SUPPLEMENTARY MATERIALS

The archaeal ATPase PINA interacts with the helicase Hjm via its carboxyl terminal KH domain remodeling and processing replication fork and Holliday junction

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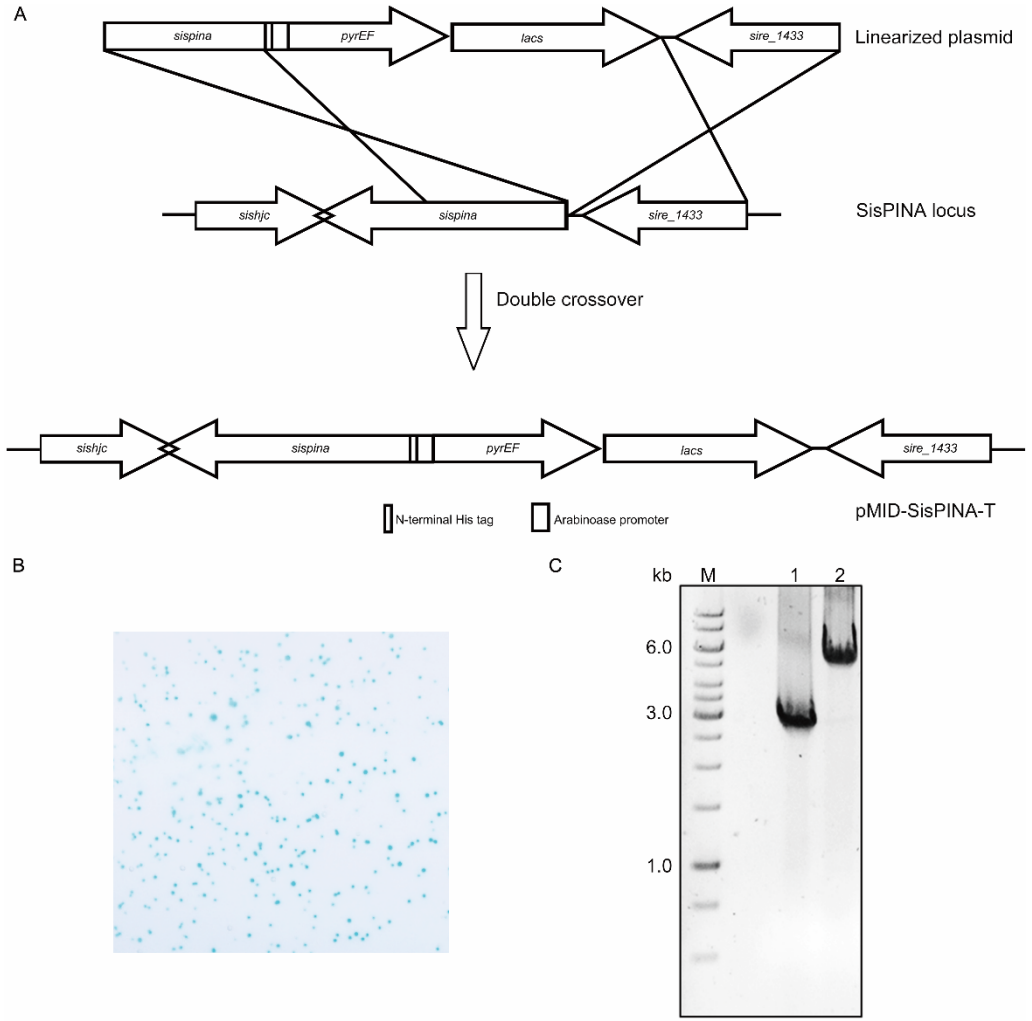
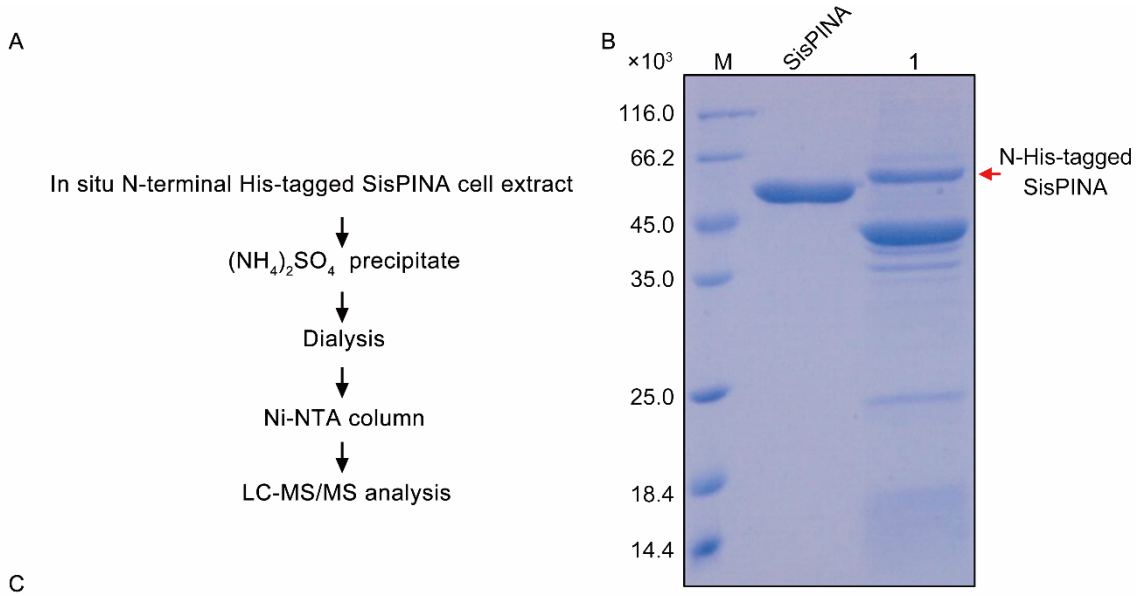


