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We use this protocol and it's working

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LRRK2 cloning, plasmid construction, and mutagenesis

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ABSTRACT

Protocol for Cloning, plasmid construction, and mutagenesis of LRRK2 and LRRK2-RCKW as done by Leschziner and Reck-Peterson Labs.

Original protocol by David Snead and Yu Xuan Lin.

MATERIALS

Materials

- Q5 Site-Directed Mutagenesis (NEB)
- DH5α competent cells.
- QIAprep Spin Miniprep Kit (Qiagen)

Equipment

- Thermocycler

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Cloning, plasmid construction, and mutagenesis

1 The DNA coding for LRRK2-RCKW residues 1327 to 2527 (taken from Mammalian Gene Collection) was PCR-amplified using the forward primer TACTTCCAATCCATGAAAA491AGGCTGTGCCTTATAACCGA and the reverse primer TATCCACCTTTACTGTCACTCAACAGATGTTCGTCTCATTTTTTCA.
The DNA coding for LRRK2 was codon-optimized for *Spodoptera frugipera* (Sf9) cells and synthesized by Epoch Life Science.

2 The DNA for either LRRK2 and LRRK2-RCKW, containing a N-terminal His6-Z-tag and TEV protease cleavage site, was cloned into a pFB-6HZB vector **(SGC)** by ligation-independent cloning, RRID:Addgene_53641

3 LRRK2 variants were generated using Q5 Site-Directed Mutagenesis Kit (NEB).

3.1 Primers for G2019S mutant:
Forward: gccaagatcgctgactacagcattgcccagtactgttgc
Reversed: gcaacagtactgggcaatgctgtagtcagcgatcttggc

Primers for I2020T mutant:
Forward: ccaagatcgctgactacggaactgcccagtact
Reversed: agtactgggcagttccgtagtcagcgatcttgg

Using the following thermocycler conditions.
98 °C 00:00:30
(98 °C 00:00:10
50-72 °C 00:00:30
72 °C 00:13:00

72 °C 00:02:00) X25 cycles
4 °C Hold.

4 PCR products were subject to a KLD reaction (NEB), followed by a transformation into DH5α competent cells and plated on LB with antibiotics.

5 Colonies were picked and grown overnight for DNA extraction using Qiagen miniprep kit.

6 Extracted DNA plasmids were submitted for sequencing.
The resulting plasmids were utilized for the generation of recombinant Baculoviruses according to the Bac-to-Bac expression system protocol (Invitrogen).