

proW_RT-PCR_2.fwd

G C C A G A

GTGCCGGGCGTGGTGGTGACGATCATCTTTGCTCTGCCGCCGATTATCCGTCTGACCATTTCTGGGGATTAACCAGGTTCCGGCGGATCTGATTGAAGCCTCGCGCTCATTCGGTGCCAGCCCCGCGCCAGA

3900

CACGGCCCGCACCACTGCTAGTAGAAACGAGACGGCGGCTAATAGGCAGACTGGTAAGACCCCTAATTGGTCCAAGGCCGCTAGACTAACTTCGGAGCGCGAGTAAGCCACGGTCGGGCGCGGTCT

210215220225230235240245250

ValProGlyValValValThrIleIlePheAlaLeuProProIleIleArgLeuThrIleLeuGlyIleAsnGlnValProAlaAspLeuIleGluAlaSerArgSerPheGlyAlaSerProArgGln

proW

Fragment proU8_NlaIII_PT

proW2

proW_RT-PCR_2.fwd

T G C T G T T C A A A G T T C A G T T A C

TGCTGTTCAAAGTTTCAGTTACCGCTGGCGATGCCGACCATTTATGGCGGGCGTTAACCAGACGCTGATGCTGGCCCTTTCTATGGTGGTCATCGCCTCGATGATTGCCGTCGGCGGGTTGGGTTCAGATGGT

4030

ACGACAAGTTTCAAGTCAATGGCGACCGCTACGGCTGGTAATACCGCCCGCAATTGGTCTGCGACTACGACCGGGAAAGATACCACCAGTAGCGGAGCTACTAACGGCAGCCGCCCAACCCAGTCTACCA

255260265270275280285290

MetLeuPheLysValGlnLeuProLeuAlaMetProThrIleMetAlaGlyValAsnGlnThrLeuMetLeuAlaLeuSerMetValValIleAlaSerMetIleAlaValGlyGlyLeuGlyGlnMetVal

proW

Fragment proU8_NlaIII_PT

proW2

CGGGAAAGATACCACCAGTAGCG

proW_RT-PCR_2.rev

ACTTCGCGGTATCGGTCGTCTGGATATGGGGCTTGCCACCGTTGGCGGGCGTCGGGATTGTGATCCTCGCCATTATCCTCGATCGTCTGACGCAGGCCGTTGGGCGCGACTCACGCAGTCGCGGGCAACCGT

4160

TGAAGCGCCATAGCCAGCAGACCTATACCCCGAACGGTGGCAACCGCCGCGAGCCCTAACACTAGGAGCGGTAATAGGAGCTAGCAGACTGCGTCCGGCAACCCGCGCTGAGTGCGTCAAGCGCCGTTGGCA

295300305310315320325330335

LeuArgGlyIleGlyArgLeuAspMetGlyLeuAlaThrValGlyGlyValGlyIleValIleLeuAlaIleIleLeuAspArgLeuThrGlnAlaValGlyArgAspSerArgSerArgGlyAsnArg

proW

Fragment proU8_NlaIII_PT

CGCTGGTACACCACTGGCCCTGTTGGTCTGCTGACCCGCCCATTCATTAAGTAACTCTGCACTTGCCCGGTGACGCCGGGCATTATCACCTGCCAAAAAAGGAATAACAATGCGACATAGCGTACTTT

4290

GCGACCATGTGGTGACCGGGACAACCAGACGACTGGGCGGGTAAGTAATTCATTGAGACGTGAACGGGCCACTGCGGCCCGTAATAGTGGGACGGTTTTTTTCTTATTGTTACGCTGTATCGCATGAAA

340345350355

ArgTrpTyrThrThrGlyProValGlyLeuLeuThrArgProPheIleLys

proW

Fragment proU8_NlaIII_PT

15

MetArgHisSerValLeu

proX

TTGCGACAGCGTTTGCCACGCTTATCTCTACACAACTTTTGCTGCCGATCTGCCGGGCAAAGGCATTACTGTTAATCCAGTTCAGAGCACCATCACTGAAGAAACCTTCCAGACGCTGCTGGTTCAGTCG

