2.1 Materials

2.1.1 Strains and bacteriophages

The *E. coli* strains used in this study with their genotypes are shown in Table 2.1. All strains other than BL21 (DE3) employed in protein overexpression experiments are derivatives of *E. coli* K12. Bacterial strains were routinely stored on solid agar plates at 4ºC and also as thick suspensions in 40% glycerol at –70ºC. Plasmid harboring strains were freshly prepared by transformation of the required plasmid.

The bacteriophage P1*kc* from the laboratory collection was used for routine transduction to move a locus from one strain to another and is referred to as P1 throughout this thesis.

Table 2.1 *E. coli* strains used in this study

Strain	**Genotype**[a]
MG1655	wild-type
MC4100	Δ*(argF-lac)U169 rpsL150 relA1 araD139 flbB5301 deoC1 ptsF25*
BL21(DE3)[b]	F– *ompT gal dcm lon hsdSB* (rB–, mB–) *gal* λ(DE3[*lacI lacUV5*-T7 RNAP*Ind1 Sam7 nin5*])
DH5α	Δ*(argF-lac)U169 supE44 hsdR17 recA1 endA1gyrA96 thi-1 relA1 (*φ *80lacZ*Δ*M15)*
GJ4652[c]	MC4100 Δ*gltBDF500*
GJ4748[d]	MC4100 *argR64 zhb-914*::Tn*10*dCm
GJ9602	MC4100 Δ*argP*
GJ9611	GJ4652 Δ*argP*
GJ9623	MC4100 Δ*lysP*
GJ9624	MC4100 Δ*argP* Δ*lysP*
GJ9647	MC4100 Δ*cadC*::Kan
GJ9648	MC4100 Δ*argP* Δ*cadC*::Kan
GJ9649	MC4100 Δ*lysP* Δ*cadC*::Kan
GJ9650	MC4100 *araD*$^+$
GJ9651	MC4100 *araD*$^+$ Δ*argP*

GJ9652	MC4100 *araD*$^{+}$ Δ*lysR*::Kan
GJ9653	MC4100 *araD*$^{+}$ Δ*argP* Δ*lysR*::Kan

[a] Genotype designations are as in Berlyn (1998). The Δ*argP*, Δ*lysP*, Δ*lysR*, and Δ*cadC* alleles were introduced as Kan^{r} deletion-insertion mutations from the Keio knockout collection (Baba *et al*., 2006) and, where necessary, the Kan^{r} marker was then excised by site-specific recombination with the aid of plasmid pCP20, as described (Datsenko and Wanner, 2000). The latter mutations are shown without the Kan^{r} designation in the table.
[b] *E. coli* B strains.
[c] Strain described earlier (Nandineni *et al*., 2004).
[d] Strain described earlier (Nandineni and Gowrishankar, 2004).

2.1.2 Plasmids

The plasmid vectors used in this study were as follows:

1. pCU22: It is a derivative of pUC19 used to prepare supercoiled DNA for *in vitro* transcription where two strong phage *fd* transcription terminators flank MCS. This ensures that the transcripts originated from vector based promoters will not interfere with the transcription from the cloned promoter and that the transcript originated from the cloned promoter will be terminated after the MCS (Ueguchi and Mizuno, 1993).

2. pMU575: It is an IncW-based, single-copy, trimethoprim resistance bearing promoter probe vector. It carries its MCS upstream of a promoterless *galK*'-*lacZ* fusion. This fusion has the first 58 codons of *galK* fused to the 8th codon of *lacZ*, and the resultant hybrid polypeptide possesses functional β-Galactosidase activity (after assembly as a tetramer). Translation of the hybrid gene is controlled by the ribosome binding site of *galK*. There are stop codons in all the three reading frames between MCS and initiation codon of *galK* so that there is no interference caused by translational read-through from inserts cloned into MCS region. A strong *pheR* terminator located upstream of the MCS prevents read through from any vector-based promoter into the *lacZ* gene (Andrews *et al*., 1991).

3. pTrc99A: It is an expression vector with ColE1 origin of replication and ampicillin resistance marker. It provides IPTG dependent induction of the cloned gene (Amann *et al*., 1988)

4. pCL1920: It is a pSC101-based, low-copy-number vector with spectinomycin and streptomycin resistance marker carrying the MCS in *lacZα* region and hence provides the advantage of screening the insertions using α-complementation (Lerner and Inouye, 1990).

5. pET21b: It is a ColE1-based, high-copy-number expression vector bearing ampicillin resistance marker. A strong T7 RNAP-recognised promoter and an efficient ribosome

binding site lie upstream of the MCS to ensure the high level expression of any gene cloned in MCS. A stretch of hexa-histidine (His_6)-encoding codons followed by stop codon is incorporated downstream of MCS to give a C-terminally His_6-tagged recombinant protein (EMD Biosciences).

6. pBAD18: It is an expression vector with a pMB9 derived origin of replication and allows for tightly regulated expression of genes cloned under the P_{BAD} promoter of the *araBAD* operon (Guzman *et al*., 1995). The vector also carries the *araC* gene, encoding the positive and negative regulator of this promoter.

7. pCP20: pSC101-based Ts replicon, chloramphenicol resistant, ampicillin resistant, for *in vivo* expression of Flp recombinase (Datsenko and Wanner, 2000)

Plasmid DNA preparations were routinely made from *recA* strain DH5α and were stored in 10 mM Tris-Cl (pH-8.0) with 1 mM EDTA at –20ºC. The plasmid constructs used in this study are given in Table 2.2.

