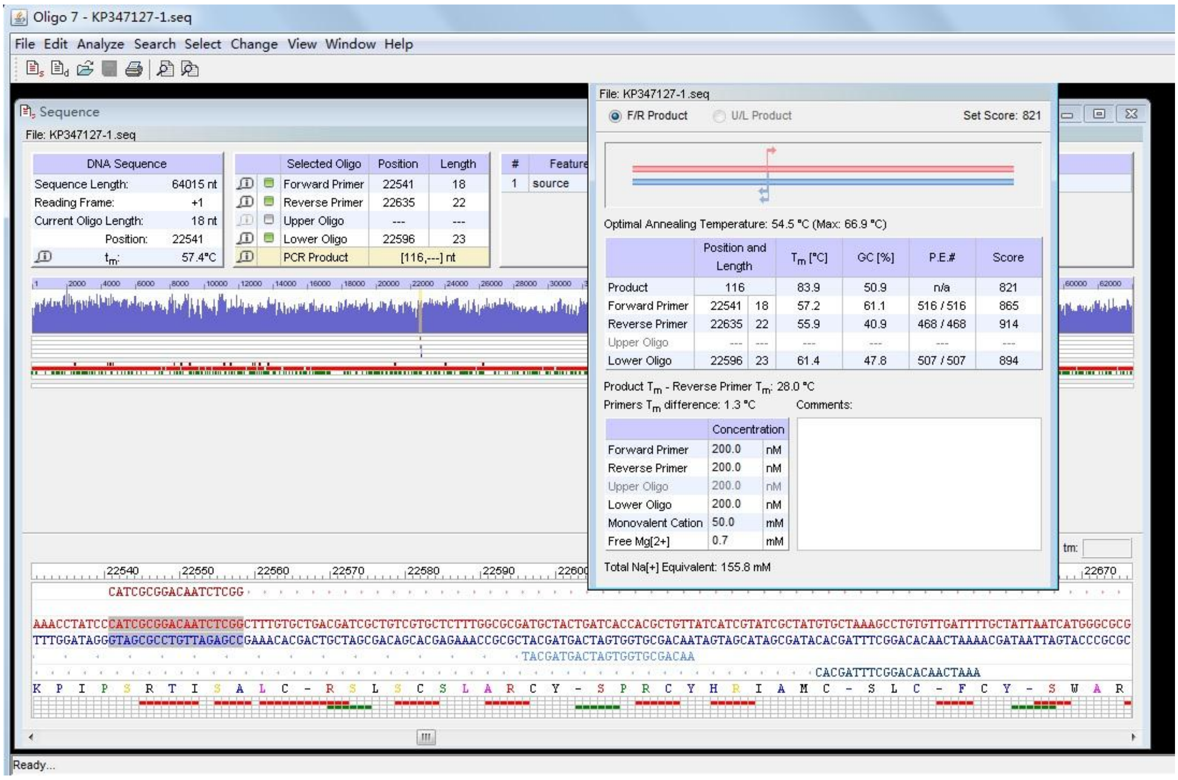
International Journal of
Environmental Research and Public Health

Supplementary Materials: The Occurrence of the Colistin Resistance Gene *mcr-1* in the Haihe River (China)

Dong Yang [1,†], Zhigang Qiu [1,†], Zhiqiang Shen [1,†], Hong Zhao [2], Min Jin [1], Huaying Li [3], Weili Liu [1] and Jun-Wen Li [1,*]



File: KP347127-1.seq

DNA Sequence	
Sequence Length:	64015 nt
Reading Frame:	+1
Current Oligo Length:	18 nt
Position:	22541
t_m:	57.4°C

Selected Oligo	Position	Length
Forward Primer	22541	18
Reverse Primer	22635	22
Upper Oligo	---	---
Lower Oligo	22596	23
PCR Product	[116,---] nt	

#	Feature
1	source

File: KP347127-1.seq

● F/R Product ○ U/L Product Set Score: 821

Optimal Annealing Temperature: 54.5 °C (Max: 66.9 °C)

	Position and Length		T_m [°C]	GC [%]	P.E.#	Score
Product	116		83.9	50.9	n/a	821
Forward Primer	22541	18	57.2	61.1	516 / 516	865
Reverse Primer	22635	22	55.9	40.9	468 / 468	914
Upper Oligo	---	---	---	---	---	---
Lower Oligo	22596	23	61.4	47.8	507 / 507	894

Product T_m - Reverse Primer T_m: 28.0 °C

Primers T_m difference: 1.3 °C

Comments:

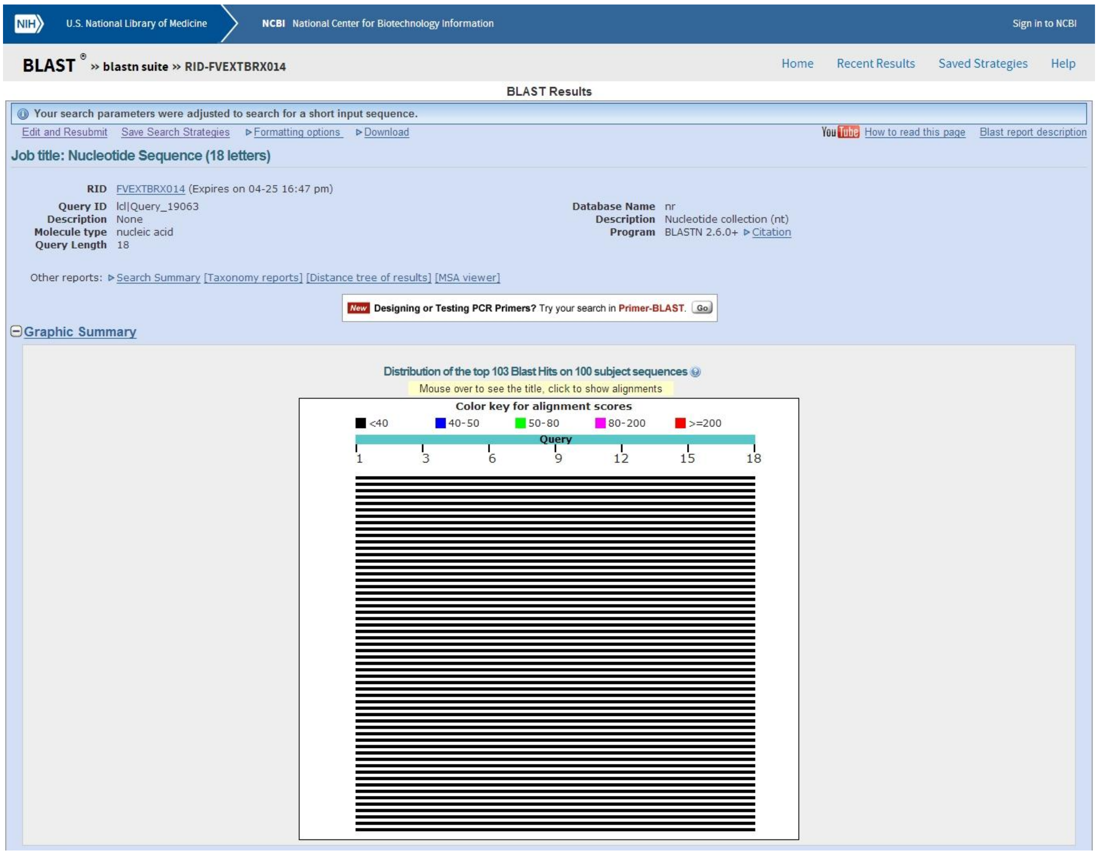
	Concentration	
Forward Primer	200.0	nM
Reverse Primer	200.0	nM
Upper Oligo	200.0	nM
Lower Oligo	200.0	nM
Monovalent Cation	50.0	mM
Free Mg[2+]	0.7	mM

Total Na[+] Equivalent: 155.8 mM

tm:

```
  CATCGCGGACAATCTCGG
AAACCTATCCCATCGCGGACAATCTCGGCTTTGTGCTGACGATCGCTGTCGTGCTCTTTGGCGCGATGCTACTGATCACCACGCTGTTATCATCGTATCGCTATGTGCTAAAGCCTGTGTTGATTTTGCTATTAATCATGGGCGCG
TTTGGATAGGGTAGCGCCTGTTAGAGCCGAAACACGACTGCTAGCGACAGCACGAGAAACCGCGCTACGATGACTAGTGGTGCGACAATAGTAGCATAGCGATACACGATTTCGGACACAACTAAAACGATAATTAGTACCCGCGC
                                                         TACGATGACTAGTGGTGCGACAA
                                                                                         CACGATTTCGGACACAACTAAA
K P I P S R T I S A L C - R S L S C S L A R C Y - S P R C Y H R I A M C - S L C - F C Y - S W A R
```

Figure S1. The scores of product, prime and probe in Oligo 7.0.



BLAST Results

Your search parameters were adjusted to search for a short input sequence.

Job title: Nucleotide Sequence (18 letters)

RID FVEXTBRX014 (Expires on 04-25 16:47 pm)

Query ID lcl|Query_19063
Description None
Molecule type nucleic acid
Query Length 18

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.6.0+ Citation

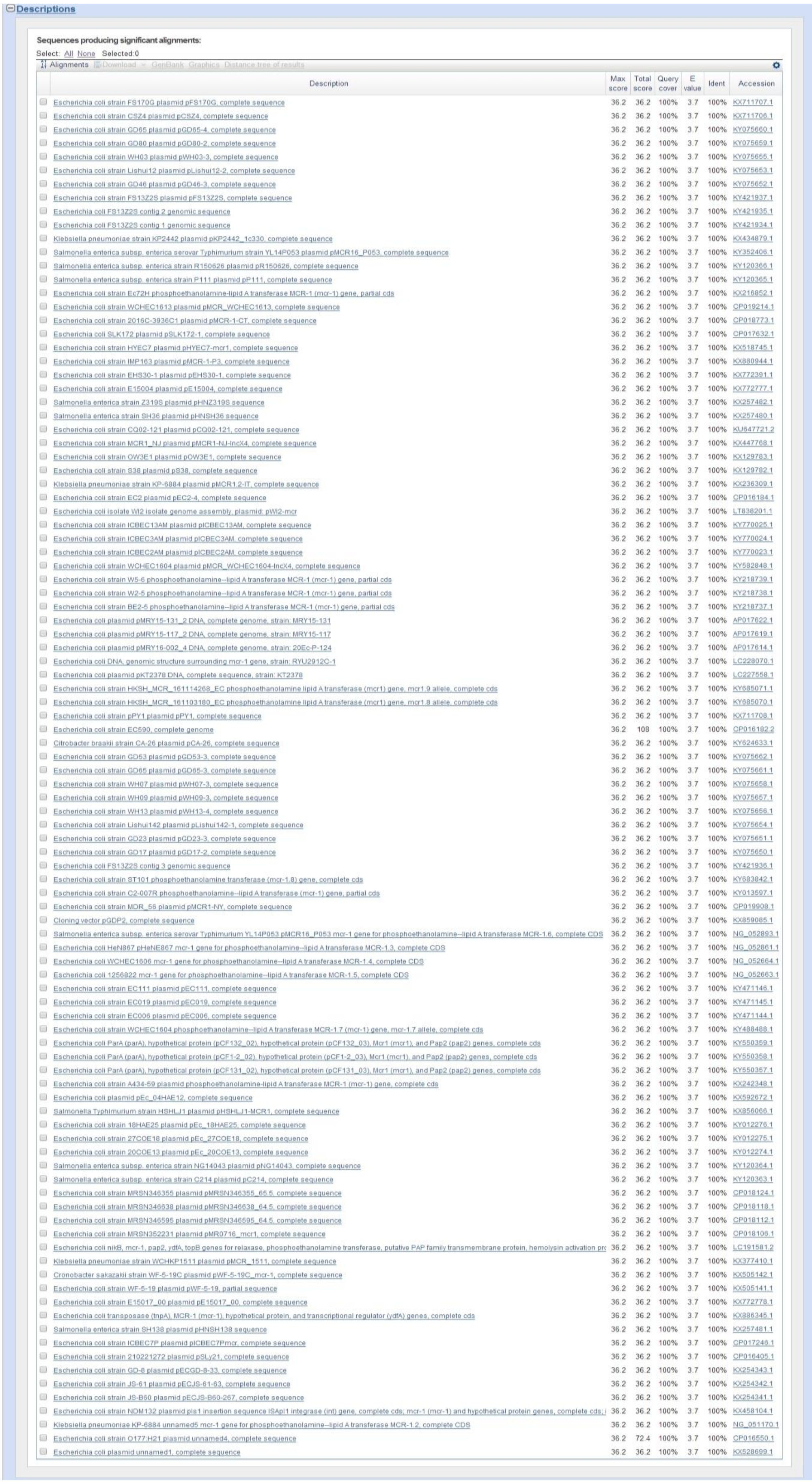
Other reports: Search Summary [Taxonomy reports] [Distance tree of results] [MSA viewer]

