2.1 Section A: Materials

2.1.1 Strains and bacteriophages

The *E. coli* strains used in this study with their genotypes are shown in the table below. Bacterial strains were routinely stored on solid agar plates at 4ºC and also as thick suspensions in 20% glycerol at –80ºC. Plasmid harbouring strains were freshly prepared by transformation of the required plasmid.

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

Bacterial Strains

Table 2.1: *E. coli* strains used and constructed in this study:

Strain	**Genotype**[a]
MG1655[b]	*F⁻ λ⁻ ilvG⁻ rfb-50 rph-1* (Wild-type *E. coli* K-12)
MC4100[b]	Δ*(argF-lac)U169 rpsL150 relA1 araD139 flb5301 deoC1 ptsF25*
AG1	*recA1 endA1 gyrA96 thi-1 hsdR17* ($r_K^- m_K^+$) *supE44 relA1*
BL21	*F⁻ ompT gal dcm lon hsdS*$_B$($r_B^- m_B^-$) [*malB*$^+$]$_{K\text{-}12}$(λ^S) (*E. coli* B strain)
BL21(DE3)	BL21 *gal* λ(DE3[*lacI lacUV5*-T7 RNAP*Ind1 Sam7 nin5*])
BW25113	Δ*(araB–D)567* Δ*(rhaD–B)568* Δ*lacZ4787*(::*rrnB-3*) *hsdR514 rph-1*
DH5α[b]	Δ*(argF-lac)U169 supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1 (Φ80 lacZ*Δ*M15)*
K37	*Hfr (PO2A) galK garB10 fhuA22 phoA4(Am) ompF627(T2R) serU132(AS) fadL701(T2R) relA1 pitA10 spoT1 rrnB-2 mcrB1 creC510* (Withey & Friedman, 1999)
W3110	F⁻ λ⁻ *rph-1* IN(*rrnD, rrnE*)1
CF1648	Same as MG1655 (strain collection, Cashel Laboratory)
CF1693	CF1648 *relA251*::Kan *spoT207*::Cm (strain collection, Cashel Lab)
CF10237	CF1648 Δ*relA* (*relA256*) Δ*spoT* (*spoT212*) (strain collection, Cashel Lab)
CF13249	CF1648 *ssrA*::Cm (strain collection, Cashel Laboratory)
CF15348	BW25113 Δs*mpB*::Kan (same as JW2601) (strain collection, Cashel Lab)
CF15394	CF10237 *ssrA*::Kan/pHR14 (strain collection, Cashel Lab)

ENS133	BL21(DE3) (*lacZ*::Tn*l0 malP*pΔA534::P_{lac}*lacZ-Arg5*) (Lopez *et al.*, 1994)
JW0093	AG1/pCA24NftsZ (ASKA collection)
JW0167	AG1/pCA24Nfrr (ASKA collection)
JW0864	AG1/pCA24NcspD (ASKA collection)
JW0974	AG1/pCA24NcspG (ASKA collection)
JW1549	AG1/pCA24NcspB (ASKA collection)
JW3525	AG1/pCA24NcspA (ASKA collection)
JW0141	BW25113 Δ*dksA*::Kan (Keio collection)
JW0187	BW25113 Δ*arfB*::Kan (Keio collection)
JW0941	BW25113 Δ*sulA*::Kan (Keio collection)
JW0429	BW25113 Δ*lon*::Kan (Keio collection)
JW2205	BW25113 Δ*rcsB*::Kan (Keio collection)
JW2755	BW25113 Δ*relA*::Kan (Keio collection)
JW3253	BW25113 Δ*arfA*::Kan (Keio collection)
JW3389	BW25113 Δ*glpD*::Kan (Keio collection)
JW5471	BW25113 Δ*rnr*::Kan (Keio collection)
GJ1158	BL21 Δ*mal*Ap510 Δ*malP*::(*proU*p-T7 RNAP) *malQ*::*lacZ*hyb11 Δ(*zhf*-900::Tn*10d*Tet) (strain collection, Gowrishankar Lab)
GJ5030	MG1655 *nadA*3052::Tn*10d*Kan *galEp3* (strain collection, Gowrishankar Lab)
GJ6504	MG1655 *lacI* $lacZ_{U118}$ *trpR55 trpE9777* (strain collection, Gowrishankar Lab)
GJ6509	GJ6504 *rho4* (strain collection, Gowrishankar Lab)
GJ6511	GJ6504 *nusG*-G146D (strain collection, Gowrishankar Lab)
GJ6804	DH5α *srl300*::Tn*10 rne* ΔCTH::Kan (strain collection, Gowrishankar Lab)
GJ7026	MC4100 *ilv*::Tn*10* (strain collection, Gowrishankar Lab)
HR202	MG1655 Δ*relA* Δ*spoT* (Lab collection)
HR597	Same as GC2553 (Vinella & D'Ari, 1994)
HR1041	HR202 Δ*lacZYAI*::FRT *ssrA*::Cm/pRC_{Sp}-spoT/pTrc-greA (Lab collection)
HR1042	HR202 Δ*lacZYAI*::FRT *ssrA*::Cm/pRC_{Sp}-spoT/pTrc-greB (Lab collection)
HR1164	HR1179 Δ*relBE*, Δ(*dinJ-yafQ*), Δ(*yefM-yoeB*), Δ*mazF*, Δ*chpB* Δ*higBA*, Δ(*prl-FyhaV*), Δ*yafNO*, Δ*mqsRA*, Δ*hicAB* (Lab collection)
HR1179	MG1655 Δ*lacZYAI*::FRT Δ*spoT* Δ*relA*::Kan *ssrA*::Cm/pRCspoT (Lab

	collection)
HR2001	HR202 Δ*lacZYAI*::FRT *rnc40*::Tn*10*/pRCspoT (Lab collection)
HR2004	HR2001 *ssrA*::Kan (Lab collection)
HR2005	HR2001 Δ*smpB*::Kan (Lab collection)
HT120	W3110 *rnc40*::Tn*10* (Takiff *et al.*, 1989)
K8619	K37 *ssrA*::Cm (Withey & Friedman, 1999)
K8637	K8619 $ssrA^{O}$ Amp^{r} (At λ*att*) (Withey & Friedman, 1999)
K8664	K8619 $ssrA^{+}$ Amp^{r} (At λ*att*) (Withey & Friedman, 1999)
K8666	K8619 $ssrA^{DD}$ Amp^{r} (At λ*att*) (Withey & Friedman, 1999)
K8668	K8619 Amp^{r} (At λ*att*) (Withey & Friedman, 1999)
MGJ5987	MG1655 Δ*mazF* Δ*chpB* Δ*relBE* Δ*(dinJ-yafQ)* Δ*(yefM-yoeB)* Δ*higBA* Δ*(prlF-yhaV)* Δ*yafNO* Δ*mqsRA* Δ*hicAB* (Maisonneuve *et al.*, 2011)
SC301467	MG1655 Δ*mazF* Δ*chpB* Δ*relBE* Δ*(dinJ-yafQ)* Δ*(yefM-yoeB)* (Christensen *et al.*, 2004)
TA370	W3110 $ssrA^{DD}$::Kan (Abo *et al.*, 2002)
Strains constructed in this study	
AN01	MG1655 *ssrA*::Cm
AN02	MG1655 Δ*relA*::FRT *ssrA*::Cm
AN03	MG1655 Δ*smpB*::Kan
AN16	MG1655 Δ*dksA*::FRT
AN20	HR202 *ssrA*::Cm
AN27	MG1655 *rpoB* L571P *btuB*::Tn*10*
AN28	MG1655 *rpoB* T563P *btuB*::Tn*10*
AN29	MG1655 *rpoB* A532Δ *btuB*::Tn*10*
AN30	HR202 *rpoB* A532Δ *btuB*::Tn*10*
AN31	HR202 *rpoB* T563P *btuB*::Tn*10*
AN32	MG1655 *btuB*::Tn*10*
AN33	HR202 *btuB*::Tn*10*
AN34	HR202 *rpoB* L533P *btuB*::Tn*10*