Table 2.2 Plasmid constructs

Plasmids[a]	**Specifications**
pHYD915	1.8 kb *SalI* fragment containing *argP* from λ-471 cloned in pCL1920
pHYD926	*argP* (S94L) mis-sense mutation derived from pHYD915
pHYD927	*argP* (P108S) mis-sense mutation derived from pHYD915
pHYD928	*argP* (V144M) mis-sense mutation derived from pHYD915
pHYD929	*argP* (P217L) mis-sense mutation derived from pHYD915
pHYD931	*argP* (R295C) mis-sense mutation derived from pHYD915
pHYD932	*argP* (A68V) mis-sense mutation derived from pHYD915
pHYD1705	His_6tagged *argP* cloned at the *Nde*I and *Xho*I sites of pET21b
pHYD1723	–293 to +109 of *argO* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2601	–322 to +113 (435-bp) of *gdhA* regulatory region cloned at *Pst*I and *Bam*HI sites in pCU22
pHYD2602	–322 to +113 (435-bp) of *gdhA* regulatory region sub-cloned from pHYD2601at *Pst*I and *Bam*HI sites in pMU575
pHYD2606	*argP* (P274S) mis-sense mutation derived from pHYD915

pHYD2610	–273 to +100 (373-bp) of *dapD* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2636	–206to +32 (238-bp) of *lysP* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2647	–114to +32 (158-bp) of *lysP* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2648	–76to +32 (120-bp) of *lysP* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2649	Derivative of pHYD2601 where two nucleotides of a putative –10 sequence of *gdhA* promoter is modified (TATAGT→CCTAGT)
pHYD2650	Derivative of pHYD2601 where two nucleotides of a putative –10 sequence of *gdhA* promoter is modified (TATAGT→TATAGC)
pHYD2653	*gdhA* regulatory region from pHYD2649 sub-cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2654	*gdhA* regulatory region from pHYD2650 sub-cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2658	–270 to +146 (416-bp) of *artJ* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2659	–192 to +96 (288-bp) of *artP* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2660	–236 to +131 (367-bp) of *argT* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2661	–187 to +119 (306-bp) of *hisJ* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2664	–187 to +119 (615-bp) of *lysC* regulatory region cloned at *Pst*I and *Xba*I sites in pMU575
pHYD2668	–207 to +136 (343-bp) of *asd* regulatory region cloned at *Sal*I and *Hind*III sites in pMU575
pHYD2669	–285to +101 (386-bp) of *dapB* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2670	–139 to +129 (268-bp) of *lysA* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2671	–460 to +208 (668-bp) of *dnaA* regulatory region cloned at *Pst*I and

	*Bam*HI sites in pMU575
pHYD2672	–311 to +170 (481-bp) of *nrdA* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2673	1340-bp of *lysA* coding region with native RBS cloned at *Kpn*I and *Xba*I sites in pBAD18
pHYD2674	–387 to +140 (527-bp) of *cadB* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2675	–127 to +141 (268-bp) of *lysR* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2676	939-bp of *lysG* coding region with native RBS cloned at *Eco*RI and *Hind*III sites in pBAD18
pHYD2677	–301 to +58 (359-bp) of *lysE* regulatory region cloned at *Pst*I and *Bam*HI sites in pMU575
pHYD2678	His_6tagged $argP^d$-S94L cloned at the *Nde*I and *Xho*I sites of pET21b
pHYD2679	His_6tagged $argP^d$-P108S cloned at the *Nde*I and *Xho*I sites of pET21b
pHYD2680	His_6tagged $argP^d$-P274S cloned at the *Nde*I and *Xho*I sites of pET21b
pHYD2681	His_6tagged *lysG* cloned at the *Nde*I and *Hind*III sites of pET21b
pHYD2685	1468-bp of *gdhA* coding region with native RBS cloned at the *Eco*RI and *Hind*III sites in pTrc99A

[a] Plasmids pHYD915 to pHYD932 (Nandineni and Gowrishankar, 2004) and pHYD1705 and pHYD1723 (Laishram and Gowrishankar, 2007) have been described earlier. Plasmids pHYD2601 to pHYD2685 were constructed in this study.

2.1.3 Primers

The primers used in this study are listed in Table 2.3.

Table 2.3 Oligonucleotide primers [a]

Oligonucleotides ID	**Sequence (5'→3')**
JGARGP1r	AGCAGACAACA*CATATG*AAACGCCCGGA
JGARGP3r	ATTATTTGAT*CTCGAG*ATCCTGACGAAG
JGARGO8f	GTGCGC*CTGCAG*GAACTTGGTG
JGARGO9r	GTATGCCC*GGATCC*ATCACAAAA
JGBPMAlacZF1	GTGGTGCAACGGGCGCTGGGTCGGTTAC

JGBPMAlacZR1	CAACTCGCCGCACATCTGAACTTCAG
JGJgdhAFnew	ATTTTGATC*CTGCAG*AACGCAGCACTG
JGJgdhAR	GTTTGATTC*GGATCC*CGCTTTTGGACATG
JGJgdhA1	AGAGAATATGTCTGATCCAT
JGJgdhA2	CATGTGCTTTTGCAGTTTTC
JGJgdhA3	ATAACGAGAGTAATCTCATA
JGJdapDF	CGCCATTTTA*CTGCAG*AAACCGAAG
JGJdapDF	CGGGTAAC*GGATCC*TGCATTGGCT
JGJlysPF	GCGCTTT*CTGCAG*TATTGCGATCC
JGJlysPR	TAGTTTC*GGATCC*CATACAAAAATGC
JGJlysPFP1	ACAA*CTGCAG*TTCGCCA GAAAA
JGJlysPFP2	ACAA*CTGCAG*CTGGGCGATCAT
JGJgdhAqc1	TTCTTGATGG*CC*TAGTCGAAAAC
JGJgdhAqc2	TTTTCGACTA*GG*CCATCAAGAATG
JGJgdhAqc3	TGATGGTATAG*C*CGAAAACTGC
JGJgdhAqc4	GCAGTTTTCG*G*CTATACCATC
JGJartJF	ACAA*CTGCAG*CAAAGCGCTGGCA
JGJartJR	ACAA*GGATCC*GGATAGGTGGCTGAAA
JGJartPF	ACAA*CTGCAG*GAATCGCTAACGCC
JGJartPR	ACAA*GGATCC*TATCGAACAGCGCC
JGJargTF	ACAA*CTGCAG*ATCTCTTTGCCCGC
JGJargTR	ACAA*GGATCC*GTAGCGCCGCATA
JGJhisJF	ACAA*CTGCAG*TGTCTACGGTGACTG
JGJhisJR	ACAA*GGATCC*TCGCAGCAAACG
JGJlysCF	ACAA*CTGCAG*GTCTGCGTTGGATT
JGJlysCR	ACAA*TCTAGA*CGGTTCATGGCGT

