VERSION 3

JUN 24, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocol.s.io.4nrgvd6

Protocol Citation: Anastasios D. Tsaousis, Eleanna Kazana 2019. Transfection of *Naegleria gruberi*. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.4nrgvd6>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Jun 24, 2019

Last Modified: Jun 24, 2019

PROTOCOL integer ID:
 25009

Transfection of *Naegleria gruberi* V.3

Anastasios D. Tsaousis¹, Eleanna Kazana²

¹University of Kent; ²University of Kent at Canterbury

Anastasios D. Tsaousis
 University of Kent

GUIDELINES

>pNaeHYG plasmid sequence

```
CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAA
ATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCAGTTTGAACAAGAGTCCACTATTAAAGAACGTGGACTCCA
ACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCACTACGTGAACCATCACCTAATCAAGTTTTTGGGGTCGAGGTGCCGTA
AAGCACTAAATCGGAACCTAAAGGGAGCCCCGATTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAA
GCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCAACACACCCGCCGCGCTTAATGCGCCGCTACAG
GGCGCGTCCCATTCGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGG
GGATGTGCTGCAAGGCGATTAAGTTGGGTAAACGCCAGGGTTTTCCAGTCACGACGTTGTAACGACGCGCCAGTGAGCGCGCGTAATAC
GACTCACTATAGGGCGAATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGATCTGACGAAAACAACATGTGGATACTCAATACTGTTGAA
ACCTTTCAAATAGGACTGTGGCAAGATAAATGATCTGGTGGTTCCAAAACACTGCTGTGATAGCAGCATTCCTCAATATTTCTTATTGTGC
CATCCAAAAGAGATAATTTACTAGTAAATGATTGCATATCCTTCACTTCAGCCTCACAAACAACCTACAGTATCCAAAGGAAGCCTTCT
CGTTTTTCCTTTCTATTTCTTGATATCCACCAAGTGGCTGATCCTTGTTCATTTCCGTTTTTCTTCCATGAATTTTAATTAAGTCCACTG
TTTGATTTTTCTTCCATGTATTGTTTAAATGAAGTCCACTGTTATTGATTCTGTTGATGGTTATTCTGTTATTATTGTTGATGATGATGCG
TTTGTTGCCAAGAGTCCAGAAATTTTATGAATCATCAGACTAGGATGATACCTCTTCTCTTTCTTGGCATTTTTTTAAACATTAAATTA
CTTTGTTACAAAGCGCGGAACAAGTTTATAGTCTTACAAAAAATGAAAAAACAGAAATGACTGCTACTAGTGTGAAAAATTTTGA
TTGAAAAATTTGATTCTGTTTCTGATTGATGCAATTGTCTGAAGGTGAAGAATCAAGAGCTTTTTCTTTGATGTTGGTGGTAGAGGTTAT
GTTTTGAGAGTTAATTTCTGTGCTGATGGTTTTATAAAGATAGATATGTTTATAGACATTTTGCTTCTGCTGCTTTGCCAATTCAGAA
TTTGATATTGGTGAATTTTCTGAATCTTTGACTTATTGTATTCAAGAAGAGCTCAAGGTGTACTTTGCAAGATTGGCCAGAACTGAAT
TGCCAGCTGTTTTGCAACCAAGTGTCTGAAGCTATGGATGCTATTGCTGCTGCTGATTGTCTCAAACCTCTGGTTTTGGTCCATTTGGTCCA
CAAGGTATTGGTCAATATACTACTTGGAGAGATTTTATTGTGCTATTGCTGATCCACATGTTTATCATTGGCAAACCTGTTATGGATGATAC
TGTTTCTGCTTCTGTTGCTCAAGCATTGGATGAATTGATGTTGTGGGCTGAAGATTGTCCAGAAGTTAGACATTTGGTTCATGCTGATTTTG
GTTCTAATAATGTTTTGACTGATAATGGTAGAATTACTGCTGTTATTGATTGGTCTGAAGCTATGTTTGGTGATTCTCAATATGAAGTTGCT
AATATTTTTTTTTGGAGACCATGGTTGGCTTGATGGAACAACAACTAGATATTTTGAAGAAGACATCCAGAATTGGCTGGTTCTCCAA
