



1 Polyacrylamide Gel Preparation

- 1.1 Select glass plates of size appropriate for the test to be run. Make sure the plate surfaces are clean, since plates that are not clean will be difficult to pour without bubbles. The shorter glass plate will be treated with gel slick. This does not need to be repeated each run. Every three or four runs is usually sufficient. Place ~2 ml of gel slick in the center of a clean plate. Using kimwipes, spread the gel slick using a circular motion covering the entire surface.
- 1.2 Allow plate to dry.
- 1.3 Treat the longer plate with bind silane. Add 3 μ l bind silane to a spot of dH₂O (~1 ml) spread on the well end of the plate. Using kimwipes, spread the bind silane on the well end of the plate. **Note:** Spread silane to bottom of place if gel is to be reused later.
- 1.4 Assemble the glass plates by placing a 0.4 mm side spacer along both edges of the longer glass plate. Place the shorter plate on top of this with the treated side toward the longer plate. Hold the plates together using clamps on each side of the assembly. Make sure the clamps are placed over the spacer. Allow room for the final clamps at the end nearest the wells. A small pipette tip may be placed between the plates at the well end for aid in pouring the gel. This is added after the first pair of clamps has been added.
- 1.5 Measure the following solutions in to a squeeze bottle according to the chart below:

Solution	Long (42 cm) gels
5 % Acrylamide	50 ml
Temed	35 μ l
10 % APS	250 μ l

- 1.6 Gently but thoroughly swirl the bottle after adding each solution.

NOTE: Do not introduce bubbles into the solution by excessive shaking as air will inhibit polymerization of the acrylamide.

- 1.7 As quickly as possible, gently pour the solution between the glass plates just until it begins to run out the other end. The plates may be tipped for ease of pouring. Lay plates flat and gently remove the pipette tip. Check for bubbles and remove the bubbles if seen. Push the

NCSBI Forensic Biology Section	DNA SOP	Effective Date: July 19, 2004
Title: Polyacrylamide Gel Preparation		Revision 04

comb between the plates and anchor with the remaining clamps.

- 1.8 Allow to polymerize for approximately 1.5 hours. Gels may be stored overnight if both ends of the plate are wrapped in paper towels soaked in Tris Borate (tank running buffer) or deionized water and placed in ziplock bags or wrap in plastic wrap. An alternative is to set up the gel in a vertical electrophoresis apparatus and cover with tank buffer. Do not allow the polymerized acrylamide to dry out.

2 Re-Use of Polyacrylamide Gels

- 2.1 After its initial use, the acrylamide gel may be stored and/or reversed prior to its next use.
- 2.2 To store the gel, it may be wrapped in wet paper towels and wrapped in Saran wrap to keep it moist. If the gel should dry out, it will not be re-used. For extended life, the wrapped or bagged gels can be placed in the refrigerator. As an alternative (for immediate re-use), the gel could be placed back into the electrophoresis tank and covered with TBE buffer.
- 2.3 Before re-use, the polarity will need to be reversed and the gel electrophoresed at 60W for at least two hours to rid the gel of previously loaded DNA. At the end of the reversal time period, return the polarity back to normal run conditions.
- 2.4 At this point, the gel is ready to be loaded with fresh samples.
- 2.5 Analysts are to re-use gels only if they are of good quality, (ie. no broken wells or distortions, etc.). A gel may be re-used until it exhibits problems.

NCSBI Forensic Biology Section	DNA SOP	Effective Date: July 19, 2004
Title: Polyacrylamide Gel Preparation		Revision 04

Revision History		
Effective Date	Revision Number	Reason
Unknown	00	Original Document for DNA Tech Procedures
January 4, 2002	01	Addition of Speed-Vac Procedure (Change in DNA Technical Procedures)
November 26, 2002	02	Procedure Updates; Collation of Procedures and use of WP Outline; change in page numbering (Change in DNA Technical Procedures)
August 7, 2003	03	Addition of Table of Contents; no substantive changes in any procedure. (Change in DNA Technical Procedures)
July 19, 2004	04	Combined "Polyacrylamide Gel Preparation" and "Re-use of Polyacrylamide Gels" SOPs a separate document titled "Polyacrylamide Gel Preparation". No technical changes in SOPs.

APPROVAL SIGNATURES		Date
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