U-HiFi PCR Master Mix (2x) (Cat#PU1000)

This protocol provided a general procedure for routine PCR using U-HiFi Master Mixes. These guidelines cover routine PCR. If target templates contains high GC content, high secondary structure, low template concentrations or long amplicons, further optimization would be need.

Protocol

1. All components should be mixed and centrifuged prior to use.
2. Set up all reaction as below table, mix the reaction well, spin down all liquid to the bottom of the tube.
3. Transfer the reactions to a thermocycler preheated to the denaturation temperature (98°C).

Component	25 µl Reaction	50 µl Reaction	Final Concentration
10 µM Forward Primer	1.25 µl	2.5 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	2.5 µl	0.5 µM
Template DNA	Genomic: 25~125 ng Plasmid:1 pg~5 ng	Genomic: 50~250 ng Plasmid:1 pg~10 ng	< 250 ng
DMSO (optional, not provided)	(0.75 µl)	(1.5 µl)	(3%)
Nuclease-free water	to 12.5 µl	to 25 µl	
2X Phusion Master Mix	12.5 µl	25 µl	1x

PCR conditions:

Step	Temp (ºC)	Time (Sec)
Initial Denaturation	98 ºC	30 Sec
25~35 Cycles	98ºC 45~72ºC 72ºC	5~10 Sec 10~30 Sec 15~30 Sec/kb
Final Extension	72ºC	10 min
Hold	4ºC	