Purification of Cas9::NLS_{SV40}::His_6

1. Transform Rosetta 2 (Millipore EMD, #71397) cells with nm2973 plasmid (Fu *et al*., 2014) and plate on LB + 50µg/mL Carbenicillin.
The nm2973 plasmid can be obtained from Addgene (Addgene ID: 67881).

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

3. Transfer 5mL of overnight culture to 1L LB + 0.1% glucose + 50 µg/mL Carbenicillin and grow at 37°C. Grow to OD_{600}=~0.5.

3. Shift culture to 18°C for 15-25 minutes, then add IPTG to 0.2 mM. Incubate overnight.

4. Pellet culture and obtain wet weight. Resuspend at ~6 mL/g cells with Buffer A + protease inhibitor (Roche, #11836170001) + 1mM PMSF. Add lysozyme to final 1mg/ml concentration (Sigma L6876).

5. Sonicate: setting at 10% amplitude, 1.5 second pulse on, 5 second pause - for a total time of 45min.

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

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

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

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

10. Elute protein with Buffer C. Determine fractions that have Cas9 protein using Bradford assay or by running a small amount on SDS-PAGE gel. Pool fractions.

11. To remove contaminating DNA in the prep. Equilibrate a 5mL Q Sepharose (Sigma, #Q1126) column with 1M KCl (25mL, this charges the column). Then equilibrate Q Sepharose column with Buffer C (25mL).

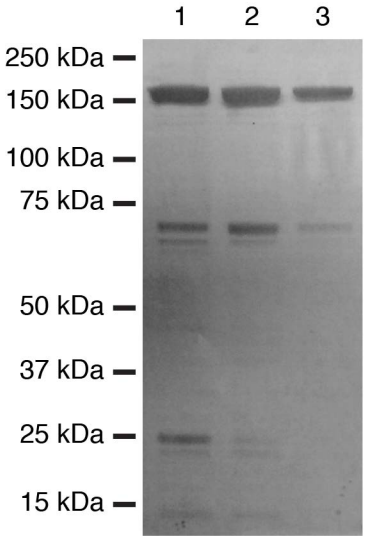
12. Flow eluent (from step 11) over Q Sepharose column. Collect flow-through and dialyze into 1L Buffer D for 5 hours at 4 C. Transfer into 1L Buffer D and dialyze overnight (Thermo Fisher Scientific, #66012).

13. **** Optional**** To remove Cas9 aggregates from the sample, the eluent (from step 12) can be further fractionated via size exclusion chromatography. First, equilibrate the 26/60 Sephacryl S-200 column (GE Healthcare Life Sciences, #17-1195-01) with Buffer D. Second, fractionate the eluent (from step 12) and pool fractions containing monomeric Cas9 (see figure below).

14. Concentrate protein (from step 12 or step 13) to ~10 mg/mL using a 100K centrifugal filter (Milipore, UFC910024). Aliquot and flash-freeze in liquid nitrogen. Store aliquots at -80°C. Typical yield is sufficient for 50-70 single-use aliquots (5µl aliquot, 10µg/µl Cas9).

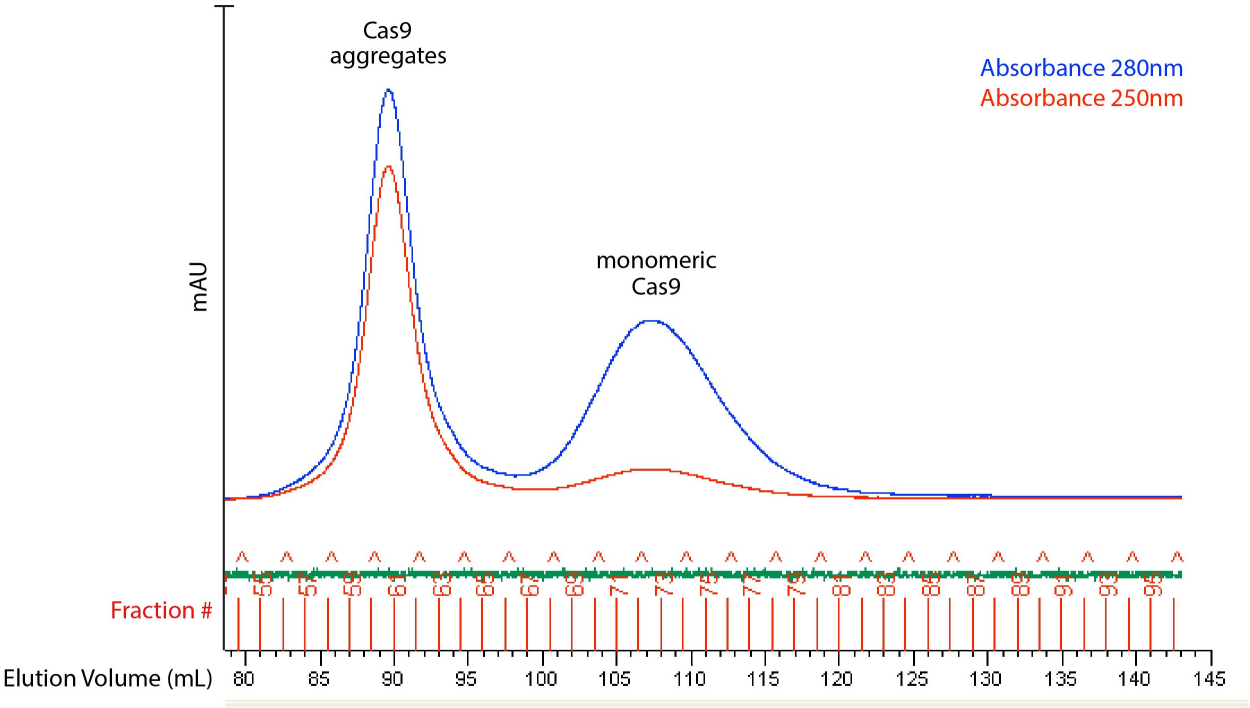
Buffers:

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 7.5**,** 500 mM KCl**,** 20% glycerol



Recombinant Cas9::NLS_{SV40}::His_6 was affinity purified using Ni-agarose (lane 1). Pooled eluent was flowed over Q sepharose to remove contaminating DNA bound to Cas9 (lane 2). To remove Cas9 aggregates the eluent was further fractionated via size exclusion chromatography (lane 3). Samples were concentrated to 10mg/mL resolved by SDS-PAGE and visualized by coomassie staining.

Reference Cas9::NLS_{SV40}::His_6 size exclusion chromatography elution profile:



Cas9 activity assay:

We recommend testing your Cas9 preparation using the method outlined in the direct delivery protocol (File S1, Section F).