AN INVESTIGATION INTO THE CORRELATION BETWEEN SPECIFIC GENETIC MUTATIONS AND COLISTIN RESISTANCE IN *ESCHERICHIA COLI*

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BACKGROUND OF COLISTIN

- Last line of defence for treating multi-drug resistant Gram negative bacterial infections
- Cationic polypeptide with a fatty acid chain attached
- Targets the lipopolysaccharide molecule
- Use in human medicine becoming more common

COLISTIN IN MEDICINE

- Manufactured as colistin sulfate or colistimethate sodium
- Colistin sulfate used in bowel decontamination and treating surface infections
- Colistimethate sodium used to treat MDR *Pseudomonas aeruginosa* infections in cystic fibrosis patients
- Associated with nephrotoxicity and neurotoxicity

CHROMOSOMAL COLISTIN RESISTANCE

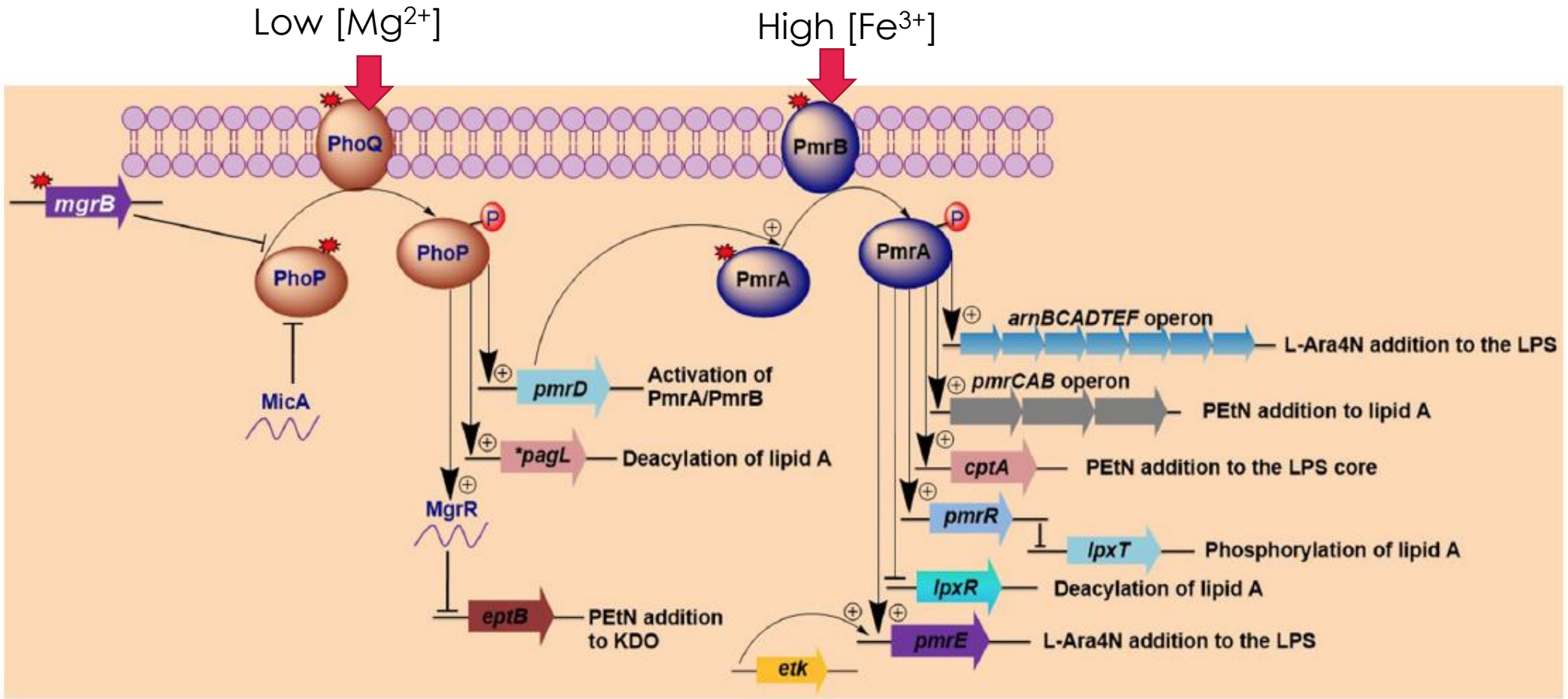

Figure 1: **Colistin resistance mechanisms (1)**

PROJECT METHODOLOGIES

1. Microbroth Dilution to investigate antibiotic minimum inhibitory concentrations

2. Lambda Red Recombination for deleting *phoP* from *E. coli* ATCC 25922 genome

1. MICROBROTH DILUTION

Table 1: Schematic table of microbroth dilution method

Antibiotic conc. (Mg/l)	S.C.	G.C.	0.0156	0.0313	0.0625	0.125	0.25	0.5	1	2	4	8
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
M6												
M73												
M33												
M52												
M4												
M44												
Control ATCC 25922												

