SUPPLEMENTARY MATERIALS

Supplementary Table S2. DNA sequences used for the PpCas9 characterization.

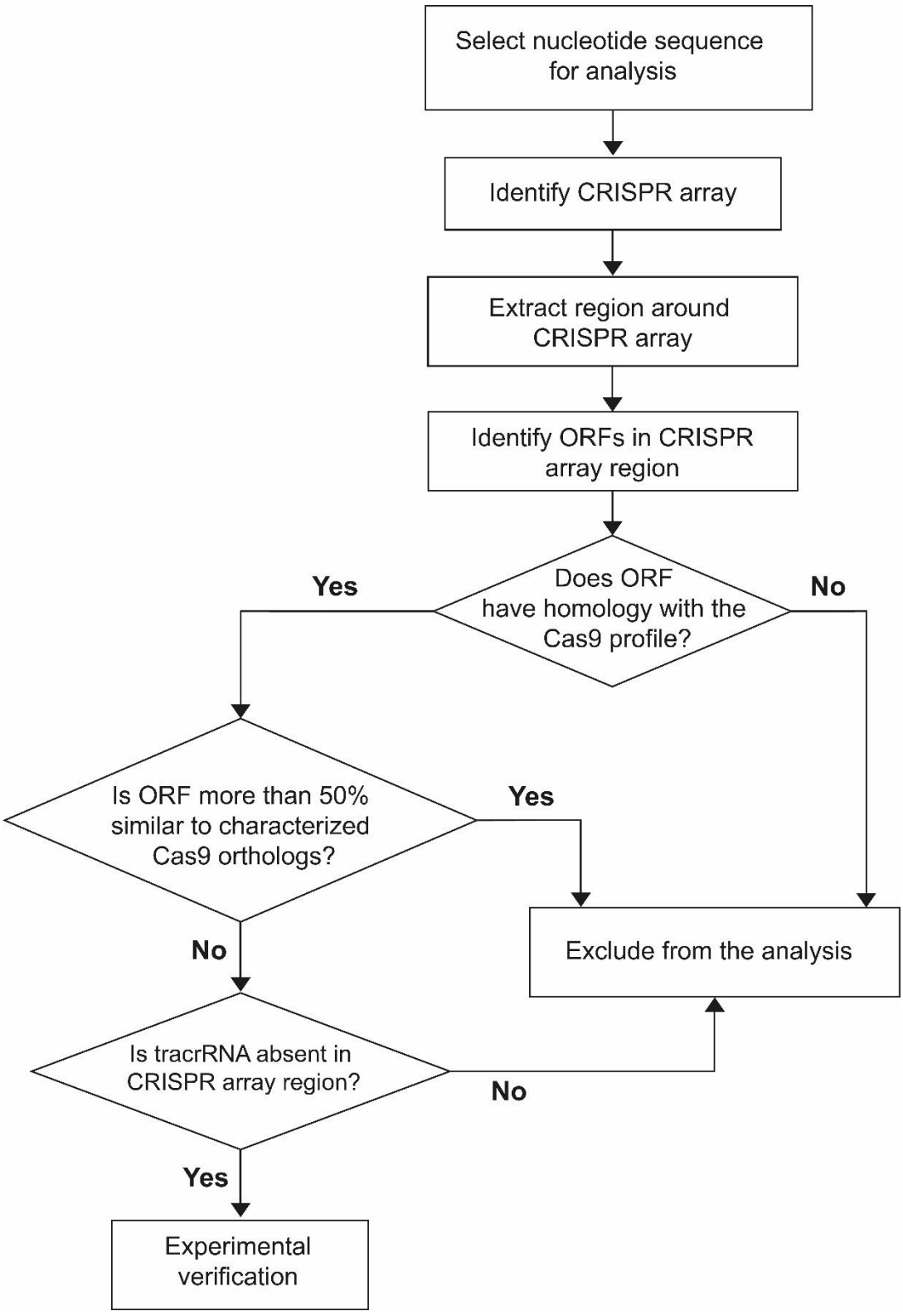
Step	Sequence, 5' → 3'	benchling.com link
Pasteurella pneumotropica ATCC 35149 CRISPR Type II locus		https://benchling.com/s/seq-CLVrTVfWkJ2m62jE3sMl?m=slm-9nEuTWu59Rv3SCmOrha9
DNA template for the crRNA synthesis	TAATACGACTCACTATAGGGTATCTCCTTTCATTGAGCACGTTGTAGCTCCCTTTTTCATTTCGC	https://benchling.com/s/seq-HzHx6SiG9sv8xtzCo0ef?m=slm-dyYeQGZTL9OwwSqtGN3b
DNA template for the tracrRNA synthesis	TAATACGACTCACTATAGGGCGAAATGAAAAACGTTGTTACAATAAGAGATGAATTTCTCGCAAAGCTCTGCCTCTTGAAATTTCGGTTTCAAGAGGCATCTTTTT	https://benchling.com/s/seq-1Ag3YluT39dXDPEFbmRu?m=slm-h96UhxlktAQDgOg4QoPV
7N *in vitro* PAM library forward primer	AATACCAGAGATAAGAGAGTAGGCTGGTAGATGGAGTTNNNNNNNGTGCTCAATGAAAGGAGATAAGGTC	
7N *in vitro* PAM library reverse primer	CCCGGGGTACCACGGAGAGATGGTG	
7N *in vitro* PAM library	AATACCAGAGATAAGAGAGTAGGCTGGTAGATGGAGTTNNNNNNNGTGCTCAATGAAAGGAGATAAGGTCCTTGAATTGCAGTATCTAGCCTCTTCTAAGACAGGTTACGTGATGTAGATCCTATTTTAACATGCTCTTTCTTTGTGTTTGCAGGGAGTCGACGAGTTGAAGATGAAGCCCAGAGCGGAGTGCTGTTCTCCCAAGTTCTGGTTGGTGTTGGCCGTCCTGGCCGTGTCAGGCAGCAGAGCTCGTTCTCAGAAGAGCCCCCCCAGCATTGGCATTGCTGTCATCCTCGTGGGCACTTCCGACGAGGTGGCCATCAAGGATGCCCACGAGAAAG	https://benchling.com/s/seq-aEAkRaY80eNFX7q2UFC1?m=slm-LpYsnBDLK1uK9nIWJHPn

