**Infecting and Processing Spinal Cord Organoids**

*Walker Orr, 02/26/2024*

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| **Steps** |  | **Helpful Hints** |
| Day One |  |  |
| 1. Before you start, thaw cell freezing media (contains DMSO and stored at -20C). |  |  |
| 1. Wash cells, trypsinize, resuspend, and count the number of cells for each cell line you are freezing. |  | Pay attention to the total volume of cells; you are counting the total number of cells here, not just the concentration. |
| 1. Centrifuge for 3 minutes at 300 x g and 4C to pellet cells. |  |  |
| 1. Resuspend cells in cell freezing mediaat 1 million cells/mL. |  |  |
| 1. Produce 1 mL aliquots of your resuspended cells; store in cryovials. |  |  |
| 1. Place cryovials in an insulated cooler at -80C for 24 hours. This prevents the cells from flash-freezing. |  |  |
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| Day Two |  |  |
| 1. Go to the QVEU liquid nitrogen inventory and identify empty positions to place your cells. |  |  |
| 1. Transport your cells to the freezer farm (in the building 33 basement) on dry ice. |  |  |
| 1. It’s very important to wear cryoprotective gloves for all sunsequent steps. Liquid nitrogen is very, very cold and can fuck you up almost instantly. |  |  |
| 1. Remove the foam plug from the liquid nitrogen tank (ours is tank #8). Press “start fill” and allow the tank to fill with nitrogen for around 10 seconds. You’ll see liquid nitrogen flow out of the tank. Press “stop fill” after this period. |  |  |
| 1. Remove our liquid nitrogen tower from the tank. Working very quickly, but very carefully, remove the appropriate box from the tower. They’re numbered from the bottom up. Quickly place your cells into the designated places, double-checking and remembering the orientation of the box in the tower. |  | It’s tough to identify which liquid nitrogen box tower is ours. Go with someone who knows what they’re doing until we figure out a better system ☹. |
| 1. Replace the box in the tower, the tower in the tank, and the foam plug on the tank. |  |  |
| 1. Phew! |  |  |

**TIPS (Adam)**

1. Wait three days between changing media; this allows the organoids to adhere slightly to the plate.
2. Make sure to use round-bottom plates (one organoid per well)
3. Dip the tip in BSA.
4. Didn’t wash organoids before adding virus.
5. Allowed the organoids to remain completely undisturbed during the 1 hour at 37C.
6. Didn’t wash after placing the DMS library on the cells. Let it go for three days. (Why would you wash without justification).

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