SI10: Nsp14

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1ab; nsp14
2	**Region/Name/Further Specification**
	nsp14 / 3'-to-5' exonuclease / guanine N7-methyltransferae (MTase)
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	AENVTGLFKDCSKVITGLHPTQAPTHLSVDTKFKTEGLCVDIPGIPKDMTYRRLISMMGFKMNY QVNGYPNMFITREEAIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTGVNLVAVPTGYVDT PNNTDFSRVSAKPPPG
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
fl	aa 1-527 (fl nsp14)
MTase	aa 288-527 (MTase domain)
5	**Ratio for construct design**
fl	fl protein
MTase	In analogy to SCoV structure (PDB 5C8U)
6	**Sequence homology (to SCoV)**
fl	Identity: 95%; similarity: 99%
MTase	Identity: 95%, similarity: 97%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 5C8U, 5C8S, 5C8T, 5NFY
8	**(Published) assignment (SCoV2 or homologue variants)**
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Table 2: Protein Expression

1	**Expression vector**
fl	pRSF-Duet1 (Novagen)
MTase	pET28a (Novagen)
2	**Purification-/Solubility-Tag**
fl	N-terminal His_6
MTase	N-terminal His_6
3	**Cleavage Site**

fl	TEV
MTase	Thrombin
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
fl	60.01 kDa / 91,660 M^{-1} cm^{-1} / 7.79
MTase	27.82 kDa / 48,970 M^{-1} cm^{-1} / 7.19
5	**Comments on sequence of expressed construct**
fl	N-terminal “GSM” three artificial residues due to construct design.
MTase	N-terminal “GSHM” four artificial residues due to construct design.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	2xTY for protein production, LB for transformation and maintenance
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	1 mM IPTG at OD_{540} 0.5-0.6
10	**Cultivation temperature and time**
	20°C for 18-20 h

Table 3: Protein Purification (fl nsp14 and nsp14 MTase)

1	**Buffer List**
A	50 mM Tris-HCl (pH 9.0), 0.5 M NaCl, 10 mM bME, 2 mM $MgCl_2$, 0.1% (v/v) Triton X-100, 10 % (v/v) glycerol, 50 mM imidazole (cell disruption).
B	50 mM Tris-HCl (pH 9.0), 0.5 M NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 50 mM imidazole (IMAC).
C	50 mM Tris-HCl (pH 9.0), 0.5 M NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 1 M imidazole (IMAC).
D	20 mM HEPES (pH 8.5), 0.5 M NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 20 mM imidazole (SEC).
E	20 mM potassium phosphate (pH 8.0), 0.25 M KCl (Screening).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** by sonication in pulse mode (0.5 s on /0.5 s off) for 10 min.
B	IMAC (gravity flow or batch Ni^{2+}-NTA) (GE Healthcare), washing with buffer **1B**, elution with **1C**.
C-fl	[Optional] Overnight incubation with TEV protease at 4°C. The ratio was 1 mg of TEV protease per 20-40 mg of nsp14 protein.

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C-MTase	[Optional] Overnight incubation with thrombin protease at 4°C. The ratio was 1-2 U of thrombin protease per 3-4 mg of MTase nsp14 protein.
D	SEC on SD 200 16/600 column (GE Healthcare) in buffer **1D** (elution volume 75-95 mL).
E-**fl**	[Optional] Separation of TEV protease and uncleaved nsp14 material with IMAC, collection of flow through in buffer **1D**.
E-MTase	[Optional] Separation of thrombin protease and uncleaved MTase nsp14 material with IMAC, collection of flow through in buffer **1D**.
F	For fragment screening the buffer is exchanged to **1E**.
G	[Optional] If higher concentrations or increased stability of full length nsp14 is desired, nsp10 should be added at 1:1 molar ratio.

Table 4: Final sample

1	**Yield**
fl	6 mg/L 2xTY medium
MTase	~ 10 mg/L 2xTY medium
1b	**A260/280 ratio**
fl	0.6
MTase	0.6
2	**Stability**
fl	The fl nsp14 construct tends to be unstable at concentrations above 3 mg/ml without reducing agent (TCEP-HCl or bME). Unstable at 4°C longer than one week. Freezing is not advisable; storage in 50% (v/v) glycerol at -20°C is preferable.
MTase	The MTase construct is even more unstable, and requires the presence of reducing agent (TCEP-HCl or bME) and NaCl at least in 400 mM concentration.
3	**Comment on applicability**
	Suitable for fragment screening and enzymatic activity assays.

Additional information

Constructs	**Conditions**	**Comments**
Fl nsp14; His_6 (pETDuet (GenScript)), no cleavage site, N-terminal "MGSSHHHHHHSQDP" 14 artificial residues.	**IMAC-buffer:** 25 mM Tris/HCl (pH 8.5), 300 mM NaCl, 5 mM imidazole, 10 mM bME, 5% (v/v) glycerol. **SEC-buffer:** 25 mM Tris/HCl (pH 8.5), 300 mM NaCl, 5 mM DTT, 5% (v/v) glycerol	Yields 14 mg/L ^{15}N-M9 medium. Tendency to aggregate.

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