	ATGATTTCCACCATCTCTCCGTGGTACCCCGGG	
NGS sample preparation: 1 round, forward primer	CTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCAAATACCAGAGATAAGAGAGTAGGCTG	
NGS sample preparation: 1 round, reverse primer	TCAGACGTGTGCTCTTCCGATCTAGGCTAGATACTGCAATTCAAGGACC	
NGS sample preparation: 2 round, forward primer	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	
NGS sample preparation: 2 round, reverse primer	CAAGCAGAAGACGGCATACGAGATNNNNNNNNNGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	

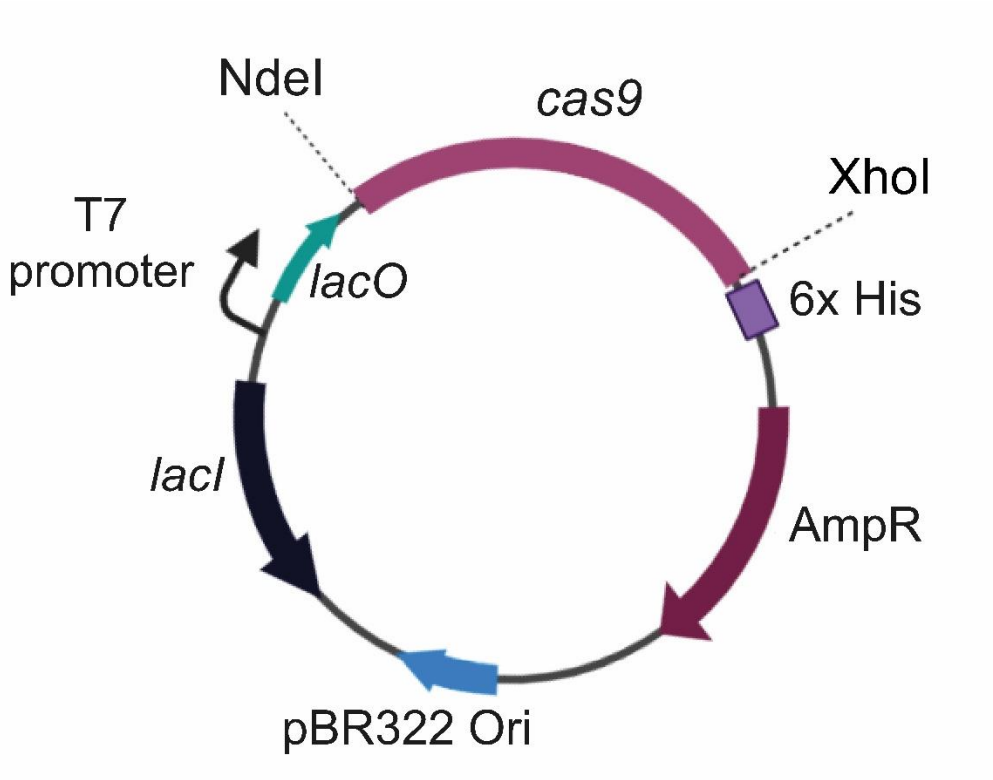
Supplementary Table S3. RNA sequences used for the PpCas9 characterization.

