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# Bi-parental conjugation protocol for non-model bacteria using E.coli WM3064 DAP auxotroph donor

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**Protocol status:** Working
**We have used this protocol successfully with Arctic marine diatom-associated bacterial recipients.**

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## Abstract

This is a protocol for using bi-parental conjugation to transfer a plasmid into non-model recipient bacteria from a DAP auxotroph donor strain.

## Guidelines

1. **Recipients : donor ratio**. For fast-growing commercial strains, the recipient : donor ratio can be 1 : 1, but non-model/slow growing strains will be rapidly out-competed by the donor. In such cases, a larger recipient : donor ratio will give significantly better results. 10 : 1 or even 100 : 1 are some variations of mixtures worth trying.
2. **Conjugation time**. This may need to be optimized based on the recipient strain used. Some may require 24+ hours of incubation. Tweak the mixture ratio and conjugation time such that the donor strain does not out-compete the recipient cells entirely. The incubation temperature can also be regulated to control this.

## Materials

1. **Donor strain** carrying the desired plasmid to be transferred. Your plasmid must contain an oriT sequence in order to be transferred. The donor strain (if using a different one than WM3064) must carry rp4-based transfer machinery and must be 2,6-Diaminopimelic acid **Thermo Scientific** auxotrophic.
2. **Recipient strain**
3. **Growth media** for individual strains. Donor growth media must contain **0.3mM DAP**.
4. **Non-selective plates** containing **DAP** (0.3 mM) for conjugation. These plates should be made from media that **both strains** can grow on, and contain **no antibiotic**.
5. **Selective plates** containing the same antibiotic as the marker on the plasmid you want to transfer, but **no DAP**. To plate dilutions, you need 3-4 plates per conjugation mixture.
6. 1X PBS (Phosphate-buffered saline )
7. 2,6-Diaminopimelic acid **Thermo Scientific**
8. Pipettes, microcentrifuge tubes, and incubator that supports growth of both donor and recipients.
9. Antibiotics for plasmid selection

| | A | B |
|---|---|---|
| | Antibiotic | Working Concentration |
| | Ampicillin | 100 µg/ml |
| | Carbenicillin | 100 µg/ml |
| | Chloramphenicol | 33 µg/ml |
| | Kanamycin | 50 µg/ml |
| | Streptomycin | 25 µg/ml |
| | Tetracycline | 15 µg/ml |

## Protocol materials

1X PBS (Phosphate-buffered saline )

2,6-Diaminopimelic acid **Thermo Scientific**

## Before start

1. Verify that the donor strain is carrying the desired plasmid to be transferred
2. Your plasmid **MUST CONTAIN an oriT sequence** in order to be transferred.
3. The donor strain (if using a different one than WM3064) must carry rp4-based transfer machinery and must be 2,6-Diaminopimelic acid **Thermo Scientific** auxotrophic in order to be eliminated during selection.
4. Ensure that appropriate growth medium is used to grow individual strains and that the donor strain growth medium contains [M] 0.3 millimolar (mM) 2,6-Diaminopimelic acid **Thermo Scientific** .
5. Non-selective plates must contain DAP. These plates should be made from media that **both** strains can grow on, and contain no antibiotic. You can set up ~4 conjugation mixtures per plate.
6. Selective plates must contain NO DAP and the appropriate antibiotic depending on the selection marker used in the transferred payload. You may also include additional counter selectable markers to minimize E. coli background.

This protocol was developed as part of a master's thesis affiliated with the Microalgae & Microbiomes Research Group at NFH, UiT, Norway.
M2RG Website : https://uit.no/research/micro

## Overnight culture preparation

1 Inoculate a loopful of donor E.coli WM3064 and recipient strains in respective culture tubes containing 5 mL of growth medium. Ensure that the donor strain is grown in presence of [M] 0.3 millimolar (mM) 2,6-Diaminopimelic acid **Thermo Scientific** and appropriate antibiotic according to the selection marker present on the carried plasmid.

2 Incubate the cells Overnight or until they have grown sufficiently. The recipient cells may take longer to grow.

## Conjugation mixture preparation

5m

3 Transfer 1 mL of donor and recipient overnight cultures to microcentrifuge tubes.

4 Gently spin down the cells at 3000 rpm, 00:05:00 and wash with 500 µL 1X PBS (Phosphate-buffered saline ) . 5m

5 Repeat go to step #4 .

6 Resuspend the cells in 500 µL 1X PBS (Phosphate-buffered saline ) .

Note

Washing removes residual antibiotic from the donor cell culture. Recipient cells can also be scraped off of plates and washed in PBS.

7 Prepare 100 µL conjugation mixture by combining the desired volume ratio of donor and recipient cells in a microcentrifuge tube. Mix by pipetting twice.

Note

For fast-growing commercial strains, the recipient : donor ratio can be 1 : 1, but non-model/slow growing strains will be rapidly out-competed by the donor. In such cases, a larger recipient : donor ratio will give significantly better results. 10 : 1 or even 100 : 1 are some variations of mixtures worth trying.

## Non-selective plating

8 Spot-plate 100 µL of the mixture onto a **non-selective plate containing DAP and no antibiotic**. DO NOT SPREAD.

Note

Up to 4-5 conjugation mixtures can be set up on the same plate by carefully plating into quadrants on the plate.

9 Let the spots dry and incubate the plates Overnight .

Note

This step may need to be optimized based on the recipient strain used. Some may require 24+ hours of incubation. Tweak the mixture ratio and conjugation time such that the donor strain does not out-compete the recipient cells entirely. The incubation temperature can also be regulated to control this.

## Washing 5m

10 Scrape each conjugation mixture from the non-selective plates and resuspend in 1 mL 1X PBS (Phosphate-buffered saline )

11 Vortex and gently spin down 3000 rpm, 00:05:00 . 5m

12 Resuspend in 1 mL 1X PBS (Phosphate-buffered saline )

13 Repeat go to step #11

14 Resuspend the mixture in 100 µL 1X PBS (Phosphate-buffered saline )

## Selective plating

15 Spread-plate the entire 100 µL or desired dilutions of washed mixtures on **selective plates containing antibiotic and no DAP.**

16 Incubate the plates according to the recipient strain requirements and pick single colonies to restreak or grow in selective media.

17 Confirm the transfer of the payload via PCR.

## Protocol references

https://barricklab.org/twiki/bin/view/Lab/ProtocolsConjugation

## Acknowledgements

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