# 1) KT Electroporation H2O

Day 1:

* inoculate 20 ml LB with KT Growth overnight
* H2O sterile and cold (0-4ºC)
* put the electroporation cuvettes at -20ºC

Day 2: ()

* put the electroporation cuvettes in ice
* Cold down centrifuge to 4ºC
* Centrifuge 2 min maximum speed 1 ml of overnight P. putida culture in 2 ml tubes. (Several tubes per electroporation just in case).
* Remove the supernatant
* Re-suspend the pellets in 1 ml H2O (sterile)
* Centrifuge 2 min maximum speed the cells
* Remove the supernatant
* Re-suspend and repeat wash x4
* Re-suspend the cell pellets in 50 μl H2O and maintain them on ice.
* Add 1-2 μl of plasmid into the cells (50-200 ng). Mix, gentle
* Put the cells with the plasmid inside the electroporation cuvettes
* Perform the electroporation.

Conditions for Ependorf electroporator. 0.2 cm cuvettes.

P2 program (2.5 Kv), pulse start. Check time constant between 5-6 mS

Conditions for Victor`s electroporator (BioRad) for 0.2 cm cuvettes: program Ec2

Pulse until sound. Check for voltage 2.5 Kv and time constant close to 5 mS

* Add immediately 950 μl of LB or SOC, 1 hour or 45 min incubation at 30ºC
* Plate: 10, 100 μl and rest in LB + Antibiotic

# 2) KT Electroporation Sucrose

Day 1:

* inoculate 20 ml LB with KT Growth overnight
* sucrose 300mM (10%)
* put the electroporation cuvettes at -20ºC

Day 2: ()

* put the electroporation cuvettes in ice
* Centrifuge 2 min maximum speed 1 ml of overnight P. putida culture per electroporation in independent 2 ml tubes
* Remove the supernatant
* Re-suspend the pellets in 1 ml sucrose solution (sterile)
* Centrifuge 2 min maximum speed the cells
* Remove the supernatant
* Re-suspend the pellets in 500 μl sucrose solution (sterile)
* Centrifuge 4 min 9000*g* the cells
* Remove the supernatant
* Re-suspend the cell pellets in 50 μl sucrose solution and maintain on ice.
* Add X μl of plasmid into the cells (200 ng). Mix, gentle
* Keep in ice 30 seconds
* Put the cells with the plasmid inside the electroporation cuvettes
* Perform the electroporation. Conditions for Victor`s electroporator (BioRad) and our cuvettes: program Ec2 (0.2 cm cuvette)
* Pulse and Check for voltage 2.5 Kv and time constant close to 5 mS
* Add immediately 1 ml of LB, 1-2 hours incubation at 30ºC
* Plate: 25, 50 μl in LB + Antibiotic