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Version 1

Karyotyping Protocol V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

Clelland Lab internal protocol for karyotyping.

Materials

Items you need to bring/find

1. Shipping cardboard box with styrofoam box inside. It needs to fit a T-25 standing
2. Small ziplock bags (that can fit a T-25)
3. FedEx slip + FedEx Slip pouch (it has an adhesive side to attach to the box)
4. Paper towels, bubble wrap or similar product (can find by asking other labs nicely)
5. Parafilm
6. Shipping Scale (located on the 1st floor. Take the service elevator down and it should be in that corridor)
7. Shipping tape (should be on the first floor next to the shipping scale)

Media/Reagent

1. Essential 8™ Medium (Thermo A1517001)
2. Fetal Bovine Serum (FBS) (Cytiva SH30071.03HI) (if you don't have this, that is ok)

Items at Gladstone Stem Cell Core

1. T-25 with sealed cap (should be in SCC, if not that is also OK)
2. Ethanol
3. 50mL Conical Tube



Instructions

- 1 Make sure the T-25 is 40-60% confluent
- 2 Warm 100mL E8 + 15% FBS (85 mL of E8 with 15mL FBS) to 37°C.
 - 2.1 I usually use the bead bath
 - 2.2 Let this incubate for 30 minutes, but you can cut down this step if you don't have time
- 3 Locate a cardboard shipping box that is small and has a styrofoam box within it.
 - 3.1 You cannot just use a styrofoam box
- 4 Clean the box with ethanol and remove old shipping information from the box
- 5 In the stem cell core, find a T-25 with sealed caps (it looks just like a regular T-25, but the cap has no filter)
 - 5.1 If you can a T-25 with a sealed cap
 - 5.2 You only need the caps of the sealed T-25. You will toss the bodies.
 - 5.3 You will swap caps with this sealed T-25 after filling your sample with media
 - 5.4 If you cannot find a T-25 with a sealed cap
 - 5.5 You can use extra parafilm



- 6 After the media is warm, aspirate spent media from T-25 in a BSC
- 7 Hold the T-25 so the cap is facing upwards, then slowly fill the T-25 with warm E8+ 15% FBS.
 - 7.1 If you have a sealed cap, fill up the T-25 until there is little room at the cap. Toss the excess plasticware
 - 7.2 If you do not have a sealed cap, leave a centimeter - 1.5 centimeters of space.
 - 7.3 NOTE: Avoid aiming the stream at cells! That can cause cells to lift.
- 8 Place excess media in a 50mL conical tube. This will be shipped as well.
- 9 Prepare items for shipping
 - 9.1 Take the T-25 and conical tube to a bench area.
 - 9.2 Use parafilm to fully seal the tops of the T25 and Conical tube. Be extra conscious of sealing when using the non-sealed T-25 cap.
 - 9.3 Place the items in small ziplock bags
- 10 Submit a Characterization request form via WiCell's website
- 11 Place items in the box
 - 11.1 Layer the shipping box with paper towels



- 11.2 Place T-25 and the excess media
- 11.3 Add bubble wrap or similar product to prevent items from shifting during shipping
- 11.4 Attach ship form from WiCell's website
- 11.5 Seal the box
- 12 Ship box: fill out the FedEx slip
 - 12.1 Enter WiCell's address
 - 12.2 Select FedEx priority overnight
 - 12.3 Other packaging
 - 12.4 Select dangerous good, but shipping declaration not required
 - 12.5 Determine the weight of the box using a scale
 - 12.6 Charge sender
- 13 You are done! You can leave the box at the FedEx pick up location on the 1st floor at Gladstone!