GATTGAGAGCTTATATGTTGAGAATTGGTTGGATCAATTGTATCAATCTTTGGTTGATGGTAATTTTATGATGCTGCTTGGGCTCAAGGT
AGATGTGATGCTATTGTTAGATCAGGTGCTGGTACTGTTGGTAGAACTCAAATTGCTAGAAGATCAGCTGCTGTTGGACTGATGGTTGTG
TTGAAGTTTTGGCTGATTCTGGTAATAGAAGACCATCTACTAGACCAAGAGCTAAAGAATAAGGGCCCTGATTAAATTTATCCAGTAATT
TTGGACGATTCTCTTTCTTATTGAAGCAATAAGGTAAGGTAAGGTAAGGTAAGGTAAGGTAAGGTAAGGTAAGGTAAGGTAAGGTAAGG
AAGAATGTGTAACAGATGTTCTTTTACAGGAATAATTATTGAAGATGAAAACCTACATGATACAAAATACACACTGCACTGGATTCA
AATTAAGGTTGACAAGGTGATACAAACCACTTGTGGCCATCCTCGTGGTCTGAAGATTGAATCAACACTTTTGTCTGCTTTGTGAATGT
CGACACTTGTTCAGTTAGTAACTAATTGGTATCATATCTAATACAAACAGCATTGGAAGGAAAATGGATTATTACCTTACCATATCTCTCTC
ATCCAATCGATTGAAGTAATGAGGTTGATGATGTTAAAAGTTGATTGGTTGTGTAATAATGGCAATAGTGTATTAATGGATTGAAAGCGAAT
ATGGATGTGGAGTTGAATCTCTTCAATCTAAACGTGCTGAAGCGAGGATGAACCTACGTTGTAACAGCAAACCGCCATTAATTCTATTTT
AACGTCCAATAACCTATTAATACAAGTACCCATCATTGATCGTCACTACAACAAATCAAAAAAATCAATAACTGCTTATTGTATACTAA
AGCATATGGTGAGTAAGGGCGAGGAGCTGTTACCCGGTGTGGTGCCTATCCTGGTCGAGCTGGATGGCGATGTAACGGCCACAAGTTCA
GTGTGTCGGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTTCACTGTACCACCGGCAAGCTGCCTGTGCCTTGGC
CAACCCTCGTGACCACCTGACCTACGGCGTGCAATGTTTCAGTCGTTACCCCGATCAGATGAAGCAACACGATTTCTTCAAGTCGGCCA
TGCCAGAAGGCTACGTCCAAGAGCGTACCATCTTCTTCAAGGATGACGGCAACTACAAGACCAGAGCCGAGGTGAAGTTGAGGGCGATA
CCCTGGTGAACCGTATCGAGCTGAAGGGCATCGACTTCAAGGAGGATGGCAACATCCTGGGCCACAAGCTGGAGTACAACACTACAACAGTC
ACAACGCTATATCATGGCGGATAAGCAAAAGAACGCGCATCAAGGTGAACCTCAAGATCAGACACAACATCGAGGATGGCAGTGTGCAAC
TCGCGGATCACTACCAACCAACACCCCTATCGGCGACGGCCCTGTGCTGCTGCCAGATAACCACTACCTGAGTACCAATCAGCCCTGA
GTAAAGATCCTAACGAGAAGCGTGATCAGATGGTCTGCTGGAGTTCTGACCAGCGCCGCGCATCACTCTCGGCATGGATGAGCTGTACA
AGGGATCCTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTACGCTTAACTCGAGATAAAAAACAGTTAACTTTTG
ATTTACAGTCAATTTTAAATCTTCCAAACATGATGGTGCAGCAGCTGCATTGTTTGGTCCAATATTACTGCCATTAATTCATTGGGTGA
AATTTATATTGCTGATAGATTGTAACGTTTTCAATAATGGTACAATTATTACAATTGCTGGAACCTGGCATAAGTGGTTACAATGGTGATT
ATCAAGATGCAACAAGTGCCAACTTGAATATCCATCAGGTATTTTATTGCTGCCCCTAATGGAGAAATGATTATTTTCAAGATTACGGAAGACA
CAGTATTCGTAGAGTATTAACCAATGGAACATCACACCACTAGCTGGTGTGGAAGTGATCTTATTGTGCAAGTATTACGGCAACTAAC
ACTCCATTGACAAATCCTAGAGGAACCTTTTTAATTCGAATGGAGATATTTATGTTGCCGAAACACAAGGTAATAGAATTAGTAGAATTT
CAAAGAATGAAAAGCTTTGATTGACTCTATTGCAAGTACAGGAAATATGGTAATAATGGAGGTGGTGGCTGGCAACAGATACTCAATT
AGCATTACCTAATTCGATTGCAACTGATCTCGATGGTCAATTACTTGTGGCAGATTGCTATAACCATGTCATTGCTAAAATTTCTAATAAT
GGAACCATGGTTATCATTGCAGGAAGTGGTGTCTCAGGTTATAATGGAGATGGTATCGATGCAAGAAGTGCAAAATTAACAATCCATTGT
TGTTGATTCTAAAAGTGGTGAGCTCTATATTGCTGATAGTAATAATTATAGGATTAGAAAGATTTTAAACAAATGGAACGATTGTTACAGCT
```

