

## **Impact of different cell penetrating peptides on the efficacy of antisense therapeutics for targeting intracellular pathogens**

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**Table S1: Bacterial isolates used for this study:**

<b>Bacterial isolate</b>	<b>Description</b>
<i>L. monocytogenes</i> F4244	Invasive strain F4244 CDC. Clinical isolate from patient's cerebrospinal fluid (CSF)
<i>L. monocytogenes</i> J0161	Isolated from a case of human listeriosis linked to a multi-state outbreak from a turkey processing plant, USA. (2000)
<i>L. monocytogenes</i> ATCC 13932	Clinical isolate from cerebrospinal fluid of child with meningitis, Germany
<i>L. monocytogenes</i> ATCC 19112	Clinical isolate from patient's cerebrospinal fluid, Scotland (1935)
<i>L. monocytogenes</i> ATCC 19111	Isolated from poultry, England (1937)
<i>L. monocytogenes</i> ATCC 19114	Isolated from bovine brain, USA (1931)
<i>Methicillin-resistant Staphylococcus aureus</i> (MRSA) USA300	Isolated from a wound in Mississippi, USA. Resistant to erythromycin, methicillin and tetracycline
<i>Escherichia coli</i> OP50	Uracil auxotroph strain with limited growth on nematode growth medium

**Table S2: The targeted sequence of the *rpoA* gene for six clinical strains of *L. monocytogenes*.**

<b>Strain</b>	<b>Sequence (5'-3')</b>
<i>L. monocytogenes</i> F4244	AAGGAGGGTAAATTTGAATGATCGAAATTGAAAAGC
<i>L. monocytogenes</i> J0161	AAGGAGGGTAAATTTGAATGATCGAAATTGAAAAGC
<i>L. monocytogenes</i> ATCC 13932	AAGGAGGGTAAATTTGAATGATCGAAATTGAAAAGC
<i>L. monocytogenes</i> ATCC 19112	AAGGAGGGTAAATTTGAATGATCGAAATTGAAAAGC
<i>L. monocytogenes</i> ATCC 19111	AAGGAGGGTAAATTTGAATGATCGAAATTGAAAAGC
<i>L. monocytogenes</i> ATCC 19114	AAGGAGGGTAAATTTGAATGATCGAAATTGAAAAGC

**Table S3. Sequence alignment of *rpoA* 5' terminal region among different *Listeria* species**

Organism	Source	Locus tag	Identity <sup>a</sup>	Sequence (5'-3') <sup>b</sup>
<b><i>Listeria monocytogenes</i></b>				
J0161	Human CSF	LMOG_01906	100%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
EGD-e	Rabbit, England	lmo2606	99%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
10403S	Human skin lesion, USA	LMRG_02150	99%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
07PF0776	Cardiac abscess, human, USA	MUO_13015	95%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
F2365	Cheese, USA	LMOf2365_2579	95%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
HCC23	Channel catfish	LMHCC_2928	94%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
M7	Cow's milk, China	LMM7_2718	94%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
<b><i>Listeria innocua</i></b>				
Clip11262	Food, Morocco	lin2755	87%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
<b><i>Listeria ivanovii</i></b>				
PAM 55	Sheep, Spain	LIV_2517	84%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
<b><i>Listeria welshimeri</i></b>				
SLCC5334	Decaying vegetation, USA	lwe2556	86%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA
<b><i>Listeria seeligeri</i></b>				
SLCC3954	Soil, Germany	lse_2511	84%	GAGGGTAAATTT <b><u>GAATGATCG</u></b> AAATTGAAA

<sup>a</sup> The identities between different *Listeria* strains were determined by using BLAST (Basic Local Alignment Search Tool).

<sup>b</sup> *rpoA* gene 5' terminal region among *Listeria* species showing the targeted area of interest in boldface type and the start codon ATG is bold and underlined.

