Troubleshooting Guide for Protein Electrophoresis & Analysis

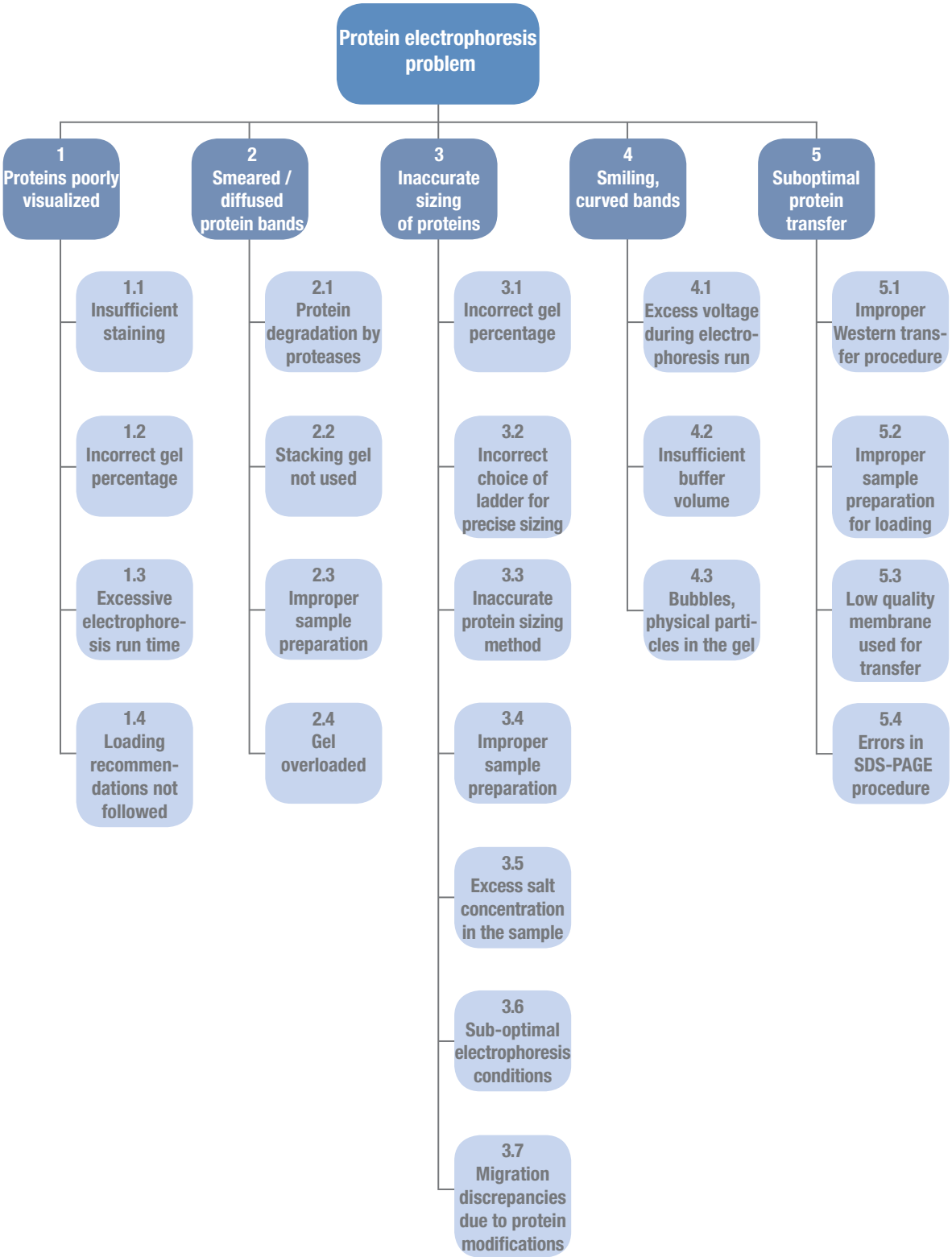

Table 8.2. Troubleshooting Guide for Protein Electrophoresis & Analysis.

