Mismatch sensitive nuclease (T7E1) assay protocol

T7 endonuclease 1 (T7E1) recognizes and cleaves non-perfectly matched DNA generated. T7E1 mismatch detection assay is a simple method to check if there has been any alteration in genome locus, such as small insertion and deletion events after CRISPR/Cas9 gene editing. Surveyor nuclease kit from Transgenomics can be used as an alternative.

1. Genomic DNA prep

Prep genomic DNA. It is important to remove all the chemicals that can affect PCR such as SDS.

2. PCR of genomic DNA at the target site
    a. It is critical to set up a robust PCR to amplify the target region. If necessary, run a nested PCR (N-PCR) to improve sensitivity .
    b. Design the primer that amplifies 400~1000 bp for PCR. Run 35~40 cycles.
    c. Make sure the band is strong and little smearing is present.

3. Denature and annealing of PCR products
    a. Complete denaturation and slow annealing allows the formation of heteroduplex DNA.
    b. Set up a program in PCR machine as below.
        i. Step1: 9 ^{o}C 4min denaturation
        ii. Step2: fast annealing by lowering temperature 2^{o}C per second to 85^{o}C
        iii. Step3: slow annealing by lowering temperature 0.1^{o}C per second to 25^{o}C
        iv. Step 4: 16^{o}C forever
    c. The reaction mixture can be stored at 4^{o}C for 1~2 days.

4. T7 endonuclease I (T7E1) reaction
    a. Prepare T7E1 reaction mixture as below.

10x NEB #2 buffer	2 ul
PCR product	10~15 ul (0.5~1 ug)
T7E1 (NEB)	0.25 ul
DI water	up to 20 ul

    b. Incubate for 15~20 min at 37^{o}C.
        - Reaction time and amount of T7E1 enzyme may need optimization. Too long incubation or excess T7E1 activity can cause non-specific degradation of DNA.

5. Agarose gel electrophoresis
    a. Prepare 2~2.5% agarose gel.
    b. Analyze the digested product in the gel.

* Make sure to run the same amount of DNA before T7E1 digest (after step 3) as a negative control.