4420

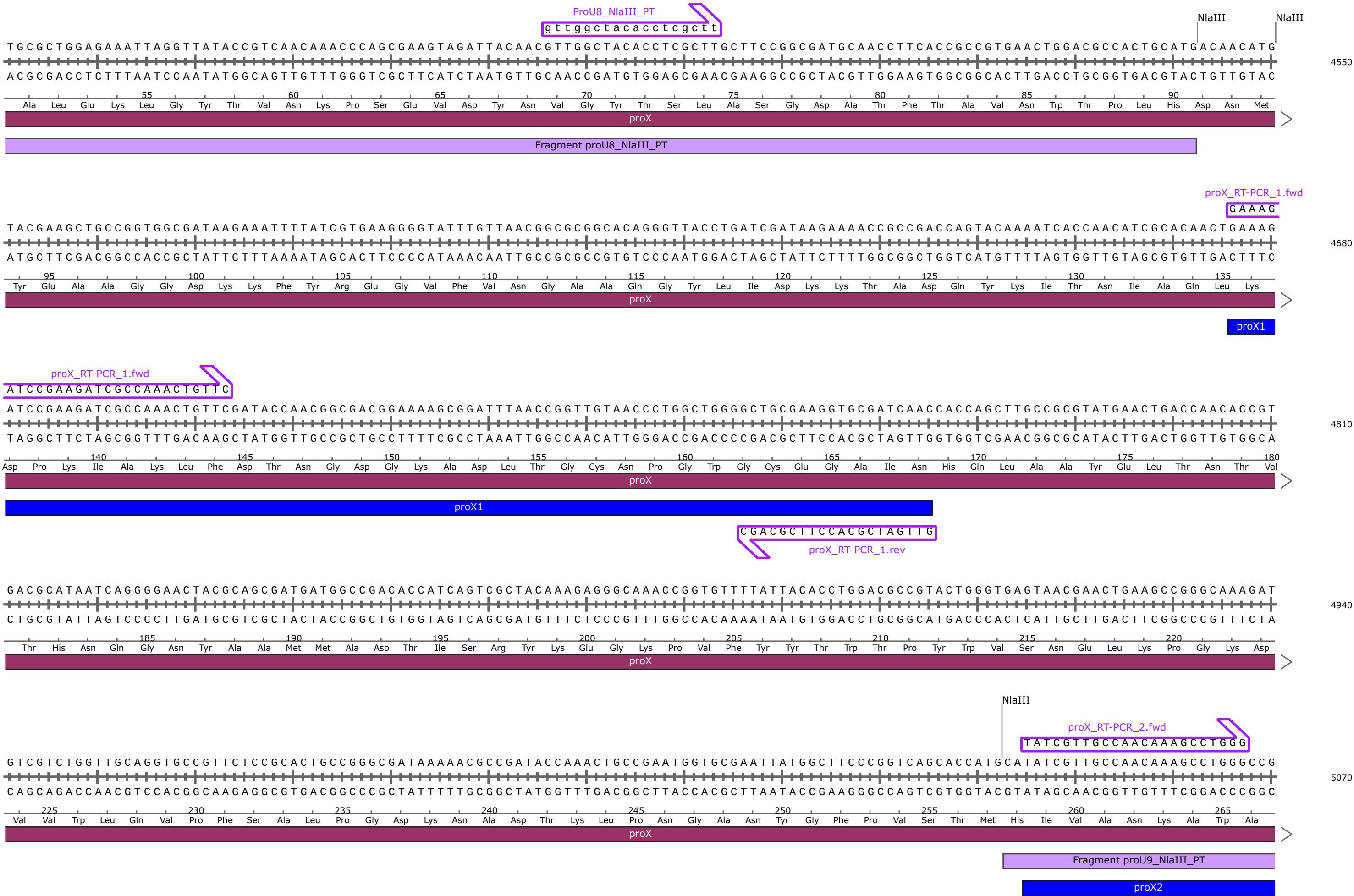
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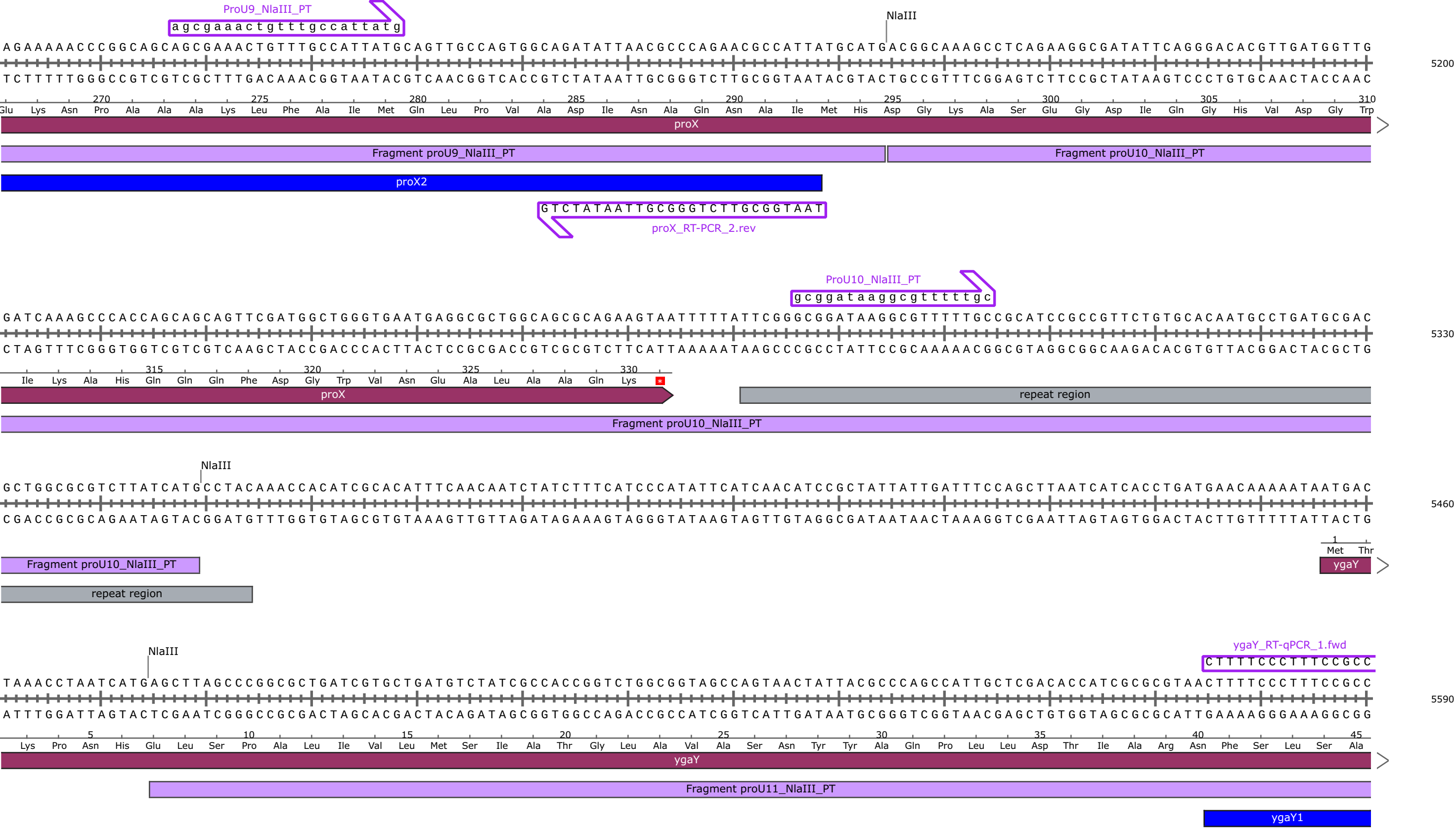
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PheAlaThrAlaPheAlaThrLeuIleSerThrGlnThrPheAlaAlaAspLeuProGlyLysGlyIleThrValAsnProValGlnSerThrIleThrGluGluThrPheGlnThrLeuLeuValSerArg

proX

Fragment proU8_NlaIII_PT





ygaY_RT-qPCR_1.fwd

AGTTTCG

CTCGGTGATATGTTTGAACGC

NlaIII

AGTTTCGGCAGGCTTTATTGTTACCGCCGCGCAGTTGGGCTATGCCGCAGGTCTACTGTTTCTTGTTCCTCTCGGTGATATGTTTGAACGCCGCCGCTGATTGTCTCGATGACCTTACTGGCGGCATGTT

TCAAGCCGTCCGAAATAACAATGGCGGCGCGTCAACCCGATACGGCGTCCAGATGACAAAGAACAAGGGGAGCCACTATACAAACTTGCGGGCGGCGGACTAACAGAGCTACTGGAATGACCGCCGTACAA

Ser Ser Ala Gly Phe Ile Val Thr Ala Ala Gln Leu Gly Tyr Ala Ala Gly Leu Leu Phe Leu Val Pro Leu Gly Asp Met Phe Glu Arg Arg Arg Leu Ile Val Ser Met Thr Leu Leu Ala Ala Cys

ygaY

Fragment proU11_NlaIII_PT

Fragment proU12_NlaIII_PT

ygaY1

GGACTAACAGAGCTACTGGAATGAC

ygaY_RT-qPCR_1.rev

GATTACCGCCAGCAGTCAGTCGCTGGCGATGATGATCCTCGGTACGGCATTAAACCGGTTTATTCTCAGTCGTGGCACAAATTCTGGTTCCGCTGGCAGCGACGCTGGCTTCACCGGATAAACGCGGCAAA

CTAATGGCGGTCGTCAGTCAGCGACCGCTACTACTAGGAGCCATGCCGTAATTGGCCAAATAAGAGTCAGCACCGTGTTTAAGACCAAGGCGACCGTCGCTGCGACCGAAGTGGCCTATTTGCGCCGTTT

