

I. 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* SP DNA Polymerase (Cat. No. 639292) 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.

II. Setting up the Reaction

Combine the following in a PCR tube on ice:

Volume	Reagent
40 µl	PCR-Grade Water
5 µl	10X Titanium <i>Taq</i> SP 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 <i>Taq</i> SP DNA Polymerase
1 µl	DNA Template (100 ng/µl)
50 µl	Total Volume

III. Recommended Cycling Conditions

Use the following guidelines when setting up your initial experiments with Titanium *Taq* SP DNA Polymerase. 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	1 min	
<u>25–30 cycles:</u>		
95°C	30 sec	}
68°C	1–3 min	
68°C	3 min	
4°C	forever	

IV. 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/Hind III

Contact Us For Assistance	
Customer Service/Ordering	Technical Support
Telephone: 800.662.2566 (toll-free)	Telephone: 800.662.2566 (toll-free)
Fax: 800.424.1350 (toll-free)	Fax: 800.424.1350 (toll-free)
Web: www.clontech.com	Web: www.clontech.com
E-mail: orders@clontech.com	E-mail: tech@clontech.com

Notice to Purchaser

General Purpose Reagent. This product is intended For Laboratory Use. Outside of the United States, this product is intended for research use only unless otherwise stated. This product is not intended for a specific application or made for any clinical use. The performance characteristics of this product have not been fully established. It is the user's responsibility to validate the performance of the product, and any component thereof, for any particular use. Resale or transfer of this product, any component thereof, or any substance produced through use of this product, or any component thereof, to any third party is expressly forbidden. To obtain additional rights, please contact BD_OEM@clontech.com.

Your use of this product is also subject to compliance with the licensing requirements listed below and described on the product's web page at <http://www.clontech.com>. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

Clontech®, the Clontech logo, and Titanium are trademarks of Clontech Laboratories, Inc. All other marks are the property of their respective owners. Certain trademarks may not be registered in all jurisdictions. Clontech is a Takara Bio Company. ©2015 Clontech Laboratories, Inc.

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