AN35	HR202 *rpoB* L571P *btuB*::Tn*10*
AN36	MG1655 *rpoB* L533P *btuB*::Tn*10*
AN37	HR202 *rpoB8 btuB*::Tn*10*
AN56	MG1655 Δ*relA*::FRT
AN76	DH5α/pAMssrA
AN78	AN56/pTrc99A
AN96	HR202/pAMssrA
AN97	AN96 *ssrA*::Cm
AN98	AN16 Δ*lacZYAI*::Kan/pRCdksA
AN101	MG1655 Δ*lacZYAI*::Kan
AN104	HR202/pRCspoT
AN105	AN101/pRCspoT
AN113	MG1655 *ssrA*::Cm/pAMssrA
AN119	AN104 Δ*lacZYAI*::Kan
AN120	AN104 Δ*lacZYAI*::FRT
AN121	AN105 *ssrA*::Cm
AN122	AN119 *ssrA*::Cm
AN123	AN98 *ssrA*::Cm
AN125	AN121 *rpoB8 btuB*::Tn*10*
AN126	AN121 *rpoB* L571P *btuB*::Tn*10*
AN127	AN121 *rpoB* T563P *btuB*::Tn*10*
AN128	AN122 *rpoB8 btuB*::Tn*10*
AN129	AN122 *rpoB* L571P *btuB*::Tn*10*
AN130	AN122 *rpoB* T563P *btuB*::Tn*10*
AN131	MG1655 Δ*lacZYAI*::FRT/pRCspoT
AN132	AN120 Δ*smpB*::Kan
AN133	AN131 Δ*smpB*::Kan
AN134	AN120 *rne*ΔCTH::Kan
AN135	AN131 *rne*ΔCTH::Kan
AN138	AN131 *ssrA*::Cm

AN139	AN120 *ssrA*::Cm
AN140	AN138 *rne*ΔCTH::Kan
AN141	AN139 *rne*ΔCTH::Kan
AN144	AN138 *btuB*::Tn*10*
AN145	AN139 *btuB*::Tn*10*
AN146	AN131 *rpoB2 btuB*::Tn*10*
AN147	AN120 *rpoB2 btuB*::Tn*10*
AN158	AN133 *btuB*::Tn*10*
AN159	AN132 *btuB*::Tn*10*
AN160	AN158 *rpoB2*
AN161	AN158 *rpoB8*
AN162	AN158 *rpoB* T563P
AN163	AN158 *rpoB* L571P
AN164	AN159 *rpoB2*
AN165	AN159 *rpoB8*
AN166	AN159 *rpoB* T563P
AN167	AN159 *rpoB* L571P
AN178	MG1655 *rpoB8 btuB::*Tn*10*
AN180	DH5α/pRC7$_{Sp}$
AN181	DH5α/pRC$_{Sp}$-spoT
AN182	DH5α/pRC$_{Sp}$-dksA
AN188	MG1655 Δ*lac*::FRT Δ*relA*::Kan/pRCspoT
AN196	AN16 Δ*lac*::FRT/pRCdksA
AN198	AN131 Δ*clpX*::Kan
AN199	AN131 Δ*clpA*::Kan
AN200	AN131 Δ*clpP*::Kan
AN201	AN120 Δ*clpX*::Kan
AN202	AN120 Δ*clpA*::Kan
AN203	AN120 Δ*clpP*::Kan
AN205	AN196 Δ*smpB*::Kan

AN207	AN131 Δ*lon*::Kan
AN222	AN131 *rpoB*35 btuB*:: Tn*10*
AN223	AN120 *rpoB*35 btuB*:: Tn*10*
AN241	AN222 *ssrA*::Cm
AN242	AN223 Δ*ssrA*::Cm
AN244	MG1655 Δ*lac*::FRT/pRC$_{Sp}$-spoT
AN245	MG1655 Δ*lac*::FRT/pRC7$_{Sp}$
AN246	HR202 Δ*lac*::FRT/pRC$_{Sp}$-spoT
AN247	HR202 Δ*lac*::FRT/pRC7$_{Sp}$
AN248	AN244 *ssrA*::Cm
AN250	AN246 *ssrA*::Cm
AN251	AN244 Δ*smpB*::Kan
AN253	AN246 Δ*smpB*::Kan
AN256	AN131 Δ*relA::*FRT
AN257	AN248/pJK537 (pdksA)
AN258	AN248/pHR53 (pdksA′)
AN259	AN251/pJK537 (pdksA)
AN260	AN251/pHR53 (pdksA′)
AN261	AN250/pJK537 (pdksA)
AN262	AN250/pHR53 (pdksA′)
AN263	AN253/pJK537 (pdksA)
AN264	AN253/pHR53 (pdksA′)
AN289	AN120 *ssrA*::Kan
AN295	AN139 Δ*argA*::Tn*10*
AN306	AN138 Δ*spoT* Δ*relA*::kan Δ*argA*::Tn*10*
AN308	AN120 *btuB*::Tn*10*
AN309	AN308 *rpoB8*
AN310	AN308 *rpoB* T563P
AN311	AN308 *rpoB* L571P
AN313	AN250/p-ssrA^{+}

AN314	AN250/p-ssrA^{0}
AN315	AN250/p-ssrA^{DD}
AN316	AN250/p-ssrA^{UG}
AN322	AN120 Δ*relBE*::Kan
AN336	AN250/pHM1684 (pdksA^{NN})
AN341	AN138 Δ*relA*::Kan
AN342	AN120 Δ*argA*::Tn*10*
AN347	AN250 Amp^{r} (At λ*att*)
AN348	AN250 *ssrA*$^{+}$ Amp^{r} (At λ*att*)
AN349	AN250 *ssrA*^{O} Amp^{r} (At λ*att*)
AN350	AN250 *ssrA*^{DD} Amp^{r} (At λ*att*)
AN351	AN342 Δ*relA*::Kan
AN352	AN223 Δ*lon*::Kan
AN353	AN311 Δ*lon*::Kan
AN356	AN131 *btuB*::Tn*10*
AN403	AN250 Δ*greA*::Kan
AN404	AN250 Δ*greB*::Kan
AN412	AN246/p-ssrA
AN413	AN246/p-ssrA^{0}
AN414	AN246/p-ssrA^{DD}
AN415	AN246/p-ssrA^{UG}
AN417	AN138 *rpoB2 btuB*::Tn*10*
AN418	AN139 *rpoB2 btuB*::Tn*10*
AN422	MG1655Δ*lacZYAI*::FRT/ pPW500
AN423	HR202 Δ*lacZYAI*::FRT/ pPW500
AN424	AN422 *ssrA*::Cm
AN427	MG1655 Δ*lacZYAI*::FRT/pTrc99A
AN428	AN427 *ssrA*::Cm
AN429	HR202 Δ*lacZYAI*::FRT/pTrc99A
AN430	AN120 Δ*rnr*::Kan

AN431	AN139 Δ*rnr*::Kan
AN435	DH5α/p30
AN443	AN250/pRS414
AN452	MG1655 Δ*lacZYAI*::FRT/pPW510F
AN454	AN452 *ssrA*::Cm
AN456	HR202 Δ*lacZYAI*::FRT/pPW510F
AN457	AN131 *rpoB369 btuB*::Tn*10*
AN459	AN120 *rpoB369 btuB*::Tn*10*
AN460	AN139 *rpoB369 btuB*::Tn*10*
AN461	AN138 *rpoB369 btuB*::Tn*10*
AN465	MG1655 Δ*lacZYAI*::FRT Δ*relBE*::FRT Δ*dinJyafQ*::FRT Δ*yefMyoeB*::FRT Δ*mazF*::Cm Δ*chpB*::FRT (Δ5TA) Δ*relA*::Kan
AN471	MG1655 Δ*lacZYAI*::FRT Δ*relA*::FRT/pPW500
AN472	MG1655 Δ*lacZYAI*::FRT Δ*relA*::FRT/pPW510F
AN490	AN465(Δ*mazF*::FRT) Δ*relA*::FRT
AN491	AN490/pRCspoT
AN495	AN491 Δ*spoT*::Cm
AN496	AN495 *ssrA*::Kan
AN512	AN246 Δ*lon*::Kan/pBAD24
AN513	AN246 Δ*lon*::Kan/pBADlon
AN514	AN246 Δ*lon*::Kan/pBADlonS679A
AN515	AN246 Δ*lon*::Kan/pBADlonK362Q
AN516	AN246 Δ*lon*::Kan/pBADlonK362Q-S679A
AN518	AN246 Δ*lon*::Kan
AN519	AN120 Δ*lon*::Kan
AN523	AN120 *ssrA*-his$_6$::Kan
AN524	AN120 *nadA3052*Tn*10*::Kan *galEP3*
AN525	MG1655 Δ*arfA*::FRT
AN526	AN525/pAM34
AN527	AN525/pAMssrA