Problem	Possible cause and recommended solution
1. Proteins poorly visualized	**1.1. Insufficient staining.** For Coomassie based staining, load ~0.5-5 µg of total protein per minigel well. 10 µg of total protein maybe required for lysates, 1-3 µg should be used to assay homogeneous protein. The staining sensitivity with PageBlue™ Protein Staining Solution (#R0571) is ~5 ng per band. Follow the protocol outlined in the manual. For silver staining procedures load ~0.1-2 ng of total protein per minigel well. The staining sensitivity with PageSilver™ Silver Staining Kit (#K0681) is ~0.05-0.6 ng per band. Unstained protein ladder/marker can be visualized with PageBlue™ Protein Staining Solution (#R0571), PageSilver™ Silver Staining Kit (#K0681) or other protein staining techniques.
	1.2. Incorrect gel percentage. Linear gradient gels allow for adequate resolution of both small and large proteins. Homogeneous low percentage gels are recommended for analysis of large proteins and high percentage gels for analysis of small proteins. In high percentage gels (14-18%) large proteins (150-250 kDa) may not separate, while in low percentage gels (4-8%) small proteins will migrate with a tracking dye. To choose the correct gel percentage for analysis of particular MW proteins, refer to the Table 8.1 on p.402.
	1.3. Excessive electrophoresis run time. Stop the electrophoresis run as soon as the tracking dye front reaches the bottom of the gel. In low percentage gels (4-8%), small proteins (10-15 kDa) migrate with the tracking dye during electrophoresis and may be not visible. Use high percentage or gradient gels to resolve low molecular weight proteins. To choose the right gel percentage for analysis of particular MW proteins, refer to the Table 8.1 on p.402.
	1.4. Loading recommendations not followed. Follow loading recommendation on p.403. Heat protein probes and Unstained Protein Molecular Weight Marker (#SM0431) as described. Do not heat other Fermentas protein ladders/marker.
2. Smeared/diffused protein bands	**2.1. Protein degradation by proteases.** Use clean tips and vials when handling proteins. Use protease inhibitors when extracting proteins. Store protein samples, ladders and markers at -20ºC.
	2.2. Stacking gel not used with the resolving gel. Placement of the stacking gel on top of the resolving gel is necessary to concentrate protein samples and to ensure accurate migration and separation into sharp bands. Follow the gel preparation recommendations on p.402.
	2.3. Improper sample preparation. To ensure proper migration during electrophoresis, protein samples should contain SDS, dithiothreitol (DTT) or 2-mercaptoethanol and must be heated prior to loading. Follow the recommendations for protein sample preparation and for protein ladders/markers on pp.403-404.
	2.4. Gel overloaded. For Coomassie based stains and Western blot applications use 0.5-5 µg of total protein per minigel well. For silver staining procedures use 0.1-2 ng of total protein per minigel well and dilute Fermentas protein ladder/marker 50 times just prior to use, *see* p.403.
3. Inaccurate sizing of proteins	**3.1. Incorrect gel percentage.** Use high percentage gels for analysis of small proteins and low percentage gels for analysis of large proteins. A gradient gel is ideal for precise determination of protein molecular weights. Refer to the table on p.402 to identify the correct percentage gel for a particular protein size.
	3.2. Incorrect choice of ladder/marker for precise sizing. For precise determination of molecular weights, only unstained protein ladders/markers should be used. Prestained standards are recommended only for approximate protein sizing, as chromophores that are covalently coupled to the prestained proteins affect their mobility in various SDS-PAGE-buffer and gel systems. However, they are suitable for approximate molecular weight determination when calibrated against unstained standards in the same system.

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Table 8.3. Troubleshooting Guide for Protein Electrophoresis & Analysis.

Problem	Possible cause and recommended solution
3. Inaccurate sizing of proteins	**3.3. Inaccurate protein sizing method.** Always create a standard curve based on the mobility of protein standards after digitizing a gel image. The standard protein mobility data can be used to prepare a graph of the relationship between the molecular weight of standard proteins and their relative mobility (R_f). Usually the functional relationship is calculated according to the formula log (MW) = a + b x R_f, where a and b are constants determined by calibration with known standards. The MW of an unknown protein is calculated by substituting its Rf in the equation outlined above. A new equation must be calculated for each gel, and data for several gels may be processed to create statistically robust results.
	3.4. Improper sample preparation. To ensure proper migration during electrophoresis, protein samples must contain SDS, dithiothreitol (DTT) or 2-mercaptoethanol and must be heated prior to loading. Follow recommendations for protein sample preparation and for protein ladders/markers on p.403.
	3.5. Excess salt concentration in the sample. High salt concentration in the sample will alter protein mobility. Remove excess salts by gel filtration.
	3.6. Suboptimal electrophoresis conditions from those used for ladder/marker calibration. The apparent molecular weights of Fermentas prestained protein standards are calibrated in classical Tris-glycine-SDS Laemmli system. Each lot of prestained protein ladder/marker is calibrated against a precisely sized unstained protein ladder/marker in Tris-glycine gel and the calculated apparent molecular weights are reported in the product's Certificate of Analysis and www.fermentas.com. However, the bands of the protein standard may have different mobilities in other electrophoresis buffer and gel systems. The migration pattern of a particular protein standard in different buffers and gels is provided on www.fermentas.com.
	3.7. Migration discrepancies due to protein modifications. Natural protein modifications such as; phosphorylation and glycosylation, may alter protein mobility. The molecular weights of modified proteins may or may not correspond to those of unmodified standard proteins of the same size.
4. Smiling, curved bands	**4.1. Excessive voltage during electrophoresis run.** Set the voltage to 250 V. Depending on a number of gels you run, use the appropriate power according the recommendations on p.405. Increase the power when dye front reaches the separating gel.
	4.2. Insufficient buffer volume. Fill the electrophoresis tank (bottom and top reservoirs) with fresh 1X Tris-glycine-SDS buffer, make sure that the gel wells are completely covered with buffer. Use cold buffer for electrophoresis.
	4.3. Bubbles, physical particles in the gel. Mix and pour all gel preparation solutions carefully to avoid formation of bubbles. If physical particles are visible in solutions, remove them by filtration.
5. Suboptimal protein transfer	**5.1. Improper Western transfer procedure.** Follow the recommendations for Western blot transfer. For semi-dry Western transfers follow the protocol on p.407. Make sure that buffer solutions completely cover the gel/membrane/paper sheets during all steps. Use unstained gels for transfer, as stained proteins are transferred with lower efficiency. Use prestained protein ladders (p.396) and the DualColor™ Protein Loading Buffer Pack (#R1011) for electrophoresis as they allow for monitoring of the transfer efficiency.
	5.2. Improper sample preparation for loading. To ensure proper migration during electrophoresis, protein samples must contain SDS, dithiothreitol (DTT) or 2-mercaptoethanol and must be heated prior to loading. Follow recommendations for protein sample preparation and for protein ladders/markers on pp.403-404.
	5.3. Low quality membrane used for transfer. Choose high quality PVDF membrane for Western blotting procedures. Low MW proteins are frequently transferred through nitrocellulose membranes and therefore may be not visible on the blot.
	5.4. Errors SDS-PAGE procedure. Follow specific recommendations for protein electrophoresis. Use prestained protein ladders (p.396) as they allow for monitoring of electrophoresis and transfer efficiency.