

Sonication Protocols

Steps to prepare PFFs before and after sonication:

- 1) Upon receiving the fibrils, store them right away at -80C.
- 2) Plan out which sonication settings they would like to try out and how much volume of fibrils they'll need to achieve that purpose.
- 3) Thaw the amount needed at room temperature for sonication experiment.
- 4) Aliquot into multiple sonication tubes and sonicate them at various times and settings as planned.
- 5) Record these sonication parameters where the fibrils are <50nm in length via AFM/EM, or run a sedimentation assay to confirm that the majority of fibrils are in the soluble phase.
- 6) Thaw out the unsonicated fibrils (the one stored at -80C upon receiving).
- 7) Sonicate at the optimal sonication setting prior to beginning experiments (the optimal situation would be sonicate right before use, but if it's not doable work flow, the alternative would be: sonicate at the optimal sonication setting, aliquot into the desired dose (volume) for use, freeze the aliquots for later use. Then they can thaw the sonicated aliquots as needed prior to injection).

Diagenode Bioruptor Pico Protocol:

We run 100-300 uL of material in 1.5 mL tubes 5 cycles (30s on 30s off) on high power setting. The manual doesn't explicitly say what frequency/power this is, although the unit specs range from 20-60kHz and 25-210 watts.

We would recommend sonicating at higher concentration and then diluting after. The sonication will increase solubility and homogeneity of material, so will be more accurate to pipette and make dilutions afterwards.

The best bet, as we'd recommend to anyone, would be to optimize your sonication protocol for your particular lab with your particular sonicator. You can look for the sonication parameters where the majority of fibrils become soluble via sedimentation assay, or use AFM/TEM to determine the sonication parameters where the fibrils are <50nm in length.

Probe Sonicator Protocol by MJJF**Equipment:**

- Fume hood (BSL2)
- Sonicator with 1/8" tip (Qsonica XL-2000)
- Stereotaxic surgery setup

Protocol:

1. Perform all sonication steps in a fume hood or biosafety cabinet. Ensure that hood is externally ducted and does not re-circulate exhaust into the laboratory space.
2. Thaw sufficient aliquots of 5 mg/mL PFFs at room temperature immediately before use. It is recommended to measure protein concentration again (See Step 1, Protocol Step 3) as freeze-thaw may change protein concentration.
3. Dilute PFFs to required concentration by adding PFFs to a sterile microcentrifuge tube containing the appropriate volume of sterile dPBS. Note that pffs are assembled in dPBS. For mouse injections, we typically use 2-2.5 mg/mL PFFs.
4. Using a probe sonicator, sonicate at power level 2 for a total of 60 pulses (~0.5 seconds each). Pause briefly between every 10-12 pulses to prevent solution from heating up excessively and to avoid frothing.

NOTE: We have also tested this protocol using select high-energy bath sonicators such as the Covaris and Bioruptor systems. Results so far indicate that they are also suitable for preparation of PFFs prior to addition/injection. These systems can sonicate closed tubes and are preferable where aerosol generation is a concern. However, be cautious when using bath sonication in place of probe sonication and be sure to verify fibrils are $\leq 50\text{nm}$ in length for proper toxicity.

<https://www.michaeljfox.org/sites/default/files/media/document/PFF%20Protocol%202017b.pdf>

Sonication Protocols from our Collaborators:

- **Branson series model M1800 bath sonicator:** sonicate approximately 10 μ L of sample (5mg/mL) in a 0.5mL centrifuge tube, continuously for 1h.
- **QSonica bath sonicator:** sonicate at 80% amplitude (30 secs OFF, 30 secs ON for 30 cycles) at 10°C.

Note: Because different labs use different models of sonicators, we cannot recommend one specific sonication protocol. We do recommend the Diagenode Bioruptor Pico bath sonicator, this is the model we use in-house and it has shown the greatest success. The recommended sonication parameters are a good starting point, but keep in mind that we always recommend validating your sonication parameters to an average fibril length of 50nm via AFM/TEM or confirming fibril solubility via sedimentation assay.