Protocol-at-a-Glance

Titanium® *Taq* PCR Kit
Protocol-at-a-Glance

(PT3304-2)

Please read the *User Manual* before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

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. *Always check your primer design before constructing or ordering primers.*

Titanium *Taq* can be used in a wide variety of PCR applications, and the constraints on primer design will vary from one application to the next. In general, primers should have a T_m of ~70°C to achieve optimal results in a two-step cycling program with a 68°C combined annealing/extension step. Therefore, whenever possible, primers should be *at least* 22 nucleotides long (25–30-mers are preferred) and should have a GC content of 45–60%. Furthermore, the 3' ends of each primer should not be complementary to each other and should have a low GC content.

Setting up the Reaction

Combine the following in a PCR tube on ice:

Volume	Reagent
40 µl	PCR-Grade Water
5 µl	10X Titanium *Taq* PCR Buffer
1 µl	50X dNTP Mix (10 mM ea.)
1 µl	5' primer (10 µM)
1 µl	3' primer (10 µM)
1 µl	50X Titanium *Taq* DNA Polymerase
1 µl	DNA Template (100 ng/µl)
50 µl	**Total volume**

Recommended Cycling Conditions

Use the following guidelines when setting up your initial experiments with Titanium *Taq*. These are general guidelines—the optimal cycling conditions may vary with different thermal cyclers and will depend on your particular primers, templates, and other experimental variables.

95°C for 1 min } 1 cycle

95°C 30 sec
68°C 1–3 min } 25–35 cycles

68°C for 3 min } 1 cycle

Recommendations for Electrophoresis

Expected insert size range	% agarose	Recommended DNA size markers
0.3–1.5 kb	1.5	ϕX174/*Hae* III
0.5–10 kb	1.2	1 kb DNA ladder
>5 kb	0.8	λ/*Hin*d III

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This document has been reviewed and approved by the Clontech Quality Assurance Department.