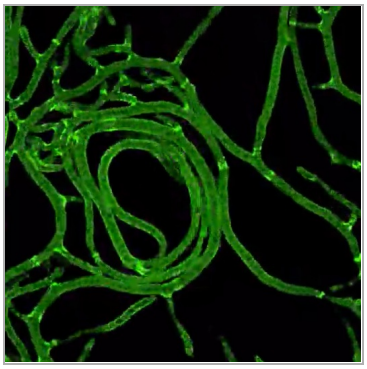

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We use this protocol and it's working

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Transient CRISPR-Cas9 Coupled with Electroporation Protocol

Amy
Gladfelter[1]

[1]Duke University

Amy Gladfelter

ABSTRACT

Transient CRISPR-Cas9 transformation of Cryptococcus neoformans.

ATTACHMENTS

CRISPR Electroporation Protocol Cryptococcus.pdf

GUIDELINES

References

1. Lin, X., et al., Generation of stable mutants and targeted gene deletion strains in Cryptococcus neoformans through electroporation. Med Mycol, 2015. **53**(3): p. 225-234.

2. Fan, Y. and X. Lin, Multiple applications of a transient CRISPR-Cas9 coupled with electroporation (TRACE) system in the Cryptococcus neoformans species complex. Genetics, 2018.

1 PCR amplification of CAS9, sgRNA, your construct.

- CAS9: Use plasmid pXL1-CAS9-HYG as template with primers CAS9-F and CAS9-R. (6985 bp)
- sgRNA: U6P and sgRNA scaffold

1. U6pPromoteris PCR amplified using serotype Dgenomic DNA astemplate with primers U6P-F and GOI-sgRNA-R. ~295 bp
2. SgRNA scaffold is PCR amplified using pYF515 astemplate with primers GOI- sgRNA-F and sgRNA-R. ~108 bp
3. sgRNA construct is PCR amplified using above two PCR product sastemplate with primers U6P-F and sgRNA-R. ~383 bp

2 Mix 2 µg your construct DNA, 100 ng sgRNA, and 170 ng CAS9 in an Eppendorf tube.

3 Vacuum dry the DNA and elute in 5 µL DNAse/RNAse free water.

Note

Notes: use the combination of 2 µg construct DNA, 1 µg CAS9 DNA, and 700 ng sgRNA can increase the transformation efficiency, but low dose is sufficient to obtain transformants.

4 Inoculate recipient strain in 5 mL YPD liquid medium, culture overnight at 30 °C with shaking at 250 rpm.

5 Use the overnight culture to inoculate 100 mL fresh YPD medium at an initial inoculum of OD600=0.2. Grow the cells for additional 04:00:00 to 05:00:00 until the cell density reached OD600 between 0.6-1.0.
From this step on, everything on ice and centrifugation at 4 °C

6 Collect cells by centrifugation at 3200g for 00:05:00 at 4 °C.

7 Wash cells with ice-cold water (EB Buffer instead of water in 2015 paper). (wash 1/2)

8 Wash cells with ice-cold water (EB Buffer instead of water in 2015 paper). (wash 2/2)

9 Suspend cells in 10 mL ice-cold EB buffer (10 mM Tris-HCl, pH 7.5, 1mM $MgCl_2$, 270 mM Sucrose) with 1mM DTT.

10 Incubate the cells on ice for an hour (01:00:00).

Note

(30 to 60 mins in 2015 paper)

11 (Optional) Wash cells with 10 mL ice-cold EB buffer once (2015 paper).

12 Collect the cells by centrifugation and resuspend in 250 µL EB buffer.

13 Mix 45 µL cells with 5 µL DNA mix from step 2 in a pre-cooled 2 mm gap electroporation cuvette.

14 Transform the DNA by electroporation using the BioRad gene pulser with settings of 0.45 kV, 125 µF, 600 Ω. (If using an Eppendorf multiporator, use the bacterial mode with V=2 kV with τ optimized for 5 ms)

15 Suspend electroporated cells in 1 mL of YPD medium and culture at 30 °C for 02:00:00 (01:30:00 in 2015 paper) before plating onto the appropriate selective agar medium.