Construction of mutant library V.2

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This protocol is used to construct mutant library of target gene with high efficiency and low false positives/negatives rate after subsequent functional screening.

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PCR tube, thermocycler, ddH2O, Nanodrop

Error-prone PCR:
Random Mutagenesis Kit by Solarbio
Plasmid template

MEGAWHOP PCR:
2×High Fidelity Master Mix

DpnI digestion:
DpnI (NEB) (20,000units/ml)
10xCutsmart

Product purification
E.Z.N.A.® Cycle Pure Kit

Please wear gloves for the experiment, don't try to touch the lid after PCR program initiation.

Make sure that the template of MEGAWHOP PCR is fresh to improve the construction efficiency.

Error-prone PCR

1 Add the following reagent to a PCR tube (50µl) (Random Mutagenesis Kit by Solarbio).

A	B
Template(10µl)	Depends on the concentration
Forward Primer (10 µM)	1µl
Reverse Primer (10 µM)	1µl
Mut Enhencer	3µl
2 x Mut Random System	25µl
ddH2O	Add to 50µl

2 Program the thermocycler as follows:

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A	B
Temperature	Time
95℃	2min
94℃	30 s
Tm-3~5℃	1min
72℃	1kbp/min
72℃	7 min
16℃	∞

3 Use the palm centrifuge to mix the solution in PCR tube.

4 Put the PCR tube into the thermocycler and Run the program.

5 Using agarose gel electrophoresis to confirm if correct construct was present.

PCR product purification

6 PCR product purified by E.Z.N.A.® Cycle Pure Kit.

7 Test the concentration and purity of DNA using NanoDrop.

MEGAWHOP PCR

8 Add the following reagent to a PCR tube (50µl).

A	B
Plasmid templete (50µl)	Depends on the concentration
Purified Production of error-prone PCR (mega primer) (500µl)	Depends on the concentration
2×High Fidelity Master Mix (Enzyme)	25 µl
ddH2O	Add to 50µl

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9 Program the thermocycler as follows:

A	B
Temperature	Time
95°C	5min
95°C	30s
Depends on the Tm	30s
72°C	2kb/min
72°C	7 min
16°C	∞

10 Use the palm centrifuge to mix the solution in PCR tube.

11 Put the PCR tube into the thermocycler and Run the program.

12 Using agarose gel electrophoresis to confirm if correct construct was present.

PCR product purification

13 PCR product purified by E.Z.N.A.® Cycle Pure Kit.

14 Test the concentration and purity of DNA using NanoDrop.

DpnI digestion

15 Add the following reagents to a PCR tube (e.g. 20µl).

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A	B
DpnI (NEB) (20,000units/ml)	Depends on the quality of DNA (20units DpnI degests 1µg DNA)
10xCutsmart	2µl
Purified Production of MEGAWHOP PCR	Moderate (e.g.400 ng)
ddH2O	Add to 20µl

16 Use the palm centrifuge to mix the solution in PCR tube.

17 Incubate at 37°C for 2 hours and heat Inactivation 80°C for 20 min.

Digestion product purification

18 Digestion product purified by E.Z.N.A.® Cycle Pure Kit.

19 Test the concentration and purity of DNA using NanoDrop.

Nick ligation (T4 ligase)

20 Add the following reagents to a PCR tube (e.g. 20µl)

A	B
T4 DNA ligase (Thermo Fisher) (Weiss U)	1U
Purified Production of DpnI digestion	50ng
10X T4 DNA Ligase Buffer (Thermo Fisher)	2µl
ddH2O	Add to 20µl

21 Use the palm centrifuge to mix the solution in PCR tube.

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22 Incubate the reaction at 16°C overnight.

Transformation

23 Transform the nick ligation product into competent cells.

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