New **Designing or Testing PCR Primers?** Try your search in **Primer-BLAST**. Go

Distribution of the top 103 Blast Hits on 100 subject sequences

Mouse over to see the title, click to show alignments

Figure S2. *Cont.*



Descriptions

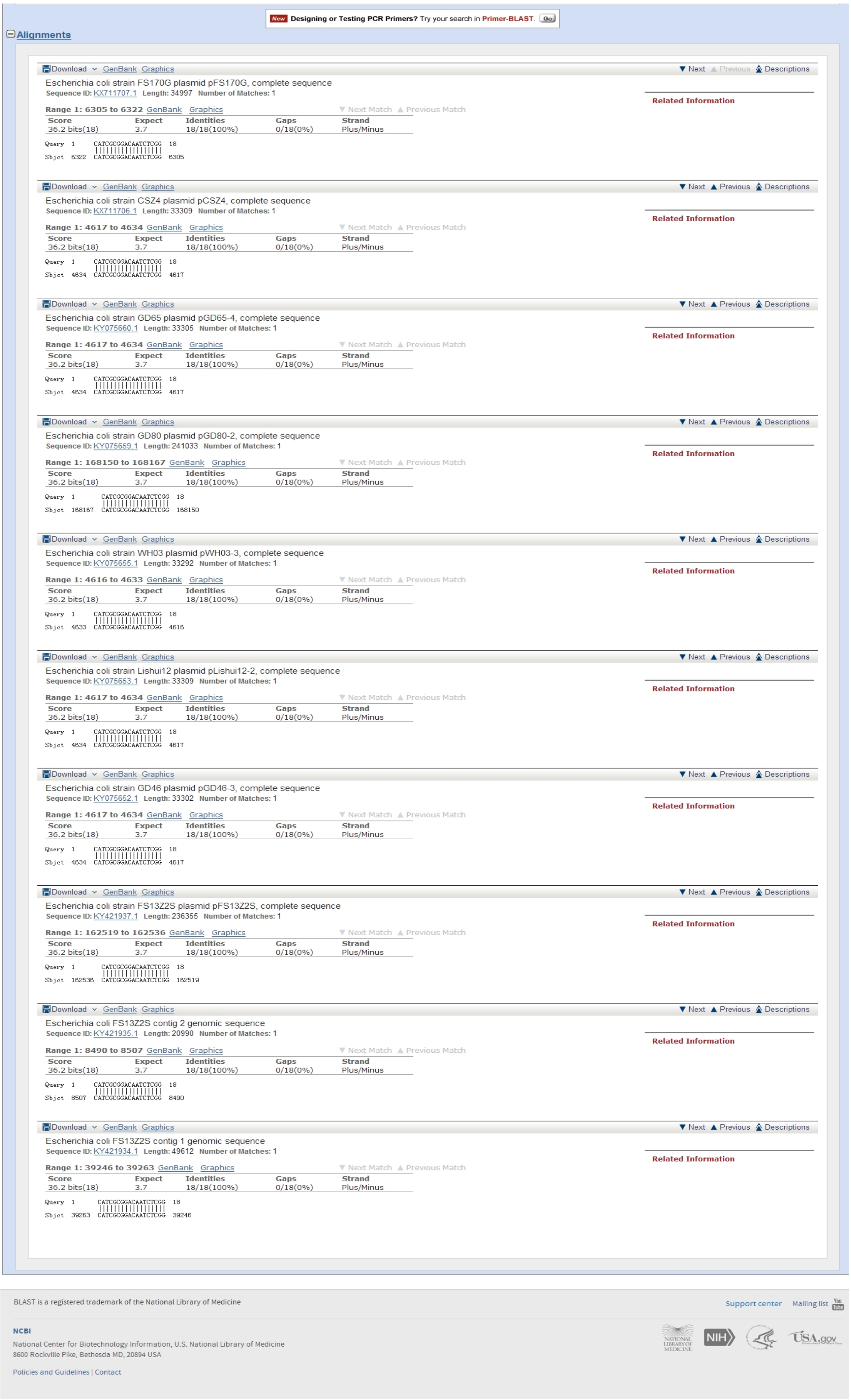
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Escherichia coli strain FS170G plasmid pFS170G, complete sequence	36.2	36.2	100%	3.7	100%	KX711707.1
☐	Escherichia coli strain CSZ4 plasmid pCSZ4, complete sequence	36.2	36.2	100%	3.7	100%	KX711706.1
☐	Escherichia coli strain GD65 plasmid pGD65-4, complete sequence	36.2	36.2	100%	3.7	100%	KY075660.1
☐	Escherichia coli strain GD80 plasmid pGD80-2, complete sequence	36.2	36.2	100%	3.7	100%	KY075659.1
☐	Escherichia coli strain WH03 plasmid pWH03-3, complete sequence	36.2	36.2	100%	3.7	100%	KY075655.1
☐	Escherichia coli strain Lishui12 plasmid pLishui12-2, complete sequence	36.2	36.2	100%	3.7	100%	KY075653.1
☐	Escherichia coli strain GD46 plasmid pGD46-3, complete sequence	36.2	36.2	100%	3.7	100%	KY075652.1
☐	Escherichia coli strain FS13Z2S plasmid pFS13Z2S, complete sequence	36.2	36.2	100%	3.7	100%	KY421937.1
☐	Escherichia coli FS13Z2S contig 2 genomic sequence	36.2	36.2	100%	3.7	100%	KY421935.1
☐	Escherichia coli FS13Z2S contig 1 genomic sequence	36.2	36.2	100%	3.7	100%	KY421934.1
☐	Klebsiella pneumoniae strain KP2442 plasmid pKP2442_1c330, complete sequence	36.2	36.2	100%	3.7	100%	KX434879.1
☐	Salmonella enterica subsp. enterica serovar Typhimurium strain YL14P053 plasmid pMCR16_P053, complete sequence	36.2	36.2	100%	3.7	100%	KY352406.1
☐	Salmonella enterica subsp. enterica strain R150626 plasmid pR150626, complete sequence	36.2	36.2	100%	3.7	100%	KY120366.1
☐	Salmonella enterica subsp. enterica strain P111 plasmid pP111, complete sequence	36.2	36.2	100%	3.7	100%	KY120365.1
☐	Escherichia coli strain Ec72H phosphoethanolamine-lipid A transferase MCR-1 (mcr-1) gene, partial cds	36.2	36.2	100%	3.7	100%	KX216852.1
☐	Escherichia coli strain WCHEC1613 plasmid pMCR_WCHEC1613, complete sequence	36.2	36.2	100%	3.7	100%	CP019214.1
☐	Escherichia coli strain 2016C-3936C1 plasmid pMCR-1-CT, complete sequence	36.2	36.2	100%	3.7	100%	CP018773.1
☐	Escherichia coli SLK172 plasmid pSLK172-1, complete sequence	36.2	36.2	100%	3.7	100%	CP017632.1
☐	Escherichia coli strain HYEC7 plasmid pHYEC7-mcr1, complete sequence	36.2	36.2	100%	3.7	100%	KX518745.1
☐	Escherichia coli strain IMP163 plasmid pMCR-1-P3, complete sequence	36.2	36.2	100%	3.7	100%	KX880944.1
☐	Escherichia coli strain EHS30-1 plasmid pEHS30-1, complete sequence	36.2	36.2	100%	3.7	100%	KX772391.1
☐	Escherichia coli strain E15004 plasmid pE15004, complete sequence	36.2	36.2	100%	3.7	100%	KX772777.1
☐	Salmonella enterica strain Z319S plasmid pHNZ319S sequence	36.2	36.2	100%	3.7	100%	KX257482.1
☐	Salmonella enterica strain SH36 plasmid pHNSH36 sequence	36.2	36.2	100%	3.7	100%	KX257480.1
☐	Escherichia coli strain CQ02-121 plasmid pCQ02-121, complete sequence	36.2	36.2	100%	3.7	100%	KU647721.2
☐	Escherichia coli strain MCR1_NJ plasmid pMCR1-NJ-IncX4, complete sequence	36.2	36.2	100%	3.7	100%	KX447768.1
☐	Escherichia coli strain OW3E1 plasmid pOW3E1, complete sequence	36.2	36.2	100%	3.7	100%	KX129783.1
☐	Escherichia coli strain S38 plasmid pS38, complete sequence	36.2	36.2	100%	3.7	100%	KX129782.1
☐	Klebsiella pneumoniae strain KP-6884 plasmid pMCR1.2-IT, complete sequence	36.2	36.2	100%	3.7	100%	KX236309.1
☐	Escherichia coli strain EC2 plasmid pEC2-4, complete sequence	36.2	36.2	100%	3.7	100%	CP016184.1
☐	Escherichia coli isolate WI2 isolate genome assembly, plasmid: pWI2-mcr	36.2	36.2	100%	3.7	100%	LT838201.1
☐	Escherichia coli strain ICBEC13AM plasmid pICBEC13AM, complete sequence	36.2	36.2	100%	3.7	100%	KY770025.1
☐	Escherichia coli strain ICBEC3AM plasmid pICBEC3AM, complete sequence	36.2	36.2	100%	3.7	100%	KY770024.1
☐	Escherichia coli strain ICBEC2AM plasmid pICBEC2AM, complete sequence	36.2	36.2	100%	3.7	100%	KY770023.1
☐	Escherichia coli strain WCHEC1604 plasmid pMCR_WCHEC1604-IncX4, complete sequence	36.2	36.2	100%	3.7	100%	KY582848.1
☐	Escherichia coli strain W5-6 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds	36.2	36.2	100%	3.7	100%	KY218739.1
☐	Escherichia coli strain W2-5 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds	36.2	36.2	100%	3.7	100%	KY218738.1
☐	Escherichia coli strain BE2-5 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds	36.2	36.2	100%	3.7	100%	KY218737.1
☐	Escherichia coli plasmid pMRY15-131_2 DNA, complete genome, strain: MRY15-131	36.2	36.2	100%	3.7	100%	AP017622.1
☐	Escherichia coli plasmid pMRY15-117_2 DNA, complete genome, strain: MRY15-117	36.2	36.2	100%	3.7	100%	AP017619.1
☐	Escherichia coli plasmid pMRY16-002_4 DNA, complete genome, strain: 20Ec-P-124	36.2	36.2	100%	3.7	100%	AP017614.1
☐	Escherichia coli DNA, genomic structure surrounding mcr-1 gene, strain: RYU2912C-1	36.2	36.2	100%	3.7	100%	LC228070.1
☐	Escherichia coli plasmid pKT2378 DNA, complete sequence, strain: KT2378	36.2	36.2	100%	3.7	100%	LC227558.1
☐	Escherichia coli strain HKSH_MCR_161114268_EC phosphoethanolamine lipid A transferase (mcr1) gene, mcr1.9 allele, complete cds	36.2	36.2	100%	3.7	100%	KY685071.1
☐	Escherichia coli strain HKSH_MCR_161103180_EC phosphoethanolamine lipid A transferase (mcr1) gene, mcr1.8 allele, complete cds	36.2	36.2	100%	3.7	100%	KY685070.1
☐	Escherichia coli strain pPY1 plasmid pPY1, complete sequence	36.2	36.2	100%	3.7	100%	KX711708.1
☐	Escherichia coli strain EC590, complete genome	36.2	108	100%	3.7	100%	CP016182.2
☐	Citrobacter braakii strain CA-26 plasmid pCA-26, complete sequence	36.2	36.2	100%	3.7	100%	KY624633.1
☐	Escherichia coli strain GD53 plasmid pGD53-3, complete sequence	36.2	36.2	100%	3.7	100%	KY075662.1
☐	Escherichia coli strain GD65 plasmid pGD65-3, complete sequence	36.2	36.2	100%	3.7	100%	KY075661.1
☐	Escherichia coli strain WH07 plasmid pWH07-3, complete sequence	36.2	36.2	100%	3.7	100%	KY075658.1
☐	Escherichia coli strain WH09 plasmid pWH09-3, complete sequence	36.2	36.2	100%	3.7	100%	KY075657.1
☐	Escherichia coli strain WH13 plasmid pWH13-4, complete sequence	36.2	36.2	100%	3.7	100%	KY075656.1
☐	Escherichia coli strain Lishui142 plasmid pLishui142-1, complete sequence	36.2	36.2	100%	3.7	100%	KY075654.1
☐	Escherichia coli strain GD23 plasmid pGD23-3, complete sequence	36.2	36.2	100%	3.7	100%	KY075651.1
☐	Escherichia coli strain GD17 plasmid pGD17-2, complete sequence	36.2	36.2	100%	3.7	100%	KY075650.1
☐	Escherichia coli FS13Z2S contig 3 genomic sequence	36.2	36.2	100%	3.7	100%	KY421936.1
☐	Escherichia coli strain ST101 phosphoethanolamine transferase (mcr-1.8) gene, complete cds	36.2	36.2	100%	3.7	100%	KY683842.1
☐	Escherichia coli strain C2-007R phosphoethanolamine--lipid A transferase (mcr-1) gene, partial cds	36.2	36.2	100%	3.7	100%	KY013597.1
☐	Escherichia coli strain MDR_56 plasmid pMCR1-NY, complete sequence	36.2	36.2	100%	3.7	100%	CP019908.1
☐	Cloning vector pGDP2, complete sequence	36.2	36.2	100%	3.7	100%	KX859085.1
☐	Salmonella enterica subsp. enterica serovar Typhimurium YL14P053 pMCR16_P053 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.6, complete CDS	36.2	36.2	100%	3.7	100%	NG_052893.1
☐	Escherichia coli HeN867 pHeNE867 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.3, complete CDS	36.2	36.2	100%	3.7	100%	NG_052861.1
☐	Escherichia coli WCHEC1606 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.4, complete CDS	36.2	36.2	100%	3.7	100%	NG_052664.1
☐	Escherichia coli 1256822 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.5, complete CDS	36.2	36.2	100%	3.7	100%	NG_052663.1
☐	Escherichia coli strain EC111 plasmid pEC111, complete sequence	36.2	36.2	100%	3.7	100%	KY471146.1
☐	Escherichia coli strain EC019 plasmid pEC019, complete sequence	36.2	36.2	100%	3.7	100%	KY471145.1
☐	Escherichia coli strain EC006 plasmid pEC006, complete sequence	36.2	36.2	100%	3.7	100%	KY471144.1
☐	Escherichia coli strain WCHEC1604 phosphoethanolamine--lipid A transferase MCR-1.7 (mcr-1) gene, mcr-1.7 allele, complete cds	36.2	36.2	100%	3.7	100%	KY488488.1
☐	Escherichia coli ParA (parA), hypothetical protein (pCF132_02), hypothetical protein (pCF132_03), Mcr1 (mcr1), and Pap2 (pap2) genes, complete cds	36.2	36.2	100%	3.7	100%	KY550359.1
☐	Escherichia coli ParA (parA), hypothetical protein (pCF1-2_02), hypothetical protein (pCF1-2_03), Mcr1 (mcr1), and Pap2 (pap2) genes, complete cds	36.2	36.2	100%	3.7	100%	KY550358.1
☐	Escherichia coli ParA (parA), hypothetical protein (pCF131_02), hypothetical protein (pCF131_03), Mcr1 (mcr1), and Pap2 (pap2) genes, complete cds	36.2	36.2	100%	3.7	100%	KY550357.1
☐	Escherichia coli strain A434-59 plasmid phosphoethanolamine-lipid A transferase MCR-1 (mcr-1) gene, complete cds	36.2	36.2	100%	3.7	100%	KX242348.1
☐	Escherichia coli plasmid pEc_04HAE12, complete sequence	36.2	36.2	100%	3.7	100%	KX592672.1
☐	Salmonella Typhimurium strain HSHLJ1 plasmid pHSHLJ1-MCR1, complete sequence	36.2	36.2	100%	3.7	100%	KX856066.1
☐	Escherichia coli strain 18HAE25 plasmid pEc_18HAE25, complete sequence	36.2	36.2	100%	3.7	100%	KY012276.1
☐	Escherichia coli strain 27COE18 plasmid pEc_27COE18, complete sequence	36.2	36.2	100%	3.7	100%	KY012275.1
☐	Escherichia coli strain 20COE13 plasmid pEc_20COE13, complete sequence	36.2	36.2	100%	3.7	100%	KY012274.1
☐	Salmonella enterica subsp. enterica strain NG14043 plasmid pNG14043, complete sequence	36.2	36.2	100%	3.7	100%	KY120364.1
☐	Salmonella enterica subsp. enterica strain C214 plasmid pC214, complete sequence	36.2	36.2	100%	3.7	100%	KY120363.1
☐	Escherichia coli strain MRSN346355 plasmid pMRSN346355_65.5, complete sequence	36.2	36.2	100%	3.7	100%	CP018124.1
☐	Escherichia coli strain MRSN346638 plasmid pMRSN346638_64.5, complete sequence	36.2	36.2	100%	3.7	100%	CP018118.1
☐	Escherichia coli strain MRSN346595 plasmid pMRSN346595_64.5, complete sequence	36.2	36.2	100%	3.7	100%	CP018112.1
☐	Escherichia coli strain MRSN352231 plasmid pMR0716_mcr1, complete sequence	36.2	36.2	100%	3.7	100%	CP018106.1
☐	Escherichia coli nikB, mcr-1, pap2, ydfA, topB genes for relaxase, phosphoethanolamine transferase, putative PAP family transmembrane protein, hemolysin activation pr	36.2	36.2	100%	3.7	100%	LC191581.2
☐	Klebsiella pneumoniae strain WCHKP1511 plasmid pMCR_1511, complete sequence	36.2	36.2	100%	3.7	100%	KX377410.1
☐	Cronobacter sakazakii strain WF-5-19C plasmid pWF-5-19C_mcr-1, complete sequence	36.2	36.2	100%	3.7	100%	KX505142.1
☐	Escherichia coli strain WF-5-19 plasmid pWF-5-19, partial sequence	36.2	36.2	100%	3.7	100%	KX505141.1
☐	Escherichia coli strain E15017_00 plasmid pE15017_00, complete sequence	36.2	36.2	100%	3.7	100%	KX772778.1
☐	Escherichia coli transposase (tnpA), MCR-1 (mcr-1), hypothetical protein, and transcriptional regulator (ydfA) genes, complete cds	36.2	36.2	100%	3.7	100%	KX886345.1
☐	Salmonella enterica strain SH138 plasmid pHNSH138 sequence	36.2	36.2	100%	3.7	100%	KX257481.1
☐	Escherichia coli strain ICBEC7P plasmid pICBEC7Pmcr, complete sequence	36.2	36.2	100%	3.7	100%	CP017246.1
☐	Escherichia coli strain 210221272 plasmid pSLy21, complete sequence	36.2	36.2	100%	3.7	100%	CP016405.1
☐	Escherichia coli strain GD-8 plasmid pECGD-8-33, complete sequence	36.2	36.2	100%	3.7	100%	KX254343.1
☐	Escherichia coli strain JS-61 plasmid pECJS-61-63, complete sequence	36.2	36.2	100%	3.7	100%	KX254342.1
☐	Escherichia coli strain JS-B60 plasmid pECJS-B60-267, complete sequence	36.2	36.2	100%	3.7	100%	KX254341.1
☐	Escherichia coli strain NDM132 plasmid pls1 insertion sequence ISApl1 integrase (int) gene, complete cds; mcr-1 (mcr-1) and hypothetical protein genes, complete cds;	36.2	36.2	100%	3.7	100%	KX458104.1
☐	Klebsiella pneumoniae KP-6884 unnamed5 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.2, complete CDS	36.2	36.2	100%	3.7	100%	NG_051170.1
☐	Escherichia coli strain O177:H21 plasmid unnamed4, complete sequence	36.2	72.4	100%	3.7	100%	CP016550.1
☐	Escherichia coli plasmid unnamed1, complete sequence	36.2	36.2	100%	3.7	100%	KX528699.1

