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Turbo Amp 2x mastermix (Without Dye)

Cat # ☐ **PCR016** ☐ **PCR017**

Pack Size: PCR016, 1.25mL, 100 PCR reaction of 25µl; **PCR017, 1.25mLx4**, 400 PCR reaction of 25µl

Storage: -20°C *Note: Make a working aliquots to minimize the number of freeze-thaw cycles . Mix well prior to use. DO NOT VORTEX*

Introduction

Molecular biology relies on precise and efficient DNA amplification techniques to drive various scientific applications, from genotyping to gene expression analysis. Turbo Amp 2X MasterMix (Without Dye) stands as an advanced solution, formulated to meet the stringent requirements of PCR (polymerase chain reaction) experiments. This master mix offers a well-balanced composition without the addition of dye, engineered to enhance and simplify the PCR process while ensuring accuracy. In this introduction, we delve into the key attributes of Turbo Amp 2X MasterMix (Without Dye), exploring its molecular biology applications and its ability to elevate DNA amplification very robustly, mainly to those which are tough targets during genotyping plants.

Protocol

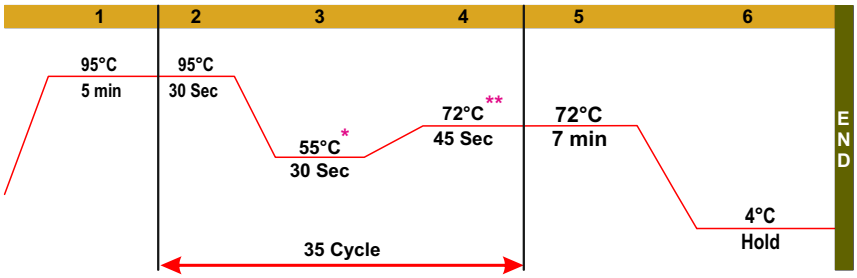
1. Gently vortex and briefly centrifuge PCR Master Mix (2X) after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 25 or 50 µL reaction:

Standard PCR Reaction mix with Turbo Amp 2x mastermix 2X

Component	For 25ul reaction	For 50ul reaction	Final Concentration
Template (DNA)	Variable	Variable	<1,000 ng
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05-1µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05-1µM)
Turbo Amp 2X MasterMix (2X)	12.5 µl	25 µl	1X
Nuclease-Free Water	to 25 µl	to 50 µl	

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below

General PCR Condition for 1 kb amplification



* Depends on the primer Tm and template used (Need to be optimized)
** Allow approximately 2 minutes for every 1kb to be amplified.

Result

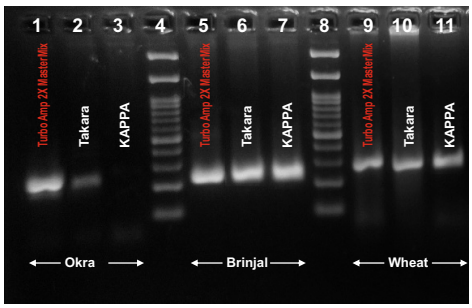


Fig: PCR amplification results are presented for different plant samples. Lanes 1, 2, and 3 depict the amplification of Okra DNA using Turbo Amp 2X MasterMix, Takara Mastermix, and KAPPA Taq Polymerase, respectively. Lane 4 shows the 100-base pair ladder (L15), which serves as a size reference. Lanes 5, 6, and 7 display the amplification of Brinjol DNA using the same three PCR reagents. Lane 8 features the 100-base pair ladder (L15) once again. Finally, Lanes 9, 10, and 11 illustrate the amplification of Wheat DNA using Turbo Amp 2X MasterMix, Takara Mastermix, and KAPPA Taq Polymerase. These results provide a comparative analysis of the performance of different polymerase master mixes in PCR amplification across various plant species.

1. No amplification or low yield

- Ensure that the Turbo Amp 2X MasterMix and other components are added correctly and mixed well
- Check that the annealing temperature is appropriate for the primers used
- Optimize the annealing temperature by performing a temperature gradient PCR
- Increase the amount of template DNA or adjust the template DNA concentration if necessary
- Check the quality of the template DNA, which may be degraded or inhibited
- Increase the extension time or number of cycles in the PCR program
- Make sure the PCR machine is functioning properly
- Use fresh Turbo Amp 2X MasterMix, as they can lose activity over time or after multiple freeze-thaw cycles

2. Non-specific amplification

- Ensure that the annealing temperature is appropriate for the primers used
- Optimize the annealing temperature by performing a temperature gradient PCR
- Check the specificity of the primers by analyzing the PCR product by gel electrophoresis and sequencing
- Check that the primer concentration is appropriate, as too high or low primer concentrations can cause non-specific amplification
- Reduce the annealing time, as a longer annealing time can increase non-specific amplification
- Increase the extension time, as a shorter extension time can lead to non-specific amplification

- **3. Smearing or multiple bands**

- Ensure that the annealing temperature is appropriate for the primers used
- Optimize the annealing temperature by performing a temperature gradient PCR
- Check the template DNA concentration, as too much or too little template DNA can cause smearing or multiple bands
- Use a lower annealing temperature, as a higher annealing temperature can cause smearing or multiple bands
- Use a higher extension time or temperature, as a shorter extension time or lower extension temperature can cause smearing or multiple bands

4. PCR inhibition

- Check the purity of the template DNA, as contaminants can inhibit PCR amplification
- Use a different extraction method for the template DNA if necessary
- Dilute the template DNA to reduce PCR inhibition
- Use of additives such as BSA or DMSO may improve PCR amplification in difficult samples