ES Cell Fixation for Split Barcoding RNA-seq

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Overview

- Cell fixation for split-seq
- Need ~500k cells for each sample
  - Ex. one well from a 6 well plate
- Freeze down a few extra samples in order to test PCR cycles for split-seq

Solutions

PBS + RI

- Make up fresh and store at 4C

Component	Volume/sample	Volume/20 samples
PBS (TC Grade)	2 mL	42 mL
RNase OUT	40 U/μL	840 U/μL
SUPERase Inhibitor	20 U/μL	420 U/μL

PBS + Formaldehyde

- Make up fresh on the day and chill

Component	Volume/sample	Volume/20 samples
PBS (TC Grade)	2.75 mL	57.75 mL
16% Formaldehyde (Pierce cat.28906)	0.25 mL	5.25 mL

Protocol

1. Collect ~500k cells from cell suspension
2. Spin down at 180 x g (RCF)
3. Resuspend pellet in 1mL of PBS + RI
4. Add 3mL of ice cold PBS + 1.33% formaldehyde and mix gently by pipetting up and down 2-3 times
5. Incubate on ice for 10min
6. Spin 300g for 3min at RT
7. Wash pellet with 1mL PBS + RI and respin 300g for 3min at RT
8. Remove supernatant and snap freeze pellet on dry ice
9. Store pellet at -80C