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Electroporation - mediated transformation protocol for marine isolated bacteria

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We use this protocol and it's working

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Abstract

Electroporation protocol specialized for transforming marine isolated bacteria strains with plasmid DNA, developed to resolve limitations of current genetic tools for marine application. The steps involve preparing competent cells for a single cycle of electroporation.

Guidelines

Optimization of electroporation

This electroporation protocol may need to be optimized depending on variability of strains.

Table 1 - Suggested control plate scheme

	A	B	C	D	E	F	G
	ID	**Culture type**	**Plate type**	**Control type**	**uL to add**	**ID number**	**Comments**
	NC1	Strain_1 no plasmid	Antibiotic selection	Negative control	200	**N1**	Should have no growth
	NC2	Strain_2 no plasmid	Antibiotic selection	Negative control	200	**N2**	Should have no growth
	NC3	Strain_3 no plasmid	Antibiotic selection	Negative control	200	**N3**	Should have no growth
	SC1	Strain_1 + plasmid	Growth media	Survival control	50	**S1**	Should have growth. Controls for survival of electroporation procedure
	SC2	Strain_2 + plasmid	Growth media	Survival control	50	**S2**	Should have growth. Controls for survival of electroporation procedure
	SC3	Strain_3 + plasmid	Growth media	Survival control	50	**S3**	Should have growth. Controls for survival of electroporation procedure

	A	B	C	D	E	F	G
	T1	Strain_1 + plasmid	Antibiotic selection	Selection control	50	**AS1**	Only transformed cells should grow
	T2	Strain_2 + plasmid	Antibiotic selection	Selection control	50	**AS2**	Only transformed cells should grow
	T3	Strain_3 + plasmid	Antibiotic selection	Selection control	50	**AS3**	Only transformed cells should grow

Materials

MATERIALS and REAGENTS

- BD Difco™ Dehydrated Culture Media: Marine Broth 2216 **Thermo Fisher Scientific**
- Peptone from casein (Tryptone) **Merck MilliporeSigma (Sigma-Aldrich)**
- Agar **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**05040
- Kanamycin sulphate **VWR International (Avantor)**
- Glycerol **Merck MilliporeSigma (Sigma-Aldrich)**

Equipment

Electroporation Cuvettes	NAME
VWR	BRAND
732-1135	SKU
1 mm Gap, 90 µl Gray Sterile	SPECIFICATIONS

Equipment

Heraeus Multifuge 1 S-R	NAME
Sentrifuge	TYPE
Thermo Scientific	BRAND
NA	SKU

Equipment	
ECM® 630 Electroporation System	NAME
Electroporation System	TYPE
BTX Harvard Apparatus	BRAND
NA	SKU

MEADIA

FMAP (1 L)

- 15 g Marine Broth
- 5 g Peptone
- 300 mL Filtered seawater
- 700 mL MilliQ water

FMAP agar

- 1 L FMAP medium
- 15 g Agar

ANTIBIOTIC SELECTION

Recommended stock and working concentrations for antibiotic selection

	Antibiotic	Recommended Stock Concentration	Recommended Working Concentration
	Ampicillin	100 mg/mL	100 µg/mL
	Bleocin	5 mg/mL	5 µg/mL
	Carbenicillin*	100 mg/mL	100 µg/mL
	Chloramphenicol	25 mg/mL(dissolve in EtOH)	25 µg/mL
	Coumermycin	25 mg/mL(dissolve in DMSO)	25 µg/mL
	Gentamycin	10 mg/mL	10 µg/mL

	Antibiotic	Recommended Stock Concentration	Recommended Working Concentration
	Kanamycin	100 mg/mL	100 µg/mL
	Spectinomycin	50 mg/mL	50 µg/mL
	Tetracycline	10 mg/mL	10 µg/mL

Citation

addgene (2016). Pouring LB Agar Plates. https://www.addgene.org/protocols/pouring-lb-agar-plates/.