JGJasdF	GTATGTTTCAGT*GTCGAC*ATGAAAATAG
JGJasdR	GGCGTCG*AAGCTT*CGCTCTTCAACCA
JGJdapBF	CCCTGTTTTG*CTGCAG*TGGAAAC
JGJdapBR	AATGCCAGC*GGATCC*TGAATCAAC
JGJlysAF	ACAA*CTGCAG*CCTCAGTCAGGCTTC
JGJlysAR	ACAA*GGATCC*CAGCCAAATTCAGC
JGJdnaAF	ACAA*CTGCAG*TTTCATGGCGATT
JGJdnaAR	ACAA*GGATCC*GCTGGTAACTCAT
JGJnrdAF	ACAA*CTGCAG*CGCCATCAACAAT
JGJnrdAR	ACAA*GGATCC*GTCGAGATTGATGC
JGJlysAfullF	ACAA*GGTACC*TTTTATGATGTGGCGT
JGJlysAfullR	ACAA*TCTAGA*AGTCATCATGCAACC
JGJcadBF	TTTAATTTAC*CTGCAG*GGGCAAAC
JGJcadBR	TGCAATACC*GGATCC*CATCATATTA
JGJlysRF	ACAA*CTGCAG*CAGCCAAATTCAGC
JGJlysRR	ACAA*GGATCC*CCTCAGTCAGGCTTC
JGJlysGF	ACAA*GAATTC*GGTTCTTAACATGGT
JGJlysGR	ACAA*AAGCTT*GCGAAGAAGTGAAA
JGJlysE1	TAGTTT*CTGCAG*GCAGCAACAC
JGJlysE2	GTCCGAT*GGATCC*TAAAAGACTGG
JGJlysGF_pet	ACAA*CATATG*AACCCCATTCAA
JGJlysGR_pet	ACAA*AAGCTT*CTAGTGGTGGTGGTGGTGGTGAGGCCGC AATC
JGJgdhAfullF	ACAA*GAATTC*CTGCAAAAGCACATG
JGJgdhAfullR	AACA*AAGCTT*TGTAGGCCTGATAAG
JGJargO5plusF	GTGCGCCTGAACGAACTTGGTG
JGJargO5plusR	CACGTTGGATATTCCGAATT

JGJargOeqF	CGCTGAGGCCAGATAATACT
JGJargOeqR	CACGGCGACTGCATCAATAA
JGJargO3plusF	CGCTGAGGCCAGATAATACT
JGJargO3plusR	CACGGCGACTGCATCAATAA
JGJlysE5plusF	CTGCTTGCACAAGGACTTCACC
JGJlysE5plusR	ACCTGTAATGAAGATTTCCAT
JGJlysEeqF	TCGAGAGCTTTAACGCGCTGAC
JGJlysEeqR	CCTTCGCGCTTAATTCCTTGTT
JGJlysE3plusF	CCAGTTGAATGGGGTTCATGA
JGJlysE3plusR	CACGATCGGCGCGGCATTGGAC
JGJpMUF	TCCCCACATCACCAGCAA
JGJgalK	CAGAGATTGTGTTTTTTCTTTCAG

[a] Primers JGARGP1r, JGARGP3r, JGARGO8f and JGARGO9r are from a previous study (Laishram and Gowrishankar, 2007). JGBPMAlacZF1 and JGBPMAlacZR1 were obtained from S. Aisha. The remaining primers were synthesised as part of this study. Restriction sites or mutated nucleotides are shown in italics.

2.1.4 Media

All media and buffers were sterilised by autoclaving at 121ºC for 15 mins. Media and buffers used in this study are given below:

Glucose /Glycerol-minimal A medium

K_2HPO_4	10.5 gm
KH_2PO_4	4.5 gm
$(NH_4)_2SO_4$	1.0 gm
Sodium citrate, $2H_20$	0.5 gm
Water to	1000 ml

After autoclaving the following solutions were added

$MgSO_4$ (1M)	1 ml
Glucose (20%)	10 ml
Or Glycerol (80%)	5 ml
Vitamin B1 (1%)	0.1 ml

Amino acids when required, were added to a final concentration of 40 μg/ml. When growth on other carbon sources was to be tested, glucose was substituted with appropriate sugar at 0.2%.

Glucose-minimal A medium, pH 7.4

This medium was same as Glucose-minimal A medium described above except for the difference in K_2HPO_4 and KH_2PO_4 which were as mentioned below:

K_2HPO_4	14.0 gm
KH_2PO_4	2.7 gm

Glucose-minimal A medium, pH 5.8

This medium was same as Glucose-minimal A medium described above except for the difference in K_2HPO_4 and KH_2PO_4 which were as mentioned below:

K_2HPO_4	1.5 gm
KH_2PO_4	12.4 gm

Glucose /Glycerol-minimal A 19 (18 or 17) amino acid medium

This medium is essentially the same as glucose/glycerol-minimal A medium described above except that all 19 or 18 or 17 other than either Lys or Lys and Arg or Lys and Arg and His amino acids were added after autoclaving at a final concentration of 40 μg/ml from autoclaved 4 mg/ml stock solutions.

Minimal A agar

It contains 1.5% bacto-agar (Difco) in minimal A medium. The plates were poured after mixing double strength minimal A with 3% agar that had been autoclaved separately.

LB medium

Tryptone	10.0 gm
Yeast Extract	5.0 gm
NaCl	10.0 gm
Water to	1000 ml

pH adjusted to 7.0 to 7.2 with 1 N NaOH

LB agar

LB medium	1000 ml
Bacto-agar	15 gm

Z broth (for P1 transduction)

LB medium	100 ml
$CaCl_2$ (0.5 M)	0.5 ml

Z agar (for P1 transduction)

Z broth	100 ml
Bacto-agar	0.75 gm

2.1.5 Buffers and solutions

Citrate buffer

Citric acid (0.1 M)	4.7 volume
Sodium citrate (0.1 M)	15.4 volume

TE buffer

Tris-Cl (pH 8.0)	10 mM
EDTA	1 mM

TBE buffer

Tris-Borate	90 mM
Tris-Borate	90 mM
EDTA (pH 8.0)	2 mM

This was prepared as 10 X stock solution and used at 1 X concentration.

TAE buffer

Tris-acetate	40 mM
EDTA (pH 8.0)	2 mM

This was prepared at 50 X concentrated stock solution. Both TBE and TAE were used as standard electrophoresis buffers.

MOPS buffer

MOPS	4.16 gm
0.5 M EDTA	1.0 ml
Sodium acetate	0.68 gm
Water (nuclease free) to	500 ml

It was filter sterilized and stored in an amber colored bottle. This was prepared as 10 X stock solution and used at 1 X concentration.

