

ES Cell Fixation for Split Barcoding RNA-seq

Callum Malcolm

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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/ μ L | 840 U/ μ L |
| SUPERase Inhibitor | 20 U/ μ L | 420 U/ μ L |

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