Genomic Modification of SNCA to Generate Cell Lines

Introduction

Locus	gene	Inheri-tance	Pathology	Clinical phenotype	Protein functions
PARK1	SNCA	dominant	LB+	Often aggressive	Vesicle/ neurotranstmitter
PARK8	LRRK2	dominant	LB+ (most)	Typical Parkinsonism	SNCA clearance, etc
16q12	VPS35	dominant	Unknown	Typical Parkinsonism	Vacuolar protein sorting
3q27	EIF4G1	dominant	Unknown	Typical Parkinsonism	Translation initiation
PARK2	parkin	recessive	LB-neg(most)	Early-onset/typical	U3 ligase/protein &organellels clearance
PARK6	PINK1	recessive	LB+(1 brain)	Early onset/typical	Mitochondrial removal
PARK7	DJ-1	recessive	Unknown	Early onset/typical	?
PARK9	ATP13A2	recessive	Unknown	Juvenile-onset/atypical	Alysosomal/+ transporter
PARK14	PLA2G6	recessive	LB+	Juvenile-onset/atypical	Phospholipase/group6
PARK17	FBX07	recessive	Unknown	Juvenile-onset/atypical	Protein ubiquitination
GBA	GBA		Unknown	susceptible	ATPase/Lysosomal function
SCA2	ATAXIN-2		Unknown		?

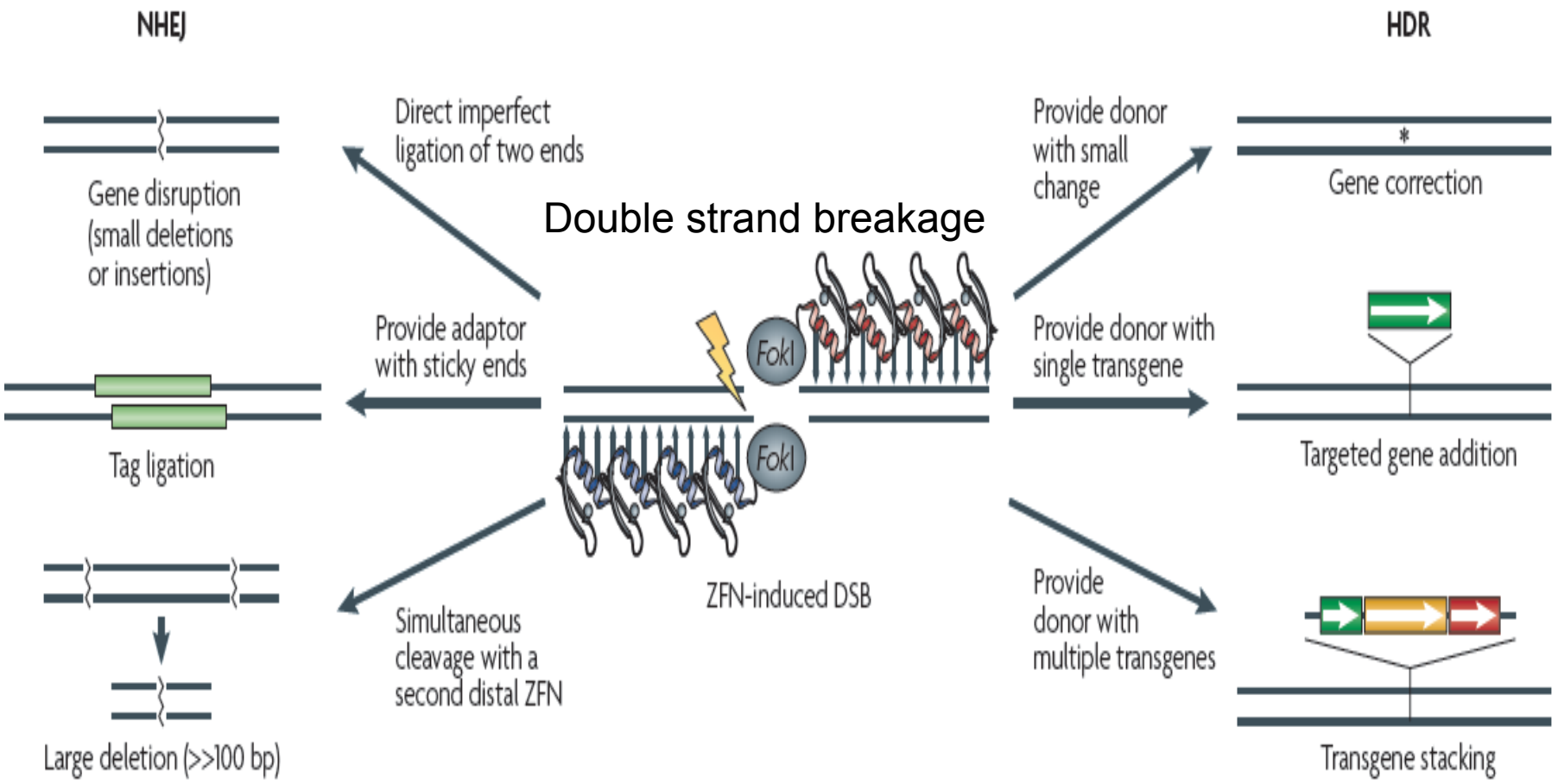
V. Bonifati (2012) Parkinsonism and Related Disorders 28S1:S4-S6

Why SNCA?

