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## Cleavage assay

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protocol .

iGEM Gifu



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Cleavage assay to validate for Cas12a activity

Yuichiroh Ikagawa 2021. Cleavage assay. **protocols.io**  
<https://protocols.io/view/cleavage-assay-bzdmp246>



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### Reagents

Cas12a

crRNA

target DNA (U94)

reaction buffer ( PBS (pH 7.5), 150 mM KCl, 10 mM MgCl<sub>2</sub>, 1% glycerol and 0.5 mM DTT)

- 1 Thawed crRNA solution on ice.
- 2 Add the following solution to the tube and gently mix by pipetting.

A	B
COMPONENT	VOLUME (μl)
Cas12a	4
crRNA	5
reaction buffer	7
Total Volume	16

If the crRNA or Cas12a solution was not added to the reaction as a negative control, the reaction buffer for it was added and the total volume was adjusted to 20 μl.

- 3 Incubate for 10 minutes.
- 4 Add 4μl of U94 solution to each tube.
- 5 Incubate the mixture at 37°C overnight.
- 6 Check for cleavage by 1.5% agarose gel electrophoresis.