

U6-based sgRNA ligation mixture

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Abstract

1 μ l 10x CutSmart buffer, 1 μ l T4 DNA ligase buffer, 0.25 μ l U6 plasmid (about 100ng), 1 μ l annealed oligos, 0.3 μ l T4 DNA ligase, 0.3 μ l BsmBI, 0.2 μ l PstI (optional), 0.2 μ l Sall (optional), 5 μ l H₂O

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Protocol

Step 1.

1 μ l 10x CutSmart buffer

Step 2.

1 μ l T4 DNA ligase buffer

Step 3.

0.25 μ l U6 plasmid (about 100ng)

Step 4.

1 μ l annealed oligos

Step 5.

0.3 μ l T4 DNA ligase

Step 6.

0.3 μ l BsmBI

Step 7.

0.2 μ l PstI (optional)

Step 8.

0.2 μ l Sall (optional)

Step 9.

5 μ l H₂O