Figure S2. *Cont.*



Escherichia coli strain FS170G plasmid pFS170G, complete sequence
Sequence ID: KX711707.1 **Length:** 34997 **Number of Matches:** 1

Range 1: 6305 to 6322 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

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Query  1     CATCGCGGACAATCTCGG  18
             ||||||||||||||||||
Sbjct  6322  CATCGCGGACAATCTCGG  6305
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Escherichia coli strain CSZ4 plasmid pCSZ4, complete sequence
Sequence ID: KX711706.1 **Length:** 33309 **Number of Matches:** 1

Range 1: 4617 to 4634 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

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Query  1     CATCGCGGACAATCTCGG  18
             ||||||||||||||||||
Sbjct  4634  CATCGCGGACAATCTCGG  4617
```

Escherichia coli strain GD65 plasmid pGD65-4, complete sequence
Sequence ID: KY075660.1 **Length:** 33305 **Number of Matches:** 1

Range 1: 4617 to 4634 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

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Query  1     CATCGCGGACAATCTCGG  18
             ||||||||||||||||||
Sbjct  4634  CATCGCGGACAATCTCGG  4617
```

Escherichia coli strain GD80 plasmid pGD80-2, complete sequence
Sequence ID: KY075659.1 **Length:** 241033 **Number of Matches:** 1

Range 1: 168150 to 168167 GenBank Graphics

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36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

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Query  1       CATCGCGGACAATCTCGG  18
               ||||||||||||||||||
Sbjct  168167  CATCGCGGACAATCTCGG  168150
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Escherichia coli strain WH03 plasmid pWH03-3, complete sequence
Sequence ID: KY075655.1 **Length:** 33292 **Number of Matches:** 1

Range 1: 4616 to 4633 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

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Query  1     CATCGCGGACAATCTCGG  18
             ||||||||||||||||||
Sbjct  4633  CATCGCGGACAATCTCGG  4616
```

Escherichia coli strain Lishui12 plasmid pLishui12-2, complete sequence
Sequence ID: KY075653.1 **Length:** 33309 **Number of Matches:** 1

Range 1: 4617 to 4634 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

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Query  1     CATCGCGGACAATCTCGG  18
             ||||||||||||||||||
Sbjct  4634  CATCGCGGACAATCTCGG  4617
```

Escherichia coli strain GD46 plasmid pGD46-3, complete sequence
Sequence ID: KY075652.1 **Length:** 33302 **Number of Matches:** 1

Range 1: 4617 to 4634 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

```
Query  1     CATCGCGGACAATCTCGG  18
             ||||||||||||||||||
Sbjct  4634  CATCGCGGACAATCTCGG  4617
```

Escherichia coli strain FS13Z2S plasmid pFS13Z2S, complete sequence
Sequence ID: KY421937.1 **Length:** 236355 **Number of Matches:** 1

Range 1: 162519 to 162536 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

```
Query  1       CATCGCGGACAATCTCGG  18
               ||||||||||||||||||
Sbjct  162536  CATCGCGGACAATCTCGG  162519
```

Escherichia coli FS13Z2S contig 2 genomic sequence
Sequence ID: KY421935.1 **Length:** 20990 **Number of Matches:** 1

Range 1: 8490 to 8507 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

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Query  1     CATCGCGGACAATCTCGG  18
             ||||||||||||||||||
Sbjct  8507  CATCGCGGACAATCTCGG  8490
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Escherichia coli FS13Z2S contig 1 genomic sequence
Sequence ID: KY421934.1 **Length:** 49612 **Number of Matches:** 1

Range 1: 39246 to 39263 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
36.2 bits(18)	3.7	18/18(100%)	0/18(0%)	Plus/Minus