AN528	MG1655 Δ*arfA*::Kan *ssrA*::Cm/pAMssrA
AN529	AN524 *rho4 ilvD*::Tn*10*
AN532	AN525/pSTV28
AN533	AN525/pSTVyhdL
AN534	AN525/pSTVyaeJ
AN551	AN289/pSTV28
AN552	AN289/pSTVyhdL
AN553	AN289/pSTVyaeJ
AN555	MC4100 Δ*spoT*::Cm/pRCspoT
AN556	AN555 Δ*relA*::FRT
AN561	AN529/pCL1920
AN562	AN529/pHYD567
AN563	AN120 Δ*sulA*::Kan
AN564	AN120 Δ*rcsB*::Kan
AN565	AN120 Δ*sulA*::FRT
AN566	AN120 Δ*rcsB*::FRT
AN567	AN565 Δ*lon*::Kan
AN568	AN566 Δ*lon*::Kan
AN570	AN120 *galEP3*Δ*argE*::Kan
AN572	AN120 *galEP3*
AN573	AN572 *rho4*
AN574	AN572 *ssrA*::Cm
AN575	AN573 *ssrA*::Cm
AN577	MC4100 *ssrA*::Kan/pRCspoT
AN578	MC4100/pRCspoT
AN579	AN555 *ssrA*::Kan
AN580	AN556 *ssrA*::Kan
AN585	AN572 *nusG*-G146D
AN591	AN585 *ssrA*::Cm
AN597	AN289/p30

AN609	AN132 *galEP3*
AN610	AN609 Δ*ilvD*::Tn*10*
AN611	AN609 Δ*argE*::Tn*10*
AN614	HR202 *rho4*
AN615	AN609 *rho4*
AN616	AN609 *nusG*-G146D
AN620	AN131 *relA* D275G Δ*spoT*::Cm
AN621	AN620 *ssrA*::Kan
AN626	AN250/pTrc99A
AN627	AN250/pBA169
AN630	AN139 Δ*ppx*::Kan
AN631	AN139 Δ*ppk*::Kan
AN632	AN248/pTrc99A
AN633	AN246/pTrc99A
AN634	AN246/pBA169
AN637	AN244/pTrc99A
AN638	AN244/pBA169
AN639	AN248/pBA169
AN647	AN120 *lexA3 malB*::Tn*9*
AN648	AN647 Δ*lon*::Kan
AN658	MG1655 Δ*lacZYAI*::FRT *sulA-lacZ*
AN660	HR202 Δ*lacZYAI*::FRT *sulA-lacZ*
AN677	AN519/pCL1920
AN678	AN519/pMN8 (pCLftsQAZ)
AN679	AN519/pMN15 (pCLsdiA)
AN680	AN519/pTB63 (pSCftsQAZ)
AN682	AN246 $ssrA^{DD}$::Kan
AN685	AN256 Δ*smpB*::Kan
AN686	MG1655 *lacZ*::Tn*10*
AN688	AN518/pHR53 (pdksA′)

AN689	AN518/pJK537 (pdksA)
AN690	AN518/pHM1684 (pdksA^{NN})
AN692	HR202 *lacZ*::Tn*10*/pRCspoT
AN696	AN250/pBAD24
AN697	AN250/pBADarfA
AN698	AN250/pBADarfB
AN699	AN686 *lacZ-Trna*(U73)^{Arg5}
AN701	HR202 *lacZ*::Tn*10 lacZ-Trna*(U73)^{Arg5}
AN705	AN701/pRCspoT
AN706	AN289/pCA24NcspA
AN707	AN289/pCA24NcspB
AN708	AN289/pCA24NcspG
AN709	AN289/pCA24NcspD
AN730	AN289/pRARE
AN731	AN120 Δ*arfA*::FRT
AN737	GJ1158 Δ*relA*::FRT
AN743	AN660 Δ*lon*::FRT
AN745	AN699/pACYC184
AN746	AN699/pRARE
AN747	AN701/pACYC184
AN748	AN701/pRARE
AN750	AN739 Δ*recA*::Kan
AN762	AN289/pRARE-ΔAP
AN763	AN289/pRARE-ΔTP
AN764	AN289/pRARE-ΔMP
AN766	AN289/pACYC184
AN767	AN289/pCA24Nfrr
AN768	AN737 *lon*$^{+}$
AN771	AN768 Δ*lacZYAI*::FRT
AN773	AN771/pRCspoT

AN774	AN658 Δ*lon*::FRT
AN775	AN773 Δ*spoT*::Cm
AN777	AN775 *ssrA*::Kan
AN792	AN519/pCA24N
AN793	AN519/pCA24NftsZ
AN801	MG1655 Δ*lacZYAI*::FRT *malB*::Tn*9*
AN802	AN801 *lexA3*
AN803	MG1655 Δ*lacZYAI*::FRT Δ*sulA*::Kan
AN804	MG1655 Δ*lacZYAI*::FRT *leu*::Tet
AN805	AN804 *ftsZ84*
AN806	AN804 Δ*sulA*::Kan
AN807	AN805 Δ*sulA*::Kan
AN808	AN120 *leu*::Tet
AN809	AN808 *ftsZ84*
AN810	AN120/pCA24N
AN811	MG1655 Δ*lacZYAI*::FRT/pCA24N
AN812	AN120/pCA24NftsZ
AN813	HR202 Δ*lacZYAI*::FRT/pCA24N
AN814	AN120/pCL1920
AN815	AN120/pMN8 (pCLftsQAZ)
AN816	HR202 Δ*lacZYAI*::FRT/pJK537 (pdksA)
AN817	HR202 Δ*lacZYAI*::FRT/pHM1684 (pdksA^{NN})
AN818	HR202 Δ*lacZYAI*::FRT/pHR53 (pdksA′)
AN819	HR202 Δ*lacZYAI*::FRT/pBADlon
AN820	HR202 Δ*lacZYAI*::FRT/pBAD24
AN821	MG1655 *lacZ*::Tn*10* P_{lac}*lacZ′-tRNA*(U73)^{Arg5}
AN825	AN196 Δ*lon*::Kan
AN826	AN308 Δ*lon*::Kan
AN835	MG1655 Δ*lacZYAI*::FRT attλ::(P_{208}-*ftsZ-gfp*, Amp^r)
AN837	AN658 *srl300*::Tn*10*

AN838	AN837 *recA56*
AN839	AN658 *lexA3* (Ind$^-$) *malB*::Tn*9*
AN840	AN518 attλ::(P_{208}-*ftsZ-gfp*, Amp^r)
AN841	AN701/pJK537 (pdksA)
AN842	AN701/pHM1684 (pdksA^{NN})
AN843	AN701/pHR53 (pdksA′)
AN844	AN256 Δ*lon*::Kan
AN845	AN701 *btuB*::Tn*10*/pRCspoT
AN846	AN845 *rpoB* L571P
AN847	AN845 *rpoB8*
AN848	AN518/pACYC184
AN849	AN518/pRARE
AN855	HR202 *lacZ*::Tn*10* P_{lac}*lacZ'-tRNA*(U73)^{Arg5}
AN856	AN855 *btuB*::Tn*10*
AN857	AN856 *rpoB* L571P
AN858	AN855/pJK537 (pdksA)
AN859	AN855/pHR53 (pdksA′)
AN860	AN139 Δ*rnb*::Kan
AN861	AN289/pCA24N

a- Genotype designations are as in (Berlyn, 1998).

b- Strains DH5α, MC4100 and MG1655 were from the laboratory stock collection.

FRT- indicates FLP recognition target, a sequence that is recognized by FLP recombinase and is left as a scar at the indicated sites after excision of an antibiotic marker that is flanked by the FRT sites.

2.1.2 Plasmids

Table 2.2 List of plasmids used in this study

Plasmid	**Specifications**	**Source or reference**
pACYC184	Medium copy-number cloning vector (~20 copies/cell) with Cm^r and Tet^r selectable markers. It carries the origin of replication from plasmid p15A	(Chang & Cohen, 1978)

pAM34	pBR322-derived cloning vector with Amp^r and Sp^r selectable markers. The replication of this plasmid is dependent on the presence of IPTG, the gratuitous inducer of the *lac* operon	(Gil & Bouche, 1991)
pBAD24	An expression vector with a pBR322 derived origin of replication and allows tightly regulated expression of the genes cloned under the P_{BAD} promoter of the *araBAD* operon. The vector also carries the *araC* gene, encoding the positive and negative regulator of this promoter.	(Guzman *et al.*, 1995)
pCL1920	A pSC101-based, low copy number vector with spectinomycin (Sp) and streptomycin (Sr) resistance marker carrying the MCS in *lacZα* region and hence provides the advantage of screening the insertions using α-complementation	(Lerner & Inouye, 1990)
pCP20	A pSC101-based Ts replicon, Cm^r Amp^r used for *in vivo* expression of Flp recombinase	(Cherepanov & Wackernagel, 1995)
pCA24N	Cloning vector used in ASKA library; Cm^r.	(Kitagawa *et al.*, 2005)
pCA24NcspA	plasmid from ASKA plasmid collection which expresses *cspA* gene	(Kitagawa *et al.*, 2005)
pCA24NcspB	Plasmid from ASKA plasmid collection which expresses *cspB* gene	(Kitagawa *et al.*, 2005)
pCA24NcspD	Plasmid from ASKA plasmid collection which expresses *cspD* gene	(Kitagawa *et al.*, 2005)
pCA24NcspG	Plasmid from ASKA plasmid collection which expresses *cspG* gene	(Kitagawa *et al.*, 2005)
pCR2.1-10Sa	A TA-vector pCR2.1 (Invitrogen) in which a 457-bp DNA fragment that contains the *ssrA* gene (coordinates from nucleotide -7 to nucleotide 450) from *E. coli* chromosomal DNA has been cloned	(Lin-Chao *et al.*, 1999)
pHM1684	pBR322-dkaA^{NN} (pdksA^{NN}), aspartic acid residues at 71 and 74 positions replaced with asparagine; Amp^r	Cashel Lab