INOUE (PIPES) buffer

PIPES (free acid)	10 mM
$CaCl_2.2H_2O$	15 mM
KCl	250 mM
$MnCl_2.4H_2O$	55 mM

pH was adjusted to 6.7 with 1 N KOH.

PIPES gets into solution when the pH is greater than 6.7. $MnCl_2$ was dissolved separately and added drop by drop with stirring. The pH was adjusted to 6.7 and filter sterilized and stored at –20ºC.

Z buffer (for β-Galactosidase assay)

Na_2HPO_4	16.1 gm
NaH_2PO_4	5.5 gm
KCl	0.75 gm
$MgSO_4.7H_2O$	0.246 gm
β-mercaptoethanol	2.7 ml
Water to	1000 ml

pH was adjusted to 7.0 and stored at 4ºC.

SDS running buffer

Tris-base	30.3 gm
Glycine	144 gm
SDS	10 gm
Water to	1000 ml

It was prepared in 10 X concentration and diluted to 1 X for running.

Storage buffer for protein

Tris-Cl (pH 8.0)	20 mM
NaCl	300 mM
DTT	10 mM
Glycerol	40 %

Hybridization buffer

Tris-Cl (pH 8.0)	9 mM
EDTA	0.35 mM

Sample buffer (for SDS-PAGE)

Tris-Cl (pH 6.8)	150 mM
SDS (20%)	6% v/v
Glycerol	30% v/v
β-mercaptoethanol (5%)	15%
Bromophenol blue	0.6% (w/v)

EMSA binding buffer

Tris-Cl (pH 7.5)	10 mM
NaCl	50 mM
EDTA	1 mM
Glycerol	5 %
DTT	5 mM

Denaturing gel loading buffer with dye

Formamide	95%
EDTA	20 mM
Xylene Cyanol	0.05 gm
Bromophenol blue	0.05 gm

Non denaturing gel loading buffer with dye

Tris-Cl (pH 7.5)	250 mM
Bromophenol blue	0.02%
Glycerol	20%

Formaldehyde agarose gel

(For 50 ml)

DEPC treated water	43 ml
MOPS buffer	5.3 ml
Agarose	0.63 gm
Formaldehyde	2.6 ml

The above mix was boiled without formaldehyde to dissolve agarose and then at around 50ºC formaldehyde was added just before casting the gel.

40% Acrylamide solution

Acrylamide	39 gm
Bis-acrylamide	1 gm
Water to	100 ml

Non denaturing gel composition (50 ml)

40% acrylamide solution	5 ml
10 X TBE	5 ml
H_2O	40 ml
10% APS	250 µl
TEMED	30 µl

SDS PAGE gel (12%)

For resolving Gel (15 ml):	
30% Acrylamide solution	6 ml
1.5 M Tris-Cl (pH 8.8)	3.8 ml
10% SDS	150 µl
10% APS	150µl
Water to	15 ml
TEMED	10 µl
For stacking gel (3 ml):	
30% Acrylamide solution	500 µl
1 M Tris Cl (pH 6.8)	380 µl
10% SDS	30 µl
10% APS	30 µl

Water to	3 ml
TEMED	10 µl

Denaturing (urea) sequencing gel (6%) composition

10 X TBE	50 ml
40% acrylamide	75 ml
Urea	210 gm (7 M)
Water to	500 ml

This was filtered through a 0.45/0.22 µ milipore filter. For casting the gel 35 ml of the sequencing gel mixure was mixed with 150 µl 10% APS and 25 µl TEMED.

2.1.6 Antibiotics

Antibiotics were used at the following final concentrations in various media as given in Table 2.4.

Table 2.4 Concentrations of antibiotics (µg/ml)

Antibiotic	Rich media	Minimal media
Ampicillin (for plasmids)	100	50
Trimethoprim (for plasmids)	60	30
Spectinomycin (for plasmids)	50	50
Streptomycin (for plasmids)	50	100
Chloramphenicol (chromosome)	25	25
Chloramphenicol (for plasmids)	50	30

2.1.7 Chemicals:

Chemicals were obtained from commercial sources. Most of the chemicals such as amino acids, antibiotics, sugars, IPTG, ONPG and X-gal were obtained from Sigma Chemical Co. The media components for the growth of bacteria were mostly from HiMedia laboratories. The materials used in the recombinant DNA experiments such as restriction endonucleases, T4-DNA ligase, DNA-polymerases and DNA size markers were obtained from companies including New England Biolabs, MBI Fermentas and Stratagene. RNA isolation chemicals like Reverse transcriptase, trizol, RNA loading buffers and dyes and RNA size markers were obtained from Invitrogen and Sigma. Protein markers were obtained from MBI Fermentas. Kits for plasmid isolation,

purification of DNA fragments were from Qiagen or HiMedia. The oligonucleotide primers used in this study were mainly synthesised by Ocimum Biosolutions or MWG Biotech. The radioactive chemicals were procured from BRIT Mumbai.

2.2 Methods

2.2.1 Genetic techniques

2.2.1.1 Phage P1 lysate preparation by broth method

0.3 ml of overnight culture of the donor strain in Z-broth was mixed with 10^7 plaque forming units (pfu) of a stock P1 lysate prepared on strain MG1655. Adsorption was allowed to occur at 37ºC for 20-mins. To 0.3 ml of infection mixture, 10 ml of Z-broth was added and incubated at 37ºC with slow shaking until the visible lysis of the culture occurred (in 4-6 hrs). The lysate was treated with 0.3 ml of chloroform, centrifuged and the clear lysate was stored at 4ºC with chloroform. Preparation of P1 lysates on *recA* mutant strains were also done similarly, but with a higher multiplicity of infection (i.e. 10^8 starter P1 phage).

To quantitate the P1 phage lysate preparation, titration was done using P1 phage sensitive indicator strain such as MG1655. 100 µl each of dilution of phage (typically 10^{-5}, 10^{-6}) were mixed with 0.1 ml of fresh culture grown in Z-broth. After 15-min of adsorption at 37ºC without shaking, each mixture was added on a soft agar overlay of Z-agar plates and incubated overnight at 37ºC. The phage titer was calculated from the number of plaques obtained on the plates.