2. LAMBDA RED RECOMBINATION

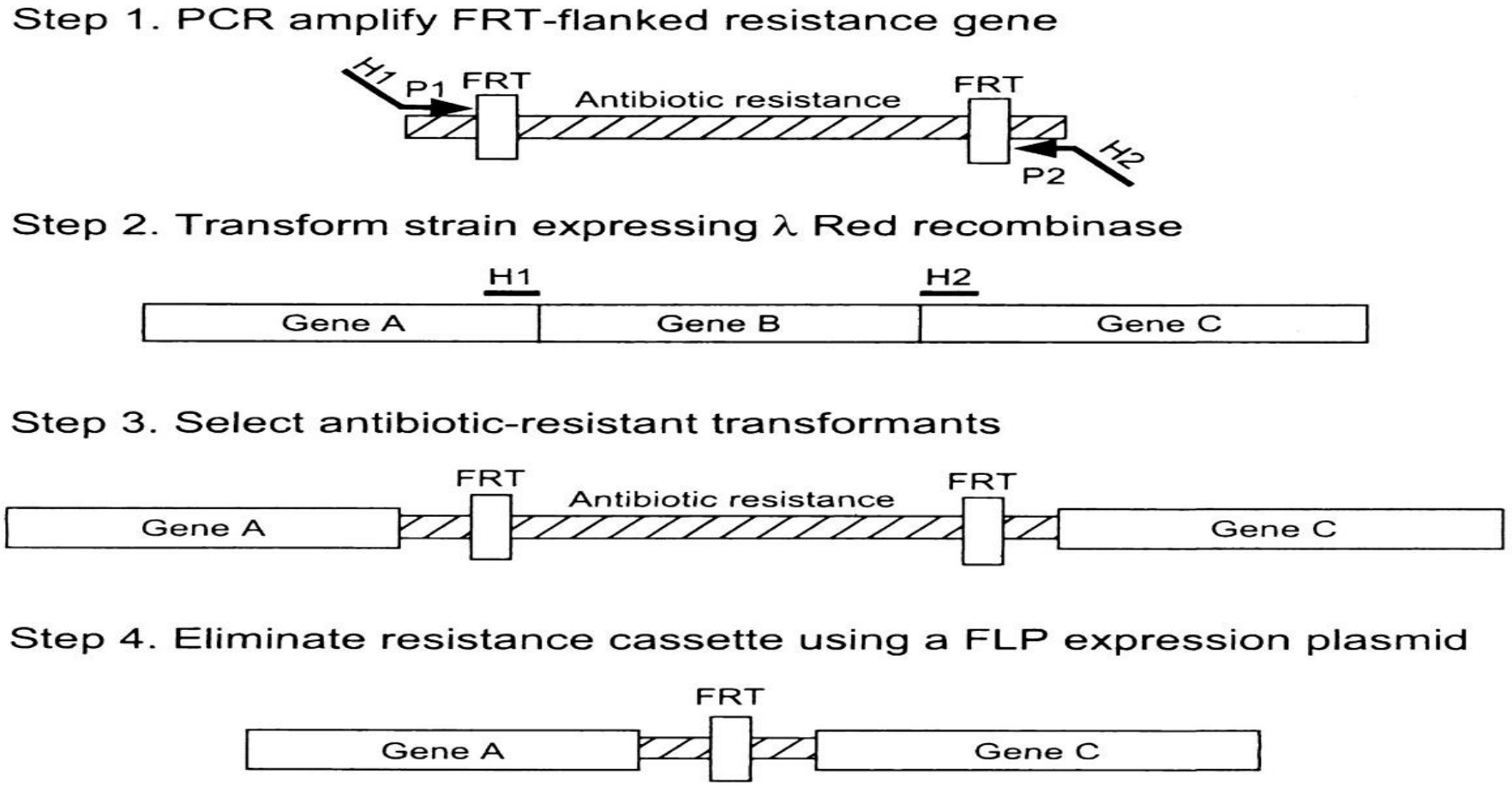

Figure 2: **Schematic demonstrating Lambda Red Recombination (2)**

Table 2: *mcr-1* status and colistin MIC interpretation of *E. coli* test isolates and control strain

Isolate	*mcr-1 Status of Isolate*	Colistin MIC Interpretation
M6	*mcr-1 negative*	RESISTANT
M73	*mcr-1 negative*	RESISTANT
M33	*mcr-1 positive*	LOW LEVEL RESISTANCE
M52	*mcr-1 positive*	SENSITIVE
M4	*mcr-1 negative*	SENSITIVE
M44	*mcr-1 negative*	SENSITIVE
Control ATCC 25922	*mcr-1 negative*	SENSITIVE

