Cell sonication (to lyse the cells): Written by Fenneke KleinJan (Ikura Lab)

Equipment in Common room (4-307)

- Prepare 100 ml lysis buffer per pellet (in a 150 ml beaker glass)
- (50 mM Tris pH 8, 150 mM NaCl, 0.1% NP-40, 10% glycerol, 10 mM imidazole, 5 mM MgCl2)

- Add just before use:
  - tip of lysozyme (-20 degrees, left fridge)
  - tip of Dnase (4 degrees, left fridge, middle shelf, blue bottle)
  - 10 mM (70 ul) b-ME (add in fume hood!)
  - 1 mM (1 mL) PMSF (-20 degrees, own box)

- Cool buffer on ice

- Resuspend pellet (f.e. on turning wheel in 50 ml falcon) in cold room

- Add everything in the beaker glass

- Screw probe/nut in the sonicator and tighten with wrench (both on Le's bench)
- Place sample in the sonicator, increase height until there is only ~1cm space left.
- Set the program:
  - time = 10 min.
  - amplitude = 40%
  - move to 2nd page
  - pulse on: 0.5 sec
  - pulse off: 2 sec (off time should be equal or larger than on time, to avoid too much heat generation)

time = on-time! (total time of the program = 50 min.)

- For times use the select buttons, for amplitude use the wheel on the right side

- Switch on centrifuge to cool down to 4 degrees
- After 20-30 min. press pause, increase the height of the bucket again and readjust ice
- press pause again to restart the program (dont press start!)
- After sonication: clean the probe with ethanol first and then water, disassemble equipment

- Sample is in solution with protease, etc. have to do the next step directly
- Keep 100 ul for SDS-PAGE sample
  - Spin down 1 min., max speed
  - Save supernatant (SN)
  - Resuspend pellet in water or lysis buffer (100 ul)

    - Take of both SN and resuspended pellet a sample of 20 ul and add 20 ul SDS loading buffer

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- Put sample in high speed centrifuge tubes 4x (hold ~30 ml)
- Balance them
- Centrifuge (Thermo Scientific in refrigerator corridor)
- 15,000 rpm, 30 min, 4 degrees
- Save supernatant
- Fill tubes with water to dissolve pellet O.N., add 10% bleach for 30 min. and rinse with water