
CONTROL OF THE UNIDIRECTIONAL MOTOR IN
RHODOBACTER SPHAEROIDES

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY AT THE UNIVERSITY OF OXFORD



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ABSTRACT

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Complementation studies revealed that CheY₃, CheY₄, and CheY₅ are functionally equivalent. The copy numbers per cell of important CheYs were found to vary greatly under the conditions tested (<1,000, ~3,000, ~60,000 for CheY₃, CheY₄, and CheY₆ respectively).

DECLARATION

The work in this thesis was undertaken at the University of Oxford; in the Microbiology Unit, Department of Biochemistry and in the Clarendon Laboratory, Department of Physics. Work was performed from October 2005 to October 2008 under the supervision of Prof. J.P. Armitage. All the work in this thesis is my own unless stated and has not been submitted for a degree at this or any other university.

PUBLICATIONS

paper 1

paper 2

ACKNOWLEDGEMENTS

Firstly, thanks to ...

ABBREVIATIONS

Ap ^R	ampicillin resistance
DIG	digoxigenin
dNTP	deoxynucleoside 5'-triphosphate
EDTA	ethylenediaminetetraacetic acid
HEPES	sodium N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
MES	2-(N-morpholino)ethanesulfonic acid
OD	optical density
PVDF	polyvinylidene fluoride
Sm ^R	streptomycin resistance
Tc ^R	tetracycline resistance

*To bla bla bla,
for bla bla bla...*

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LIFE AT LOW REYNOLDS NUMBER

bla bla bla

1.1 LIFE FROM A BACTERIUM'S PERSPECTIVE

bla bla bla

Bacteria are so small that they are affected by natural forces in ways we are unaccustomed to. For instance, unlike us, bacteria do not coast when they stop swimming; they stop almost instantaneously (0.004 nm) [1]. The Reynolds number ($\frac{\text{inertial forces}}{\text{viscous forces}} = \frac{\text{Size} \cdot \text{Velocity} \cdot \text{Density}}{\text{Viscosity}}$) is used as a measure of which of the two types of force, inertia (arising from the object's momentum) or viscosity (the amount of resistance the fluid exerts on the object), is the dominant factor that limits movement through fluids.¹ Examples of some characteristic Reynolds numbers for self-propelled organisms are shown in Table 1.1.

- bla bla bla
- bla bla bla
- bla bla bla.
- bla bla bla

¹An excellent informal paper, Life at Low Reynolds Number (Purcell, 1976), is available online at <http://dx.doi.org/10.1119/1.10903>

Organism type	Reynolds number
A large whale swimming at 10 m/s	300,000,000.0
A large dragon fly going 7 m/s	30,000.0
Flapping wings of the smallest flying insects	30.0
A sea urchin sperm swimming at 0.2 mm/s	0.03
A bacterium, swimming at 0.01 mm/s	0.00001

Table 1.1: A spectrum of Reynolds numbers for self-propelled organisms taken from [2]. The Reynolds numbers range from very large values, for a swimming whale, to extremely small values for swimming bacteria. That means that a whale, where inertial effects dominate, will continue to ‘coast’ for a substantial distance and time after it has stopped actively swimming. For a bacterium on the other hand, inertial effects are irrelevant and viscous effects dominate, so that a bacterium will stop almost instantaneously.

1.2 MOTILITY AND TAXIS IN MODEL SPECIES

1.2.1 MOTILITY

Unlike swimming animals [3, 4].

1.2.2 THE BACTERIAL FLAGELLAR MOTOR

The motor, shown in Figure 1.1, is formed from the products of ~ 40 genes, which are expressed in a particular sequence that results in self-assembly across the cell membrane (reviewed in [5]). The motor consists of a central rotor surrounded (in *E. coli* at least) by approximately eleven stator complexes [6]. Torque is generated at the periphery of the rotor by the translocation of ions into the cytoplasm through the stator complexes.

