Aug 12, 2021

SaCas9 protein purification

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dx.doi.org/10.17504/protocols.io.bxbupinw

Protist Research to Optimize Tools in Genetics (PROT-G)

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ABSTRACT

Developing transfection protocol for *Bodo saltans*, using SaCas9/sgRNA ribonucleoprotein (RNP) complex in conjunction with DNA repair template to disrupt the Paraflagellar rod 2 gene (*BsPFR2*) and increase the efficiency of targeted homologous recombination when a repair template DNA is provided. The exogenous repair template is double stranded DNA and it consists of *eGFP* fused with the drug selection gene *nptII/neo* and flanked by 500 bp of the untranslated regions (UTRs) upstream and downstream of the targeted *BsPFR2* as homologous repair arms.

DOI

dx.doi.org/10.17504/protocols.io.bxbupinw

PROTOCOL CITATION

Fatma Gomaa, Zhu-Hong Li, Roberto Docampo, Virginia Edgcomb 2021. SaCas9 protein purification. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bxbupinw

KEYWORDS

Bodo saltans transfection, SaCas9/RNP complex, homologous recombination

CREATED

Aug 12, 2021

LAST MODIFIED

Aug 12, 2021

PROTOCOL INTEGER ID

52308

MATERIALS TEXT

Protocol 1: for SaCas9 protein purification

SaCas9 protein was prepared following methods described in Medeiros et al. (Medeiros et al., 2017) with modifications. Briefly, the bacterial expression vector p6XHis_NLS-SaCas9 (Addgene #101086) was transformed into *Escherichia coli* Rosetta 2(DE3) competent cells (Novagen) and grown as an overnight preculture with shaking at 37°C. Subsequently, 1 ml of preculture was used to inoculate 100 ml of terrific broth media which was incubated with shaking at 37°C until it reached an optical density at of 0.6 at 600 nm. Protein expression was induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 200 µM and cells were grown overnight with vigorous shaking at 18°C. Following incubation, cells were collected by centrifugation, and the pellet was resuspended in 2 ml / 100 mg cell weight of xTractor Buffer (Takara Bio) plus 5 mg of lysozyme. Cells were lysed by sonication, then 1 µl / 2ml of DNAse I was added. The soluble fraction was purified using the His60 Ni Superflow Resin & Gravity Columns (Takara Bio) following the manufacturer's recommendations. The concentration of SaCas9 containing fractions were determined by optical density at 280 nm and then pooled. Pooled fractions were concentrated and desalted into Cytomix (120 mM KCl, 0.15 mM $CaCl_2$, 10mM KH_2PO_4, 25 mM HEPES, 2 mM EGTA, 5 mM $MgCl_2$, pH 7.6) using Amicon Ultra-15 centrifugal filter devices (Millipore Sigma). The protein concentration was determined using a BCA Protein Assay Kit (Pierce).

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Citation: Fatma Gomaa, Zhu-Hong Li, Roberto Docampo, Virginia Edgcomb (08/12/2021). SaCas9 protein purification. https://dx.doi.org/10.17504/protocols.io.bxbupinw