

CONTENTS

| | Page |
|-------------------------|-------------|
| ACKNOWLEDGEMENTS | i |
| ABSTRACT | ii |
| ABSTRAK | iii |
| CONTENTS | iv |
| ABBREVIATIONS | viii |

| | | |
|-------------------|---|----|
| CHAPTER 1: | General Introduction | |
| 1.1 | Site-specific recombination | 1 |
| 1.2 | Xer-site specific recombination system | 3 |
| 1.3 | Arginine repressor of <i>Escherichia coli</i> K-12 (ArgR) | 4 |
| 1.4 | ArgR homologues | 8 |
| 1.5 | ArgR mutants | 8 |
| 1.6 | Fusion protein system | 10 |
| 1.7 | Objectives of study | 10 |
| CHAPTER 2: | Materials and Methods | |
| 2.1 | Bacterial strains and plasmids | 12 |
| 2.2 | Chemicals and reagents | 12 |
| 2.3 | Bacterial growth media | 12 |
| 2.4 | Growth and maintenance of bacterial culture | 13 |
| 2.5 | Amino acids, antibiotics, <i>lac</i> inducer, indicator, and vitamin | 13 |
| 2.6 | Isolation and purification of covalently closed circular plasmid DNA | 14 |
| 2.6.1 | Small-scale plasmid DNA preparation | 14 |
| 2.6.2 | Midi-scale plasmid DNA preparation | 15 |

| | | |
|----------|---|----|
| 2.6.3 | Large-scale plasmid DNA preparation | 16 |
| 2.6.4 | Purification of plasmid DNA with Cesium chloride/Ethidium bromide (CsCl-EtBr) density gradient centrifugation | 17 |
| 2.7 | <i>In vitro</i> DNA manipulation | 18 |
| 2.7.1 | Restriction endonuclease digestion of DNA | 18 |
| 2.7.2 | Dephosphorylation of DNA restriction fragment | 19 |
| 2.7.3 | Phenol extraction and ethanol precipitation of DNA | 19 |
| 2.7.4 | Ligation of DNA fragment | 19 |
| 2.8 | Bacterial transformation | 20 |
| | (a) Preparation of competent cells | 20 |
| | (b) Transformation | 20 |
| 2.9 | Rapid screening of plasmid DNA | 20 |
| | (a) Phenol-choloroform-isoropanol method | 20 |
| | (b) SCFSB method | 21 |
| 2.10 | DNA sequencing | 21 |
| 2.11 | Gel electrophoresis of DNA | 22 |
| 2.11.1 | Staining of DNA gel | 22 |
| 2.11.2 | Extraction of DNA from agarose gel | 22 |
| 2.12 | Protein expression and detection | 23 |
| 2.12.1 | Cell growth and induction | 23 |
| 2.12.2 | Cell lysis | 24 |
| 2.13 | SDS-PAGE analysis | 24 |
| 2.14 | Western blotting | 25 |
| 2.14.1 | Preparation of the gel for protein transfer | 26 |
| 2.14.2 | Preparation of the transfer membrane | 26 |
| 2.14.3 | Assembling of the transfer stack | 26 |
| 2.14.4 | Protein transfer | 26 |
| 2.14.5 | Visualization of the proteins | 27 |
| 2.14.6 | Drying of the blotted membrane | 27 |
| 2.14.7 | Detection of biotinylated protein | 28 |
| 2.14.7.1 | Blocking of the blotted membrane | 28 |

| | | |
|----------|--|----|
| 2.14.7.2 | Chromogenic substrate incubation | 29 |
| 2.15 | <i>In vivo</i> Xer-site specific recombination assay | 29 |
| 2.16 | Protein purification | 30 |
| 2.17 | Photography | 31 |

| | | |
|-------------------|--|----|
| CHAPTER 3: | Construction of ArgRWT and ArgRNV-Biotinylated peptide fusion protein | |
| 3.1 | Introduction | 32 |
| 3.2 | Preparation of cloning vector and DNA insert | 33 |
| 3.2.1 | Isolation of plasmid DNA | 33 |
| 3.2.2 | Purification of plasmid DNA | 34 |
| 3.2.3 | Digestion of plasmid DNA | 35 |
| 3.3 | Construction of ArgRWT-biotinylated peptide fusion protein | 36 |
| 3.3.1 | Subcloning of <i>argRWT</i> gene into PinPoint TM Xa-3 cloning vector | 36 |
| 3.3.2 | Analysis of <i>argRWT</i> -biotinylated peptide fusion recombinant DNA | 36 |
| 3.4 | Construction of ArgRNV-biotinylated peptide fusion protein | 37 |
| 3.4.1 | Subcloning of <i>argRNV</i> gene into PinPoint TM Xa-3 cloning vector | 38 |
| 3.4.2 | Analysis of <i>argRNV</i> -biotinylated peptide fusion recombinant DNA | 38 |
| 3.5 | Characterization of recombinant plasmids | 39 |
| 3.5.1 | Restriction endonucleases analysis for <i>argRWT</i> fusion derivative | 39 |
| 3.5.2 | Restriction endonuclease analysis for <i>argRNV</i> fusion derivative | 40 |
| 3.5.3 | DNA sequence analysis | 41 |