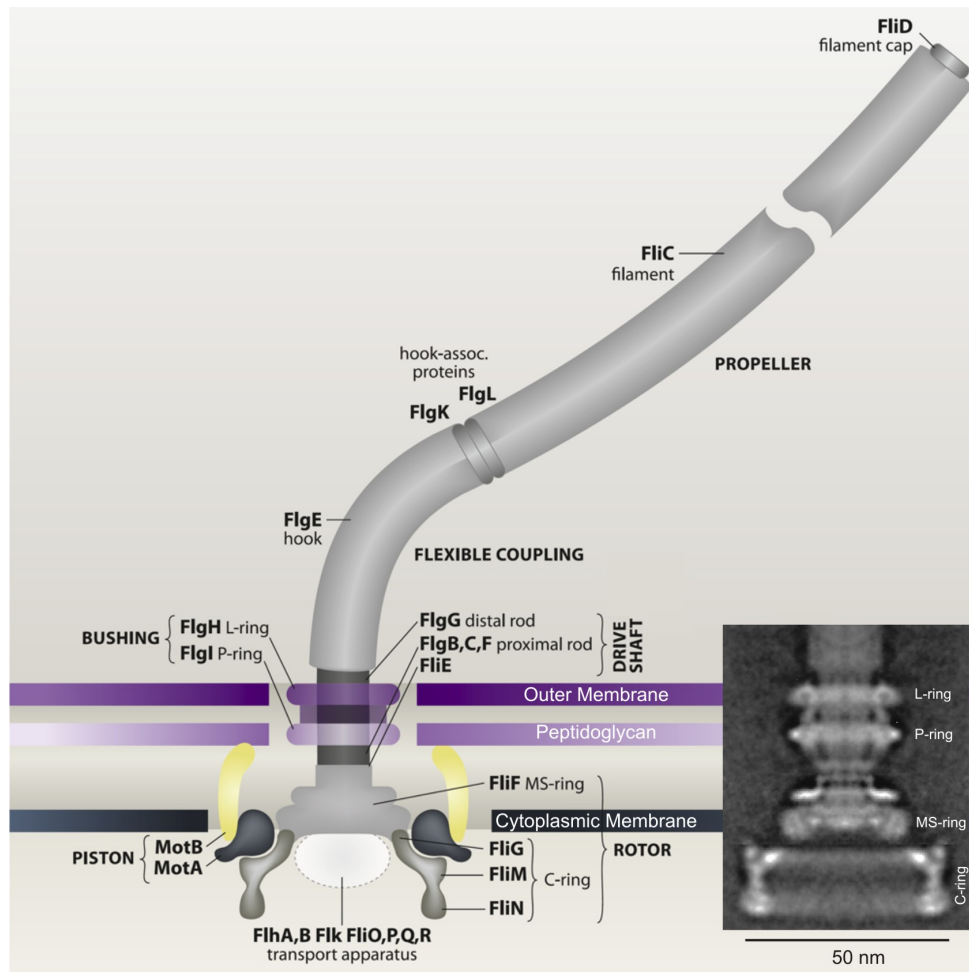


Figure 1.1: A schematic of the bacterial flagellar motor based on an electron micrograph (EM) (adapted from [7] and [8]). The core of the motor is called the ‘basal body’ that spans the cell envelope. The L and P rings are thought to be embedded in the outer lipopolysaccharide membrane and peptidoglycan cell wall, respectively. They may work as a bushing between the rotor and the outer parts of the cell envelope. The flow of ions from the periplasm into the cytoplasm through channels in the stator complexes, drives the rotation of the motor. The switch complex (FliG, FliM, and FliN) is responsible for generating torque of the appropriate sign, dependent on cytoplasmic CheY-P levels. See [9] and [10] for full flagellar motor reviews.

