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Sonication of α-synuclein Fibrils for injection into the mouse brain V.3

Generation and sonication of α-synuclein fibrils

Version 1 is forked from Generation and Sonication of α-synuclein Fibrils

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We use this protocol and it's working

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Abstract

Animal models that accurately recapitulate the accumulation of alpha-synuclein (α-syn) inclusions, progressive neurodegeneration of the nigrostriatal system and motor deficits can be useful tools for Parkinson's disease (PD) research. The preformed fibril (PFF) synucleinopathy model in rodents generally displays these PD-relevant features, however, the magnitude and predictability of these events is far from established. We therefore have optimized the sonication protocol of α-syn fibrils to ensure reliable, robust results. This protocol includes steps for sonication of PFFs on the day of injection into mice.

Guidelines

This protocol is a modification from previously published manuscripts (Patterson et al., 2019; Polinski et al., 2018; Stoyka et al., 2020; Volpicelli-Daley, Luk, & Lee, 2014).

For safe handling of fibrils please read Bousset L et al. (2016) An Efficient Procedure for Removal and Inactivation of alpha-Synuclein Assemblies from Laboratory Materials *J Parkinsons Dis.*6:143-51
https://pubmed.ncbi.nlm.nih.gov/26639448/

When opening tubes and pipetting, perform in a BSL2 safety hood to prevent contamination.

References:

CITATION

Patterson, J. R., Polinski, N. K., Duffy, M. F., Kemp, C. J., Luk, K. C., Volpicelli-Daley, L. A., . . . Sortwell, C. E. (2019). Generation of Alpha-Synuclein Preformed Fibrils from Monomers and Use In Vivo. J Vis Exp(148).
LINK
10.3791/59758

CITATION

Polinski, N. K., Volpicelli-Daley, L. A., Sortwell, C. E., Luk, K. C., Cremades, N., Gottler, L. M., . . . Dave, K. D. (2018). Best Practices for Generating and Using Alpha-Synuclein Pre-Formed Fibrils to Model Parkinson's Disease in Rodents. J Parkinsons Dis, 8(2), 303-322.
LINK
10.3233/JPD-171248

CITATION

Stoyka, L. E., Arrant, A. E., Thrasher, D. R., Russell, D. L., Freire, J., Mahoney, C. L., . . . Volpicelli-Daley, L. A. (2020). Behavioral defects associated with amygdala and cortical dysfunction in mice with seeded alpha-synuclein inclusions. Neurobiol Dis, 134, 104708.
LINK
10.1016/j.nbd.2019.104708

CITATION

Volpicelli-Daley, L. A., Luk, K. C., & Lee, V. M. (2014). Addition of exogenous alpha-synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous alpha-synuclein to Lewy body and Lewy neurite-like aggregates. Nat Protoc, 9(9), 2135-2146.
LINK
10.1038/nprot.2014.143

Materials

Equipment:

- -80 °C freezer
- Benchtop centrifuge
- **Q700 Sonicator and sound enclosure with chiller** at 10 °C

Materials:

- α-synuclein Fibrils (PFFs)
- Monomeric α-synuclein, which is used for control injections

Note

For in vivo mouse models in which α-synuclein is endogenously expressed, we use PFFs generated from recombinant mouse α-synuclein because human α-synuclein is not as efficient in seeding α-synuclein inclusions from endogenously expressed mouse α-synuclein.

- Ice
- PBS
- Deionized water
- 1% SDS
- Polystyrene sonication tube (Active Motif, Cat No. 53071)

Safety warnings

Please see the Safety Data Sheet (SDS) for safety warnings and hazards before start.

When opening tubes and pipetting, perform in a BSL2 safety hood to prevent contamination.

