**Protocol for expression**

*Stock solutions:*

***Antibiotics****: -* ***Ampicilin (Amp)****: 100 mg/mL - 1.5 g in 15 mL milliQ*

***- Chloramphenicol (Chl)****: 34 mg/mL in ethanol - 340 mg in 10 mL ethanol*

***Inducers: - IPTG*** *1 M 2.38 g in 10 mL milliQ*

***- Ara 20%****: 2g in 10 mL milliQ*

* *Weight the compound and put in a greiner tube*
* *Dissolve completely*

*Work sterile:*

* *Take close greiner tube to open next to the flame*
* *Filter the solution through the 0.2 µm filter to sterilize*
* *Close greiner tube*
* *Aliquot solution in 1.5 mL sterile eppendorfs*
* *Freeze and keep frozen until use (re-freeze after use)*

**Day 1** needed - sterile medium

- sterile tubes

- antibiotic stock solution (Ampicillin, Chloramphenicol)

- sterile tips

* Prepare o/n culture
* 5-10 mL media
* Add antibiotic - dilute X1000 (5-10 µL antibiotic for 5-10 mL media)
* Grow overnight cultures at 37°C
* Prepare Large scale media for next day:

Always grow in bigger Erlenmeyer than needed volume of media

1. L Erlenmeyer for 500-750-(max 1000) mL media

1L Erlenmeyer for 200-250 mL media

Always close Erlenmeyer with cotton and alluminium foil - autoclave tape on top

**Day 2**  needed - Sterile erlenmeyer with medium

- antibiotic stock solution

- inducer stock solution (1M IPTG, 20% arabinose)

- non-canonical amino acid (pAzF\*HCl)

- sterile tips

* Add antibiotic to large scale media diluting X 1000.

*For example: 100 µL of antibiotic in 100 mL media*

* Inoculate overnight cultures in large scale medium diluting 100 or 200 times at least

*For example: 1 mL of o/n culture in 100 mL LB/antibiotic medium*

* Grow cells until OD 0.5-0.9 (check OD with 1 mL of culture) (note: Reuben used OD=0.8-0.9)
* Induce - dilute inducer X 1000

*For example: 100 µL of inducer in 100 mL media*

* Express protein - usually at 30°C

**Day 2/3**

* Centrifuge bacteria from culture (6000 rpm, 25 minutes, rotor JLA 9.1 in big centrifuge in building 16 or JA10.5 in centrifuge in building 14.)
* Freeze cell pellet (transfer into a greiner tube) or proceed to purification