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DNA-Blot genotyping of Trypanosoma cruzi Discrete Typing Units (DTU) with radiolabeled probes

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We use this protocol and it's working

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Abstract

DNA-Blot genotyping of *Trypanosoma cruzi* Discrete Typing Units (DTU) with radiolabeled probes

Guidelines

- Use gloves, mask and eye protection to prepare solutions.
- Use gloves during the whole procedure.

Before start

1. Prepare solutions:
- EDTA 0.5M, pH8.
- TBE 10X: Tris 0.89M + Boric Acid 0.89M+ EDTA 0.5M 40 ml + distilled H2O to complete 1000 ml
- TBE 1X:Tris (0.089M) - Borate (0.089M) - EDTA (0.002M). Use 100 ml of TBE 10X + 900 ml distilled H2O.
- Denaturation solution: NaCl 1.5M NaOH 0.5M
- Neutralization solution: Tris 1M pH 7.4 NaCl 1.5M
- Hybridization solution SSC 20X: NaCl 3M Na3C6H5O7 0.3M pH7
- SSC 2X: Use 50 ml of SSC 20X + distilled H2O to complete 500 ml.

2. Amplify *Trypanosoma cruzi*'s kinetoplastidic DNA by PCR using primers 121 and 122 (Wincker P, Britto C, Pereira JB, Cardoso MA, Oelemann W, Morel CM. Use of a simplified polymerase chain reaction procedure to detect *Trypanosoma cruzi* in blood samples from chronic chagasic patients in a rural endemic area. Am J Trop Med Hyg. 1994;51(6):771-777).

DNA Blotting

1 Use a 20 µl variable volume micropipette with a filtered tip to mix 10 µl of each PCR product with gel loading buffer according to the manufacturer's recommendation. Load this mix in one well of an 2% agarose TBE 1X gel placed inside an electrophoresis chamber, covered with TBE 1X solution. This procedure will be repeated, changing the tip, until loading the same sample in 4 different gels, or in 4 rows of wells from one big gel. Use the same well number in each of the gels/rows for the same sample. In each gel/row, load a lane with a 100 bp DNA ladder, and another with the negative control.

2 Submit the gels to a 45 min electrophoresis at 100 volts.

3 Cut a little piece of the left corner of the gel using a scalpel, to identify the position of the gel later on.

4 Using a plastic sheet below the gel to move it; place it in a glass or plastic recipient just a bit wider than the gel itself (one recipient per gel). Cover the gel with denaturation solution; remove the plastic sheet carefully, and place the recipient with the gel in a 3D agitator platform for 20 min to agitate the solution over the gel.

5 Remove the denaturation solution; since the gel can rupture, when turning the recipient to discard the buffer, use the plastic sheet to hold the gel in place. Cover the gel with new denaturation solution. Place it in the 3D agitator platform for 20 min, to obtain some single-stranded DNA in the gel.

6 Wash the gel five times with distilled water (app. 500 ml each time), agitating it over the gel several times. Discard the water and renew it each time; remember to use the plastic sheet to hold the gel. Remove any remaining distilled water.

7 Wash the gel with 500 ml of neutralization solution, placing the recipient in the 3D agitator, for 20 min. Remove the neutralization solution and wash it again with new neutralizing solution for 20 min. Remove the solution, using the plastic sheet to hold the gel.

8 Prepare a glass recipient with 500 ml of hybridization solution SSC 2X. Over that recipient, place a square glass across it, like a bridge. Change gloves to new, dry ones.

9 Cut a filter paper the width of the gel, long enough to place it over the bridge with both sides hanging down to the glass recipient, and submerged in the hybridization solution.

10 Using the plastic sheet to hold it, place the agarose gel inverted over the filter paper.

11 Over it, place a nylon membrane (Amersham Hybond™ N+, General Electric HealthCare Life Sciences, Budapest, Hungary) cut with scissors to the same size of the gel, labelled using a pencil with the equivalent position of each sample in the agarose gel and its identification code. Always cut the left corner of the nylon membrane, and place it inverted over the gel, matching the cutted corners.

12 Cover the membrane with another filter paper cutted to the same size of the gel. Over that filter paper, place a pile of absorbent paper napkins, allowing the hybridization solution to arise by capillarity from the inferior filter paper through the agarose gel, impregnating the membrane, and dragging the DNA overnight.

13 The next day, expose the membranes to UV light (254 nm) for 2 min, in a Cross-linker (e.g. Bio-Link BLX Vilber Lourmat®, Marne-la-Vallée, France) to fix the DNA. Store in a labeled paper envelope until genotyping.

Genotyping

14 Probe preparation and radiolabeling follows Veas et al. 1991. Four *T. cruzi* clones (sp 104 cl 1, CBB cl 3, NR cl 3, and V195 cl 1, corresponding to TcI, TcII, TcV and TcVI, respectively), are used to prepare the probes, as described (Rojo et al. 2017).

15 Pre-hybridize and then hybridize the nylon membranes (following Rojo et al. 2017), with the specific 32P marked probes. Record in a diagram or with a photo the exact position of each membrane in the plate, to be able to identify the results for each sample. Reveal the results in a Molecular Imager FX (Bio-rad®, California, USA).