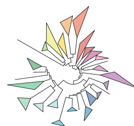 ARCHAEA.bio

Transformation of electrocompetent *S. acidocaldarius*

Corresponding author: Gabriel Tarrason Risa
List of authors: Gabriel Tarrason Risa, Marleen Van Wolferen, Buzz Baum, Sonja Albers
Group leader: Sonja Albers
Institution: University of Freiburg

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Protocol Category: Genetic manipulation
Model organism(s): Sulfolobus acidocaldarius
Tags: Transformation, electroporation

Abstract

Sulfolobus acidocaldarius is one of few genetically tractable archaeal species. This protocol outlines how to transform *S. acidocaldarius* with electroporation.

Materials

Product name	Brand	Manufacturer	Catalogue number	Notes
MW001 *S. acidocaldarius*				Uracil auxotroph
50 mM sucrose				
BROCK I				See BROCK media protocol
BROCK II + III				See BROCK media protocol
$FeCl_3$				
NZ-amine (10%)				
Dextrin (20%)				
Uracil				

Equipment

Equipment name	Brand	Manufacturer	Catalogue number	Notes
DNA (plasmid)				See plasmid protocols

Equipment name	Brand	Manufacturer	Catalogue number	Notes
Ice				
Bunsen burner				
Electroporator				
Gelrite plates				See gelrite plate protocol
Centrifuge				Should be chilled to 4℃
Shaking incubator				Must go to 70℃

Protocol

[Maintain a sterile (using the bunsen burner) lab bench environment throughout]

A. Make electrocompetent MW001 S. acidocaldarius

1. Inoculate MW001S. acidocaldarius from a glycerol stock at -80℃ in Brock medium, adding the dextrin, NZ-amine, and Uracil to achieve a final concentration of 0.2%, 0.1% and 10 µg/mL, respectively.
    - NB: *S. acidocaldarius MW001* has a deletion in pyrEF and can only grow when supplemented with uracil (10 µg/mL).
2. Within 1-2 days OD_{600} should be between 0.3 and 0.7. At that point, transfer an aliquot into fresh medium so that the culture can be harvested the next day at an OD of 0.2-0.3. A doubling time of about 4.5 hours can be assumed.
3. Chill the aliquot on ice (cells should be on ice from this point on), then centrifuge for 10 min at 2500 x g. Wash 2 or 3 times (depending on culture volume) with 30 ml of ice-cold 20 mM sucrose.
4. Resuspend the pellet in 1 ml 20 mM sucrose and transfer the suspension to an 1.5 mL eppendorf tube.
5. Centrifuge for 10 min at 2500 x g at 4°C.
6. Resuspend the pellet to a theoretical OD_{600} of 20 in 20 mM sucrose.
7. Store 50 µL aliquoted cells at –80°C.

B. Transform electrocompetent MW001 *S. acidocaldarius*

1. Thaw the competent MW001 cells on ice
2. Add DNA (plasmids) to the cells
    - About 200ng of DNA needed per 50ul aliquot
    - Plasmid should contain PyrEF, so to confer the ability to make Uracil

2. Incubate 30 min on ice

2. Transfer the contents to a chilled cuvette
3. Electroporate
    - Be fast as the cells do not enjoy being electroporated
4. Add 400ul recovery solution

5. Incubate 30 min at 75°C with shaking (300rpm)
6. Plate 150µL of the transformed cells on Gelrite plates (without Uracil)

Recipes

Electroporation recovery solution

- BROCK I (1:100 of a 100x stock)
- BROCK II + III (1:100 of a 100x stock)
- $FeCl_3$ (1:100 of 2g/L stock)
- NZ-Amine (10% solution) (1:100 of 100g/L stock)

Competing interests

The authors declare that they have no conflict of interest.