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Query  1      CATCGCGGACAATCTCGG  18
              ||||||||||||||||||
Sbjct  39263  CATCGCGGACAATCTCGG  39246
```

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8600 Rockville Pike, Bethesda MD, 20894 USA

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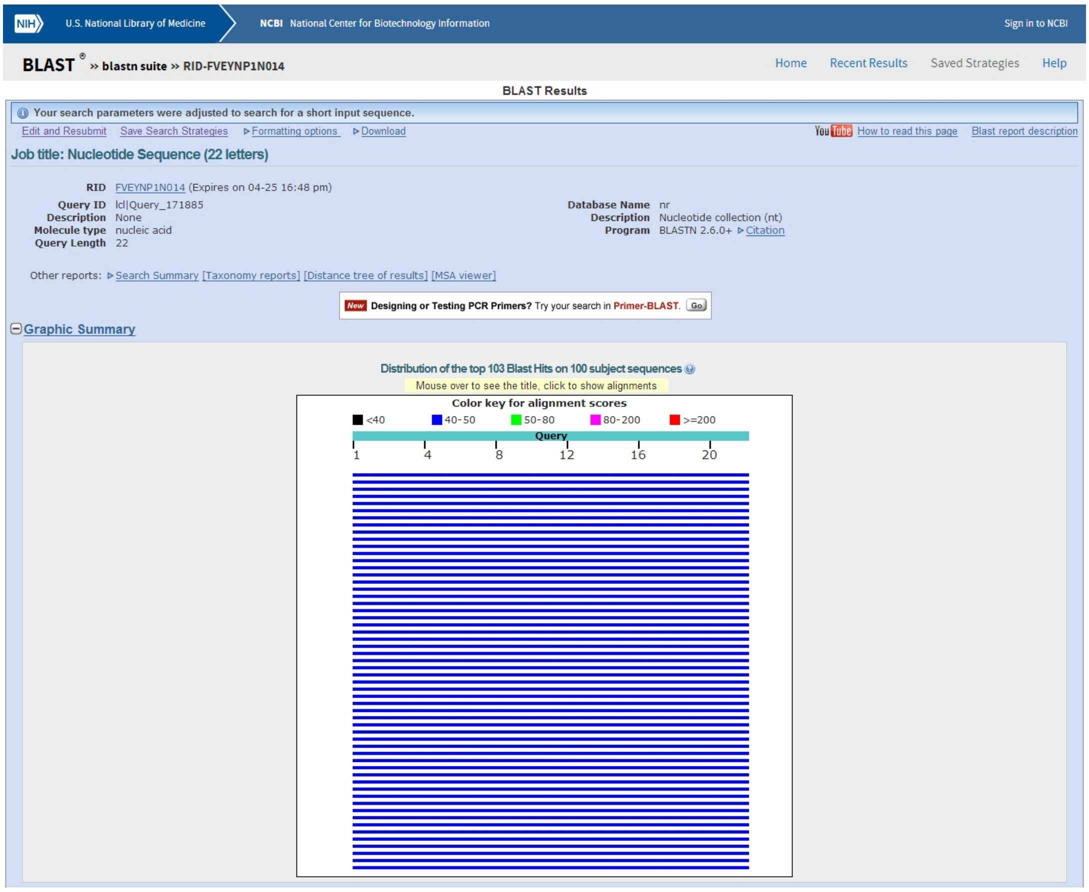
Figure S2. BLASTN analysis of primer M-F.



BLAST Results

Your search parameters were adjusted to search for a short input sequence.

Job title: Nucleotide Sequence (22 letters)

RID FVEYNP1N014 (Expires on 04-25 16:48 pm)

Query ID lcl|Query_171885
Description None
Molecule type nucleic acid
Query Length 22

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.6.0+ Citation

Other reports: Search Summary [Taxonomy reports] [Distance tree of results] [MSA viewer]

Designing or Testing PCR Primers? Try your search in Primer-BLAST.

Distribution of the top 103 Blast Hits on 100 subject sequences

Mouse over to see the title, click to show alignments

Color key for alignment scores

■ <40 ■ 40-50 ■ 50-80 ■ 80-200 ■ >=200

Figure S3. *Cont.*

Sequences producing significant alignments:

Select: All None Selected:0

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Escherichia coli isolate WI2 isolate genome assembly, plasmid: pWI2-mcr	44.1	44.1	100%	0.023	100%	LT838201.1
☐	Escherichia coli strain ICBEC13AM plasmid pICBEC13AM, complete sequence	44.1	44.1	100%	0.023	100%	KY770025.1
☐	Escherichia coli strain ICBEC3AM plasmid pICBEC3AM, complete sequence	44.1	44.1	100%	0.023	100%	KY770024.1
☐	Escherichia coli strain ICBEC2AM plasmid pICBEC2AM, complete sequence	44.1	44.1	100%	0.023	100%	KY770023.1
☐	Escherichia coli strain WCHEC1604 plasmid pMCR_WCHEC1604-IncX4, complete sequence	44.1	44.1	100%	0.023	100%	KY582848.1
☐	Escherichia coli strain W5-6 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds	44.1	44.1	100%	0.023	100%	KY218739.1
☐	Escherichia coli strain W2-5 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds	44.1	44.1	100%	0.023	100%	KY218738.1
☐	Escherichia coli strain BE2-5 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds	44.1	44.1	100%	0.023	100%	KY218737.1
☐	Escherichia coli plasmid pMRY15-131_2 DNA, complete genome, strain: MRY15-131	44.1	44.1	100%	0.023	100%	AP017622.1
☐	Escherichia coli plasmid pMRY15-117_2 DNA, complete genome, strain: MRY15-117	44.1	44.1	100%	0.023	100%	AP017619.1
☐	Escherichia coli plasmid pMRY16-002_4 DNA, complete genome, strain: 20Ec-P-124	44.1	44.1	100%	0.023	100%	AP017614.1
☐	Escherichia coli DNA, genomic structure surrounding mcr-1 gene, strain: RYU2912C-1	44.1	44.1	100%	0.023	100%	LC228070.1
☐	Escherichia coli plasmid pKT2378 DNA, complete sequence, strain: KT2378	44.1	44.1	100%	0.023	100%	LC227558.1
☐	Escherichia coli strain HKSH_MCR_161114268_EC phosphoethanolamine lipid A transferase (mcr1) gene, mcr1.9 allele, complete cds	44.1	44.1	100%	0.023	100%	KY685071.1
☐	Escherichia coli strain HKSH_MCR_161103180_EC phosphoethanolamine lipid A transferase (mcr1) gene, mcr1.8 allele, complete cds	44.1	44.1	100%	0.023	100%	KY685070.1
☐	Escherichia coli strain pPY1 plasmid pPY1, complete sequence	44.1	44.1	100%	0.023	100%	KX711708.1
☐	Escherichia coli strain EC590, complete genome	44.1	132	100%	0.023	100%	CP016182.2
☐	Citrobacter braakii strain CA-26 plasmid pCA-26, complete sequence	44.1	44.1	100%	0.023	100%	KY624633.1
☐	Escherichia coli strain GD53 plasmid pGD53-3, complete sequence	44.1	44.1	100%	0.023	100%	KY075662.1
☐	Escherichia coli strain GD65 plasmid pGD65-3, complete sequence	44.1	44.1	100%	0.023	100%	KY075661.1
☐	Escherichia coli strain WH07 plasmid pWH07-3, complete sequence	44.1	44.1	100%	0.023	100%	KY075658.1
☐	Escherichia coli strain WH09 plasmid pWH09-3, complete sequence	44.1	44.1	100%	0.023	100%	KY075657.1
☐	Escherichia coli strain WH13 plasmid pWH13-4, complete sequence	44.1	44.1	100%	0.023	100%	KY075656.1
☐	Escherichia coli strain Lishui142 plasmid pLishui142-1, complete sequence	44.1	44.1	100%	0.023	100%	KY075654.1
☐	Escherichia coli strain GD23 plasmid pGD23-3, complete sequence	44.1	44.1	100%	0.023	100%	KY075651.1
☐	Escherichia coli strain GD17 plasmid pGD17-2, complete sequence	44.1	44.1	100%	0.023	100%	KY075650.1
☐	Escherichia coli FS13Z2S contig 3 genomic sequence	44.1	44.1	100%	0.023	100%	KY421936.1
☐	Escherichia coli strain ST101 phosphoethanolamine transferase (mcr-1.8) gene, complete cds	44.1	44.1	100%	0.023	100%	KY683842.1
☐	Escherichia coli strain C2-007R phosphoethanolamine--lipid A transferase (mcr-1) gene, partial cds	44.1	44.1	100%	0.023	100%	KY013597.1
☐	Escherichia coli strain MDR_56 plasmid pMCR1-NY, complete sequence	44.1	44.1	100%	0.023	100%	CP019908.1
☐	Cloning vector pGDP2, complete sequence	44.1	44.1	100%	0.023	100%	KX859085.1
☐	Salmonella enterica subsp. enterica serovar Typhimurium YL14P053 pMCR16_P053 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.6, complete CDS	44.1	44.1	100%	0.023	100%	NG_052893.1
☐	Escherichia coli HeN867 pHeNE867 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.3, complete CDS	44.1	44.1	100%	0.023	100%	NG_052861.1
☐	Escherichia coli WCHEC1606 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.4, complete CDS	44.1	44.1	100%	0.023	100%	NG_052664.1
☐	Escherichia coli 1256822 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.5, complete CDS	44.1	44.1	100%	0.023	100%	NG_052663.1
☐	Escherichia coli strain EC111 plasmid pEC111, complete sequence	44.1	44.1	100%	0.023	100%	KY471146.1
☐	Escherichia coli strain EC019 plasmid pEC019, complete sequence	44.1	44.1	100%	0.023	100%	KY471145.1
☐	Escherichia coli strain EC006 plasmid pEC006, complete sequence	44.1	44.1	100%	0.023	100%	KY471144.1
☐	Escherichia coli strain WCHEC1604 phosphoethanolamine--lipid A transferase MCR-1.7 (mcr-1) gene, mcr-1.7 allele, complete cds	44.1	44.1	100%	0.023	100%	KY488488.1
☐	Escherichia coli ParA (parA), hypothetical protein (pCF132_02), hypothetical protein (pCF132_03), Mcr1 (mcr1), and Pap2 (pap2) genes, complete cds	44.1	44.1	100%	0.023	100%	KY550359.1
☐	Escherichia coli ParA (parA), hypothetical protein (pCF1-2_02), hypothetical protein (pCF1-2_03), Mcr1 (mcr1), and Pap2 (pap2) genes, complete cds	44.1	44.1	100%	0.023	100%	KY550358.1
☐	Escherichia coli ParA (parA), hypothetical protein (pCF131_02), hypothetical protein (pCF131_03), Mcr1 (mcr1), and Pap2 (pap2) genes, complete cds	44.1	44.1	100%	0.023	100%	KY550357.1
☐	Escherichia coli strain A434-59 plasmid phosphoethanolamine-lipid A transferase MCR-1 (mcr-1) gene, complete cds	44.1	44.1	100%	0.023	100%	KX242348.1
☐	Escherichia coli plasmid pEc_04HAE12, complete sequence	44.1	44.1	100%	0.023	100%	KX592672.1
☐	Salmonella Typhimurium strain HSHLJ1 plasmid pHSHLJ1-MCR1, complete sequence	44.1	44.1	100%	0.023	100%	KX856066.1
☐	Escherichia coli strain 18HAE25 plasmid pEc_18HAE25, complete sequence	44.1	44.1	100%	0.023	100%	KY012276.1
☐	Escherichia coli strain 27COE18 plasmid pEc_27COE18, complete sequence	44.1	44.1	100%	0.023	100%	KY012275.1
☐	Escherichia coli strain 20COE13 plasmid pEc_20COE13, complete sequence	44.1	44.1	100%	0.023	100%	KY012274.1
☐	Salmonella enterica subsp. enterica strain NG14043 plasmid pNG14043, complete sequence	44.1	44.1	100%	0.023	100%	KY120364.1
☐	Salmonella enterica subsp. enterica strain C214 plasmid pC214, complete sequence	44.1	44.1	100%	0.023	100%	KY120363.1
☐	Escherichia coli strain MRSN346355 plasmid pMRSN346355_65.5, complete sequence	44.1	44.1	100%	0.023	100%	CP018124.1
☐	Escherichia coli strain MRSN346638 plasmid pMRSN346638_64.5, complete sequence	44.1	44.1	100%	0.023	100%	CP018118.1
☐	Escherichia coli strain MRSN346595 plasmid pMRSN346595_64.5, complete sequence	44.1	44.1	100%	0.023	100%	CP018112.1
☐	Escherichia coli strain MRSN352231 plasmid pMR0716_mcr1, complete sequence	44.1	44.1	100%	0.023	100%	CP018106.1
☐	Escherichia coli nikB, mcr-1, pap2, ydfA, topB genes for relaxase, phosphoethanolamine transferase, putative PAP family transmembrane protein, hemolysin activation pr	44.1	44.1	100%	0.023	100%	LC191581.2
☐	Klebsiella pneumoniae strain WCHKP1511 plasmid pMCR_1511, complete sequence	44.1	44.1	100%	0.023	100%	KX377410.1
☐	Cronobacter sakazakii strain WF-5-19C plasmid pWF-5-19C_mcr-1, complete sequence	44.1	44.1	100%	0.023	100%	KX505142.1
☐	Escherichia coli strain WF-5-19 plasmid pWF-5-19, partial sequence	44.1	44.1	100%	0.023	100%	KX505141.1
☐	Escherichia coli strain E15017_00 plasmid pE15017_00, complete sequence	44.1	44.1	100%	0.023	100%	KX772778.1
☐	Escherichia coli transposase (tnpA), MCR-1 (mcr-1), hypothetical protein, and transcriptional regulator (ydfA) genes, complete cds	44.1	44.1	100%	0.023	100%	KX886345.1
☐	Salmonella enterica strain SH138 plasmid pHNSH138 sequence	44.1	44.1	100%	0.023	100%	KX257481.1
☐	Escherichia coli strain ICBEC7P plasmid pICBEC7Pmcr, complete sequence	44.1	44.1	100%	0.023	100%	CP017246.1
☐	Escherichia coli strain 210221272 plasmid pSLy21, complete sequence	44.1	44.1	100%	0.023	100%	CP016405.1
☐	Escherichia coli strain GD-8 plasmid pECGD-8-33, complete sequence	44.1	44.1	100%	0.023	100%	KX254343.1
☐	Escherichia coli strain JS-61 plasmid pECJS-61-63, complete sequence	44.1	44.1	100%	0.023	100%	KX254342.1
☐	Escherichia coli strain JS-B60 plasmid pECJS-B60-267, complete sequence	44.1	44.1	100%	0.023	100%	KX254341.1
☐	Escherichia coli strain NDM132 plasmid pls1 insertion sequence ISApl1 integrase (int) gene, complete cds; mcr-1 (mcr-1) and hypothetical protein genes, complete cds;	44.1	44.1	100%	0.023	100%	KX458104.1
☐	Klebsiella pneumoniae KP-6884 unnamed5 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.2, complete CDS	44.1	44.1	100%	0.023	100%	NG_051170.1
☐	Escherichia coli strain O177:H21 plasmid unnamed4, complete sequence	44.1	88.2	100%	0.023	100%	CP016550.1
☐	Escherichia coli plasmid unnamed1, complete sequence	44.1	44.1	100%	0.023	100%	KX528699.1
☐	Escherichia coli strain 59 plasmid pECJS-59-244, complete sequence	44.1	44.1	100%	0.023	100%	KX084394.1
☐	Escherichia coli strain 61 plasmid pECJS-61-63, complete sequence	44.1	44.1	100%	0.023	100%	KX084393.1
☐	Escherichia coli strain B65 plasmid pECJS-B65-33, complete sequence	44.1	44.1	100%	0.023	100%	KX084392.1
☐	Escherichia coli strain BA76 plasmid pBA76-MCR-1, complete sequence	44.1	44.1	100%	0.023	100%	KX013540.1
☐	Escherichia coli strain BA77 plasmid pBA77-MCR-1, complete sequence	44.1	44.1	100%	0.023	100%	KX013539.1
☐	Escherichia coli strain ABC149 plasmid pABC149-MCR-1, complete sequence	44.1	44.1	100%	0.023	100%	KX013538.1
☐	Kluyvera ascorbata strain WCH1410 plasmid pMCR_1410, complete sequence	44.1	44.1	100%	0.023	100%	KU922754.1
☐	Escherichia coli strain KP81 plasmid pKP81-BE, complete sequence	44.1	44.1	100%	0.023	100%	KU994859.1
☐	Escherichia coli plasmid pKH457-3-BE, complete sequence	44.1	44.1	100%	0.023	100%	KU353730.1
☐	Escherichia coli strain H226B plasmid pH226B, complete sequence	44.1	44.1	100%	0.023	100%	KX129784.1
☐	Escherichia coli strain S2.14 plasmid pS2.14-2, complete sequence	44.1	44.1	100%	0.023	100%	CP016187.1
☐	Escherichia coli strain EC13 plasmid pEC13-1, complete sequence	44.1	44.1	100%	0.023	100%	CP016186.1
☐	Escherichia coli strain EC5 plasmid pEC5-1, complete sequence	44.1	44.1	100%	0.023	100%	CP016185.1
☐	Escherichia coli strain EC2_1 plasmid pEC2_1-4, complete sequence	44.1	44.1	100%	0.023	100%	CP016183.1
☐	Escherichia coli strain FS170G plasmid pFS170G, complete sequence	44.1	44.1	100%	0.023	100%	KX711707.1
☐	Escherichia coli strain CSZ4 plasmid pCSZ4, complete sequence	44.1	44.1	100%	0.023	100%	KX711706.1
☐	Escherichia coli strain GD65 plasmid pGD65-4, complete sequence	44.1	44.1	100%	0.023	100%	KY075660.1
☐	Escherichia coli strain GD80 plasmid pGD80-2, complete sequence	44.1	44.1	100%	0.023	100%	KY075659.1
☐	Escherichia coli strain WH03 plasmid pWH03-3, complete sequence	44.1	44.1	100%	0.023	100%	KY075655.1
☐	Escherichia coli strain Lishui12 plasmid pLishui12-2, complete sequence	44.1	44.1	100%	0.023	100%	KY075653.1
☐	Escherichia coli strain GD46 plasmid pGD46-3, complete sequence	44.1	44.1	100%	0.023	100%	KY075652.1
☐	Escherichia coli strain FS13Z2S plasmid pFS13Z2S, complete sequence	44.1	44.1	100%	0.023	100%	KY421937.1
☐	Escherichia coli FS13Z2S contig 2 genomic sequence	44.1	44.1	100%	0.023	100%	KY421935.1
☐	Escherichia coli FS13Z2S contig 1 genomic sequence	44.1	44.1	100%	0.023	100%	KY421934.1
☐	Klebsiella pneumoniae strain KP2442 plasmid pKP2442_1c330, complete sequence	44.1	44.1	100%	0.023	100%	KX434879.1
☐	Salmonella enterica subsp. enterica serovar Typhimurium strain YL14P053 plasmid pMCR16_P053, complete sequence	44.1	44.1	100%	0.023	100%	KY352406.1
☐	Escherichia coli strain EHS30-1 plasmid pEHS30-1, complete sequence	44.1	44.1	100%	0.023	100%	KX772391.1
☐	Escherichia coli strain E15004 plasmid pE15004, complete sequence	44.1	44.1	100%	0.023	100%	KX772777.1
☐	Salmonella enterica strain Z319S plasmid pHNZ319S sequence	44.1	44.1	100%	0.023	100%	KX257482.1
☐	Salmonella enterica strain SH36 plasmid pHNSH36 sequence	44.1	44.1	100%	0.023	100%	KX257480.1

Figure S3. *Cont.*

Escherichia coli isolate WI2 isolate genome assembly, plasmid: pWI2-mcr
Sequence ID: LT838201.1 Length: 33304 Number of Matches: 1

Range 1: 25333 to 25354

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1      AAATCAACACAGGCTTTAGCAC  22
              ||||||||||||||||||||||
Sbjct  25354  AAATCAACACAGGCTTTAGCAC  25333
```