**Table S4: Effect of PNAs on pure culture of *L. monocytogenes* F4244**

PNA Concentrations												
PNA	1 µM		2 µM		4 µM		8 µM		16 µM		32 µM	
	Log CFU/ml	Log CFU reduction	Log CFU/ml	Log CFU reduction	Log CFU/ml	Log CFU reduction	Log CFU/ml	Log CFU reduction	Log CFU/ml	Log CFU reduction	Log CFU/ml	Log CFU reduction
<b>PKFF</b>	9.40±0.08	-0.009	9.35±0.05	0.041	9.26±0.07	0.129	8.93±0.35	0.46	8.40±0.16	0.98*	6.63±0.59	2.78*
<b>PANT</b>	9.30±0.06	0.095	9.27±0.21	0.119	9.14±0.05	0.25	8.09±0.95	1.30*	6.73±0.48	2.66*	Cleared	9.39*
<b>PTAT</b>	8.28±0.37	1.1*	5.94±0.42	3.45*	Cleared	9.39*	Cleared	9.39*	Cleared	9.39*	Cleared	9.39*
<b>PRXR</b>	6.87±0.84	2.51*	3.15±0.21	6.24*	Cleared	9.39*	Cleared	9.39*	Cleared	9.39*	Cleared	9.39*
<b>PRFR</b>	9.26±0.09	0.13	7.48±0.69	1.91*	6.10±0.63	3.29*	Cleared	9.39*	Cleared	9.39*	Cleared	9.39*
<b>Control</b>	9.39±0.04	0										

Asterisks indicate values found to be significantly different from water by statistical analysis.

**Table S5: Selected genes used for this study and their proposed function**

<b>Gene</b>	<b>Locus tag</b>	<b>Proposed function</b>
<i>rpoA</i>	lmo2606	DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates [1]
<i>hly</i>	lmo0202	Encodes Listeriolysin O (LLO), a sulfhydryl-activated toxin that causes cytolysis by forming pores in cholesterol-containing host membranes [2]
<i>plcA</i>	lmo0201	Encodes phosphatidylinositol-specific phospholipase C (PI-PLC) which cleaves glycosylphosphatidylinositol (GPI) and phosphatidylinositol (PI) anchors but not PI phosphates. Important factor in Listeria pathogenesis that works in conjunction with LLO lyse vacuoles inside host cells [3-6]
<i>plcB</i>	lmo0205	Encodes phosphatidylcholine-phospholipase C (PC-PLC) which plays an important role in the infection process. It assists LLO in efficient lysis of the two-membrane vacuoles that surround the bacteria after direct cell-to-cell spread [3-5, 7]
<i>16s rRNA</i>	M645_14975	Housekeeping gene [34, 35]

**Table S6: Effect of PNAs on growth of *L. monocytogenes* J0161 in infected *C. elegans*.**

Treatment	Concentrations			
	16 $\mu$ M		32 $\mu$ M	
	Log CFUs/worm	Log CFUs reduction	Log CFUs/worm	Log CFUs reduction
<b>PKFF</b>	3.43 $\pm$ 0.2	0.71*	3.35 $\pm$ 0.25	1.03*
<b>PANT</b>	3.88 $\pm$ 0.15	0.27	2.49 $\pm$ 0.48	1.89*
<b>PTAT</b>	3.04 $\pm$ 0.36	1.11*	1.7 $\pm$ 0.6	2.67*
<b>PRXR</b>	2.24 $\pm$ 0.99	1.91*	0	4.38*
<b>PRFR</b>	2.85 $\pm$ 0.60	1.3*	1.86 $\pm$ 0.26	2.52*
<b>Free PNA</b>	4.29 $\pm$ 0.17	-0.13	4.39 $\pm$ 0.18	-0.012
<b>Gentamicin</b>	3.52 $\pm$ 0.55	0.63*	2.18 $\pm$ 0.6	2.2*
<b>Water</b>	4.15 $\pm$ 0.06	0	4.38 $\pm$ 0.12	0

Asterisks indicate values found to be significantly different from water by statistical analysis.

**Table S7: Primers used in this study**

<b>Primer name</b>	<b>Target gene</b>	<b>Sequence (5'-3')</b>	<b>Amplicon size (bp)</b>
RpoA-seqF	<i>rpoA</i>	ACAAGCAGCTGGTCTTGAAGTAAC	386
RpoA-seqR	<i>rpoA</i>	ACCTTCAATTACAGAAAACATG	
RpoA-F	<i>rpoA</i>	GTCCTGGTGTAGTAACTGCAG	145
RpoA-R	<i>rpoA</i>	GCAGGTGTGTAACCACGACC	
Hly-F	<i>hly</i>	CATGGCACCACCAGCATCTC	136
Hly-R	<i>hly</i>	CACTGCATCTCCGTGGTATAC	
PlcA-F	<i>plcA</i>	CAAGATGACTACAATGGTCCG	149
PlcA-R	<i>plcA</i>	GCTGCAGCATACTGACGAGG	
PlcB-F	<i>plcB</i>	ATCGGTGACTGATTACCGAG	147
PlcB-R	<i>plcB</i>	TATGCACAGTGGTAGCCTGG	
HK-F	<i>16s rRNA</i>	GCCTACCAAGGCAACGATGC	145
HK-R	<i>16s rRNA</i>	CATACACGCGGCGTTGCTC	