

Template Amplification Protocol

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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 μ M Dev_Round2.Fwd.Fixed Primer
- 100 μ 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/ μ 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/ μ L.
 - (b) 2X Phusion Master Mix: 1X.

- (c) 100 μ M Dev_Round2_Fwd_Fixed Primer: .5 μ M.
- (d) 100 μ M Dev_Round2_Rev_2_Try2 Primer: .5 μ 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.

OPTIONAL STOP POINT

3. Validate a successful PCR by running samples on a 2% gel.
4. Use a PCR purification kit on validated samples to extract pure DNA.

6 Analysis

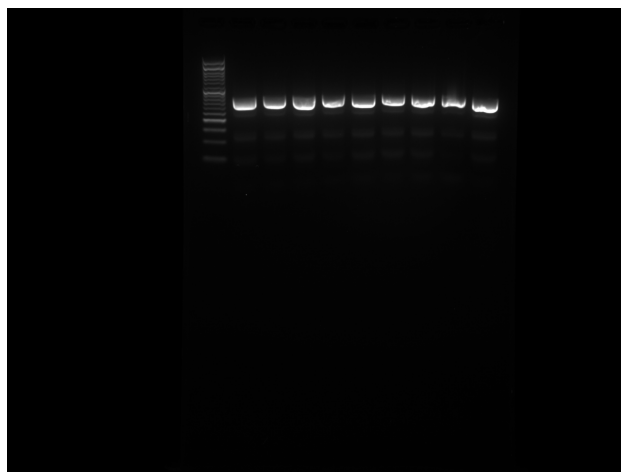


Figure 1: An example of a successful PCR amplification of the AD template.

References

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

