# DD cDNA Amplification Protocol

**PT5154-2**

## Table of Contents



## 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 $H_2O$, 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 $MgCl_2$.

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<br>67°C 25 sec |

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

## Notice to Purchaser



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