Triparental mating (conjugation)

DAY 1

1. Start ON of several strains
    - pRW50 donor (DH5α pRW50 *oriT*)
    - *tra* donor (DH5α pRK2013)
    - Recipient strain (desired *Vibrio cholerae* strain)
    - Optional→ DH5 α wild type (to use as a negative control)

DAY 2

1. Take 1 mL of each ON culture and spin it down for 1 min (13300 rpm)
2. 2-3 washes with 1/0.5mL 0.9% NaCl (to wash the antibiotics)
3. Resuspend in 1mL of LB and mix in a 1:1:1 ratio in a 15mL Falcon tube
4. Filter the 3mL mixture in a sterile conjugation filter (from the stores)
5. Incubate the filter on LB agar plate for 4 hours at 37ºC
6. Take the filter out, put it in an universal with 2mL of 0.9% NaCl and vortex it properly
7. Prepare serial dilutions of the mixture and plate in Minimal Media plates with the appropriate antibiotic
    - For pRW50 and *Vibrio cholerae*, 5 μg/mL tetracycline will be enough
    - Minimal media will avoid DH5 α growth as it lacks thiamine, and DH5 α are *thi-*

Minimal media plates

For 100mL

M9 components

- 5x M9 → 20mL
- 20% furctose → 1.5mL
- 20% casaminoacids→ 0.5 mL
- 1M MgSO2 → 0.2mL
- 0.1M CaCl2 → 0.1mL

Agar technical (1.2% final concentration)

- 77.7 mL dH20
- 1.2 g agar technical

→ Autoclave it, cool it and mix it with the rest of the components + desired antibiotic