The Michael J. Fox Foundation's Efforts to Generate, Characterize, and Promote the Use of a Variety of Preclinical Models of Parkinson's Disease

Poster 131.06
Booth 3337

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Introduction

Preclinical models are important tools for investigating the pathogenesis and potential therapeutic strategies for diseases like Parkinson's disease (PD). As the precise etiology of PD is currently unknown and appears to vary among individuals, numerous preclinical models are available to study this disease. To ensure the research community has access to well-validated models of PD, The Michael J. Fox Foundation (MJFF) has taken an active role in designing, validating, and distributing various models of PD that rely on different genetic or interventional manipulations that can be used to investigate mechanisms of PD neurodegeneration or strategies for preventing, slowing, or halting disease progression. Ultimately, MJFF's investment in providing the research community with robust, well-characterized animal models and information on choosing an appropriate model will hopefully lead to advancements in PD research.

Alpha-Synuclein Pre-Formed Fibrils

The use of alpha-synuclein pre-formed fibrils (aSyn PFFs) to generate an *in vitro* or *in vivo* model of PD is gaining traction in PD research. Investigators may opt to use the aSyn PFF model as it is an inducible model that allows spatiotemporal control of aSyn pathology and nigrostriatal degeneration resulting from pathological alterations in endogenous aSyn after introduction of the recombinant aSyn PFFs. Although many groups have successfully adopted the aSyn PFF model, issues with generating consistent pathology have been reported. To improve the replicability of this model and minimize these issues, MJFF is providing the research community with guidelines and practical tips for generating and using aSyn PFFs. A summary of common pitfalls and solutions can be found below, along with information on the aSyn PFF generation protocol in Figure 1, recommended validation experiments in Figure 2, and a list of aSyn PFF species made available by MJFF through Proteos in the table below.

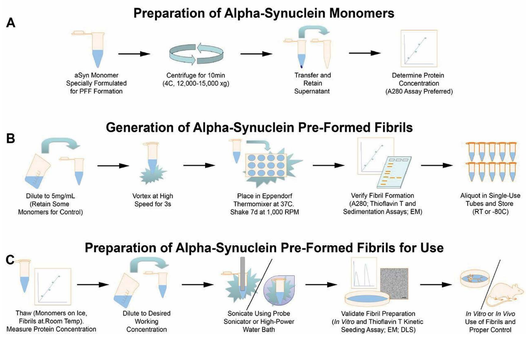


Figure 1. Schematic depiction of the protocol for generating aSyn PFFs. The protocol for generating aSyn PFFs from monomers includes three main stages, (A) preparation of aSyn monomers, (B) generation of aSyn PFFs from monomers, and (C) preparation of aSyn PFFs for use. When using the PFFs to model PD, please note that the species of aSyn chosen will impact pathogenesis (mouse aSyn seeds pathology more rapidly than human aSyn). The protocol corresponding to this schematic can be downloaded from the MJFF online Tools Catalog and is included in orders of the monomeric starting material from Proteos, Inc.

Key Messages for Avoiding Common Pitfalls in the Generation of aSyn PFFs for a Preclinical Model of PD

Pitfall A: Incompatible Protein	Pitfall B: Incompatible Buffer	Pitfall C: Incompatible Temperature	Pitfall D: Inadequate Sonication	Pitfall E: Lack of Validation	Pitfall F: Inattention to Control
Use a well-validated source of aSyn monomeric protein specially-formulated for PFF generation	Monitor the ionic strength and pH of the buffers during PFF formulation and dilution (Keep pH ~7.0, NaCl ~100mM)	Store the monomers at -80C and PFFs at -80C or RT. Keep monomers on ice and PFFs at RT during use	Sonicate the PFFs to an average of 50nm or smaller prior to use using validated protocols	Verify fibril formation and size prior to use. Compare PFFs to monomeric starting material	Choose the best control for the study. If monomers are used, factor this in prior to PFF generation and remove endotoxins

MJFF aSyn PFFs	Availability	Product Name
Human aSyn PFFs	Currently Available at Proteos	Human aSyn monomer protein for making PFFs
Mouse aSyn PFFs	Early 2018 at Proteos	Mouse aSyn monomer protein for making PFFs
Rat aSyn PFFs	Proteos rat aSyn PFFs did not seed robust pathology in initial validation studies in rat brain and mouse primary neurons. MJFF will not make this protein available due to the suboptimal seeding capacity.	N/A

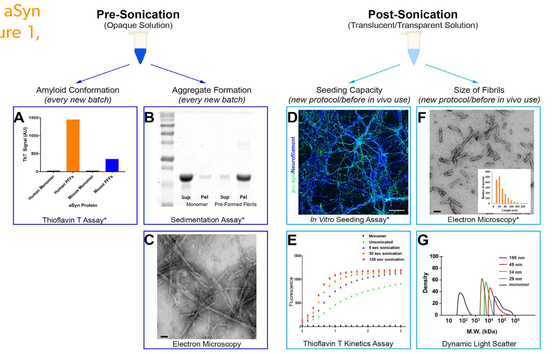
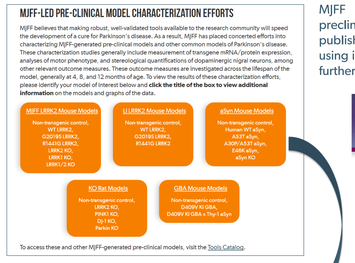


