Confirmation that CRISPR samples where cut with PvuII

Restriction Digest

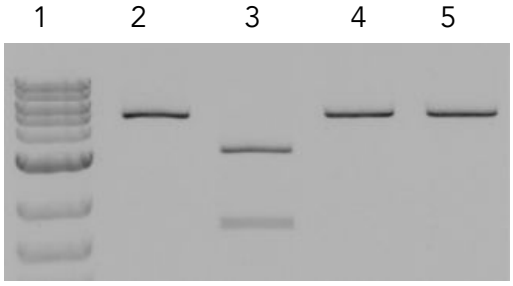
1. Label 4 microreaction tubes
2. Add all components to each tube in the order shown:

2 µL 10x buffer
2 µL restriction enzyme
10 µL sterile water

Additionally in tube 2 add 3ul of mutated SERPINA
In tube 3 add 1ul of SERPINA, 1ul of sgRNA & 1ul of CAS9 nuclease
In tube 4 add 2ul of SERPINA & 1 ul of sgRNA
In tube 5 add 2ul of SERPINA & 1ul of CAS9 nuclease

Total volume 17ul per tube

3. Incubate the restriction digest at 37°C for 1 hour.
4. Add 2ul of loading dye and place samples into prepared 8% agarose gel with 1x TBE buffer.
5. Run for 30 min at 150V
6. Remove from Electrphoresis Chamber
7. Place Blue Istastain Card on top of gel for 10min
8. Destain with distilled water
9. Take picture , analyze and confirm



Lane 1 DNA standard markers
Lane 2: Mutated SERPINA
Lane 3: Mutated SERPINA & sGRNA & Cas9 Nuclease
Lane 4: Mutated SERPINA & Cas9 Nuclease
Lane 5: Mutated SERPINA & sGNRA

Shows confirmation that samples were successfully cut