2.2.1.2 Phage P1 transduction

To 2 ml of fresh overnight culture of recipient strain, 10^8 pfu equivalent of phage lysate was added and incubated at 37ºC without shaking for 15-min to facilitate phage adsorption. The un-adsorbed phage particles were removed by centrifugation at 4000 rpm for 5-min and pellet of bacterial cells was re-suspended in 5 ml of LB-broth containing 20 mM sodium citrate to prevent further phage adsorption. This was incubated for 30-min at 37ºC without shaking to allow the phenotypic expression of the antibiotic resistance gene. The mixture was then centrifuged, and the pellet was resuspended in 0.3 ml citrate buffer. 100 µl aliquots were plated on appropriate antibiotic containing plates supplemented with 2.5 mM sodium citrate. A control tube without addition of P1 lysate was also processed in the same way. In the case of selection of nutritional requirement,

the infection mixture was centrifuged, washed in 5 ml of citrate buffer and plated without phenotypic expression.

2.2.1.3 Transformation

A. Calcium chloride method

For routine plasmid transformations, following method which is modification of that described by Cohen *et al*. (1972) was used. An overnight culture of recipient strain was sub-cultured 1:100 in fresh LB medium and grown till mid-exponential phage. The culture was chilled on ice for 15-min, and the steps thereafter were performed at 4ºC. 20 ml of culture was centrifuged and pellet was re-suspended in 10 ml of 0.1 M $CaCl_2$. After 15-min of incubation on ice, the cells were again centrifuged and re-suspended in2 ml of 0.1 M $CaCl_2$. The suspension was incubated on ice for 30-min. To the 200 µl aliquot of the cell suspension plasmid DNA (20 to 200 ng in less than 10 µl volume) was added, incubated for half an hr on ice and given a heat shock for 90-sec at 41ºC.The cultures was rapidly chilled, mixed with 0.8 ml of LB-broth and incubated at 37ºC for 1-hr, and plated on an appropriate selective medium at various dilutions. An aliquot of cell suspension to which plasmid DNA was not added served as a negative control.

B. Preparation of high efficiency competent cells

Competent cells for high efficiency transformations were prepared by a method of Inoue *et al*. (1990) with few modifications. An overnight culture of the strain (routinely DH5α) was sub-cultured into fresh sterile LB-broth in 1:100 dilutions and grown at 18ºC to an A_{600} of 0.55. The cells were harvested by centrifugation at 2500 rpm for 10-min at 4ºC. This was re-suspended in 0.4 volumes of INOUE buffer and incubated in ice for 10 min. The cells were recovered by centrifugation at 2500 rpm at 4ºC for 10-min and finally re-suspended in 0.01 volume of the same buffer. Sterile DMSO was added to a final concentration of 7%. After incubating for 10-min in ice, the cells were aliquoted in 100 µl volumes, snap frozen in liquid nitrogen and stored at –70ºC.

2.2.1.4 Scoring for phenotypes

A. *lac* phenotype

Lac^+ colonies were distinguished from Lac^- on X-gal containing plate or MacConkey lactose plate. X-gal is a non-inducing colorless substrate of β-galactosidase enzyme which upon hydrolysis yields dark blue indolyl group and hence the Lac^+ colonies on X-gal plate will appear as dark blue colonies. Similarly, on the MacConkey

agar plates Lac^+ colonies will appear dark pink colonies whereas Lac^- will remain colourless.

B. NaCl-sensitivity testing

The colonies to be tested were streaked on the surface of minimal A-glucose plates containing either 0.4-0.7 M NaCl with 1 mM glycine betaine, and incubated at 37^{o}C. NaCl-tolerant strains grew to form single colonies in 36-60 hrs whereas NaCl-sensitive ones did not. As controls, MC4100 (WT) and other previously identified NaCl sensitive mutants were streaked for comparison.

C. Test for canavanine (CAN) sensitivity

CAN is a toxic analog of Arg and is an inhibitor of bacterial growth. Strains were tested for sensitivity/resistance to CAN by streaking them on minimal A-glucose plates supplemented without and with 40 μg/ml CAN (or other concentrations as indicated) and 40 μg/ml uracil.

D. Test for ArgR$^+$/$^-$ phenotype

For testing ArgR$^+$/$^-$ phenotype, the colonies were streaked on minimal A-glucose plates containing uracil (40 μg/ml) and CAN (65 μg/ml). Uracil was added to the medium to sensitize an *argR*$^+$ strain to CAN. An *argR*$^+$ strain is inhibited at 65 μg/ml CAN on a uracil-containing plate, whereas on a plate without uracil, *argR*$^+$ would grow even at 700-800 μg/ml CAN. Uracil represses the *carAB* transcription, which encodes the carbamoyl phosphate synthase enzyme (CarAB). This results in reduced amounts of carbamoyl phosphate, which is the common intermediate between pyrimidine and Arg biosynthetic pathways. Reduced carbamoyl phosphate levels would result in decreased flux through the Arg biosynthetic pathways. This in turn would result in decrease in Arg pools inside the cell. An *argR* mutant would be derepressed for the Arg biosynthetic pathway and is resistant even to 300 μg/ml CAN in a uracil-containing plate.

E. Test for thialysine resistance

Thialysine or thiosine (S-Aminoethyl-L-cysteine) is a toxic analog of Lys. Strains were tested for sensitivity/resistance to thialysine by streaking them on minimal A-glucose plates supplemented without and with100-200 μg/ml thialysine (Steffes *et al*., 1992).

2.2.1.5 β-galactosidase assay

β-galactosidase assay was performed according to Miller (1992). An overnight grown culture of the bacterial strain was sub-cultured in glucose Minimal A medium

supplemented with amino acids and appropriate antibiotic and grown at 37ºC to an A_{600} of 0.5-0.6. Around 0.1-0.5 ml of culture was made up to 1 ml with Z-buffer and lysed with addition of one drop of chloroform and 1-2 drops of 1% SDS solution. 0.2 ml of freshly prepared 4 mg/ml ONPG was added to start the reaction and incubated at room temperature till the color of the reaction mixture turned yellow. 0.5 ml of 1 M Na_2CO_3 was added to stop the reaction and the time duration from initial addition of ONPG to the stopping of reaction was noted. The absorbance of reaction mix was taken at 420 nm and 550 nm. The A_{600} of the culture used was also noted. The enzyme specific activity (in Miller units) was calculated using following equation:

β-galactosidase specific activity = [1000 X A_{420}- (1.75 X A_{550})] / t X v X A_{600}

Where t is the time period in mins and v the volume of culture used in ml.

Each value reported is the average of at least three independent experiments, and the standard error was <10% of the mean in all cases.