Sample	Sequence[a], 5'→3'	benchling.com link
crRNA	**GGG**uaucuccuuucauugagcacGUUG UAGCUCCCUUUUUCAUUUCGC	https://benchling.com/s/seq-HzHx6SiG9sv8xtzCo0ef?m=slm-dyYeQGZTL9OwwSqtGN3b
tracrRNA	**GGG**CGAAAUGAAAAACGUUGUUACAAU AAGAGAUGAAUUUCUCGCAAAGCUCUG CCUCUUGAAAUUUCGGUUUCAAGAGGC AUCUUUUU	https://benchling.com/s/seq-1Ag3YluT39dXDPEFbmRu?m=slm-h96UhxlktAQDgOg4QoPV

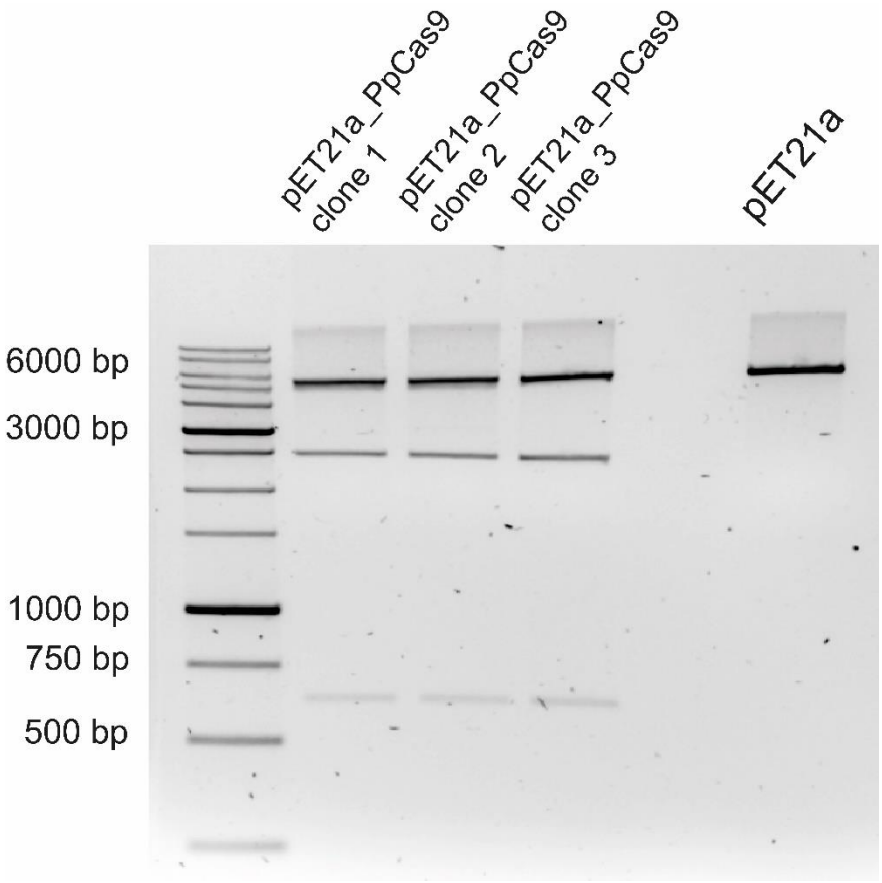
[a]The 5'-terminal G nucleotides added during T7 RNA polymerase synthesis are shown in bold. crRNA contains spacer sequence followed by direct repeat.