Fig. S1 Construction of a strain coding an N-terminal 6 × His-tagged SisPINA by the chromosomal gene. (A) A scheme for the construction of the strain. (B) Screening for positive transformants by X-gal staining. (C) Analysis of the genotype of the strain by PCR. The flanking primers described in reference (1) were used for the analysis. Lanes 1, genomic DNA of *S. islandicus* REY15A(E233S); 2, genomic DNA of Sis/pMID-SisPINA-in-situ-N-His.



Protein names	Gene number	Protein score	Sequence coverge (%)	Unique peptides
SisPINA	SiRe_1432	16296.25	91.09	60
Hjm (Hel308)	SiRe_0250	2142.89	64.06	42
RFCs	SiRe_1235	470.48	30.3	8
DnaG	SiRe_1914	467.83	23.38	8
SSB	SiRe_0161	265.32	37.16	5
XPB1	SiRe_1129	163.36	9.64	5
RFCl	SiRe_1234	126.14	9.38	3
DNA ligase	SiRe_1818	111.11	3.83	3

Fig. S2 Identification of proteins that have potential interaction with SisPINA. (A) Scheme for the purification of the N-terminal His-tagged SisPINA and its associated proteins from the modified strain of *S. islandicus* REY15A(E233S). (B) SDS-PAGE of the proteins. The His-tagged SisPINA was indicated with a red arrow. Purified SisPINA (no tag) from *E. coli* was used as a positive control. (C) The peptide coverage of SisPINA and DNA processing related proteins identified by LC-MS/MS. Proteins with scores of more than 100 are listed.

