# Template Amplification Protocol

Written by **Alan Tomusiak**

**Written:** May 19, 2019, **Printed:** 19th May, 2019

## 1 Procedure Purpose

In order to obtain useful Sanger sequencing results for testing single-basepair addition, large quantities of template DNA are necessary. This protocol uses an existing template and PCR to generate a large quantity of template DNA useful for testing the backspace synthesis method.

## 2 Safety Information

1. **SYBR Gold** has no data available addressing the mutagenicity or toxicity of SYBR® Gold nucleic acid gel stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.[1]

## 3 Materials

- AD Template
- 100$\mu$M Dev_Round2_Fwd_Fixed Primer
- 100$\mu$M Dev_Round2_Rev_2_Try2 Primer
- 2X Phusion Master Mix
- 100% DMSO
- De-ionized Water
- Agarose
- 10,000X SYBR Green I

## 4 Dilutions

1. Dilute the AD template such that its final concentration in the PCR reaction will be 1ng/$\mu$L.

## 5 Procedure

1. Mix the following reagents in either a PCR tube or a 96-well plate such that the final concentrations match the following:
   (a) AD Template: 1ng/$\mu$L.
   (b) 2X Phusion Master Mix: 1X.

(c) 100$\mu$M Dev_Round2_Fwd_Fixed Primer: .5$\mu$M.

(d) 100$\mu$M Dev_Round2_Rev_2_Try2 Primer: .5$\mu$M.

(e) 100% DMSO: 3%.

**HELPFUL TIP**

You can, and should, create a large master mix with enough materials for multiple reactions and then aliquot smaller volumes into either PCR tubes or 96-well plate wells.

2. Place the tubes or plate into a thermocycler. Set up the following PCR cycle:

   (a) 98°C for 30 seconds.

   (b) 98°C for 10 seconds.

   (c) 57°C for 20 seconds.

   (d) 72°C for 15 seconds.

   (e) Repeat B-D 35 times.

   (f) 72°C for 40 seconds.

3. Validate a successful PCR by running samples on a gel.

4. Use a PCR purification kit on validated samples to extract pure DNA.

# 6 Analysis

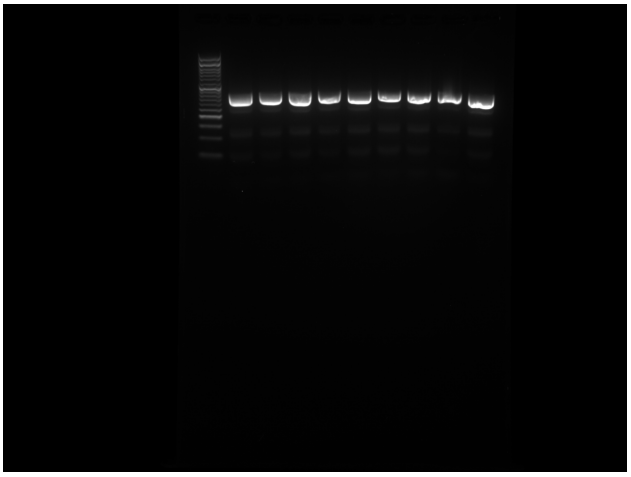

Figure 1: An example of a successful PCR amplification of the AD template.

# References

[1] Invitrogen, "SYBR® Gold Nucleic Acid Gel Stain — 2 Working with the SYBR® Gold Gel Stain,"