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Long Primer PCR (for Trypanosoma brucei)

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Protocol status: Working
We use this protocol in our group and it is working!

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36974

1 PRC Mix

- 1 uL pPOT (25 ng/uL)
- 0.2 mM dNTPs
- 1 uM for primer
- 1 uM rev primer
- 1 uL PCR grade DMSO
- 5uL 10x buffer 2 (Roche)
- XX uL ddH2O for total volume of 49 uL
- Add 1 uL Expand High Fidelity polymerase (Roche) once mixture has reached 94 C.

1.1 PCR conditions

- 94 C 5 mins
- 94 C 15 sec
- 65 C 30 sec (30 cycles)
- 72 C 2 min
- 72 C 7 min

- 2 Maintain procyclic form SMOX P9 cells [31] between 1×10^6 - 1×10^7 cells ml^{-1} for at least 72 hours prior to transfection to ensure they are in log growth phase.
- 3 Centrifuge 1×10^7 log phase procyclic cell per transfection at 800 g for 10 min at room temp
- 4 remove all supernatant
- 5 Resuspend cells in 500 mL of room temperature cytomix per transfection, and add to a 4 mm gap electroporation cuvette
- 6 Add 50 mL of unpurified PCR to the cell suspension

- 7 Electroporate the cell once with 1.7 kV, 25 mF (gene pulser (Bio-Rad) or three times 1.7 kV for 100 Ms, 200 ms interval (BTX ECM830 (hardvard Apparatus))
- 8 Recover the cells for 8-16 hours in 10 ml SDM-79 at 28 C
- 9 Add the appropriate selective drug to the final concentrations:
 - Blasticidin(Melford)20mg/ml(<20mg.ml-1 is not sufficient to kill off all non transformed cells).
 - Hygromycin b Gold (Invivogen)25mg/ml(>25mg.ml-1 reduces transfection efficiency due to low readthrough transcription of the resistance cassette).
- 10 If clones are required, dilute 5 ml of recovered cells into 50 ml of selective medium and distribute 1 ml aliquots into a 48 well plate.
- 11 Resistant populations of cells emerge after 7 – 10 days, and clones emerge after 10 – 14 days.
- 12 FOLLOW UP

Transfection of bloodstream form T. brucei using the Amaxa Nucleofector II

T. brucei can be efficiently transfected using the Amaxa Nucleofector II using the human T-cell kit (VPA-1002 Lonza).

Step 12 includes a Step case.

step case

untitled case

- 13 1. Complete the human T-cell Nucleofector solution by addition of supplement 1. The combined solution and supplement can be stored and is stable for 3 months at 4°C.

- 14 Purify 100ml of long primer PCR (~8mg) with one phenol chloroform extraction followed by ethanol precipitation at -80°C for 1 hour with two 70% ethanol washes (the pellet should be easily visible). Note that using a silica membrane column instead of phenol chloroform to purify the DNA will reduce transfection efficiency by 10 – 50 fold.
- 15 Resuspend the dried pellet in 10ml 5mM Tris pH8.
- 16 Maintain bloodstream form SMOX B4 cells [\[31\]](#) between 1×10^5 – 1×10^6 cells.ml⁻¹ for at least 72 hours prior to transfection to ensure they are in log growth phase.
- 17 Centrifuge 2×10^7 log phase ($< 1.3 \times 10^6$ cells.ml⁻¹) bloodstream form cells per transfection at 800 g for 10 minutes at room temperature.
- 18 Carefully remove all supernatant.
- 19 Resuspend the cell pellet in 100ml of complete AmaxaT cell buffer per transfection, and transfer to an Amaxa cuvette.
- 20 Add the purified DNA to the cell suspension, and electroporate once using Program X-001.
- 21 Recover cells for 8 - 16 hours in 50 ml HMI-9 at 37°C with 5% CO₂.
- 22 Add the appropriate selective drug to the final concentrations:

Blasticidin(Melford)5mg/ml. Hygromycin B Gold (Invivogen)1.5mg/ml.

23 Distribute 1ml aliquots of cells into two 48 well plates.

24 Resistant clones emerge after 6 – 8 days.