NA LINK

Safety warnings

Refer to the Safety Data Sheets (SDS) for information on health and environmental hazards. Be sure to follow your institution's Health and Safety (HMS) guidelines, wear appropriate personal protective equipment (PPE), and adhere to all established safety protocols to minimize risks

Before start

Widely available genetic techniques are primarily designed for model organisms, which exhibit inability to transfer genes or low transformation efficiencies in marine bacteria. This protocol aims to improve transformation efficiency and cell viability, overcoming the challenges with conventional methods in marine application (Zahraa Zeaiter Et al., 2018).

For marine bacteria, high salt concentrations in the growth medium can lead to increased conductivity in the electroporation buffer, which may cause electrical arcing and damage cells, thereby reducing transformation efficiency. However, by simply reducing the salt concentration, issues such as osmotic shock can occur, leading to decrease cell viability (Katrina Christi et al., 2024). Consequently, as osmolytes like glycerol are natural counters for osmotic stress in marine bacteria, 10 % glycerol is utilized in this protocol to mitigate this effect.

As many strains and species can have very different growth rates, the protocol differs between fast and slow growers. This definition is vague but the rule of thumb is:

Fast growers reach a stationary-like phase after 12-20 hours in 5 mL of growth medium with shaking at optimal growth temperature (similar to E. coli DH5α e.g.).

Slow growers reach a stationary-like phase after 24-48 hours in growth medium with shaking at optimal growth temperature.

FMAP media was utilized as an all-purpose marine growth media (see Materials for media composition), however it is highly recommended to establish optimal growth media for each bacterial strain. This ensures the highest efficiency in electroporation and recovery of transformants. Additionally, the plasmids utilized in this protocol where based on double kanamycin ([M] 100 µg/ml) selection, however, selecting the appropriate antibiotics corresponding to the antibiotic selection markers on your plasmids is crucial for proper selection (see Materials for concentration recommendations).

It is also advisable before beginning the procedure, to measure the plasmid concentration (ng/µL) and calculate the necessary volume of plasmid DNA (50-100 ng) required to create competent cells.

Note that preparing and creating competent cells is the most time-consuming step in this protocol. As the protocol provides enough competent cells for a single electroporation cycle, scaling up steps 1 through 9 is recommended to generate enough cells for multiple cycles. Competent cells can be stored at -80°C for future use.

Although transformation has been highly successful for the marine isolated strain *Sulfitobacter marinus,* with a range of plasmids 1000-4000 base-pairs in size, the electroporation protocol may need to be optimized depending on variability of other marine strains.

This protocol was developed as part of a master's thesis affiliated with **Microalgae & Microbiomes - The M2 Research Group** at **Norges Fiskerihøgskole**, University of Tromsø, Norway

Preparation of strains and media

1h 5m

1 Inoculate strains in 7 mL of preferred marine growth media in a 15 mL sterile culture tubes and shake Overnight for fast growers or longer for slow growing strains at the strain's optimal growth temperature. 5m

Note

The inoculation time may vary depending on the growth dynamics of your strains, and it is typically considered acceptable for electroporation once the concentration reaches 0.8-1 at OD600.

2 Prepare and transfer 10 % glycerol (sterilized), growth medium, growth media plates, growth medium agar plates with appropriate antibiotics, and 1 mm electroporation cuvettes in fridge Overnight 1h

Day 1 - Reviving culture

3h 5m

3 Using a spectrophotometer, measure the OD600 of the culture. 5m

Note

It is recommended in combination with this step to ensure optimal cell density (0.8 -1 OD600) by measuring OD600.