TORQUE-GENERATING UNITS

MOTOR ‘FUEL’

TORQUE-SPEED RELATIONSHIP

MATERIALS AND METHODS

Strain	Description	Source
XL-1 Blue	General <i>E. coli</i> cloning strain. <i>lacI^q</i> , Tc ^R	Stratagene
M15pREP4	<i>E. coli</i> expression host containing pREP4. Kn ^R	Qiagen
S17-1λ-pir	<i>E. coli</i> strain capable of mobilizing the suicide vector pK18 <i>mobsacB</i> into <i>R. sphaeroides</i> . Sm ^R .	[11]
WS8N	Spontaneous nalidixic acid resistant mutant of wild-type WS8	[12]
JPA410	WS8N derivative that carries a <i>cheY₃</i> gene that has been insertionally inactivated (named <i>cheY₃*</i>) [†]	[13]
JPA421	Δ <i>cheY₄</i> derivative of WS8N	[13]
JPA425	<i>cheY₃*</i> , Δ <i>cheY₄</i> derivative of WS8N	[13]
JPA1025	Δ <i>cheY₁</i> Δ <i>cheY₂</i> Δ <i>cheY₃</i> Δ <i>cheY₄</i> Δ <i>cheY₅</i> derivative of WS8N	[14]
JPA1337	Δ <i>cheY₁</i> Δ <i>cheY₂</i> Δ <i>cheY₃</i> Δ <i>cheY₄</i> Δ <i>cheY₅</i> Δ <i>cheY₆</i> derivative of WS8N	[14]
JPA443	Δ <i>cheOp₁</i> and Δ <i>cheY₅</i> derivative of WS8N	S.L. Porter
JPA1349	Δ <i>cheBRA</i> , ΔRSP2230 derivative of WS8N	S.L. Porter
JPA1278	Δ <i>fliM₂</i> (RSP6099) derivative of WS8N	S.L. Porter
<i>fliM₁</i> (E12D)	<i>fliM₁</i> (E12D) derivative of WS8N*	R.E. Sockett
<i>fliM₁</i> (E12Q)	<i>fliM₁</i> (E12Q) derivative of WS8N*	R.E. Sockett
<i>fliM₁</i> (S9F)	<i>fliM₁</i> (S9F) derivative of WS8N*	R.E. Sockett
JPA419	Δ <i>cheOp₂</i> and Δ <i>cheY₄</i> derivative of WS8N	[13]
JPA1301	Δ <i>cheOp₃</i> derivative of WS8N	[15]
JPA1336	Δ <i>cheY₆</i> derivative of WS8N	[15]
JPA1314	Δ <i>cheA₃</i> derivative of WS8N	[15]
JPA1353	Δ <i>cheOp₁</i> (<i>cheY₅</i> , <i>cheD</i> , <i>cheP₁</i> , <i>cheY₁</i> , <i>cheA₁</i> , <i>cheW₁</i> , <i>cheR₁</i> , <i>cheY₂</i>), Δ <i>cheOp₂</i> (<i>cheP₂</i> , <i>cheY₃</i> , <i>cheA₂</i> , <i>cheW₂</i> , <i>cheW₃</i> , <i>cheR₂</i> , <i>cheB₁</i> , <i>tlpC</i>), Δ <i>cheOp₃</i> (<i>cheA₄</i> , <i>cheR₃</i> , <i>cheB₂</i> , <i>cheW₄</i> , <i>ppfA</i> , <i>tlpT</i> , <i>cheY₆</i> , <i>cheA₃</i>), Δ <i>cheBRA</i> , Δ <i>cheY₄</i>	S.L. Porter

Continued...

Strain	Description	Source
JPA1319	$\Delta cheA_2$, $\Delta cheA_3$ derivative of WS8N	S.L. Porter
JPA1213	<i>cheY</i> ₆ (D56N) derivative of WS8N	[14]
JPA1216	<i>cheY</i> ₆ (D56A) derivative of WS8N	[14]
JPA1218	<i>cheY</i> ₃ (D53N) derivative of WS8N	[14]
JPA1220	<i>cheY</i> ₄ (D53N) derivative of WS8N	[14]
JPA917	<i>cheY</i> ₃ [*] , <i>cheY</i> ₄ (D53A) derivative of WS8N (parental = JPA425)	This study
JPA918	<i>cheY</i> ₃ [*] , <i>cheY</i> ₄ (D53N) derivative of WS8N (parental = JPA425)	This study
JPA919	<i>cheY</i> ₃ (D53A), <i>cheY</i> ₄ (D53A) derivative of WS8N (parental = JPA917)	This study
JPA920	<i>cheY</i> ₃ (D53N), <i>cheY</i> ₄ (D53N) derivative of WS8N (parental = JPA918)	This study

