



OCT 25, 2023

OPEN ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.kxygx35ddg8j/v1

Protocol Citation: David Snead, Yu Xuan Lin 2023. LRRK2 cloning, plasmid construction, and mutagenesis. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.kxygx35ddg8j/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Oct 23, 2023

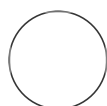
🌐 LRRK2 cloning, plasmid construction, and mutagenesis

Yu Xuan

David Snead^{1,2}, Lin^{1,2}

¹Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA



Amalia Villagran Suarez

Department of Cellular and Molecular Medicine, University of...

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

Last Modified: Oct 25, 2023

PROTOCOL integer ID:
89780

Funders
Acknowledgement:

ASAP
Grant ID: ASAP-000519
MJFF
Grant ID: 18321

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 frugiper*a (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: gccaagatcgctgactacagcattgccagctactgttgc

Reversed: gcaacagtactgggcaatgctgtagtcagcgatcttggc



Primers for I2020T mutant:

Forward: ccaagatcgctgactacggaactgccagctact

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).