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Marine Bacteria Plasmid Conjugation

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ABSTRACT

This protocol can be used to mate broad host range plasmids (including pBTK and other plasmids containing RSF1010 origins of replication) into diverse marine bacteria.

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MATERIALS TEXT

Media

- **Plates to streak out Plasmids contained in E.coli mating strains** (SM10, S-17, MFD): LB plates containing 100 µg/mL of the appropriate antibiotic(s) (plus 0.3mM DAP for MFD cells)
- **Liquid media for incoluations:** LB, Natural Sea Water Tryptone (NSWT) and/or Marine Broth (Difco 2216) media
- **Plates to streak out the marine bacteria:** Natural SWT and/or Marine Agar plates
- **Plates to perform the mating:** Natural SWT and/or Marine Agar plates [containing 0.3mM DAP if the plasmids are in MFD cells]
- **Plates for selection:** Natural SWT and/or Marine Agar plates containing 200 µg/mL Kanamycin
- **Storing stocks:** 50% glycerol
- **Antibiotic Stocks:** 100mg/mL filter sterilized antibiotic stocks for the appropriate selectable markers
- **Media Supplements:** 2,6 Diaminopimelic Acid (DAP) filter sterilized at 30mM working stock

Dry materials:

- Sterile sticks
- Beads
- 1.5 mL microcentrifuge tubes
- Petri dishes

Confirmation materials

- GoTaq for colony PCR
- Primers to confirm plasmid in colony PCR
- For Fluorescent plasmids: Microscope with Fluorescence filters for visual confirmation of colonies

Day 1

1 Streak out the marine bacteria and *E.coli* strains containing the plasmids to be mated using the streak plate method.

Be sure to check the library to determine the location of the strains and which media, antibiotics, and/or media supplements (i.e. Diaminopimelic Acid [DAP]) should be used for each strain/plasmid.

2 Incubate the plates overnight in a plastic bag in the incubator.
E.coli strains are incubated at **37 °C**

Marine bacteria strains are incubated at **25 °C**

Day 2 2m

3 [Morning] Inoculate 3 colonies of the marine strains into 5mL NSWT or Marine Broth. Incubate at **25 °C** shaking at **200 rpm** .

Some marine bacteria may take longer to grow than others, which could slightly shift the timeline of this protocol. If the marine bacteria is a slower grower, you can opt to inoculate the marine bacteria in the morning of day two and the plasmids at night for day two, and spot mate on the morning of Day 3. This will increase the protocol to 7 days total.

4

[Morning] Inoculate a single colony of plasmid into 5mL LB broth + 100 µg/mL of appropriate antibiotic(s) (i.e. 5µL of 100mg/mL stock) and/or media supplements (i.e. 0.3mM DAP = 50µL of 30mM stock). Incubate at **37 °C** shaking at **200 rpm** .

5 [Evening] **Marine bacteria:** Remove 1mL of culture per each plasmid being mated and put into a 1.5mL microcentrifuge tube. Include an additional 1mL of culture as a negative control (i.e. If you are mating 1 marine bacterium with 3 different plasmids [GFP/mRuby/Nanoluc] you will need (4) 1mL aliquots of culture).

6 [Evening] **Plasmid mating strains:** Remove 1mL of culture for each marine bacterium being mated and put into a 1.5 mL microcentrifugre tube. Include an additional 1mL of culture as a negative control (i.e. If you are mating 1 marine bacteria with 3 different plasmids [GFP/mRuby/Nanoluc], you will need (2) 1mL aliquots of **each plasmid**).

7 Centrifuge all culture aliquots **4000 x g** for **00:02:00** 2m

8 Remove all of the supernatant of the plasmid aliquots.
Remove all but 100µL of the supernatant for the marine bacteria aliquots.

9 Resuspend the marine bacteria in the remaining 100µL of supernatant. Put the marine bacteria negative control to the side for now.

10 Pipet up the 100µL of resuspended cells and transfer it to a tube containing the plasmid cell pellet. Pipet mix to homogenize the plasmid cells and marine bacteria cells together. Repeat process for all different plasmids + marine bacteria being mated.

11 For the Plasmid negative controls, add 100µL of marine bacteria media to the plasmid cell pellet and resuspend.

12 After all strains are resuspended and/or mixed, plate (2) 50µL spots onto mating plates.
- Marine bacteria negative control plate.
- Plasmid negative control plate(s).
- 1 experimental plate for each marine bacteria + plasmid combo.

The media plates used for mating should be determined by the type of E.coli cells used for mating.
SM10 and S17 cells can be mated on regular NSWT/Marine Broth
MFD cells should be mated on NSWT/Marine Broth containing 0.3mM DAP.

13 Incubate all plates at **25 °C** overnight with their lids facing up.

Day 3

14 Aliquot 500µL of NSWT or Marine Broth into a 1.5 mL microcentrifuge tube for each spot.
One spot can be plated for each negative control.
Both spots should be mated for each experimental mating plate.

15 Pick up the spot with a pipette tip and place into the microcentrifuge tube containing the media. Resuspend the bacteria in the media. Shake the pipette tip vigorously then remove the tip and pipette mix or vortex to homogenize the bacteria in the media.

16 Plate 100µL of the cells onto selection plates [NSWT or Marine agar plates containing antibiotics at the appropriate concentration determined by the marine bacteria's MIC--**insert MIC protocol here]**. Spread with beads. Incubate overnight at **25 °C**

Day 4

17 Select 3-6 colonies per spot to patch plate and perform a Colony PCR [**insert colony PCR Protocol**]. Patch onto a new selection plate.

From here forward, the strains must be grown on media containing the appropriate concentration of antibiotics to retain the plasmid.

18 Run a gel to confirm colony PCR

19 Streak out positive clones onto NSWT or Marine Agar selection plates and incubate at **25 °C** overnight. Be sure to include at least one clone from each spot mating to create 2 copies for storage.

Day 5

20 Inoculate a single colony of each strain into 5mL NSWT or Marine Broth media containing antibiotics. Incubate overnight at **25 °C** shaking at **200 rpm** .

Day 6

21 Make a glycerol stock **[insert glycerol stock protocol]** of the new strains (2 copies/strain, 2 strains/mating) and store them in the the strain library.