HYBRID
LAB NETWORK

Gel Electrophoresis, run and analysis

The objective of this step is to run the synthesized purified product in a gel to see if the purification worked. Also, to allow a rough quantification of the sgRNA by the intensity of fluorescence of the bands. It will be used as a standard lab procedure (Agarose gel electrophoresis) to resolve linear RNA/DNA fragments on the basis of their molecular weight (length in base pairs, bp) for visualization/ purification.

Agarose Gel Electrophoresis - 15min + 60 min = 1.15h

Agarose gel electrophoresis is a standard lab procedure that can be used to resolve linear RNA/DNA fragments on the basis of their molecular weight (length in base pairs, bp) for visualization/purification.
Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode, with smaller fragments moving through the gel faster than larger ones. When the gel is stained with a RNA/DNA-binding dye, the fragments can be seen as bands in the gel, each representing a group of same-sized fragments. The size of fragments can be determined by comparing the travelled distances with standards of known size in the same run.

Agarose Gel Electrophoresis - Needed Materials:

- Agarose powder;
- 1x TAE (Tris-acetate-EDTA) solution;
- GreenSafe Premium (nzytech);
- 6x TriTrack DNA Loading Dye (Thermo Scientific);
- GeneRuler 1 kb DNA Ladder (Thermo Scientific);

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- Pipette tips and Micropipettes;
- Gel casting tray (UV-transparent plastic) and well comb;
- Microwave;
- Electrophoresis chamber;
- Voltage source;
- Transilluminator (UV light source).

Agarose Gel Electrophoresis - General Protocol:

a) Add 1g of agarose powder to 100 mL of 1xTAE solution in a microwavable flask (for a 1% agarose gel).

b) Microwave until the agarose is completely dissolved.
NOTE: Do not let agarose boil or overheat. The agarose solution can easily boil over. It can also become superheated and NOT boil over until taken out.

c) Let the agarose solution cool down until it is no longer hot to the touch (about 50°C) and add about 3µL of GreenSafe Premium, mix gently.
GreenSafe is a fluorescent dye used for staining nucleic acids. The DNA/RNA-bound dye emits visible light when exposed to ultraviolet light.

d) Pour the agarose into a gel tray with the well comb in place and wait until the gel is completely solidified. When solidified, carefully remove the comb.
The molten agarose is poured around the comb to form the sample wells in the gel.

e) Place the agarose gel, in the tray, in the electrophoresis tank, submerged in 1xTAE solution.

f) Add 1µL of TriTrack DNA Loading Dye (6X) per 5µL of DNA/RNA sample;
The loading dye contains glycerol to allow the sample to "fall" into the sample wells, and three tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.

g) Carefully load 3 µL the molecular weight ladder, GeneRuler 1 kb DNA Ladder, into the first well of the gel, followed by the remaining samples in the following wells.
The DNA ladder contains several DNA fragments of known size, which are used to calibrate the gel.

h) Run the 1% gel at 100V for 10-15 min (RNA samples) / 30-60 min (DNA samples).
The DNA/RNA in the gel will degrade over time, with RNA being destroyed much faster than DNA, therefore, run times should be very short for RNA samples.

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Higher voltages reduce the run time, as RNA/DNA-migration becomes faster, but can cause the gel to overheat resulting in sample degradation and/or gel melting.

i) Visualize the RNA/DNA fragments in the gel under an UV light.
Always wear protective eyewear when observing nucleic acids on a Transilluminator to prevent damage to the eyes from UV light.

The expected sizes of the sgRNAs / DNA fragments can be found in the SUMMARY OF SEQUENCES.

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