2 MATERIALS

2.1 Bacterial strains

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

E. coli strain	Genotype	Reference	Growth medium[a]
BL21(DE3)pLysS	*E. coli* B F^- *dcm ompT hsdS*(r_B- m_B-) *gal* λ(DE3) [pLysS Cm^r]	(96)	NZCYM
CC191	F128 *lacI*^{q} Δ(*lacZ*)m15 *traD*/ Δ(*ara-leu*)7697 *araD*139 Δ(*lac*)X74 *galE galK thi rpsE phoA20 rpoB argE(am) recA*1	(59)	YT
K12 HB101	*supE44 ara14 galK2 lacY1* Δ(*gpt-proA*)*62 rpsL20* (Str^r) *xyl-5 mtl-1 recA13* Δ(*mcrC-mrr*) *hsdS*(r_B- m_B-)	(10)	YT+
K12 HB101 Nx^r	spontaneous, nalidixic acid-resistant derivative of HB101	(10)	YT+
K12 SCS1	*recA1 endA1 gyrA96 thi-1 hsdR17* (r_k^-, m_k^+) *supE44 relA1*	(33)	YT+
SG13109	F^- *his sulA*	(67)	YT
XK1200	F^- *lac*Δ*U124* Δ(*nadA aroG gal attλ bio) gyrA*	(65)	YT
K12 XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacI*^{q}*Z*Δ*M15* Tn*10* (Tc^r)]	(11)	YT

[a] see chapter 2.3 for composition of media

2.2 Plasmids and phages

Table 2.2. Plasmids and phages used in this study

Plasmid / phage	Description	Relevant Genotype	Selective marker(s)[a]	Reference
λTn*lacZ*/in	λ28ΩTn*lacZ*/in [b]	-	-	(59)
λTn*phoA*/in	λ28ΩTn*phoA*/in	-	-	(59)
M13 mjF182	M13 mp18Ω[pJF142 *Bam*HI-*Bam*HI, 0.8 kb fragment]	*oriT*	-	(113)
M13 mp18	F-specific bacteriophage, circular ssDNA (replicative form)	-	-	(113)
pBS140	pJF119HEΔ[*Hin*cII-*Sph*I]Ω[RP4 48710-46495 [c]]	$traG^+$	Ap	(108)
pBS140(K187T)	pJF119HEΔ[*Hin*cII-*Sph*I]Ω[RP4 48710-46495 (T 47936 G)]	$traGK187T^+$	Ap	(108)
pDB127	pDB126Δ[RP4 *Sfi*I-*Ssp*I 48374-46670]	$(trbB\text{-}trbM)^+$ $(traF\text{-}traM)^+$ $traG^-$	Cm	(4)
pDB173	pMS119HEW[T7 gene *10* SD, *Nde*I-*Ear*I adaptor, RP4 *Ear*I-*Ssp*I 50656-48374]	$traI^+$	Ap	(70)
pET14b	vector for production of His_6 fusion proteins	-	Ap	(78)
pFS141	pMS470Δ8Δ[*Nde*I-*Hin*dIII]Ω[RP4 48495-46588, his_6 [d]]	$traG\text{-}his_6^+$	Ap	This work
pFS241	pMS470Δ8Δ[*Nde*I-*Hin*dIII]Ω[his_6, RP4 48495-46588]	$his_6\text{-}traG^+$	Ap	This work
pFS241M	pMS470Δ8Δ[*Nde*I-*Hin*dIII]Ω[his_6, RP4 48495-46588 (T 47936 G)]	$his_6\text{-}traGK187T^+$	Ap	This work
pGS002	pMS470Δ8Δ[*Nde*I-*Hin*dIII]Ω[his_6 linker]	-	Ap	This work
pGS003Δ1	pMS470Δ8Δ[*Eco*RI-*Sma*I; *Nde*I-*Hin*dIII]Ω[*Nde*I/*Nsi*I/*Bcl*I/*Hin*dIII/*Sac*I linker]	-	Ap	This work
pGS006Δ1	pGS002Δ[*Nde*I-*Hin*dIII]Ω[RP4 48387-46588]	$his_6\text{-}traG\Delta1^+$	Ap	This work
pGS006Δ2	pGS002Δ[*Nde*I-*Hin*dIII]Ω[RP4 48201-46588]	$his_6\text{-}traG\Delta2^+$	Ap	This work
pGS007	pGS003Δ1Ω[*Nsi*I-*Nsi*I R388 16-1638 [e]]	$trwB^+$	Ap	This work
pGS011	pGS006Δ2Δ[*Sfi*I-*Sfi*I]Ω[pFS241M *Sfi*I-*Sfi*I 1467-bp fragment]	$his_6\text{-}traG\Delta2K187T$	Ap	This work
pGS012Δ1	pGS002Δ[*Nde*I-*Hin*dIII]Ω[R388 229-1638]	$his_6\text{-}trwB\Delta1^+$	Ap	This work
pHY524Δ1	pET14bΔ[*Nde*I-*Bam*HI]Ω[*Hp* 550217-551951 kb [f]]	$his_6\text{-}hp0524\Delta1^+$	Ap	This work
pJF142	pBR329Ω[*Bam*HI, *Bam*HI-*Xma*III linker, RP4 *Xma*III-*Xma*III 50995-51770 kb, *Xma*III-*Bam*HI linker]	*oriT*	Ap, Cm	(29)
pJF143	pBR329Ω[*Bam*HI, *Bam*HI-*Xma*III linker, RP4 *Xma*III-*Acc*I 50995-51269 kb, *Acc*I-*Bam*HI linker]	*oriT*	Ap, Cm	(29)
pKI410	pSPORTΩ[pMP1 *Hpa*I-*Bam*HI fragment]	$traD^+$	Ap	(58)
pMS119EH	Vector, $P_{tac}/lacI^q$	-	Ap	(95)
pMS470Δ8	Vector, pMS119EHΔ[*Xba*I-*Pst*I]Ω[pT7-7 *Xba*I-*Nde*I, 40bp-fragment, R751 *traC Ava*I-*Sph*I, 1.4 kb], $P_{tac}/lacI^q$	-	Ap	(5)
pOX38*traD411*	*In vivo* recombinant of pOX38 × pKI411	$traD^-$	Km	(58)
pSK410	pMS470Δ8Δ[*Nde*I-*Hin*dIII]Ω[F 23779-25937 kb [g]]	$traD^+$	Ap	This work
pSK410CH	pMS470Δ8Δ[*Nde*I-*Hin*dIII]Ω[F 23779-25937 kb, his_6]	$traD\text{-}his_6^+$	Ap	This work
pSK410NH	pMS470Δ8Δ[*Nde*I-*Hin*dIII]Ω[his_6, F 23779-25937 kb]	$his_6\text{-}traD^+$	Ap	This work
pSK470	pMS470Δ8Δ[*Nde*I-*Hin*dIII]Ω[RP4 48495-46588 kb]	$traG^+$	Ap	This work
pSK470ΔB	pSK470Δ[*Bam*HI]Ω[GATC]	$traG^+$	Ap	This work
pSU4054	pHG329Ω[*Eco*RI-*Hin*dIII R388 *oriT*, *trwA-trwC*, *eex*, *trwD-trwM*]	$(trwA\text{-}trwM)^+$	Ap	(9)

