Transfection of Naegleria gruberi

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Abstract

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Protocol

Harvest Naegleria cells that have been "transformed" to flagellates, via the protocol (see below).
Step 1.

Use a 100% confluent 25cm^2 flask for each transformation.

https://www.protocols.io/view/flagellar-transformation-of-naegleria-gruberi-hnhb5b6

Pellet the cells by centrifugation at 1000 x g for 5 minutes
Step 2.

Wast the pellet with ice-cold sterile 1X PBS
Step 3.

Pellet the cells by centrifugation at 1000 x g for 5 minutes
Step 4.

Wash the pellet with SM ice cold buffer. Filter sterilise an aliquot before use.
Step 5.

1X SM BUFFER: 500 ml

5 Mm KCl: 0.18 g

15 mM $MgCl_2$: 710 mg

120 mM Na_2HPO_4/NaH_2PO_4, pH 7.2 : 60 ml

25 mM succynil acid: 1.475 g

25 mM mannitol: 2.275 g

Filter aliquots before electroporation

Pellet the cells by centrifugation at 1000 x g for 5 minutes

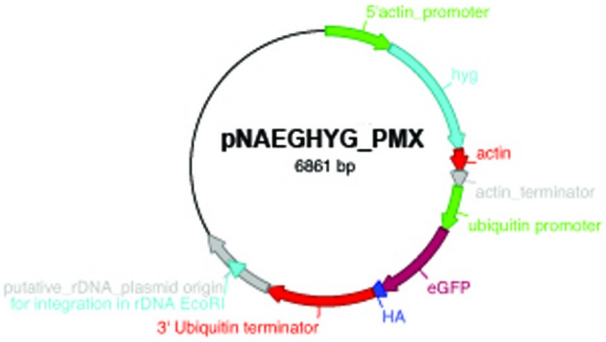
Step 6.

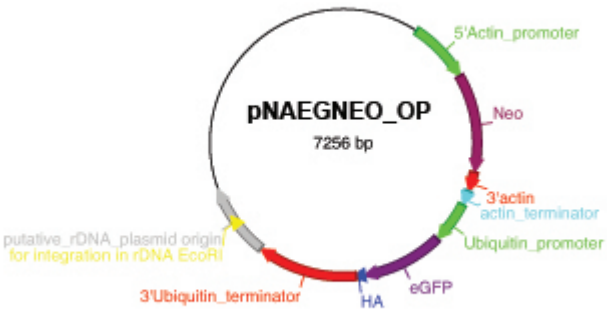
Resuspend the pellet in 400 µl of SM ice-cold buffer per electroporation sample

Step 7.

In an 1.5 ml eppendorf tube add 10 µl of plasmids (pNaegNEO and pNaegHYG plasmids; concentration 400 ng/µl) and the 400 µl of cells in SM ice-cold buffer (above)

Step 8.