Leu Pro Pro Ala Val Ser Arg Trp Arg * * Ser Ser Val Arg His * Pro Val Tyr Ser Gln Ser Trp His Lys Phe Trp Phe Arg Trp Gln Arg Arg Trp Leu His Arg Ile Asn Ala Ala Lys

ygaY

Fragment proU12_NlaIII_PT

GTGGTTGGCACTATTATGAGCGGTCTGCTGTTGGGGATCTTGCTGGCACGGACAGTTGCCGGATTGCTGGCGAATCTCGGCGGCTGGCGCACCGTCTTTTGGGTTGCTTCGGTGTTAATGGCACTGATGG

CACCAACCGTGATAATACTCGCCAGACGACAACCCCTAGAACGACCGTGCTGTCAACGGCCTAACGACCGCTTAGAGCCGCGCGACCGCGTGCGCAGAAAACCCAACGAAGCCACAATTACCGTGACTACC

Trp Leu Ala Leu Leu * Ala Val Cys Cys Trp Gly Ser Cys Trp His Gly Gln Leu Pro Asp Cys Trp Arg Ile Ser Ala Ala Gly Ala Pro Ser Phe Gly Leu Leu Arg Cys * Trp His * Trp

ygaY

Fragment proU12_NlaIII_PT

CGCTGGCATTATGGCGTGGTCTGCCACAAATGAAATCAGAAACCCACCTCAACTACCCACAGTTGTTGGGTTCCGTTTTTCAGTATGTTTATCAGCGATAAAATTCTGCGTACCCGCGCGTTGCTGGGCTG

GCGACCGTAATACCGCACCAAGACGGTGTTTACTTTAGTCTTTGGGTGGAGTTGATGGGTGTCAACAACCCAAGGCAAAAGTCATACAAATAGTCGCTATTTTAAAGACGCATGGGCGCGCAACGACCCGAC

Arg Trp His Tyr Gly Val Val Cys His Lys * Asn Gln Lys Pro Thr Ser Thr Thr His Ser Cys Trp Val Pro Phe Ser Val Cys Leu Ser Ala Ile Lys Phe Cys Val Pro Ala Arg Cys Trp Ala

ygaY

Fragment proU12_NlaIII_PT

ygaY_RT-qPCR_2.fwd

GCCGCTCCACCTTTTAACTACAG

CCTGACCTTTGCCAATTTTCAGCATTCTCTGGACCTCAATGGCCTTTTGTCTGCGCTCCACCTTTTAACTACAGCGATGGTGTCATTGGTCTGTTTGGACTTGCGGGAGCTGCCGGAGCGTTGGGCGCT

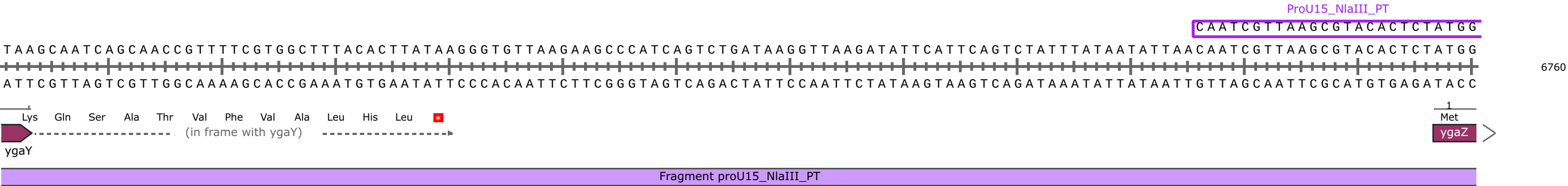
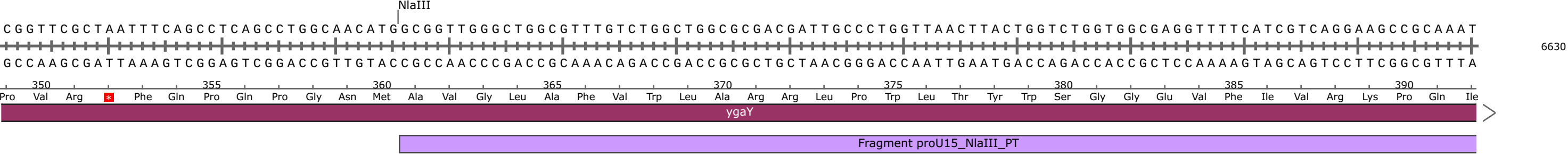
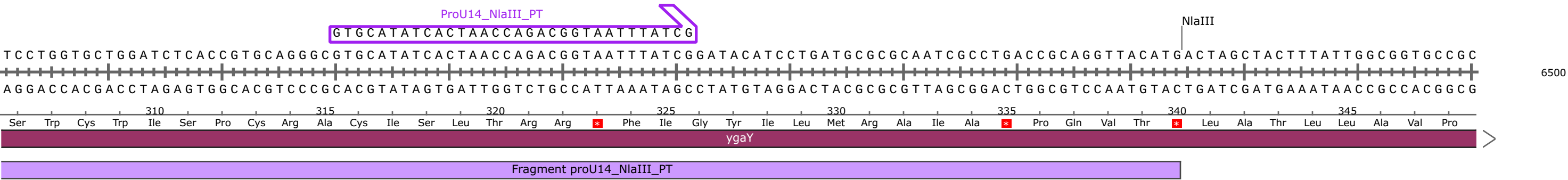
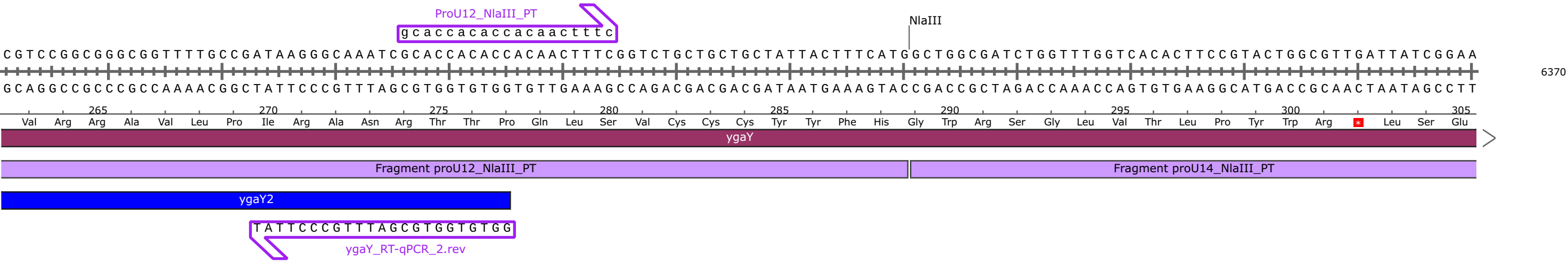
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Ala * Pro Leu Pro Ile Ser Ala Phe Ser Gly Pro Gln Trp Pro Phe Cys Leu Pro Leu His Leu Leu Thr Thr Ala Met Val Ser Leu Val Cys Leu Asp Leu Arg Glu Leu Pro Glu Arg Trp Ala Leu

