LM047-2 collagen gel contraction assay with MLF WT and ALDH2-/-

Previous attempt didn’t work because I made a dumb mistake and cast gels in 12-well plate. Don’t do that

1. NaOH titration
   1. Add 0.4 mL of media that is being used to tubes
   2. Add 0.2mL of collagen
   3. Add 2-6 L of 1M NaOH and pipet three times
   4. Sit for 20 minutes
   5. Whichever amount of NaOH creates the most rigid gel (just enough to make it light pink) is the amount that should be used for the experiment.
2. Prepare cells
   1. Trypsinize and count cells
   2. Spin
   3. Resuspend so that final concentration is 3x105 cells per mL; resuspend enough volume for all the wells you need
3. Pour Gels
   1. Add 400 L of cell suspension to each tube
   2. Add 200 L of collagen and appropriate volume of NaOH to each tube
   3. Pipet up and down 3 times
   4. Immediately transfer to wells of 24 well plate
   5. Leave at RT for 20 minutes to allow to solidify
   6. Cover gels with 500L of full media
4. Release Gels
   1. Rim gels with pipet tip
   2. Put into incubator
5. At 24 hours, scoop and move gels to 12 well plates and put on serum starve media
6. At 48 hours, add +TGFb media or vehicle media (1uL TGFb/4mL media)
7. Monitor Contraction with daily images