# Site Directed Mutagenesis 2016

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[1]In-house protocol

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1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

Site--directed Mutagenesis

PROTOCOL CITATION

Elizabeth Fozo 2021. Site Directed Mutagenesis 2016. **protocols.io** https://protocols.io/view/site-directed-mutagenesis-2016-brgim3ue

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GUIDELINES

- Primers
- QuikChange PCR (enzyme from Stratagene)
- DpnI Digest
- Transformation

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ABSTRACT

Site--directed Mutagenesis

BEFORE STARTING

**Primers**: Design 40-mer primers with mutation in center. Can mutate up to several aa's in a row (make longer

primers then). Order the forward and reverse complement.

**QuikChange PCR (enzyme from Stratagene):**

| A | B |
|---|---|
| Template: | 250 ng |
| 10 μM fwd primer: | 2 μL |
| 10 μM rev primer: | 2 μL |
| 10x LA Taq or Pfu buffer: | 5 μL |
| 10mM dNTPs: | 1 μL |
| dH20: | to 49 μL |
| LA Taq or Pfu Turbo polymerase: | 1 μL (We use LA taq) |
| PCR program: | 95ºC - 3 minutes |
| | *95ºC - 30 seconds |
| | *55 ºC - 1 minutes |
| | 68 ºC - 2-2.5 min/kb |
| | 4ºC - hold |

**Note**:
1. Newer QuikChange kit says 1 min/kb, but I always still use 2 min/kb and it works well.
2. If making two separate mutations with two sets of primers, can try using both sets in one reaction (as well as separately). I have had this work before and it is much faster.
* 18x

DpnI Digest:

1 Transfer PCR to Epp tube.

2 Remove 2 μL from PCR.

3 Add 2 μL DpnI enzyme to remaining 48μL.

4 Incubate 1+ hrs 37ºC (1st digestion).

5 Add another 2 μL DpnI enzyme to remaining 48 μL (2nd digestion).

6 Use PCR purification kit to purify the 2nd digestion product.

After PCR purification, you will lose around 20% of your product. I always try to concentrate my 2nd digestion PCR product to a volume of around 40 μL before I do the transformation in order to keep the same concentration between the undigested product and the digested product.

7 Store at -20 ºC.

You can go ahead to do the transformation using the undigested PCR product and the 2nd digestion product. If you don't get quite good result after the transformation, such as no significant difference between the undigested one and the 2nd digestion one, you can do the 3rd digestion. But when you do the 3rd digestion, you need to add restriction enzyme buffer 4.

Transformation:

8 Make TOP10 electrocompetent cells as usual.

9 Transform 1μL DpnI-digested plasmid and 1μL non-DpnI plasmid as control.

10 Plate 1/10, 9/10 on LB + antibiotic plates.

11 Should see many more colonies on the -DpnI plate if the digest of the parental unmutated plasmid worked well.

12 Streak a minimum of 6.

13 Sequence 6.

14 Check that
1. the desired mutation is present .
2. the primer region did not get duplicated.

Sometimes you will see that somehow the 60-mer of the primer is present in two or more tandem repeats.