GTAGGAAGTGGTTCTTCAGGTTATAATGGAGATGGCTGCAGCAATAGGACTCTCTAACAAAAAAATTTGCTTTATTTTTGTTGCGTTAATA
AAACCTATCAACTCGTCTTAATTGACAAGTGGTAGGGTTTTTTTTTCGAAAAATTTTCAATCCTGACTTGGATATTTTTTCAAGTTCGGG
AAAGTATTTTCTTTTTTAATATAATAAAACCTATCAACTCGTCTTAATTGACAAGTGGTAGGGTTTTTTTTTCGAAAAATTTTCAATC
CTGACTTGGATATTTTTTCAAGTTCGGGAAAGTATTTTCTTTTTTAAATATAATAAAACCTATCAACGAATTCTCGTCTTAATTGACAAG
TGGTAGGGTTTTTTTTTCGAAAAATTTTCAATCCTGACTTGGATATTTTTTCAAGTTCGGGAAAGTATTTTCTTTTTTAAATATAATAAA
ACCTATCAACTCGTCTTAATTGACAAGTGGTAGGGTTTTTTTTTCGAAAAATTTTCAATCCTGACTTGGATATTTTTTCAAGTTCGGGAA
GTATTTTCTTTTTTAAATATAATAAAACCTATCAACTCGTCTTAATTGACAAGTGGTAGGGTTTTTTTTTCGAAAAATTTTCAATCCTGA
CTTGGATATTTTTTCAATTTGGGGATATTTGGTACCCAGCTTTTGTCCCTTAGTGAGGGTAATTGCGCGCTTGGCGTAATCATGG
TCATAGCTGTTTCTGTGTGAAATGTTATCCGCTCACAATCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCT
AATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGG
CCAACGCGCGGGGAGAGGCGGTTTGGTATTGGGCGCTCTCCGCTTCTCGCTCACTGACTCGCTCGCTCGGTGCTTGGCTGCGGCG
AGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTAGCAAAAGGCCAGCA
AAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGCGGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAGAAAAATCGACGCTCAAG
TCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCC
GCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGGTGAGGT
GTTGCTCCAGCTGGGCTGTGTGCACGAACCCCGCTTCAGCCGACCGCTGCGCTTATCCGGTAACATCTGCTTGAAGTCCAACCCG
GTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCCTGAAG
TGGTGGCCTAACTACGGCTACACTAGAAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCT
CTTGATCCGGCAAAACAAACACCGCTGGTAGCGGTGGTTTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAA
ATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTT
CACCTAGATCCTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATAGAGTAACTTGGTCTGACAGTTACCAATGCTTAATC
AGTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCCCTGACTCCCGCTGCTGTAGATAACTACGATACGGGAGGGCT
TACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCAGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGG
CCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAA
TAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTGGTTGGTATGGCTTCATTACAGTCCGGTCCCAACGA
TCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGACG
TGTTATCACTCATGGTTATGGCAGCACTGCATAATCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAAC
CAAGTCATTCTGAGAATAGTGATGCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGATAAATCCGCGCCACATAGCAGAATTT
AAAAGTGCTCATCTTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCAGTGTGAGATCAGTTCGATGTAACCCACTCGT
GCACCCAACTGATCTTCAGCATCTTTACTTTACCAGCGTTTTCTGGGTGAGCAAAACAGGAAGCAAAATGCCGCAAAAAAGGGAATA
AGGGCGACACGGAATGTTGAATACTTCACTCTTCTTTTTCAATATTGGAAGCAATTTATCAGGGTATTGTCTCATGAGCGGATACA
TATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC

