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DNA polyacrylamide gel electrophoresis

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We use this protocol and it's working

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Abstract

To separate and visualize DNA fragments of varying sizes, using a gel matrix and an electrical current.

Materials

MATERIALS

- Blue Loading Buffer Pack - 8.0 ml **New England Biolabs Catalog #**B7703S
- SYBR Gold **Thermo Scientific Catalog #**S-11494
- Ammonium Persulfate, 25g (Ammonium Persulphate) **Promega Catalog #**V3131
- Bisacrylamide, 25g (N,N'-Methylenebisacrylamide) **Promega Catalog #**V3141
- TEMED (Tetramethyl-ethulenediamine) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T9281
- Acrylamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A9099
- Safe Imager™ 2.0 Blue Light Transilluminator **Thermo Fisher Scientific Catalog #**G6600

1 Assemble the glass plates with spacers in gel caster

2 Prepare the gel solution with the desired polyacrylamide percentage

Gel 8%

	Component	Volume	Initial Conc.
	Acrylamide	1,86 mL	30%
	TBE	1,4 mL	5x
	APs	100 µl	10 %
	TEMED	7 µl	
	Nuclease-Free Water	3,74 mL	

3 Work quickly after addition of TEMED to complete the gel before the acrylamide polymerizes.

4 Immediately insert the appropriate comb into the gel, being careful not to allow air bubbles to become trapped under the teeth. If necessary, use the remaining acrylamide gel solution to fill the gel mold completely. Make sure that no acrylamide solution is leaking from the gel mold.

5 Allow the acrylamide to polymerize for 30 minutes at room temperature.

6 When polymerized, remove gels from gel caster, and insert gels into gel box. Add TBE 1x buffer and carefully pull the combs from the polymerized gel.

7 Mix 10 µl of DNA samples with 2 µl of 6x gel loading buffer. Load the mixture into the wells using a micropipette.

8 Connect the electrodes to a power pack, turn on the power, and begin the electrophoresis run at 100 V for 1 hour.

9 Turn off the electric power, disconnect the leads, and discard the electrophoresis buffer from the reservoirs.

10 Detach the glass plates. Remove the spacers. Use a spacer to lift a corner of the upper glass plate. Check that the gel remains attached to the lower plate. Pull the upper plate smoothly away.

11 Stain gels with 1x SYBR gold (Invitrogen) in 70 µl of Miliq Water, for two gels, for 30 minutes.

12 Analyse the gel using the Safe Imager™ 2.0 Blue-Light Transilluminator (Invitrogen).