

Pro

Enterococcus faecalis

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<i>Pro</i>	<i>E. faecalis.</i>
<i>in vitro</i>	-
<i>Pro</i>	-
<i>in vitro,</i>	-

• - , *in vitro*

• - , - , - , *in vivo.*

lpp- : *in vivo* (, -)

[1] *in vitro.*

[2].

• - , 7, *Pro*

• - , 5'- , 7- *Pro* [3].

	[4]	[5-7].	DH5 ,	[14],	<i>E. coli</i>
5'-			<i>Bst</i> NI,		
5'-	[8,9],		<i>in vitro</i>		7
e			-HCl, 8,0, 30	MgCl ₂ , 5	100
	():	[10]	4		50
	[11].		0,5	1 8	
Pro	<i>E. faecalis</i> .		Toyopearl 650M (1)	DEAE-
			10	-HCl, 8,0, 10	MgCl ₂ .
				20	
				1	NaCl.
	<i>E. faecalis</i> –				
[12, 13].			20	-HCl, pH 7,5, 30	MgCl ₂ ,
				4	/
<i>Escherichia coli</i>	DH5 (<i>F</i> ⁻ 80 <i>dlacZ</i>			14	
<i>M15</i> (<i>lacZYA-argF</i>)U169	<i>deoR</i> , <i>recA1</i> <i>endA1</i>			95 °	30
<i>hsdR17</i> (<i>r</i> _k ⁻ <i>m</i> _k ⁺ <i>phoA</i> <i>supE44</i> <i>thi-1</i> <i>gyrA96</i> <i>relA1</i>)	«Life Technologies» () ,		<i>t</i> = 60 °	9	
	«Difco»			<i>t</i> = 70 °	3
() ,				70	
	« . . . » « . . . » ,				
	«Reanal» () ,			c	
«Q-BIOgen» () ,	«Roche»		ProSwift Monolith WAX-1S (DEAE-		
() ,	DEAE-Toyopearl 650M)	0,73	
«Toyo Soda» () ,	ProSwift	57 °		1 /	
Monolith WAX-1S	«Dionex» () ,			250	
	«Amersham»		(50	-HCl, pH7,5, 4	MgCl ₂ ,
() ,		10 %-)		
«Whatman» () .			NaCl	0,2	1
<i>in vitro</i> .	<i>Pro E. faecalis</i>				<i>Pro</i>
<i>calis</i> , -	<i>Pro E. fae-</i>				e
7-			130	100	-HCl, pH 8,0, 20
			MgCl ₂ , 0,5 /	, 3	, 3
	<i>pUC18</i>		20	14	(268 /) ;
<i>Bam</i> HI <i>Eco</i> RI (. 1) .			1	Pro 50	

a

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PEFokA2 AGCTTGCATGCCTGCAGGTCGACTCTA
PEFokA1   ACGTACGGACGTCCAGCTGAGATCCTACCTCCTAG

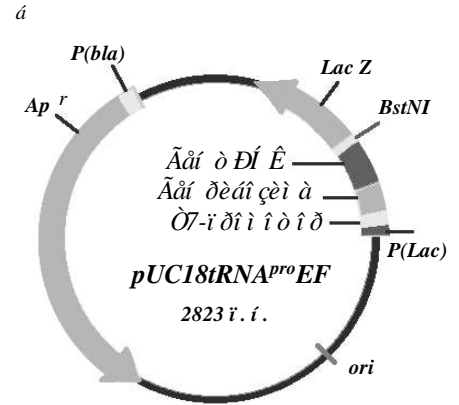
PE10Fok  GGATGGAGGATCCTGGTCGGGAAGACAGGATT
PE5      GACCAGCCCTTCTGTCTTAAGCTTGGACGG

