TagMaster® Site-Directed Mutagenesis Kit Catalog # 7001, # 7002

GM Biosciences.

QUICK-REFERENCE PROTOCOL

1. Prepare sample reaction(s) and control reactions as indicated below:

Sample Reactions	Point Mutation (1-3bp)	Other Mutation*
10X Reaction Buffer	5 µl	5 µl
dsDNA template plasmid (20-100ng)	*X µl*	*X µl*
dNTP Mixture (2mM each)	5 µl	5 µl
$MgSO_4$ (25mM)	4 µl	3 µl
Forward Mutagenic Primer (20µM)	1 µl	1 µl
Reverse Mutagenic Primer (20µM)	1 µl	1 µl
50x TagMaster Supplement	----	1 µl
ddH_2O to a final volume of 49µl	*Y ul*	*Y ul*
TagMaster Enzyme (1.0U/µl)	1 µl	1 µl

Control Reactions	Negative Control	Substitute 2bp	Insert 6xHis Tag
10X Reaction Buffer	5 µl	5 µl	5 µl
pUC19 Control Template (50ng/µl)	1 µl	1 µl	1 µl
dNTP Mixture (2mM each)	5 µl	5 µl	5 µl
$MgSO_4$ (25mM)	4 µl	4 µl	3 µl
Control Primer #1 (20µM)	----	1 µl	----
Control Primer #2 (20µM)	----	1 µl	----
Control Primer #3 (20µM)	----	----	1 µl
Control Primer #4 (20µM)	----	----	1 µl
50x TagMaster Supplement	----	----	1 µl
ddH_2O to a final volume of 49µl	34 µl	32 µl	32 µl
TagMaster Enzyme (1.0U/µl)	1 µl	1 µl	1 µl

*** Long-Range Mutation (4-60bp), 4-660bp Deletion, Multiple Sites Mutation (2 sites)**

2. Perform reaction using the following parameters:

	Temperature	Time
1 cycle	95°C	2 minutes
20 cycles	95°C	20 seconds
	60°C	10 seconds
	68°C	30 seconds/ kb of plasmid length
1 cycle	68°C	5 minutes
1 cycle	4°C	forever

3. Directly transform 5 µl of each reaction into 50 µl of TagMaster Competent cells:

Note *Any enzymatic treatment of the reaction mixture is unnecessary. The reaction mixture should be directly transformed into TagMaster Competent cells.*

- ► Add 5 µl of ice-chilled each reaction into 50 µl TagMaster Competent cells, gently swirl the tube
- ► Incubate on ice for 30min
- ► Heat-shock the tube in a 42°C water bath for 30 seconds, put on ice for 2 minutes
- ► Add 250 µl of SOC medium (pre-warm to room temperature or to 37°C) to each tube
- ► Shake at 250rpm for 1 hour. Spread appropriate volume on agar plate, and culture at 37°C overnight