BstXI Restriction Digestion Protocol

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Written: May 20, 2019, **Printed:** 21st May, 2019

1 Procedure Purpose

Perform BstXI restriction digestion on AD template DNA in order to create a free 3' overhang. This overhang can be used for basepair addition in order to test the backspace 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
- BstXI (10000 units/mL)
- 10X NEBuffer 3.1
- Nuclease-free Water
- Agarose
- 1X Tris-Borate-EDTA (TBE) Buffer
- Thermocycler
- Gel-running apparatus.
- PCR 8-strip tubes OR 96-well plate.

4 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) 1μg AD template
   (b) 1X NEBuffer 3.1
   (c) 10 units BstXI
2. Fill remaining volume with nuclease-free water.

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.

3. Incubate at 37°C for twenty minutes, and then at 80°C for 20 minutes.

OPTIONAL STOP POINT

4. Validate successful restriction digestion utilizing a 2% agarose gel.
5. If necessary, further validate successful restriction digestion by sending in a purified sample to be Sanger sequenced.

References

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