>pNaeNEO plasmid sequence

CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAA
ATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGAACAAGAGTCCACTATTAAAGAACGTGGACTCCA
ACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCACTACGTGAACCATCACCTAATCAAGTTTTTGGGGTCGAGGTGCCGTA
AAGCACTAAATCGGAACCTAAAGGGAGCCCCGATTAGAGCTTGACGGGGAAGCCGGCGAACGTGGCGAGAAAGGAAGGAAGAAA
GCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGAGCGGTACGCTGCGCGTAACCAACACACCCGCGCGCTTAATGCGCCGCTACAG
GGCGGTCCCATTCGCCATTACGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCTCTTCGCTATTACGCCAGCTGGCGAAAGGG
GGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAACGACGCGCCAGTGAGCGCGCGTAATAC
GACTCACTATAGGGCGAATTGGAGCTCCACCGGGTGGCGGCGCTCTAGAATCTGACGAAAAACAATGTGGATACTCAATACTGTTGA
AACCTTTCAAATAGGACTGTGGCAAGATAAATGATCTGGTGGTTCCAAACACTGCTGTGATAGCAGCATTCCTCAATATTTCTATTGTG
CCATCCAAAAGAGATAATTTACTAGTAAATGATTGCATATCCTTTCACTTACGCTCACAACAACCTCTACAGTATCCAAAGGAAGCCTTC
TCGTTTTCTTTCTATTCTTGATATCCACAGTGGCTGTATCCTTGTTCATTTTCGGTTTTCTTCCATGAATTGTTAATTAAGTCCACT
GTTTGATTTTTCTTCCATGTATTGTTAATGAAGTCCACTGTTATTGATTCTTGTGATGGTTATTCTGTTATTATTGTTGATGATGCA
GTTTGTGCAAGAGTCAGAAATTTATGAATCATCAGACTAGGATGATACCTCCTTTCTTTCTTGGCATTTTTTTAAACATTAATAATTA
ACTTTGTACAAAGGCGGGAACAAGTTCATAGTCTTACAAAAAAATGTCTGATCCAATCAGTTTTAGATCTGATCAAGAGACAGTAGGA
GGATCGTTTAGAATGATTGAACAAGATGGATTGCACGCAGGATCTCCTGCCGCTTGGGTAGAGAGACTATTCGGCTATGATTGGGCACAAC
AAACAATCGGCTGTTCTGATGCCGCCGTGTTACAGGCTGTGAGCTCAAGGAAGACCAGTTCTTTTTGTAAAGACCGATCTGTCTGGTGCCT
GAATGAAGTGAAGATGAGGCAGCTCGACTATCGTGGCTGGCCACTACAGGAGTTCTTGTGACGCTGTGCTCGATGTTGTCACTGAAGC
CGGAAGGGATTGGCTGCTATTGGGCGAAGTGCCAGGACAGGATCTCCTGTATCTCACCTTGCTCCTGCCGAGAAAGTATCTATCATGGCT
GATGCAATGAGAAGGCTGCATACTCTTGATCCTGCTACCTGTCCATTGATCACCAAGCAAAACATAGAATCGAGCGAGCAAGAACTAGA
ATGGAAGCCGGTCTTGTGATCAGGATGATCTGGATGAAGAGCATCAAGGTCTCGCTCCAGCCGAAGTTCGCGCAGGCTCAAGGCCCTGA
ACTGTGGCCGCTCGGGTGTGGCAGACAGATATCAAGCAATGCTTTGGCTACCCGCTGATATTGCTGAAGAGCTTGGCGGCAATGGGCTGA
CCGTTTTCTCGTCTTACGGTATCGCGCTCCAGATCTCAACGATACGCTTCTATCGTCTTCTTGACGAGTCTTCTAAGGGCCCTGA
TTTAAATTATTCCAGTAATTTTGGACGATTCTTCTTCTTATTGAAGCAATAAGGTAAAAAAGCACCAACAACATTGGAAGCCACA
AATGGCTGTCTGAACACAAAAAGATGTGTAACAGATGTTCTTTCTAACAGGAATAATTATTGAAGATGAAAACCTACATGATACAAAAT
ACACACTGCACTGGATTCAAATTAAGGTTGACAAGGTGATACAAACCACTTGTGGCCATCCTCGTGGTCTGAAGATTGAATCAACACTTT
TGTCTGCTTTGTGAATGTCGACACTTGTTCAGTTAGTAACATAATTGGTATCATATCTAATACAACAGCATTGGAAGGAAAATGGATTATT
ACCTTACCATATCTCTCATCCAATCGATTGAAGTAATGAGGTTGATGATGTTAAAGTTGATTGGTTGTGTAATGGCAATAGTGTAT

