt the samples by gel filtration (NAP column) or by dialyzing against sample buffer.
Precipitates at the edge of the slots.	Gradient gel: the gradient was poured the wrong way round and the samples applied in the part with narrow pores.	Make sure that the slots are in the area with the lower acrylamide concentration.
	Electro-decantation, the slots are too deep.	Make the slots less deep (decrease the number of layers of Tesa film), they should not be more than 1/3 of the thickness of the gel. If larger sample volumes must be applied, it is preferable to increase the surface of the slots.
	A few proteins are too large to penetrate the gel pores.	Use gradient gels which allow separate of a broader molecular weight spectrum.
	Mistake during sample application; the free SH groups were not protected and disulfide bridges have formed between the polypeptides which are now too large for the pores.	Protect DTT from oxidation with EDTA; after reduction, heating and cooling, add the same amount of DTT as for the reduction to the samples; or else alkylate them, with iodoacetamide for example.

Symptom	*Cause*	*Remedy*
High molecular weight "ghost bands".	Mistake during sample preparation, see above.	See above.
Protein precipitate at the edge of the sample well; a narrowing then widening trace stretches below the sample well through the separation trace.	Overloading; the polypeptides form complexes and aggregate when entering the gel matrix; protein dissolves intermittently and migrates towards the anode.	Dilute the sample, apply less.
The bands streak.	There are particles in the sample.	Centrifuge or filter the sample.
There are individual streaks which sometimes begin in the middle of the gel.	Dust particles or dandruff etc. have fallen on the surface.	Do not leave the lid of the chamber open too long; apply the samples as quickly as possible; do not lean over the gel



Symptom	*Cause*	*Remedy*
Smudged bands, the bands tail.	There is grease in the sample.	Remove lipophilic substances completely during sample preparation.
	Insufficient loading with SDS.	The sample buffer should contain at least 1% SDS; the SDS/sample ratio should be higher than 1.4:1.
	Incomplete stacking of some highly concentrated protein fractions.	Use a complete discontinuous buffer system either by casting a resolving and a stacking gel in the traditional way, or by equilibrating the stacking zone of a film supported gel selectively in a vertical buffer chamber (see page 228).
	Very acidic and basic proteins, nucleoproteins do not react well in the SDS system.	Try CTAB electrophoresis (cationic detergent in an acid buffer system) (Eley *et al.* 1979, Atin *et al.* 1985).
The molecular weights do not agree with other experimental results.	The molecular weights of non-reduced samples were determined with reduced marker proteins.	An estimation of the molecular weights is possible by comparison with non-reduced globular polypeptides.

Symptom	*Cause*	*Remedy*
	Glycoproteins migrate more slowly than polypeptides with the same molecular weight, because the sugar moieties are not charged with SDS.	Use a Tris-borate-EDTA buffer; borate binds to the sugar moieties and the mobility increases because of the additional negative charge. Also use a gradi-ent gel.
Background in the separation lanes.	Protease activity in the sample.	Proteases are also active in the presence of SDS; add inhibitor if necessary (e.g. PMSF).

Trouble shooting: SDS-electrophoresis

4- Specific staining problems..

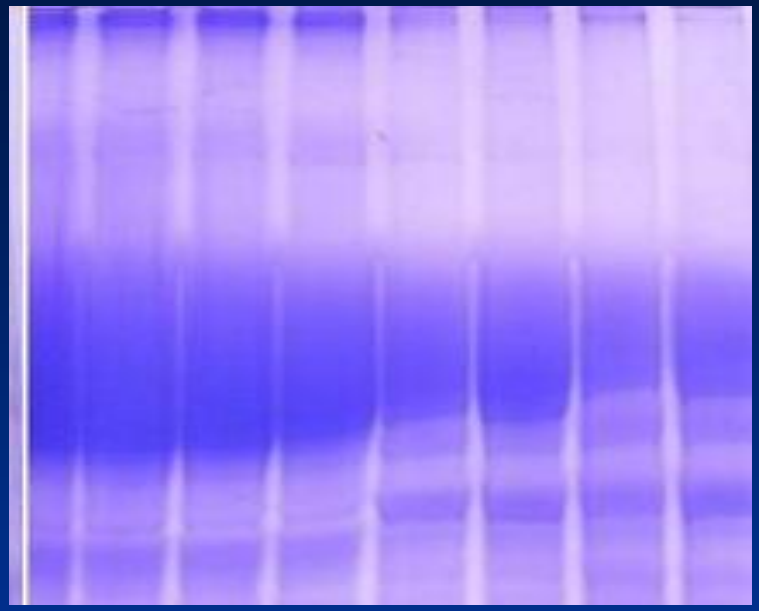
Symptom	*Cause*	*Remedy*
Coomassie Blue: insufficient staining power.	SDS was not properly removed from the sample.	Only use pure SDS, low percentages of 14-C and 16-C sulfate bind more strongly to proteins; wash out SDS with 20% TCA; stain for longer than native electrophoresis.
	The alcohol content of the destaining solution is too high.	Avoid alcohol containing staining and destaining solutions; or else: use colloidal development methods.
Silver staining: negative bands.	Impure SDS.	See above.

