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Version 1

## Triparental mating with pSEVA protocol V.1

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**Protocol status:** Working

We use this protocol and it's working

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## Abstract

This process involves bacterial conjugation, where a conjugative plasmid found in one bacterial strain facilitates the transfer of a mobilizable plasmid from a second bacterial strain to a third bacterial strain.

In the method from our lab, CC118 $\lambda$ pir containing a pSEVA plasmid works as the donor, the *E. coli* 1047 pRK2013 strand is the helper, and the receiver is a *C. rodentium* strand.



## Insertion of the suicide plasmid by three partner conjugation

3d 2h 55m

- 1 Inoculate Overnight cultures of strains:

1d

**DONOR** CC118λpir pSEVA Gm (10 µg/ml)

**HELPER** *E. coli* 1047 pRK2013 Km (50 µg/ml)

**RECEIVER** *C. rodentium* pACBSR Sm (50 µg/ml)

### Note

We have a pACBSR plasmid encoding Cm resistance.

- 2 Place 20 µl spots of the helper and donor strains onto an LB plate (no antibiotics) and an additional spot of **20 µl of the helper on top of 20 µl of the donor (D+H)**.

10m

- 3 Leave the plate open at the flame until the spots get dry. Incubate at 37 °C

2h

02:00:00 , facing up.

- 4 Add **40 µl of the receiver strain on top of the D+H spot (D+H+R)** and an additional 20 µl spot of this strain alone. Wait for the spot to get dry and incubate for Overnight

1d 0h 20m

37 °C facing up.

- 5 Collect the 4 patches using a sterile loop and resuspend each of them in 1 ml of LB in an eppendorf.

10m

- 6 Plate 100 µl of each tube in LB plates supplemented with **Gm + Sm**.

10m

- 7 Centrifuge the rest of the D+H+R tube at 2000 x g, 00:02:00 to pellet the cells, resuspend in 100 µl and plate as well.

5m

- 8 Incubate the plates Overnight at 37°C.

1d



## Second recombination for a scarless genomic modification

2d 10h 10m

- 9 Pick two colonies of the D+H+R plate and grow them in **LB+Sm + L-arabinose broth at 0.4%** for a minimum of  06:00:00 for the induction of the I-SceI endonuclease of the pACBSR plasmid.

6h

### Note

In the  Overnight plates with only donor, helper or receiver no colonies should grow.

- 10 Insert the inoculation loop in the culture and streak on LB+Sm plates to obtain individual colonies. Incubate the plates  Overnight at 37°C.

1d 0h 10m

- 11 The next day, pick some colonies and patch them on a **LB+Sm** plate and on a **LB+Sm+Gm** plate. Incubate the plates  Overnight at 37°C.

1d 0h 30m

### Note

Colonies that do not grow on the plate with Gm are those that have recombined after treatment with the endonuclease I-SceI.

- 12 Analyze by PCR and gel electrophoresis those colonies which have grown on LB+Sm but not on LB+Sm+Gm to differentiate the modified colonies from the ones which have reverted to the Wild-Type genotype.

3h 30m

**NOTE:** The primers should hybridise outside of the homology regions selected. Upon analysis of 10 colonies you should get a about 50% of modified colonies.

- 13 **NOTE:** To remove the pACBSR plasmid, make 8-9 passes of the strain without Sm in liquid LB. Plate the last culture in LB plates and patch individual colonies the following day on LB and LB+Sm plates to select those sensitive to the antibiotic.