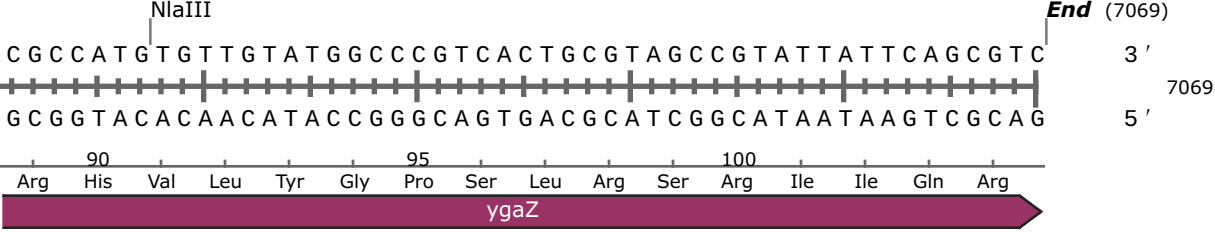
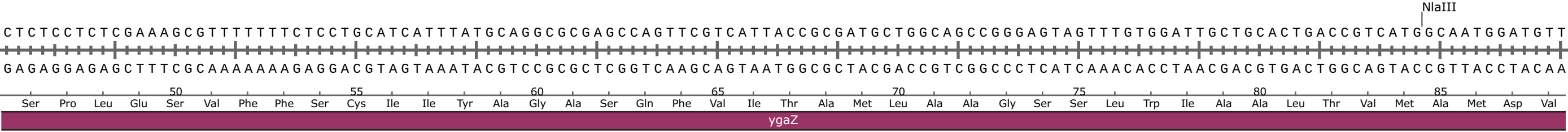
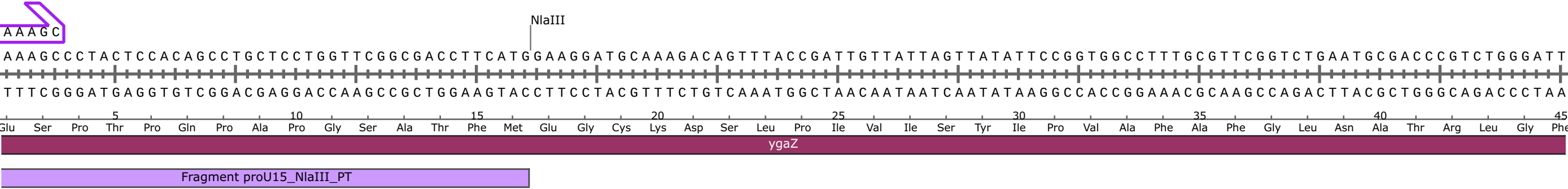
ygaY

Fragment proU12_NlaIII_PT

ygaY2















































ProU15_NlaIII_PT


























Enzymes	Sites
NlaIII	25 279 456 900 945 1262 1840 1957 2134 2641 3041 3710 3752 4542 4550 5042 5154 5349 5474 5718 6320 6474 6535 6804 7008 7027

SI_1A.dna (Linear / 7069 bp)									
Feature		Location			Size				Type
✓	nrdE	1	..	632	632 bp				CDS
	<div><div>/codon_start = 1</div><div>/transl_table = 11 (Bacterial, Archaeal and Plant Plastid)</div><div>/gene = nrdE</div><div>/gene_synonym = ECK2669; JW2650</div><div>/locus_tag = b2675</div><div>/product = ribonucleoside-diphosphate reductase 2, alpha subunit</div><div>/function = enzyme; Central intermediary metabolism: Nucleotide interconversions</div><div>/EC_number = 1.17.4.1</div><div>/db_xref = GI: 16130589</div><div>/db_xref = ASAP: ABE-0008806</div><div>/db_xref = UniProtKB/Swiss-Prot: P39452</div><div>/db_xref = EcoGene: EG20257</div><div>/db_xref = GeneID: 947155</div><div>/protein_id = NP_417161.1</div><div>/translation = LRTSMLLARERGETFAGFKQSRYPASGEYFSQYLQGNWQPKTAKVGELFTRSGITLPTREMWAQLRDDVMRYGIYNQNLQAVPPTGSISYINHATSSIHPIVAKVEIRKEGKTGRVYYPAPFMTNENLALYQDAYEIGAEEKIIDTYAEATRHVDQGLSLTLFFPDTATTRDINKAQIYAWRKGIKTLYYIRLRQMALEGTEIEGCVSCAL* 209 amino acids = 23,8 kDa</div></div>								
✓	Fragment proU17_NlaIII_PT	1	..	279	279 bp				misc_feature
✓	Fragment proU16_NlaIII_PT	280	..	456	177 bp				misc_feature
✓	Fragment proU13_NlaIII_PT	457	..	900	444 bp				misc_feature
✓	nrdF	642	..	1601	960 bp				CDS
	<div><div>/product = ribonucleoside-diphosphate reductase 2, beta subunit, ferritin-like protein</div><div>/translation = MKLSRISAINWNKISDDKDLEVWNRLTSNFWLPEKVPLSNDIPAWQTLTVVEQQLTMRVFTGLTLLDRTLQNVIGAPSLMPDALTPHEEAVLSNISFMEAVHARSYSSIFSTLCQTKDQVDAAYAWSEENAPLQRKAQIIQQHYRGDDPLKKKIASVFLESFLFYSGFWLPMYFSSRGKLTNTADLIRLIIRDEAVHGYIIGYKYQKNMEKISLGQREELKSFAFDLLLELYDNELQYTDELYAETPWADDVKAFLCYNANKALMNLGYEPLFPAEMAEVNPAILAALSPNADENHDDFFSGSGSSYVMGKAVETEDEDWNF* 319 amino acids = 36,4 kDa</div></div>								
✓	nrdF1	712	..	840	129 bp				misc_feature
✓	Fragment proU1_NlaIII_PT	946	..	1262	317 bp				misc_feature
✓	Fragment proU2_NlaIII_PT	1263	..	1840	578 bp				misc_feature
✓	nrdF2	1457	..	1557	101 bp				misc_feature
✓	Intrinsically curved region	1536	..	1815	280 bp				misc_feature
	<div><div>/note = This region is predicted to be a curved DNA sequence. <i>In vitro</i> studies have shown that H-NS exhibits a preferential binding to this site [1]. <i>In vivo</i> studies do not corroborate this observation [2].</div><div>[1] Tanaka, K. I., Muramatsu, S., Yamada, H., & Mizuno, T. (1991). Systematic characterization of curved DNA segments randomly cloned from Escherichia coli and their functional significance. <i>Molecular and General Genetics MGG</i>, 226(3), 367-376.</div><div>[2] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. <i>Journal of Biological Chemistry</i>, 269(9), pp. 6578-6578.</div></div>								
✓	NRE	1643	..	3075	1433 bp				misc_feature
	<div><div>/note = Dattananda,C.S.,Rajkumari,K. and Gowrishankar,J. (1991) Multiple mechanisms contribute toosmotic inducibility of proU operon expression in Escherichia coli:Demonstration of two osmoresponsive promoters and of a negative regulatoryelement within the first structural gene. <i>J. Bacteriol.</i>,10.1128/jb.173.23.7481-7490.1991.</div></div>								
✓	URE	1666	..	1848	183 bp				regulatory
	<div><div>/regulatory_class = silencer</div></div>								
✓	H-NS bound region	1666	..	1685	20 bp				misc_feature
	<div><div>/note = In vitro DNase-I protection assay. Fig. 6 in [1]</div><div>[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. <i>Journal of Biological Chemistry</i>, 269(9), pp. 6578-6578.</div></div>								






