C) sgRNA synthesis

Kit used:
https://international.neb.com/products/e3322-engen-sgrna-synthesis-kit-s-pyogenes#Product%20Information

Materials not included in the Kit:
Target-specific DNA oligo(s) → based on the original DNA
Nuclease-free water
Spin columns for RNA cleanup
Kit used: https://www.neb.com/products/t2040-monarch-rna-cleanup-kit-50-ug#Product%20Information

RNase-free tubes, aerosol tips
Cas9 Nuclease, S. pyogenes
EnGen Spy Cas9, NLS

Storage Temperature
-20°C

Lab equipement:
Pipets
Gelelectrophoresis machine
Microcentrifuge
Thermocycler/37°C heat block/incubator

sgRNA synthesis protocol
We strongly recommend wearing gloves and using nuclease-free tubes and reagents. Reactions should be assembled in microfuge tubes or PCR strip tubes.

1. Thaw EnGen 2X sgRNA Reaction Mix, S. pyogenes, 0.1 M DTT and customer-supplied target-specific oligo (1 µM). Mix and pulse-spin each component in a microfuge prior to use. Store enzyme mix on ice but assemble reaction at room temperature.
2. Assemble the reaction at room temperature in the order listed. Avoid master mixes, and add the enzyme last to each reaction:

REAGENT	AMOUNT
Nuclease-free water	2 µl
EnGen 2X sgRNA Reaction Mix, S. pyogenes	10 µl (salts for the enzyme to work)

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Target-specific DNA Oligo (1 µM)	5 µl (primers we designed)
DTT (0.1 M)	1 µl
EnGen sgRNA Enzyme Mix	2 µl (T7 RNA polymerase, Taq polymerase)
Total volume	20 µl

1. Mix thoroughly and pulse-spin in a microfuge. Incubate at 37°C for 30 minutes.
2. Transfer reaction to ice.
3. For DNase treatment bring volume to 50 µl by adding 30 µl of nuclease-free water. Add 2 µl of DNase I (RNase-free, provided), mix and incubate at 37°C for 15 minutes.
4. Proceed with purification of RNA or analysis by gel electrophoresis.

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