Protocol for purification of dCas9 fusion protein

Transformants of E.coli strain BL21 are grown overnight at 37℃ with shaking in LB medium supplemented with suitable concentration of antibiotic. The culture is diluted 50-fold into fresh LB medium with antibiotic and grown at 37℃ for 8h to 12h. Then the culture is diluted 100-fold into fresh LB medium with antibiotic and grown in a shaking incubator at 37℃. When the OD600 of the culture reaches 0.6 to 0.8, IPTG is added to a final concentration of 200μM. The induced cells are incubated 12h at 16℃ with shaking.

Cells are harvested by centrifugation at 6000rpm for 10min at 4℃. The cells are resuspended in buffer A (20mM Tris, 500mM NaCl, pH 8.0) and disrupted by ultrasonic cell disrupter. The crude cell lysate is separated into soluble and insoluble fractions by centrifugation at 15000rpm for 50min at 4℃. The soluble fraction pass through 0.22 μm syringe-driven filter units (Millex).

The cleared supernatant is applied to a 5mL bed volume HisTrap™ column equilibrated with 5 bed volumes of buffer A. Unbound protein is removed by washing the column with buffer A. Once the base line is reached, the target protein is eluted by applying a stepped 0 to 0.5M imidazole gradient.

Fractions containing the target protein are concentrated to proper volume, and applied to 5mL bed volume desalting column equilibrated with 5 bed volumes of buffer D (20mM HEPES, 150mM KCl) to remove imidazole. Then the fractions containing the target protein are concentrated to proper volume.

Protein purity is checked by SDS-PAGE, and the resulting protein is quantified by spectrophotometry.