Figure 2. Recommended quality control experiments to verify proper aSyn PFF formation and preparation. After the aSyn PFFs are generated from monomers, samples should be validated to confirm (A) amyloid conformation and (B-C) formation of long fibrillary protein aggregates. A second round of validation should be performed after aSyn PFFs have been sonicated to confirm (D-E) seeding capacity and (F-G) proper size of sonicated aSyn PFFs. Recommended quality control experiments are denoted with an asterisk. **Expected results:** A) Higher levels of Thioflavin T (ThT) fluorescence in PFFs vs monomers. B) More protein in the pellet (pel) vs the supernatant (sup) for aSyn PFFs. C) PFFs should appear as elongated fibrils; scale bars = 50nm. D) High levels of pS129 staining after incubation with sonicated aSyn PFFs; scale bar = 50μm. E) A positive correlation between PFF sonication time and rate of ThT fluorescence. F) The majority of fibrils should be 50nm or smaller (graph inset indicates average fibril size); scale bars = 50nm. G) The majority of fibrils should be 50nm or smaller (graph depicts data for fibrils separated by size).

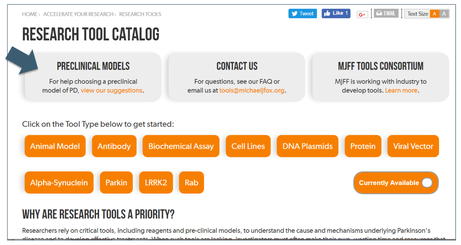
MJFF Preclinical Model Resources

In 2017, MJFF added a new webpage to provide investigators with information on various preclinical models used in PD research. In addition to highlighting common preclinical models of PD, this webpage hosts a variety of resources aimed at helping investigators choose an appropriate model for their research. These resources include a link to the PD research models page on the Alzforum website, results of MJFF-led efforts to characterize a variety of transgenic rodent models of PD, and information on our preclinical biospecimen repository.

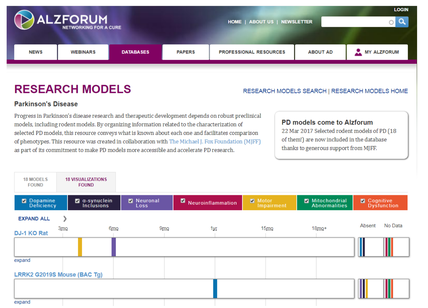


MJFF has led efforts to characterize common transgenic models of PD. To access this data, click on the orange boxes for the study of interest in the Preclinical Models webpage or find a link to the data in the "Characterization Data" banner at the top right of the webpage.

To access a list of molecular tools and preclinical models generated and validated by MJFF, visit the MJFF online Research Tools Catalog at www.michaeljfox.org/toolscatalog. Through this page you will be able to browse the currently available tools as well as those in development. You can access the MJFF preclinical models webpage by clicking the "view our suggestions" link in the Preclinical Models box at the top of the page.



MJFF has partnered with Alzforum to curate data on genetically-modified preclinical models used in PD research. The Alzforum interactive database presents published information on PD-related pathology and behaviors in these models using interactive timelines and summary descriptions with links to publications for further reading and repository websites for ordering.



MJFF offers tissue from many of the models characterized by MJFF. Fresh frozen tissue is available for various peripheral organs and brain regions. More information and order forms can be accessed through our Preclinical Models webpage or the Research Tools Catalog FAQs.

Alpha-Synuclein Knockdown Viral Vectors

In 2015, the MJFF Industry Tools Consortium embarked on the generation and validation of viral vectors expressing micro-RNA (miR) to knock down expression of mouse or human aSyn--including wildtype and common pathogenic mutants (A30P, E46K, A53T) of this protein. Viral vectors also express GFP as a non-toxic reporter protein to enable easy analysis of transduction efficiency. Expression for all viral vectors is driven by the chicken beta-actin promoter hybridized with the cytomegalovirus early enhance sequence (CAG) to ensure transduction of various cell types, with enhancement by the woodchuck post-transcriptional regulatory element (WPRE) and bovine growth hormone polyadenylation sequence (BGH-polyA) to drive high expression levels. Viral vectors were designed, generated, and validated by GeneDetect. MJFF is pleased to announce that these viral vectors will be made available for purchase in early 2018 by Vigene Biosciences, the new MJFF AAV repository and domestic production partner.