Escherichia coli strain ICBEC13AM plasmid pICBEC13AM, complete sequence
Sequence ID: KY770025.1 Length: 34975 Number of Matches: 1

Range 1: 29166 to 29187

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1      AAATCAACACAGGCTTTAGCAC  22
              ||||||||||||||||||||||
Sbjct  29187  AAATCAACACAGGCTTTAGCAC  29166
```

Escherichia coli strain ICBEC3AM plasmid pICBEC3AM, complete sequence
Sequence ID: KY770024.1 Length: 33304 Number of Matches: 1

Range 1: 27406 to 27427

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1      AAATCAACACAGGCTTTAGCAC  22
              ||||||||||||||||||||||
Sbjct  27427  AAATCAACACAGGCTTTAGCAC  27406
```

Escherichia coli strain ICBEC2AM plasmid pICBEC2AM, complete sequence
Sequence ID: KY770023.1 Length: 33051 Number of Matches: 1

Range 1: 27153 to 27174

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1      AAATCAACACAGGCTTTAGCAC  22
              ||||||||||||||||||||||
Sbjct  27174  AAATCAACACAGGCTTTAGCAC  27153
```

Escherichia coli strain WCHEC1604 plasmid pMCR_WCHEC1604-IncX4, complete sequence
Sequence ID: KY582848.1 Length: 31229 Number of Matches: 1

Range 1: 3666 to 3687

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1     AAATCAACACAGGCTTTAGCAC  22
             ||||||||||||||||||||||
Sbjct  3687  AAATCAACACAGGCTTTAGCAC  3666
```

Escherichia coli strain W5-6 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds
Sequence ID: KY218739.1 Length: 1518 Number of Matches: 1

Range 1: 178 to 199

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1    AAATCAACACAGGCTTTAGCAC  22
            ||||||||||||||||||||||
Sbjct  199  AAATCAACACAGGCTTTAGCAC  178
```

Escherichia coli strain W2-5 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds
Sequence ID: KY218738.1 Length: 1518 Number of Matches: 1

Range 1: 178 to 199

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1    AAATCAACACAGGCTTTAGCAC  22
            ||||||||||||||||||||||
Sbjct  199  AAATCAACACAGGCTTTAGCAC  178
```

Escherichia coli strain BE2-5 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds
Sequence ID: KY218737.1 Length: 1518 Number of Matches: 1

Range 1: 178 to 199

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1    AAATCAACACAGGCTTTAGCAC  22
            ||||||||||||||||||||||
Sbjct  199  AAATCAACACAGGCTTTAGCAC  178
```

Escherichia coli plasmid pMRY15-131_2 DNA, complete genome, strain: MRY15-131
Sequence ID: AP017622.1 Length: 60722 Number of Matches: 1

Range 1: 19923 to 19944

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1      AAATCAACACAGGCTTTAGCAC  22
              ||||||||||||||||||||||
Sbjct  19944  AAATCAACACAGGCTTTAGCAC  19923
```

Escherichia coli plasmid pMRY15-117_2 DNA, complete genome, strain: MRY15-117
Sequence ID: AP017619.1 Length: 61223 Number of Matches: 1