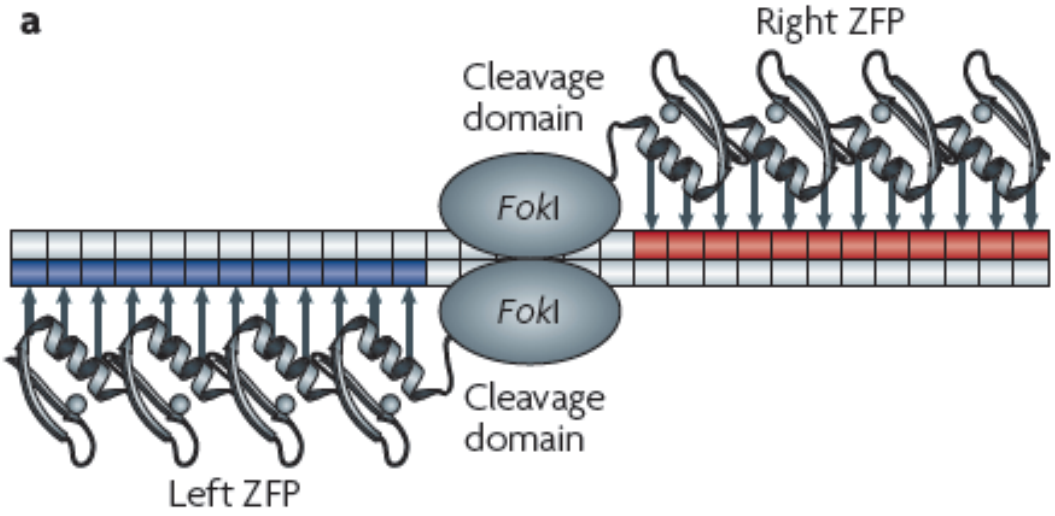
- SNCA is the gene that expresses α-synuclein which is an important factor in causing PD even in its wt stage.
- PD patients, animal models, and cell line models (transient transfection, LRRK2 linked PD iPSC derived neurons, brain tissues of PD linked GBA mutations, etc.) showed general increased levels of α-synuclein
- Goals:
  - specific intracellular regulation of α-synuclein metabolism and expression
  - Screen for compound(s) that can decrease the levels of α-synuclein at either the promoter or translation level.

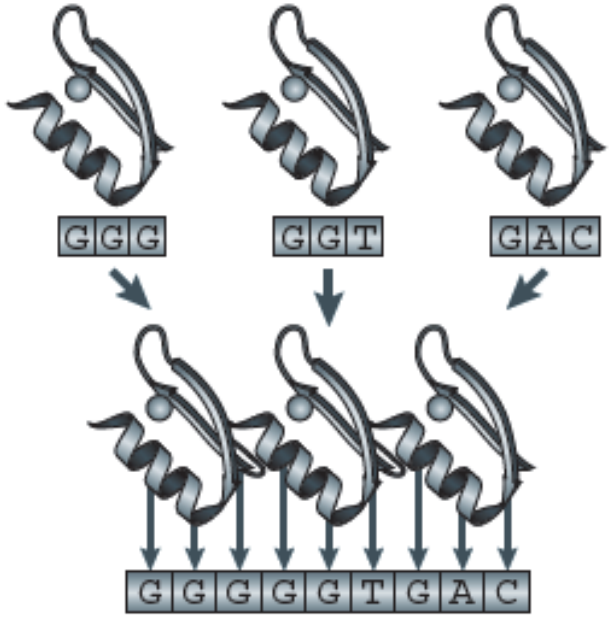
Zinc Finger Nuclease (ZFN)

Types of genome editing made possible by ZFN or Talen



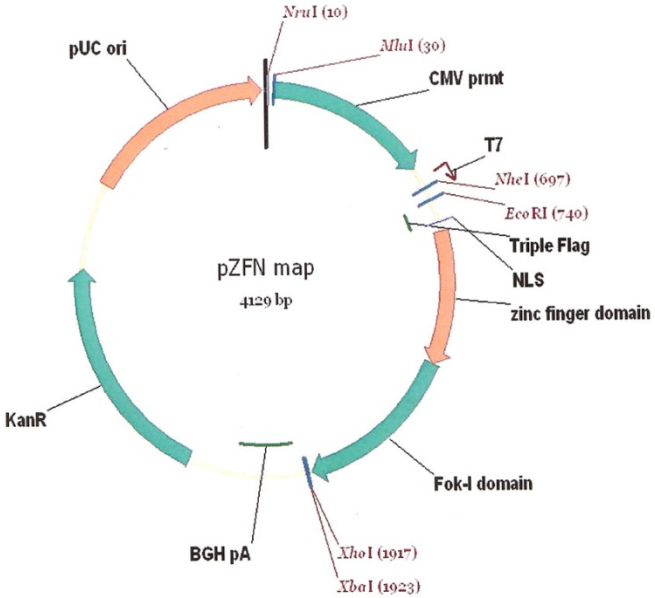
Structure of Zinc Finger Nucleases





If the double strands breakage is NOT repaired by the introduced donor plasmid, then it will heal by random nucleotide insertion through DNA polymerase and available nucleotides.