[a] ampicillin (Ap), chloramphenicol (Cm) and kanamycin (Km)
[b] λ28: non-replicative λ phage b221(Δ*att*) *cI*857 *P*am3
[c] RP4 bp-coordinates of inserted fragments are given (accession L27758) (73).
[d] his_6 sequence: CAC CAT CAC CAT CAC CAT
[e] R388 bp-coordinates of inserted fragments are given according to accession X63150 (56).
[f] *Hp* bp-coordinates of *H. pylori* genome are given (accession AE000511) (100).
[g] F bp-coordinates of inserted fragments are given (accession U01159) (28).

2.3 Media

Bacteria (Table 1) were grown in YT medium (62), YT medium with additives (YT+), NZCYM medium (80), LB medium (62) or TYE medium. The composition of the media is indicated for one liter of medium:

YT

10 g tryptone
5 g yeast extract
5 g NaCl

LB

10 g tryptone
5 g yeast extract
10 g NaCl

TYE

10 g tryptone
5 g yeast extract
8 g NaCl

YT+

10 g tryptone
5 g yeast extract
5 g NaCl
25 mg thiamine-HCl *
25 mg thymine *
1 g glucose *
25 mM MOPS (pH 8.0) *
(*) the indicated compounds were autoclaved separately before addition

NZCYM

10 g tryptone peptone (NZ amine)
5 g NaCl
5 g yeast extract
1 g casamino acids
8 mM $MgSO_4$
25 mM MOPS (pH 8.0)

15 g/l agar was added to the media for preparation of solid media. When appropriate, antibiotics or other supplements were added as follows:

ampicillin (sodium salt)	100 µg/ml
chloramphenicol	10 µg/ml
kanamycin sulfate	40 µg/ml
nalidixic acid	30 µg/ml
epicillin (dihydroampicillin)	100 µg/ml
tetracycline·HCl	15 µg/ml
X-gal or X-phos	40 µg/ml
IPTG	0.2 mM

