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Wound Healing Assay

Part I. Experimental procedure

1. In the morning, plate 600,000 cells/well in six-well plate wells.
   Note: this density is optimal for parental H1666 cells. Different plating densities may be required for different cell lines or with the same cell line engineered with a knockdown/expression construct or treated with an inhibitor. For example, H1666 cells with SHP2 kd should be plated at 800,000/well.
2. The evening of the next day, make two scratches per well using a 200 µL pipet tip. Use a sterilized razor blade as a straight edge to make the scratch as straight as possible. Also, use the same part of the tip each time in order to create similar diameter scratches.
3. Swirl plate, aspirate, wash once with PBS or media and aspirate again to remove floating cells that were scraped off. Add 2 mL of fresh media.
4. Using a black marker, make two lines perpendicular to each scratch. There should now be 4 points on each well to image. Additionally, make marks parallel to each scratch (but not on top of the scratch) in order to find the scratch when it is close to fully healed.
5. Take 10X pictures of scratches (4x/well) to use as the baseline condition.
6. 12 hr later, take pictures again of the same positions. To get as close as possible to the same position each time, use the black lines you drew and compare your live image against previous pictures before taking a new picture. Continue taking pictures approximately every 2 hr until the scratches have fully healed. Timing may need to be optimized for different purposes or cell lines.
7. Save all images as tif files.

Part II. Analysis

Method based on http://www2.le.ac.uk/colleges/medbiopsych/facilities-and-services/cbs/AIF/tips-and-tricks-1/wound-healing-assay

8. Open ImageJ.
9. Import image sequence (all images in a given folder).
10. Find edges.
    - *Process ----> Find Edges*
    - *Process ----> Sharpen*
11. Adjust threshold until there is a clear difference between the scratch and the surrounding cells. We have generally used a threshold between 20 and 30.
    - *Image ----> Adjust ----> Threshold*
    - *Select Black/White*
    - *Set upper slider to 0 (left)*
    - *Set lower slider in such a way that it is clear where the cells are.*
    - *Click 'apply' and close window.*
12. Analyze particles. Use minimum particle size of at least 100000.
    - *Process ----> Find Edges*
    - *Image ----> Lookup Tables ----> Invert LUT*
    - *Analyze ----> Analyze Particles*
      Size: 100000-inf
      Circularity: 0.00-1.00
      Show: Outlines
      Flag: Summarize
13. Copy data from the Summary window and analyze in Excel.
14. The resulting drawing of your tif file (the outline of the scratch) can be saved and layered with the original image to display the ImageJ-determined wound area.