Sonicating α-synuclein Fibrils (PFFs)

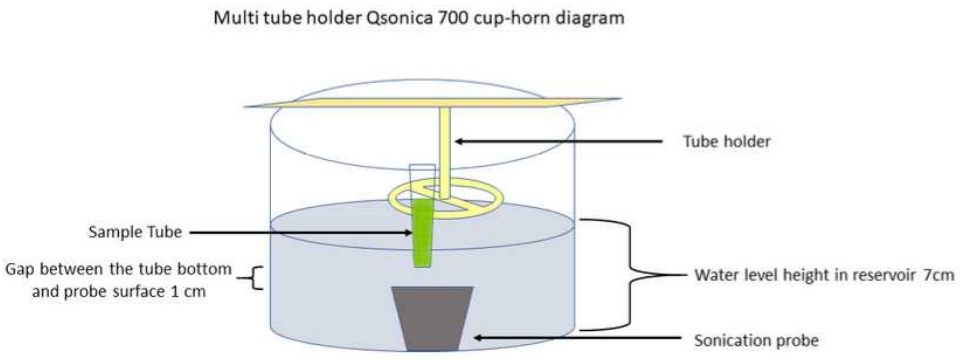
1 Fill Qsonica Sonicator (Q700 Sonicator) water reservoir with about 900 mL deionized water.

2 Turn on the heating system of the Q700 Sonicator and set the temperature at 10 °C.

3 After the temperature inside Q700 Sonicator water reservoir is equilibrated to 10 °C, thaw and pipette 25-22 µL fibrils (5 mg/mL) PFFs stored at -80 °C and transfer to polystyrene sonication tube (Active Motif, Cat No. 53071) inside a biosafety cabinet. Screw the cap of the polystyrene sonication tube tightly after transferring, but avoid overtightening.

> **Note**
>
> A proper lab coat, gloves, sleeve guards, and an N95 or FFP2 mask should be worn while working with PFFs to prevent inhalation or exposure. PFF-contaminated items, such as pipette tips, tubes, gloves, and any spills, should be decontaminated with 1% SDS before disposal.

4 Fit the polystyrene sonication tube containing PFFs to the Q700 Sonicator tube holder and transfer it into the Q700 Sonicator water reservoir. Ensure the reservoir water level is maintained up to the level of the upper meniscus of the PFFs (PFFs: green in the figure below). Operate the sonication for 00:15:00 at an amplitude of 45%, with pulse on and off durations of 00:00:03 and 00:00:03 respectively, which should generate a power of around 110 watts for each pulse. 15m 6s



5 Take the sample tube after sonication and wipe it to remove any water from the reservoir. Spin the tube at 1000 rpm for a few seconds to settle PFF droplets inside. Inside the biosafety hood, remix the PFF sample by pipetting up and down 5 times, ensuring to avoid introducing bubbles. After remixing, re-sonicate the sample for 00:07:30 using the same sonication parameters as described above in step 4. 7m 30s

Note

We also receive a bulk of sonicated PFFs aliquoted at a volume of 22-25 µl, with an optimal size of around 50 nm. We store them at -80 °C for months, and on the day of injection, we re-sonicate using the above-mentioned sonication parameters for 00:15:00 .

PFFs Injection 8h 15m

6 Inject the sonicated PFFs in the mouse brain as soon as possible within 08:00:00 . PFFs should be kept at room temperature until all mice are injected. The monomer, serving as a control, should be spun down at 0 °C at 20000 x g for 00:15:00 to obtain supernatant for injection and then kept on ice until all mice are injected. Any leftover PFFs should be properly disposed of after SDS treatment as indicated earlier. 8h 15m

Note

We transfer 1 µl of sonicated PFFs into an Eppendorf tube before the first injection and another 1 µl after the last injection into another Eppendorf tube. Both samples are diluted in 1X PBS (to a final volume of 500 µl), snap-frozen on dry ice if a dynamic light scattering detector is not immediately available, and then sent for dynamic light scattering analysis to check if the PFFs are fragmented to the optimal fibril sizes of around 50 nm after sonication, and to ensure that this size is maintained during the injection sessions. Larger sizes indicate that the injected PFFs may not develop the extent of pathology seen with fibrils of optimal size.