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# Flag-TBK1 N455S and Flag-TBK1 M559R cloning (NEB Site directed mutagenesis kit, #E0554S)

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

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## Abstract

site-directed mutagenesis of TBK1 variant vectors

## Materials

- Template DNA: TBK1 expression plasmids (OmicsLink™ Expression Clone EX-U0617-M09/EX-U0617-M11; GeneCopoeia, Inc., Rockville, MD)
- Flag-tagged EGFP expression plasmid (OmicsLink™ Expression Clone EX-EGFP-M11; GeneCopoeia, Inc., Rockville, MD), will not go through mutagenesis
- HA-tagged EGFP expression plasmid (OmicsLink™ Expression Clone EX-EGFP-M09; GeneCopoeia, Inc., Rockville, MD), will not go through mutagenesis
- Q5® Site-Directed Mutagenesis Kit (NEB, #E0554S)
- Q5 Hot Start High-Fidelity 2X Master Mix (New England BioLabs, #M0494S)
- Forward and reverse primers:

TBK1-N455S mutation:
Forward Primer: GATGATTACA**g**TGAAACTGTTCAC (Invitrogen)
Reverse Primer: TTTAATTAATTCAATCAGCCATC (Invitrogen)

TBK1-M559R mutation:
Forward Primer: TTAAATTGCA**g**GACAGAGATTTAC (Invitrogen)
Reverse Primer: CAGGACTTGTAGTTTTTCTAC (Invitrogen)

- Thermocycler ()
- MAX Efficiency DH5α competent *E. coli* cells (Thermo Fisher Scientific, #18258012)
- SOC Medium (Thermo Fisher Scientific, #15544034)
- Miller's LB Broth (Corning, #46-050-CM)
- 14 mL falcon tube (Corning, #352059)
- carbenicillin ()
- NanoDrop Eight Spectrophotometer (ThermoFisher)

## Prepare template DNA (Flag- or HA-TBK1 wild-type)

1 Follow the general protocol for transforming, inoculating and isolating purified GeneCopoeia ORF cDNA Clones in E. coli:

Protocol

NAME

Transforming, inoculating and isolating purified GeneCopoeia ORF cDNA Clones in E. coli

CREATED BY

Jailyn Izu

Preview

Protocol-specific parameters:

- For **Step 1**, the obtain the following plasmids from GeneCopoeia:

-Flag-tagged TBK1 expression plasmid (OmicsLink™ Expression Clone EX-U0617-M11; GeneCopoeia, Inc., Rockville, MD)

-HA-tagged TBK1 expression plasmid (OmicsLink™ Expression Clone EX-U0617-M09; GeneCopoeia, Inc., Rockville, MD)

-Flag-tagged EGFP expression plasmid (OmicsLink™ Expression Clone EX-EGFP-M11; GeneCopoeia, Inc., Rockville, MD), will not go through mutagenesis

-HA-tagged EGFP expression plasmid (OmicsLink™ Expression Clone EX-EGFP-M09; GeneCopoeia, Inc., Rockville, MD), will not go through mutagenesis

- For **Step 2**

-Add 1 µL of the 465 ng/µl purified stock Flag-TBK1 plasmid to 999 µL 10 Mm Tris pH 7.4 to obtain 1 mL of Flag-TBK1 (465 pg/mL)

-For the HA-TBK1 plasmid, add 1 µL of the 262 ng/µl purified stock plasmid to 499 µL 10 Mm Tris pH 7.4 to obtain 0.5 mL of HA-TBK1 (524 pg/mL).

-Dilute EGFP purified stocks in 10 Mm Tris pH 7.4 to obtain a concentration of 1-10 ng per 5 µl.

## Cloning Flag- or HA-TBK1 N455S and M559R

2 Follow the general protocol for cloning using the NEB Site-Directed Mutagenesis Kit

Protocol

NAME

Cloning using the NEB Q5® Site-Directed Mutagenesis Kit

CREATED BY

Jailyn Izu

Preview

Protocol-specific parameters:

- For **Step 1**, the template DNA is the TBK1 expression plasmid (OmicsLink™ Expression Clone EX-U0617-M09 or EX-U0617-M11; GeneCopoeia, Inc., Rockville, MD)

- For **Step 1**, the forward and reverse primer sequences are:

- For the TBK1-N455S mutation:

Forward Primer: GATGATTACA**g**TGAAACTGTTCAC (Invitrogen)
Reverse Primer: TTTAATTAATTCAATCAGCCATC (Invitrogen)

- For the TBK1-M559R mutation:

Forward Primer: TTAAATTGCA**g**GACAGAGATTTAC (Invitrogen)
Reverse Primer: CAGGACTTGTAGTTTTTCTAC (Invitrogen)

## Sequence-verify wild-type TBK1 and the mutagenic clones

3 Follow the general protocol for plasmid sequencing with GeneWiz:

Protocol

NAME

GeneWiz sequencing

CREATED BY

Jailyn Izu

Preview

Protocol-specific parameters:

- For **Step 1**, TBK1 is about 8 kB, so prepare 80 ng/µl template DNA
- For **Step 2**, primer sequences are:

For TBK1 plasmids:
For beginning:

- CMV: CGCAAATGGCGGTAGGCGTG (Invitrogen)

For middle:
For **TBK1 wild-type**:

- Forward: AGGGGAAGATGGACAGTCTGTGT (Invitrogen)
- Reverse: CCACCTGGAGATAATCTGCTGTCG (Invitrogen)

For **TBK1 N455S**:

- Forward: GATGATTACAGTGAAACTGTTCAC (Invitrogen)
- Reverse: TTTAATTAATTCAATCAGCCATC (Invitrogen)

For **TBK1 M559R**:

- Forward: TTAAATTGCAGGACAGAGATTTAC (Invitrogen)
- Reverse: CAGGACTTGTAGTTTTTCTAC (Invitrogen)

For end:

- T7: TAATACGACTCACTATAGGG (Invitrogen)