PE9      CGAACCTGCGACCCCTTGGTCCCAAACCAAGT
PE4      TGGGGAACCAGGGTTTGGTTTCACGAGATGGTTC

PE8      GCTCTACCAAGCTGAGCTACTTCCAGGACGG
PE3      GACTCGATGAAGGGCTGCCATGGCCCATGG

PE7      TACCGGGTACCGTTTTCGTCCTCACGGACTCAT
PE2      CAAAGCAGGAGTGCCTGAGTAGTCGCCCTTCAG

PE6      CAGCGGGAAGTCTCCCTATAGTGAGTCGTATTAG
PE1      AGGGATATCACTCAGCATAATCTTAA
    
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a

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AATTCTAATACGACTCACTATAGGGAGACTTCCCGCTGATGAGTCCGTGAGGACGAAACGGTACCCGGTA
CATTATGCTGAGTGATATCCCTCTGAAGGGCGACTACTCAGGCACTCCTGCTTTGCCATGGGCCAT
    Ö-ı ð ï ï ð ï ð      Ä ä í ð è á í ç è ì à

CCGTCCGGGAAGTAGCTCAGCTTGGTAGAGCACTTGGTTTGGGACCAAGGGGTGCGAGGTTCGAATCCTG
GGCAGGCCCTTCATCGAGTCGAACCATCTCGTGAACCAAACCCTGGTTCCCGAGCGTCCAAGCTTAGGAC
    Ä ä í ð ð Í Ê

TCTTCCCGACC*AGGATCCTCCATCCTAGAGTCGACCTGCAGGCATGCA
AGAAGGGCTGGT*CCTAGGAGGTAGGATCTCAGCTGGACGTCCGTACGTTTCCA
    BstNI
    
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. 1. - Pro *E. faecalis* in vitro: -

pUC18; - ; - , -

E. faecalis. 37° ad) -

20 , (tobacco ringspot virus) [9], -

10 %- 7- -

200 , -

50 5 %- , -

Pro *E. faecalis* 1,) Pro *E. faecalis* in vitro (7- -

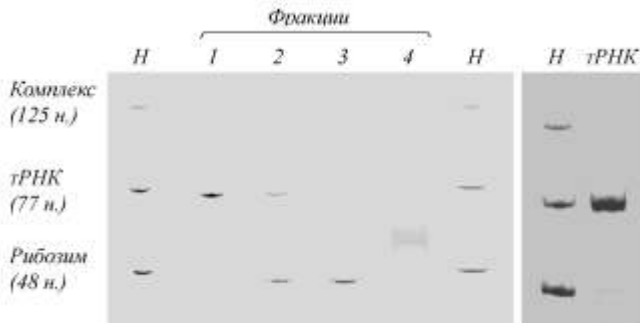
5'- , -

3'- -

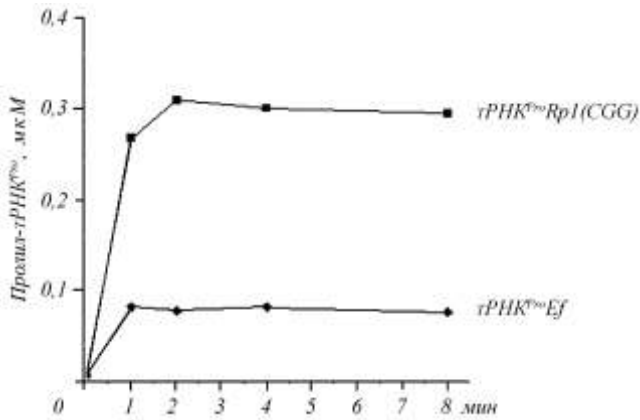
e - 7. - BstNI, -

7- 5'- -

(hammerhe- (. 1,). -



4. Pro *E. faecalis* (Pro⁺); 1 — Pro⁻; 2 — Pro⁺; 3 — Pro⁻; 4 — Pro⁺



5. Pro *E. faecalis* (CGG), *Rhodopseudomonas palustris* (*in vivo*)

WAX-1S, DEAE-NaCl

(. 3, 4). 0,8 (. 5).

in vivo,

Pro [15]. 95 % (. 4). DEAE-«Dionex» (WAX-1S)

K. S. Boyarshin, I. A. Krikliyvi, A. D. Yaremchuk, . . . Tukalo Cloning, expression and purification of tRNA^{Pro} from bacteria *Enterococcus faecalis* Summary

Aim. To elaborate the method of expression and purification of bacteria *Enterococcus faecalis* tRNA^{Pro} transcript. **Methods.** tRNA, co-expressed *in vitro* with *cis*-hydrolytical ribozyme, was purified by high performance liquid chromatography using anion-exchange chromatographic column. **Results.** A satisfactory yield of high purity preparation was obtained. A transcript of tRNA^{Pro} exhibits acceptor activity in aminoacylation reaction. **Conclusions.** The method developed may be introduced in laboratory practice including the obtaining of other tRNAs.