SI_1A.dna (Linear / 7069 bp)							
Feature		Location		Size			Type
✓	H-NS bound region	1697	.. 1718	22 bp			misc_feature
/note = In vitro DNase-I protection assay. Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	σS ProU TSS	1704	.. 1705	2 bp			misc_feature
/note = σS ProU TSS between the two bases [1].							
[1] Dattananda, C. S., Rajkumari, K., & Gowrishankar, J. (1991). Multiple mechanisms contribute to osmotic inducibility of proU operon expression in Escherichia coli: demonstration of two osmoreponsive promoters and of a negative regulatory element within the first structural gene. <i>Journal of bacteriology</i> , 173(23), 7481-7490.							
✓	H-NS bound region	1721	.. 1749	29 bp			misc_feature
/note = In vitro DNase-I protection assay. Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	proU1	1747	.. 1872	126 bp			misc_feature
/note = RT-qPCR proU1 amplicon							
✓	H-NS bound region	1755	.. 1784	30 bp			misc_feature
/note = In vitro DNase-I protection assay. Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	H-NS bound region	1791	.. 1819	29 bp			misc_feature
/note = In vitro DNase-I protection assay. Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	H-NS bound region	1830	.. 1851	22 bp			misc_feature
/note = In vitro DNase-I protection assay. Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	EMSA_fragment	1841	.. 2134	294 bp			misc_feature
✓	Fragment proU3_NlaIII_PT	1841	.. 1957	117 bp			misc_feature
✓	NT644_DRE	1855	.. 2071	217 bp			misc_feature
/note = DRE Sequence in NT644:							
AGGGGTTGCCTCAGATTCTCAGTATGTTAGGGTAGAAGCAAGTGACGCTTTCCATTGGGTAGTACATCGACATAGACAAATAAAGGAATCTTTCTATTGCATGGCAATCAAACCTCGAGATCAAGAACCTCTACAAGATCTTCGGCGAGCATCCACAGCGAGCGTTCAAGTACATCGAGCAGGGGCTCTCG AAGGAGCAGATCCTGGAGAAGACGGGG							
✓	DRE	1855	.. 2071	217 bp			regulatory
► 5 segments							
/note = H-NS nucleation sites (Green) described in [1].							
[1] Bouffartigues, E., Buckle, M., Badaut, C., Travers, A., & Rimsky, S. (2007). H-NS cooperative binding to high-affinity sites in a regulatory element results in transcriptional silencing. <i>Nature structural & molecular biology</i> , 14(5), 441-448.							
/regulatory_class = silencer							
✓	H-NS bound region (Weak binding)	1856	.. 1873	18 bp			misc_feature
/note = In vitro DNase-I protection assay. Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
















SI_1A.dna (Linear / 7069 bp)							
Feature		Location		Size		Type	
✓	-35 promoter sequence	1860	.. 1865	6 bp			misc_feature
/note = Determined from Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	IHF binding site	1862	.. 1919	58 bp			misc_feature
/note = Khodr,A., Fairweather,V.,Bouffartigues,E. and Rimsky,S. (2015) IHF is a trans-acting factor implicatedin the regulation of the proU P2 promoter. <i>FEMS Microbiol. Lett.</i> ,10.1093/femsle/fnu049.							
✓	-10 promoter sequence	1882	.. 1887	6 bp			misc_feature
/note = Determined from Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	Initial IHF protection site	1883	.. 1914	32 bp			misc_feature
/note = Khodr,A., Fairweather,V.,Bouffartigues,E. and Rimsky,S. (2015) IHF is a trans-acting factor implicatedin the regulation of the proU P2 promoter. <i>FEMS Microbiol. Lett.</i> ,10.1093/femsle/fnu049.							
✓	H-NS bound region	1888	.. 1909	22 bp			misc_feature
/note = In vitro DNase-I protection assay. Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	σ70 ProU TSS	1894	.. 1895	2 bp			misc_feature
/note = σ70 proU TSS (between the two bases); determined from Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	H-NS bound region	1912	.. 1924	13 bp			misc_feature
/note = In vitro DNase-I protection assay. Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							
✓	Shine Dalgarno sequence	1927	.. 1957	31 bp			misc_feature
/note = http://regulondb.ccg.unam.mx/gene?term=ECK120000762&organism=ECK12&format=jsp&type=gene							
✓	H-NS bound region	1928	.. 1949	22 bp			misc_feature
/note = In vitro DNase-I protection assay. Fig. 6 in [1]							
[1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.							




















SI_1A.dna (Linear / 7069 bp)									
Feature		Location		Size			Type		
✓	proV	1955	.. 3157	1203 bp			CDS		
	/transl_table = 11 (Bacterial, Archaeal and Plant Plastid)								
	/gene = proV								
	/gene_synonym = ECK2671; JW2652; osrA; proU								
	/locus_tag = b2677								
	/product = glycine betaine transporter subunit								
	/function = transport; Transport of small molecules: Amino acids, amines								
	/note = ATP-binding component of transport system for glycine, betaine and proline								
	/db_xref = GI: 16130591								
	/db_xref = ASAP: ABE-0008814								
	/db_xref = UniProtKB/Swiss-Prot: P14175								
	/db_xref = EcoGene: EG10771								
	/db_xref = GeneID: 947148								
	/protein_id = NP_417163.1								
	/translation = MAIKLEIKNLYKIFGEHPQRAFKYIEQGLSKEQILEKTGLSLGVKDASLAIEEGEIFVIMGLSGSGKSTMVRLLNRLIEPTRGQVLIDGVDIAKISDAELREVRKKIAMVFQSFALMPHMTVLDNTAFGMELAGINAEERREKALDALRQVGLENYAHSYPDELSGGMRQRVGLARALAINPDILLMDEAF SALDPLIRTEMQDELVKLQAKHQRTIVFISHDLDEAMRIGDRIAIMQNGEVVQVGTPEILNNPANDYVRTFFRGVDISQVFSAKDIARRTPNGLIRKTPGFGPRSAKLLQDEGREYGYVIERGNKFVGAVSIDSLKTALTQQQGLDAALIDAPLAVDAQTPLSELLSHVGQAPCAVPVVEDDQQYVGIIS KGMLLRALDREGVNNG* 400 amino acids = 44,2 kDa								
✓	Fragment proU4_NlaIII_PT	1958	.. 2134	177 bp			misc_feature		
✓	H-NS bound region (Strong binding)	1958	.. 2003	46 bp			misc_feature		
	/note = In vitro DNase-I protection assay. Fig. 6 in [1] [1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.								
✓	H-NS bound region	2014	.. 2035	22 bp			misc_feature		
	/note = In vitro DNase-I protection assay. Fig. 6 in [1] [1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.								
✓	H-NS bound region	2043	.. 2075	33 bp			misc_feature		
	/note = In vitro DNase-I protection assay. Fig. 6 in [1] [1] Lucht, J.M., Dersch, P., Kempf, B. and Bremer, E., 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled proU operon of Escherichia coli. Journal of Biological Chemistry, 269(9), pp. 6578-6578.								
✓	conserved RNase III degradation site	2100	.. 2189	90 bp			misc_feature		
✓	Fragment proU5_NlaIII_PT	2135	.. 2641	507 bp			misc_feature		
✓	proV1	2240	.. 2339	100 bp			misc_feature		
	/note = proV1 RT-qPCR amplicon								
✓	Fragment proU6_NlaIII_PT	2642	.. 3041	400 bp			misc_feature		
✓	proV2	2722	.. 2837	116 bp			misc_feature		
	/note = proV2 RT-qPCR amplicon								
✓	Fragment proU7_NlaIII_PT	3042	.. 3710	669 bp			misc_feature		