VERIFICATION OF *phoP* DELETION

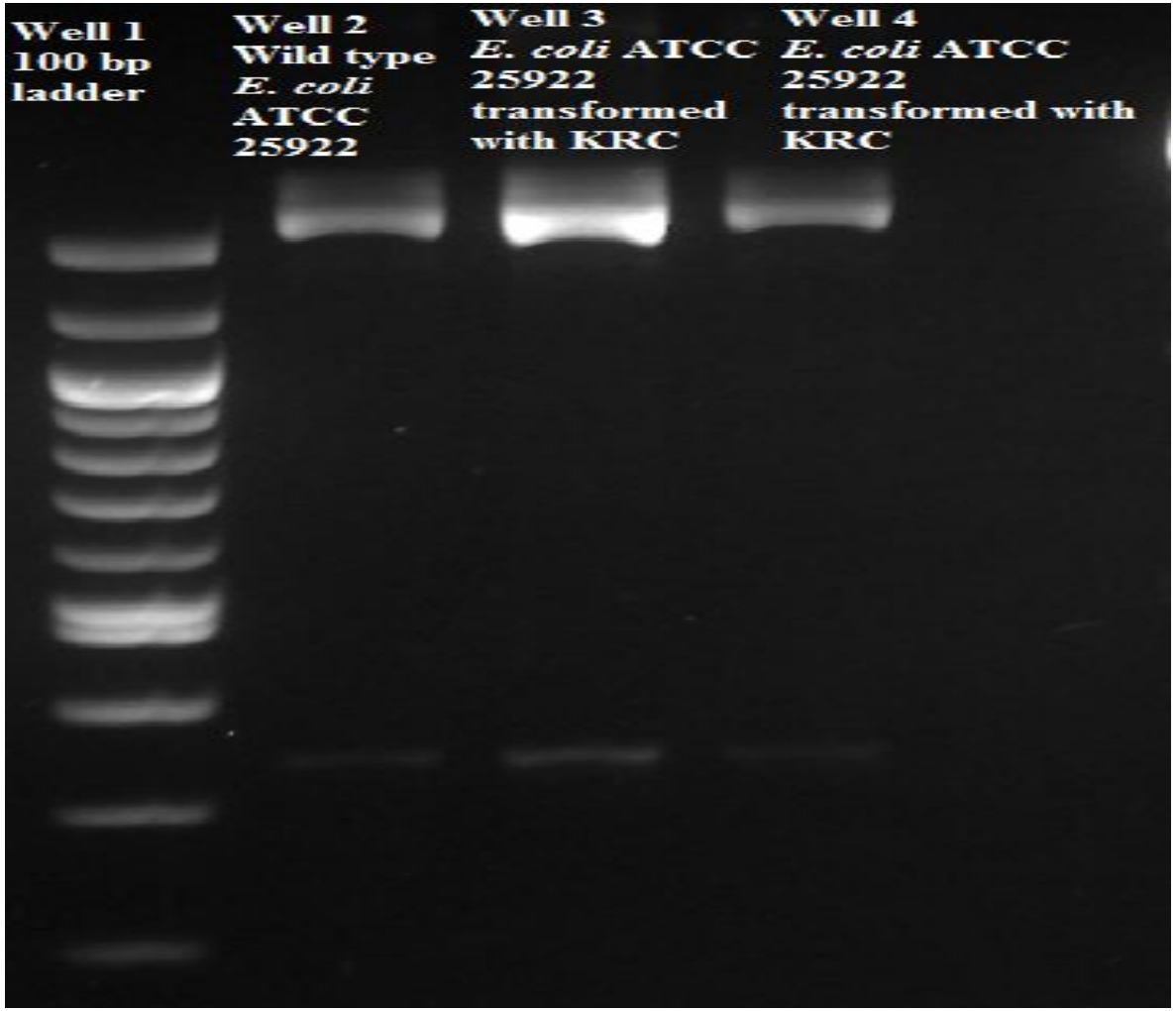

Figure 4: **Electrophoresis demonstrating the presence of kanamycin resistance cassette in Well 2 and *phoP* in Wells 2 and 4**