Keywords: tRNA, transcript, gene expression *in vitro*, high performance liquid chromatography.

Enterococcus faecalis

in vitro

Pro

E. faecalis.

in

Pro

in vitro,

1. Meinnel T., Mechulam Y., Fayat G. Fast purification of a functional elongator tRNA^{Met} expressed from a synthetic gene *in vivo* // Nucl. Acids Res.–1988.–**16**, N 16.–P. 8095–8096.
2. Sherlin L. D., Bullock T. L., Nissan T. A., Perona J. J., Lariviere F. J., Uhlenbeck O. C., Scaringe S. A. Chemical and enzymatic synthesis of tRNAs for high-throughput crystallization // RNA.–2001.–**7**, N 11.–P. 1671–1678.
3. Milligan J. F., Groebe D. R., Witherell G. W., Uhlenbeck O. C. Oligoribonucleotide synthesis using T7 RNA-polymerase and synthetic DNA templates // Nucl. Acids Res.–1987.– **15**, N 21.–P. 8783–8798.
4. Crepin T., Yaremchuk A., Tukalo M., Cusack S. Structures of two bacterial prolyl-tRNA synthetases with and without a *cis*-editing domain // Structure.–2006.–**14**, N 10.–P. 1511–1525.
5. An S., Musier-Forsyth K. Cys-tRNA(Pro) editing by *Haemophilus influenzae* YbaK via a novel synthetase. YbaKtRNA ternary complex // J. Biol. Chem.–2005.–**280**, N 41.–P. 34465–34472.

6. Boyarshin S., Krikliy I., ukal . . tRNA-dependent editing of errors by prolyl-tRNA synthetase from bacteria *Enterococcus faecalis* // Ukr. biochem. J.–2008.–**79**, N 6.–P. 40–44.
7. Boyarshin K. S., Krikliy I. A., Rayevsky V., Himin . . , Yaremchuk A. D., Tukalo . . Putative active site of *Enterococcus faecalis* prolyl-tRNA synthetase // Biopolymers and Cell.–2009.–**25**, N 1.–P. 39–43.
8. Taira K., Nakagawa K., Nishikawa S., Furukawa K. Construction of a novel RNA-transcript-trimming plasmid which can be used both *in vitro* in place of run-off and (G)-free transcriptions and *in vivo* as multi-sequences transcription vectors // Nucl. Acids Res.–1991.–**19**, N 19.–P. 5125–5130.
9. Price S. R., Ito N., Oubridge C., Avis J. M., Nagai K. Crystallization of RNA-protein complexes I. Methods for the large-scale preparation of RNA suitable for crystallographic studies // J. Mol. Biol.–1995.–**249**, N 2.–P. 398–408.
10. Kim I., McKenna S. A., Puglisi E. V., Puglisi J. D. Rapid purification of RNAs using fast performance liquid chromatography (FPLC) // RNA.–2007.–**13**, N 2.–P. 289–294.
11. Shields T. S., Mollova E., Marie L. S., Hansen M. R., Pardi A. High-performance liquid chromatography purification of homogenous-length RNA produced by *trans* cleavage with a hammerhead ribozyme // RNA.–1999.–**5**, N 9.–P. 1259–1267.
12. Sood S., Malhotra M., Das B. K., Kapil A. Enterococcal infections & antimicrobial resistance // Ind. J. Med. Res.–2008.–**128**, N 2.–P. 111–121.
13. Arias C. A., Murray B. E. Emergence and management of drug-resistant enterococcal infections // Exp rt Rev. Anti-Infect. Ther.–2008.–**6**, N 5.–P. 637–655.
14. Birnboim H. C., Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA // Nucl. Acids Res.–1979.–**7**, N 6.–P. 1513–1523.
15. Liu H., Peterson R., Kessler J., Musier-Forsyth K. Molecular recognition of tRNA^{Pro} by *Escherichia coli* proline tRNA synthetase *in vitro* // Nucl. Acids Res.–1995.–**23**, N 1.–P. 165–169.

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