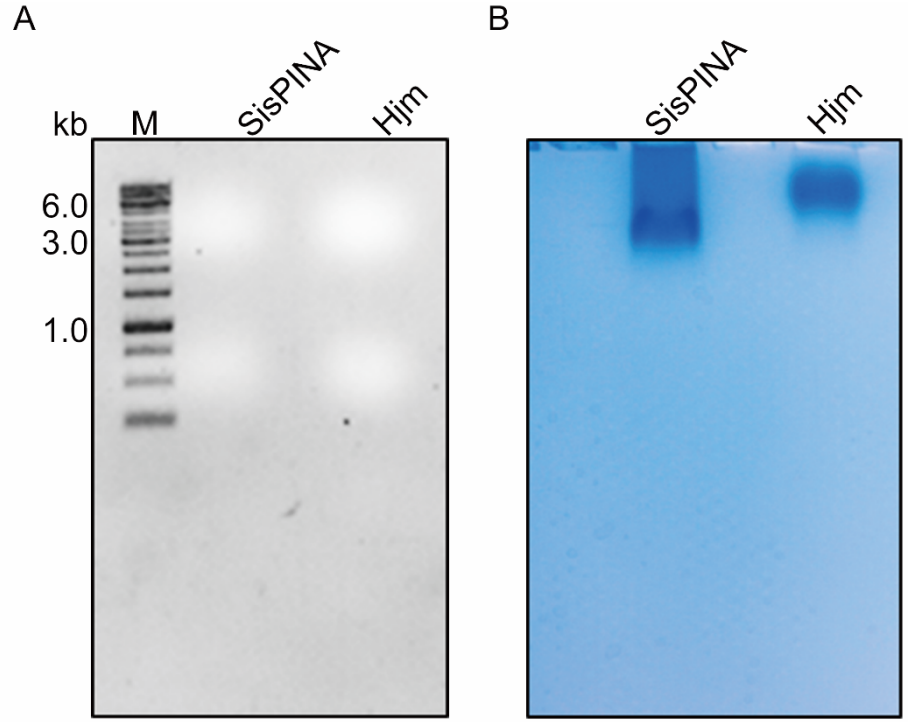


Fig. S3 Purified SisPINA and Hjm samples do not contain DNA or RNA. Purified SisPINA (10 μg) and Hjm (10 μg) were loaded into a 1% agarose gel. After electrophoresis, the gel was stained with ethidium bromide and observed under UV light (A). The proteins were checked by Coomassie brilliant blue staining (B). M, DNA size marker. The bright spots in (A) correspond to bromophenol blue and xylene cyanol present in the loading dye.

A

Protein conentration (nmol)	Elution volume (ml)	
	10	11
SisPINA (Hexamer)	0.26	0.33
Hjm (Monomer)	0.34	0.41

B

Protein conentration (nmol)	Elution volume (ml)	
	13	14
SisPINA-R206A/R147K/ I199S (Monomer)	0.63	1.49
Hjm (Monomer)	0.67	1.36

Fig. S4 Estimation of the molar ratios of Hjm and PINA in the complexes of Hjm-SisPINA (A) and Hjm-SisPINA-R206A/R147K/I99S (B). The percentages of Hjm and SisPINA or SisPINA-R206A/R147K/I99S in the elution fractions (10 and 11 in Fig. 1C, right panel; 13 and 14 in Fig. 4B lower panel) were estimated from the band intensity on the SDS-PAGE gels using Image J software. As the amount of loaded protein was known, the protein amount and concentrations in the fractions could be determined.