2.4 Reagents and materials

Table 2.3. Reagents or materials and their suppliers

Compound	Supplier
Materials	
Ni-NTA Superflow	Qiagen
Phosphocellulose P11	Whatman
Superdex™ 200 columns	Amersham Pharmacia Biotech
nitrocellulose membrane BA85	Schleicher&Schuell
PVDF membrane	Amersham Pharmacia Biotech
cellulose MN300, polyethyleneimine impregnated	Machery-Nagel
dialysis bags	Roth
HAWP filters (0.2 µm / 0.45 µm pore size, sterile)	Millipore
Minisart filters (0.2 µm pore size, sterile)	Sartorius
PhosphorImager scanning device	Molecular Dynamics
Personal Densitometer scanning device	Molecular Dynamics
FluorImager 575 scanning device	Molecular Dynamics
ImageQuant Software (version 5.0)	Molecular Dynamics
Biacore 2000 optical biosensor system	Biacore AB (Uppsala, Sweden)
BIAevaluation software, version 3.1	Biacore AB
Reagents	
radioactive nucleotides	Amersham Pharmacia Biotech
nucleotides	Roche Molecular Biochemicals /Sigma
TNP-ATP [a]	Molecular Probes
TNP-ADP [b]	Molecular Probes
ATPγS [c]	Jena Bioscience
AppNp [d]	Jena Bioscience
BSA	Roche Molecular Biochemicals
FITC-labelled goat-anti-rabbit antibodies	Dianova
enzymes	New England Biolabs
Chemicals	
Brij-58	Fluka
Zwittergent 3-14	Fluka
Zwittergent 3-16	Fluka
Triton X-100	Sigma
Coomassie Brilliant Blue R250 (Serva Blue R)	Serva
Spermidine-HCl	Serva
ethidiumbromide	Sigma
Ficoll 400	LKB
PEG 6,000	Serva
PEG 20,000	Serva
IPTG	Biomol
MOPS	Biomol
urea	United States Biochemical
Kits	
Gel filtration standard	Bio-Rad
Plasmid Maxi Kit	Qiagen
QIAprep Spin Miniprep Kit	Qiagen
QIAEX II Gel Extraction Kit	Qiagen
BIAcore amine coupling kit	Biacore AB
HBS-EP buffer	Biacore AB

[a] 2',3'-O-(2,4,6-trinitrophenyl)adenosine 5'-triphosphate, trisodium salt
[b] 2',3'-O-(2,4,6-trinitrophenyl)adenosine 5'-diphosphate, disodium salt
[c] Adenosine-5'-(γ-thio)-triphosphate, sodium salt
[d] Adenosine-5-[(β,γ)-imido]triphosphate, triethylammonium salt

Reagents and materials used in this work are listed in Table 2.3. Chemicals that are not listed in Table 2.3 were from Merck or Sigma (*p.a.* grade). Oligonucleotides were synthesized by the service group of the Max-Planck-Institut für Molekulare Genetik.

2.5 Buffers

Buffers are listed below. Indicated percentages (%) refer to weight per volume (w/v) unless noted otherwise.

A 100 mM Tris-HCl (pH 7.6)
40 mM NaCl
8 % sucrose
0.44 mg/ml lysozyme
0.15 % Brij-58

B 20 mM Tris-HCl (pH 7.6)
100 mM NaCl

C 100 mM Tris-HCl (pH 7.6)
100 mM NaCl
0.25 % Brij-58

D 50 mM KH_2PO_4/K_2HPO_4 (pH 8.0)
300 mM NaCl
1 mM DTT

E 50 mM Tris-H_3PO_4 (pH 7.0)
40 mM NaCl
1 mM DTT

F 40 mM Tris-HCl (pH 7.6)
50 mM NaCl
1 mM DTT
0.1 mM EDTA

G 50 mM CHES-NaOH (pH 9.5)
500 mM NaCl

H 50 mM Tris-HCl (pH 8.7)
1 M NaCl
10 mM Zwittergent 3-14
1 mM DTT
0.1 mM EDTA
10% glycerol

I 50 mM Tris-HCl (pH 7.6)
500 mM NaCl
1 mM DTT
0.1 mM EDTA
10 % glycerol
0.01 % Brij-58

J 20 mM Tris-HCl (pH 7.6)
100 mM NaCl
10 mM $MgCl_2$
1 mM DTT
0.05% Brij-58
50 µg/ml bovine serum albumin (BSA)