Creation of α-Synuclein Truncation Variants to Test Parkinson's Disease Relevance in Yeast

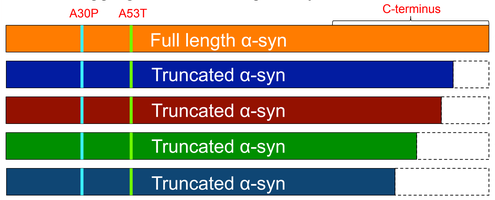
Charles Alvarado '16, Sarah Chiren '16, Jyothis James '16, Alex Roman '16, Katrina Campbell '14 and Shubhik DebBurman
Neuroscience Program, Lake Forest College, Lake Forest, IL 60045

ABSTRACT

Parkinson's disease (PD) is a hypokinetic neurodegenerative disorder characterized by the death of midbrain dopaminergic neurons. This selective cell death is linked to the misfolding and aggregation of the brain protein α-synuclein that accumulates as Lewy bodies. The full-length α-synuclein (140 amino acids long) associates with membranes and its carboxyl-terminus keeps it soluble. In PD, this full-length form is the major component of Lewy bodies, although several carboxyl-terminal truncation variants (α-syn 103, 110, 120, and 123) were also recently found in them (Lewis *et al.,* 2010). While these variants can increase the aggregation of the full-length α-synuclein *in vitro* and enhance toxicity in specialized cell cultures (Li *et al.*, 2005; Liu *et al.*, 2005), the individual properties of each variant towards aggregation, membrane association, and toxicity in free living organisms is not well studied. In this first-year Richter Scholar project, we sought to test the hypothesis that the larger the truncations, the more the variants would reduce α-synuclein solubility and membrane association, and increase toxicity in organisms. Our goal was to create these four variants of α-synuclein (in both wild-type and two familial PD mutant versions- A30P and A53T) and characterize their properties in a budding yeast (*Saccharomyces cerevisiae)* model for PD. In this poster, we report the creation of all twelve variants and their successful transformation into yeast. The next goal is to evaluate several properties of these variants by comparing them to the full-length form: their cellular localization (GFP imaging), expression/accumulation (Western blotting), and toxicity (serially diluted growth on plates).

INTRODUCTION

Cause: loss of midbrain dopaminergic neurons
Symptoms: bradykinesia, muscle rigidity, resting tremors, posture impairment, sometimes depression, dementia
Classification: familial and sporadic; at least eight genes linked to familial PD, including α-synuclein; three α-synuclein mutations cause familial PD (A30P, E46K, A53T)
Pathology: misfolding and aggregation of α-synuclein in Lewy bodies, oxidant accumulation, mitochondrial dysfunction, calcium dysregulation; C-terminus of α-synuclein keeps it soluble
α-Synuclein truncation variants: present in Lewy bodies; increase aggregation of full-length α-synuclein



Gap: Truncation variants have only been evaluated *in vitro* and in specialized cell culture. The relative pathogenic potential of each truncation is unclear. Effects on membrane localization are not known. Truncated A53T has been the only familial mutant evaluated.

HYPOTHESIS & AIMS

Hypothesis:
The larger the truncation, the more likely it enhances pathogenicity by increasing aggregation and toxicity; truncated familial mutants are even more pathogenic than the wild-type form.

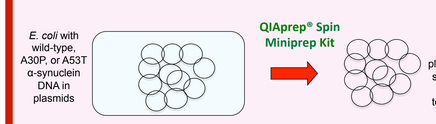
Model organism: Budding yeast (*S. cerevisiae*)

Aim: To create truncation variants of α-synuclein (α-syn 103, 110, 120, and 123) in wild-type and two familial mutants (A30P & A53T)

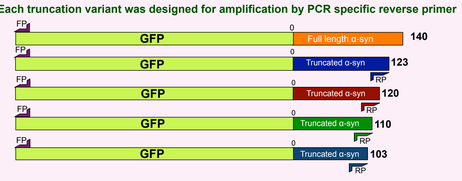
STRATEGY AND RESULTS

STEP 1

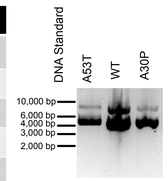
PCR: Primer & Template Preparation



Each truncation variant was designed for amplification by PCR specific reverse primer



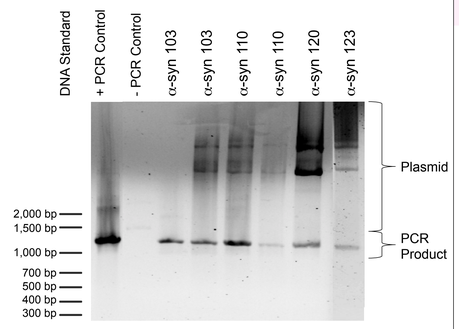
PRIMER	SEQUENCE
FP	GAGTCTAGAATGGCTAGACACCATGG CCAGCAAAGGAGAAG
RP-103	ATTCTTGCCCAACTGGTCCTTTTTGAC
RP-110	TTCCTGTGGGGCTCCTTCTTCATTCTT
RP-120	AGGATCCACAGGCATATCTTCCAGAAT
RP-123	CTCATTGTCAGGATCCACAGGCATATC



STEP 2

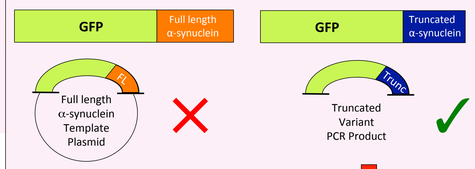
PCR: Synthesis of Truncation Variant

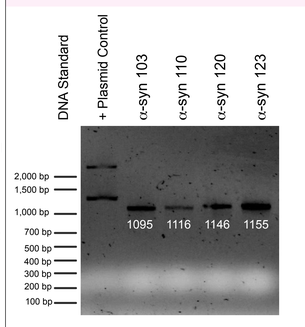
Polymerase Chain Reaction (amplify section of DNA)
Temp. cycles of 94°C, 50°C, 72°C and 4°C