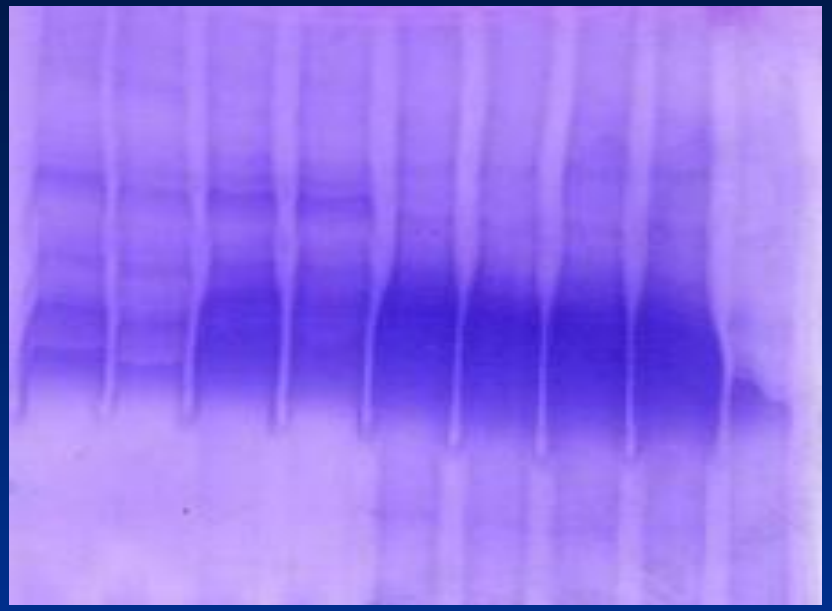
	Problem		Cause		Solution
1.	"Smile effect" - band pattern curves upward at both sides of the gel.	a.	Center of the gel running hotter than either end.	a.	Buffer not mixed well or buffer in upper chamber too concentrated. Remake buffer, insuring thorough mixing, especially when diluting 5x or 10x stock.
		b.	Power conditions excessive.	b.	Decrease power setting from 200 V to 150 V or fill lower chamber to within 1 cm of top of Short Plate.
2.	Vertical streaking of protein.	a.	Sample overload.	a.	Dilute sample, selectively remove predominant protein in the sample, or reduce voltage by about 25% to minimize streaking.
		b.	Sample precipitation.	b.	Centrifuge sample before addition of SDS sample buffers, or decrease % T of resolving gel.*
				c.	The ratio of SDS to protein should be enough to coat each protein molecule with SDS, generally 1.4:1. It may require more SDS for some membrane protein samples. For example, SDS in sample can be increased to 4% and/or in running buffer increased to 0.4%.

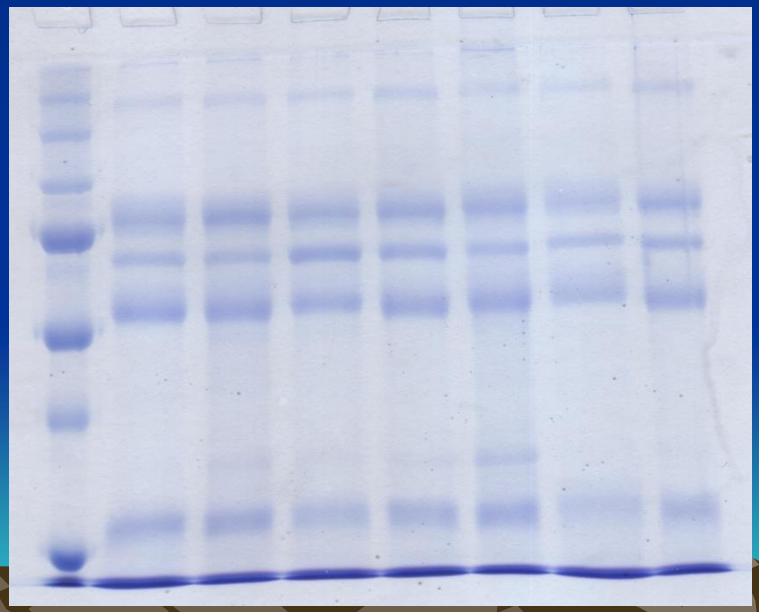
	Problem		Cause		Solution
3.	Lateral band spreading.	a.	Diffusion out of the wells prior to turning on the current	a.	Minimize the time between sample application and power start up.
		b.	Ionic strength of sample lower than that of gel.	b.	Use same buffer in sample as in gel or stacking gel.
4.	Skewed or distorted bands.	a.	Poor polymerization around sample wells.	a.	Degas stacking gel solution thoroughly prior to casting; increase ammonium persulfate and TEMED concentrations by 25%; for stacking gel or low%T, leave APS the same and double the TEMED concentration.
		b.	Salts in sample.	b.	Remove salts by dialysis, desalting column, Micro Bio-Spin columns, etc.
		c.	Uneven gel interface.	c.	Decrease the polymerization rate. Overlay gels very carefully.
5.	Lanes constricted at bottom of gel.	a.	Ionic strength of sample higher than that of surrounding gel.	a.	Desalt sample and neighboring samples.
6.	Run taking unusually long time.	a.	Running buffer too concentrated.	a.	Check buffer protocol, dilute if necessary.
		b.	Excessive salt in sample.	b.	Desalt sample.

