Assay Development Proof of Concept I

Written by **Alan Tomusiak**

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1 Procedure Purpose

Perform a full validation of whether the technique employed by the assay development team is capable of testing backspace synthesis. Test every step in the assay to troubleshoot any problematic components. Determine whether a 5-bp or a 6-bp complementary strand will lead to more efficient nucleotide addition. Create positive and negative control results for future comparison.

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 Sample Explanations

4 Materials

- BstXI-Digested, Verified AD Template
- Verified AD Positive Control
- 100μM Dev_Seq_Fwd2 Primer
- 100μM Dev_Comp_5_Pure Primer
- 100μM Dev_Comp_6_Pure Primer
- Agarose
- Nuclease-free Water
- 1X Tris-Borate-EDTA (TBE) Buffer
- 10,000X SYBR Green I
- Thermocycler
- 10X TdT Buffer Pack
- 10X CoCl2
- Exonuclease T

- NEBuffer 4
- EDTA
- Qiagen PCR Purification Kit
- 100mM dATP
- 100mM dTTP
- 10mM dNTPs
- 10X Taq Reaction Buffer Pack
- Oligo d(T)18 Primer

5 Procedure

1.

References

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