SI_1A.dna (Linear / 7069 bp)									
	Feature		Location		Size				Type
✓	proW		3150	.. 4214	1065 bp	<div></div>	→		CDS
	/transl_table	=	11 (Bacterial, Archaeal and Plant Plastid)						
	/gene	=	proW						
	/gene_synonym	=	ECK2672; JW2653; osrA; proU						
	/locus_tag	=	b2678						
	/product	=	glycine betaine transporter subunit						
	/function	=	transport; Transport of small molecules: Amino acids, amines						
	/note	=	high-affinity transport system for glycine betaine and proline; membrane component of ABC superfamily						
	/db_xref	=	GI: 16130592						
	/db_xref	=	ASAP: ABE-0008816						
	/db_xref	=	UniProtKB/Swiss-Prot: P14176						
	/db_xref	=	EcoGene: EG10772						
	/db_xref	=	GeneID: 947145						
	/protein_id	=	NP_417164.1						
	/translation	=	MADQNNPWDTTPAADSAAQSADAWGTPTTAPTGGGADWLTSTPAPNVEHFNILDPFHKTLIPLDSWVTEGIDWVVTHERPVFQGVVPVDYILNGFQQLLLGMPAPVAIIVFALIAWQISGVGMGVATLVSLIAIGAIGAWSQAMVTLALVLTALLFCIVIGLPLGIWLARSPRAAKIIRPLLDAMQTTPAFVYLVPIVMLFGIGNVPGVVVTIIFALPPIIRLTILGINQVPADLIEASRSFGASPRQMLFKVQLPLAMPTIMAGVNQTLMLALSMVVIASMIAVGGLGQMVLRGIGRLDMGLATVGGVGIVILAIILDRLTQAVGRDSRSRGNRRWYTTGPVGLLTRPFIK*						
	354 amino acids = 37,6 kDa								
✓	proW1		3380	.. 3500	121 bp	<div></div>	⇐		misc_feature
	/note	=	proW1 RT-qPCR amplicon						
✓	rG4 site		3571	.. 3620	50 bp	<div></div>	⇐		misc_feature
	/note	=	Class: 2 quartet Reverse Transcriptase Stalling: 0.276786 Strand: + Reference: Shao, X., Zhang, W., Umar, M.I., Wong, H.Y., Seng, Z., Xie, Y., Zhang, Y., Yang, L., Kwok, C.K. and Deng, X., 2020. RNA G-Quadruplex structures mediate gene regulation in bacteria. <i>MBio</i> , 11(1).						
✓	rG4 site		3575	.. 3624	50 bp	<div></div>	⇐		misc_feature
	/note	=	Class: 2 quartet Reverse Transcriptase Stalling: 0.003488 Strand: + Reference: Shao, X., Zhang, W., Umar, M.I., Wong, H.Y., Seng, Z., Xie, Y., Zhang, Y., Yang, L., Kwok, C.K. and Deng, X., 2020. RNA G-Quadruplex structures mediate gene regulation in bacteria. <i>MBio</i> , 11(1).						
✓	rG4 site		3579	.. 3628	50 bp	<div></div>	⇐		misc_feature
	/note	=	Class: 2 quartet Reverse Transcriptase Stalling: 0.002725 Strand: + Reference: Shao, X., Zhang, W., Umar, M.I., Wong, H.Y., Seng, Z., Xie, Y., Zhang, Y., Yang, L., Kwok, C.K. and Deng, X., 2020. RNA G-Quadruplex structures mediate gene regulation in bacteria. <i>MBio</i> , 11(1).						
✓	Fragment proU8_NlaIII_PT		3753	.. 4542	790 bp	<div></div>	⇐		misc_feature
✓	proW2		3895	.. 3994	100 bp	<div></div>	⇐		misc_feature
	/note	=	proW2 RT-qPCR amplicon						








