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SapphireAmp PCR Master Mix -- CHEM 584

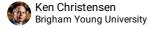
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Works for me

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ABSTRACT

SapphireAmp Fast PCR Master Mix contains a hot start PCR enzyme, optimized buffer, dNTP mixture, gel loading dye (blue), and a density reagent as a 2X premix. SapphireAmp Fast PCR Master Mix is optimized for fast PCR and offers a rapid extension rate (10 sec. per kb). The inclusion of blue dye and a density reagent allows direct loading of PCR products on an agarose gel for electrophoresis. The master mix format simplifies workflows and sample handling; simply add primers, template, and water and then begin PCR. SapphireAmp Fast PCR Master Mix is ideal for fast colony PCR screening. Fast colony PCR amplification of a 5 kb insert can be completed in approximately 1 hr 15 min. Furthermore, it is possible to amplify fragments up to 6 kb from genomic DNA templates.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://www.takarabio.com/assets/documents/User%20Manual/RR350A_DS.v1902Da.pdf

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GUIDELINES

Storage: -20 °C for long-term storage. 4 °C for short-term storage (up to 3 months). (Note) If used frequently, store at 4 °C; the activity of the Master Mix may decrease with repeated freezing and thawing. Gently mix well before use and centrifuge briefly.

Application:

MDNA amplification by PCR MColony PCR

 $\textbf{Citation:} \ \ \text{Ken Christensen (09/23/2020)}. \ \ \text{SapphireAmp PCR Master Mix-CHEM 584}. \ \ \underline{\text{https://dx.doi.org/10.17504/protocols.io.bmc7k2zn}}$

PCR Products: Since most PCR products amplified with SapphireAmp Fast PCR Master Mix have an A overhang added at 3'-termini, the obtained PCR product can be used directly for cloning into a T-vector. Additionally, it is possible to clone the product in a blunt-end vector after blunting and phosphorylation of the end.

Dye Migration During Electrophoresis: When 5 μ l of the PCR sample is loaded on a 1% gel made with Agarose L03 [TAKARA] (Cat. #5003) and subjected to electrophoresis, the blue dye fronts are detected at positions corresponding to 1 kb and 3 - 5 kb. The absorption maxima for the dyes are 260 nm and 620 nm, respectively. The dyes may be removed by isolating and purifying the DNA fragment from the gel or extracting DNA with NucleoSpin Gel and PCR Clean-Up (Cat. #740609.50/.250), if necessary

Setup Reaction

1 To a 12.5 μl aliquot of SapphireAmp PCR Master Mix, add template (4 μl cleared lysate for colony PCR or < 1 ng of purified DNA for typical PCR), forward and reverse primers to a final concentration of [M]200 Nanomolar (nM). Adjust final volume to 25 μl with nuclease free water or autoclaved water.

Sapphire Master Mix	12.5 ul (pre-
	aliquoted and
	stored in the
	freezer)
Template	4 ul of bacterial
	lysate or <1 ng
	DNA
Forward Primer	0.5 ul of 10 uM
	primer dilution
Forward Primer	0.5 ul of 10 uM
	primer dilution
ddH2O	to a final
	volume of 25 ul

Run Reaction

2 followed by 30 cycles of 98°C, 5 sec; 55°C, 5 sec; and 72°C, 40 sec.

Initial denature	98C	1 minute
Denature	94C	10 seconds
Anneal	55C	10 seconds
Extension	72C	30 seconds
Repeat steps 2-4		30-40x
Final extension	72	1 minute
Cool	4C	Until cancelled

A typical thermocycling program for a quick PCR for amplicons less than 1 kb. For longer amplicons, adjust the program to 10 seconds per kb for the extension and final extension times.