Protocol-at-a-Glance

Diversify® PCR Random Mutagenesis Kit
Protocol-at-a-Glance
(PT3393-2)

Please read the *User Manual* (PT3393-1) before using this abbreviated protocol. The Protocol-at-a-Glance is provided for your convenience, but is not intended for first-time users.

Setting Up and Running Diversify® Mutagenesis Reactions

1. Consult the User Manual for guidelines on choosing the optimal buffer condition(s) for your experiment. Prepare reactions on ice; combine reagents in the order shown:

TABLE III: MUTAGENESIS REACTIONS

	Volumes by Buffer Condition (µl)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Std.**[a]
Mutations per 1,000 bp	2.0	2.3	2.7	3.5	4.6	4.8	5.8	7.2	8.1	0.4
PCR Grade Water	40	39	38	37	36	35	34	33	32	41
10X TITANIUM *Taq* Buffer	5	5	5	5	5	5	5	5	5	5
$MnSO_4$ (8 mM)	0	1	2	3	4	4	4	4	4	0
dGTP (2 mM)	1	1	1	1	1	2	3	4	5	0
50X Diversify dNTP Mix	1	1	1	1	1	1	1	1	1	0
50X dNTP Mix	0	0	0	0	0	0	0	0	0	1
Primer mix[b]	1	1	1	1	1	1	1	1	1	1
Template DNA[c]	1	1	1	1	1	1	1	1	1	1
TITANIUM *Taq* Polym.	1	1	1	1	1	1	1	1	1	1
Total volume	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**

[a] Standard PCR reaction using TITANIUM *Taq* DNA Polymerase

[b] Experimental or Control Primer Mix (10 µM each primer)

[c] Experimental or Control PCR Template (~1 ng/µl)

2. Mix well and spin briefly to collect all liquid at the bottom of the tubes.
   Note: If you are not using a hot-lid thermal cycler, overlay contents with mineral oil.
3. Commence thermal cycling using the following parameters for either hot-lid or non-hot-lid thermal cyclers:
   - 94°C for 30 sec
   - 25 cycles:
     94°C 30 sec
     68°C 1 min*
   - 68°C for 1 min
   - 4°C soak

   * For experimental mutagenesis reactions with templates longer than 1 kb, add 1 min of extension time per additional kb.

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