Range 1: 19923 to 19944

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.023	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1      AAATCAACACAGGCTTTAGCAC  22
              ||||||||||||||||||||||
Sbjct  19944  AAATCAACACAGGCTTTAGCAC  19923
```

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8600 Rockville Pike, Bethesda MD, 20894 USA

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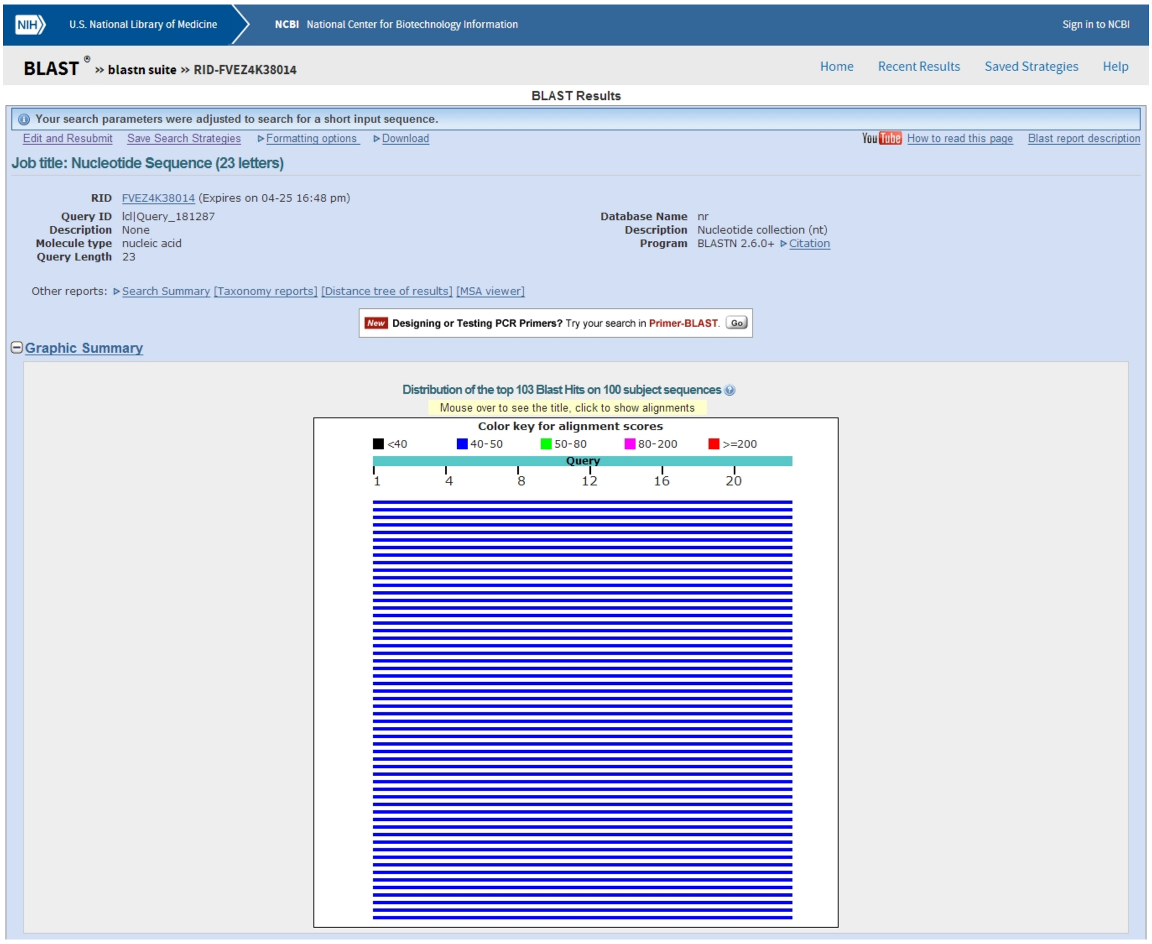
Figure S3. BLASTN analysis of primer M-R.



BLAST Results

Your search parameters were adjusted to search for a short input sequence.

Job title: Nucleotide Sequence (23 letters)

RID FVEZ4K38014 (Expires on 04-25 16:48 pm)

Query ID lcl|Query_181287
Description None
Molecule type nucleic acid
Query Length 23

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.6.0+ Citation

Other reports: Search Summary [Taxonomy reports] [Distance tree of results] [MSA viewer]

Designing or Testing PCR Primers? Try your search in **Primer-BLAST**.

Distribution of the top 103 Blast Hits on 100 subject sequences

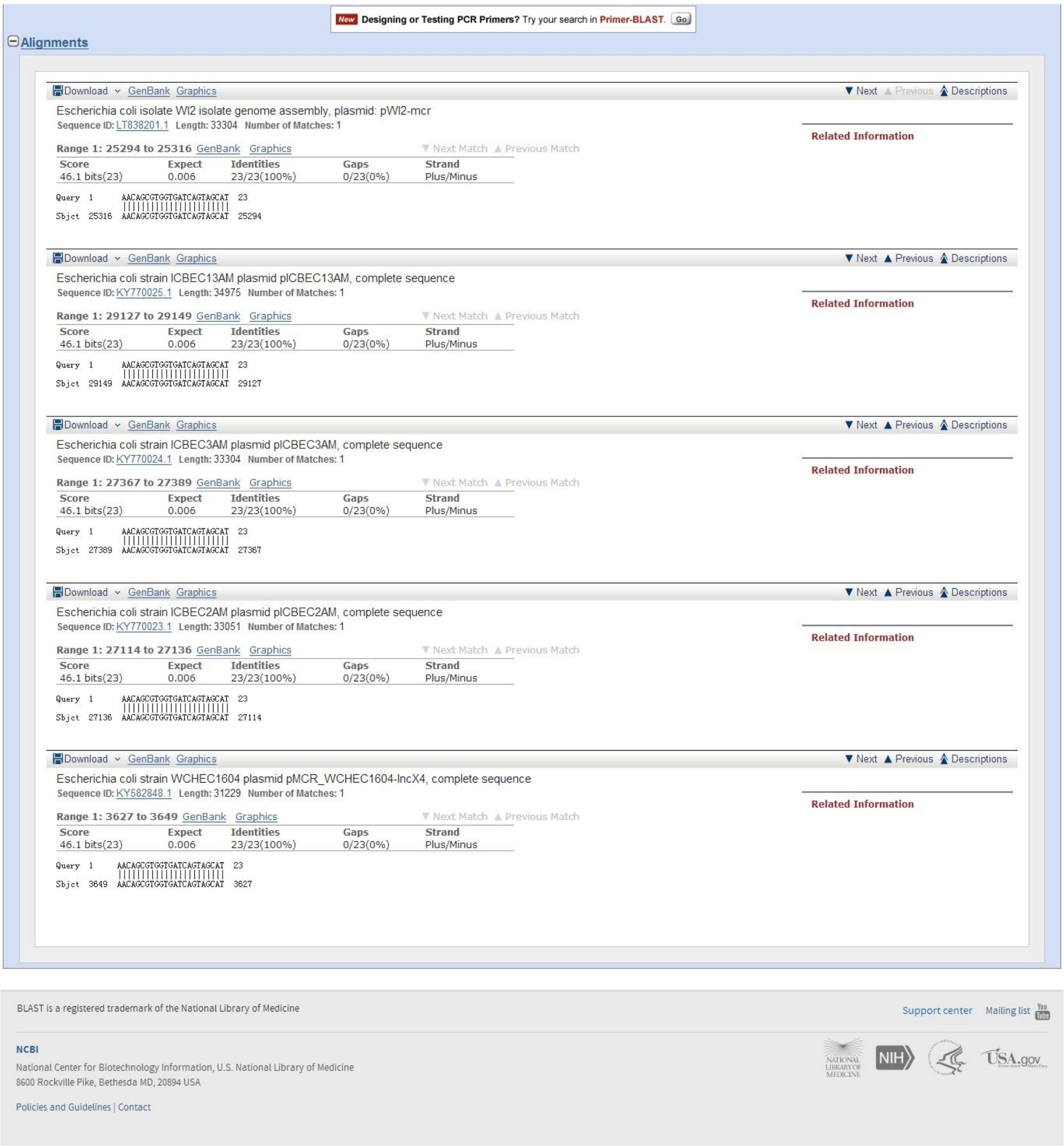
Mouse over to see the title, click to show alignments

Figure S4. *Cont.*

Sequences producing significant alignments:

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Escherichia coli isolate WI2 isolate genome assembly, plasmid: pWI2-mcr	46.1	46.1	100%	0.006	100%	LT838201.1
☐	Escherichia coli strain ICBEC13AM plasmid pICBEC13AM, complete sequence	46.1	46.1	100%	0.006	100%	KY770025.1
☐	Escherichia coli strain ICBEC3AM plasmid pICBEC3AM, complete sequence	46.1	46.1	100%	0.006	100%	KY770024.1
☐	Escherichia coli strain ICBEC2AM plasmid pICBEC2AM, complete sequence	46.1	46.1	100%	0.006	100%	KY770023.1
☐	Escherichia coli strain WCHEC1604 plasmid pMCR_WCHEC1604-IncX4, complete sequence	46.1	46.1	100%	0.006	100%	KY582848.1
☐	Escherichia coli strain W5-6 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds	46.1	46.1	100%	0.006	100%	KY218739.1
☐	Escherichia coli strain W2-5 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds	46.1	46.1	100%	0.006	100%	KY218738.1
☐	Escherichia coli strain BE2-5 phosphoethanolamine--lipid A transferase MCR-1 (mcr-1) gene, partial cds	46.1	46.1	100%	0.006	100%	KY218737.1
☐	Escherichia coli plasmid pMRY15-131_2 DNA, complete genome, strain: MRY15-131	46.1	46.1	100%	0.006	100%	AP017622.1
☐	Escherichia coli plasmid pMRY15-117_2 DNA, complete genome, strain: MRY15-117	46.1	46.1	100%	0.006	100%	AP017619.1
☐	Escherichia coli plasmid pMRY16-002_4 DNA, complete genome, strain: 20Ec-P-124	46.1	46.1	100%	0.006	100%	AP017614.1
☐	Escherichia coli DNA, genomic structure surrounding mcr-1 gene, strain: RYU2912C-1	46.1	46.1	100%	0.006	100%	LC228070.1
☐	Escherichia coli plasmid pKT2378 DNA, complete sequence, strain: KT2378	46.1	46.1	100%	0.006	100%	LC227558.1
☐	Escherichia coli strain HKSH_MCR_161114268_EC phosphoethanolamine lipid A transferase (mcr1) gene, mcr1.9 allele, complete cds	46.1	46.1	100%	0.006	100%	KY685071.1
☐	Escherichia coli strain HKSH_MCR_161103180_EC phosphoethanolamine lipid A transferase (mcr1) gene, mcr1.8 allele, complete cds	46.1	46.1	100%	0.006	100%	KY685070.1
☐	Escherichia coli strain pPY1 plasmid pPY1, complete sequence	46.1	46.1	100%	0.006	100%	KX711708.1
☐	Escherichia coli strain FS170G plasmid pFS170G, complete sequence	46.1	46.1	100%	0.006	100%	KX711707.1
☐	Escherichia coli strain CSZ4 plasmid pCSZ4, complete sequence	46.1	46.1	100%	0.006	100%	KX711706.1
☐	Escherichia coli strain EC590, complete genome	46.1	138	100%	0.006	100%	CP016182.2
☐	Citrobacter braakii strain CA-26 plasmid pCA-26, complete sequence	46.1	46.1	100%	0.006	100%	KY624633.1
☐	Escherichia coli strain GD53 plasmid pGD53-3, complete sequence	46.1	46.1	100%	0.006	100%	KY075662.1
☐	Escherichia coli strain GD65 plasmid pGD65-3, complete sequence	46.1	46.1	100%	0.006	100%	KY075661.1
☐	Escherichia coli strain GD65 plasmid pGD65-4, complete sequence	46.1	46.1	100%	0.006	100%	KY075660.1
☐	Escherichia coli strain GD80 plasmid pGD80-2, complete sequence	46.1	46.1	100%	0.006	100%	KY075659.1
☐	Escherichia coli strain WH07 plasmid pWH07-3, complete sequence	46.1	46.1	100%	0.006	100%	KY075658.1
☐	Escherichia coli strain WH09 plasmid pWH09-3, complete sequence	46.1	46.1	100%	0.006	100%	KY075657.1
☐	Escherichia coli strain WH13 plasmid pWH13-4, complete sequence	46.1	46.1	100%	0.006	100%	KY075656.1
☐	Escherichia coli strain WH03 plasmid pWH03-3, complete sequence	46.1	46.1	100%	0.006	100%	KY075655.1
☐	Escherichia coli strain Lishui142 plasmid pLishui142-1, complete sequence	46.1	46.1	100%	0.006	100%	KY075654.1
☐	Escherichia coli strain Lishui12 plasmid pLishui12-2, complete sequence	46.1	46.1	100%	0.006	100%	KY075653.1
☐	Escherichia coli strain GD46 plasmid pGD46-3, complete sequence	46.1	46.1	100%	0.006	100%	KY075652.1
☐	Escherichia coli strain GD23 plasmid pGD23-3, complete sequence	46.1	46.1	100%	0.006	100%	KY075651.1
☐	Escherichia coli strain GD17 plasmid pGD17-2, complete sequence	46.1	46.1	100%	0.006	100%	KY075650.1
☐	Escherichia coli strain FS13Z2S plasmid pFS13Z2S, complete sequence	46.1	46.1	100%	0.006	100%	KY421937.1
☐	Escherichia coli FS13Z2S contig 3 genomic sequence	46.1	46.1	100%	0.006	100%	KY421936.1
☐	Escherichia coli FS13Z2S contig 2 genomic sequence	46.1	46.1	100%	0.006	100%	KY421935.1
☐	Escherichia coli FS13Z2S contig 1 genomic sequence	46.1	46.1	100%	0.006	100%	KY421934.1
☐	Escherichia coli strain ST101 phosphoethanolamine transferase (mcr-1.8) gene, complete cds	46.1	46.1	100%	0.006	100%	KY683842.1
☐	Escherichia coli strain C2-007R phosphoethanolamine--lipid A transferase (mcr-1) gene, partial cds	46.1	46.1	100%	0.006	100%	KY013597.1
☐	Escherichia coli strain MDR_56 plasmid pMCR1-NY, complete sequence	46.1	46.1	100%	0.006	100%	CP019908.1
☐	Cloning vector pGDP2, complete sequence	46.1	46.1	100%	0.006	100%	KX859085.1
☐	Salmonella enterica subsp. enterica serovar Typhimurium YL14P053 pMCR16_P053 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.6, complete CDS	46.1	46.1	100%	0.006	100%	NG_052893.1
☐	Escherichia coli HeN867 pHeNE867 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.3, complete CDS	46.1	46.1	100%	0.006	100%	NG_052861.1
☐	Escherichia coli WCHEC1606 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.4, complete CDS	46.1	46.1	100%	0.006	100%	NG_052664.1
☐	Escherichia coli 1256822 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.5, complete CDS	46.1	46.1	100%	0.006	100%	NG_052663.1
☐	Escherichia coli strain EC111 plasmid pEC111, complete sequence	46.1	46.1	100%	0.006	100%	KY471146.1
☐	Escherichia coli strain EC019 plasmid pEC019, complete sequence	46.1	46.1	100%	0.006	100%	KY471145.1
☐	Escherichia coli strain EC006 plasmid pEC006, complete sequence	46.1	46.1	100%	0.006	100%	KY471144.1
☐	Escherichia coli strain WCHEC1604 phosphoethanolamine--lipid A transferase MCR-1.7 (mcr-1) gene, mcr-1.7 allele, complete cds	46.1	46.1	100%	0.006	100%	KY488488.1
☐	Klebsiella pneumoniae strain KP2442 plasmid pKP2442_1c330, complete sequence	46.1	46.1	100%	0.006	100%	KX434879.1
☐	Escherichia coli ParA (parA), hypothetical protein (pCF132_02), hypothetical protein (pCF132_03), Mcr1 (mcr1), and Pap2 (pap2) genes, complete cds	46.1	46.1	100%	0.006	100%	KY550359.1
☐	Escherichia coli ParA (parA), hypothetical protein (pCF1-2_02), hypothetical protein (pCF1-2_03), Mcr1 (mcr1), and Pap2 (pap2) genes, complete cds	46.1	46.1	100%	0.006	100%	KY550358.1
☐	Escherichia coli ParA (parA), hypothetical protein (pCF131_02), hypothetical protein (pCF131_03), Mcr1 (mcr1), and Pap2 (pap2) genes, complete cds	46.1	46.1	100%	0.006	100%	KY550357.1
☐	Escherichia coli strain A434-59 plasmid phosphoethanolamine-lipid A transferase MCR-1 (mcr-1) gene, complete cds	46.1	46.1	100%	0.006	100%	KX242348.1
☐	Escherichia coli plasmid pEc_04HAE12, complete sequence	46.1	46.1	100%	0.006	100%	KX592672.1
☐	Salmonella enterica subsp. enterica serovar Typhimurium strain YL14P053 plasmid pMCR16_P053, complete sequence	46.1	46.1	100%	0.006	100%	KY352406.1
☐	Salmonella Typhimurium strain HSHLJ1 plasmid pHSHLJ1-MCR1, complete sequence	46.1	46.1	100%	0.006	100%	KX856066.1
☐	Escherichia coli strain 18HAE25 plasmid pEc_18HAE25, complete sequence	46.1	46.1	100%	0.006	100%	KY012276.1
☐	Escherichia coli strain 27COE18 plasmid pEc_27COE18, complete sequence	46.1	46.1	100%	0.006	100%	KY012275.1
☐	Escherichia coli strain 20COE13 plasmid pEc_20COE13, complete sequence	46.1	46.1	100%	0.006	100%	KY012274.1
☐	Salmonella enterica subsp. enterica strain R150626 plasmid pR150626, complete sequence	46.1	46.1	100%	0.006	100%	KY120366.1
☐	Salmonella enterica subsp. enterica strain P111 plasmid pP111, complete sequence	46.1	46.1	100%	0.006	100%	KY120365.1
☐	Salmonella enterica subsp. enterica strain NG14043 plasmid pNG14043, complete sequence	46.1	46.1	100%	0.006	100%	KY120364.1
☐	Salmonella enterica subsp. enterica strain C214 plasmid pC214, complete sequence	46.1	46.1	100%	0.006	100%	KY120363.1
☐	Escherichia coli strain MRSN346355 plasmid pMRSN346355_65.5, complete sequence	46.1	46.1	100%	0.006	100%	CP018124.1
☐	Escherichia coli strain MRSN346638 plasmid pMRSN346638_64.5, complete sequence	46.1	46.1	100%	0.006	100%	CP018118.1
☐	Escherichia coli strain MRSN346595 plasmid pMRSN346595_64.5, complete sequence	46.1	46.1	100%	0.006	100%	CP018112.1
☐	Escherichia coli strain MRSN352231 plasmid pMR0716_mcr1, complete sequence	46.1	46.1	100%	0.006	100%	CP018106.1
☐	Escherichia coli strain EHS30-1 plasmid pEHS30-1, complete sequence	46.1	46.1	100%	0.006	100%	KX772391.1
☐	Escherichia coli nikB, mcr-1, pap2, ydfA, topB genes for relaxase, phosphoethanolamine transferase, putative PAP family transmembrane protein, hemolysin activation pr	46.1	46.1	100%	0.006	100%	LC191581.2
☐	Klebsiella pneumoniae strain WCHKP1511 plasmid pMCR_1511, complete sequence	46.1	46.1	100%	0.006	100%	KX377410.1
☐	Cronobacter sakazakii strain WF-5-19C plasmid pWF-5-19C_mcr-1, complete sequence	46.1	46.1	100%	0.006	100%	KX505142.1
☐	Escherichia coli strain WF-5-19 plasmid pWF-5-19, partial sequence	46.1	46.1	100%	0.006	100%	KX505141.1
☐	Escherichia coli strain E15017_00 plasmid pE15017_00, complete sequence	46.1	46.1	100%	0.006	100%	KX772778.1
☐	Escherichia coli strain E15004 plasmid pE15004, complete sequence	46.1	46.1	100%	0.006	100%	KX772777.1
☐	Escherichia coli transposase (tnpA), MCR-1 (mcr-1), hypothetical protein, and transcriptional regulator (ydfA) genes, complete cds	46.1	46.1	100%	0.006	100%	KX886345.1
☐	Salmonella enterica strain Z319S plasmid pHNZ319S sequence	46.1	46.1	100%	0.006	100%	KX257482.1
☐	Salmonella enterica strain SH138 plasmid pHNSH138 sequence	46.1	46.1	100%	0.006	100%	KX257481.1
☐	Salmonella enterica strain SH36 plasmid pHNSH36 sequence	46.1	46.1	100%	0.006	100%	KX257480.1
☐	Escherichia coli strain ICBEC7P plasmid pICBEC7Pmcr, complete sequence	46.1	46.1	100%	0.006	100%	CP017246.1
☐	Escherichia coli strain 210221272 plasmid pSLy21, complete sequence	46.1	46.1	100%	0.006	100%	CP016405.1
☐	Escherichia coli strain CQ02-121 plasmid pCQ02-121, complete sequence	46.1	46.1	100%	0.006	100%	KU647721.2
☐	Escherichia coli strain GD-8 plasmid pECGD-8-33, complete sequence	46.1	46.1	100%	0.006	100%	KX254343.1
☐	Escherichia coli strain JS-61 plasmid pECJS-61-63, complete sequence	46.1	46.1	100%	0.006	100%	KX254342.1
☐	Escherichia coli strain JS-B60 plasmid pECJS-B60-267, complete sequence	46.1	46.1	100%	0.006	100%	KX254341.1
☐	Escherichia coli strain NDM132 plasmid pls1 insertion sequence ISApl1 integrase (int) gene, complete cds; mcr-1 (mcr-1) and hypothetical protein genes, complete cds;	46.1	46.1	100%	0.006	100%	KX458104.1
☐	Escherichia coli strain MCR1_NJ plasmid pMCR1-NJ-IncX4, complete sequence	46.1	46.1	100%	0.006	100%	KX447768.1
☐	Klebsiella pneumoniae KP-6884 unnamed5 mcr-1 gene for phosphoethanolamine--lipid A transferase MCR-1.2, complete CDS	46.1	46.1	100%	0.006	100%	NG_051170.1
☐	Escherichia coli strain O177:H21 plasmid unnamed4, complete sequence	46.1	92.2	100%	0.006	100%	CP016550.1
☐	Escherichia coli plasmid unnamed1, complete sequence	46.1	46.1	100%	0.006	100%	KX528699.1
☐	Escherichia coli strain 59 plasmid pECJS-59-244, complete sequence	46.1	46.1	100%	0.006	100%	KX084394.1
☐	Escherichia coli strain 61 plasmid pECJS-61-63, complete sequence	46.1	46.1	100%	0.006	100%	KX084393.1
☐	Escherichia coli strain B65 plasmid pECJS-B65-33, complete sequence	46.1	46.1	100%	0.006	100%	KX084392.1
☐	Escherichia coli strain BA76 plasmid pBA76-MCR-1, complete sequence	46.1	46.1	100%	0.006	100%	KX013540.1
☐	Escherichia coli strain BA77 plasmid pBA77-MCR-1, complete sequence	46.1	46.1	100%	0.006	100%	KX013539.1
☐	Escherichia coli strain ABC149 plasmid pABC149-MCR-1, complete sequence	46.1	46.1	100%	0.006	100%	KX013538.1
☐	Kluyvera ascorbata strain WCH1410 plasmid pMCR_1410, complete sequence	46.1	46.1	100%	0.006	100%	KU922754.1
☐	Escherichia coli strain KP81 plasmid pKP81-BE, complete sequence	46.1	46.1	100%	0.006	100%	KU994859.1
☐	Escherichia coli plasmid pKH457-3-BE, complete sequence	46.1	46.1	100%	0.006	100%	KU353730.1
☐	Escherichia coli strain H226B plasmid pH226B, complete sequence	46.1	46.1	100%	0.006	100%	KX129784.1

Figure S4. *Cont.*



Escherichia coli isolate WI2 isolate genome assembly, plasmid: pWI2-mcr
Sequence ID: LT838201.1 Length: 33304 Number of Matches: 1

Range 1: 25294 to 25316

Score	Expect	Identities	Gaps	Strand
46.1 bits(23)	0.006	23/23(100%)	0/23(0%)	Plus/Minus

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              |||||||||||||||||||||||
Sbjct  25316  AACAGCGTGGTGATCAGTAGCAT  25294
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Escherichia coli strain ICBEC13AM plasmid pICBEC13AM, complete sequence
Sequence ID: KY770025.1 Length: 34975 Number of Matches: 1

Range 1: 29127 to 29149

Score	Expect	Identities	Gaps	Strand
46.1 bits(23)	0.006	23/23(100%)	0/23(0%)	Plus/Minus

