SI3: Nsp3Y

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	Nsp3-Y / Cov-Y
3	**Sequence of "fl" protein (aa 1638-1945 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	DTFCAGSTFISDEVARDLSLQFKRPINPTDQSSYIVDSVTVKNGSIHLYFDKAGQKTYERHSLSHF VNLDNLRANNTKGSLPINVIVFDGKSKCEESSAKSASVYYSQLMCQPILLLDQALVSDVGDSAE VAVKMFDAYVNTFSSTFNVPMEKLKTLVATAEAELAKNVSLDNVLSTFISAARQGFVDSDVET KDVVECLKLSHQSDIEVTGDSCNNYMLTYNKVENMTPRDLGACIDCSARHINAQVAKSHNIAL IWNVKDFMSLSEQLRKQIRSAAKKNNLPFKLTCATTRQVVNVVTTKIALKGG
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1,638-1,945 of complete nsp3
5	**Ratio for construct design (detailed and comprehensible)**
	We took the C-terminal part of nsp3 after predicted transmembrane region and Y1 domain that consists of two sequential zinc finger motifs.
6	**Sequence homology (to SCoV)**
	Identity: 89%; similarity: 96%
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
	pET28b(+) (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	34 kDa / 17,420 M^{-1} cm^{-1} / 6.66
5	**Comments on sequence of expressed construct**

 doi:10.3389/fmolb.2021.653148

	N-terminal „G" one artificial residue due to TEV-cleavage.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labeling)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.5 mM IPTG at OD_{600} 0.7
10	**Cultivation temperature and time**
	18°C for 15-16 h

Table 3: Protein Purification

1	**Buffer List**
A	20 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole, 0.1 mM PMSF, 5 mM bME, 0.1 mg/mL lysozyme, cOmplete EDTA-free inhibitor (Cell disruption).
B	20 mM Tris-HCl (pH 8.0), 300 mM NaCl, 20 mM imidazole (IMAC).
C	50 mM Tris-HCl (pH 8.0), 200 mM NaCl, 2 mM DTT (TEV-cleavage).
D	50 mM HEPES (pH 6.9), 200 mM LiBr, 5 mM DTT.
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA) (Thermo Scientific), wash with buffer **1B** and elution with 250 mM imidazole in buffer **1B**.
C	TEV-cleavage (5% (w/w) TEV protease per approximate amount of the protein) in buffer **1C** o.n. at rt.
D	Inv. IMAC (gravity flow Ni^{2+}-NTA) in buffer **1C**.
E	SEC on 10/300 GL SD 200 (GE Healthcare) in buffer **1D**.

Table 4: Final sample

1	**Yield**
	12 mg/L ^{13}C,^{15}N-M9 medium
2	**Stability**
	Stable at 25°C at protein concentration below 0.4 mM for 3 to 5 days or at 30°C o.n.. The protein gradually degrades at rt. After one week, we observe an additional band on SDS gel at ~27 kDa.
3	**Comment on applicability**

	The protein is suitable for NMR assignment and protein interaction studies at low temperature (20-25°C) and reasonably low concentration (< 0.2 mM).