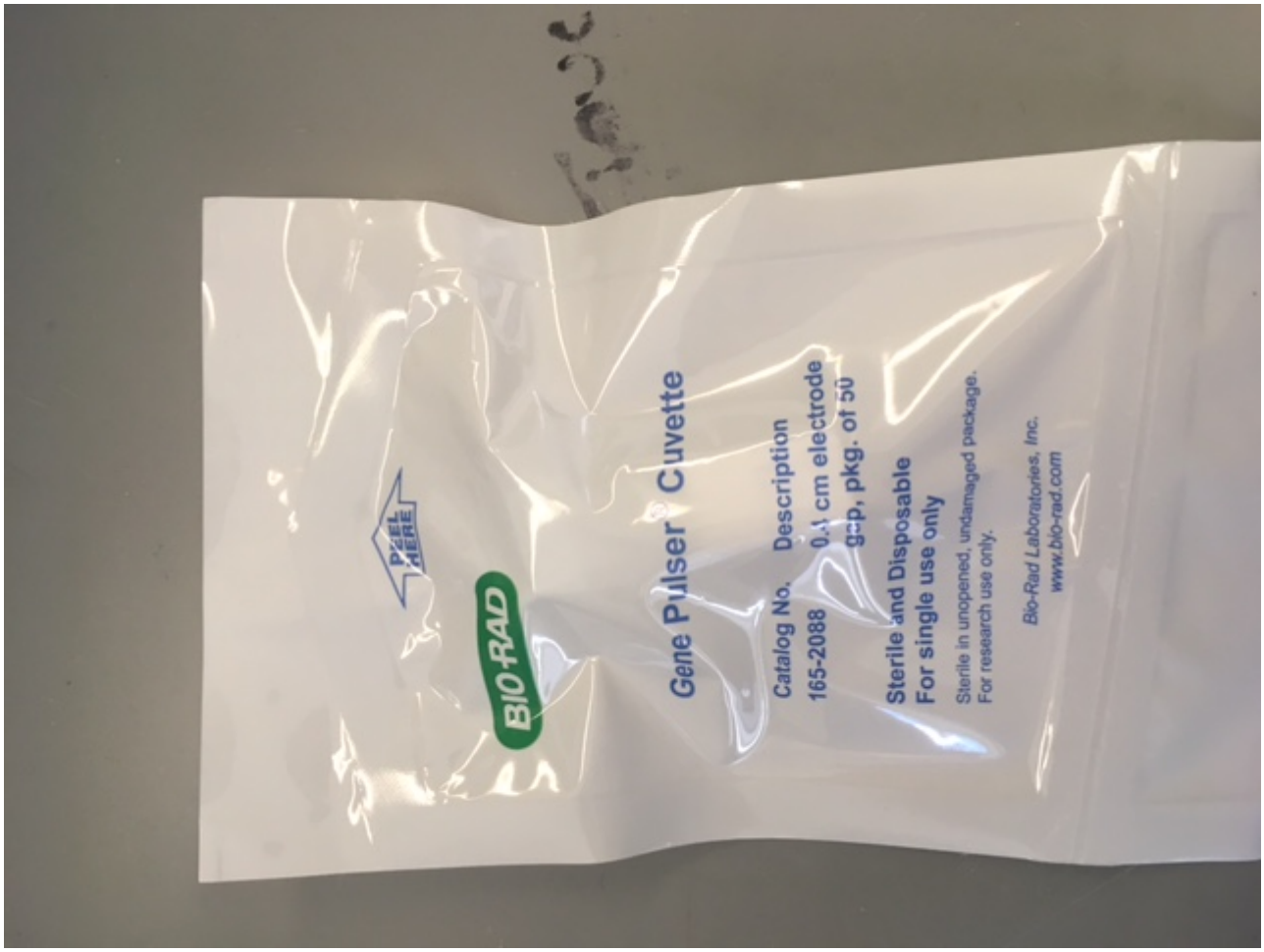


Incubate on ice fo 5 minutes

Step 9.

Transfer the suspension in an ice-cold bio-rad electroporation cuvette; 0.4 cm.

Step 10.



Electroporation shock: clean the ice and water of the cuvette with tissue and put them in the chamber of the BioRad Gene Pulser xCell (see attached

Step 11.

Electroporation conditions for the BioRad Gene Pulser xCell: 175 V, 500 µF, 400 Ω.

Step 12.

Remove the cuvette from the chamber and put back on ice and incubate fo 5 minutes

Step 13.

In a 24 well plate, add 400 µl of the electroporation (above) per well and 1,400 µl of M7 media supplemented with 10% FBS and 1% penicillin/streptomycin

Step 14.

M7 rich media for growing *Naegleria gruberi* axenically contains 0.54 % glucose (Fisher Scientific – Product No. G/0500/61), 0.5 % yeast extract (Melford), 0.0045% L-methionine (Duchefa Biochemie – Product No. M0715.0100) and 2% KPi buffer solution containing 0.18% KH_2PO_4 (Melford – Product No. P0574) and 0.25 Na_2HPO_4 (Melford – Product No. S2002) , 10% FBS (Sigma – Product No. 7524 non-USA origin, sterile-filtered, suitable for cell culture) and 1% penicillin/streptomycin (Gibco from Fisher Scientific – Product No. 11548876).

Seal the 24 well plate with parafilm and incubate at 32oC for 24-48 hours.

Step 15.

After maximum of 48 hours add the corresponding selectivedrug to the transected cells (300 µg/ml of Hygromycin B or 700 µg/ml of Neomycin (G-418))

Step 16.

Incubate at 32oC for 24 hour

Step 17.

Carefully remove the supernatant (along with dead cells) and add 2 ml of fresh media with the corresponding selective drug (Hygromycin B or G418) to the final concentrations shown above.

Step 18.

Incubate at 32oC for 72 hours

Step 19.

Transfer the cells in a 25cm2 flask and incubate at 32oC and analyse them by fluorescence microscopy (alive or fixed) to determine the gene expression.

Step 20.

The protocol for fluoresence microscopy can be found at:

https://www.protocols.io/view/transfected-naegleria-fluorescence-microscopy-hpvb5n6