Table 2.1: Strains used in this study. †JPA410 was made by cloning a *Mlu*I linker (5'-GACGCGTC-3') into the *Eco*RV site near the beginning of *cheY*₃, introducing a stop codon at the site of insertion with a new *Mlu*I restriction site, which is expected to produce a truncated peptide of 26 N-terminal amino acids (named CheY₃^{*}) [13]. *For future reference, strains made by R.E. Sockett were assigned JPA numbers so they can be located in the freezers (*fliM*₁(E12D), *fliM*₁(E12Q), *fliM*₁(S9F) = JPA1249, JPA1250, JPA1251 respectively). *lacI*^q denotes the gene encoding the high expression mutant of the *lacI* gene. Tc^R, Kn^R, Sm^R denote tetracycline, kanamycin, streptomycin resistance respectively.

Plasmid	Description	Source
pUC19	High copy number cloning vector. Ap ^R	Pharmacia
pREP4	Plasmid carrying the <i>lacI</i> gene.	Qiagen
pIND4	<i>R. sphaeroides</i> inducible expression plasmid [†] , P _{A1/04/03} , <i>lacI</i> ^q , Kn ^R	[16]
pK18 <i>obsacB</i>	Allelic exchange suicide vector. Kn ^R , Sucrose sensitive	[17]
pK18Y3D53A	Construct for replacing <i>cheY</i> ₃ with <i>cheY</i> ₃ (D53A) in <i>R. sphaeroides</i> genome; pK18 <i>obsacB</i> derivative	[14]
pK18Y3D53N	Construct for replacing <i>cheY</i> ₃ with <i>cheY</i> ₃ (D53N) in <i>R. sphaeroides</i> genome; pK18 <i>obsacB</i> derivative	[14]
pK18Y4D53A	Construct for replacing <i>cheY</i> ₄ with <i>cheY</i> ₄ (D53A) in <i>R. sphaeroides</i> genome; pK18 <i>obsacB</i> derivative	[14]
pK18Y4D53N	Construct for replacing <i>cheY</i> ₄ with <i>cheY</i> ₄ (D53N) in <i>R. sphaeroides</i> genome; pK18 <i>obsacB</i> derivative	[14]
pQEY1	CheY ₁ expression plasmid. pQE30 derivative	[18]
pQEY2	CheY ₂ expression plasmid. pQE30 derivative	[18]
pQEY3	CheY ₃ expression plasmid. pQE30 derivative	[18]
pQEY4	CheY ₄ expression plasmid. pQE30 derivative	[18]
pQEY5	CheY ₅ expression plasmid. pQE30 derivative	[19]
pQEY6	CheY ₆ expression plasmid. pQE30 derivative	[15]
pQEY6D56N	CheY ₆ (D56N) expression plasmid. pQE30 derivative	[14]
pQEYFP-Y6	YFP-CheY ₆ expression plasmid. pQE30 derivative	[14]
pIND-empty	As pIND4 above, i.e. empty vector used as a control	[16]
pIND-Y1	<i>cheY</i> ₁ cloned into pIND4	This study
pIND-Y2	<i>cheY</i> ₂ cloned into pIND4	This study

Continued...

Plasmid	Description	Source
pIND-Y3	<i>cheY</i> ₃ cloned into pIND4	This study
pIND-Y3D53A	<i>cheY</i> ₃ (D53A) cloned into pIND4	This study
pIND-Y3D53N	<i>cheY</i> ₃ (D53N) cloned into pIND4	This study
pIND-Y4	<i>cheY</i> ₄ cloned into pIND4	This study
pIND-Y4D53A	<i>cheY</i> ₄ (D53A) cloned into pIND4	This study
pIND-Y4D53N	<i>cheY</i> ₄ (D53N) cloned into pIND4	This study
pIND-Y5	<i>cheY</i> ₅ cloned into pIND4	This study
pIND-Y6	<i>cheY</i> ₆ cloned into pIND4	This study
pIND-Y6D56A	<i>cheY</i> ₆ (D56A) cloned into pIND4	This study
pIND-Y6D56N	<i>cheY</i> ₆ (D56N) cloned into pIND4	This study
pIND-YFPY6	<i>yfp-cheY</i> ₆ cloned into pIND4	This study
pIND-YFPY6D56A	<i>yfp-cheY</i> ₆ (D56A) cloned into pIND4	This study
pIND-ecY	<i>E. coli cheY</i> cloned into pIND4	This study
pBAD-ecYD13K	<i>E. coli cheY</i> (D13K) expression plasmid. pBAD24 derivative	Y. Sowa
pIND-ecY**	<i>E. coli cheY</i> (D13K, Y106W) cloned into pIND4	This study
pIND-ecYZ	<i>E. coli cheY, cheZ</i> cloned into pIND4	This study
pIND-ecZ	<i>E. coli cheZ</i> cloned into pIND4	This study

