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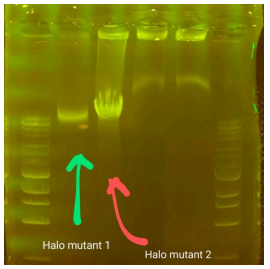
Quick and dirty sequencing microbial genome extraction v1



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External link: https://naturepoker.wordpress.com/2023/02/26/halobacteria-mutant-sequencing-3-years-in/

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Protocol status: Working
The protocol works and is currently being improved. Different microbes will require optimization.

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Protocol Integer ID: 83293

Keywords: dirty microbial genome extraction protocol, sequencing microbial genome extraction v1, microbial genome extraction v1, difficult microbes in the future, lab archaeal evolution study, difficult microbe, deinococcus, rapid sequencing kit, certain microbe, such as deinococcus, ont deinococcus, rad004 rapid sequencing kit, read capable dna, using edwards buffer, microbe, adding initial lysozyme incubation step, sequencing project, initial lysozyme incubation step in the very beginning, binomica lab, edwards buffer, capable dna

Abstract

Quick and dirty microbial genome extraction protocol using Edwards buffer.

I've been using this method for in-lab Archaeal evolution study for the last four years or so and it's been working fantastically well, especially for short read sequencing.

Certain microbes (such as Deinococcus) can be difficult to process properly using just this protocol - standard caveats and fixes tend to improve the output, such as adding initial lysozyme incubation step in the very beginning, sometimes combined with a freeze-thaw cycle for especially difficult samples (I'll update and upload a separate version of this protocol for difficult microbes in the future).

This particular protocol is aimed at getting as much intact, long-read capable DNA out from a microbe as cheaply as possible, as shown in the included gel picture.

Originally written up for my **lab note** at **https://naturepoker.wordpress.com/2023/02/26/halobacteria-mutant-sequencing-3-years-in/**

Ideation and initial testing performed with Sebastian S. Cocioba at Binomica Labs for ONT Deinococcus radiophilus genome sequencing project, using RAD004 rapid sequencing kit.

https://www.ncbi.nlm.nih.gov/assembly/GCF_020889625.1

Image Attribution

Image taken by author - Halobacterium mutant strain genome extraction

1 Spin down 1 ml of sample for 1 minute at max speed and decant

2 Resuspend vigorously with 100ul Edward's buffer

3 Transfer carefully to PCR tube - mixture will be viscous

4 Add 2ul of RNase A and mix vigorously, vortex for 10 seconds

5 Incubate at 37C for 15 minutes

6 Add 2ul of Proteinase K and mix vigorously, vortex for 10 seconds

7 Incubate at 55C for 1 hour, and deactivate via incubation at 95C for 10 minutes

8 Transfer to 1.5ml eppendorf tube

9 Add 10% 3M (pH 5.4) sodium acetate, and 1:1 volume of 100% isopropyl alcohol

10 Invert tube 10 times - precipitates should begin to form

11 Spin down at max speed for 5 minutes

12 Decant the supernatant carefully

13 Add 1ml of 70% EtOH and resuspend the pellet

14 Spin down at max speed for 5 minutes

15 Repeat 70% EtOH resuspension and washing step at least 2 more times

16 Decant completely and dry the pellet for 5 minutes - do not let the pellet overdry

17 Resuspend in storage buffer of choice or dH2O to wanted volume

18 Incubate in a 37C shaker for 1 hour - the extract is likely to be not dissolved fully

19 Incubate in 4C overnight - depending on yield, the extract will be extremely viscous

20 Check extraction quality using both standard gel electrophoresis and UV-Vis method such as Nanodrop.