TAATGGATTGAAAGCGAATATGGATGTGGAGTTGAATCTCTTCAATCTAAACGTGCTGAAGCGAGGATGAACCTCACGTTGTAACAGCAAAC
CGCCATTAATTCTATTTTTAACGTCCAATAACCTATTAATACAAGTACCCATCATTGATCGTCACTACAACAAATCAAAAAAATCAAATA
ACTGCTTATTGTATACTAAAGCATATGGTGAGTAAGGGCGAGGAGCTGTTACCGGTGTGGTGCCTATCTGGTCGAGCTGGATGGCGATG
TAAACGGCCACAAGTTCAGTGTGTGGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGTACCACCGGCA
AGCTGCCTGTGCCTTGGCCAAACCTCGTGACCACCTGACCTACGGCGTGCAATGTTTCAGTCGTTACCCCGATCACATGAAGCAACACG
ATTTCTTCAAGTCGGCCATGCCAGAAGGCTACGTCCAAGAGCGTACCATCTTCTTCAAGGATGACGGCAACTACAAGACCAGAGCCGAGG
TGAAGTTCGAGGGCGATACCCTGGTGAACCGTATCGAGCTGAAGGGCATCGACTTCAAGGAGGATGGCAACATCTGGGCCACAAGCTGG
AGTACAACCTACAACAGTCACAACGTCTATATCATGGCCGATAAGCAAAAGAACGGCATCAAGGTGAACCTCAAGATCAGACACAACATCG
AGGATGGCAGTGTGCAACTCGCCGATCACTACCAACAAAACACCCCTATCGGCGACGGCCCTGTGCTGCTGCCAGATAACCACTACCTGA
GTACCCAATCAGCCCTGAGTAAAGATCCTAACGAGAAGCGTGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCCGGCATCACTCTCG
GCATGGATGAGCTGTACAAGGATCCTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTACGCTTAACTCGAGAT
AAAAACAGTTAACTTTTGATTTACAGTCAATTTTAATTTCTTCCAACATGATGGTCGAGCAGCTGCATTGTTGGTCCAATATTTCACTGC
CATTAACTCATTGGGTGAATTTATATTGCTGATAGATTCTGAACGTTTTCAATAATGGTACAATTATTACAATTGCTGGAACCTGGCATAA
GTGGTTACAATGGTGATTATCAAGATGCAACAAGTGCCAACCTGAATATTCCATCAGGTATTTTGATTGCCCTAATGGAGAAATGATTAT
TTCAGATTACAGGAAGACACAGTATTCGTAGAGTATTAACCAATGGAACATCACACCACTAGCTGGTGATGGAAGTGCATCTTATTGTGCA
GGTATTACGGCAACTAACACTCCATTGACAAATCTAGAGGAACTCTTTTTAATTCGAATGGAGATATTTATGTTGCCGAAACACAAGGTA
ATAGAATTAGTAGAATTTCAAAGAATGGAAAAGCTTTGATTGACTCTATTGCAGGTACAGGAAATTATGGTAATAATGGAGGTGGTCGGCT
GGCAACAGATACTCAATTAGCATTACCTAATTCGATTGCAACTGATCTCGATGGTCAATTACTTGTGGCAGATTTCGTATAACCATGTCATT
CGTAAAATTCTTAATAATGGAACCATGGTTATCATTGCAGGAACTGGTGTCTCAGGTTATAATGGAGATGGTATCGATGCAAGAAGTGCAA
AATTAACAATCCATTGTTGTTGATTCTAAAAGTGGTGAGCTCTATATTGCTGATAGTAATAATTATAGGATTAGAAAAGATTTTAAACAAAT
GGAACGATTGTTACAGCTGTAGGAACCTGGTCTTCAGGTTATAATGGAGATGGCTGCAGCAATAGGACTCTCTAACAAAAAAATTTGCTTT
ATTTTTGTTTCGCTTAATAAAAACCTATCAACTCGTCTTAATTGACAAGTGGTAGGGTTTTTTTTTCGAAAATTTTTCAATCCTGACTTGG
TATTTTTTCAAGTTCGGGAAAGTATTTTCTTTTTTTAATATAATAAAACCTATCAACTCGTCTTAATTGACAAGTGGTAGGGTTTTTTT
TTCGAAAATTTTCAATCTGACTTGGATATTTTTTCAAGTTCGGGAAAGTATTTTCTTTTTTTAATATAATAAAACCTATCAACGAATT
C
TCGTCTTAATTGACAAGTGGTAGGGTTTTTTTTTCGAAAATTTTTCAATCCTGACTTGGATATTTTTTCAAGTTCGGGAAAGTATTTTCTT
TTTTTAATATAATAAAACCTATCAACTCGTCTTAATTGACAAGTGGTAGGGTTTTTTTTTCGAAAATTTTTCAATCCTGACTTGGATATT
TTTTCAAGTTCGGGAAAGTATTTTCTTTTTTTAATATAATAAAACCTATCAACTCGTCTTAATTGACAAGTGGTAGGGTTTTTTTTTCGA
AAATTTTTCAATCCTGACTTGGATATTTTTTCATAATTTGGGGGATATTTTGGTACCCAGCTTTTGTCCCTTATGAGGGGTAAATTCGC
GCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTTGTTATCCGCTCACAAATCCACACAACATACGAGCCGGAAGCATAAAGTG