pHR53	Also called as pdksA′ in this study, contains DksA deleted after 36th amino acid, Amp^{r}; a derivative of pJK537 and contains only a1.2-kb PvuII fragment of *dksA* fragment	(Nazir & Harinarayanan, 2015)
pHYD567	pCL1920 carrying 3.3 *Nsi*I fragment with *rho*$^{+}$ gene cloned at *Pst*I site	(Harinarayanan & Gowrishankar, 2003)
pJK537	Also called pdksA in the present study, contains a 1.7-kb fragment of *dksA* inserted into pBR322	(Kang & Craig, 1990)
pJW28	pRS415 carrying *ssrA*$^{+}$ (called as p-ssrA^{+} in the present study)	Withey & Friedman, 1999
pJW29	pRS415 carrying *ssrA*^{0} (called as p-ssrA^{0} in the present study)	Obtained from S. Gottesman lab
pJW30	pRS415 carrying *ssrA*^{DD} (called as p-ssrA^{DD} in the present study)	Obtained from S. Gottesman lab
pJW34	pRS415 carrying *ssrA*^{UG} (called as p-ssrA^{UG} in the present study)	Withey & Friedman, 1999
plon	(Same as pBADlon), a pBAD24 plasmid expressing *lon*	(Van Melderen & Gottesman, 1999)
plonS679A	pBAD24 expressing *lon* carrying S679A mutation in proteolytic active site	(Van Melderen & Gottesman, 1999)
plonK362Q	pBAD24 expressing *lon* carrying K362A mutation in ATPase domain	(Van Melderen & Gottesman, 1999)
plonS679A-K362Q	pBAD24 expressing *lon* carrying both S679A K362A mutations	(Van Melderen & Gottesman, 1999)
pMN8	pCL1920 carrying *ftsQAZ* (also called as pCLftsQAZ in the present study)	(Reddy, 2007)
pMN15	pCL1920 carrying *sdiA* (also called as pCLsdiA in the present study)	(Reddy, 2007)
pPW500	A pBR322-derivative containing N-terminal domain of the λ-repressor gene lacking in-frame stop-codon under the control of the P_{trc} promoter	(Keiler *et al.*, 1996)
pPW510F	A derivative of pPW500 that contains a recombinant P_{trc}-*ssrA* DNA fragment expressing *ssrA* RNA cloned upstream of the N-terminal domain of the λ-repressor lacking terminal codons	(Lin-Chao *et al.*, 1999)

pRARE	A commercially available from Novagen, harbours p15A ori and genes for rare tRNAs *proL* (reads CCC andCCU), *leuW* (reads UUG), *metT* (reads AUG), *argW* (reads AGG), *thrT*(reads ACC and ACU), *glyT* (reads GGA and GGG), *tyrU* (reads UAC and UAU), *thru* (reads ACA, ACU, and ACG), *argU* (reads AGA), and *ileX* (reads AUA)	Novagen
pRC7	A low copy-number, mini-F derivative of pFZY1; the multiple cloning sites (MCS) in the *lac* promoter of pRC7 contain restriction sites for *Eco*RI, *Bam*HI, *Sal*I and *Hin*dIII	(Bernhardt & de Boer, 2004)
pRCspoT	pRC7 which contains a minimal *spoT* ORF PCR amplified from the MG1655 and ligated in its *Eco*RI and *Hin*dIII sites, the expression of which is under *lac* promoter, therefore IPTG-inducible	(Nazir & Harinarayanan, 2015)
pRCdksA	pRC7 carrying *dksA* gene in *Eco*RI and *Hin*dIII sites expressed from *lac* promoter	(Nazir & Harinarayanan, 2015)
pRS414	A protein fusion vector with *lacZ* starting from 9th codon, rest of the features similar to pRS415	(Simons *et al.*, 1987)
pRS415	A derivative of pBR322 contains entire *lacZYA* to which trancriptional fusions can be made, Amp^{r}	(Simons *et al.*, 1987)
pSTV28	A p15A derived cloning vector	Chadani *et al.*, 2010
pSTVarfA	a derivative of pSTV28 expressing ArfA (YhdL)	Chadani *et al.*, 2010
pSTVarfB	A derivative of pSTV28 expressing ArfB (YaeJ)	Chadani *et al.*, 2011b
pTB63	pSC101-based carrying *ftsQAZ* (also called as pSCftsQAZ in the present study), Tet^{r}	(Bernhardt & de Boer, 2005)
pTrc99A	An expression vector with ColE1 origin of replication and Ampicillin resistance marker. It provides IPTG-dependent induction of the insert	(Amann *et al.*, 1988)
p30	Suppressor clone from pACYC184 based plasmid library	Radman lab

Plasmids constructed in this study		
pAMssrA	457-bp DNA fragment containing ssrA gene obtained as *Eco*RI and *Hind*III fragment from pCR2.1-10Sa plasmid and cloned in the respective sites of pAM34	This study
$pRC7_{Sp}$	*bla* of pRC7 replaced with *aadA* gene from pAM34; Sp^r	This study
pRC_{Sp}-spoT	*bla* of pRCspoT replaced with *aadA*; Sp^r	This study
pBADarfA	*Eco*RI-*Hin*dIII fragment from pSTVarfA cloned in respective sites in pBAD24	This study
pBADarfB	*Eco*RI-*Hin*dIII fragment from pSTVarfB cloned in respective sites in pBAD24	This study
p30Δ*ydaW*	*ydaW* deleted and replaced by Kan in p30 by recombineering	This study
p30Δ′*ydaV*	*′ydaV* deleted and replaced by Kan in p30 by recombineering	This study
p30Δ*rzpR-trkG′*	*Both rzpR* and *trkG′* deleted and replaced by Kan in p30 by recombineering	This study

Plasmid DNA preparations were routinely made from the *recA* strain DH5α and were stored in water at –20ºC.

2.1.3 Primers

Table 2.3: Oligonucleotide primers.

Oligonucleotide ID	**Sequence (5′→3′)**
JGO_spec_pRChom_FP	ACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAG TATGCGCTCACGTAACTGGTCC
JGO_spec_pRChom_RP	AATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAG TTATTTGCCGACTACCTTGG
JGOpkaFP	GCCAATATTGTACTGCCGAGG
JGOssrATS	GATTCTGGATTCGACGGG
JGOssrALS	AGCCTGATTAAGTTTTAACGC
JGOC1Pr	ATGAGCACAAAAAAGAAACC
JGOEPlacZFP	GGATTCACTGGCCGTCGT
JGOEPlacZRP	TCACGTTGGTGTAGATGGG
JGOLPlacZFP	ACCCCGTACGTCTTCCCG

JGOLPlacZRP	TTATTATTTTTGACACCAGACC
JGOydaVSalFP	ATAAGTCGACAGATCAACCGTCAGAATCG
JGOydaVBamFP	ATAAGGATCCAGATCAACCGTCAGAATCG
JGOtrkSalRP	ATAAGTCGACATCTGTAACTATCTTCCCTCC
JGOtrkBamRP	ATAAGGATCCATCTGTAACTATCTTCCCTCC
JGOydaWFP	ATAAGAATTCCGAATGTCGTCCAGCC
JGOydaWRP	ATAAAAGCTTGCCGCGTTCGCCAG
JGOrzpRFP	ATAAGAATTCGAGAACTGAAGCTGGCG
JGOrzpRRP	ATAAAAGCTTAAATGAATCATATTGTAGTTAAGATT
JGP_ydaV_P1	TAAATTTTTCTCCCGGTAAAAATTACTTCGCAATTCCTGG GTGTAGGCTGGAGCTGCTTC
JGP_184_P4	CTATCGACTACGCGATCATGGCGACCACACCCGTCCTGTG ATTCCGGGGATCCGTCGACC
JGP_P30_P1	ATAATGGTACCGGGAAGAAC
JGP_P30_P2	ATAATGGTACCAAACAATGTAGC
JGP_P30IN_P3	GAGAAAAATTTATTGGAGACTGTTT
JGP_P30IN_P4	AAACAGTCTCCAATAAATTTTTCTC
JGP_P30IN_P5	GAGAAAAATTTACTGGAGACTGTTTT
JGP_P30IN_P6	AAACAGTCTCCAGTAAATTTTTCTC
JGP_arfA_EcoRIFP	TTATAGAATTCACCATGAGTCGATATCAGC
JGP_arfA_Hind3RP	ATAAAAGCTTGAATAACCGCTCTTAACA
JGP_arfB_EcoRIFP	TTATAGAATTCACCATGATTGTGATTTCCC
JGP_arfB_Hind3RP	TATTTAAGCTTTTATTCCCGACCGCTG
JGO5sRNA Probe	ACTACCATCGGCGCTACGGC
JGOU73 Probe	TGGAGTCCCCTGCAG
JGP_PrxtRNA_P4F	ATAGAATGCTGGCCGTCGTTTTACAACGTCGTGACATTCC GGGGATCCGTCGACC
JGP_tRNA_P1_RP	TACTGCTGGCGCACCGGCGTTAAAACAAAAGCCTAGTGT AGGCTGGAGCTGCTTC
JGP_lacZPE_RP1	GTTTTCCCAGTCACGAC
JGPlac_startFP	GGAATTGTGAGCGGATAAC
16SFP	GAAGCTTGCTTCTTTGCT
16SFP	GAGCCCGGGGATTTCACAT
JGP_PROXtRNA_SEQ	CACGACAGGTTTCCCGAC

