# BM Stroma Cell Production and Harvest

## Generating an immortal murine bone marrow cell line

## Materials

* Alpha-MEM media
	+ With 20% FBS
	+ With Pen/Strep
* PBS
* Trypsin
* 15cm dishes
* 10cm dishes
* Polybrene
* SV40 virus

## Preparation

* Conduct CBCs on all mice being taken down (always)
* Collect plasma from terminal RO bleed (if blood remaining)
* Label all tubes for dissection/ spin flush the day before

## Protocol

Day 1:

1. Sac and dissect mice (WT and KO)
2. Remove bone marrow cells via spin flush
3. Reserve 25 million cells for plating
4. Plate each sample in a 15cm dish with 75ml Alpha-MEM 20% FBS with P/S
5. Grow for 1 week, checking periodically on growth

Day 7:

1. Conduct full media change with Alpha-MEM 20% FBS
2. Grow for another week, checking periodically on growth
	1. Should see fibroblasts forming, adherent on bottom of plate

Day 14:

1. Conduct full media change with Alpha-MEM 20% FBS
2. Grow for another week, checking periodically on growth
	1. Should see more fibroblasts forming, adherent on bottom of plate

Day 21:

1. Rinse with PBS 3X to remove dead cell debris and non-adherent cells
2. Add 3ml Trypsin, incubate 5’ at 37°C
3. Top up to 45ml (add 42ml media) and split evenly between six 10-cm dishes
	1. Three uninfected, three stock
4. Rinse plate with 5ml media to remove all cells and add to 10cm dishes

Day 22:

1. Observe condition of uninfected and stock plates of cells
2. Infect cells with SV40 virus
	1. Thaw 10ml virus on ice
	2. Dilute with 5ml alpha-MEM media (final vol. 15ml)
	3. Add 5ml to each of the three plates
	4. Incubate at 37°C overnight

Day 23:

1. Remove all media from infected dishes
2. Replace with 10ml alpha-MEM
3. Expand over the weekend (or at least 2 days)

Day 26:

1. Add Puro (antibiotic resistance in SV40 virus) to all infected plates at 2ug/ml
2. Observe for the following 7 days

Immortalized cells will have survived the antibiotic treatment and are adherent on the plate. Remove all media, rinse, and Trypsinize cells before freezing or using for further experimentation.