CT26 CULTURE AND INJECTION

- Check tumor cells under the microscope. Confirm there is no contamination, they are not overgrown, and you have enough cells for your experiment.
- Aspirate the medium and add ~2 ml of undiluted trypsin:EDTA to the cells. Incubate for about a minute at 37°.
- Dislodge the tumor cells. Rinse the bottom of the flask with 10 ml of medium. Transfer into a 15 ml conical tube.
- Remove 10 ul of cells for counting (or you can count during one of the washes). Note the total volume. Dilute sample 1:1 with trypan blue.
- Spin cells in swinging bucket rotor at 1200 rpm (335 x g) for 5 minutes.
- Resuspend in 10 ml of HBSS, spin again.
- Wash 1 more time to remove trypsin and serum.
- Resuspend in HBSS, in a total volume that will give you a cell concentration of 1e5 (for assays) or 5e4 (for tumor growth) per 100 ul. Inject 100 ul of tumor cells subcutaneously.
- To continue growing tumor cells in culture, transfer 1 ml of these cells (1:20-1:10 dilution, 0.5-1e6 cells/T75) into a new T75 flask, add 10-15 ml medium.

CT26 CELL MEDIUM

900 ml RPMI-1640 with L-glutamine
100 ml FCS
10 ml Pen/Strep (100x, 10,000 IU/ml Pen, 10,000 ug/ml Strep)
10 ml MEM non-essential amino acids solution (100x)
10 ml L-Glutamine (200 mM)
10 ml Sodium pyruvate (100 mM)
10 ml 1 M Hepes
1 ml 0.1 M ß-mercaptoethanol