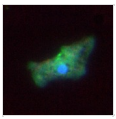

Jun 24, 2019

# Transfection of Naegleria gruberi V.3

Anastasios D.. Tsaousis[1], Eleanna Kazana[2]

[1]University of Kent, [2]University of Kent at Canterbury

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Anastasios D.. Tsaousis
University of Kent

GUIDELINES

>pNaeHYG plasmid seqeunce

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

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

2

Wast the pellet with ice-cold sterile 1X PBS

3

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

4

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

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

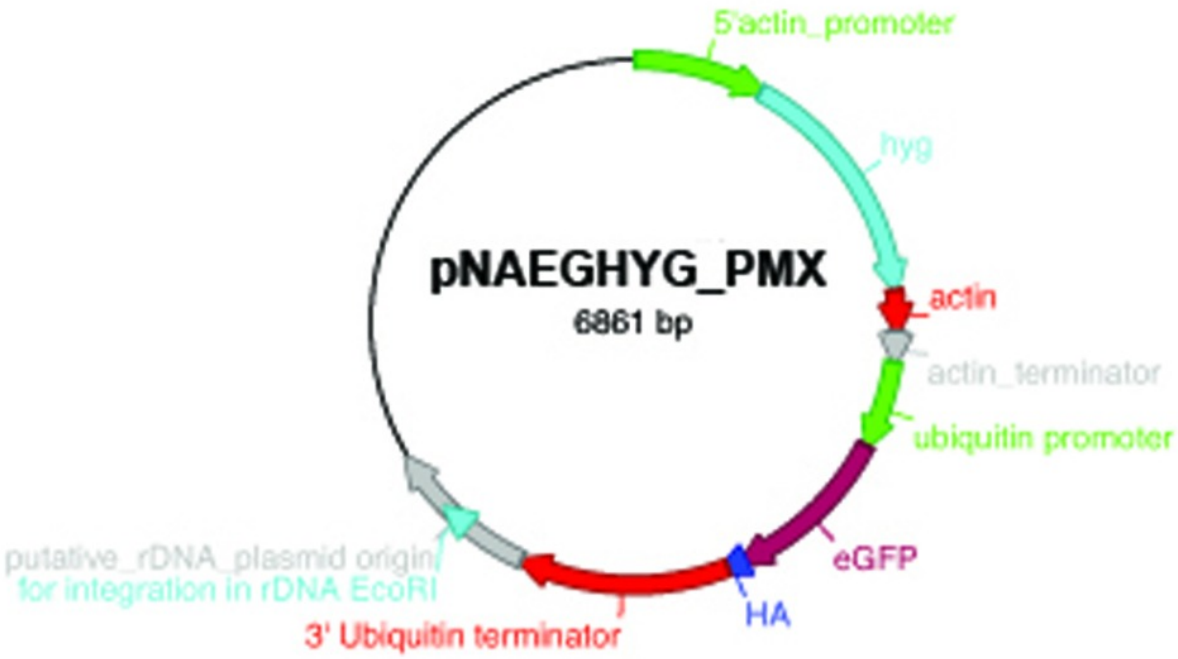
6

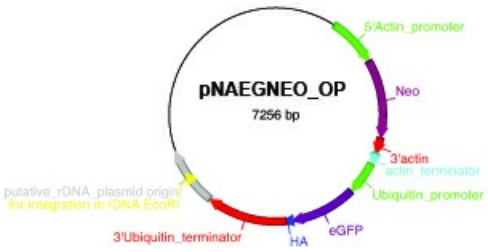
Resuspend the pellet in 400 µl of SM ice-cold buffer per electroporation sample

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)

8



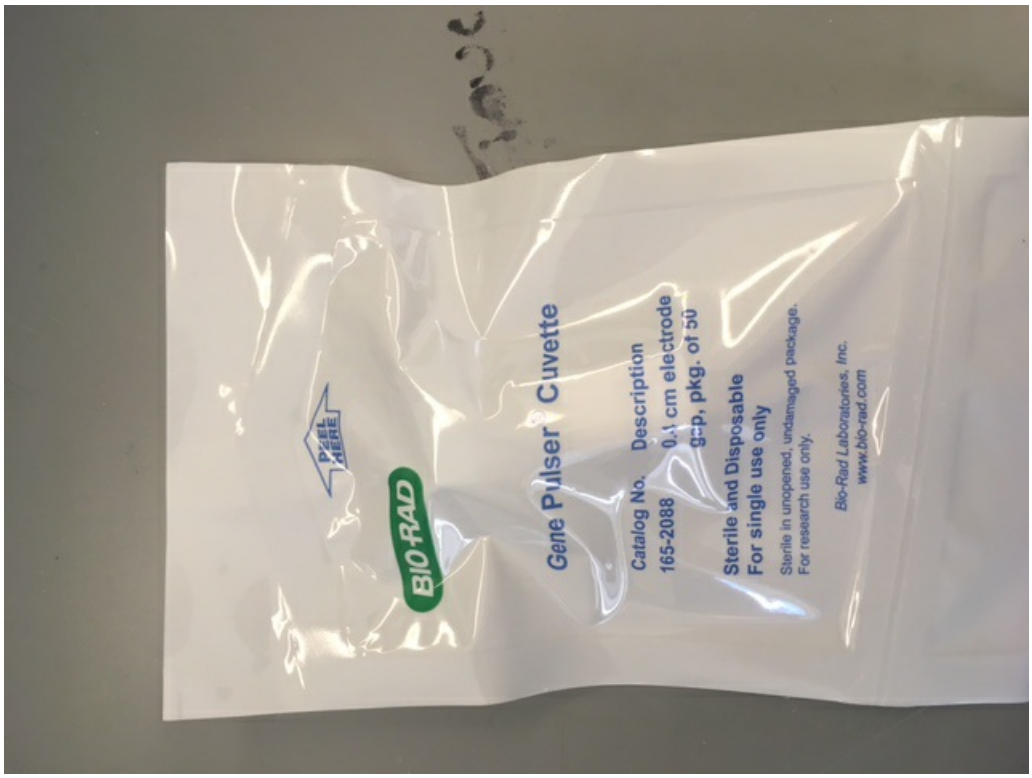




Incubate on ice fo 5 minutes

9

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

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

11

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

12

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

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

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.

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))

16

Incubate at 32oC for 24 hour

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.

18

Incubate at 32oC for 72 hours

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.

20 The protocol for fluoresence microscopy can be found at:
https://www.protocols.io/view/transfected-naegleria-fluorescence-microscopy-hpvb5n6