**LM044-9: HSAEC-KT yIR senescence induction with SA-B-Gal readout**

**A549 grows too fast in full media and apparently doesn’t become senescent under low-serum media, and bleo doesn’t induce senescence in KT cells. Soooo, I’ll try irradiation.**

**Day 0-14: plate cells, treat, incubate, harvest**

1. HSAEC-KT cells were trypsinized and resuspended two tubes of 200k cells in 4mL full SAEC medium.
2. One tube was irradiated with 10Gy yIR and the other was mock irradiated by placing next to the irradiator with a Geiger counter for the duration of the irradiation.
3. Thoroughly mix cell suspensions and plate 1mL/well in 3 wells of each.
4. Cells were incubated overnight to adhere.
5. Prepare 2x 4mL aliquots of media and add 66.67uL bleo to one and 66.67uL ultrapure H2O to the other.
6. Pipet 1mL media onto respective wells.
7. Every three days, aspirate media and replace with the same formulations applied on treatment day 1.

**Day 6: SA-B-Gal harvest**

1. Dilute the 10X fixative solution with water.
   1. Make 25mL: 2.5mL 10x + 22.5mL H2O
2. Remove media from cells on 3cm plates.
3. Wash wells/plates with PBS.
4. Add 1mL 1x Fixative Solution to wells/plates.
5. Fix 10-15min at RT.
6. Rinse wells/plates 2x with 1X PBS. Leave at 4C until stain is performed.

**SA-B-Gal staining and WB**

**Solution preparation**

1. Resuspend the 10x staining solution by heating to 37C with agitation. Dilute the solution to 1x with H2O. Make 12.09mL: 1.209mL 10x + 10.881mL H2O
2. Dissolve 20mg X-gal in DMSO to make 20mg/mL stock. Store excess at -20C for 1 month. **Must prepare in polypropylene plastic or glass.**
3. B-gal staining solution: For each well to be stained, prepare the following:
   1. 930uL 1x staining solution
   2. 10uL 100x Solution A
   3. 10uL 100x Solution B
   4. 50uL 20mg/mL X-gal stock solution
   5. **pH the final solution. pH should be 5.9-6.1. DO NOT SKIP THIS!!**

**Staining procedure**

1. Aspirate PBS from each plate/well.
2. Add 0.5mL B-gal staining solution to each well. Seal plate with parafilm.
3. Incubate 37C at least O/N in dry incubator.
4. Check blue staining under a microscope.
5. Make a 1:600 dilution of DAPI.
6. Incubate cells in DAPI 5min RT in a dark drawer.
7. Wash 2x 5min in PBS.
8. Cover with 500uL PBS.

**Quantitation**

1. Measure DAPI intensity on a fluorometric plate reader in a 7x7 grid (ex 354 nm em 456 nm).
2. Measure SA-B-Gal stain absorbance on absorbance plate reader in the same 7x7 grid (max abs = 615nm)
3. Average readings for each well.
4. Divide each well’s SA-B-Gal signal by its DAPI signal to get well signal.