2.2.1.6 Microarray details

A differential gene expression microarray with respect to *argP* was performed by Genotypic Technology Pvt. Ltd., Bengaluru. The experiment was performed on an oligonucleotide microarray having 10828 probes for coding region (on average three probes were designed for each 4294 coding regions) and 4380 probes for non-coding region (on average two probes were designed for 2240 non-coding regions). The RNA was labelled using Cy3 and single channel detection was used. Data was analysed using GeneSpring GX Version 7.3.

2.2.2 Recombinant DNA techniques

2.2.2.1 Isolation of plasmid DNA

1.5 ml of cells from an overnight culture was pelleted by centrifuging in cold (4ºC) for10-min at 6000 rpm. The cells were re-suspended in 200 µl solution I (50 mM glucose; 25 mM Tris-Cl, pH-8; 10 mM EDTA, pH-8) with vortexing. 400 µl of freshly prepared solution II (0.2% NaOH, 1% SDS) was added and mixed by gently inverting the tubes. Subsequently, 300 µl of solution III (prepared by mixing 60 ml of 5 M CH_3COOK,11.5 ml glacial acetic acid, 28 ml water) was added and the tubes were inverted repeatedly and gently for homogeneous mixing followed by incubation for 5-min on ice. After centrifuging at 12,000 rpm for 15-min, supernatant was decanted into a fresh tube, an equal volume of iso-propanol was added, the precipitated nucleic acids

were then recovered by centrifugation at 12,000 rpm for 30-min. The pellet was washed once with 70% ethanol, air-dried and re-suspended in 100 µl of TE-buffer. It was treated with RNase at a concentration of 20 µg/ml by incubating at 37ºC for 1-hr. It was further extracted with an equal volume of phenol:chloroform mixture followed by chloroform:isoamyl alcohol (24:1) mixture. After centrifugation, the clear supernatant was used for recovering the nucleic acids. The nucleic acids were precipitated with 200µl of alcohol in presence of 0.3 M sodium acetate (Sambrook and Russell, 2001). In case where high purity plasmid preparations are required (DNA sequencing) the plasmid isolation was carried out with the commercially available kits following the manufacturer's instruction. Plasmids were observed on 1% agarose gel.

2.2.2.2 Isolation of chromosomal DNA

1.5 ml of stationary phase culture was centrifuged and cell pellet was re-suspended in 567 µl of TE buffer. To this 30 µl of 10% SDS, and 3 µl of proteinase K (20 mg/ml) were added in that order and the cell suspension was mixed and incubated at 37ºC for 1-hr. When the suspension was clear, 100 µl of 5 M NaCl was added and thoroughly mixed followed by the addition of 80 µl CTAB/NaCl (10% cetyl trimethyl ammonium bromide in 7 M NaCl). The suspension was incubated at 65ºC for 10-min, brought to room temperature and extracted with an equal volume (780 µl) of chloroform isoamyl alcohol (24:1), and aqueous phase transferred to fresh tube. The aqueous phase was further extracted successively, first with phenol:chloroform:isoamyl alcohol (25:24:1) and then with chloroform isoamyl alcohol (24:1). DNA was precipitated from the clear supernatant by the addition of 0.6 volumes of iso-propanol. The chromosomal DNA was either spooled out or pelleted at this stage and washed with 70% ethanol air dried and dissolved in 100 µl of TE-buffer.

2.2.2.3 Agarose gel electrophoresis

The DNA samples were mixed with appropriate volumes of 6 X loading dye (0.25% bromophenol blue and 0.25% xylene cyanol and 30% glycerol in water) and subjected to electrophoresis through 0.8 to 1 % agarose gel in TAE buffer. The gel was stained in 1 µg/ml ethidium bromide solution for 15-min at room temperature and visualised by fluorescence under UV-light in a UV-transilluminator.

2.2.2.4 Restriction enzyme digestion and analysis

Around 0.5 to 1 µg of DNA was regularly used for each restriction digestion. 2 to 5units of restriction enzyme were used in the total reaction volume of 20 µl containing 2µl of the corresponding buffer supplied at 10 X concentration by the manufacturer. The reaction was incubated for 2 hrs at the temperature recommended by the manufacturer. The DNA fragments were visualised by ethidium bromide staining after electrophoresis on a 0.8 to 1% agarose gels. Commercially available DNA size markers were run along with the digestion samples to compare with and to estimate the sizes of the restriction fragments.

2.2.2.5 Purification of DNA by gel elution

DNA fragments to be used for specific purposes like ligation or radioactive labeling were eluted from the agarose gel after electrophoresis. The gel piece containing the desired band was sliced out from the gel and the DNA was purified using commercially available purification kits for this purpose. The efficiency of elution was determined by checking a small aliquot of DNA sample on the gel.

2.2.2.6 Purification of PCR products

PCR products were purified using the PCR Purification Kit (Qiagen) as per the manufacturer's instructions.

2.2.2.7 Ligation of DNA

Typically 200-300 ng of DNA was used in each ligation reaction. The ratio of vector to insert was maintained between 1:3 to 1:5 for cohesive end ligation and 1:1 for blunt end ligation. The reaction was generally performed in 10 µl volume containing ligation buffer (provided by the manufacturer) and 0.05 Weiss unit of T4-DNA ligase, at 16ºC for 14- to 16-hrs. On using the rapid ligation kit from Fermentas, incubation was at 22ºC for 1-2 hrs.

2.2.3 Biochemical techniques

2.2.3.1 Overexpression and purification of ArgP and ArgP^{d} proteins

For preparing ArgP and ArgP^{d}-S94L, -P108S and -P274S proteins, derivatives (designated as pHYD1705, pHYD2678, pHYD2679 and pHYD2680 respectively) of the plasmid vector pET21b (Novagen) was constructed which carries the PCR-amplified