Table 2.2: Plasmids used in this study. Kn^R , Ap^R denote ampicillin and kanamycin resistance respectively. $\text{P}_{\text{A1/04/03}}$ denotes an isopropyl -D-1-thiogalactopyranoside (IPTG) inducible promoter. †See Appendix B.1, Page 21 for pIND4 plasmid map.

STANDARD DNA TECHNIQUES

Preparation of Plasmid DNA - Carried out using the QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer's instructions.

Restriction Digests - Carried out using enzymes from New England Biolabs (NEB); 0.5 μl of each enzyme, 2 μl 10X buffer, 2-4 μl DNA to be digested, made up with sterile water to a total volume of 20 μl .

Gel Electrophoresis - Multi purpose agarose (Roche) was made up at between 0.8 and 2 % in 0.5X TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.0) and electrophoresed at between 100 and 150 V. The gels were stained for 20 mins in 1 ng/ml

ethidium bromide and visualised on a UV transilluminator.

Purification of DNA Fragments - DNA fragments were cleaned from agarose gels or from solution using the Genelute Gel Extraction Kit (Sigma) according to the manufacturer's instructions.

Ligations - 2 μ l T4 DNA ligase (NEB) was used in a final volume of 50 μ l with vector and insert DNA in a ratio of about 1:3 and the supplied reaction buffer and incubated at 15 °C for at least 16 hours.

Competent E. coli Cells - Prepared using the calcium chloride method of Sambrook and Russell [20] and stored in 200 μ l aliquots at -80 °C.

Transformation - DNA was transformed by incubation with 200 μ l of the competent cells on ice for 1 hour, followed by 2 min at 42 °C and 2 min on ice. 1 ml of LB was added and incubated at 37 °C for 1 hour. The mixture was spread on LB-agar plates containing appropriate antibiotics.

Polymerase Chain Reaction (PCR) - Reactions consisted of DNA template (~100 ng), 100 pmol of each primer (Sigma-Genosys), 12.5 nmol dNTPs, and 1 unit of *pfu* DNA polymerase (Promega), made up to a final volume of 25 μ l. Thermocycling was performed in a Thermal MiniCycler (MJ Research) as follows:

Step 1	98°C x 5 min
Step 2.1	98°C x 2 min
Step 2.2	55°C x 1.5 min
Step 2.3	72°C x 4 min
Step 3	repeat step 2, 25 times
Step 4	72°C x 5 min

DNA Sequencing - All constructs were sequenced before use by the Automated DNA Sequencing Service (Geneservice, Source BioScience) using BigDyeTM dye terminators (PE Biosystems) on a 3730 DNA sequencer (Applied Biosystems). Sequences were assembled using the Staden Software Package and analysed using Clone Manager Software (Scientific and Educational Software).

DIG Labelling of DNA Probes for Southern Blot - DNA for use as a probe in Southern blots was purified from agarose gels as described previously and labelled using

the random hexanucleotide priming technique with the DIG DNA Labelling and Detection Kit (Roche) according to the manufacturer's instructions.

Southern Blotting - DNA for Southern blotting was separated on an agarose gel, depurinated, denatured, neutralised and transferred to Hybond-N membrane (Amersham) by capillary transfer as described in [20]. The membrane was dried, UV fixed, prehybridised for at least 3 hours. Hybridisation of the probe was carried out at 68 °C in a Hybaid oven over night. Detection was performed using the DIG colour detection kit (Roche) according to the manufacturer's instructions.

