Cleavage Assay

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Abstract

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Protocol

Step 1.

If needed, dilute cleavage template, gRNA, and Cas9 stock solutions in molecular-grade H2O to working concentrations.

For example, I often dilute 6 ug/uL Cas9 1:11 to create 500 n

Calculate amounts

Step 2.

Decide on amounts of each component per reaction. For example, my PCSK9 experiments were optimized to run with 300 ng Cas9, 300 ng gRNA, and 400 ng cleavage template.

Set up a matrix in your lab notebook with amounts and volumes of each component. For proper controls, each combination of components should be run (ex. include tubes with only Cas9, tubes with gRNA and cleavage template, etc. etc.).

Magnesium is also vital for cleavage. I usually run with 2 mM MgCl2 (1 uL of 20 mM stock), but running with as low as .5 mM never affected cleavage.

Calculate amount of water added to each tube to create a 10uL reaction volume per tube

Heat gRNA

Step 3.

Place the volume of gRNA calculated in the previous step into all tubes that need gRNA, and heat on thermocycler using "GRNA HEAT" protocol.

(GRNA HEAT = 95* for 5 min, cooled to 20* at -1

Incubate Cas9, gRNA, and MgCl2

Step 4.

Step 5.

Step 6.

Step 7.