

### PCR Master Mix- Flexi Buffer (Promega) – All primer sets

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<sub>2</sub>O**, **PCR buffer**, **MgCl<sub>2</sub>** (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.
270	10	Buffer w/o MgCl <sub>2</sub>	5X	1X
135	5	MgCl <sub>2</sub>	25 mM	2.5 mM
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
722.25	26.75	Water		
1215	<b>45</b>	<b>Total volume</b>		
	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.