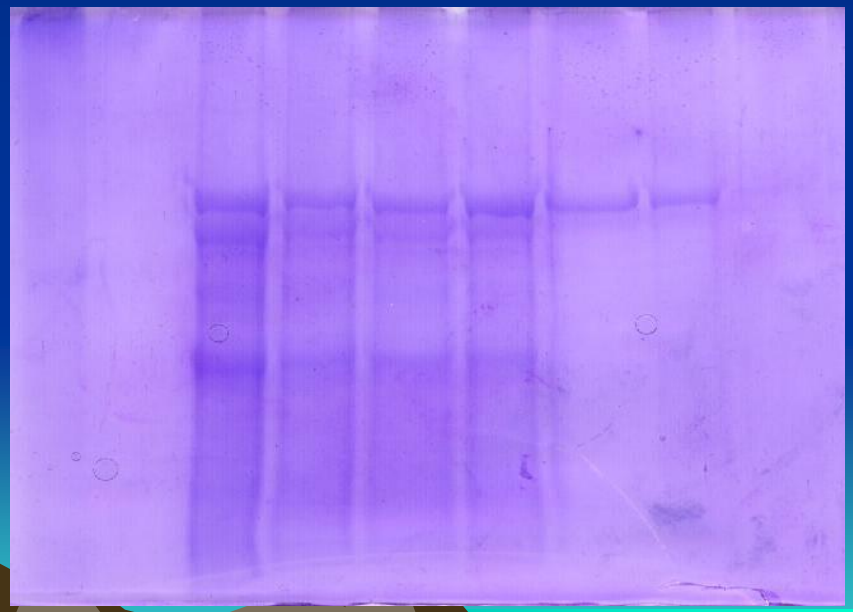
	Problem	Cause	Solution
7.	Run too fast, poor resolution.	a. Running or reservoir buffer too dilute.	a. Check buffer protocol, concentrate if necessary.
		b. Voltage too high.	b. Decrease voltage by 25–50%.
8.	Doublets observed where a single protein species is expected (SDS-PAGE)	a. A portion of the protein may have been reoxidized during the run or may not have been fully reduced prior to run.	a. Prepare fresh sample buffer solutions if over 30 days old; increase 2-mercaptoethanol concentration in the sample buffer; substitute DTT for BME.
9.	Observe fewer bands than expected and one heavy band at dye front.	a. Protein(s) migrating at the dye front.	a. Increase % T of resolving gel.*
		b. Protein degradation.	b. Use protease inhibitors, *e.g.* PMSF, etc.
10.	Upper buffer chamber leaks.	a. Upper buffer chamber over filled.	a Keep level of buffer below the top of the Spacer Plates.
		b. Improper assembly.	b. Be sure u-shaped electrode core gasket is clean, free of cuts, and lubricated with buffer. Be sure Short Plate is *under* the notch on the gasket, not on top of it and press down on electrode assembly when closing cams of the frame.

	Problem		Cause		Solution
11.	Leaking during gel casting.	a.	Chipped glass plates.	a.	Insure glass plates are free of flaws.
		b.	Spacer Plate and Short Plate not level.	b.	Insure cassette is aligned correctly.
		c.	Casting Stand gasket is flawed or worn out.	c.	Replace casting stand gaskets.
12.	Poor end well formation.	a.	Incorrect catalyst concentration.	a.	Prepare fresh catalyst solution, or increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED.
		b.	Monomer solution not degassed. Oxygen inhibits polymerization.	b.	Degas monomer solution immediately prior to casting the stacking gel.
13.	Webbing/excess acrylamide behind the comb.	a.	Incorrect catalyst concentration.	a.	Prepare fresh catalyst solution, or increase catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED.
14.	The pressure cams on the casting frame are difficult to close or make a noise when closed.	a.	A build up of a powder residue at the pivot point of the pressure cams.	a.	Rinse or wipe off the powder residue before each use.










Questions