```
Query  1      AACAGCGTGGTGATCAGTAGCAT  23
              |||||||||||||||||||||||
Sbjct  29149  AACAGCGTGGTGATCAGTAGCAT  29127
```

Escherichia coli strain ICBEC3AM plasmid pICBEC3AM, complete sequence
Sequence ID: KY770024.1 Length: 33304 Number of Matches: 1

Range 1: 27367 to 27389

Score	Expect	Identities	Gaps	Strand
46.1 bits(23)	0.006	23/23(100%)	0/23(0%)	Plus/Minus

```
Query  1      AACAGCGTGGTGATCAGTAGCAT  23
              |||||||||||||||||||||||
Sbjct  27389  AACAGCGTGGTGATCAGTAGCAT  27367
```

Escherichia coli strain ICBEC2AM plasmid pICBEC2AM, complete sequence
Sequence ID: KY770023.1 Length: 33051 Number of Matches: 1

Range 1: 27114 to 27136

Score	Expect	Identities	Gaps	Strand
46.1 bits(23)	0.006	23/23(100%)	0/23(0%)	Plus/Minus

```
Query  1      AACAGCGTGGTGATCAGTAGCAT  23
              |||||||||||||||||||||||
Sbjct  27136  AACAGCGTGGTGATCAGTAGCAT  27114
```

Escherichia coli strain WCHEC1604 plasmid pMCR_WCHEC1604-IncX4, complete sequence
Sequence ID: KY582848.1 Length: 31229 Number of Matches: 1

Range 1: 3627 to 3649

Score	Expect	Identities	Gaps	Strand
46.1 bits(23)	0.006	23/23(100%)	0/23(0%)	Plus/Minus

