JUN 06, 2018

DNA polyacrylamide gel electrophoresis

Izabela M
Lívia Sacchetto, Rezende¹

¹Stanford University School of Medicine

MRCA

Izabela M Rezende
Stanford University School of Medicine

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.pxadpie

Protocol Citation: Lívia Sacchetto, Izabela M Rezende 2018. DNA polyacrylamide gel electrophoresis. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.pxadpie>

MANUSCRIPT CITATION:
Persistence of Yellow fever virus outside the Amazon Basin, causing epidemics in Southeast Brazil, from 2016 to 2018.
Rezende IM de*, Sacchetto L,* Mello ÉM de, Alves PA, Iani FC de M, Adelino TÉR, Duarte MM, Cury, ALF, Bernardes AFL, Santos TA, Pereira LS, Dutra MRT, Ramalho DB, Thoisy B, Kroon EG, Trindade GS, Drumond BP
PLoS Neglected Tropical Disease 2018;12: e0006538.
doi:10.1371/journal.pntd.0006538

*These authors contributed equally to this work

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-ethylenediamine) Sigma-aldrich Catalog #T9281
- Acrylamide Sigma Catalog #A9099
- Safe Imager™ 2.0 Blue Light Transilluminator Thermo Fisher Scientific Catalog #G6600

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: May 05, 2018

Last Modified: Jun 06, 2018

PROTOCOL integer ID:
11970

- 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).