Conjugation - Plasmids were conjugated into *R. sphaeroides* from the donor strain *E. coli* S17-1 λ -pir. A stationary phase culture of S17-1 containing the appropriate plasmid was diluted 1:30 into fresh medium and grown at 37 °C to an optical density (OD) of OD₆₀₀ ~0.1. 1 ml of the *E. coli* culture and 1 ml of stationary phase *R. sphaeroides* were centrifuged at 4000 g, washed gently in succinate medium and finally resuspended in 100 μ l of succinate medium. 10 μ l of *E. coli* was mixed with 100 μ l of *R. sphaeroides* cells and pipetted onto a 0.45 μ m filter (Whatman) on an LB-agar plate. After overnight incubation at 30 °C the filter was washed with 1 ml of succinate medium and plated on 1.5 % LB-agar plates containing kanamycin.

R. sphaeroides Chromosomal DNA Preparation - 1.5 ml of cells were spun down and frozen in liquid nitrogen. The pellet was resuspended in 0.5 ml of 65 °C lysis buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 1 % SDS) and 0.1 mg proteinase K added. This was incubated at 42 °C for 2 hours and extracted twice with 0.5 ml phenol:chloroform:isoamyl alcohol (25:24:1 v/v). The DNA was precipitated with 100 % ethanol, washed in 70 % ethanol and dried. The pellet was resuspended in 30 μ l of water with RNAase and stored at 4 °C until required.

WILD-TYPE MOTOR OUTPUT:
SPEED, TORQUE AND STOPS

FUNDAMENTAL MOTOR CONTROL

MOTOR CONTROL AT THE MOLECULAR LEVEL

5.1 OVERVIEW AND AIMS

5.1.1 COPY NUMBERS OF CHEY_{3/4/6} PER CELL

bla bla bla

THE STOP MECHANISM

DISCUSSION AND FUTURE WORK

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MEDIA RECIPES AND REAGENTS

LB Medium

Bacto-tryptone 10g/l
Yeast extract 5g/l
NaCl 5g/l
pH 7.0

Succinate Medium

1M Phosphate buffer 20ml
Concentrated base 20ml
Ammonium sulphate 0.5g
Growth factors 2ml
Sodium succinate 2g
NaCl 0.5g
Casamino acids 1g
Made up to 1l, pH 7.2

M22 Medium

As for succinate medium but omitting sodium succinate and casamino acids.

Concentrated Base

Nitriloacetic acid (disodium salt) 5.94g
Metals 44 solution 25ml
MgSO₄·7H₂O 14.5g
CaCl₂·6H₂O 2.5g
FeSO₄·7H₂O 50mg
Ammonium molybdate (4H₂O) 4.6mg

Made up to 1l, pH 6.8. Store at 4 °C.

Metals 44 Solution

EDTA 2.5g

ZnSO₄·7H₂O 11g

FeSO₄·7H₂O 5g

MnSO₄·4H₂O 2g

CuSO₄·5H₂O 0.39g

CoCl₂·6H₂O 0.2g

Boric acid 0.12g

Sulphuric acid 1.5ml

Made up to 1l and filter sterilised. Store at 4 °C.

Growth Factors

NaHCO₃ 50mg

Biotin 2mg

Niacin 100mg

Thiamine 50mg

Made up to 100ml and filter sterilised. Store at 4 °C.

1M Phosphate Buffer

Buffer A 136.8g K₂HPO₄·3H₂O in 600ml water

Buffer B 68g KH₂PO₄ in 500ml water

Mix 61.5ml Buffer A with 38.5ml Buffer B, adjust to pH 7.0 and store at 4 °C.

