Rev. 0

EmeraldNStart Site-Directed Mutagenesis Kit with Competent Cells (ESDMutateWC)

Catalog #MB6138WC, 20 reactions

Product Description

ScienCell's EmeraldNStart Site-Directed Mutagenesis Kit is a versatile and efficient tool for introducing *in vitro* site-directed mutagenesis, including point mutations, deletions or insertions in the plasmid DNA. DNA isolated from almost all *E. coli* strains is dam methylated and is a suitable starting template. By using the EmeraldNStart HiFi Marathon PCR Master Mix and user designed primers, the dsDNA plasmid is exponential PCR amplified with desired mutations. The "hot-start" property in the master mix achieved through ScienCell's unique chemically modified DNA polymerase provides maximal inhibition of primer dimer formation. The advanced buffer formulation provides superior specificity and efficiency to various plasmid templates (up to 15 kb for simple templates, and 10 kb for complex templates). This kit does not require unique restriction sites and gel recovery. Mutagenesis will be generated rapidly in three steps.

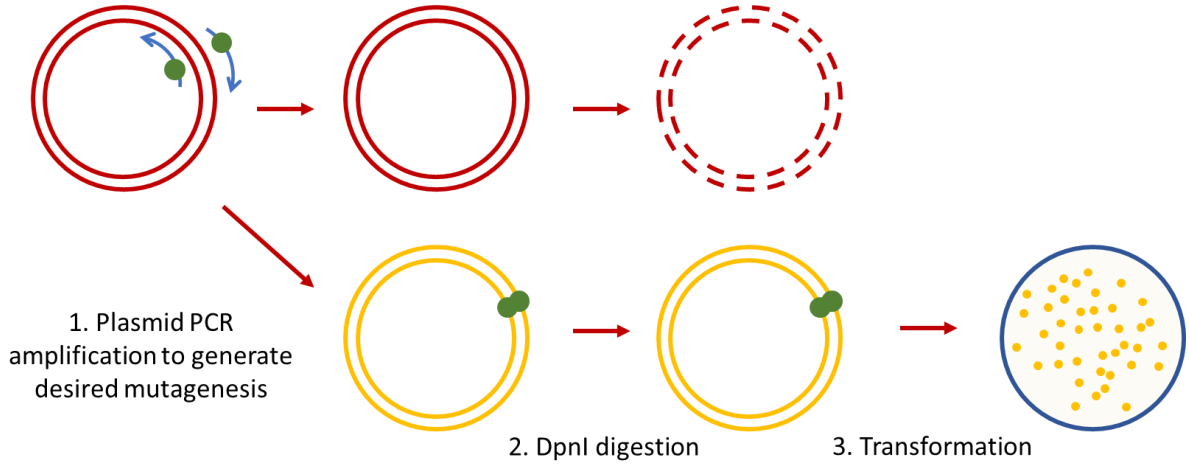

Figure 1. The three steps of the EmeraldNStart Site-Directed Mutagenesis Kit.

Kit Components

Catalog #MB6138WC

Cat #	Item	Quantity	Storage
MB6038a	EmeraldNStart HiFi Marathon PCR Master Mix	0.2 mL	-20°C
MB6138a	DpnI Enzyme	12 µL	-20°C
MB6138b	Control Plasmid (20 ng/ µL)	12 µL	-20°C
MB6138c	Control Primer Mix (5 µM)	12 µL	-20°C
MB6138d	Competent Cells (200 µL per tube)	10 tubes	-80°C

Materials Not Provided

SOC media
LB media
X-Gal LB agar plate
Antibiotics
1.7 mL (or 1.5 mL) microcentrifuge tubes (DNase/RNase free)
PCR tubes

Quality Control

The performance of ESDMutateWC is verified by using the control plasmid and the control primer mix for PCR amplification. The mutagenesis efficiency is evaluated by blue/white colonies ratio.

Product Use

ESDMutateWC is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The products are shipped on dry ice. Upon receipt, store Competent Cells (MB6138d) at -80^{o}C immediately**.** All other components are stored at -20^{o}C in a manual defrost freezer.

Procedure

***Important: Only** use nuclease-free reagents in PCR amplification.*

1. Primer Design Guidelines
   According to the desired mutation (point mutation, deletion or insertion), the primer set used with this protocol must be designed individually.