2.1.4 Media

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

Glucose Minimal A medium

Minimal A salts (1X)

K_2HPO_4	10.5 g
KH_2PO_4	4.5 g
$(NH_4)_2SO_4$	1.0 g
$CH_3COONa.2H_2O$	0.5 g
H_20	to 1000 ml

After autoclaving the following solutions were added to Min A salts:

$MgSO_4$ (1M)	1 ml
Glucose (20%)	10 ml
Vitamin B1 (1%)	0.1 ml

Amino acids when required were added to a final concentration of 40 μg/ml or casaminoacids were added at a concentration of 0.2% whenever needed.

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.

M9 minimal medium (1X)

$Na_2HPO_4•7H_2O$	7.0 g
KH_2PO_4	3.0 g
NaCl	0.5g
NH_4Cl	1.0 g
H_20	to 1000 ml

Sterilize the solution by autoclaving.

Glucose-M9 minimal medium was made in a similar way to that of Glucose Minimal A medium.

LB medium

Tryptone	10.0 g
Yeast Extract	5.0 g
NaCl	10.0 g
H_2O	to 1000 ml

pH was adjusted to 7.0 – 7.2 with 1 N NaOH.

LB agar

LB medium	1000 ml
Bacto-agar	15.0 g

LB soft agar

LB medium	100 ml
Bacto-agar	0.6 g

Z broth (for P1 transduction)

LB medium	100 ml
0.5 M $CaCl_2$	0.5 ml

Buffered LB agar

Tryptone	10.0 g
Yeast extract	5.0 g
Min A salts	1X
Bacto-agar	15.0 g
H_2O	to 1000 ml

Buffered Yeast extract agar

Yeast Extract	5.0 g
Min A salts	1X
Bacto-agar	15.0 g
H_2O	to 1000 ml

Yeast extract broth

Yeast Extract	5.0 g
NaCl	10.0 g
Bacto-agar	15.0 g

H_2O	to 1000 ml

LBON medium (LB medium without NaCl)

Tryptone	10.0 g
Yeast Extract	5.0 g
H_2O	to 1000 ml

pH was adjusted to 7.0-7.2 with 1N NaOH.

LBON agar

LBON medium	1000 ml
Bacto-agar	15.0 g

MacConkey Agar

MacConkey agar (Difco)	51.5 g
H_2O	to 1000 ml

2.1.5 Buffers and solutions

Citrate Buffer

Citric Acid (0.1 M)	4.7 volumes
Sodium citrate (0.1 M)	15.4 volumes

TE Buffer

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

TBE Buffer

Tris-Borate	90 mM
EDTA	2 mM

This was prepared as 5X solution and used at 0.5X concentration.

TAE Buffer

Tris-Acetate	40 mM

EDTA	2 mM

This was prepared at 50X concentration and used at 1X concentration.

Both TBE and TAE were used as standard electrophoresis buffers.

Gel loading buffer with dye

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

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 1N KOH.

PIPES gets into solution when the pH is greater than 6.7. MnCl2 was dissolved separately and added with stirring. The pH was then adjusted to 6.7 and solution was filter sterilized and stored at –20ºC.

Z Buffer (for β-galactosidase assay)

Na_2HPO_4	16.1 g
NaH_2PO_4	5.5 g
KCl	0.75 g
$MgSO_4.7H_2O$	0.246 g
H_2O	to 1000 ml

pH was adjusted to 7.0 and stored at 4ºC.

Pre-Hybridization Buffer

20X Saline-sodium citrate (SSC)	3ml
50% dextran sulphate	2ml
50X Denhardt’s solution	1ml

20% SDS	250 µl
10 mg/ml Salmon sperm DNA	100 µl
DEPC water	to 10ml

Hybridization Buffer

Same as pre-hybridisation buffer but contains the radio-labelled probe.

SDS sample Buffer (1X)

Tris-HCl, pH 6.8	50mM
Glycerol	10%
EDTA	12.5 mM
SDS	2%
Bromophenol blue	0.02%
β-mercaptoethanol	1%

Running buffer for western blotting

Glycine	14.4 g/l
Tris base	3.05 g/l
SDS	1.0 g/l

Transfer buffer for western blotting

Glycine	14.4 g/l
Tris base	3.03 g/l

The above salts were dissolved in 800 ml of milliQ water and 200 ml of methanol was then added. The buffer was chilled before use.

TBST buffer for Western blot

10X of TBS (1000ml)	
Sodium chloride	80 g
Potassium chloride	2 g
Disodium hydrogen phosphate (Na_2HPO_4)	14.1 g
Potassium dihydrogen phosphate (KH_2PO_4)	2.49 g
MilliQ water	to 1000ml

1litre of 1X TBS +1 ml of Tween-20

40% Acrylamide solution (29:1)

Acrylamide	39 g
Bis-acrylamide	1 g
H_2O	to 100 ml

7.5M Urea 10% acrylamide composition

40% Acrylamide	12.5ml
Urea	22.5g
5X TBE	10 ml
DEPC treated H2O	to 50 ml

The gel mixtures were filtered through a 0.45 µ Millipore filter before adding APS and TEMED.

2.1.6 Antibiotics

The antibiotics were used at the below concentrations (µg/ml) unless otherwise stated:

Antibiotics	**LB medium**	**Minimal A medium**
Ampicillin (for high copy number plasmids)	100	50
Ampicillin (for low copy number plasmids)	50	25
Ampicillin (for chromosome)	50	25
Chloramphenicol (for plasmids)	30	30
Chloramphenicol (for chromosome)	15	7.5
Kanamycin	25	12.5
Tetracycline	15	7.5
Nalidixic acid	50	25
Rifampicin	100	50
Spectinomycin	25	12.5

2.1.7 Chemicals

Chemicals were obtained from different 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 Difco laboratories and Himedia.

The materials used in the recombinant DNA experiments such as restriction endonucleases, T4-DNA ligase, DNA polymerases, polynucleotide kinase and DNA size markers were obtained from companies including New England Biolabs, Invitrogen, Promega, Bangalore Genei, Sigma and MBI Fermentas.

Kits used for plasmids and genomic DNA isolation, purification of DNA fragments and PCR amplification were obtained from Qiagen and Invitrogen. High fidelity enzymes for PCR amplification were purchased from Sigma.

The oligonucleotide primers used in this study were synthesized by Xcelris genomics or MWG Biotech. The radioactive chemicals were procured from Jonaki.

2.2 Section B: 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 phage lysate prepared on strain MG1655. Adsorption was allowed to occur at 37ºC for 30 minutes and the lysate was prepared by broth method.

To 0.3 ml of infection mixture, 8-10 ml of Z-broth was added and incubated at 37ºC with slow shaking until the visible lysis of the culture occurred (in 4-6 hours). The lysate was treated with 0.2 ml of chloroform, centrifuged and the clear lysate was stored at 4ºC with chloroform.

Quantitation of Plaque forming units (pfu)

To quantitate the titre of P1 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 30 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 titre was calculated from the number of plaques obtained on the plates as follows:

Phage titre (pfu) per ml = No. of plaques × dilution factor × 1000/vol. of lysate added (in µl)

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 30 minutes to facilitate phage adsorption. The unadsorbed phage particles were removed by centrifugation at 6000 rpm for 5 minutes and the pellet of bacterial cells was resuspended in 5 ml of LB broth containing 20 mM sodium citrate to prevent further phage adsorption. This was incubated for 25-60 minutes at desired temperature without shaking to allow the phenotypic expression of the antibiotic resistance gene. The mixture was then centrifuged and the pellet was resuspended in 300 µl of 0.1M citrate buffer. 100 µl aliquots were spread 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, resuspended in 300 µl of 0.1M citrate buffer and plated without phenotypic expression.

