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Version 3

🌐 Transfection of *Naegleria gruberi* V.3

📖 [Nature Methods](#)

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Anastasios D.. Tsaousis¹, Eleanna Kazana²

¹University of Kent; ²University of Kent at Canterbury



Anastasios D. Tsaousis

University of Kent

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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 25009

Keywords: transfection of naegleria gruberi, naegleria gruberi, transfection

Guidelines

>pNaeHYG plasmid sequeunce

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>pNaeNEO plasmid sequence

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

- 1 Use a 100% confluent 25cm² 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

Wash 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 MgCl₂ : 710 mg
120 mM Na₂HPO₄/NaH₂PO₄, pH 7.2 : 60 ml
25 mM succinyl 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

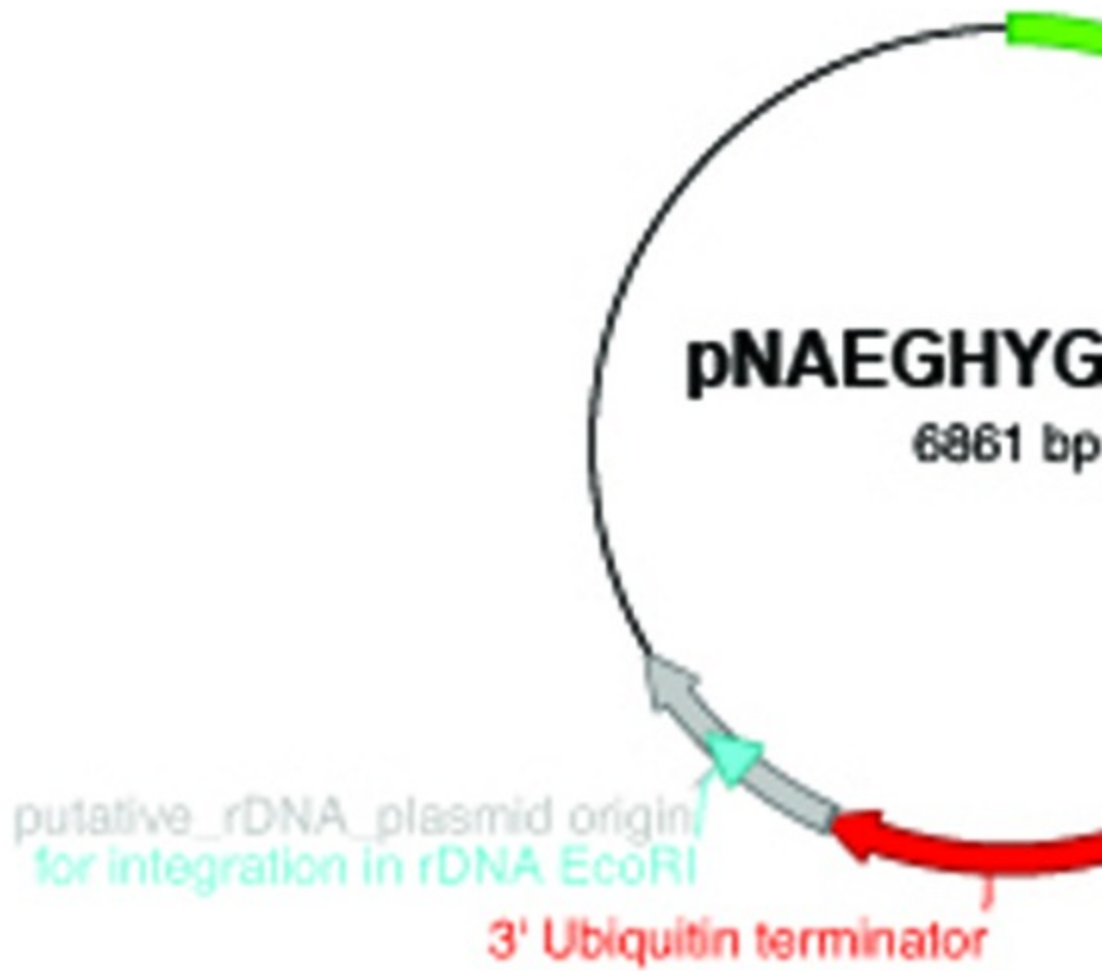


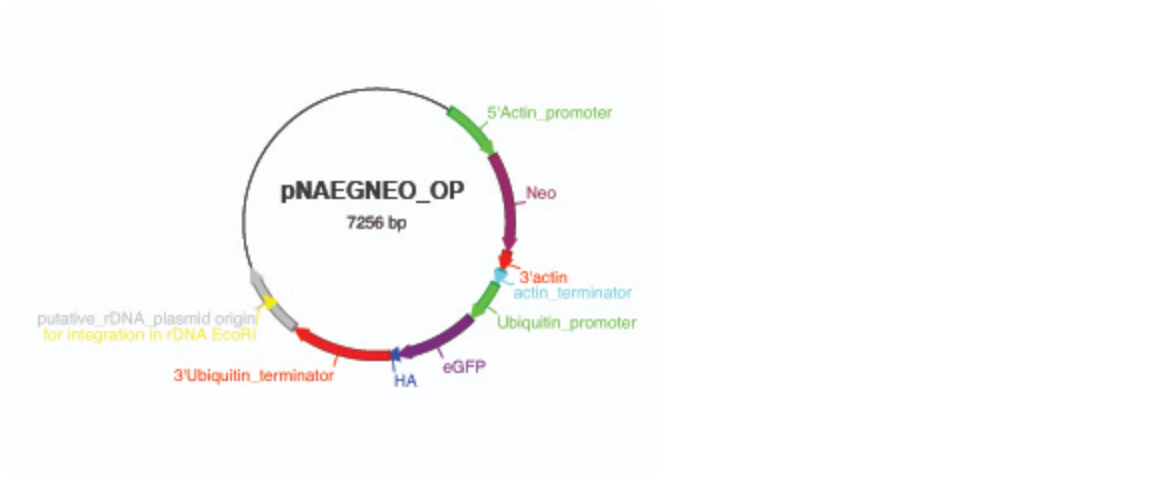
Resuspend the pellet in 400 µl of SM ice-cold buffer per electroporation sample

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



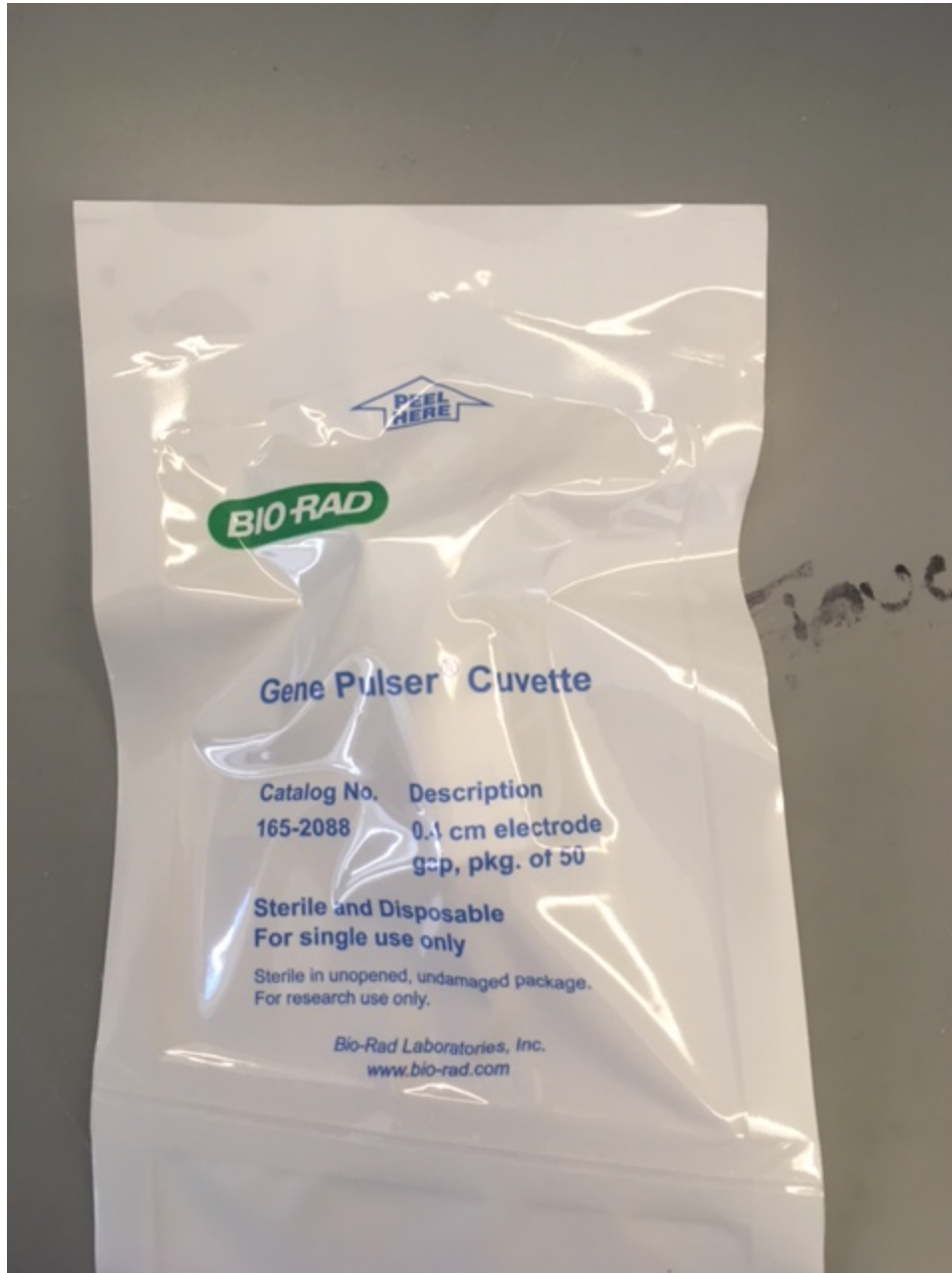


Incubate on ice for 5 minutes

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



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

12

Remove the cuvette from the chamber and put back on ice and incubate for 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 selective drug 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 32°C and analyse them by fluorescence microscopy (alive or fixed) to determine the gene expression.

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