4 To revive the overnight cultures, ensuring that the cells transition back into the growth phase, add 3 mL growth medium, and return to shaker to let them grow for 2-4 hours 3h

Day 1 - Creating competent cells

58m

5 Prepare an ice tray and cool a large centrifuge to 4 °C. From this point everything should be kept on ice. 5m

6 Centrifuge overnight cultures at 4500 x g, 4°C, 00:10:00 10m

7 Discard supernatants and resuspend pellets in 5 mL fridge-cooled 10 % glycerol 3m

8 Centrifuge cultures for another 4500 x g, 4°C, 00:10:00 and discard supernatants 10m

9 go to step #7 and repeat until a total 3 washes has been carried out with 10 % glycerol. On the final wash, resuspend in 100 µL 10 % glycerol in sterile microcentrifuge tubes. Store at -80 °C, or proceed for electroporation. 30m

Note

Preparing and creating competent cells is the most time-consuming step in this electroporation protocol. Since the protocol provides enough competent cells for a single electroporation cycle, it is recommended to scale up steps 1 through 9 to generate enough cells for multiple cycles. The competent cells can be stored at -80 °C for later use.

Day 1 - Electroporation 2h 40m

10 Transfer 80 µL competent cells (from step 9) to 1 mm electroporation cuvettes. 5m

Note

Remember to include a negative control where plasmid-DNA is not added.
A recommendation for the controls to be included can be found under Guidelines & Warnings.

11 Add 50-100 ng plasmid-DNA to the competent cells. Flick the cuvette 3-4 times to ensure homogeneity, and incubate on ice for 25 minutes. 30m

12 Insert electroporation cuvettes and electroporate with 1.25 kV, 200 ohms and 25 µF 5m

13 Immediately after electroporation add 1 mL cold growth medium, homogenize by pipetting up and down. 2h

For fast growers: Transfer to a microcentrifuge tube and incubate for 01:30:00 at optimal growth temperature with shaking.

For slow growers: Transfer to a 15 mL sterile culture tube, add additional 4 mL of growth media and incubate Overnight at optimal growth temperature with shaking.

Note

It might be difficult to extract all the culture from the cuvette. It is recommended to tilt the cuvette to one side allowing the culture to pool to the side while aspirating with a 100 or 200 uL pipette tip.

Day 1 or 2 - Plating 15m

14 After recovery, plate the following: 15m

Survival control (SC): Plate 50 µL of the transformants on an agar plate with growth medium and no antibiotics. This controls that cells survives the electroporation procedure.

Transformants (T) and negative controls (NC): Plate 100 µL of recovery culture on an agar plate with growth medium and appropriate antibiotics.

[Optional]:

If low transformation efficiency is expected, the rest of the culture should be centrifuged at 4500 x g, 00:05:00 . Decant supernatant so a little medium remains, re-dissolve cell pellet and plate on appropriate agar plates.

Suggested scheme for plating can be found in Table 1 under Guidelines section.

Note

Alternatively, for calculating transformation efficiency:

Dilute the culture 1000-fold for plating.

Transformation efficiency calculation:

Number of transformants per ug=

(Number of transfomants/ ug of DNA) * (Final volume of recovery (ml) / Volume plated (ml))

15 Incubate and culture transformants Overnight (or longer if needed) in your preferred marine growth media supplemented with your choice of antibiotics.

Day 2 or 3 - Verification of transformants

16 Transformants should be verified using a method of choice; for the purpose of this protocol, colony PCR was selected.

Protocol references

Katrina Christi, Jennifer Hudson, Suhelen Egan,
Current approaches to genetic modification of marine bacteria and considerations for improved transformation efficiency,
Microbiological Research,
Volume 284, 2024,

Zahraa Zeaiter, Francesca Mapelli, Elena Crotti, Sara Borin, Methods for the genetic manipulation of marine bacteria, Electronic Journal of Biotechnology, Volume 33, 2018

Piekarski, T., Buchholz, I., Drepper, T. *et al.* Genetic tools for the investigation of *Roseobacter* clade bacteria. *BMC Microbiol* **9**, 265 (2009)

Kyoung-Hee Choi, Ayush Kumar, Herbert P. Schweizer, A 10-min method for preparation of highly electrocompetent Pseudomonas aeruginosa cells: Application for DNA fragment transfer between chromosomes and plasmid transformation, Journal of Microbiological Methods, Volume 64, Issue 3, 2006

Citations

addgene. Pouring LB Agar Plates
NA