```
Query  1     AACAGCGTGGTGATCAGTAGCAT  23
             |||||||||||||||||||||||
Sbjct  3649  AACAGCGTGGTGATCAGTAGCAT  3627
```

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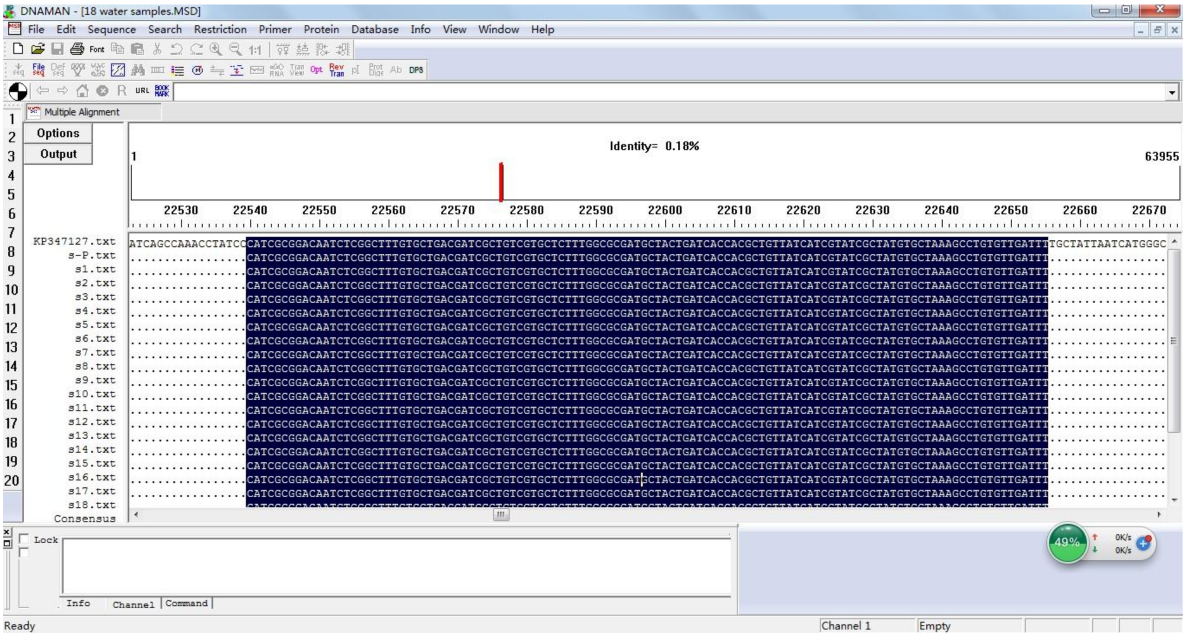
Figure S4. BLASTN analysis of primer M-Probe.



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       s-P.txt .................CATCGCGGACAATCTCGGCTTTGTGCTGACGATCGCTGTCGTGCTCTTTGGCGCGATGCTACTGATCACCACGCTGTTATCATCGTATCGCTATGTGCTAAAGCCTGTGTTGATTT.................
        s1.txt .................CATCGCGGACAATCTCGGCTTTGTGCTGACGATCGCTGTCGTGCTCTTTGGCGCGATGCTACTGATCACCACGCTGTTATCATCGTATCGCTATGTGCTAAAGCCTGTGTTGATTT.................
        s2.txt .................CATCGCGGACAATCTCGGCTTTGTGCTGACGATCGCTGTCGTGCTCTTTGGCGCGATGCTACTGATCACCACGCTGTTATCATCGTATCGCTATGTGCTAAAGCCTGTGTTGATTT.................
        s3.txt .................CATCGCGGACAATCTCGGCTTTGTGCTGACGATCGCTGTCGTGCTCTTTGGCGCGATGCTACTGATCACCACGCTGTTATCATCGTATCGCTATGTGCTAAAGCCTGTGTTGATTT.................
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        s5.txt .................CATCGCGGACAATCTCGGCTTTGTGCTGACGATCGCTGTCGTGCTCTTTGGCGCGATGCTACTGATCACCACGCTGTTATCATCGTATCGCTATGTGCTAAAGCCTGTGTTGATTT.................
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       s13.txt .................CATCGCGGACAATCTCGGCTTTGTGCTGACGATCGCTGTCGTGCTCTTTGGCGCGATGCTACTGATCACCACGCTGTTATCATCGTATCGCTATGTGCTAAAGCCTGTGTTGATTT.................
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       s18.txt
     Consensus
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Figure S5. Alignment of the sequences of qPCR products and the *mcr-1* gene sequence: (KP347127: the *mcr-1* gene sequence reported by Liu and colleagues, s-P: positive control, s-1–s-18: water samples).

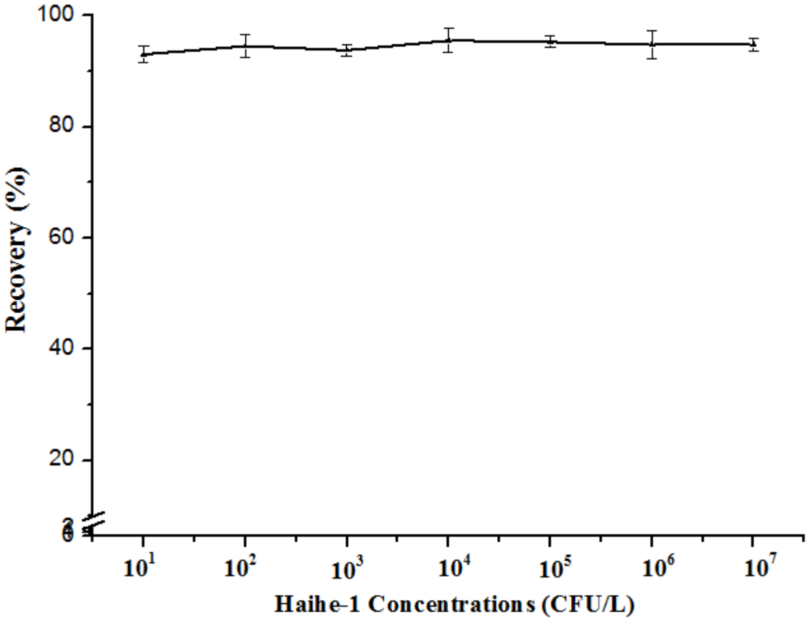


Figure S6. Recovery of Haihe-1 strain (10^1 CFU/L–10^7 CFU/L) spiked into 10L PBS

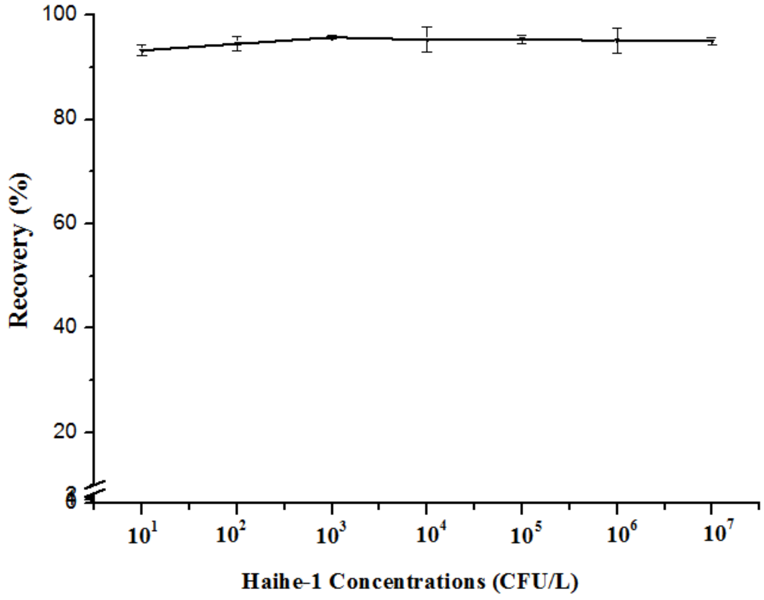
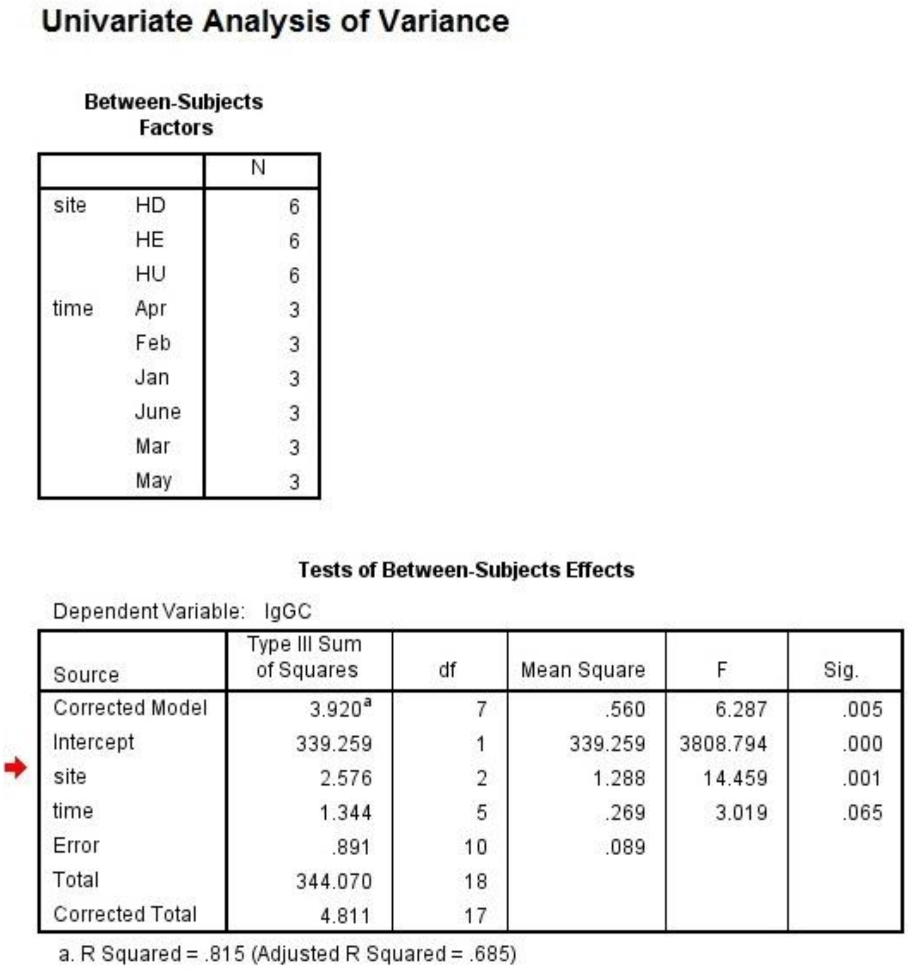


Figure S7. Recovery of Haihe-1 strain (10^1CFU/L–10^7CFU/L) spiked into 10L sterilized waster sample.



Univariate Analysis of Variance

Between-Subjects Factors

		N
site	HD	6
	HE	6
	HU	6
time	Apr	3
	Feb	3
	Jan	3
	June	3
	Mar	3
	May	3

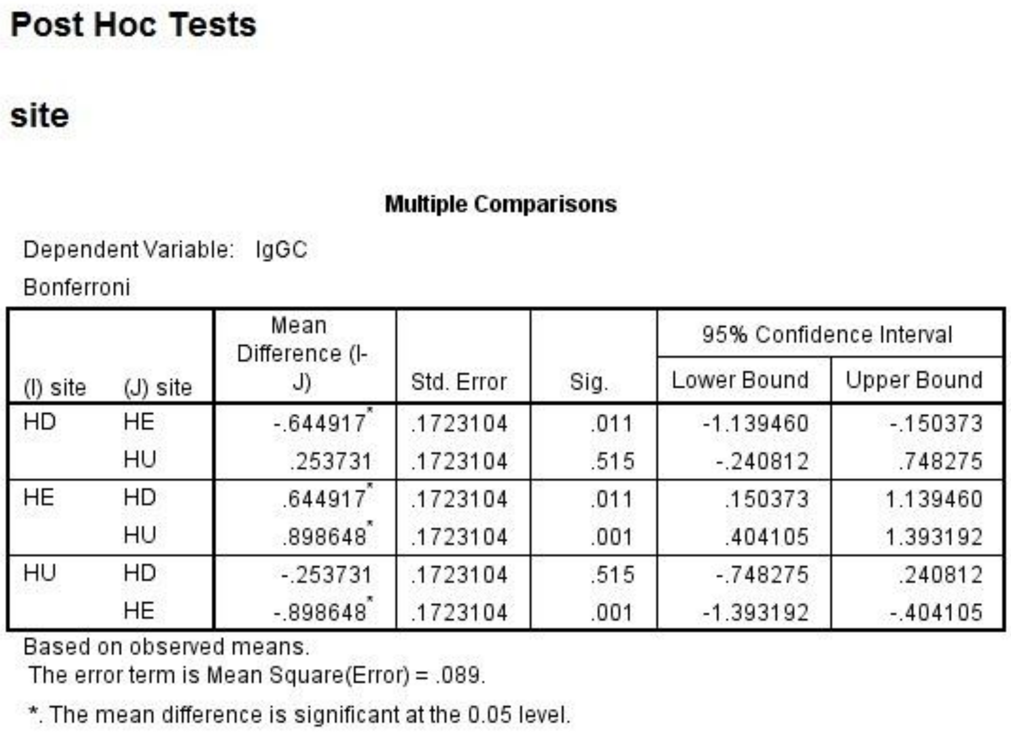
Tests of Between-Subjects Effects

Dependent Variable: lgGC

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.920[a]	7	.560	6.287	.005
Intercept	339.259	1	339.259	3808.794	.000
site	2.576	2	1.288	14.459	.001
time	1.344	5	.269	3.019	.065
Error	.891	10	.089		
Total	344.070	18			
Corrected Total	4.811	17			

a. R Squared = .815 (Adjusted R Squared = .685)

Figure S8. The multiple comparisons result of ANOVA.



Post Hoc Tests

site

Multiple Comparisons

Dependent Variable: lgGC

Bonferroni

(I) site	(J) site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
HD	HE	-.644917*	.1723104	.011	-1.139460	-.150373
	HU	.253731	.1723104	.515	-.240812	.748275
HE	HD	.644917*	.1723104	.011	.150373	1.139460
	HU	.898648*	.1723104	.001	.404105	1.393192
HU	HD	-.253731	.1723104	.515	-.748275	.240812
	HE	-.898648*	.1723104	.001	-1.393192	-.404105

Based on observed means.
The error term is Mean Square(Error) = .089.

*. The mean difference is significant at the 0.05 level.

Figure S9. The deviance analysis result of ANOVA.