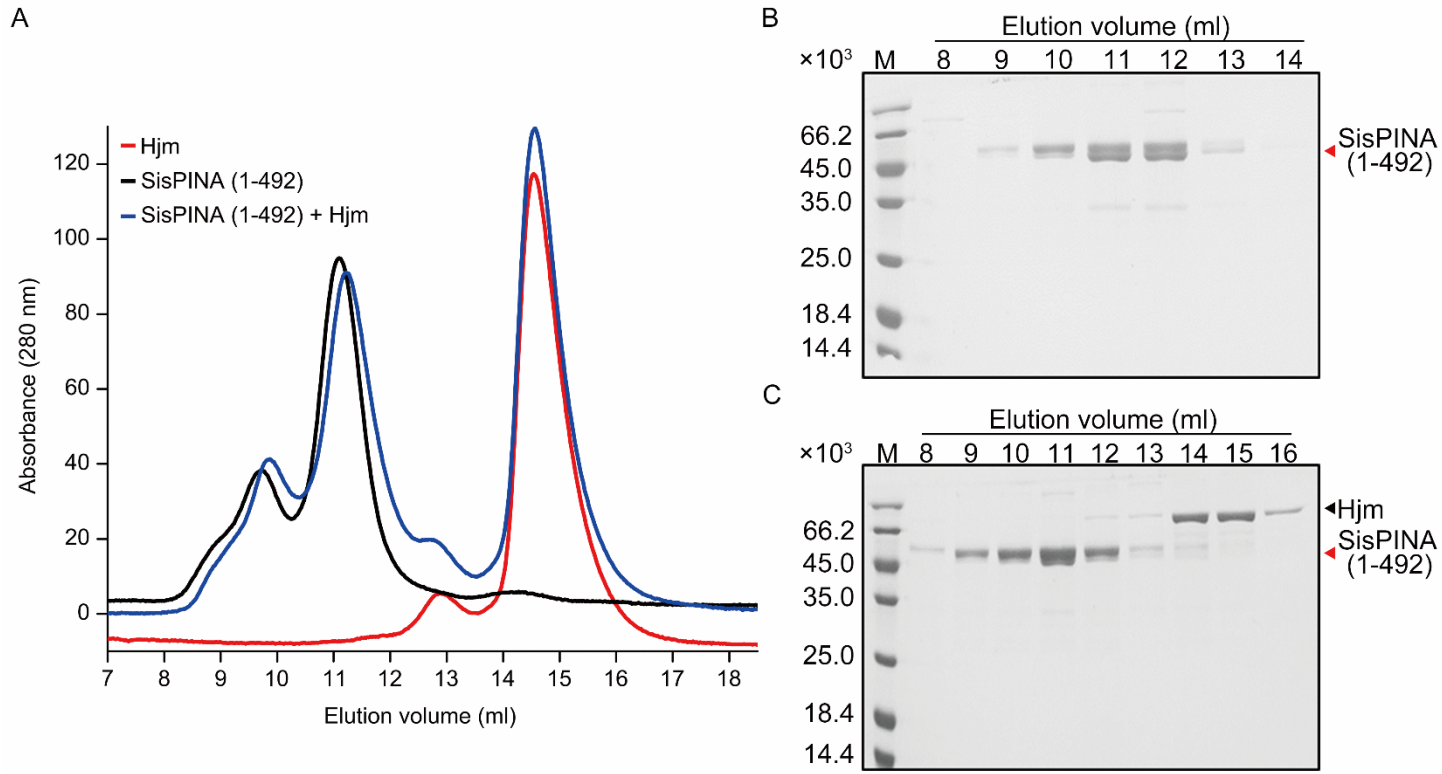


Fig. S5 SisPINA(1-492) is unable to form a complex with SisHjm. (A) Gel filtration profile of Hjm, SisPINA(1-492), and Hjm/SisPINA(1-492) mixture. The sample (500 µl) of SisPINA(1-492) (350 µg), Hjm (230 µg), or their mixture was loaded onto the Superdex 200 column which was equilibrated with a buffer containing 25 mM Tris-HCl, pH 8.0, 200 mM NaCl, and 5% glycerol. (B) SDS-PAGE analysis of the fractions of SisPINA(1-492). (C) SDS-PAGE analysis of the fractions of Hjm/SisPINA(1-492) mixture. The elution fractions (1 ml each) were collected and analyzed.