Map of pZFN and products



Cut

ZFN1
5 binding site

1 2 3 4 5
TGT-TCC-ATC-CTG-TAC

aagtc

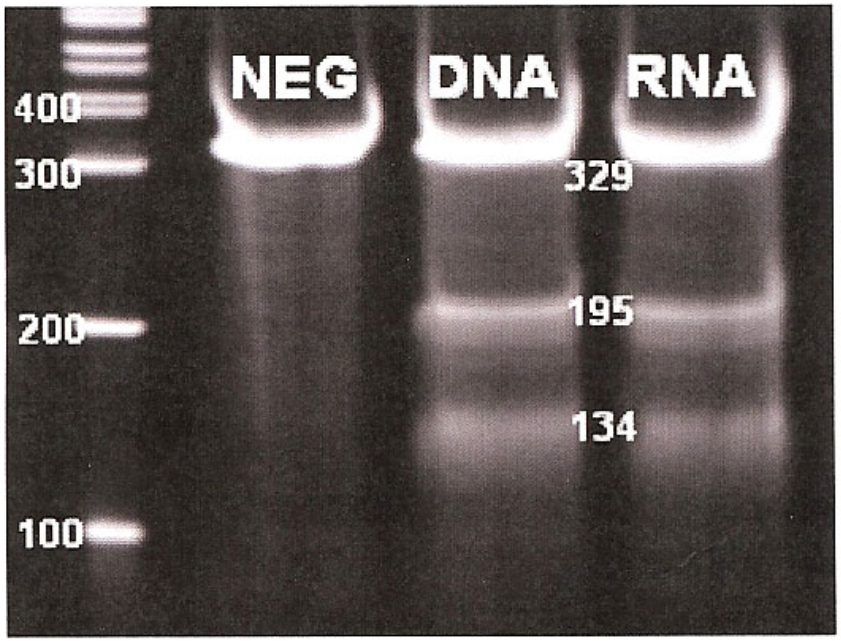
ZFN2
6 binding sites

1 2 3 4 5 6
TCA-GTT-CCA-ATG-TGC-CCA-G

zfp1 fokI

fokI zfp2

Surveyor Mutation Assay:Cel-1 Assay



Cel-1 assay resolved on 10% TBE-PAGE

PCR, reannealing, then treated with Surveyor nuclease (Cel-1). PCR product 329 bp.
Mismatch is cut by Surveyor nuclease (Cel-1), an enzyme found in celery.

If the double strands breakage is NOT repaired by the introduced donor plasmid, then it will heal by random nucleotide insertion through DNA polymerase and available nucleotides.

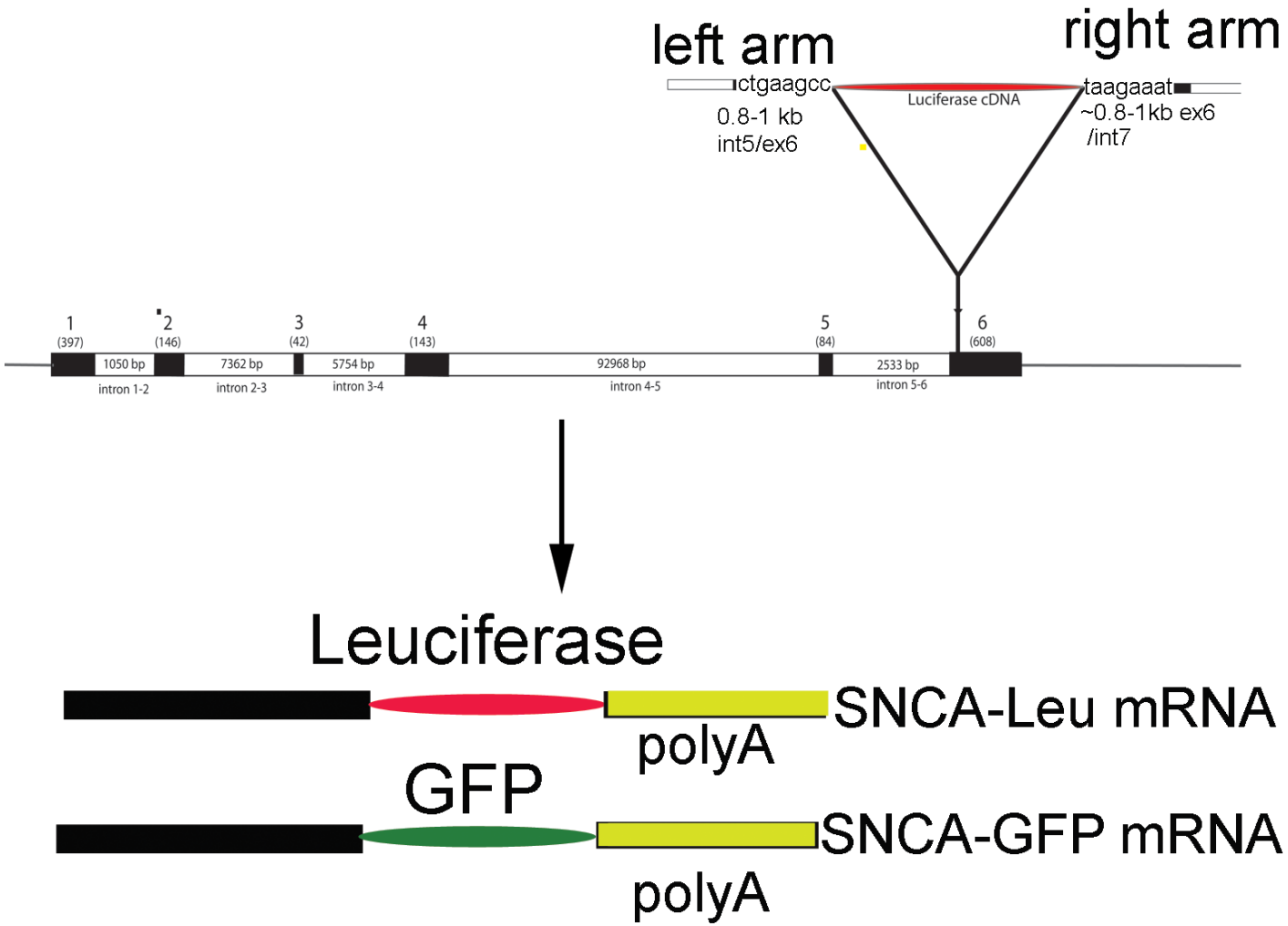
Zinc Finger Nuclease Binding/cutting site