aSyn Target	Viral Vector Nomenclature	*in vitro* Knockdown (Fig 3)	*in vivo* Knockdown (Fig 4)	Availability
miR to Human aSyn	AAV1/2-CAG-**Human *SNCA* 3xmiR/GFP-**WPRE-BGH-polyA	~75%	~100%	Early 2018 at Vigene
miR to Mouse aSyn	AAV1/2-CAG-**Mouse *SNCA* 3xmiR/GFP-**WPRE-BGH-polyA	~75%	~100%	Early 2018 at Vigene
Scrambled Control miR	AAV1/2-CAG-**Scrambled Control 3xmiR/GFP-**WPRE-BGH-polyA	~0%	~0%	Early 2018 at Vigene

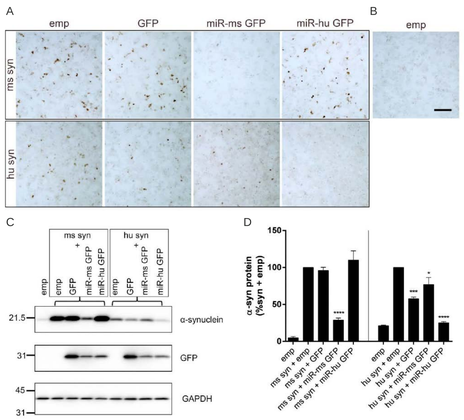


Figure 3. Knockdown efficiency of the constructs in transiently transfected HEK293 cells. A-B) aSyn immunoreactivity in HEK cells 24hrs after co-transfection with human or mouse aSyn-expressing constructs and empty constructs, GFP constructs, or *SNCA* miR-GFP constructs. Scale bars = 250μm. A) HEK293 cells successfully overexpress human or mouse aSyn, with knockdown of this expression resulting from co-transfection with the associated *SNCA* miR. B) Transfection with an empty plasmid instead of an aSyn-expressing plasmid does not result in aSyn expression. C-D) Western blot detection and quantitation of aSyn and GFP in lysates from the HEK293 cells in panels A and B. The mouse *SNCA* miR construct significantly reduces expression of mouse aSyn without affecting human aSyn expression. The human *SNCA* miR construct significantly reduces human aSyn expression with some cross-reactivity resulting in a reduction of mouse aSyn. The GFP construct reduced human aSyn expression but not mouse aSyn expression, indicating that high levels of GFP expression may attenuate the low levels of human aSyn expression. GAPDH was the loading control. Bars represent mean ± SEM (n=3 per treatment). *p<0.05, ***p<0.001, ****p<0.0001 by one-way ANOVA with Tukey's post-hoc analysis.

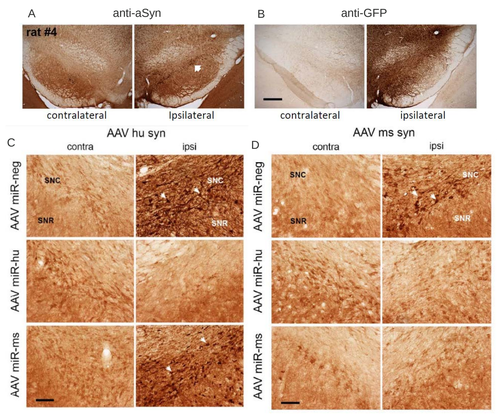


Figure 4. Transduction efficiency and aSyn knockdown after co-infusion of aSyn-overexpressing viral vectors with *SNCA* miR/GFP viral vectors in rat substantia nigra pars compacta (SNC). A-B) Validation of transduction efficiency with the human aSyn-overexpressing viral vector and the scrambled control miR viral vector at three weeks post-co-infusion. Scale bar = 500μm, arrow indicates aSyn overexpression in the SNC. A) aSyn-overexpressing viral vectors result in detectable increases in aSyn protein in the SNC three weeks post-injection. B) The scrambled control miR/GFP viral vector robustly expresses the transgene in the SNC. C-D) Co-infusion of the human or mouse aSyn-overexpressing viral vector with the various miR viral vectors. Scale bars = 100μm, arrows indicate aSyn-positive cells in the SNC adjacent to the substantia nigra pars reticulata (SNR). C) Human aSyn overexpression was abolished by the human *SNCA* miR/GFP viral vector, slightly reduced with the mouse *SNCA* miR/GFP viral vector, and unaltered with the scrambled control miR/GFP viral vector. D) Mouse aSyn overexpression was abolished by the mouse *SNCA* miR/GFP viral vector as well as the human *SNCA* miR/GFP viral vector, with no appreciable decreases in mouse aSyn overexpression observed with the scrambled control miR/GFP.

Summary and More Information

MJFF is invested in providing the PD research community with high-quality tools and models to support rapid new discoveries and encourage reliable, reproducible data. The tools described in this poster are the result of recent collaborative efforts aimed at generating molecular tools for aSyn-related research in particular.

Information on other aSyn tools or tools for other PD-related targets can be found in the Research Tools Catalog at www.michaeljfox.org/toolscatalog. Information on the MJFF Industry Tools Consortium that was involved in generating the aSyn knockdown viral vectors can be found at www.michaeljfox.org/toolsconsortium. Questions regarding MJFF preclinical tools can be sent to tools@michaeljfox.org.