

SI16: ORF6

Table 1: General Information

1	Protein Name (according to NCBI Reference Sequence NC_045512.2)
	ORF6
2	Region/Name/Further Specification
3	Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)
	MFHLVDFQVTIAEILLIIMRTFKVSIWNLDYIINLIKNLSKSLTENKYSQLDEEQPMEID
4	Protein boundaries - amino acid numbering (according to NCBI Reference Sequence NC_045512.2):
	aa 1-61 (fl ORF6)
5	Ratio for construct design (detailed and comprehensible)
	fl protein
6	Sequence homology (to SCoV)
	Identity: 68.9%; similarity: 93.4%
7	Published structures (SCoV2 or homologue variants)
	-
8	(Published) assignment (SCoV2 or homologue variants)
	-

Table 2: Cell-free Protein Synthesis

1	Expression vector
	pEU-E01-MCS (Cell-Free Sciences)
2	Purification-/Solubility-Tag
	C-terminal Strep tag II (WSHPQFEK)
3	Cleavage Site
	-
4	Molecular weight / Extinction coefficient / pI - of cleaved protein
	8.47 kDa / 13,980 M ⁻¹ cm ⁻¹ / 4.89
5	Comments on sequence of expressed construct
	C-terminal "SAWSHPQFEK" ten artificial residues due to construct design.
6	Feeding buffer

	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT and 6 mM (average concentration) amino acid mix
7	Translation mix
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 µg/mL creatine kinase, and 6 mM (average concentration) amino acid mix
8	Protein synthesis temperature and time
	22°C for 16 h without agitation (bilayer method).

Table 3: Protein Purification

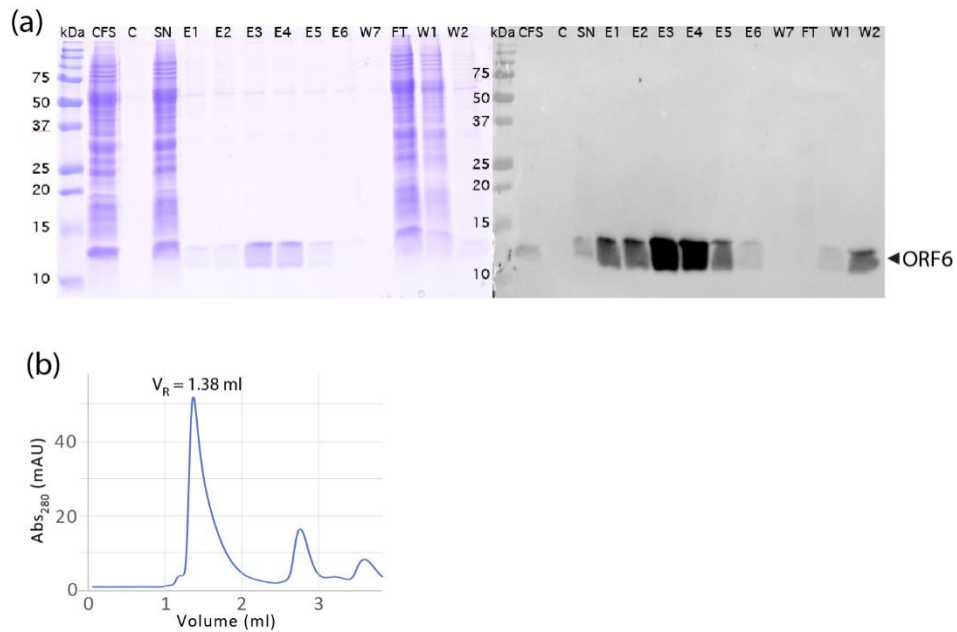
1	Buffer List
A	20 mM NaPi (pH 6.5), 50 mM NaCl (wash buffer).
B	20 mM NaPi (pH 6.5), 50 mM NaCl, 2.5 mM desthiobiotin (elution buffer).
2	Purification steps (with corresponding buffer(s) and incubation times)
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of 1A (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of 1A .
H	Elute the protein of interest with 1B .

Table 4: Final sample

1	Yield
	0.27 mg/mL of WGE and total production of 875 µg for NMR samples
1b	A260/280 ratio
	1.36
2	Stability
	stable
3	Comment on applicability
	Positioning the Strep tag at the N-terminus abolished synthesis.

Additional information

Constructs	Conditions	Comments
F1 ORF6; Strep tag II (pEU-E01-MCS (Cell-Free Sciences)), no cleavage site, N-terminal "WSHPQFEK" eight artificial residues.		No expression observed.



(a) WG-CFPS and Strep-tag purification of ORF6. SDS-PAGE (left panel) and WB (right panel). (b) SEC profile of ORF6.