TGTTCCATCCTGTACaagtgcTCAGTTCCAATGTGCCCAG

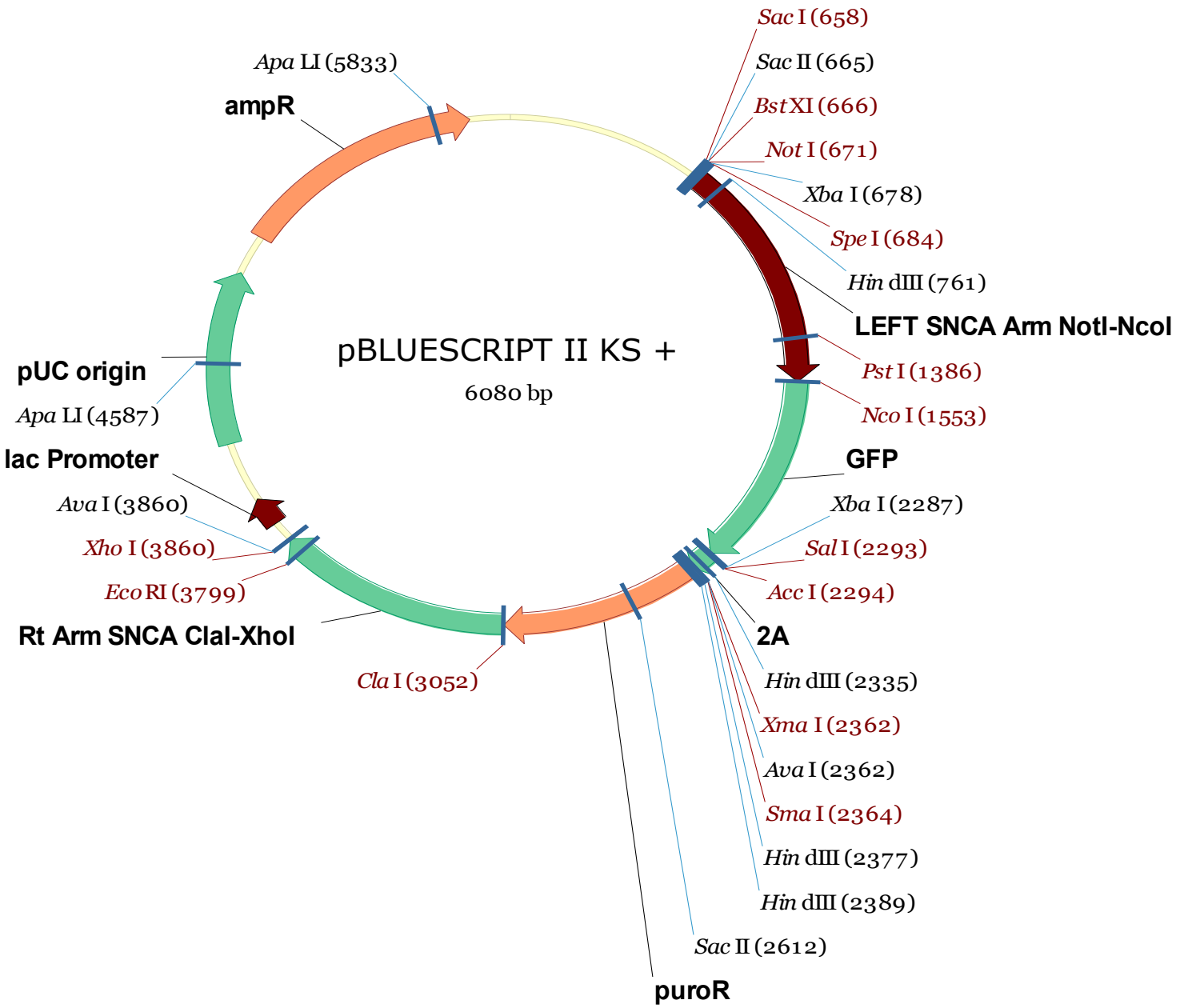
Sigma

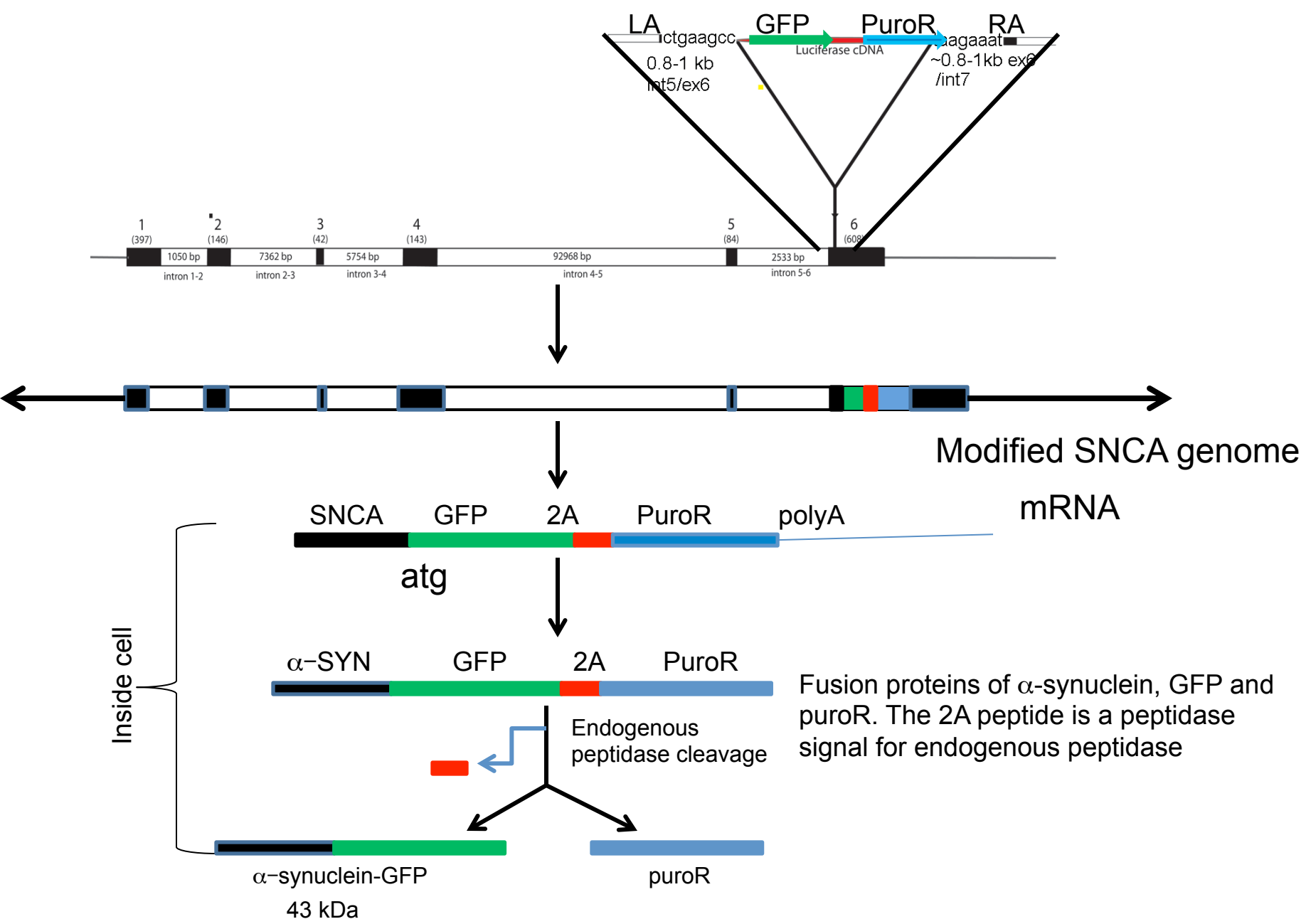
Donor plasmid

Proposed structures of alpha Synuclein-luciferase and where GFP or Luciferase will integrate



