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SapphireAmp PCR Master Mix -- CHEM 584 V.1

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We use this protocol and it's working

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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.

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:
・DNA amplification by PCR
・Colony PCR

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 µl 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.