In vitro cleavage assay using Cas9 protein

1. Set up the reaction mixture as below. Instead of 10x Cas9 Reaction Buffer (Cat No CB01), NEB Buffer 3.1, or NEB Buffer 3 and BSA can be used. Add target DNA the last for the best result.

Components	Amount	Range
Cas9 protein	150 ng	50~200 ng
sgRNA	100 ng	30~200 ng
Target DNA	60 ng PCR product	50~100 ng
	or 100 ng plasmid	50~200 ng
10x Cas9 Reaction Buffer (Cat No CB01)	1 ul	
Nuclease-free water	to 10 ul	

2. Incubate the reaction mixture at 37 ^{o}C for 1 hr.
3. Heat to inactivate Cas9 protein at 65 ^{o}C for 10 min.
4. Analyze on an agarose gel.
5. In rare cases, Cas9/sgRNA complex is still bound to the DNA template and result in abnormal migration. If it is the case, add 4 ug of RNase and incubate for 15 min at 37 ^{o}C, followed by addition of 1ul of STOP solution (30% glycerol, 1% SDS, 250 mM EDTA, pH 8.0) to the reaction mixture. Incubate for 15 min at 37 ^{o}C before gel running.

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