| | | |
|-------------------|---|----|
| CHAPTER 4: | Determination of the Xer phenotype of ArgRWT- and ArgRNV-biotinylated peptide fusion protein | |
| 4.1 | Introduction | 42 |
| 4.2 | <i>In vivo cer</i> -mediated recombination using pCS202 as reporter plasmid | 43 |
| 4.3 | <i>In vivo cer</i> -mediated recombination using pSH10 as reporter plasmid | 44 |
| CHAPTER 5: | Protein expression and analysis | |
| 5.1 | Introduction | 46 |
| 5.2 | Small-scale expression of ArgRWT-biotinylated peptide fusion protein | 46 |
| 5.3 | Small-scale expression of ArgRNV-biotinylated peptide fusion protein | 47 |
| 5.4 | Partial purification of fusion proteins | 48 |
| 5.4.1 | Expression conditions | 48 |
| 5.4.2 | Fractionation of cellular proteins | 48 |
| 5.4.2.1 | Purification by Batch capture method | 49 |
| 5.4.2.2 | Purification by Column capture method | 49 |
| CHAPTER 6: | Discussion | |
| 6.1 | Introduction | 51 |
| 6.2 | Effects of additional amino acids residues to ArgR structure and activity | 53 |
| 6.3 | Expression of <i>argR</i> fusion genes and the related properties of the fusion products | 55 |
| 6.3.1 | Protein degradation systems | 56 |
| 6.3.2 | Potential toxicity of the protein | 58 |
| 6.4 | Use of ArgR fusion protein | 58 |
| 6.5 | Possible experiments to be carried out | 60 |
| 6.6 | Conclusions and suggestions | 60 |

REFERENCES

ABBREVIATIONS

(a) Buffers/Chemicals/Enzymes/Reagents

| | |
|---------|--|
| APS | ammonium persulphate |
| ATP | adenosine triphosphate |
| BSA | bovine serum albumin |
| DNA | deoxyribonucleic acid |
| DMF | dimethylformamide |
| DNase I | deoxyribonuclease I |
| dNTP | deoxynucleoside triphosphate |
| DTT | dithiothreitol |
| EDTA | ethylenediaminetetraacetic acid (disodium salt) |
| EtBr | ethidium bromide |
| EtOH | ethanol |
| FSB | final sample buffer |
| IPTG | isopropyl- β -D-thiogalactopyranoside |
| NaCl | sodium chloride |
| PMSF | phenylmethanesulfonylfluoride |
| RNA | ribonucleic acid |
| RNase A | ribonuclease A |
| SCFSB | single colony final sample buffer |
| SDS | sodium dodecyl sulphate |
| TAE | Tris-acetate-EDTA buffer |
| TBE | Tris-borate-EDTA buffer |
| TE | Tris-EDTA buffer |
| TEMED | NNN'N'- tetramethylethylenediamine |
| TM | Tris-magnesium buffer |
| Tris | tris (hydroxymethyl) amino ethane |
| X-gal | 5-bromo-4-chloro-3-indolyl- β -D galactoside |

(b) Antibiotics

| | |
|----|-----------------|
| Ap | ampicillin |
| Cm | chloramphenicol |
| Km | kanamycin |
| Tc | tetracycline |

(c) Units

| | |
|--------------|-----------------------------|
| bp | base pair |
| $^{\circ}$ C | degree Celsius |
| Da | dalton |
| g | gram |
| hr | hour |
| kb | kilobase pairs (10^3 bp) |
| kDa | kilodalton |

| | | |
|---------------|--|------------------------|
| l | | litre |
| M | | molar |
| mA | | milliampere |
| min | | minutes |
| mg | | milligram |
| μg | | microgram |
| ml | | millilitre |
| μl | | microlitre |
| mol | | moles |
| nm | | nanometer |
| rpm | | revolutions per minute |
| sec | | seconds |
| V | | volts |

(d) Amino acids and genetic code

| | | | |
|---|-----|---------------|------------------------------|
| A | Ala | alanine | GCT, GCC, GCA, GCG |
| C | Cys | cysteine | TGT, TGC |
| D | Asp | aspartic acid | GAT, GAC |
| E | Glu | glutamic acid | GAA, GAG |
| F | Phe | phenylalanine | TTT, TTC |
| G | Gly | glycine | GGT, GGC, GGA, GGG |
| H | His | histidine | CAT, CAC |
| I | Ile | isoleucine | ATT, ATC, ATA |
| K | Lys | lysine | AAA, AAG |
| L | Leu | leucine | TTG, TTA, CTT, CTC, CTA, CTG |
| M | Met | methionine | ATG |
| N | Asn | asparagine | AAT, AAC |
| P | Pro | proline | CCT, CCC, CCA, CCG |
| Q | Gln | glutamine | CAA, CAG |
| R | Arg | arginine | CGT, CGC, CGA, CGG, AGA, AGG |
| S | Ser | serine | TCT, TCC, TCA, TCG, AGT, AGC |
| T | Thr | threonine | ACT, ACC, ACA, ACG |
| V | Val | valine | GTT, GTC, GTA, GTG |
| W | Trp | tryptophan | TGG |
| Y | Tyr | tyrosine | TAT, TAC |

(e) Genotype and phenotype

| | |
|-----------------|--|
| Xer^+ | strain proficient in Xer site-specific recombination |
| Xer^- | strain deficient in Xer site-specific recombination |
| argR^- | argR null mutant |

(f) Miscellaneous

| | |
|-----------------|---|
| ~ | approximately |
| i.e. | (Latin <i>id est</i>) that is to say, in other words |
| LB | Luria-Bertani |
| MW | molecular weight |
| OD _x | optical density at x nm |
| <i>ori</i> | origin of replication |
| ORF | open reading frame |
| % | percentage |
| PAGE | polyacrylamide gel electrophoresis |
| <i>Tn</i> | transposon |
| UV | ultra violet |
| (v/v) | volume to volume ratio |
| WT | wild-type |
| (w/v) | weight to volume ratio |
| X ^r | resistance to X |
| X ^s | sensitivity to X |