argP^{+}, *argP*^{d}-S94L, *argP*^{d}-P108S, *argP*^{d}-P274S fragment downstream of the phage T7-promoter, such that the encoded proteins bear a C-terminal His_6-tag provided by the vector DNA sequence. The resultant plasmid was transformed into strain BL21 (DE3) which has the T7 RNA Polymerase under the isopropyl thio-β-D-galactoside (IPTG) inducible *lacUV5* promoter. The resultant strains were grown in LB (500-1000 ml) to an A_{600} of around 0.6 and were then induced with 1 mM IPTG and harvested after 4-hrs of induction. Bacterial cells were recovered by centrifugation, resuspended in 20 ml of lysis buffer (20 mM Tris-Cl, pH-8; 300 mM NaCl; 10 mM DTT and 10 mM imidazole) containing 20 μg/ml lysozyme, and lysed by sonication with 30-sec pulses for 10-min. The protocol for His_6-ArgP (ArgP^{d}s) protein purification involved (i) passing the lysate through a 5ml Ni-NTA (Qiagen) chromatographic column equilibrated with lysis buffer, (ii) washing the column with 100 ml of washing buffer (20 mM Tris-Cl, pH-8; 300 mM NaCl; 10 mM DTT; 30 mM imidazole), and (iii) elution of His_6-ArgP (ArgP^{d}s) from the column with elution buffer (20 mM Tris-Cl, pH-8; 300 mM NaCl; 10 mM DTT and 250 mM imidazole) and collection of 1.5 ml eluate fractions (10 fractions). The fractions were tested for protein by Bradford method and the protein-carrying fractions (generally tubes 2 to 5) were pooled and dialysed in a 1:200 volume ratio against 20 mM Tris-Cl, pH-8 with 10 mM DTT, 300 mM NaCl for 5 hrs followed by a change to buffer of composition 20 mM Tris-Cl, pH-8 with 10 mM DTT, 300 mM NaCl and 40% glycerol for 24 hrs. The proteins were concentrated by centrifugation to around 1 mg/ml by using Amicon filter (pore size 10-KDa) and stored at −20ºC or −70ºC.

2.2.3.2 Protein estimation

Protein concentrations were estimated by the method of Bradford (1976). The A_{595} was measured after complexation with Bradford reagent. Bovine serum albumin was used as standard against which the unknown protein concentrations were estimated.

2.2.3.3 Sodium dodecyl sulphate-polyacrlyamide gel electrophoresis (SDS-PAGE)

The method followed was as described in Sambrook and Russell (2001). Gels of 1.0 mm thickness were casted in the commercially available small gel apparatus. Resolving gel of 12% (15 ml) and stacking gel (4 ml) was made. Gels were polymerised by the addition of TEMED and APS (1 % v/v of the gel mix). Sample preparation for gel loading was done as follows. Cell lysate or pure protein fractions (around 30 μg) was mixed with the sample buffer to 1 X and heated at 95ºC for 2-min. To check expression

directly from lysed cells, log and stationary phase cultures were spun down, sample buffer (1 X final concentration) was added to the cell pellet and boiled for 10 min, cooled to room temperature, and after a second spin, the clear supernatant was loaded. The gel run was started at constant current of 20 mA. When the dye front crossed the stacking gel the current was increased to 40 mA.

2.2.3.4 Gel-filtration chromatography

Gel-filtration chromatography was performed at room temperature on a BioLogic LP protein purification system (Biorad) with an in-house packed Sephadex G-100 column of size 1.5 X 43 cm; each protein sample was loaded in 0.8-ml volume, and the buffer used for chromatography was 20 mM Tris-Cl (pH 8) with 200 mM NaCl at a flow rate of 0.1 ml per min with 1.5-ml fractions being collected for analysis. Protein elution was detected by measurement of A_{295}.The void volume, V_0 was determined using blue dextran (2 X 10^6 Daltons) and the elution parameter K_{av} for each protein was calculated from elution volume V_e and total bed volume V_t using the equation:

$K_{av} = (V_e - V_0)/(V_t - V_0)$

Initially, a calibration curve was derived from a semilogarithmic plot of K_{av} of protein standards albumin (67 kDa), ovalbumin (43 kDa), chymotrypsinogen (25 kDa) and ribonuclease A (13 kDa) on the Y-axis against $\log_{10}$ of their molecular masses on the X-axis. The K_{av} of the $ArgP^d$ proteins were calculated based on their elution volume and then the molecular masses were derived from the corresponding point on the calibration curve.

2.2.3.5 Native Isoelectric Focusing

Native isoelectric focusing was done using Pharmacia Phast Gel Apparatus and precast IEF gel (pH 3-9) from GE healthcare. The samples were prepared in 50 mM sodium buffer (pH 8.0) and applied in the middle portion of the gel. Gels were run as previously described (Olsson *et al.*, 1988) that is at 15°C, pre-focusing at 2000 V (75 Vh), sample loading at 200 V (15 Vh) and run at 2000 V (500 Vh). Staining was done using Coomassie Blue G-250.

2.2.4 Molecular techniques

2.2.4.1 Polymerase chain reaction (PCR)

For amplification of short length (100-200-bp) DNA fragments or that do not

require high fidelity, Taq DNA Polymerase from MBI Fermentas was used. However, for precise amplifications either Herculase Fusion or *Pfu* DNA polymerase from Stratagene was used. Approximately, 10-20 ng of plasmid or 100 to 200 ng of chromosomal DNA was used as a template in a 50 µl reaction volume containing 200 µM of each dNTP, 20 picomole each of forward and reverse primer and 1.5 units of DNA polymerase.

In the case of colony PCR performed to examine multiple colonies for presence of the plasmid clones, *E. coli* cells from a freshly grown plate were resuspended in 50 µl of sterile Milli-Q water to get a cell suspension (~10^9cells/ml) and 4 µl from this was used as the source of DNA template. To verify various pMU575 clones described in this study, by colony PCR, the vector specific primer pairs JGJpMUF and JGJgalK were used. The expected amplicon for pMU575 alone is ~300-bp, while that carrying the cloned fragment would be >300-bp.

For each PCR reaction, the samples were subjected to 30-cycles of amplification and the typical conditions were as follows (although there were slight alterations from one set of template/primer to another):

The initial denaturation was carried out at 95°C for 4-min and the cycle conditions were as given below:

Annealing 45ºC to 50°C 1-min

Extension 68°C (1-min/kb of DNA template to be amplified)

Denaturation 95°C 1-min

After 30 cycles of PCR, the final extension step was carried out again for 10-min at 68°C.

