How to setup and perform a PCR experiment using GoTaq® G2 Master Mixes

Promega Corporation

Abstract

GoTaq® G2 Green Master Mix(a,b) is a premixed ready-to-use solution containing GoTaq® G2 DNA Polymerase, dNTPs, MgCl2 and reaction buffers at optimal concentrations for efficient amplification of a wide range of DNA templates by PCR. GoTaq® G2 Green Master Mix contains two dyes (blue and yellow) that allow monitoring of progress during electrophoresis. Reactions assembled with GoTaq® G2 Green Master Mix have sufficient density for direct loading onto agarose gels. GoTaq® G2 DNA Polymerase exhibits 5 →3 exonuclease activity.

GoTaq® G2 Green Master Mix is recommended for any amplification reaction that will be visualized by agarose gel electrophoresis and ethidium bromide staining. The master mix is not recommended if any downstream applications use absorbance or fluorescence excitation, as the yellow and blue dyes in the reaction buffer may interfere with these applications. The dyes absorb wavelengths in the range of 225–300nm, making standard A260 readings to determine DNA concentration unreliable. The dyes have excitation peaks at 488nm and in the range of 600–700nm that correspond to the excitation wavelengths commonly used in fluorescence detection instrumentation. For some instrumentation, such as a fluorescent gel scanner that uses a 488nm excitation wavelength, there will be minimal interference since it is the yellow dye that absorbs at this wavelength. Gels scanned by this method will have a light grey dye front (corresponding to the yellow dye front) below the primers.

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Guidelines

General Guidelines for Amplification by PCR

A. Denaturation

- Generally, a 2-minute initial denaturation step at 95°C is sufficient.
- Subsequent denaturation steps will be between 15 seconds and 1 minute.

B. Annealing

- Optimize the annealing conditions by performing the reaction starting approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 1°C to the annealing temperature.
- The annealing step is typically 15 seconds to 1 minute.

C. Extension

- The extension reaction is typically performed at the optimal temperature for Taq DNA polymerase, which is 72–74°C.
- Allow approximately 1 minute for every 1kb of DNA to be amplified.
- A final extension of 5 minutes at 72–74°C is recommended.

D. Refrigeration

- If the thermal cycler has a refrigeration or ”soak” cycle, the cycling reaction can be programmed to end by holding the tubes at 4°C for several hours.
- This cycle can minimize any polymerase activity that might occur at higher temperatures, although this is not usually a problem.

E. Cycle Number

- Generally, 25–30 cycles result in optimal amplification of desired products.
- Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.

General Considerations

A. GoTaq® G2 Green Master Mix Compatibility

GoTaq® G2 Green Master Mix is compatible with common PCR additives such as DMSO and betaine. These additives neither change the color of GoTaq® G2 Green Master Mix nor affect dye migration.

If both agarose gel analysis and further downstream applications involving absorbance or fluorescence will be used, the two dyes can be removed from reactions using standard PCR clean-up systems such as the Wizard® SV Gel and PCR Clean-Up System (Cat.#A9281).

B. Primer Design

PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Primers should contain 40–60% (G + C), and care should be taken to avoid sequences that might produce internal secondary structure. The 3 -ends of the primers should not be complementary to avoid the production of primer-dimers. Primerdimers unnecessarily deplete primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3 -end of the primer, as this may result in nonspecific primer annealing, increasing the synthesis of undesirable reaction products. Ideally, both primers

should have nearly identical melting temperatures (Tm); in this manner, the two primers should anneal roughly at the same temperature. The annealing temperature of the reaction is dependent upon the primer with the lowest Tm. For assistance with calculating the T_m of any primer, a T_m Calculator is provided on the BioMath page of the Promega web site at: **www.promega.com/biomath/**

C. Amplification Troubleshooting

To overcome low yield or no yield in amplifications (e.g., mouse tail genotyping applications), we recommend the following suggestions:

- Adjust annealing temperature. The reaction buffer composition affects the melting properties of DNA. See BioMath Calculator to calculate the melting temperature for primers in the GoTaq® reaction (www.promega.com/biomath/).
- Minimize the effect of amplification inhibitors. Some DNA isolation procedures, particularly genomic DNA isolation, can result in the copurification of amplification inhibitors. Reduce the volume of template DNA in reaction or dilute template DNA prior to adding to reaction. Diluting samples even 1:10,000 has been shown to be effective in improving results, depending on initial DNA concentration.
- Increase template DNA purity. Include an ethanol precipitation and wash step prior to amplification to remove inhibitors that copurify with the DNA.
- Add PCR additives. Adding PCR-enhancing agents (e.g., DMSO or betaine) may improve yields. General stabilizing agents such as BSA (Sigma Cat.# A7030; final concentration 0.16mg/ml) also may help to overcome amplification failure.

D. More Information on Amplification

More information on amplification is available online at the Promega web site: **www.promega.com/products/pcr/**

Materials

GoTaq® G2 Green Master Mix M7822 by Promega

- template DNA by Contributed by users
- upstream primer by Contributed by users
- downstream primer by Contributed by users
- mineral oil (optional) by Contributed by users

Protocol

Step 1.

Thaw the GoTaq® G2 Green Master Mix at room temperature.

TEMPERATURE
20 °C Additional info: Thawing

REAGENTS
GoTaq® G2 Green Master Mix M7822 by Promega

NOTES
Trevor Wagner 12 Dec 2017

Minimize the number of freezethaw cycles by storing in working aliquots.

Product may be stored at 4°C for up to 18 weeks.

Mix well prior to use.

Step 2.

Vortex the Master Mix, then spin it briefly in a microcentrifuge to collect the material at the bottom of the tube.

Step 3.

Prepare one of the following reaction mixes on ice:

For a 25µl reaction volume:

Component	Volume	Final Conc.
GoTaq® G2 Green Master Mix, 2X	12.5µl	1X
upstream primer, 10µM	0.25–2.5µl	0.1–1.0µM
downstream primer, 10µM	0.25–2.5µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	25µl	N.A.

NOTES
Trevor Wagner 05 Dec 2017

For a 50µl reaction volume:

Component	Volume	Final Conc.
GoTaq® G2 Green Master Mix, 2X	25µl	1X
upstream primer, 10µM	0.5–5.0µl	0.1–1.0µM
downstream primer, 10µM	0.5–5.0µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	50µl	N.A.

Step 4.

If using a thermal cycler without a heated lid, overlay the reaction mix with 1–2 drops (approximately 50µl) of mineral oil to prevent evaporation during thermal cycling.

AMOUNT
50 µl Additional info: Mineral oil

Step 5.

Centrifuge the reactions in a microcentrifuge for 5 seconds.

Step 6.

Place the reactions in a thermal cycler that has been preheated to 95°C.

TEMPERATURE
95 °C Additional info: Preheated thermal cycler

Step 7.

Perform PCR using your standard parameters.