SI_1A.dna (Linear / 7069 bp)									
	Feature	Location			Size			Type	
✓	proX	4272	..	5264	993 bp	<div></div>	→	CDS	
	<div> <div>/transl_table = 11 (Bacterial, Archaeal and Plant Plastid)</div> <div>/gene = proX</div> <div>/gene_synonym = ECK2673; JW2654; osrA; proU</div> <div>/locus_tag = b2679</div> <div>/product = glycine betaine transporter subunit</div> <div>/function = transport; Osmotic adaptation</div> <div>/note = high-affinity transport system for glycine betaine and proline; periplasmic-binding component of ABC superfamily</div> <div>/db_xref = GI: 16130593</div> <div>/db_xref = ASAP: ABE-0008818</div> <div>/db_xref = UniProtKB/Swiss-Prot: P0AFM2</div> <div>/db_xref = EcoGene: EG10773</div> <div>/db_xref = GeneID: 947165</div> <div>/protein_id = NP_417165.1</div> <div>/translation = MRHSVLFATAFATLISTQTFAADLPGKGITVNPVQSTITEETFQTLVSRALEKLGYSVKNPSEVDYNVGYTSLASGDATFTAVNWTPLHDNMYEAAGGDKKFYREGVFVNGAAQGYLIDKKTADQYKITNIAQLKDPKIAKLFDTNNGDGKADLTGCNPGWGCEGAINHQLAAYELTNTVTHNQGNYAAMMA DTISRYKEGKPVFYTWTPYWVSNELKPGKDVVWLQVPFSALPGDKNADTKLPNGANYGFPVSTMHIVANKAWAEKNPAAAKLFAIMQLPVADINAQNAIMHDGKASEGDIQGHVDGWIKAHQQFDGWVNEALAAQK*</div> <div>330 amino acids = 36,0 kDa</div> </div>								
✓	proX1	4676	..	4775	100 bp	<div></div>	⇕	misc_feature	
	/note = proX1 RT-qPCR amplicon								
✓	Fragment proU9_NlaIII_PT	5043	..	5154	112 bp	<div></div>	⇕	misc_feature	
✓	proX2	5045	..	5148	104 bp	<div></div>	⇕	misc_feature	
	/note = proX2 RT-qPCR amplicon								
✓	Fragment proU10_NlaIII_PT	5155	..	5349	195 bp	<div></div>	⇕	misc_feature	
✓	repeat region	5271	..	5354	84 bp	<div></div>	⇕	repeat_region	
	/note = REP193 (repetitive extragenic palindromic) element; contains 2 REP sequences								
✓	ygaY	5456	..	6633	1178 bp	<div></div>	→	CDS	
	<div> <div>/transl_table = 11 (Bacterial, Archaeal and Plant Plastid)</div> <div>/gene = ygaY</div> <div>/gene_synonym = ECK2674; JW2655; ygaX</div> <div>/locus_tag = b2681</div> <div>/note = predicted transporter (pseudogene);putative transport; Not classified; putative transport protein</div> <div>/db_xref = ASAP: ABE-0008822</div> <div>/db_xref = ASAP: ABE-0008824</div> <div>/db_xref = UniProtKB/Swiss-Prot: P76628</div> <div>/db_xref = EcoGene: EG13527</div> <div>/db_xref = GeneID: 2847696</div> <div>/translation = MTKPNHELSPALIVLMSIATGLAVASNYAQPLLDTIARNFSLSASSAGFIVTAAQLGYAAGLLFLVPLGDMFERRRLIVSMTLLAAC*LPPAVSRWR**SSVRH*PVYSQSWHKFWFRWQRRWLHRINAAKWLALL*AVCCWGWSCWHGQLPDCWRISAAGAPSFGLLR*WH*WRWHYGVVCHK*NQKPTS TTHSCWVPFSVCLSAIKFCVPARCWAA*PLPISAFSGPQWPFCPLPLHLLTTAMVSLVCLDLRELPERWALVRRAVLP IRANRTTPQLSVCCCYFHHGWRSGLVTLPYWR*LSESWCWISPCRACISL TRR*FIGYILMRAIA*PQVT*LATLLAVPPVR*FQPQPGNMAVGLAFVWLARRLPWLTYWSGGEV FIVRKPQI</div> <div>392 codons (14 internal stop codons)</div> </div>								
✓	Fragment proU11_NlaIII_PT	5475	..	5718	244 bp	<div></div>	⇕	misc_feature	
✓	ygaY1	5575	..	5710	136 bp	<div></div>	⇕	misc_feature	
	/note = ygaY1 RT-qPCR amplicon								
✓	Fragment proU12_NlaIII_PT	5719	..	6320	602 bp	<div></div>	⇕	misc_feature	
✓	ygaY2	6163	..	6285	123 bp	<div></div>	⇕	misc_feature	
	/note = ygaY2 RT-qPCR amplicon								
✓	Fragment proU14_NlaIII_PT	6321	..	6474	154 bp	<div></div>	⇕	misc_feature	

SI_1A.dna (Linear / 7069 bp)							
Feature		Location		Size			Type
✓	Fragment proU15_NlaIII_PT	6536	.. 6804	269 bp			misc_feature
✓	ygaZ	6757	.. 7069	313 bp			CDS
/transl_table = 11 (Bacterial, Archaeal and Plant Plastid)							
/gene = ygaZ							
/gene_synonym = ECK2676; JW2657							
/locus_tag = b2682							
/product = probable L-valine exporter, norvaline resistance							
/db_xref = GI: 16130594							
/db_xref = ASAP: ABE-0008829							
/db_xref = UniProtKB/Swiss-Prot: P76630							
/db_xref = EcoGene: EG13528							
/db_xref = GeneID: 945093							
/protein_id = NP_417167.1							
/translation = MESPTPQPAPGSATFMEGCKDSLPIVISYIPVAFAGFLNATRLGFSPLESVFFSCIIYAGASQFVITAMLAAGSSLWIAALTMAMDVRHVLYGPSLRSRIIQR							
104 amino acids = 11,1 kDa							

SI_1A.dna (Linear / 7069 bp)									
Primer		Length		Binding Sites			Tm	Date Added	
✓	ProU17_NlaIII_PT	28-mer		210	.. 237		61°C		
	/sequence = CGCTACGGCATATACAACCAGAATCTTC 46% GC / 8501,6 Da								
✓	ProU16_NlaIII_PT	27-mer		386	.. 412		61°C		
	/sequence = GCTGTATCAGGACGCTTACGAAATTGG 48% GC / 8339,5 Da								
✓	nrdF_RT-qPCR_1.fwd	20-mer		712	.. 731		58°C		
	/sequence = ATCGCCTGACCAGCAATTTC 50% GC / 6037,0 Da								
✓	nrdF_RT-qPCR_1.rev	20-mer		821	.. 840		64°C		
	/sequence = CGAGCAGCGTCAGGCCAGTA 65% GC / 6152,0 Da								
✓	ProU13_NlaIII.pt	21-mer		842	.. 862		57°C		
	/sequence = cacgctgcaaaatgttatcgg 48% GC / 6430,3 Da								
✓	ProU1_NlaIII_PT	18-mer		1212	.. 1229		57°C		
	/sequence = gatgaagcagtccacggt 56% GC / 5548,7 Da								
✓	nrdF_RT-qPCR_2.fwd	23-mer		1457	.. 1479		61°C		
	/sequence = CGCAGAAATGGCGGAAGTGAATC 52% GC / 7146,7 Da								
✓	nrdF_RT-qPCR_2.rev	23-mer		1535	.. 1557		60°C		
	/sequence = TCACATAAGAGGAGCCTGAACCG 52% GC / 7066,7 Da								
✓	proU_RT-qPCR_1.fwd	33-mer		1747	.. 1779		60°C		
	/sequence = CGCTATCTTTGACAAAAAATATCAACTTTCTCG 33% GC / 10.030,6 Da								
	/note = The proU operon can be expressed from two Transcription Start Sites: σ70 proU TSS and σS proU TSS. The proU_RT-qPCR_1 primer pair is positioned between these promoter sites. This way, it can be used to quantify the relative contribution of each promoter to the expression of the operon								
✓	ProU2_NlaIII_PT	19-mer		1778	.. 1796		58°C		
	/sequence = cgatttgctctcagccctt 53% GC / 5705,8 Da								
✓	proU3_NlaIII.fwd	22-mer		1841	.. 1862		61°C		
	/sequence = CCACATTTGCCATCAGGGGTTG 55% GC / 6726,4 Da								
✓	DRE_fwd	59-mer		1849	.. 1907		70°C	17 Jan 2021	
	/sequence = GCCATCAGGGGTTGCCTCAGATTCTCAGTATGTTAGGGTAGAAAAAAGTGA CTATTTCC 44% GC / 18.239,9 Da								
✓	proU_RT-qPCR_1.rev	23-mer		1850	.. 1872		61°C		
	/sequence = GAATCTGAGGCAACCCCTGATGG 57% GC / 7073,7 Da								
	/note = The proU operon can be expressed from two Transcription Start Sites: σ70 proU TSS and σS proU TSS. The proU_RT-qPCR_1 primer pair is positioned between these promoter sites. This way, it can be used to quantify the relative contribution of each promoter to the expression of the operon								