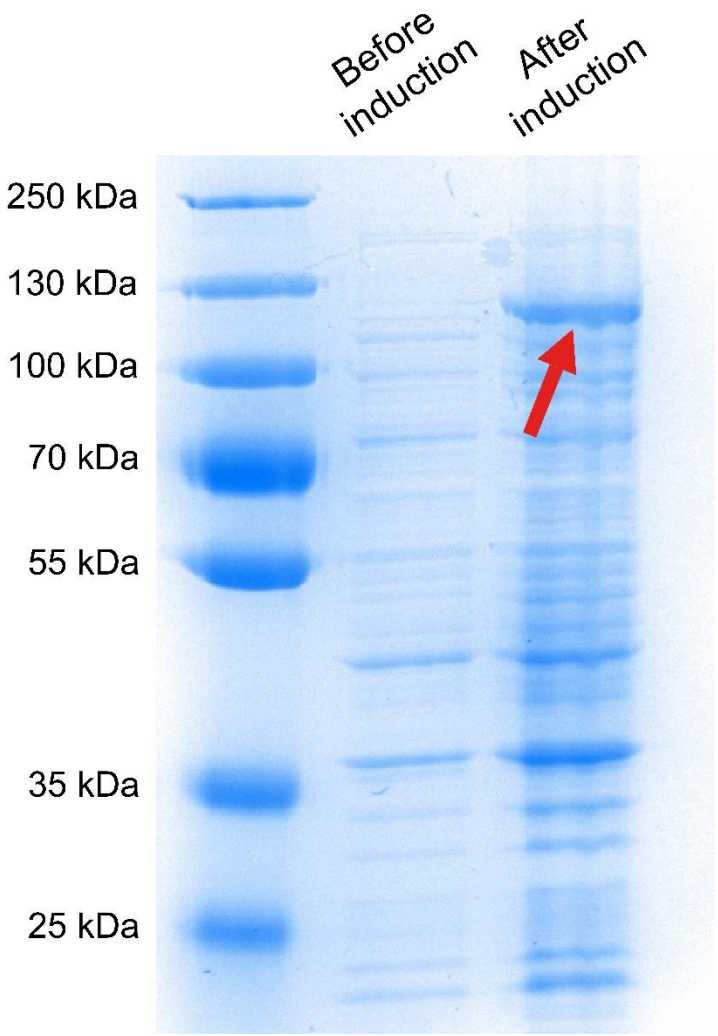
Supplementary Figure S1. Schematic representation of the bioinformatical pipeline for new type II CRISPR-Cas system nucleases characterization.



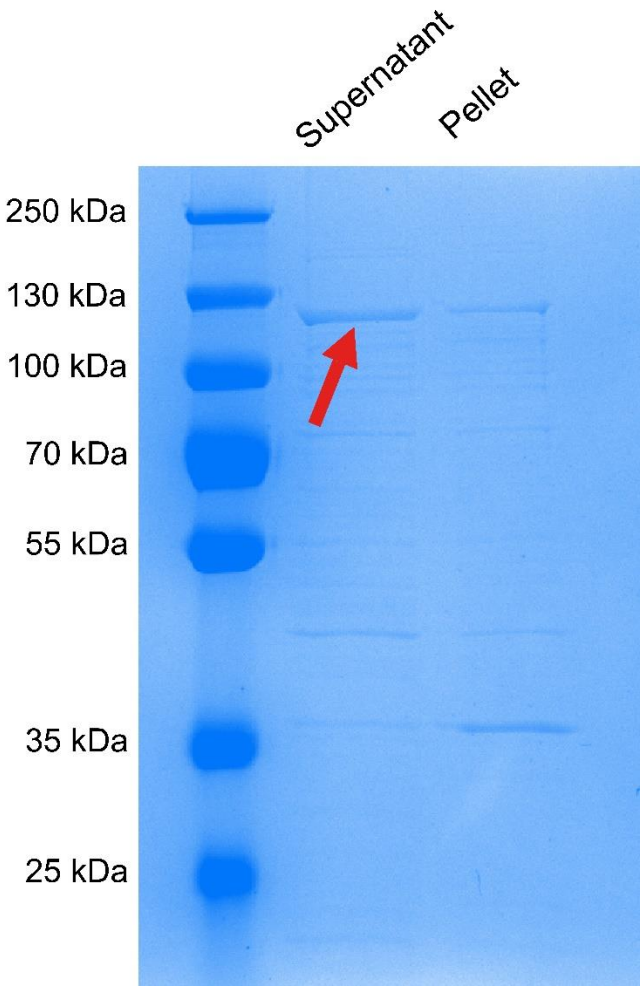
Supplementary Figure S2. The schematic representation of nuclease gene cloning into the pET21a vector.



