Seydoux lab Cas9 preparation

Alexandre Paix, Andrew Folkmann, Dominique Rasoloson, Geraldine Seydoux

Abstract

This protocol is for purification of Cas9::NLS_{SV40}::His_6 from:
Paix A, Folkmann A, Rasoloson D, and Seydoux G (2015) High Efficiency, Homology-Directed Genome Editing in Caenorhabditis elegans Using CRISPR/Cas9 Ribonucleoprotein Complexes. Genetics genetics.115.179382; Early online July 17, 2015. doi:10.1534/genetics.115.179382

Please see the full manuscript for detail.

Citation: Alexandre Paix, Andrew Folkmann, Dominique Rasoloson, Geraldine Seydoux Seydoux lab Cas9 preparation. **protocols.io**
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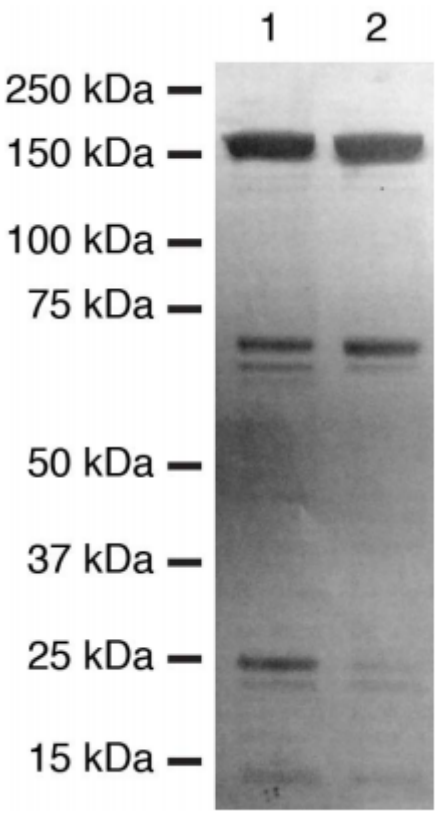
Guidelines

Protocol updates will be posted on the Seydoux lab website.
Main protocol: Direct delivery CRISPR-HDR editing protocol for *C. elegans*

Buffers:
Buffer A: 20mM Tris ph 8.0, 250 mM KCl, 20 mM imidazole, 10% glycerol, 1 mM TCEP
Buffer B: 20mM Tris ph 8.0, 800 mM KCl, 20 mM imidazole, 10% glycerol, 1mM TCEP
Buffer C: 20mM Hepes ph 8.0, 500 mM KCl, 250 mM imidazole, 10% glycerol
Buffer D: 20mM Hepes ph 8.0, 500 mM KCl, 20% glycerol

Purified Cas9::NLS_{SV40}::His_6 resolved by SDS-PAGE:



Recombinant Cas9::NLS_{SV40}::His_6 was affinity purified using Niâ□□agarose (lane 1). Pooled eluent was flowed over Q sepharose to remove contaminating DNA bound to Cas9 (lane 2). Samples were resolved by SDSâ□□PAGE and visualized by coomassie staining.Â Â

Cas9 activity assay: We recommend testing your Cas9 preparation using this method.

Protocol

Step 1.
Transform DE3 GOLD (Agilent, #230132) cells with nm2973 plasmid (Fu et al 2014) and plate on LB + 50µg/mL Carbenicillin

REAGENTS
BL21-Gold (DE3) Competent Cells 230132 by Agilent Technologies

Step 2.
Inoculate 25mL LB + 50 µg/mL Carbenicillin with bacteria from the fresh transformation and incubate at 37 °C overnight.

DURATION
18:00:00

Step 3.
Transfer 5mL of overnight culture to 1L LB + 0.1% glucose + 50 µg/mL Carbenicillin.

Step 4.
Grow at 25 °C to OD600=0.5.

Step 5.

Shift culture to 18 °C for 15-25 minutes.

DURATION
00:15:00

Step 6.
Add IPTG to 0.2 mM and incubate overnight.

DURATION
18:00:00

Step 7.
Pellet culture and obtain wet weight.

Step 8.
Resuspend at 6 mL/g cells with Buffer A + protease inhibitor (Roche, #11836170001) + 1mM PMSF.

REAGENTS
cOmplete™, Mini, EDTA-free (Protease Inhibitor) #11836170001) by Roche

Step 9.
Sonicate 6 x 45s (setting 3 at 30%, 1 second pulse-2 second pause) with 1 minute cooling in between.

Step 10.
Spin lysate 30 minutes at 16000xg and transfer supernatant to a fresh tube.

DURATION
00:30:00

Step 11.
Equilibrate a 5mL Ni-agarose (Qiagen, #30410) with column with Buffer A (at least 25mL).

REAGENTS
Ni-NTA Superflow 30410 by Qiagen

Step 12.
Batch bind clarified lysate with Ni-agarose 45 minutes at 4 °C.

DURATION
00:45:00

Step 13.
Wash Ni-agarose column with 100mL of Buffer B.

Step 14.
Elute protein with Buffer C.

Step 15.
Determine fractions that have Cas9 protein using Bradford assay or by running a small amount on SDS-PAGE gel. Pool fractions.

Remove contaminating DNA in the prep

Step 16.
Equilibrate a 5mL Q Sepharose (Sigma, #Q1126) column with 1M KCl (25mL, this charges the column).

AMOUNT
5 ml Additional info:

REAGENTS
Q Sepharose® Fast Flow Q1126 by Sigma Aldrich

Remove contaminating DNA in the prep

Step 17.

Equilibrate Q Sepharose column with Buffer C (25mL).

Step 18.
Flow eluent (from step 17) over Q Sepharose column. Collect flow-through and dialyze into 1L Buffer D for 5 hours at 4 °C.

DURATION
05:00:00

Step 19.
Transfer into 1L Buffer D and dialyze overnight.

DURATION
18:00:00

Step 20.
Concentrate protein to 10 mg/mL using a 100K centrifugal filter (Milipore, UFC910024).

REAGENTS
Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-100 membrane UFC910024 by Emd Millipore

Step 21.
Aliquot and flash-freeze in liquid nitrogen. Store aliquots at -80°C.

NOTES
Alexandre Paix 04 Sep 2015
Typical yield is sufficient for 50-70 single-use aliquots (5µl aliquot, 10µg/µl Cas9).