Version 2

Oct 04, 2022

Transfer of plasmid DNA to Rhodobacter sphaeroides via Conjugal mating V.2

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dx.doi.org/10.17504/protocols.io.ewov1oydolr2/v2

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ABSTRACT

Rhodobacter is not capable of being transformed with pure, double-stranded DNA containing sites for endogenous restriction enzymes. The most efficient way of transfer of plasmid DNA to expression hosts is conjugation where single stranded DNA is transferred bypassing the hosts restriction system. The common *E. coli* strains used in cloning do not contains components necessary for the mobilization and transfer of plasmids via conjugal mating. In order to enable transfer of the expression vector to Rhodobacter hosts, an *E coli* host that is capable of conjugal mating (For e.g. S17-10 must be utilized).

DOI

dx.doi.org/10.17504/protocols.io.ewov1oydolr2/v2

PROTOCOL CITATION

Jaya K Yakha, laible, Deborah K Hanson, Rosemarie Wilton 2022. Transfer of plasmid DNA to Rhodobacter sphaeroides via Conjugal mating. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1oydolr2/v2
Version created by Jaya K Yakha

CREATED

Oct 04, 2022

LAST MODIFIED

Oct 04, 2022

PROTOCOL INTEGER ID

70831

MATERIALS TEXT

- 15 ml sterile tubes
- Flask
- 1 ml microcentrifuge tubes
- Sterile glass beads
- Competent cells (*E.coli* S17-1 cells)
- Ice
- Shaker incubator at 37^0C
- Incubator at 37^0C
- Media plate with appropriate Antibiotic selection
- 2xTY plates
- ^{G}YCC plates
- MR26 plates
- Centrifuge
- Filter paper
- Sterile tooth pick
- Sterile forceps

1 Two days prior to the conjugation, inoculate 25 ml of ^{G}YCC with desired host strain and grow at **33 °C** with shaking at 125 rpm. If culture becomes too turbid prior to the conjugation, sub culturing may be necessary.

2 The night before the conjugation, inoculate the donor strain of S17-1 (pXYZ) in **3 mL** of growth medium containing antibiotic specific to the plasmid vector. We use the S17-1 strain of *E coli* because it has the genes necessary for the mobilization and transfer of plasmids.

3 The morning of the conjugation, pre warm 2xTY plates in **37 °C** incubator (typically use 1.5'' small round petri dishes). Sub-culture the overnight S17-1 (pXYZ) culture in the fresh medium without drug; Let it grow to log phase (approx. 2 hours). Dilute cells 1:50 into fresh medium (take 40 ul of cell and in 2ml of 2xTY medium). After subculture has reached the log phase, conjugation procedure is ready to begin.

4 In a sterile microfuge tube mix 1ml of the recipient host strain with 35 ul of donor S17-1(pXYZ) culture and spin for about **00:00:30** at max speed. Decant the supernatant. 30s

5 In a sterile hood, place a 13mm nitrocellulose filter on the surface of the pre warmed 2XTY 3h

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plate by using sterile tweezers. Resuspend the cells in the remaining medium using a filtered pipette tip and spot the mixture on top of the filter paper. Incubate at **37 °C** for at least **03:00:00** (Note time). While these are incubating it is good idea to pre warm the agar plates to be used later.

6 At the end of the incubation period, take the plate back to the sterile hood and remove filter from the plate piercing with a sterile tooth pick and transfer into the falcon 2059 (15 ml) tube that contains **1 mL** of MR26 medium. Vortex to dislodge all the cells from the filter paper.

7 Transfer the resuspended cell mixture into microcentrifuge tube.

8 Dilute the resuspended cell mixtre to 1:50 (2ul of cell and 98 ul of medium) to the fresh MR26 medium and plate **50 µL** of ^{G}YCC agar plate containing medium with antibiotic selection at the desired concentration.

9 Incubate the plate(s) in a **33 °C** incubator. After about 3 days colonies should be observable.

10 Check colonies under microscope to ensure no contamination.

Media required and components

11 **^{G}YCC**

A	B
Ingredients	**g/L**
Yeast extract	
Casamino acids	6 g
Concentrated base (see below for preparation)	5 ml

Adjust PH to 7.1 with NaOH Autoclave 30 mins in liquid cycle

2xTY

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A	B
Ingredients	**g/L**
Tryptone	16 g
Yeast extract	10 g
NaCl	5 g

Fill to 1L with ddH_2O Autoclave 30 mins in liquid cycle

MR26

(A) Potassium phosphate buffer, 1M $_pH$ = 6.8 adjusted with KOH or H_3PO_4

K_2HPO_4	115g/L
KH_2PO_4	44.9g/L

(B) Ammonium succinate, 1M P^H= 6.8

Dissolve 118 g of succinic acid in 500 ml H_2O. Adjust the P^H to 6.8 with ammonium hydroxide (It will take quite a bit) and add H_2O.

(C) Concentrated base: Add the following in order to ~500 ml H_2O, then when everything is dissolved, fill with H_2O to 1L. P^H = 6.8 adjusted with NH_4OH.

EDTA-Na_2 (dihydrate)	11.16 g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.0093 g
$FeSO_4.7H_2O$	0.099 g
“Metals 44”	50 ml
$MgSO_4$	14.5 g
$CaCl_2$	2.5 g

Use 20 ml of **A, B** and **C** per L of MR26 medium.

*Vitamin stock solution is usually made up separately.

Vitamin solution for 50 ml

*nicotinic acid	0.075 g
Nicotinamide	0.075 g
*Thiamine0.	150 g
Biotin	0.003 g
Filter sterilize	

Metals 44 – per L of stock solution

$FeSO_4.7H_2O$	5 g
Na_2-EDTA (Dihydrate)	6.5 g
$ZnSO_4$	10.9 g

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$MnCl2.4H_2O$	1.3 g
$CuSO_4.5H_2O$	0.392 g
$CoCl_2.6H_2O$	0.2 g
H_3BO_3	0.114 g

Autoclave 30 min in liquid cycle
Add vitamins with antibiotic when needed

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