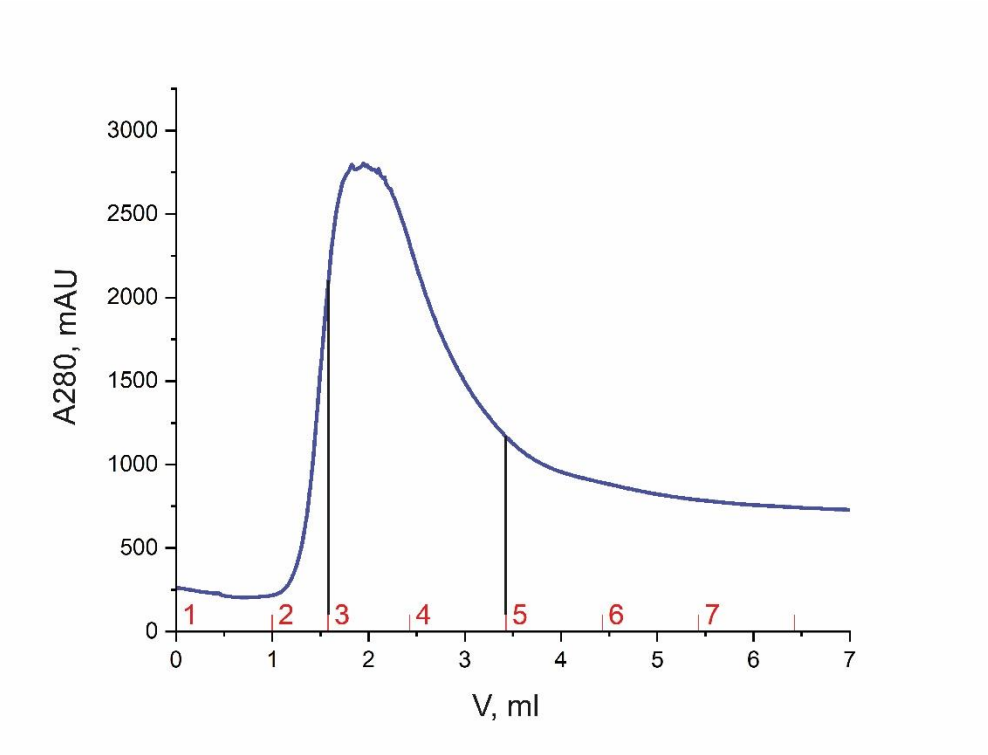
Supplementary Figure S3. The result of restriction enzyme digestion analysis. The expected size of DNA fragments generated by HindIII and XbaI – 5400 bp, 2500 bp and 630 bp. The gene of interest was successfully cloned into the pET21a vector.



