Oct 13, 2024

Site-Directed Mutagenesis

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

Carolina Lopez[1]

[1]Washington University

Cecilia Escudero
wustl

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

Protocol Citation: Carolina Lopez 2024. Site-Directed Mutagenesis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbzxjygpk/v1

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)
H20	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
H20	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 µL of product to 50 µL 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 µL 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://nebasechanger.neb.com/**
- Just insert vector sequence and indicate residues that need to be deleted, inserted, or mutated.