SNCA-GFP-2A-Puro donor plasmid

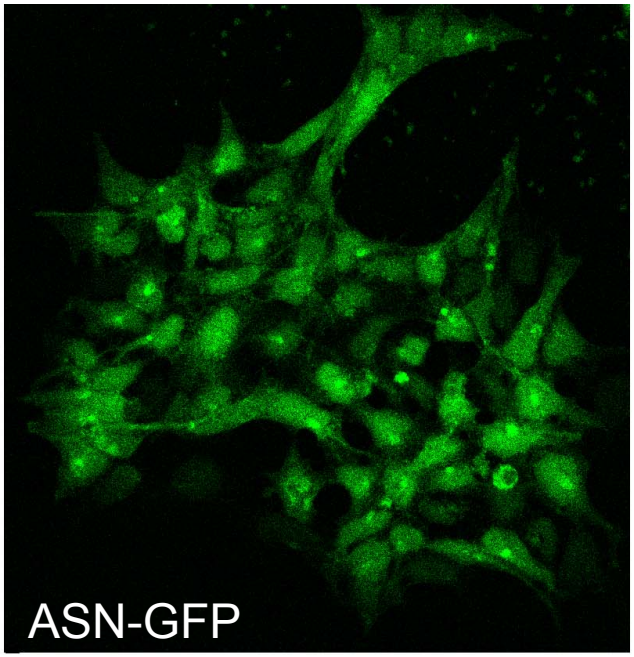


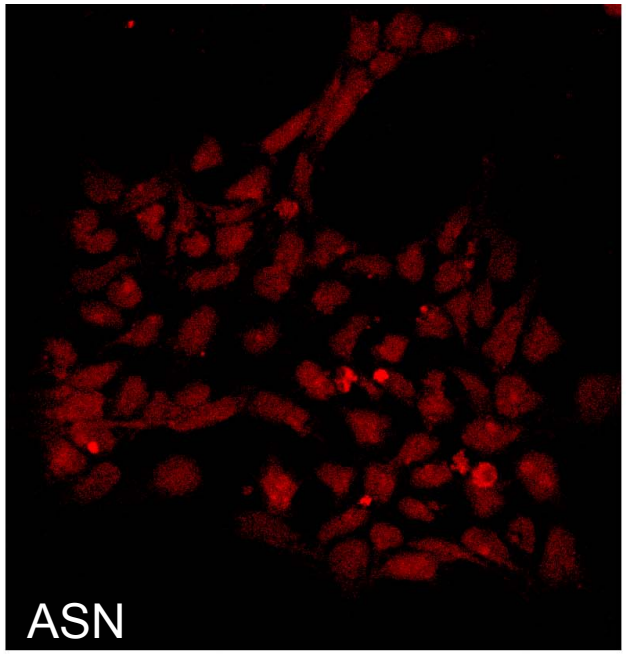


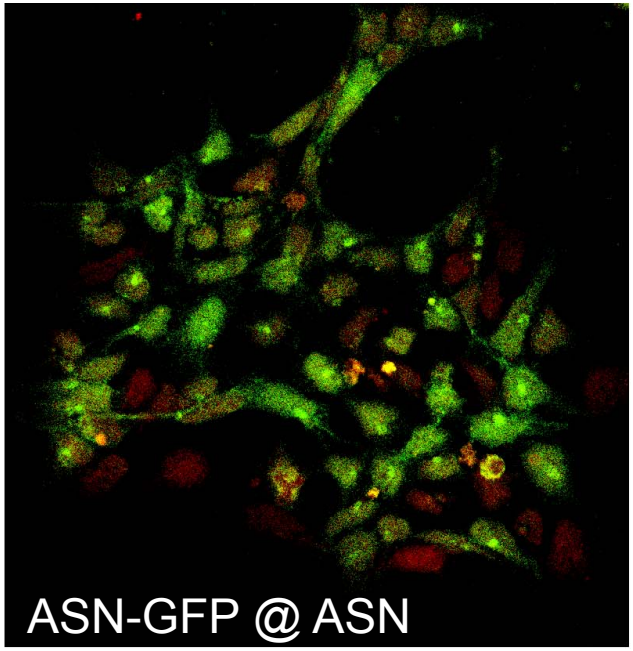
Fusion proteins of α-synuclein, GFP and puroR. The 2A peptide is a peptidase signal for endogenous peptidase

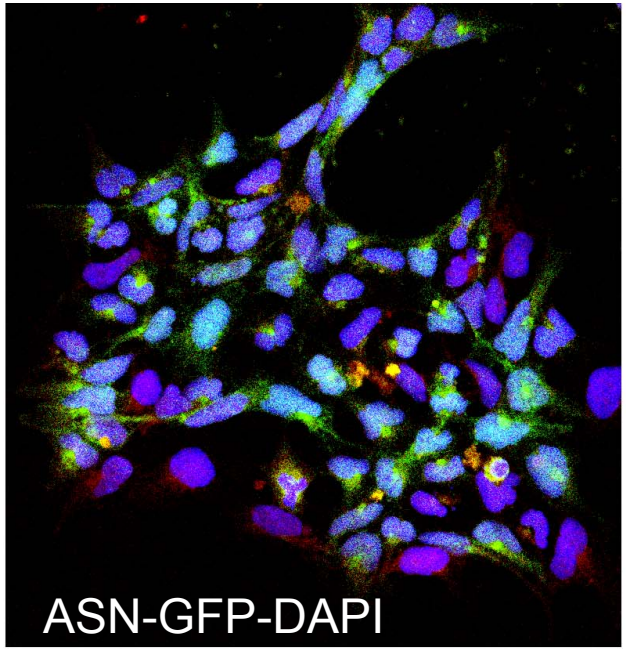
SH-SY5Y cellls were transfected, selected with 1 ug/ml puromycin, and grown as a total population. Four different pools of puromycin-selected lines were obtained.

Cells grown in slide culture dishes, then stained with 10 ug/ml rabbit anti-a-synuclein antibody and visualized with 1/200 dil of anti-rabbit IgG conjugated to TRITC.

