

PylSn and the homologous N-terminal domain of pyrrolysyl-tRNA synthetase bind  
the tRNA that is essential for the genetic encoding of pyrrolysine\*

## Supplemental materials

Ruisheng Jiang<sup>1</sup> and Joseph A. Krzycki<sup>1,2,3</sup>

<sup>1</sup>The Ohio State Biochemistry Program, <sup>2</sup> Center for RNA Biology, and <sup>3</sup>Department of  
Microbiology,  
The Ohio State University, Columbus, OH, 43210

\*Running title: *A protein specifically binding the pyrrolysine tRNA*

Name	Sequence
PylSnF	CCATGGGCATGAGAGGAGTGTCCCAGGCTCTGAGGAAAAG
PylSnR	GCGGCCGCTTAGTGGTATGATGGTGATGCCGCCGAAGATGTGA GCCATAATGCTGGC
92SF	CATATGGTAAAGTTAGGGTAG
92SR	CTCGAGCAGATTGGTTGAAATCCC
FusCF	CCATGGAGGGAGGATATCTCTGTTCTGCAAAGGC
FusCR	GGATCCTAGTGGTATGATGGTGATG CAGATTGGTTGAAATCCC
FusNF	CCATGGAGAGAGGAGTGTCCCAGG
FusNR	GATATCAAGATGTGAGCCATAATGCTGG
PylTF	TCTAGACCCATTCCAGCTGCTCC
PylTR	TCTAGACCCATTCCAGCTGCTCC

**Supplementary Table 1.** Primers used in this report.

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PylSn  1 MRGVSQASEEKKRYYRKNVDF FNLVEKIKLWPSRSGTLHGIKAMTRRGNTAEIVTHCNRRFIIYNSKHSRAARW
PylS   1 -----MDKKPLDV--LISATGLGMSRTGTLHKIKHHEVSRSKIYIEMACGDHLVVNNNSRSCRTARA
PylSc  1 -----


PylSn  75 LRNKLHFGVCPHCRIPEWKLQKYSSSTVMSQHYGSHL-----
PylS   60 FRHHKYRKTCRCSVDEDINNFLTRSTESKNSVKVRVSAPKVKKAMPKSVSRAPKPLENSVSAKASTNTSRS
PylSc  1 -----


PylSn 110 -----
PylS  134 VPSPAKSTPNSSVPASAPAPSILTRSQLDRVEALLSPEDKISLNMAKPFPRELEPELVTRRKNDFQRLYTNDREDY
PylSc  1 -----LSS--FWTKVQYQRLKELNASGEQLEMGFSDALSRDRAFGQIEHQQLMSQGKRHLEQLRTVKHRPA


PylSn  -----
PylS  208 LGKLERDITKFFVDRGFLIKEIPIPAEYVERMGINNDTELSKQIFRVDKNLCLRPMLAPTLNYLRKLDRIL
pylSc  64 LLELEEGLAALKALHQQGFVQVVTPTIITKSALAKMTIGEDHPLFSQVFWLGDGKKCLRPMLAPNLYTLWRELRLW


PylSn  -----
PylS  282 PGPIKIFEVGPCYRKESDGKEHLEEFTMVNFQCMGSGCTR--ENLEALIKEFLDYLEI-DFEIVGDSCMVGDT
pylSc 137 DKPIRIFEIGTCYRKESQGAQHILNEFTMLNLTTELGTPLEERHQRLEDMARWVLEAAGIREFELVTESSVVYGD



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**Supplementary Figure 1.** Protein sequences alignment of *D. hafniense* PylSn, PylSc and *M. barkeri* MS PylS. Sequences were aligned with clustalW2. Parts of the alignments were then adjusted and optimized manually. Identical residues were shown in red, conserved residues were shown in blue.