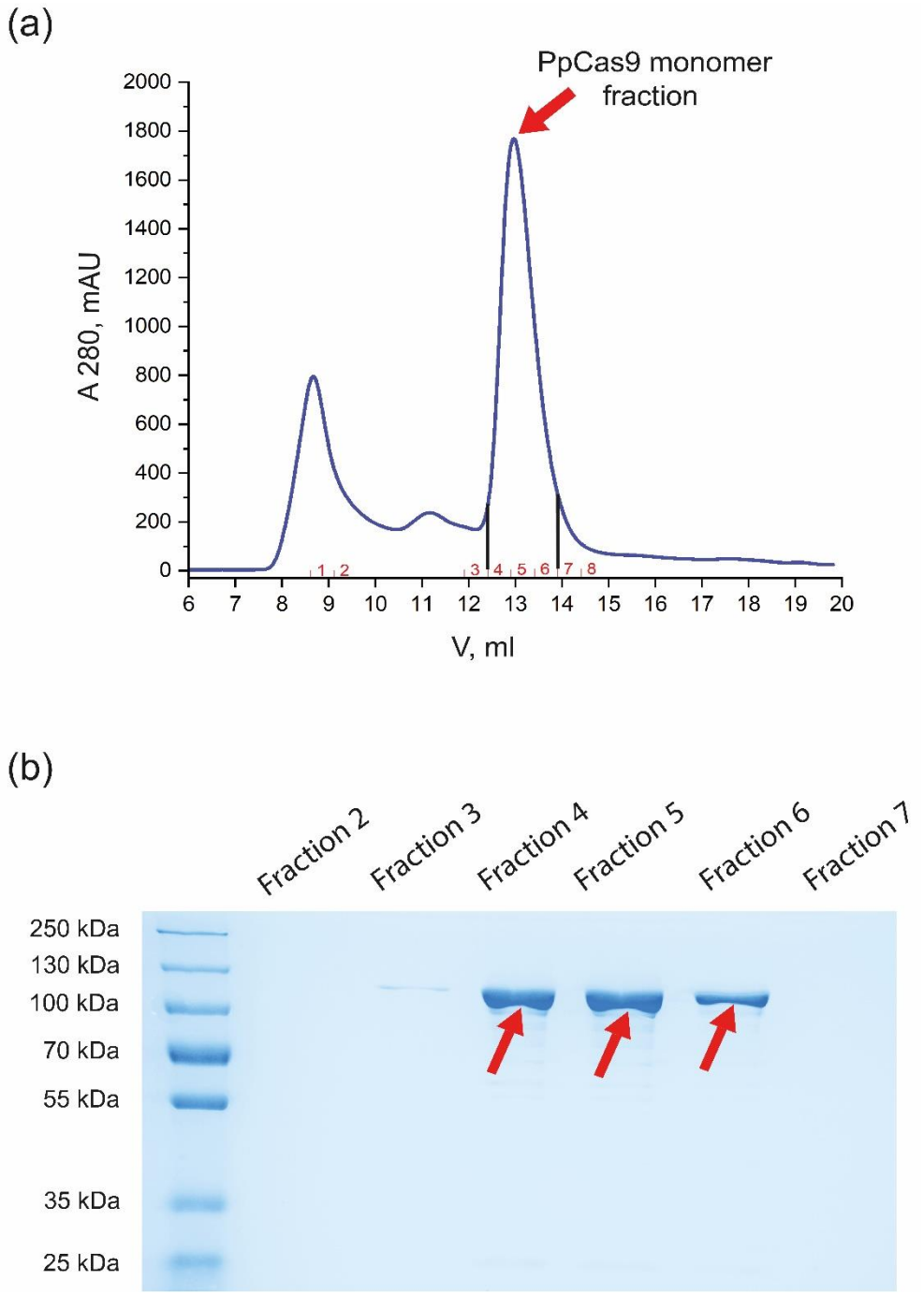
Supplementary Figure S4. The SDS-PAGE analysis of *E. coli* cells carrying the pET21a_PpCas9 plasmid. Total cell extracts before and after protein expression induction were analyzed. The PpCas9 is marked with a red arrow (expected molecular weight – 121 kDa).



Supplementary Figure S5. The SDS-PAGE analysis of *E. coli* cells carrying the pET21a_PpCas9 plasmid. Supernatant and pellet were obtained after protein induction and cells lysis. The PpCas9 is marked with a red arrow (expected molecular weight – 121 kDa).



Supplementary Figure S6. The elution profile for an affinity chromatography of the PpCas9. Fraction numbers are shown in read in the bottom of figure. Protein containing fractions – 3 and 4.



Supplementary Figure S7. The size exclusion chromatography of PpCas9. (a) – an elution profile. Fraction numbers are shown in read in the bottom of figure. Adapted from I. Fedorova et al [14], Creative Commons Attribution License. (b) – the SDS-PAGE analysis of fractions. The PpCas9 expected molecular weight – 121 kDa. Protein containing fractions – 4, 5 and 6.