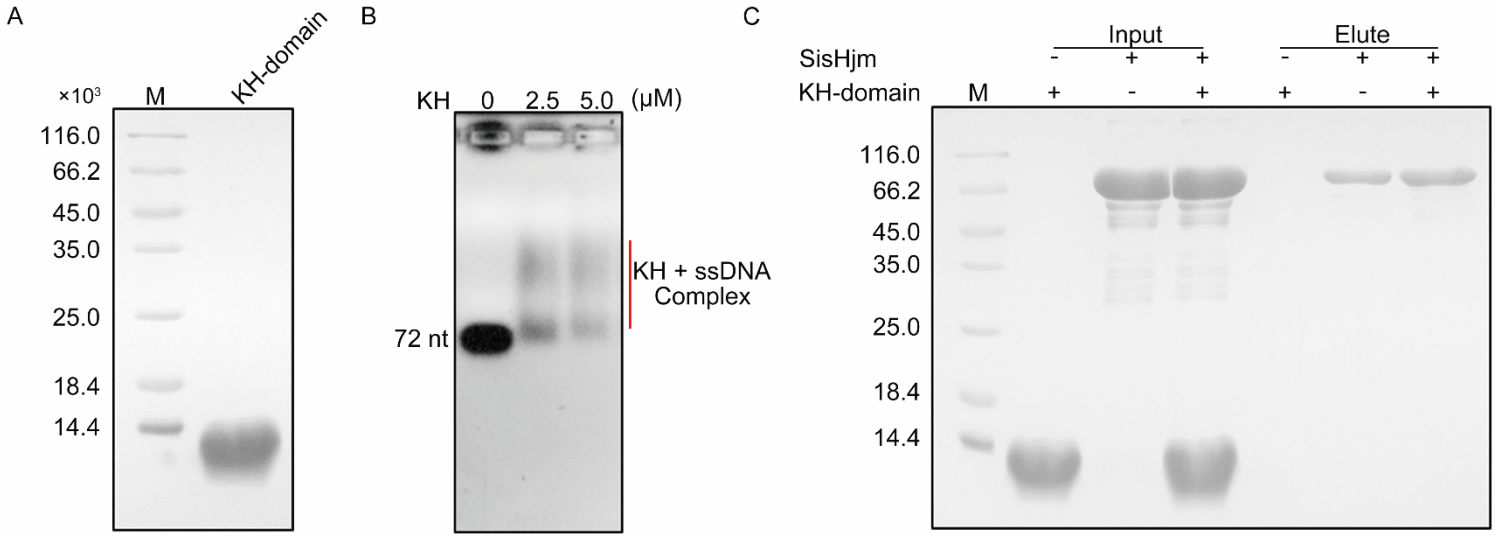


Fig. S6 The KH domain of SisPINA alone is able to bind ssDNA but not Hjm *in vitro*. (A) SDS-PAGE analysis of the KH domain of SisPINA. The recombinant protein was purified by HiTrap-Heparin and Superdex^{TM} 200 10/300 GL columns. The gel was stained with Coomassie brilliant blue. (B) ssDNA binding of the KH domain. DNA binding assay was performed in a 20 μl mixture containing 25 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin (BSA), 12% Glycerol, 1 mM DTT, 2.5 μM ssDNA (72 nt), and the indicated amount of the protein. The samples were incubated at 30℃ for 30 min and aliquots (20 μl) were loaded onto a 1% agarose gel. The gel was stained with ethidium bromide and observed under UV light. (C) Analysis of the interaction between Hjm and the KH domain by pull-down. The non-tagged KH domain was incubated with the N-terminal His-tagged Hjm. The procedure was the same as for the analysis of the interaction between SisPINA and Hjm. The detailed procedure was described in the Materials and Methods.

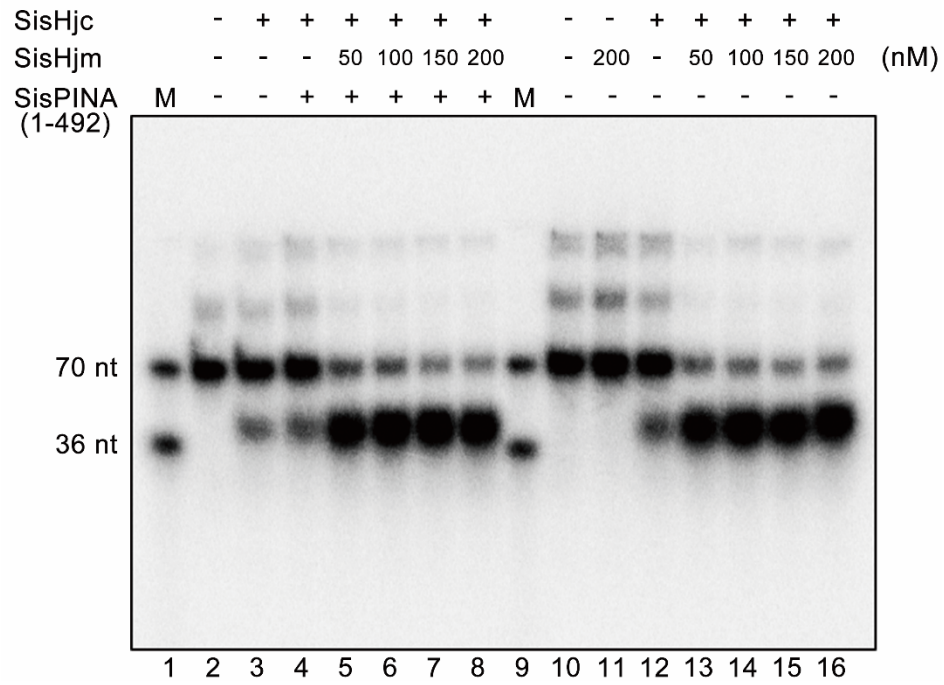


Fig. S7 Holliday junction processing by SisPINA (1-492), SisHjm, and SisHjc complex. The cleavage of HSL (mobile) HJ substrate by SisHjc in the presence of SisPINA (1-492) and SisHjm at indicated concentrations was analyzed by denatured gel electrophoresis. All of the reactions were carried out at 55^{o}C for 30 min.

