SI7: Nsp9

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp9
2	**Region/Name/Further Specification**
	Nsp9
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	NNELSPVALRQMSCAAGTTQTACTDDNALAYYNTTKGGRFVLALLSDLQDLKWARFPKSDGT GTIYTELEPPCRFVTDTPKGPKVKYLYFIKGLNNLNRGMVLGSLAATVRLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-113 (fl nsp9)
5	**Ratio for construct design (detailed and comprehensible)**
	In analogy to the available crystal structure (PDB 1QZ8) of nsp9 SCoV, fl sequence.
6	**Sequence homology (to SCoV)**
	Identity: 97%; similarity: 97%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 3EE7 (G104E), 1UW7, 1QZ8 SCoV2: PDB 6WXD, 6W4B, 6W9Q
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 6501 SCoV2: BMRB 50621, 50622, 50513

Table 2: Protein Expression

1	**Expression vector**
	pKM263 (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-GST
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	12.7 kDa / 13,075 M^{-1} cm^{-1} / 9.1
5	**Comments on sequence of expressed construct**
	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} 0.7
10	**Cultivation temperature and time**
	20-22°C for 18-20 h

Table 3: Protein Purification

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole, 4 mM DTT (cell disruption / IMAC/ dialysis after IMAC / TEV-cleavage).
B	25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl, 0.02% (w/v) NaN_3 (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 (Carl Roth)), Elution with 150-500 mM imidazole in buffer **1A**.
C	Dialysis o.n. in in buffer **1A**.
D	TEV-cleavage (0.5 mg TEV protease per 1 L culture) in buffer **1A**.
E	SEC on HiLoad 16/600SD 75 (GE Healthcare) in buffer **1B**. See relevant peak in attached SEC profile.
F	NMR sample preparation in buffer **1B**.

Table 4: Final sample

1	**Yield**
	4.5 mg/L ^{13}C, ^{15}N-M9 medium
1b	**A260/280 ratio**
	0.7
2	**Stability**
	Stable dimer. Storage at 4°C possible.
3	**Comment on applicability**
	Conditions for NMR structure determination may need to be optimized (concerning line width due to dimeric state). Backbone assignment and screening successful.

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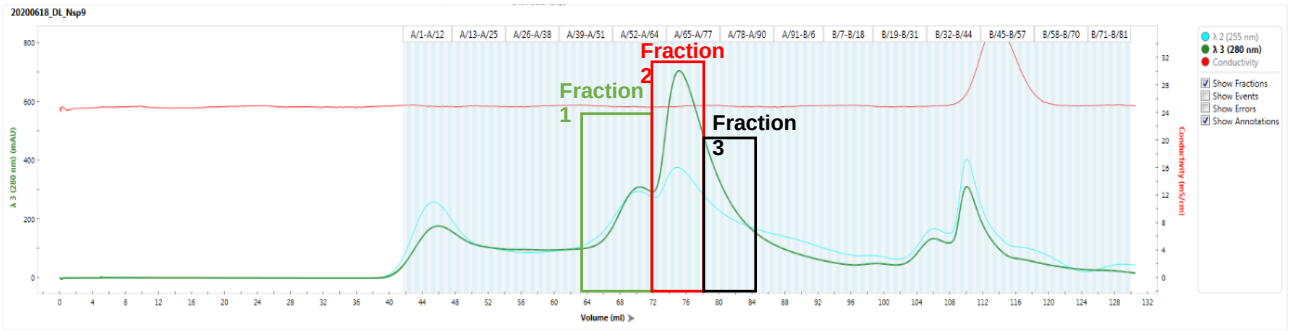
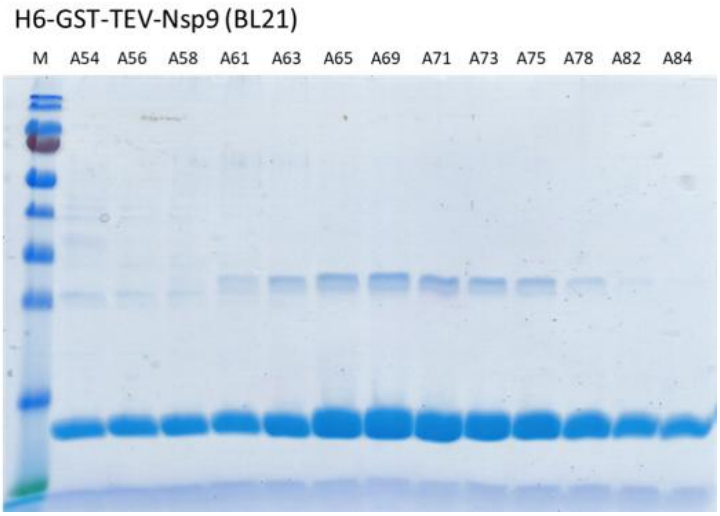
Additional information

	Constructs	Conditions	Comments
A	aa 1-113 (fl nsp9); His_7 (pET-TEV-Nco (GenScript)), TEV-cleavage site, N-terminal "GAMG" four artificial residues.	As above.	Expression and purification as for GST-tagged fl nsp9, but lower expression and yield.
B		**IMAC buffer:** 25 mM NaPi (pH 7.4), 300 mM NaCl, 20 mM imidazole, 1 mM DTT. **Cleavage buffer:** 25 mM NaPi (pH 7.4), 150 mM NaCl, 1 mM DTT. **SEC/NMR buffer A:** 25 mM NaPi (pH 7.0), 150 mM NaCl, 1 mM DTT, 150 mM NaCl, 2 mM TCEP-HCl. **SEC/NMR buffer B:** 25 mM NaAc (pH 5.0), 150 mM NaCl, 2 mM TCEP-HCl.	3 mg/L ^{13}C,^{15}N-M9 medium. Sample in Buffer **A** looked degraded (from the ^{15}N HSQC) after 5 days of ^{13}C 3D NMR experiments at 298 K. Less degradation was observed for sample in Buffer **B** after same period. Suitable for NMR studies, fragment-based screening, interaction studies.





SEC profile of TEV-cleaved His_6-GST-fl_nsp9 (HiLoad 16/600 SD 75, GE Healthcare) **and SDS gel of corresponding fractions.** (Ladder: PageRuler™ prestained, Thermo Fischer)
Main peak (fraction 2 - corresponding to SEC fractions A 61 to A73) was subsequently used for NMR.

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