HEPES

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid 2.38g in 1l water, pH 7.2 (NaOH)

5X SDS-PAGE Loading Dye

Glycerol 25ml

0.5M Tris pH 6.8 22.5ml

SDS 2.5g

β-mercaptoethanol 2.5g

Phosphate Buffered Saline (PBS) Buffer

NaCl 8g

KCl 0.2g

Na₂HPO₄·12H₂O 1.44g

KH₂PO₄ 0.24g

Made up to 1l, pH 7.4

MOLECULAR MICROBIOLOGY

B.1 PLASMID MAP - pIND4

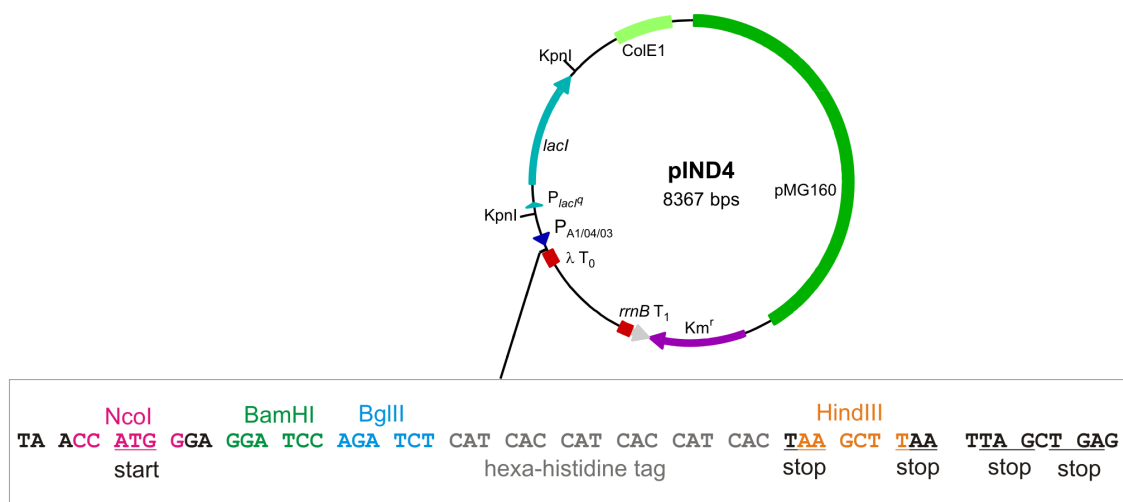


Figure B.1: pIND4 plasmid map showing important features, taken from [16]. Expanded section shows the cloning site - inserts must start with the ATG codon which forms part of the *NcoI* restriction site. If the stop codon of the inserted gene is omitted, a C-terminal histidine tag will be added. All the pIND4 derivatives made in this work included the stop codon so no tag was added.

B.2 PRIMERS

In the tables below, restriction cut sites are underlined and deliberate mutations are marked in **bold**.

Name	Sequence	Restriction Site
Y6-A	AATAC <u>CC</u> CATGGTGAGCAAGGGCGAGGAG	<i>Nco</i> I
Y6-B	CATCTCGAT GGC GAGCAGGAT	-
Y6-C	ATCCTGCTC GCC ATCGAGATG	-
Y6-D	AATAC <u>GGATCCT</u> CAGGCGGCCATCAGCGTC	<i>Bam</i> HI

Table B.1: Primers for making *yfp-cheY*₆(D56A) using pIND-YFPY6 as a template. The *cheY*₆(D56A) gene was later amplified from this plasmid to give pIND-Y6D56A using primers Y6-F and Y6-R (Table B.3).

Name	Sequence	Restriction Site
Ec-Y-A	TACACCATGGCGGATAAAGAACTT	<i>Nco</i> I
Ec-Y-B	CTTCACCA CCC AGCCACTGGC	-
Ec-Y-C	GCCAGTGGCT TGGG TGGTGAAG	-
Ec-Y-D	ATCG <u>GGATCCT</u> CAAAATCCAAGACTATC	<i>Bam</i> HI

Table B.2: Primers for introducing the Y106W mutation in *E. coli cheY*. The AB PCR was carried out on a pBAD-ecYD13K template. The CD PCR was amplified from *E. coli* RP437 genomic DNA. The overlap PCR (AD) yields a 1053 bp product containing *cheY* (including the two point mutants) and the neighbouring *cheZ* gene. Following digestion, this product was ligated into cut pIND4. After sequencing the correct clone was used as a template to amplify just *cheY*(D13K, Y106W) (commonly known as CheY**) with primers Ec-Y-F and Ec-Y-R (Table B.3).

