

Oct 13, 2024

## Site-Directed Mutagenesis

DOI

<https://dx.doi.org/10.17504/protocols.io.81wgbzxjygpk/v1>

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**Protocol Citation:** Carolina Lopez 2024. Site-Directed Mutagenesis. **protocols.io**

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** May 28, 2024



**Last Modified:** October 13, 2024

**Protocol Integer ID:** 100757

**Keywords:** mutagenesis this protocol, directed mutagenesi, procedure for site, pcr

## Abstract

This protocols describes the procedure for site-directed mutagenesis by PCR

## Materials

### Reagents:

- Q5 SDM Kit (NEB, E0554S)
- Competent E. coli cells (NEB, C3040H)
- LB plates with necessary antibiotic



## SDM PCR Protocol

1h 39m

### 1 1. Mix in 0.2 mL PCR Tube:

1h 39m

| Component           | Volume (uL) |
|---------------------|-------------|
| H2O                 | 9           |
| Fwd Primer          | 1.25        |
| Rev Primer          | 1.25        |
| Template DNA (20ng) | 1           |
| Q5 HS 2x Master Mix | 12.5        |

### 2. Run PCR program:

| Step                 | Temp   | Time      |
|----------------------|--------|-----------|
| Initial denaturation | 98C    | 30 sec    |
| 25 cycles            | 98C    | 10 sec    |
|                      | 50-72C | 30 sec    |
|                      | 72C    | 30 sec/kb |
| Final Extension      | 72C    | 2 min     |
|                      | 4C     | Hold      |

### Kinase, Ligase, and Dpn1 (KLD) Reaction:

#### 3. Mix in 0.2 mL PCR Tube:

| Component              | Volume (uL) |
|------------------------|-------------|
| PCR Product            | 1           |
| 2x KLD Reaction Buffer | 5           |
| 10x KLD Enzyme         | 1           |
| H2O                    | 3           |

4. Mix well by pipetting up and down and incubate for  00:15:00 at room temperature.

#### 5. Transform Product into E. coli

a) Add  2  $\mu$ L of product to  50  $\mu$ L of TOP10 cells and pipette up and down slowly to mix.

b) Incubate for  00:20:00 on ice.

c) Heat shock bacteria in  42 °C waterbath for  00:01:00 .

d) Incubate on Ice for  00:03:00 .



- e) Add  100  $\mu\text{L}$  of SOC media and shake in warm room for  01:00:00 .
- f) Plate bacteria onto LB-Antibiotic Plate and spread cells with plate spreader to get individual colonies.
- g) Incubate overnight in  37 °C warm room.
- h) Pick colonies for miniprep growth and sequencing.

## Protocol references

### Site-Directed Mutagenesis:

[Q5® Site-Directed Mutagenesis Kit | NEB](#)

### Primer design:

- Helpful tool for designing primers is <https://nebbasechanger.neb.com/>
- Just insert vector sequence and indicate residues that need to be deleted, inserted, or mutated.