VERIFICATION OF KRC DELETION

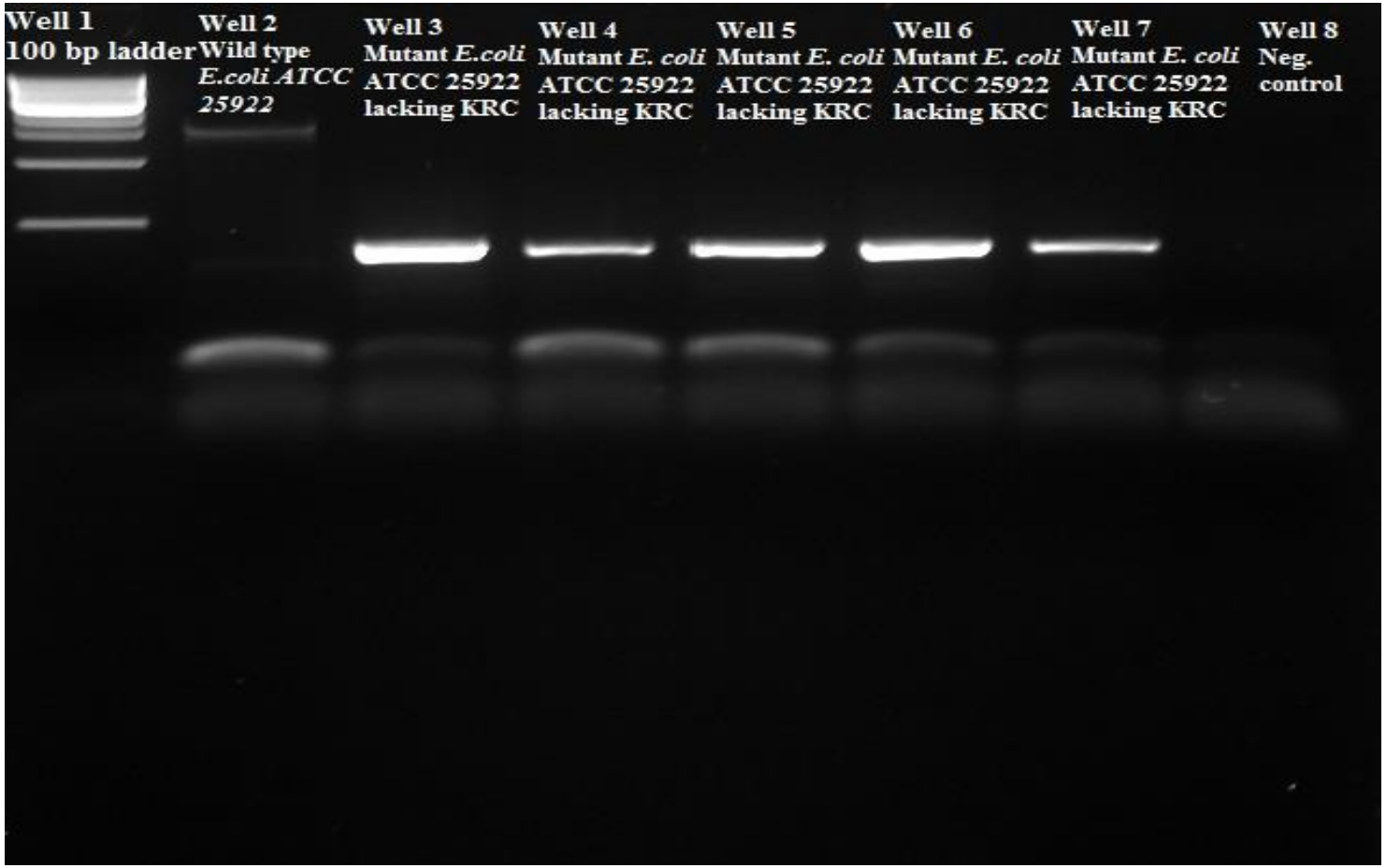

Figure 5: **Electrophoresis graph demonstrating the successful deletion of the KRC from mutant *E. coli* ATCC 25922**

Table 3: Colistin MIC of wild type *E. coli* ATCC control strain and mutant control strain

	Colistin MIC (mg/l)	Interpretation
Wild type E. coli ATCC 25922	0.125	**SENSITIVE**
Mutant E. coli ATCC 25922	**0.25**	**SENSITIVE**

CONCLUSION

- One fold increase in MIC value is significant
- Demonstrates other two-component systems compensate for loss of *phoP* upon colistin exposure
- Also implies *phoP* mutation alone cannot mediate colistin resistance
- Multiple mutations in two-component systems are required for the development of a colistin resistant phenotype

FUTURE WORK

- Clone *mcr-1* into:
  - Susceptible *E. coli* control strain ATCC 25922
  - Mutant *E. coli* ATCC 25922
- Investigate the true function of the *mcr-1* gene – does it actually confer colistin resistance or serve to augment colistin resistance mechanisms?

REFERENCES

- (1) Olaitan AO, Morand S, Rolain J-M. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Frontiers in Microbiology. 2014; 5: 634.
- (2) Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97(12): 6640 – 6645.

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