SI3: Nsp3a

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	Nsp3a Ubiquitin-like domain (Ubl) + IDR
3	**Sequence of "fl" protein (aa 1-206 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	APTKVTFGDDTVIEVQGYKSVNITFELDERIDKVLNEKCSAYTVELGTEVNEFACVVADAVIKT LQPVSELLTPLGIDLDEWSMATYYLFDESGEFKLASHMYCSFYPPDEDEEEGDCEEEEFEPSTQY EYGTEDDYQGKPLEFGATSAALQPEEEQEEDWLDDDSQQTVGQQDGSEDNQTTTIQTIVEVQP QLEMELTPVVQTIE
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
Ubl+ IDR	aa 1-206 of complete nsp3
Ubl	aa 1-111 of complete nsp3
5	**Ratio for construct design**
Ubl+ IDR	Based on homologous structure from SCoV.
Ubl	Based on disorder prediction, folded domain and SCoV Ubl1.
6	**Sequence homology (to SCoV)**
Ubl+ IDR	Identity: 58%; Similarity: 75%
Ubl	Identity: 79%; Similarity: 89%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 2GRI; 2IDY
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 7019 SCoV2: BMRB 50446

Table 2: Protein Expression

1	**Expression vector**
Ubl+ IDR	pET-TEV-Nco (GenScript)
Ubl	pKM263 (GenScript)
2	**Purification-/Solubility-Tag**
Ubl+ IDR	N-terminal His_6

Ubl	N-terminal His_6-GST
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
Ubl+ IDR	23.50 kDa / 24,410 M^{-1} cm^{-1} / 3.62
Ubl	12.72 kDa / 14,440 M^{-1} cm^{-1} / 4.08
5	**Comments on sequence of expressed construct**
Ubl+ IDR	N-terminal "GAM" three artificial residues due to TEV-cleavage and construct design.
Ubl	N-terminal "GAMG" four artificial residues due to TEV-cleavage and construct design.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	1 mM IPTG at OD_{600} 0.6-0.8
10	**Cultivation temperature and time**
Ubl+ IDR	37ºC for 5 h
Ubl	18°C for 18 h

Table 3a: Protein Purification (Ubl + IDR)

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 150 mM NaCl and complete EDTA-free tablet (cell disruption).
B	50 mM Tris-HCl (pH 8.0) and 150 mM NaCl (wash buffer).
C	50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 500 mM Imidazole (elution buffer).
D	50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 5 mM bME (TEV cleavage).
E	50 mM NaPi (pH 6.5), 250 mM NaCl (final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Resuspension of cell pellet in 50 mL per liter of culture of **1A** at 4°C.
B	Cell disruption by sonication on ice.

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C	Clarification of lysate by centrifugation at 16,000 g for 30 min at 4°C.
D	Loading of lysate on Ni^{2+}-loaded IMAC resin (ThermoFisher scientific) pre-equilibrated with **1B** at 22°C.
E	Wash IMAC resin with 50 bed volumes of **1B**.
F	Elute protein from IMAC resin with 5 bed volumes of **1C**.
G	TEV cleavage with 1 mg TEV per 50 mg protein by dialysis against **1D** for 18 h at 4°C.
H	Removal of uncleaved protein and tag by elution through Ni^{2+}-loaded IMAC resin pre-equilibrated with **1B** at 22°C.
I	Wash with 5 bed volumes of **1B**.
J	SEC with HiLoad SD 75 pg column (GE Healthcare) pre-equilibrated with **1E** at 4°C.

Table 3b: Protein Purification (Ubl)

1	**Buffer List**
A	50 mM NaPi, 300 mM NaCl, 10 mM imidazole, 2 mM TCEP-HCl, pH6.5 (Cell disruption / IMAC)
B	25 mM NaPi, 150 mM NaCl, 2 mM DTT, 0.02 % NaN_3, pH7 (dialysis after IMAC / TEV-cleavage)
C	25 mM NaPi, 150 mM NaCl, 2 mM TCEP-HCl, 0.02 % NaN_3, pH7 (SEC / final NMR buffer)
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus 100 µL protease inhibitor (Serva)) by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA), Elution with 150-500 mM imidazole in buffer **1A**
C	Dialysis o.n. in in buffer **1B**
D	TEV-cleavage (0.5 mg TEV protease per 1 L culture) in buffer **1B**
E	SEC on HiLoad 16/600SD 75 (GE Healthcare) in buffer **1C**
F	NMR sample preparation in buffer **1C**

Table 4: Final sample

1	**Yield**
Ubl+ IDR	0.7 mg/L ^{15}N-M9 medium
Ubl	2-3 mg/L ^{15}N-M9 medium
1b	**A260/280 ratio**
Ubl+ IDR	0.57
Ubl	0.6
2	**Stability**
Ubl+	2 weeks at 25°C.

IDR	
Ubl	Very stable over weeks.
3	**Comment on applicability**
Ubl+ IDR	Stable for NMR assignments and screening
Ubl	Stable for NMR assignments and screening (spectra overlay with folded part of nsp3a Ubl + IDR above.)

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