

## (i) Minimal information to provide in publications

The aim is to provide sufficient information on the **protein identity, expression and purification parameters** such that the experiment can be replicated reliably in any laboratory.

- **Protein name and full primary structure**, by providing a NCBI (or UniProt) accession number, cloning strategies, and the source of the DNA (species).
- **Expression vector and host strain**, including the tags and cleavage sites used, accompanied by the full amino acid sequence of the final protein, or sufficient details to derive the full amino acid sequence of the final protein.
- **Expression and Purification protocol**, namely the detailed description of all the protein production steps.
- **Protein concentration** (specifying the method used for quantification and the molar extinction coefficient at 280nm, if applicable. Extinction coefficient can be obtained for any sequence by using open access resources -<https://web.expasy.org/protparam/>).
- **Storage conditions**, i.e. final buffer composition (pH, buffers, salts and additives), storage temperature and, where applicable, freezing or lyophilization conditions.

### **Example of description**

*eSpCas9 (including both N and C-terminal NLS sequences) was amplified from plasmid eSpCas9(1.1) (Addgene plasmid 71814) using the high fidelity KOD polymerase (Merck) using the following primers:*

*fwd-AGGAGATATACCATGGCCCCAAGAAGAAGCGGAAGG*

*rev-CCACGAAAAAGCCGGCCAGGCCAAAAAGAAAAAGAAACATCACCATCAC*

*The resulting 4227 b.p. PCR product was DpnI treated to remove the template and purified using Ampure (Beckman Coulter) before In-Fusion (Clontech) into NcoI-PmeI –cut pOPINE (Addgene plasmid 26043). The resulting plasmid will express eSpCas9 with an N-terminal NLS and a C-terminal NLS-His-tag). The plasmid was fully sequenced before E. coli Rosetta(DE3) pLysS cells were transformed with it, and transformants were selected on LB agar plates supplemented with 1% glucose, Carbenicillin (50µg/ml) and Chloramphenicol (35µg/ml). A starter culture was grown overnight at 37°C with shaking at 250rpm in LB supplemented with 1% glucose, Carbenicillin (50µg/ml) and Chloramphenicol (35µg/ml). The culture was then diluted 1:50 in TB Overnight Express media auto-induction media (Merck), grown for a further 3 hours at 37°C until the OD<sub>600</sub> reached approximately 0.5, the temperature was then reduced to 25°C and the culture maintained for a further 24 hours. Cells were collected by centrifugation at 5000g for 10 minutes and the pellets (15 g/L) stored at -80°C before processing. Pellets were lysed in 5 volumes of **Lysis/Loading Buffer** (20mM Tris pH8, 500mM NaCl, 30mM Imidazole supplemented with 1 complete EDTA free tablet [Roche] and 1000Kunits of DNase [Merck]/10g of pellet) at 20Kpsi using a cell disruptor (Constant Systems Ltd. UK). The lysate was cleared by centrifugation for 20 minutes at 30,000xg at 4°C and filtration (cut-off 0.4 µm) before loading onto a 5ml HisTrap HP column (GE Healthcare). After sample loading the column was washed with 10CV of loading buffer before the eSpCas9 was eluted using **Elution Buffer** (20mM Tris pH8, 500mM NaCl, 500mM Imidazole, 2mM DTT, 10% glycerol). Peak fractions were analyzed by SDS-PAGE before pooling and buffer exchange into **DESALT/Storage Buffer** (20mM Tris pH8, 200mM KCl, 10mM MgCl<sub>2</sub>) using a HiPrep Desalting*

*column (GE Healthcare). eSpCas9 was then concentrated to a maximum of 3mg/ml using a ultrafiltration device with a 30kDa MW cut-off, concentration was assayed using a Nanodrop instrument (Thermo Scientific) and a calculated  $A_{280}$  of 0.69  $A_{280nm}/mg/ml$ ). eSpCas9 fraction was then separated into single-use aliquots that were flash-frozen in liquid nitrogen and stored at  $-80^{\circ}C$  until further use.*