Table S1 Primers used for gene cloning and genotype analysis.

Primer	Sequence (5’ to 3’)
SisPINA-MluI-Forward	GTTCACGCGTTATTGAATGATCTGATGTTAGATA
SisPINA-SalI-Reverse	GCTGGTCGACCTAATCGGATAGTTTTATC
SisPINA-NcoI-Forward	GACGCCATGGATGTTAAACAAGTTAGGTATAC
SisPINA-XhoI-Reverse	GATTCTCGAGCCATCACTTAAGGGTGGTCTAG
SisPINA-Flanking-SalI-Forward	CGTAGTCGACATGAAGATAGGTATAATAGC
SisPINA-Flanking-MluI-Reverse	ATCAACGCGTAATCCTTATCCCCACTTTTCAATC
SisPINA-NdeI-Forward	GCCGCATATGTTGAATGATCTGATGTTAGAT
SisPINA(1-492)-SalI-Reverse	GATCGTCGACCTACTCGAGTCTCTTTAGT
SisPINA-KH-NdeI-Forward	GTGCCATATGCCGGTGAATAGAGGAATAACAAT

The restriction enzyme sites are underlined. Other primers used in this study were as previously described in Zhai et al (1).

Table S2 Oligonucleotides used for 3'- and 5'-overhang substrate preparation.

Name	Sequence (5' to 3')
Strand 1 (34 nt)	CAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGA
Strand 2 (34 nt)	TCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTG
Strand 3 (84 nt)	TCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCCTTTTACAACGTCGTGACTGGGAAAACC CTGGCGTTAC
Strand 4 (84 nt)	GTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAAGGACGGCCAGTGCCAAGCTTGCATGCCTGCAGGTCG ACTCTAGAGGA

The 3'-overhang DNA (34/84 nts) contained strand 1 and strand 3. The 5'-overhang DNA (34/84 nts) contained strand 2 and strand 4. The oligonucleotides were used for other substrates were as previously described in Zhai et al (1).

Table S3 Plasmids used and constructed in this study.

Plasmid	Description	Sources
pSeSD-PINA-1	pSeSD carrying SisPINA (N-terminal 6 × His tag) and used as the MluI/SalI fragment for pMID-SisPINA-N-His	This study
pMID-SisPINA-1	pMID carrying SisPINA with fused N-terminal 6 × His tag and arabinose promoter	This study
pMID-SisPINA-N-His	pMID-SisPINA-1 carrying the flanking sequence down-stream of SisPINA for replacing the chromosomal SisPINA gene	This study
pET15bm-SisPINA	pET15bm carrying SisPINA (no tag) as a NdeI/SalI fragment	(1)
pET15b-SisHjm	pET15b carrying SisHjm (N-terminal His tag) insterted at NdeI/SalI	(2)
pET15bm-SisPINA(1-492)	pET15bm carrying SisPINA(1-492) (no tag) as NdeI/SalI fragment	This study
pET22b- SisPINAR206A/ R147K/I199S	pET22b carrying SisPINAR206A/R147K/I199S (no tag) as NdeI/SalI fragment	This study
pET22b-KH domain	pET22b carrying SisPINA gene for the KH domain (no tag) as a NdeI/SalI fragment	This study
pRSFDuet-1-SisHjc	pRSFDuet-1 carrying SisHjc (no tag) as a NdeI/XhoI fragment	(1)

Table S4 *Sulfolobus islandicus* strains used and constructed in this study.

Strain	Description	Sources
S. islandicus REY15A	Wild type	(3)
S. islandicus E233S	REY15A without *pyrEF* and *lacS*	(4)
Sis/pMID-SisPINA-in-situ-N-His-T	E233S double-crossover transformant generated with pMID-SisPINA-N-His via downstream insertion, containing N-terminal His-tagged SisPINA, *pyrEF* and *lacS*	This study

References

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3. Guo L., Brugger K., Liu C., Shah S.A., Zheng H., Zhu Y., Wang S., Lillestol R.K., Chen L., Frank J., Prangishvili D., Paulin L., She Q., Huang L., GArrett R.A. Genome analyses of Icelandic strains of *Sulfolobus islandicus*, model organisms for genetic and virus-host interaction studies. *J. Bacteriol*. 2011; 193: 1672-1680.
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