Project Title

Primer Design for the Detection of the *blaTEM* Gene in *Escherichia coli* (Strain MRC3) Using PCR

Objective

This project aims to design a specific pair of primers targeting the *blaTEM* gene in *Escherichia coli*, which is commonly associated with resistance to β-lactam antibiotics such as ampicillin. The designed primers are intended for use in standard PCR protocols to amplify a specific region of the gene for detection purposes.

Rationale for Sequence Selection

The selected *blaTEM* gene sequence corresponds to *Escherichia coli* strain MRC3 (GenBank Accession Number: **KJ923009.1**), which contains a complete coding sequence of the *blaTEM-116* variant. This particular strain was chosen due to its documented association with β-lactam resistance. Using a well-characterized and publicly available sequence ensures both the reliability of the primer design and its applicability in diagnostic and research settings.

Gene Information

- **Gene Name**: *blaTEM*
- **Organism**: *Escherichia coli* (Strain MRC3)
- **Accession Number**: KJ923009.1
- **Gene Variant**: *blaTEM-116*

Target Sequence (FASTA Format)

>KJ923009.1 Escherichia coli strain MRC3 blaTEM (blaTEM-116) gene, complete cds

ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTC
ACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACT
GGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT
AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATAC
ACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGT
AAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATC
GGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGG
AACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAAC
GTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAG
GCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTG
GAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT
AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCC
TCACTGATTAAGCATTGGTAA

Primer Design:

Feature	Forward Primer	Reverse Primer
Sequence (5'→3')	CCTTCCTGTTTTTGTCACCC	CATGCCATCCGTAAGATGCTTT
Length (nt)	21	22
Melting Temp (Tm)	59.66 °C	59.38 °C
GC Content (%)	52.38%	45.45%
Self-Complementarity	2.00	5.00
3’ Self-Comp.	0.00	2.00
Amplicon Size	292 bp	

The selected primer pair (Primer Pair 9) was chosen due to its balanced melting temperature, low self-complementarity, and high binding specificity, which reduces the risk of primer-dimer formation and enhances amplification efficiency.

Proposed PCR Conditions

Step	Temperature	Time
Initial Denaturation	95 °C	30 seconds
Denaturation	95 °C	30 seconds
Annealing	59 °C	30 seconds
Extension	72 °C	30 seconds
Number of Cycles		**30**
Final Extension	72 °C	5 minutes

> The annealing temperature was set ~1 °C below the lower Tm of the primers to optimize binding specificity.

Specificity Assessment

Primer-BLAST was used to validate the specificity of the designed primers against the NCBI nucleotide (nt) database, with the search restricted to *Escherichia coli* sequences. The primers showed high specificity, successfully aligning to multiple *blaTEM*-carrying plasmids, including:

- **CP116048.1** – *E. coli* strain EC812A1, plasmid p812A1-69K
- **CP116183.1** – *E. coli* strain DETEC-E480, plasmid pDETEC56
- **CP116147.1** – *E. coli* strain DETEC-P622, plasmid pDETEC60
- **LC620534.1** – *E. coli* strain K38, plasmid pK38
- **LC603215.1** – *E. coli* strain NIPH17_0036, plasmid pNIPH17_0036_2

No off-target binding was observed in unrelated regions or organisms, confirming the primers' high specificity for the *blaTEM* gene.

Conclusion

A highly specific primer pair was successfully designed for the detection of the *blaTEM* gene in *Escherichia coli*. The primers exhibit:

- Optimal melting temperature and GC content
- Low self-complementarity and minimal secondary structure risk
- An ideal 3' end structure for efficient polymerase binding
- High specificity for *blaTEM*-containing plasmids
- No off-target amplification

These primers can be confidently utilized in molecular diagnostics, environmental surveillance, and antibiotic resistance studies involving *Escherichia coli*.