RNA data generation workflow

M9 cyrostock preparation

LB glycerolstock → Streak on LB + cmp plate → Pick single colony → Inoculate in M9 + cmp over night → Inoculate to **OD = 0.1** → Growth until **OD = 1** → Make M9 glycerolstock

Pre and main culture

M9 glycerol stock @ OD = 1 → Thraw M9 glycerolstock → Inoculate **25 ml** M9 ONC with **200 µl** M9 glycerolstock (preculture) → Growth for **16 h** → Inoculate **40 ml** M9 to **OD = 0.1** (main culture)

OD = 0.4 → Induction, t=0 hr

T = 3 hr

- Sample #1 RNA → **3 ml** culture + 2X ml RNA protect buffer (**6 ml**) → Centrifuge, 8000xg, 10 mins, 4°C → Decant, incubate upside down for 5 mins in cold room → Resuspend in a**ctual resuspension volume** (RNA protect) → Aliquot in **2x 200 µl** → 5000xg, 3 mins, 4°C → Decant + flash freeze in dry ice → QIAcube for RNA extraction
- Sample #2 OD → 100 µl culture + 900 µl medium in cuvette → Measure OD at sampling time **Note for plotting** → =Volume*(OD*cells_per_OD)/target_cells_per_OD
- Sample #3 WB → **2x 1 ml** culture → Centrifuge, 8000xg, 10 mins, 4°C → Supernatant → Take **75 µl** → Western Blotting workflow
  - Lysis → Take **75 µl** → Western Blotting workflow