2.2.4.2 Isolation of total cellular RNA

For isolation of RNA, cells were grown in minimal A medium supplemented with 0.2% glucose upto A_{600} of 0.6. Cells were harvested by centrifugation and total RNA was isolated by using Trizol (Invitrogen) according to manufacturer's instructions. 1 ml of Trizol was used to lyse cells equivalent of approximately 4 ml of overnight culture. Homogeneous lysis was achieved by gentle pipetting repeatedly. The homogenized samples were incubated at room temperature for 5-min to permit complete dissociation of nucleoprotein particles. Following homogenization, 0.2 ml of chloroform for each 1ml Trizol reagent was added and vigorously shaken with hand for 15-sec and incubated further for 3-min at RT. It was then centrifuged at 12000 rpm for 10-min at 4ºC, which separates out the homogenate into lower phenol chloroform phase (red), an interphase

and a colourless upper aqueous phase. The upper aqueous phase in which RNA exists exclusively, was transferred to a fresh microfuge tube and RNA was precipitated by adding 0.5 ml of isopropyl alcohol for each ml of Trizol used. Samples were incubated at 15 to 30ºC for 10-min and centrifuged at 12000 rpm for 10-min at 4ºC. RNA formed a gel like precipitate at the bottom of the tube. Supernatant was removed and RNA was washed with 75% ethanol (by adding 1 ml of ethanol per ml of Trizol employed). RNA could be stored after this step in –20 or –70ºC for more than a year. RNA pellet was air dried for 15- to 30-min following which it was dissolved in nuclease free water. The concentrations and purity of RNA samples were determined spectroscopically as well as by visual inspection on formaldehyde-agarose gel in MOPS buffer (Good *et al*., 1996). Before loading onto the gel, RNA was mixed with loading buffer and heated at 90ºC for 3-min.

2.2.4.3 DNA sequencing

Automated DNA sequencing on plasmid templates or on PCR products was carried out with dye terminator cycle sequencing kits from Perkin-Elmer on an automated sequencer (model 377, Applied Biosystems), following the manufacturer's instructions. Manual sequencing was achieved using the Sequenase Version 2.0 DNA Sequencing Kit from USB Corp. as described in manufacturer's instructions and the sequencing reaction products were resolved by electrophoresis on a 6% sequencing gel.

2.2.4.4 Site directed mutagenesis

Site directed mutagenesis of plasmid DNA was carried out by using QuikChange kit (Stratagene) with a pair of complementary oligonucleotide primers carrying the necessary sequence modifications. In this process, the plasmid (around 20-100 ng) containing the fragment of DNA where nucleotide has to be altered, was used as template and "linear PCR" of 20 cycles was set up using *Pfu Turbo* DNA polymerase to amplify the whole plasmid with extension time calculated according to a rate of 500-bp/min. The reaction mix was digested with *Dpn*I for 1-hr (to destroy the original input plasmid DNA) following which it was transformed directly to a highly competent DH5α cells. The mutated plasmid was confirmed by sequencing.

2.2.4.5 Radiolabelling of oligonucleotides and PCR products

Oligonucleotides and PCR products were end labeled using phage T4-polynucleotidekinase (PNK, New England Biolabs) with ^{32}P-γ-ATP. The radiolabelling reaction mixture (50 µl) contained 1 X of buffer provided by the company, 10 units of T4-PNK and 50 µCi of ^{32}P-γ-ATP. The reaction mix was incubated for 1-hr at 37ºC and the reaction was stopped by adding 10 µl of 0.5 M EDTA. The labeled oligonucleotides and DNA fragments were purified either by the Qiagen PCR purification or nucleotide removal kit. Labelling efficiency was checked by scintillation counting.

2.2.4.6 Primer extension analysis

Primer extension analysis to map the transcription start site was carried out as described by Conway *et al*. (1987) and Rajkumari *et al*. (1997). 20 pmol of primer was labelled at its 5′-end with ^{32}P-γ-ATP as described above. 10^6 cpm equivalent of labelled primer was mixed with 10 µg of total cellular RNA. Sodium acetate pH-5.5 was added to a final concentration of 0.3 M and the nucleic acids were precipitated with ethanol, washed with 70% alcohol, air-dried and dissolved in hybridization buffer (9 mM Tris-Cl, pH-8 and 0.35 mM EDTA) and incubated overnight at 43ºC for annealing. Reverse transcriptase reaction was performed by the addition of 5 mM $MgCl_2$, 1 mM dNTP's, 1 X RT buffer, high concentration (10 units) of Superscript III Reverse Transcriptase (Invitrogen) to the mixture of annealed labelled primer and RNA. The reaction was incubated at 43ºC for 1-hr following which the nucleic acids were precipitated with absolute alcohol and 0.3 M CH_3COONa, pH-5.5. The precipitate was air dried and dissolved in water and gel-loading dye (95% formamide, 20 mM EDTA, 0.05% each of xylene cyanol and bromophenol blue) was added. The samples were heated at 90ºC for 2-min before loading on a 6% denaturing polyacrylamide gel for electrophoretic resolution alongside a sequencing ladder.

2.2.4.7 Electrophoretic mobility shift assay (EMSA)

The DNA templates were obtained by PCR from *E. coli* genomic DNA. After 5′-end labeling, the PCR fragments were purified by electroelution following electrophoresis on 6% native polyacrylamide gels (Sambrook and Russell, 2001).

EMSA reactions were performed in 20 µl reaction volume in EMSA binding buffer (10 mM Tris-Cl at pH 7.5, 1 mM EDTA, 50 mM NaCl, 5 mM dithiothreitol, and

5% glycerol) containing (i) 5′-end-labeled DNA fragment of 1200 cpm radioactive count (ii) 1 μg each of bovine serum albumin and poly(dIdC) (iii) the protein at the indicated monomer concentrations and (iv) when required, co-effectors at specified concentrations. The reaction mixture was incubated at room temperature for 30-mins and the complexes were resolved by electrophoresis on a non-denaturing 5% polyacrylamide gel (39:1 acrylamide:bisacrylamide) in 0.5X TBE buffer pH 8.3, at 12.5 V/cm for 3 hrs at 18°C. The gels were then dried on a gel drier at 80°C for 45 mins and the radioactive bands were visualised with a Fujifilm FLA-9000 scanner.

For DNA bending EMSA, co-effectors were not added in the binding reaction but at a concentration of 0.1 mM in both the gel and running buffer.

2.2.4.8 Densitometry

Band intensities in gel autoradiograms were determined by densitometry with the aid of the Fujifilm Multi Gauge V3.0 imaging system. Equal areas of radioactive bands (preferably the unbound probe) were boxed and the PSL (Photo stimulated luminescence) values were further considered. For K_d (dissociation constant) calculations, the values thus obtained for each lane were expressed as a percentage with respect to the PSL for the lane without any protein taken as 100%.