2.2.1.3 Transformation

A. Calcium chloride ($CaCl_2$) method

For routine plasmid transformation, following method which is a modification of that described by (Cohen *et al.*, 1972) was used. An overnight culture of recipient strain was subcultured 1:100 in fresh LB medium and grown till mid-exponential phase. The culture was chilled on ice for 20 minutes, and the steps thereafter performed at 4ºC. 10 ml of culture was centrifuged and pellet was resuspended in 5 ml of 0.1M $CaCl_2$. After 5 minutes of incubation on ice, the cells were again centrifuged and resuspended in 1ml of 0.1M $CaCl_2$. The suspension was incubated on ice for 45 minutes. To the 100µl aliquot of the cell suspension plasmid DNA (20-200ng in less than 10µl volumes) was added, incubated for 30-40 minutes on ice and given a heat shock for 90 seconds at 42ºC. The cultures were rapidly chilled for 1 minute, mixed with 0.9ml of LB broth and incubated at

desired temperature for 45 minutes 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. Inoue method

i. Preparation of high efficiency competent cells

Competent cells for high efficiency transformation were prepared by the method of (Inoue *et al.*, 1990) with few modifications. An overnight culture of the strain (routinely DH5α) was subcultured 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 2500rpm for 10 minutes at 4ºC. These cells were resuspended in 0.4 volumes of INOUE buffer and incubated in ice for 10 minutes. The cells were recovered by centrifugation at 2500rpm at 4ºC for 10 minutes and finally resuspended in 0.01 volume of the same buffer. Sterile DMSO was added to a final concentration of 7%. After incubating for 10 minutes in ice, the cells were aliquoted in 100µl volumes, snap frozen in liquid nitrogen and stored at –80ºC.

ii. Transformation protocol

For transformation, the required number of vials was thawed on ice and the transformation protocol as described for $CaCl_2$ method was employed.

2.2.1.4 Scoring for Phenotypes

A. *lacZ* phenotype

lacZ$^+$ colonies were distinguished from *lacZ*$^-$ colonies on X-gal containing plate or MacConkey lactose plate. X-gal is non-inducing colourless substrate of β-galactosidase enzyme which upon hydrolysis yields dark blue indolyl group and hence the *lacZ*$^+$ colonies on X-gal plate appear as dark blue colonies. Similarly, on the MacConkey agar plates *lacZ*$^+$ colonies appear dark pink whereas *lacZ*$^-$ colonies remain colourless.

B. UV-sensitivity

To check the UV-sensitivity of the strains qualitatively, the strains were streaked on duplicate LB-agar plates and one of the plates was UV-irradiated with a 15-W UV-germicidal lamp at a distance of 70cm for 30 seconds. The UV-exposed and unexposed plates were incubated overnight in the dark after wrapping with aluminium foil and then growth was scored. This test could differentiate a *recA*$^-$ strain (UV^s) from a *recA*$^+$ strain (UV^r).

C. LBON temperature-sensitivity

Strains were streaked on LBON agar plates and after an overnight incubation at 42°C, growth was monitored (compared to that on LBON at 30°C as control). Absence of single colony growth was taken to reflect temperature sensitivity.

D. *In vivo* transcription termination phenotypes

The rationale for each phenotype is described in the relevant section.

SMG-sensitivity

The *E. coli relA* mutants exhibit SMG-sensitive (SMG^S) phenotype, that is, growth-inhibition in the presence of serine, methionine and glycine at 1mM concentration each (Uzan & Danchin, 1978) and is proposed to be a consequence of transcriptional polarity exerted by a frameshift mutation in the *ilvG* gene on the expression of downstream genes of the *ilvGMEDA* operon (Lopes & Lawther, 1989). This test was therefore used to distinguish $relA^+$ from $relA^-$ strains. Growth in the presence of amino acids serine, methionine, and glycine (SMG) was scored on glucose-minimal A plates supplemented with each of the amino acids at 40μg/ml and compared with the growth on non-supplemented glucose-minimal A plates to score for SMG phenotype.

***galEp3* assay**

This assay was used to test for relief of transcriptional polarity in the *rho* and *nusG* mutants. The *galEp3* (*galE490**) mutation represents a 1.3kb IS*2* insertion in the *gal* leader region (between the promoter and structural genes of the *galETKM* operon). The mutation causes transcriptional polarity on the structural genes due to Rho-dependent transcription termination within IS*2*. In this assay, the *gal* operon expression in a *galEp3* mutant or its derivatives was monitored by using MacConkey galactose indicator plates (with 1% galactose), where Gal^+ colonies are red, and Gal^- colonies are white. Therefore, the depth of color serves as an indicator of relative levels of *gal* expression, i.e., the extent of transcriptional polarity relief.

2.2.1.5 Efficiency of plating (EOP)

Efficiency of plating (EOP) is a measure of the ratio of number of colonies (obtained from a given volume of a suitable culture dilution) on a test medium to those on a control or permissive medium, and is a measure of cell viability on the former. It is a very sensitive test and is often used for determining the viability of a strain in the presence

or absence of a metabolite or a particular temperature. An EOP of ≤0.01 suggests lethality of the strain on the test medium.

For strains carrying IPTG-dependent plasmids, EOP was determined by growing the strains overnight in medium containing IPTG and appropriate antibiotic, and spotting serial dilutions (10^0 or 10^{-1} to 10^{-6}) on +IPTG (permissive) and –IPTG (test) plates to observe growth. The viability is scored by taking ratio of the colony forming units per ml (cfu/ml = No. of colonies × dilution factor × 1000/volume of culture spotted (in µl) obtained on the –IPTG plate to that on the +IPTG plate and determines the EOP.

Likewise, strains carrying Ts plasmids were cultured overnight at 30°C with the appropriate antibiotic and the serial dilutions of this culture were spotted at two temperatures 30°C (permissive) and 42°C (non-permissive or test). The ratio of cfu/ml obtained on the test temperature to that on the permissive temperature determined the efficiency of plating at the test temperature.

2.2.1.6 Blue-white screening for viability or lethality phenotype

To determine whether a particular mutation conferred lethality in the ppGpp^0 or Δ*dksA* background, an assay was devised based on the use of an unstable, easy to cure shelter plasmid pRC7, similar to that described previously (Bernhardt & de Boer, 2004). In the wild-type strain carrying pRC7, this plasmid can be lost at a frequency of 20-30% in the absence of the selection. However, this will not be seen if the plasmid loss leads to cell death. Since the plasmid pRC7 confers a *lac*$^+$ phenotype, in the absence of the selection plasmid loss can be visualized on X-gal IPTG containing plates as white colonies in a Δ*lac* strain whereas the colonies that retain the plasmid will appear blue.

In order to carry out synthetic lethal screen in the ppGpp^0 or Δ*dksA* strains, the *spoT* or *dksA* genes cloned in pRC7 under the control of *lac* promoter were used. These shelter plasmids, namely, pRCspoT or pRCdksA, respectively were transformed into the ppGpp^0 or Δ*dksA* strain. To test the synthetic growth phenotypes, the mutations of the genes to be tested were introduced by phage P1 transductions. The resulting strains were grown overnight in LB containing the antibiotic selection for the shelter plasmid and IPTG for expression of *spoT* or *dksA*, subsequently washed in minimal A medium and dilutions (usually 10^{-5} or 10^{-6}) of these cultures were spread on X-gal and IPTG containing plates without antibiotic selection for the shelter plasmid. The phenotypes of the white colonies in comparison with the blue colonies were noted. Viability of the strains was inferred when

white colonies were recovered and purified to give growth. If the mutation caused synthetic lethality then white colonies (that lack the shelter plasmid) would not be observed since plasmid loss would result in growth arrest. Therefore, lethality was inferred when either white colonies were not recovered or were recovered but failed to purify further.

2.2.1.7 β-Galactosidase assay

β-Galactosidase assay was performed according to (Miller, 1992). Cultures were grown to A_{600} of 0.4-0.6 from a 1:100 dilution of overnight cultures. Around 0.1-0.5 ml of culture was made up to 1 ml with Z-buffer and lysed with the addition of 100µl of chloroform and 50µl of 0.01% SDS solution. 0.2ml of freshly prepared 4mg/ml ONPG was added to start the reaction and incubated at 28^{o}C till the colour of the reaction mixture turned yellow. 0.5ml of 1M Na_2CO3 was added to stop the reaction and the time duration from initial addition of ONPG to the stopping of the reaction was noted. The absorbance of reaction mix was taken at 420 nm (A_{420}) after spinning down the mix at 12000rpm for 3 minutes. The A_{600} of the cultures used was also noted. The enzyme's specific activity (in Miller units) was calculated using the following equation:

β-Galactosidase specific activity (Miller units) = $(1000 \times A_{420}) / t \times v \times A_{600}$

Where,'t' is the time period in minutes and 'v', the volume of culture used in ml.