Figure 2. Primer set design

As shown in Figure 2, each primer should contain three parts:

1) The upstream (5') of the mutation set. It should anneal to the sequence on opposite strands of the plasmid downstream (3') of the mutation set. The length is 10-15 bases, as shown in Figure 2 with the primers with the solid underline.
2) The desired mutation. The recommended length is 1-3 bases (including 1-3 bases deletion), as shown in Figure 2 with the primers in the red box. Longer desired mutation might reduce the PCR efficiency, the conditions (annealing temperature and the repeat cycles) should be further optimized. The two primers should have the same desired mutation.
3) The downstream (3') of the mutation set. It should anneal to the sequence on opposite strands of the plasmid upstream (5') of the mutation set. The length is 20-30 bases, as shown in Figure 2 with the primers with the dashed underline.

2. Place EmeraldNStart HiFi Marathon PCR Master Mix on ice.
3. Prepare 20 µL PCR reactions in PCR tubes or plates as shown in Table 1 or control reactions as shown in Table 2.

Table 1. Preparation of 20 µL PCR reaction

Component	Volume	Final concentration
EmeraldNStart HiFi Marathon PCR Master Mix	10 µL	1X
Template DNA	variable	5-20 ng
Forward and reverse primers	variable	0.2 – 0.5 µM
Nuclease-free water	variable	
Total volume per reaction	20 µL	20 µL

Table 2. Preparation of 20 µL control PCR reaction

Component	Volume	Final concentration
EmeraldNStart HiFi Marathon PCR Master Mix	10 µL	1X
Control plasmid (20 ng/ µL)	0.5 µL	10 ng
Control primer mix (5 µM)	0.5 µL	0.25 µM
Nuclease-free water	9 µL	
Total volume per reaction	20 µL	20 µL

4. Refer to Table 3 for a typical 3-step PCR program setup or Table 4 for a typical 2-step PCR program setup. Adjust properly according to the optimized PCR conditions for the reactions to run. Load the PCR tubes into the PCR instrument and run the program. An optimized PCR program for Control PCR reaction is shown below in Table 5.

Table 3. A typical 3-step PCR program setup

Step	Temperature	Time	Cycles
DNA polymerase activation	95°C	10 min	1
Denaturation	95°C	20 sec	12-18
Annealing	50 - 72°C	20 sec	
Extension	72°C	1.5 kb/min	
Optional	72°C	5-10 min	1
Hold	20°C	Indefinite	1

Table 4. A typical 2-step PCR program setup

Step	Temperature	Time	Cycles
DNA polymerase activation	95°C	10 min	1
Denaturation	95°C	30 sec	12-18
Annealing	68°C	1.2 kb/min	
Optional	72°C	5-10 min	1
Hold	20°C	Indefinite	1

Table 5. Optimized condition for control PCR program setup

Step	Temperature	Time	Cycles
DNA polymerase activation	95°C	10 min	1
Denaturation	95°C	20 sec	16
Annealing	55°C	20 sec	
Extension	72°C	2 min	
Optional	72°C	5 min	1
Hold	20°C	Indefinite	1

5. Add 0.5 µL DpnI (MB6138a) into the PCR reaction, mix well. Incubate at 37°C for 1 hour.
6. Start the transformation immediately, or keep the reaction at 4°C until ready for transformation.
7. Thaw ScienCell's Competent Cells (MB6138d) on ice and aliquot 100 µL to a 1.5 mL Eppendorf tube for one transformation. The leftover cells can be refrozen at -80°C for future use. Keep the competent cells on ice until the reaction is ready.
8. Add 5 µL of DpnI (MB6138a) treated PCR reaction into the competent cells and mix gently.
9. Keep the mixture on ice for 30 minutes.
10. Heat-shock the transformation mixture at 42°C for 1.5 minutes.
11. Chill the transformation mixture on ice for 1.5 minutes.
12. Add 200 µL of SOC or LB media to the transformation mixture.

13. Shake at 37°C for about 40 minutes.
14. Spreading the mixture onto LB plate with suitable antibiotics. For control reaction, spreading the mixture onto an X-Gal LB plate with 50 µg/mL ampicillin.
15. Incubate upside down at 37°C overnight.
16. Analyze transformations on the second day.
    a. For the control reaction, the efficiency of mutagenesis is estimated by the ratio of blue colonies (unmutated) to white colonies (mutated).
    b. The mutants can be screened by direct sequencing. In general, screening three colonies by sequencing will give a high probability of finding the desired mutation.