BBa_K1179002

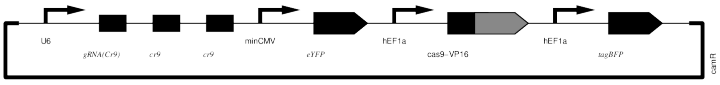
Part Summary

This part encodes for the constitutive expression of a Cas9-VP16 fusion protein. The Cas9 has been mutated in such a way as to remove its nuclease activity while retaining its ability to selectively bind DNA mediated by an appropriate guide RNA (gRNA). The Cas9-VP16 complexes with an expressed gRNA and together target a DNA sequence as defined by the gRNA. Once the Cas9-VP16 is bound, the VP16 domain can activate a downstream inducible promoter like minimal CMV.

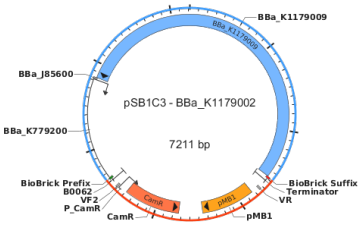
Part Type

Composite Part

Pigeon Image



Plasmid Map



Designer Information

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Date September 17, 2013

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Design Details

Type Generator

Design Components K779200, J85600, K1179009

Assembly Information

Assembly Method(s)	Gateway Technologies
Chassis	Homo sapiens
Strain	Human Embryonic Kidney 293
Scars	y

Flow Cytometry Experiment

Purpose	Characterization
Location	Massachusetts Institute of Technology
Transfer Curve Caption	On the Y axis, we see the output fluorescence in the FITC channel and our transfection marker fluorescence in the Pacific A channel on the X axis. As expected, we see activation when all components of the system are present, and increasing the amount of transfected guide RNA increased the ability of the Cas9- VP16 to bind to the synthetic upstream Cr9 regulatory sites of the reporter construct. The graph indicates that saturation hasn't occurred, but due to limitations of the Lipofectamine transfection protocol, transfecting the amount needed for saturation would likely be toxic to the cells.
Transfer Curve Graph