2.2.1.8 Estimation of growth rates

Growth curves were generated to compare the growth rates of *E. coli* test strains with control strains manually. The appropriate dilutions of the overnight cultures in desired media were made and allowed to grow at required temperature till faint turbidity was visible. At this point samples were collected every 30 minutes until stationary phase was attained. The growth curves were generated using Microsoft Excel or SigmaPlot software and growth rates were calculated from the slope of the graph which, in turn, was used to calculate generation time.

2.2.2 Recombinant DNA techniques

2.2.2.1 Isolation of plasmid and chromosomal DNA

3 ml (for high copy number) or 10 ml (for low-copy number) of cells from an overnight culture were pelleted by centrifuging for 5 minutes at 6000rpm for the plasmid isolation which was carried out with the commercially available kits (Qiagen or Invitrogen)

following the manufacturer's instructions. For genomic DNA, 1ml culture was used for DNA isolation using Qiagen or Invitrogen kits. The quality of plasmid/genomic DNA preparations was assessed following electrophoresis on 0.8% agarose gels.

2.2.2.2 Agarose gel electrophoresis

The DNA samples were mixed with appropriate volumes of 6X loading dye (0.25% bromophenol blue and 0.25% xylene cyanol and 30% glycerol in water) and subjected to electrophoresis through 0.8 to 1.5 % agarose gel in TAE buffer. The Goodview nucleic acid stain (supplied as 20000X; Beijing SBS Genetech Co. Ltd.) was added to the gel at the time of casting or 6X EZ-Vision One DNA dye (Amresco) was used as loading buffer, both being commercially available non-carcinogenic dyes to aid visualization of bands. The visualization was done by fluorescence under UV light in a UV transilluminator.

2.2.2.3 Restriction enzyme digestion and analysis

Around 0.5-1µg DNA was regularly used for each restriction digestion. 2 to 5 units of restriction enzyme were used in the total reaction volume of 20µl containing 2µl of the corresponding buffer supplied at 10X concentration by the manufacturer. The reaction was incubated for 3 hours at the temperature recommended by the manufacturer. The DNA fragments were visualized after electrophoresis on 0.8 to 1.5% 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.

2.2.2.4 Purification of DNA by gel elution

DNA fragments to be used for specific purposes like ligation or radioactive labelling 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 kit (Qiagen) for this purpose. The efficiency of elution was determined by checking a small aliquot of DNA sample on the gel.

2.2.2.5 Ligation of DNA

Typically 400-500ng of DNA was used in each ligation reaction. The ratio of vector to insert was maintained between 1:3 and 1:5 for cohesive end ligation. The reaction was generally performed in 15µl volume containing ligation buffer (provided by the manufacturer) and 0.075 Weiss unit of T4 DNA ligase at 16ºC overnight (14-16 hours).

2.2.2.6 Recombineering

Recombineering was performed as described in (Yu *et al.*, 2000) for engineering the linear DNA on the chromosome. The oligonucleotide primers were designed to amplify the DNA cassette to be engineered. Oligonucleotides used for recombination contained 30–50nt homology at the 5′ end to the sequences at the target site and 20nt homology to the DNA cassette at the 3′ end. The DNA cassette for recombination was generated by PCR and would contain 30-50 bp homologies to the target site. A strain with the target DNA and carrying a defective λ-prophage with *gam*, *beta* and *exo* genes (that facilitate homologous recombination) under the control of a temperature-sensitive λ cI-repressor was grown at 30^{o}C. At an A_{600} of 0.4, the culture was shifted to 42^{o}C for 15 minutes to express *gam*, *beta* and *exo* genes. Cells become capable of recombining linear DNA introduced into the cell by electroporation. 50-100ng of amplified DNA cassette was used for electroporation which was performed using the Bio-Rad Gene Pulser set at 1.8 kV, 25 μF with Pulse controller of 200 ohms.

2.2.3 Molecular techniques

2.2.3.1 Polymerase Chain Reaction (PCR)

The PCRs were normally performed using Taq polymerase from Roche or Fermentas. Approximately 1-5ng of plasmid or 5-100ng of chromosomal DNA was used as a template in a 50μl reaction volume containing 200μM of each dNTP, 20pM each of the forward and reverse primers and 1 unit of Taq DNA polymerase.

For colony PCR *E. coli* cells from a freshly grown plate were resuspended in 10μl of sterile Milli-Q water to get a cell suspension and this was used as a template in a PCR reaction at a final volume of 50μl.

The samples were typically subjected to 30 cycles of amplification with the following general conditions:

Initial denaturation	95ºC	5 minutes
Denaturation	95ºC	1 minute
Annealing	55ºC	1 minute
Extension	72ºC	1 minute/kb of DNA template to be amplified
Final extension	72ºC	10 minutes

For high fidelity PCR, Herculase II fusion DNA polymerase (Agilent Technologies) was used. Approximately 0.5µg of chromosomal DNA was used as a template in a 50µl reaction volume.

2.2.3.2 Isolation of total cellular RNA

Total RNA extraction from *E. coli* cells was done using Qiagen RNeasy mini kit. Cells were grown to an A_{600} of 0.6 and harvested (a maximum of 10^7cells) at 6000rpm for 5 min at room temperature to prevent cells for encountering any stress in cold. Rest of the steps were followed exactly as mentioned in the manufacturer's protocol. The quality of RNA preparations was assessed following electrophoresis on 1.4% agarose-formaldehyde-MOPS gels. In general, for a wild-type strain RNA yield would be ~0.5-1µg.

2.2.3.3 Estimation of DNA and RNA concentrations

Concentrations of DNA preparations were estimated by nanodrop or by gel electrophoresis followed by densitometric analysis. Concentration of RNA preparations were estimated by nanodrop.

2.2.3.4 Reverse transcription (RT)-PCR

The semi-quantitative reverse transcription-PCR (RT-PCR) involves the synthesis of complementary DNA (cDNA) from RNA. For this, 1µg of RNA was treated with 1µl (1 unit) DNase I enzyme (Sigma, amplification grade) for 20 min to remove DNA contamination. DNase I was inactivated by heating at 70^{o}C for 10 min. Next, 5pmol reverse primer was added along with dNTPs and volume made to 10µl with DEPC-treated water; the mix was heated at 65^{o}C for 5 min and incubated on ice for at least 1 min. The reverse transcription reaction was set up with this mix using the Superscript III RT kit (Invitrogen) as per manufacturer's protocol to obtain cDNA. The cDNA served as the template for setting up a PCR for required number of cycles. The samples were finally run on agarose gels.

2.2.3.5 DNA sequencing

Automated DNA sequencing on plasmid templates or on PCR products was carried out with dye terminator cycle sequencing kits on an automated sequencer following the manufacturer's instructions by an outsourced sequencing facility.

2.2.3.6 Radiolabelling of oligonucleotides

Oligonucleotides and PCR products were end-labelled using phage T4-polynucleotidekinase (PNK, New England Biolabs or Fermentas or Sigma) with ^{32}P-γ-ATP. The radiolabelling reaction mixture (20µl) contained 1X of buffer provided by the company, 10 units of T4-PNK and 40µCi of 32P-γ-ATP. The reaction mix was incubated for 1 hr at 37ºC and the reaction was heat-inactivated at 65^{o}C for 20 minutes. The labelled oligonucleotides and DNA fragments were purified by the Qiagen nucleotide removal kit. Labelling efficiency was checked either by using Geiger-Muller (GM) counter or using liquid scintillation counter. For scintillation counting, 1µl of radioactive sample was added to the 5ml scintillation cocktail, and radioactivity count was determined in the ^{32}P channel of scintillation counter (Perkin Elmer, Liquid Scintillation analyzer, Tri- Carb 2910 TR, USA). Liquid scintillation cocktail consists of 5g PPO (2,5- diphenyloxazol) and 0.3g POPOP (1,4- bis (5 phenyl 1,2-oxazole) Benzene, adjusted to a volume of 1L in toluene.

2.2.3.7 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 were boxed and the PSL (Photo stimulated luminescence) values were further considered. Background signal (obtained from equal area as that of the radioactive band but from other part of the gel/blot) is subtracted from the signal intensities obtained from radioactive bands to get the final values.

2.2.4 Northern Blotting

The method followed was as described in (Lopez *et al.*, 1997) with few modifications. The steps are as described.

