I. Introduction

SuperPlex Premix (Cat. Nos. 638543 & 638544) is a hot-start PCR formulation based on Titanium® *Taq* DNA Polymerase, a 5′- to 3′-exonuclease-deficient *Taq* polymerase, and the TaqStart® Antibody. This 2X premix contains polymerase, reaction buffer, and dNTPs. SuperPlex Premix is ideal for multiplex PCR where unbiased amplification of different targets and high amplicon yield are important.

II. Primer Design

Primer design is the single largest variable in PCR applications and the single most important factor in determining the success or failure of PCR reactions. In general, primers should have a T_m of ~60°C to achieve optimal results in a two-step cycling program with a 68°C combined annealing/extension step. Whenever possible, primers should have a GC content of 45–60%. Furthermore, the 3' ends of the primers should not be complementary to each other, and the absence of primer pairing between assays should be validated before multiplex PCR.

NOTE: Always check your primer design before constructing or ordering primers.

III. Reaction Setup

Combine the following in a PCR tube on ice:

25 µl	2X SuperPlex Premix
2.5 µl	5' primer (4 µM)
2.5 µl	3' primer (4 µM)
1 µl	DNA Template (variable)
Up to 50 µl	PCR-Grade Water
50 µl	Total Volume

IV. Recommended Cycling Conditions for Singleplex PCR

Use the following guidelines when setting up your initial experiments with SuperPlex Premix. These are general guidelines; the optimal cycling conditions may vary with different thermal cyclers and will depend on your specific primers, templates, and other experimental variables.

95°C	1 min	
95°C	30 sec	25–30 cycles
68°C	1–3 min	
68°C	3 min	
4°C	Hold	

V. Recommended Cycling Conditions and Input DNA Amount for Multiplex PCR

Due to the high DNA yields resulting from the polymerase used in the SuperPlex Premix, we recommend shorter PCR extension and annealing steps when performing multiplex PCR experiments. Start with a 15-sec extension time for the largest size fragment in the multiplex and increase the time if necessary. A 15-sec extension time has been used successfully in a 20-plex setup with targets ranging from 70–700 bp. Please note that increasing the number of targets may require a longer extension time. Extension times should be optimized for each multiplex reaction. At least 10 ng of input DNA is recommended for a multiplex reaction.

95°C	2 min	
95°C	10 sec	⎫
60°C	10 sec	⎬ 25–35 cycles
72°C	15 sec	⎭
4°C	Hold	

VI. Recommendations for Electrophoresis

Expected insert size range	% agarose	Recommended DNA size markers
<0.3 kb	2–4	AmpliSize Molecular Ruler 50–2,000 bp ladder (BioRad)
0.3–1.5 kb	1.5	φX174/HaeIII
0.5–10 kb	1.2	1 kb DNA ladder
>5 kb	0.8	λ/HindIII

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