

Karyotyping Protocol

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Instructions

1. Confirm flask confluency.

- a. Make sure the T-25 flask is 40–60% confluent before proceeding.

2. Warm the media.

- b. Warm 100 mL of E8 + 15% FBS (85 mL E8 combined with 15 mL FBS) to 37°C.
- c. A bead bath works well for this.
- d. Incubate for 30 minutes; this step can be shortened if you're short on time.

3. Locate a shipping box.

- e. Find a small cardboard shipping box that contains a styrofoam box inside it.

Note: A styrofoam box alone is not sufficient — it must be inside a cardboard shipping box.

4. Clean the box.

- f. Wipe down the box with ethanol and remove any old shipping labels or information.

5. Locate a sealed-cap T-25.

- g. In the stem cell core, find a T-25 flask with a sealed cap. It looks like a standard T-25, but the cap has no filter.
- h. If you find one: you only need the cap itself — discard the flask body. You'll swap this cap onto your sample flask after filling it with media (see Step 7).
- i. If you cannot find one: use extra parafilm instead.

6. Aspirate spent media.

- j. Once the media is warm, aspirate the spent media from your T-25 in a biosafety cabinet (BSC).

7. Fill the T-25 with fresh media.

- k. Hold the T-25 with the cap facing upward and slowly fill it with warm E8 + 15% FBS.
- l. If using a sealed cap: fill until there is very little headspace left, then discard the excess plasticware.
- m. If using a standard (non-sealed) cap: leave about 1–1.5 cm of headspace.

Note: Avoid aiming the media stream directly at the cells — this can cause them to lift off the flask.

8. Save the excess media.

- n. Place any excess media into a 50 mL conical tube. This tube will be shipped along with the flask.

9. Prepare items for shipping.

- o. Bring the T-25 and the conical tube to a bench area.
- p. Seal the tops of both the T-25 and the conical tube with parafilm. Be extra thorough when sealing a non-sealed T-25 cap.

- q. Place each item in its own small ziplock bag.

10. Submit the characterization request.

- r. Submit a Characterization Request Form through WiCell's website.

11. Pack the shipping box.

- s. Line the shipping box with paper towels.
- t. Place the T-25 flask and the conical tube of excess media inside.
- u. Add bubble wrap (or similar padding) to prevent the items from shifting during shipping.
- v. Attach the shipping form obtained from WiCell's website.
- w. Seal the box.

12. Complete the FedEx shipping label.

- x. Enter WiCell's shipping address.
- y. Select FedEx Priority Overnight.
- z. Choose "Other Packaging."
- aa. Select "Dangerous Goods" (a shipping declaration is not required).
- bb. Weigh the box on a scale and enter its weight.
- cc. Charge the shipment to the sender.

13. Ship the box.

- dd. You're done! Leave the box at the FedEx pickup location on the 1st floor at Gladstone.