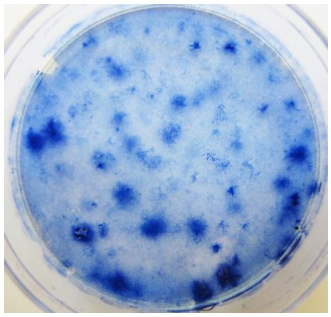
Clonogenic Survival Analysis

Reagents required

- Complete media
- For primary fibroblasts only: MEM+ NBCS
- Trypsin
- PBS



Cell line and feeder plate preparation

Day 1:

- Set up 1xT25cm flask per cell line @ $2x10^5$
- For primary fibroblasts set up 15x10cm feeder dishes per cell line containing $6x10^4$ feeder cells/ 10cm dish. (Feeder cells can either be the same as the line you are testing or a WT line. They must be given 35Gy before being plated into complete MEM + new born calf serum (NBCS).
- N/B. MEFs do not normally require a feeder layer.

Treatment

Day 2:

- Trypsinize and count T25cm^2 flasks.
- Dilute, treat, and plate onto either 10cm feeder plates for primary fibroblasts or 6cm dishes for weed cells.
- e.g.: for primary cells post exposure to IR, we would plate the following onto 10cm dishes:

For a WT line :	
0Gy	100 cells
1Gy	200 cells
3Gy	600 cells
5Gy	3,000 cells
7Gy	20,000 cells

For a sensitive line :	
0Gy	200 cells
1Gy	400 cells
3Gy	2,000 cells
5Gy	10,000 cells
7Gy	30,000 cells

Colony staining and counting

Day 12/21 (weeds/primary lines respectively):

- Add approx. 1ml of methylene blue to medium of each dish and leave for ~45 min at RT.
- Pour off, wash with water and leave to dry before scoring.

N/B. it is always advisable to carry out a straight forward platting efficiency first to determine the clonogenic ability of each individual cell line.

For advice please see Lisa/Elena