Name	Target	Sequence	Restriction Site
Ec-Y-F	<i>E. coli cheY</i>	TACACCATGGGGATAAAGAACTT	<i>NcoI</i>
Ec-Y-R	<i>E. coli cheY</i>	ATCGGATCCTCACATGCCAGTTTCTC	<i>BamHI</i>
Ec-YZ-F	<i>E. coli cheY cheZ</i>	Same as Ec-Y-F	<i>NcoI</i>
Ec-YZ-R	<i>E. coli cheY cheZ</i>	ATCGGATCCTCAAATCCAAGACTATC	<i>BamHI</i>
Ec-Z-F	<i>E. coli cheZ</i>	AGCTAGGGTCTCCCATGATGCAACCATCAATC	<i>BsaI</i>
Ec-Z-R	<i>E. coli cheZ</i>	Same as Ec-YZ-R	<i>BamHI</i>
Y1-F	<i>cheY₁</i>	AGCTAGGGTCTCCCATGCCGCTGACCGTTCTTGCC	<i>BsaI</i>
Y1-R	<i>cheY₁</i>	AATACGGATCCTCACGGACCGCCACGGG	<i>BamHI</i>
Y2-F	<i>cheY₂</i>	AGCTAGACCTGCATATCATGCGACTCAGGGACAGCATT	<i>BfuAI</i>
Y2-R	<i>cheY₂</i>	AATACAAGCTTTCATAGAGCGCCTACGAC	<i>HindIII</i>
Y3-F	<i>cheY₃</i>	AGCTAGACCTGCATATCATGAGCAGGACGGTTCTCGCC	<i>BfuAI</i>
Y3-R	<i>cheY₃</i>	AATACGGATCCTCATCCGAGCACCTTCTTG	<i>BamHI</i>
Y4-F	<i>cheY₄</i>	AGCTAGACCTGCATATCATGACGAAACCGTCCCTCGCA	<i>BfuAI</i>
Y4-R	<i>cheY₄</i>	AATACGGATCCTCAGCCAAGAAGCTTCTTC	<i>BamHI</i>
Y5-F	<i>cheY₅</i>	AGCTAGACCTGCATATCATGAGCAAGACGATCCTCGCG	<i>BfuAI</i>
Y5-R	<i>cheY₅</i>	AGCTAGACCTGCATATCATGAGCAAGACGATCCTCGCG	<i>BamHI</i>
Y6-F	<i>cheY₆</i>	AGCTAGACCTGCATATCATGCCCTACAATGTCATGATC	<i>BfuAI</i>
Y6-R	<i>cheY₆</i>	AATACGGATCCTCAGGGGCCATCAGCGTC	<i>BamHI</i>
YFP-Y6-F	<i>yfp-cheY₆</i>	AATACCCATGGTGAGCAAGGGCGAGGAG	<i>NcoI</i>
YFP-Y6-R	<i>yfp-cheY₆</i>	AATACGGATCCTCAGGGGCCATCAGCGTC	<i>BamHI</i>

Table B.3: Primers for cloning genes into pIND4. All genes were cloned into *NcoI*/*BamHI* cut pIND4 (except for *cheY₂* which was cloned into *NcoI*/*HindIII*) cut pIND4. Note *BsaI* and *BfuAI* are compatible with *NcoI*.

APPENDIX C

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ONLINE RESOURCES

The digital appendix divided into the following sections, is available in a CD at the back of the paper copy or online:

<http://databank.ouls.ox.ac.uk/objects/dataset:3.html>

D.1 THESIS

L^AT_EX files including the style file and figures used to compile this document.

D.2 VIDEOS

Videos relevant to this project.

D.3 MY PROTOCOLS

Step by step protocols used in this project, from molecular biology fundamentals to *R. sphaeroides* swarm plates and the bead assay.

D.4 BEAD ROTATION DATA

Divided into the following sections - (1) Wild-type Bead Rotation Data, (2) JPA1353 Bead Rotation Data, (3) Flow Cell Bead Data, and (4) Analysis Programs.