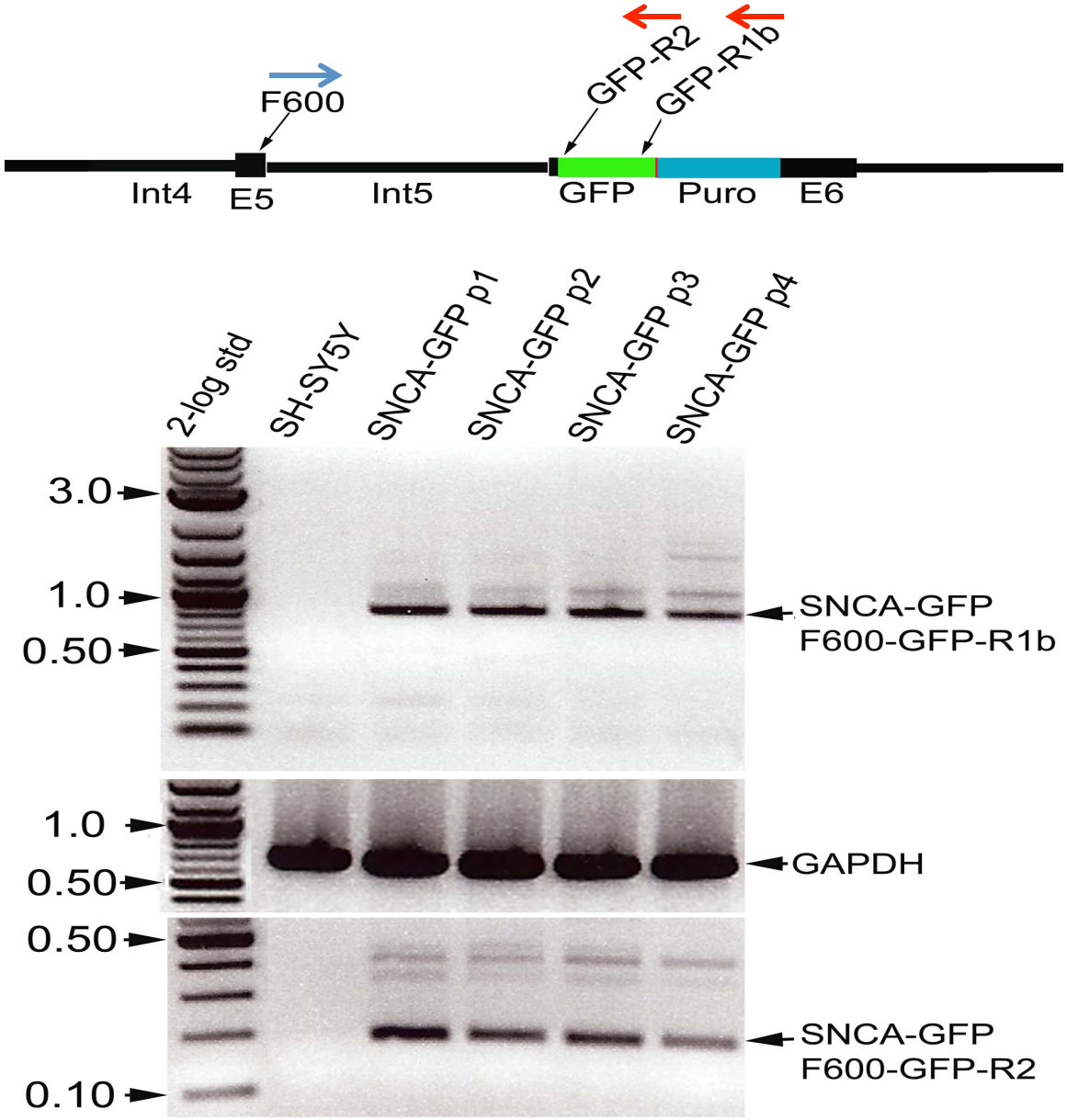




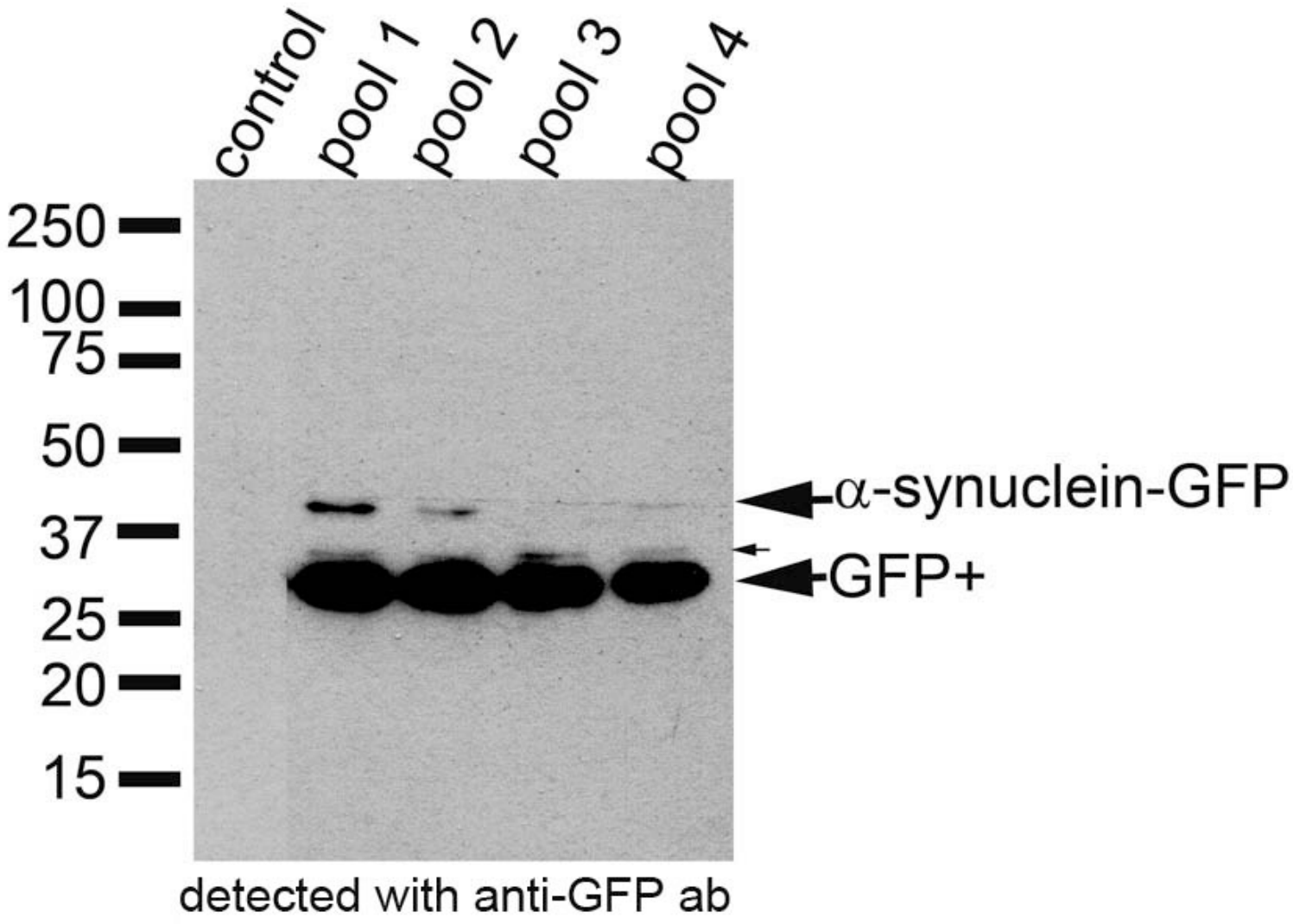




RT-PCR of SNCA-GFP lines



Western blot of 4 pools of SNCA-GFP cell lines with anti-GFP antibody



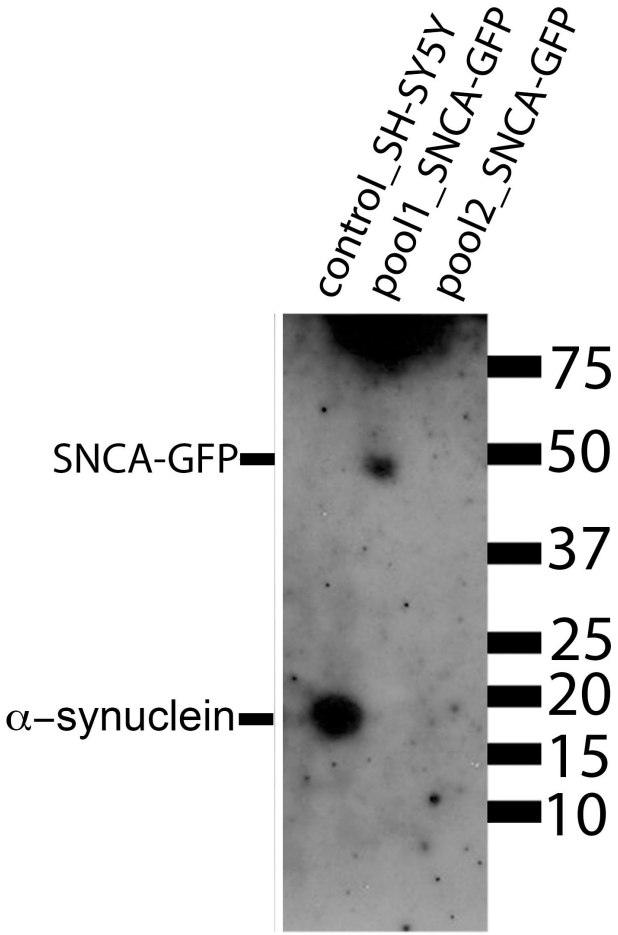
Future

Generate single colony to identify lines that produce only the fusion synuclein-GFP protein.

Generate SNCA-Leu lines

Besides RT-PCR and Western blots, we will also perform Southern blots (Warunnee) to determine whether there are additional, non-specific insertion of the marker proteins at other location.

Western blot of SNCA-GFP cell lines with rabbit anti-SNCA N-terminus



WB filter was detected with 1 ug/ml rabbit anti-α-synuclein antibody (Sigma). Previous attempts to detect with two other anti-α-synuclein abs failed to detect the fusion band.