

Freeze cells

Via the Zhang Lab

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Freezing Media: 50% FBS, 10% DMSO and 40% DMEM (no PS or puromycin!)

FRZ 3 mL: 1.5 mL FBS, 300 μ L DMSO, 1.2 mL DMEM

Cell growth media: DMEM with 10% FBS and 1% PS. Huaiying also recommends 1 μ g/mL puromycin to make sure cells don't lose the GFP over time.

1. Grow cell in 10cm dishes, one plate to make 2 tubes (Aim for 90% confluency)
 - 11 mL growth media, add 500 μ L - 1 mL confluent cells for growth in 2 days
 2. Trypsinize to resuspend cells (See Split Cells)
 - Use 2mL trypsin
 - Quench reaction with 2 mL media
 3. Centrifuge at 500g for 5mins
 4. Keep pellet; remove supernatant
 5. Add FRZ (freezing media) 1ml to the pellet and resuspend.
 6. Take 500 μ L put in freezing tube, place on ice IMMEDIATELY
 7. Put tube in freezing container
 8. Put in -80 overnight
 9. Put in liquid nitrogen container
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- Cells can be stored at -80 for short periods; store in cryo for longer term

