SCRAPE-LOADING/DYE TRANSFER ASSAY FOR GAP JUNCTIONAL INTERCELLULAR COMMUNICATION

Solutions needed:

- HBSS + 1% BSA (50 ml; 0.5 g BSA/HBSS)
- PBS + 1% Lucifer Yellow (10 ml for 6 well plates, 0.1 g LY/10 ml PBS)
- PBS
- 2-4% paraformaldehyde

For small cover slips:
Move the cover slip from the 24 well to a 3 cm plate with media. Make sure it's face up, you can label it with a pen.

1. Remove culture medium from a confluent monolayer and save the culture medium in a 10 ml tube. Put it at 37 degrees!

2. Rinse cells three times with Hank's balanced salt solution containing 1% bovine serum albumin (HBC).

3. Move the coverslip on parafilm face up.

4. Use 30G 1/2 needle to create two longitudinal scratches through the cell monolayer in the presence of a solution of Dulbecco's phosphate buffered saline containing ***100 ul of 0.5% LY and 0.5 % Dextran.***

5. ***After exactly 1 min move back the coverslip in the plate*** and quickly rinse the culture three times with HBC

6. Incubate for an additional 8 min (Lucifer yellow) *or 2 min (biocytin)* **in the saved culture medium** to allow the loaded dye to transfer to adjoining cells.

7. Rinse cells three times with HBSS and fix cells 20 minutes in 4% pfa..

8. Rinse cells with PBS 4 times, mount for LM.

9. Visualize with fluorescence microscopy.

12/5/2003 G.E.S.

11-2012 Cinzia Ambrosi