Protocols

PCRs

Colony PCRs

We used a buffer mix with polymerase (either Mango Mix (forhandler) or 2x High Fidelity) and the appropriate primers and template DNA.

	For a 10 µL reaction
2x Mango Mix/2x High Fidelity Master Mix	5 µL
Template DNA dissolved in MiliQ H_2O	4 µL
Forward primer, 10 µM	0.5 µL
Reverse primer, 10 µM	0.5 µL

Program		
Step	Temperature	Time
1	98°C	01.00
2c	98°C	00.15-30
3c	55°C	00.15-30
4c	72°C	00.15-45
5	72°C	07.00
6	15°C	--.--

Steps 2-4 were repeated 25-35 times.

PCR for amplification of the MamC gene, eGFP or eFbFP

We used Phusion polymerase with the GC buffer.

	For a 50 µL reaction
5x Phusion buffer	10 µL
3% DMSO	1.5 µL
dNTPs, 10 mM	1 µL
Forward primer, 10 µM	2.5 µL
Reverse primer, 10 µM	2.5 µL
Template DNA	2.5 µL
Phusion polymerase	0.5 µL
MiliQ H_2O	29.5 µL

Program PCR		
Step	Temperature	Time
1	98°C	01.00
2c	98°C	00.15-30
3c	55°C	00.15-30
4c	72°C	00.15-45
5	72°C	07.00
6	15°C	--.--

Gradient PCR

We used the Hot Master polymerase for gradient PCRs. Gradient PCRs can be useful for finding the optimal temperature for the PCR reactions.

	For a 50 µL reaction
10 x Hot Master buffer	5 µL
dNTPs, 10 mM	1 µL
Forward primer, 10 µM	2 µL
Reverse primer, 10 µM	2 µL
Template DNA	2.5 µL
Hot Master polymerase	0.5 µL
MiliQ H_2O	37 µL

Program Gradient PCR		
Step	Temperature	Time
1	94°C	02.00
$2c^1$	94°C	00.20
$3c^1$	65°C	01.00
$4c^2$	94°C	
$5c^2$	Gradient 56-65°C	00.10
$6c^2$	65°C	01.00
7	65°C	07.00
8	15°C	--.--

c^1 for 5 repeats, c^2 for 35 repeats.

Purifications

Concentration of the purified PCR product or plasmid was checked with a NanoDrop Spectrophotometer from Thermo Scientific? Followed by sequencing kindly sponsored by Macrogen.

Gel purification

Was performed with the QIAquick Gel Extraction Kit from QIAgen.

Miniprep

Plasmids were purified from bacteria using the QIAprep Spin Miniprep Kit from QIAgen.

Purification of PCR products

Was performed with the QIAquick PCR Purification Kit from QIAgen.

Cloning

Restriction digestions

The vector and the intended insert were both digested with the same two restriction enzymes (BamHI + EcoRI or XhoI+NdeI) to ensure directionality of the insertion.

Digestion of vector	
Vector (pJET/pDrive/pBBR1MCS-2)	5 µL
10x CutSmart Buffer	5 µL
BamHI/XhoI	1 µL
EcoRI/NdeI	1 µL
MiliQ H_2O	38 µL

Digestion of insert	
Insert	5 µL
10x CutSmart Buffer	5 µL
BamHI/XhoI	1 µL
EcoRI/NdeI	1 µL
MiliQ H_2O	38 µL

The digestion reaction was inactivated with 65°C for 10 min or QIAquick PCR Purification Kit was performed immediately.

Gateway Cloning

BP reaction:

x ul pDONR 207 (approx. 150 ng)

x ul PCR fragment with prefix attB1 and suffix attB2 (approx. 150 ng)

x ul Water

2 ul BP Clonase Enzyme mix

Total 10ul

Leave the mix at the bench for min. 1 hour (no maximum). Inactivate reaction using Proteinase K for 10 min. at 37 °C. Transform the whole mixture using the E. cloni system.

LR reaction:

Linearize entry-clone (pDONR207 with insert) using restriction enzyme that only cut one time (and not in the insert or pre- and suffix).

x ul pJAM1786 (approx. 150 ng)

x ul Linearized entry-clone (approx. 150 ng)

x ul Water

2 ul LR Clonase Enzyme mix

Total 10ul

Leave the mix at the bench for min. 1 hour (no maximum). Inactivate reaction using Proteinase K for 10 min. at 37 °C. Transform the whole mixture using the E. cloni system.

Ligation

Insert and cut vector were ligated together using the T4 DNA ligase kindly sponsored by New England BioLabs.

Transformation

The protocol for the E. cloni system by Lucigen was used. Herunder 400 µL of recovery medium was added before recovery incubation.

Culturing anaerobes

Medium recipes

Standard LB medium containing appropriate selection (AMP, KAN, CAM or GEN) was used when growing *E.Coli*. The following to kinds of media was used when growing anaerobic bacteria:

ATCC medium: http://www.atcc.org/~/media/3180B81383704F779CE290819FA539EE.ashx

Activated Charcoal medium (adapted from Shultheiss and Schüler, 2003):

Recipe for 0.5 L solid medium:
500 mL water, 1.19 g HEPES, 1.50 g sodium pyruvate, 50 mg yeast extract, 1.50g peptone, 170 mg sodium nitrate, 50 mg potassium dihydrogenphosphate, 75 mg magnesium sulfate heptahydrat, 1.5 g activated charcoal.

Adjust to pH=7.0 using NaOH and add 7.5 g agar.

Autoclave media

Before pouring plates: Add 500 µM ferric citrate and 1mM DTT.

Growing anaerobic bacteria

When growing liquid cultures, Falcon tubes were flushed with the nitrogen before use and incubated at 28 degrees. Plates were kept in a closed container with an Oxoid AnaeroGen pad.

Enrichment of magnetotactic bacteria

http://www.jove.com/video/50123/collection-isolation-enrichment-naturally-occurring-magnetotactic

Protein analysis

SDS-PAGE:

Approx. 2 mL of cell culture was spun down (17900 g, 10 min) and the supernatant was discarded. Cells were resuspended in 200 µL SDS loading buffer and heated (95°C, 10 min) to lyse the cells. Cell debris were spun down (17900 g, 10 min) and discarded. Lysate was loaded on 12 % polyacrylamide gel and run for 1 h @ 100 V. The gel was stained in Coomassie Brilliant Blue for ~1h and destained for ~3 h.

Western blot:

SDS-PAGE procedure done without staining. Proteins were transferred to membrane (PVFD, activated 2 min in methanol) via a wet WB "sandwich" (cathode - pad - Whatmann paper – gel – membrane - Whatmann paper – pad – anode). The transfer was carried out at 100 V for 30-45 min.

The membrane was blocked for min 1 h in 5 % m/v milk in PBS-T. The membrane was the washed with PBS-T for 3 x 10 min. The membrane was the incubated in primary antibody solution (5 % m/v milk in PBS-T containing primary antibody). Remaining primary antibodies were removed by washing the membrane for 3 x 10 min in PBS-T.

The membrane was incubated with HRP-conjugated secondary antibodies (in 5% m/v milk in PBS-T) for min. 1 h. Remaining antibodies were removed in 3 x 10 min PBS-t washes.
The western blot was then visualized using SuperSignal West Pico Chemiluminescent Substrate from Thermo Scientific.