SI_1A.dna (Linear / 7069 bp)							
Primer		Length		Binding Sites		Tm	Date Added
✓	proU3_NlaIII.probe.IC	30-mer		1853 .. 1882		64°C	
/sequence = AACATACTGAGAATCTGAGGCAACCCCTGA 47% GC / 9193,1 Da							
✓	ProU3_NlaIII_PT	24-mer		1862 .. 1885		57°C	
/sequence = gcctcagattctcagtatgtagg 46% GC / 7358,9 Da							
✓	ProU3_NlaIII_PT.probe	36-mer		1895 .. 1930		60°C	
/sequence = tctatgtcgatatattacccaatggaaatagtcact 33% GC / 11.017,3 Da							
✓	proU3_NlaIII_PT.probe.2	38-mer		1920 .. 1957		60°C	
/sequence = CATGCAATAGAAAGATTCCTTTATTTGTCTATGTCGAT 32% GC / 11.656,7 Da							
✓	proU3_NlaIII.rev	36-mer		1922 .. 1957		60°C	
/sequence = CATGCAATAGAAAGATTCCTTTATTTGTCTATGTCG 33% GC / 11.039,3 Da							
✓	ProU4_NlaIII_PT	19-mer		2064 .. 2082		58°C	
/sequence = aaactgggctatcgcttgg 53% GC / 5843,9 Da							
✓	proU4_NlaIII_PT.2	22-mer		2069 .. 2090		61°C	
/sequence = GGGCTATCGCTTGGCGTAAAAG 55% GC / 6815,5 Da							
✓	proV_RT-PCR_1.fwd	17-mer		2240 .. 2256		63°C	
/sequence = TCCGACGCCGAACTCCG 71% GC / 5116,4 Da							
✓	proV_RT-PCR_1.rev	20-mer		2320 .. 2339		60°C	
/sequence = ACGCAGTATTGTCCAGCACG 55% GC / 6102,0 Da							
✓	ProU5_NlaIII_PT	21-mer		2580 .. 2600		56°C	
/sequence = aattacaggcgaaacatcagc 43% GC / 6432,3 Da							
✓	proV_RT-PCR_2.fwd	28-mer		2722 .. 2749		61°C	
/sequence = GGCGAATGATTATGTCCGTACCTTCTTC 46% GC / 8545,6 Da							
✓	proV_RT-PCR_2.rev	22-mer		2816 .. 2837		60°C	
/sequence = CGAAGCCAGGGGTTTTACGAAT 50% GC / 6799,5 Da							
✓	ProU6_NlaIII_PT	19-mer		2931 .. 2949		56°C	
/sequence = tcgattcgcttaaaaccgc 47% GC / 5747,8 Da							
✓	proW_RT-PCR_1.fwd	22-mer		3380 .. 3401		61°C	
/sequence = CCATTTCCGTCCCGTCTTCAG 59% GC / 6573,3 Da							

SI_1A.dna (Linear / 7069 bp)							
Primer		Length		Binding Sites		Tm	Date Added
✓	proW_RT-PCR_1.rev	25-mer		3476 .. 3500		59°C	
/sequence	= GGCGATGAGAGCGAAAACGATAATC 48% GC / 7773,1 Da						
✓	ProU7_NlaIII_PT	19-mer		3649 .. 3667		57°C	
/sequence	= GGATATGGCTGGCGAGAAG 58% GC / 5957,9 Da						
✓	proW_RT-PCR_2.fwd	27-mer		3895 .. 3921		60°C	
/sequence	= GCCAGATGCTGTTCAAAGTTCAGTTAC 44% GC / 8274,5 Da						
✓	proW_RT-PCR_2.rev	23-mer		3972 .. 3994		62°C	
/sequence	= GCGATGACCACCATAGAAAGGGC 57% GC / 7091,7 Da						
✓	ProU8_NlaIII_PT	18-mer		4476 .. 4493		57°C	
/sequence	= gttggctacacctcgctt 56% GC / 5441,6 Da						
✓	proX_RT-PCR_1.fwd	28-mer		4676 .. 4703		62°C	
/sequence	= GAAAGATCCGAAGATCGCCAAACTGTTTC 46% GC / 8590,7 Da						
✓	proX_RT-PCR_1.rev	20-mer		4756 .. 4775		61°C	
/sequence	= GTTGATCGCACCTTCGCAGC 60% GC / 6069,0 Da						
✓	proX_RT-PCR_2.fwd	23-mer		5045 .. 5067		62°C	
/sequence	= TATCGTTGCCAACAAGCCTGGG 52% GC / 7048,6 Da						
✓	ProU9_NlaIII_PT	22-mer		5087 .. 5108		57°C	
/sequence	= agcgaaactgtttgccattatg 41% GC / 6749,5 Da						
✓	proX_RT-PCR_2.rev	27-mer		5122 .. 5148		61°C	
/sequence	= TAATGGCGTTCTGGGCGTTAATATCTG 44% GC / 8336,5 Da						
✓	ProU10_NlaIII_PT	19-mer		5276 .. 5294		57°C	
/sequence	= gcggataaggcggtttttgc 53% GC / 5874,9 Da						
✓	ygaY_RT-qPCR_1.fwd	22-mer		5575 .. 5596		60°C	
/sequence	= CTTTTCCTTTCCGCCAGTTCTG 55% GC / 6579,3 Da						
✓	ProU11_NlaIII_PT	21-mer		5660 .. 5680		56°C	
/sequence	= CTCGGTGATATGTTTGAACGC 48% GC / 6452,3 Da						
✓	ygaY_RT-qPCR_1.rev	25-mer		5686 .. 5710		58°C	
/sequence	= CAGTAAGGTCATCGAGACAATCAGG 48% GC / 7724,1 Da						

SI_1A.dna (Linear / 7069 bp)							
Primer		Length		Binding Sites		Tm	Date Added
✓	ygaY_RT-qPCR_2.fwd	23-mer		6163 .. 6185		61°C	
/sequence = GCCGCTCCACCTTTTAACTACAG 52% GC / 6919,5 Da							
✓	ygaY_RT-qPCR_2.rev	23-mer		6263 .. 6285		63°C	
/sequence = GGTGTGGTGCGATTTGCCCTTAT 52% GC / 7092,7 Da							
✓	ProU12_NlaIII_PT	19-mer		6276 .. 6294		56°C	
/sequence = gcaccacaccacaactttc 53% GC / 5661,7 Da							
✓	ProU14_NlaIII_PT	32-mer		6400 .. 6431		60°C	
/sequence = GTGCATATCACTAACCAGACGGTAATTTATCG 41% GC / 9807,5 Da							
✓	ProU15_NlaIII_PT	30-mer		6736 .. 6765		61°C	
/sequence = CAATCGTTAAGCGTACACTCTATGGAAAGC 43% GC / 9199,1 Da							