2.2.4.1 Denaturing polyacrylamide gel electrophoresis of RNA

The following solutions were used to cast and run denaturing PAGE gels:

i. 40% acrylamide stock solution
ii. 7.5M Urea
iii. 5X TBE
iv. Ammonium persulphate (APS) stock: 10% (w/v) solution made fresh
v. TEMED (N,N,N′, N′-tetramethyl ethylene diamine)
vi. Gel running buffer (0.5X TBE)

vii. RNA buffer II from Ambion (1-2X Xylene cyanol + Bromophenol blue) used for loading the samples.

RNA isolation for Northern blotting for *lacZ* transcript was done after growing cultures till A_{600} of 0.6 in LB in the presence or absence of 1mM IPTG at 30^{o}C while for *lacZ-lacY'*-tRNA(U73)^{Arg5} or *lacZ'*-tRNA(U73)^{Arg5} transcripts, cultures were grown in LB upto A_{600} of 0.3 and induced with 1mM IPTG for 30 min followed by RNA extraction.

30ml of 10% polyacrylamide gels of 1.5mm thickness were cast in the Broviga slab vertical gel electrophoresis apparatus. Gels were polymerized by the addition of TEMED and APS (1/100th volume of gel mix). The gel was pre-run at 300V for 15-20 minutes prior to loading.

Sample preparation for gel loading was done as follows. The normalized amounts of RNA samples to be analyzed were mixed with the equal volumes of 2X gel loading buffer II (Ambion) making a final concentration of 1X. The samples were then heated at 80 degrees in a thermoblock (eppendorf) for 10 minutes and loaded on the gel when still warm. The gel was run at constant voltage of 300V for 3-4 hours till xylene cynol covered 2/3rd distance.

2.2.4.2 Transfer of RNA to the membrane

Semi-dry transfer apparatus (Bio-Rad trans blot semi dry transfer cell) was used for the transfer of RNA from the gel to the membrane. The Hybond-N+ membrane from Amersham biosciences was used which was cut as per dimensions of the gel containing the RNA samples. For each transfer 6 pieces of Whatman 3mm sheets of the size of the membrane were used. The membrane was soaked for 30-60 minutes in 0.5X TBE before transfer. The transfer apparatus was set up as described by the manufacturer. Transfer was done in 0.5X TBE buffer at 20V, 400mA and 100W for 1.15 hours.

2.2.4.3 UV-crosslinking of the RNA

The RNA was cross-linked onto the membrane after transfer by exposing it to the UV light of 200KJ/cm^2 energy in a UV-crosslinker.

2.2.4.4 Pre-hybridization of the membrane

The pre-hybridization buffer contained the following constituents:

i. 6X SSC
ii. 5X Denhardt's solution

iii. 10% Dextran Sulphate
iv. 0.5% SDS
v. 100 µg/ml Salmon Sperm DNA
vi. DEPC water

5ml of pre-hybridisation buffer was used per blot in 150×35mm hybridisation bottles (Labnet). Salmon sperm DNA was heated at 95^{o}C for 5 minutes and snap-chilled for 5 minutes prior to adding to the rest of the mix. Blot was inserted into the bottle such that it stuck to the walls and the surface containing the RNA faced the inner side of bottle. Pre-hybridisation was carried out at 50^{o}C for 3 hours in hybridisation chamber (Labnet Problot 12S hybridisation oven).

2.2.4.5 Hybridisation of the membrane

For hybridisation, probe was heated at 95^{o}C for 5 minutes and snap-chilled for 5 minutes and then added to the hybridisation bottles containing the blot. Hybridisation was carried out overnight at 50^{o}C. The probes used and their radioactivity counts (in parentheses) were 5s RNA probe (2.5×10^{6} cpm), U73 probe (5×10^{6} cpm) and *lacZ* probes (10^{6} cpm).

2.2.4.6 Washing of the membrane, exposure and scanning

Non-stringent washes were carried out in 2X SSC and 0.25-0.5% SDS in DEPC water. Stringent washing was done in 1X SSC and 0.5% SDS in DEPC water. Washing was carried out at 55-56^{o}C for 20 minutes. After washing, the blot was covered in the saran-wrap and exposed to the phosphoimager film. After the desired time of exposure, the film was then scanned in phosphoimager and the picture saved. The densitometric analysis of the bands was carried out as described in the section 2.2.3.7.

Normalization of the signal intensities in northern blotting experiments using probe against tRNA(U73)^{Arg5} was done as follows. The intensity of the tRNA(U73)^{Arg5} signal in the WT or the parent strain in the absence of IPTG was taken as 1 and the relative change in the other strain/growth condition calculated. The value thus obtained was corrected using the change in the corresponding 5S rRNA intensity relative to that in the WT/parent strain in the absence of IPTG.

2.2.5 Dot-blotting

The method followed was as described in Miller (1992). Samples for dot-blotting were prepared by mixing 5µg of RNA (in10µl H_20) with 30µl of RNA denaturing solution consisting of 1X MOPS, 7% formaldehyde and 50% deionised formamide. The samples were heat denatured at 65^oC for 5 min and mixed with equal volume of 20X SSC. The samples were loaded into the slots of the dot-blot apparatus (Bio-Rad) containing the membrane (pre-soaked in 20X SSC) and gentle suction was applied using Millipore vacuum pump. The slots were rinsed twice with 10X SSC. This was followed by the UV-crosslinking, pre-hybridization, hybridization, washing and exposure of the membrane identical to that done in Northern blotting.

2.2.6 RNA polymerase elongation rate measurement

The method followed was similar to that described previously with slight modifications (Jin *et al.*, 1992; Schleif *et al.*, 1973). Overnight bacterial cultures were grown in LB and subcultured 1:500 in the same medium in a volume of 20 ml at 30^oC. Cultures were induced with 1mM IPTG at A_{600}=0.4. 0.9ml samples were aliquoted at time intervals of 0 sec, 20 sec, 40 sec, 1 min, 1.5 min, 2 min, 2.5 min, 3 min, 3.5 min, 4 min, 4.5 min, 5 min, 5.5 min and 6 min into 0.1ml of 1mg/ml ice cold chloramphenicol and the samples were put on ice. After sampling, 0.5ml of each culture was taken for β-galactosidase assay.

Square root of β-galactosidase activity (activity at time T_t–T_0) was plotted against time. In the graph, the point of inflection of the curve on the X-axis determines the rate of elongation of RNAP whereas slope represents the promoter clearance, *lacZ* mRNA stability and factors affecting translation of *lacZ* (Burova *et al.*, 1995).

2.2.7 Microscopy

2.2.7.1 Sample preparation

Fresh overnight cultures grown in LB containing appropriate antibiotics to select for plasmids were sub-cultured 1:100 (or lower dilutions for some strains) in the same medium. The cells from these cultures were taken for microscopy at exponential phase of growth (A_{600} of 0.5-0.6), as such or after concentrating the cells 10-fold.

2.2.7.2 Preparation of microscopic slides

The slides for microscopy were prepared as described in Dajkovic *et al*., (2008) with slight modifications. After wiping the glass slide with ethanol, 200µL of 1% molten agarose was layered on it between two strips of tape and clean cover-slip placed on it to obtain levelled surface. The agarose was allowed to solidify and the cover-slip was carefully removed and 5µl of sample was put on top of the agarose and carefully covered with a cover-slip.

2.2.7.3 Viewing slides under microscope

A drop of immersion oil was put on top of the cover-slip before viewing it under microscope. The cells were viewed at 100X resolution of Nikon Eclipse 80i microscope. The differential interference contrast images of the cells were captured using NIS-Elements D3.0 software also used to find out mean cell size using at least 100 randomly selected cells. Fluorescence images were captured on Zeiss LSM 710 Meta inverted confocal microscope.

2.2.8 Gel Electrophoresis and Western blotting

Cultures in mid-exponential phase normalized using A_{600} and solubilized in 1X sample buffer at 99°C for 5 min were subjected to electrophoresis on 12% sodium dodecyl sulfate (SDS) - polyacrylamide gels. Cell extracts equivalent to $0.04A_{600}$ (1X) and $0.02A_{600}$ (0.5X) were loaded and run using Tris-glycine- (SDS) buffer. Separated proteins were electrotransferred to PVDF polyvinyledene difluoride) membrane (Amersham) electrophoretically by a semi-dry method using Bio-Rad apparatus. The transfer was done for 2-3 hrs using a voltage of 75V at 4^{o}C and membrane was probed using anti-FtsZ primary antibody at 1:5000 dilution (rabbit, polyclonal), washed and probed with anti-rabbit IgG conjugated to horseradish peroxidase (HRP) at 1:20000 dilution, as described (Sambrook & Russell, 2001). Membranes were developed with chemiluminescence reagent (Amersham ECL Prime) and visualized with the aid of a chemiluminescence detection system according to the manufacturer's protocol (Sigma Chemical Co., St. Louis, MO). Quantification of band intensity and subtraction of background was done using Fujifilm Multi Gauge V3.0 imaging system (Image Quant software).