TAAAGCCTGGGGTGCTAATGAGTGAGCTAAGCTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCTGCGCAG
CTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTGCGTATTGGGCGCTCTCCGCTTCTCGCTCACTGACTCGCTGCGCTCG
GTCGTTTCGGCTGCGCGGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATG
TGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAC
AAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCTGGAAGCTCCCTCGTGCCTCT
CCTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATC
TCAGTTCGGTGTAGGTGTTGCTCCAGCTGGGCTGTGTGCACGAACCCCGCTTACGCCCAGCCGCTGCGCTTATCCGGTAACATATC
GTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTG
CTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGG
AAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACACCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAA
AAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACCTACGTTAAGGGATTTTGGTCATGAGA
TTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACA
GTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCCTGACTCCCGCTCGTGTAGATAAC
TACGATACGGGAGGGCTTACCATCTGGCCCCAGTCTGCAATGATACCGCGAGACCCACGCTACCCGGCTCCAGATTTATACGAATAAAA
CCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGT
AAGTAGTTGCGCAGTTAATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACAGCTCGTCTGTTGGTATGGCTTCATTC
AGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCTCCGATCGTTGTCA
GAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTGATGCCATCCGTAAGATGCTTTTCTGT
GACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGATGCGGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAATACCGC
GCCACATAGCAGAACTTTAAAGTGCTCATCTTGGAAAACGTTCTTCGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGT
TCGATGTAACCACTCGTGACCCCACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATG
CCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTATCAGGGTTATTG
TCTCATGAGCGGATACATATTTGAATGTATTGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC

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

- 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; co.

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...

11

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.

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 32°C for 24-48 hours.

15

After maximum of 48 hours add the corresponding selective drug to the transfected cells (3.

16

Incubate at 32°C for 24 hours

17

Carefully remove the supernatant (along with dead cells) and add 2 ml of fresh media with...

18

Incubate at 32°C for 72 hours

19

Transfer the cells in a 25cm² flask and incubate at 32°C and analyse them by fluorescence.

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