PCR Master Mix- Thermopol Buffer (NEB)- All primer sets except Mu

A Master Mix is made and used to save time, reagents, and maximize reproducibility and reliability of the protocol and hence, the results.

The PCR Master Mix consists of PCR grade **H_2O**, **PCR buffer**, **$MgCl_2$** (may be included in the PCR buffer or added separately**), dNTPs**, **primers** (The primers may or may not be included in the Master Mix, depending on the total number of reactions and primer sets used.) and **thermostable polymerase** (i.e. Taq).

A Master Mix is made by multiplying each component by the total number of PCR reactions per primer set (including + and negative controls, and duplicates). In addition, an extra reaction should be added for every 10 reactions to correct for volume errors due to repetitive pipetting.

***Example – 25 rxns** (25 +2 = **27**)

Vol. (µl)/X rxns.	Vol. (µl)/1 rxn.	Reagent	Stock Conc.	Final Conc.
135	5	Buffer w/$MgCl_2$	10X	1X
27	1	dNTPs	10 mM	0.2 mM
27	1	Forward primer	10 µM	0.2 µM
27	1	Reverse primer	10µM	0.2 µM
6.75	0.25	Polymerase	5 units/µl	1.25 units
992.25	36.75	Water		
1215	**45**	**Total volume**		
	5	DNA		5 µl/rxn.

45 µl/rxn. 50 µl total

*** Note:** Total PCR reaction volume and the volume of DNA added to a reaction may vary. These volumes are dependent on the protocol.