

DD cDNA Amplification Protocol

PT5154-2

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I. Introduction

The following protocol produces PCR-amplified, ProteoTuner™ DD cDNA suitable for In-Fusion® cloning into any Living Colors® fluorescent protein-C vector.

II. Recommendations

1. We strongly recommend using the Advantage® HD PCR Kit (Cat. No. 639241) with this protocol.
2. Thaw all reagents on ice.

III. Protocol

1. Prepare the PCR reaction mix at room temperature by adding each of the components indicated in Table I (below) to PCR tubes or plates.

NOTE: If you are using a thermostable DNA polymerase other than Advantage HD, adjust the volumes of buffer, enzyme, and water added to the reaction mix according to your enzyme's specifications.

Table I: PCR Reaction Mix	
Reagent	Volume (µl)
PCR-Grade H ₂ O, Sterile	32.5
5X Advantage HD Buffer*	10
dNTP Mix (2.5 mM each)	4.0
Primer Mix (10 µM)	2.0
Advantage HD Polymerase (2.5 U/µl)	0.5
pDD Vector (template; 20 ng/µl)	1.0
Total volume per rxn	50.0

*5X Advantage HD Buffer contains 5mM MgCl₂.

2. Mix well and spin down briefly.

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3. Program your thermal cycler with the following cycling conditions:

Denaturation (1 Cycle):	94°C 15 sec
PCR (25 Cycles):	98°C 10 sec 67°C 25 sec

4. Place your samples in the thermal cycler and begin cycling.

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