β-Galactosidase *in situ* assay for cellular senescence

This protocol was modified from Dimri [Dimri GP, et al. *A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A. 1995 Sep 26;92(20):9363-7.]* ***by*** **Zdenka Matijasevic of the Jones Lab.**

Begin by making the requisite Citric acid/sodium phosphate buffer and X-Gal stocks in order to make the Staining Solution, then compose the Fixative Solution. Once all solutions are in hand, the protocol is rather straightforward.

Protocol:

1. Rinse media from cells with PBS (phosphate buffered saline -low Mg+).
2. Fix the cells in **G/F fixative** mix. Be sure to prepare enough G/F fixative to cover all cells.
3. Incubate fixed cells @ room temperature for 3-5 minutes.
4. Rinse the fixed cells twice with PBS.
5. Stain the cells in freshly made **Staining solution** for 2 hours (to overnight) **in the dark** @37°C (do **not** use a CO_2 incubator).
6. Visualize/count by light microscopy using inverted tissue culture scope.

G/F Fixative

40ul of a 50% Glutaraldehyde stock solution (*stored @ -20°C),*
500ul of a 37% Formaldehyde stock solution,
10mls Phosphate Buffered Saline (PBS).

Staining Solution:

Add to **10ml *Citrate/sodium phosphate buffer**:
250ul of 200mM Potassium Ferricyanide (stock= 3.3 g/50ml) (final concentration: 5mM)
250ul of 200mM Potassium Ferrocyanide (stock= 4.2 g/50ml) (final concentration: 5mM)
100ul of 200mM $MgCl_2$ (stock= 2g/50ml) (final concentration: 2mM)
250ul of 6M NaCl (stock= 17.5 g/50ml) (final concentration: 150mM)
200ul of 50mg/ml X-gal in DMSO (final: 1mg/ml) (*Dissolve X-Gal in DMSO to make a 50 mg/ml stock (50X), store aliquots at -20°C,* ***DARK!)***

Citric acid/sodium phosphate buffer for Staining Solution (40mM, pH6)

- 39.4ml of 0.1M citric acid (19.2 g/l, M.W. 192)
- 60.6ml of 0.2 M sodium phosphate (53.6 g/l heptahydrate, M.W. 268)
- 100ml of deionized water.