



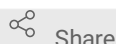
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Transfer of plasmid DNA to *Rhodobacter sphaeroides* via Conjugal mating V.2

Jaya K Yakha¹, laible¹, Deborah K Hanson¹, Rosemarie Wilton¹¹Argonne National Laboratory

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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 contain 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).

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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 37⁰C
- Incubator at 37⁰C
- Media plate with appropriate Antibiotic selection
- 2xTY plates
- GYCC plates
- MR26 plates
- Centrifuge
- Filter paper
- Sterile tooth pick
- Sterile forceps

- 1 Two days prior to the conjugation, inoculate 25 ml of GYCC 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)^{30s} culture and spin for about 00:00:30 at max speed. Decant the supernatant.
- 5 In a sterile hood, place a 13mm nitrocellulose filter on the surface of the pre warmed 2XTY^{3h}

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 mixture to 1:50 (2ul of cell and 98 ul of medium) to the fresh MR26 medium and plate **50 µL** of ^GYCC 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 ^GYCC

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

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

Fill to 1L with ddH₂O Autoclave 30 mins in liquid cycle

MR26

(A) Potassium phosphate buffer, 1M pH = 6.8 adjusted with KOH or H₃PO₄

K₂HPO₄ 115g/L

KH₂PO₄ 44.9g/L

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

Dissolve 118 g of succinic acid in 500 ml H₂O. Adjust the P^H to 6.8 with ammonium hydroxide (It will take quite a bit) and add H₂O.

(C) Concentrated base: Add the following in order to ~500 ml H₂O, then when everything is dissolved, fill with H₂O to 1L. P^H = 6.8 adjusted with NH₄OH.

EDTA-Na₂ (dihydrate) 11.16 g

(NH₄)₆Mo₇O₂₄·4H₂O 0.0093 g

FeSO₄·7H₂O 0.099 g

"Metals 44" 50 ml

MgSO₄ 14.5 g

CaCl₂ 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

FeSO₄·7H₂O 5 g

Na₂-EDTA (Dihydrate) 6.5 g

ZnSO₄ 10.9 g

MnCl ₂ ·4H ₂ O	1.3 g
CuSO ₄ ·5H ₂ O	0.392 g
CoCl ₂ ·6H ₂ O	0.2 g
H ₃ BO ₃	0.114 g

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