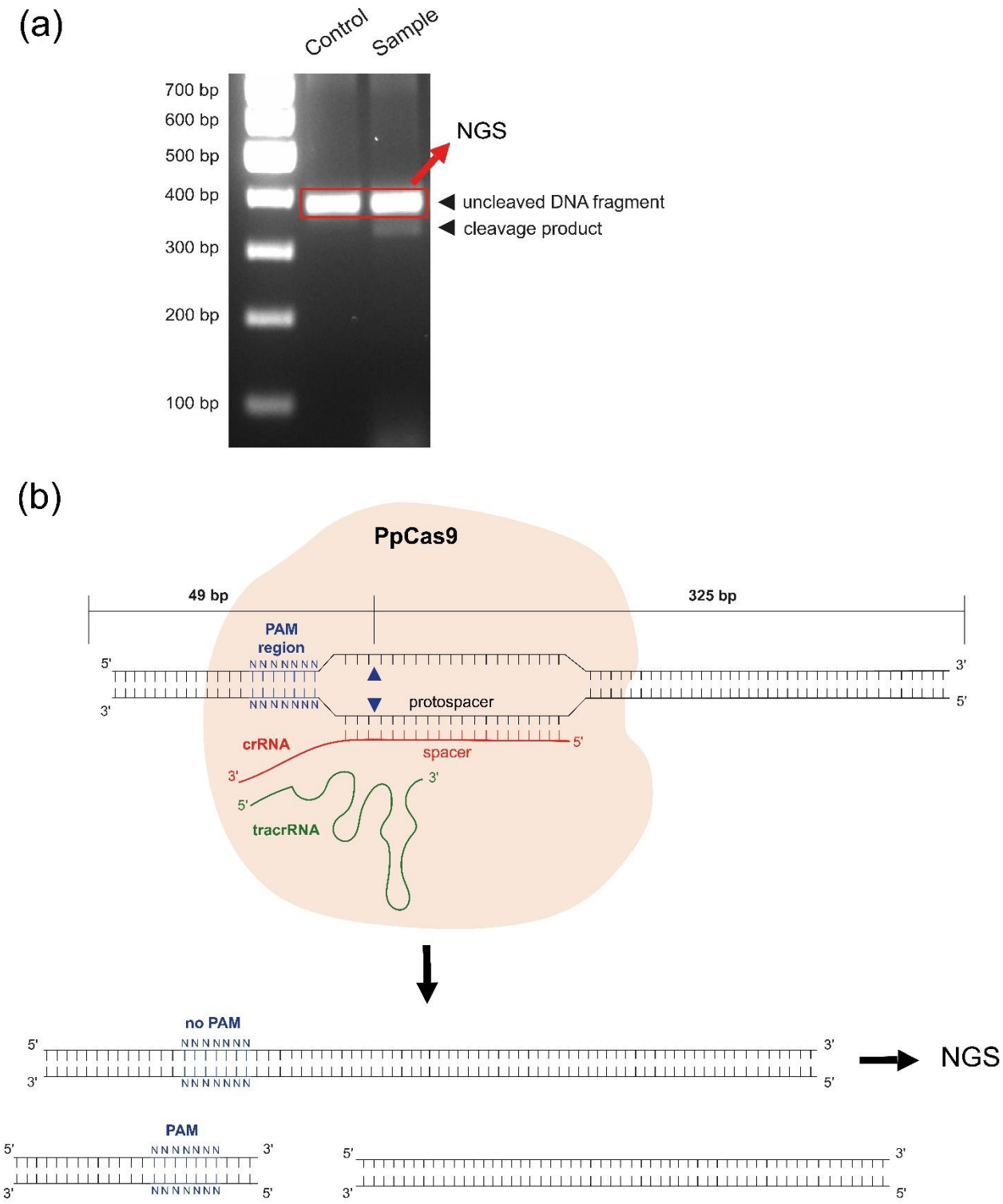


Figure S8. 7N *in vitro* PAM library cleavage by the PpCas9 and 7N *in vitro* depletion test. (a) – a gel showing results of 7N *in vitro* PAM library cleavage. Only part of the library DNA fragments had been cleaved. (b) – schematic representation of the 7N *in vitro* depletion test. DNA fragments containing PAM sequences were cleaved by the PpCas9. Undigested sequences were analyzed using high-throughput sequencing (NGS).