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Taq PCR (Protocol for ZymoTaq™ PreMix)

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Protocol status: Working
We use this protocol and it's working

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Guidelines

ZymoTaq™ PreMix is supplied as a 2X concentrated “master mix” containing all the reagents needed to perform “hot start” PCR. The inclusion of a heat-activated, thermal-stable DNA polymerase reduces primer dimer and non-specific product formation that can occur when performing conventional PCR. This unique product is specifically designed for the amplification of bisulfite-treated DNA for methylation detection, realtime and quantitative PCR that are SYBR Green and probe based. The ZymoTaq™ PreMix yields specific amplicon formation with little or no byproducts. Simple and easy to use, just add water, primers, and template DNA to the ZymoTaq™ PreMix and then heat at 95oC for 10 minutes to initiate polymerization.

ZymoTaq™ DNA polymerase is a heat-activated, “hot start” polymerase that has 3′-terminal transferase activity. The addition of “A” overhangs to amplified DNA makes it ideal for use in TA-cloning.

Store ZymoTaq™ PreMix at -20ºC for up to 12 months. Avoid repeated freeze/thawing of reagents. Prolonged storage is at -80ºC

Materials

MATERIALS

ZymoTaq™ PreMix - 50 rxns **Zymo Research Catalog #**E2003

ZymoTaq™ PreMix - 200 rxns **Zymo Research Catalog #**E2004

	Reagent	**Volume**	**Final concentration**
	ZymoTaq™ PreMix	25 µL	1X
	Forward Primer (10 µM)	Variable	0.3 to 1 µM
	Reverse Primer (10 µM)	Variable	0.3 to 1 µM
	Template	Variable	< 200 ng/50 µL
	ddH20	to 50 µL	
	Total volume	50 µL	

1 Add all components in a 250 µL tube making up to a 25 or 50 µl reaction. If performing various PCR with different templates, a Master Mix is recommended to be done.

	Reagent	Volume	Final Concentration
	Zymo Taq™ PreMix	25 µL	1X
	Forward Primer (10 µM)	Variable	0.3 to 1 µM
	Reverse Primer (10 µM)	Variable	0.3 to 1 µM
	Template	Variable	< 200 ng/50 µl
	ddH2O	to 50 µL	
	Total Volume	50 µL	

2 Gently mix the PCR reactions and transfer the tubes to a thermocycler. Thermocycling conditions for a routine PCR:

	Step	Temperature	Time	Cycles
	Initial Denaturation		10 minutes	1
	Denaturation	94-96ºC	30 sec	30-40
	Annealing	Variable	30-40 sec	
	Extension	72ºC	30 sec - 1 minute*	

	Final Extension	72ºC	7 minutes	1
	Hold	4ºC	> 4 minutes	1

* For ≤ 1kb. Add an additional 15-30 seconds to the extension time for each kb > 1 kb. Make adjustments to the temperature and/or time if necessary.