STEP 3

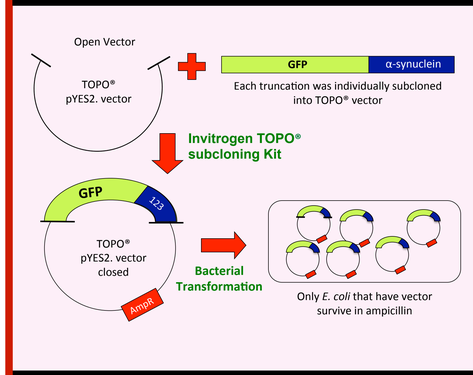
Variant DNA Purification

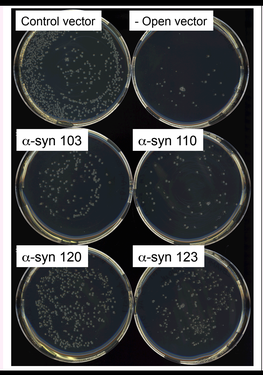




STEP 4

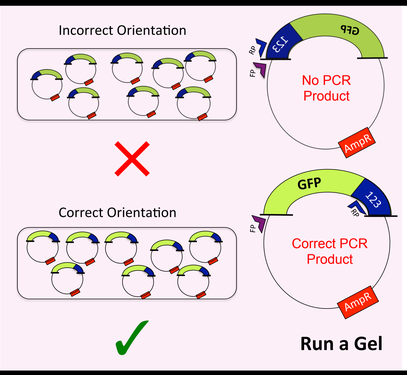
Subcloning & Bacterial Transformation

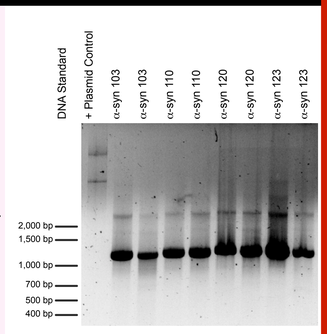




STEP 5

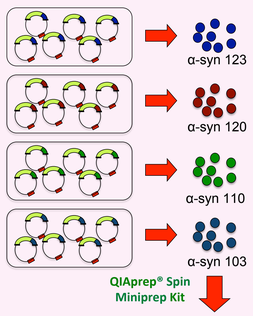
Gene Orientation Verification

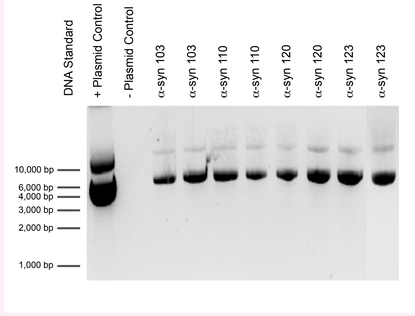




STEP 6

Truncation Variant Plasmid Preparation





1. Confirm sequence **2. Store cells in -80°C** **3. Put in yeast**

STEP 7

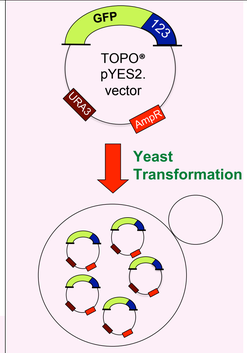
Sequencing

WORK IN PROGRESS

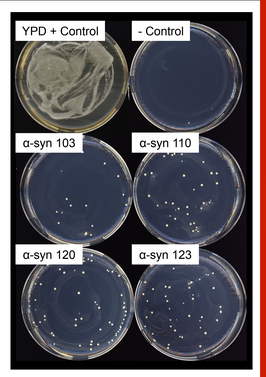
University of Chicago
CRC DNA Sequencing Facility

STEP 8

Yeast Transformation



Grow in SC-Ura to select plasmid



CONCLUSION

✔ **We successfully created all twelve α-synuclein truncation variants by PCR and put them into yeast pYES2. vectors.**

STEPS	WT				A30P				A53T			
	103	110	120	123	103	110	120	123	103	110	120	123
Orientation Confirmation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Yeast Transformation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sequence Validation	*awaiting results*											
Yeast Expression	*in the future*											

DISCUSSION

This three-week project was designed as a prototype original research experience for first-year science majors at Lake Forest College chosen as Richter Scholars. This scholarship program selects the top 10% of the first-year class and selected students work with a faculty member on an original research project during the summer.

For the past twenty years, the Richter program has been ten weeks long. This year, the college additionally piloted a three-week program to expand opportunities to top scholars during a phase of institutional enrollment growth, but at the same time cut costs and provide more summer research flexibility to both students and faculty.

In the DebBurman lab, four Richter Scholars worked on this project. They were mentored by a senior student (Katrina Campbell), as the tools created will aid her senior thesis.

Students met once before the summer began to design and order primers, while the rest of the project was successfully completed within the three weeks.

It's possible for first-year students to pursue an original hypothesis-driven scientific research project within such a limited time and make significant contributions to the discovery process.

FUTURE

These tools can be used for multiple avenues of research in yeast:

Properties
- Localization
- Aggregation
- Accumulation
- Toxicity

Dysfunctional Pathways
- Degradation
- Protein folding
- Oxidative stress

REFERENCES

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