3. Materials and methods

3.1 Materials

3.1.1 Equipment

Table 3.1 List of used devices

Device	Name	Manufacturer
Absorbance Reader	a)Tecan Sunrise	Tecan
	b)Tecan Spektramax	Tecan
Autocrosslinker	UV Stratalinker	Stratagen
Centrifuge	a) Zentrifuge 5415R	Eppendorf
	b) Sorvall RC-5 Superspeed Refrigerated Centrifuge	Du Pont Instruments
	c) Labofuge400	Heraeus
Colony Counter	Acolyte	Synbiosis
Electrophoresis Chamber	a) Mini ReadySubb Cell GT	Bio-Rad Laboratories
	b) PowerPack 300 Supply	Bio-Rad Laboratories
Electroporator	Cell Porator	Life Technologies Gibco BRL
FPLC	ÄKTAprime	Amersham Biosciences
Hemacytometer	Neubauer Zählkammer	Roth
Incubator	a) B20	Heraeus
	b) CO_2-Inkubator	Heraeus
Microscope	a) DM IL Typ 090.135.002	Leica
	b) IMT	Olympus
pH-Electrode	Toledo MP225 pH Meter	Mettler
Photo Printer	Video Graphic Printer UP-895CE	Sony
Photometer	a) Beckman Photometer DU520	Beckman Coulter
	b) Ultrospec UV/Vis Spectrophotometer	Amersham Biosciences
Plater	Whitley Spiral Plater	dw Scientific
Protein Blotter	Mini Trans-Blot Transfer Cell	Bio-Rad Laboratories

Table 3.1 List of used devices

Device	Name	Manufacturer
Protein Gel Electrophoresis	a) horizontal: Multiphor II	Amersham Biosciences
	b) vertical: Mini Protean 3	Bio-Rad Laboratories
Refrigerator	+4°C/ -20°C/ -80°C	
Shaker (for liquid cultures)	a) G25 Incubator Shaker	New Brunswick Scientific Co.
	b) TH25	Edmund Bühler
	c) Innova™ 4230 Refrigerated Incubator Shaker	New Brunswick Scientific Co.
Shaker	Unimax 1010	Heidolph
Sonicator	Sonoplus	Bandelin
Sterile Bench	a) HeraSafe	Heraeus
	b) *mikrobiologische Sicherheitskabine*	Karl Bleymehl Reinraumtechnik
Stirrer	Variomag Mono	NeoLab
Thermocycler	Thermocycler T-Gradient	Biometra
Thermomixer	Thermomixer Comfort	Eppendorf
TLC Chamber	Glass Chamber, dimensions: 35×25×12 cm	Desaga
Ultrafiltration Device	Pellicon XL 50	Millipore
UV-Transluminator	Gel Doc 2000	Bio-Rad Laboratories
Vacuum concentrator		Bachofer
Vacuum Pump	Typ N86 KN.18	KNF Neuberger Laboport
Vacuum Blotter with Regulator	Model 785	Bio-Rad Laboratories
Vortex Mixer		NeoLab
Weighing Machine	a) CP64	Sartorius AG
	b) 1212 MP	Sartorius AG

3.1.2 Special materials

Table 3.2 List of special materials

Description	Name or Properties	Order Number	Manufacturer
Anionexchange Column (prepacked)	a)Resource Q 1ml	17-1177-01	Amersham Biosciences
	a)Resource Q 6ml	17-1179-01	Amersham Biosciences
Chromatography Paper	17 CHR	3017915	Whatman
Centrifugal Filter Devices	a)Microcon XM-3	42404	Millipore
	b)Microcon XM-10	42407	Millipore
	c)Microcon XM-50	42409	Millipore
	d)Amicon 4, 10 kDa	UFC801024	Millipore
	e)Amicon Ultra-15, 30 kDa	UFC 903024	Millipore
Gelfiltration Column (prepacked)	HiLoad 26/60 Superdex 200 Column	17-1071-01	Amersham Biosciences
Silica Gel Plates	*Kieselgel* 60 WF_{254s}	034.5715	VWR
Electroporation Chambers	For Cell Porator (0.2 cm)	11608031	Biometra
Hybridization Bags		1 666 646	Roche
Membran for Ultrafiltration Device	Ultracell Regenerated Cellulose, 30 kDa	PXC0 30C 50	Millipore
Microtiter Plates	96 well	650001	Anicrin
Microtiter Plates for Cell Culture	24 well	3911925	Greiner
Nylon Membrane	Positively Charged	1 209 299	Roche
Glass Test-Tubes (for bacterial liquid cultures)	12.5×100 0.9 mm ARG grade	192772119	Neolab
Glass beads	0.45-0.5 mm	432N4035	Merck
Syringe	Omnifix 1 ml	612-0110	VWR

Table 3.2 List of special materials

Description	Name or Properties	Order Number	Manufacturer
Transfer Membrane	Immobilon P SQ 10×10 (PVDS)	ISEQ 10100	Millipore
Cassette for X-Ray Film	Cassette 30×40	X267.1	Roth
X-Ray Film	Lumi-Film Chemiluminescent Detection Film	1 666 916	Roche
Ready Protein Gels	a) horizontal: Tris-HCl Gel 12%	161-1102	Bio-Rad
	b) vertical: Excel Gel SDS, 12.5 (plastic backed) & Buffer Strips	80-1261-01&17-1342-01	Amersham Biosciences

3.1.3 Chemicals and lipids

Table 3.3 List of used chemicals

Name	Catalog Number	Manufacturer
Acetic Acid	1.00063.1000	VWR
Agar	1.01615.1000	VWR
Agarose	V3125	Promega
Albumin Bovine Serum	A7638	Sigma-Aldrich
Anti Digoxigenin AP 150 U	1 093 274	Roche
Blocking Reagent	1 096 176	Roche
Calcium Chloride Dihydrate	1.02382.0500	VWR
Casein	C 8654	Sigma-Aldrich
Chloroform	1.02445.1000	VWR
CSPD	1 655 884	Roche
(D-)Glucose 95% Powder	G-7021	Sigma-Aldrich
Diethyl Ether	1.00921.1000	VWR
Dig DNA Labelling Mix	1277065	Roche
Dig-Easy-Hyb Granules	1 796 895	Roche
Dimethyl Sulfoxid (DMSO)	20385	VWR

Table 3.3 List of used chemicals

Name	Catalog Number	Manufacturer
Disodium Hydrogen Phosphate Heptahydrate	1.06575.1000	VWR
dNTPs PCR Nucleotide Mix 10 mM	US77212-500	Amersham Biosciences
Ethanol (100%)	1.00974.1011	VWR
Ethidium Bromide	1.11608.0030	VWR
Ferric Ammonium Sulfate	F-1543	Sigma-Aldrich
Fetal Calf Serum (FCS)	Ch.-Nr. A01122-471 PAA/A-15-043	PAA Laboratories Linz
Glycerol 86%	7533.1	Roth
Glycine (for electrophoresis)	G-8898	Sigma-Aldrich
IPTG (Isopropyl-β-D-thiogalactoside)	I-6758	Sigma-Aldrich
Isopropanol	109634	VWR
Legionella Agar Plate BCYEα	1860e	Heipha-Dr. Müller GmbH
Legionella Basis Agar	1.10242.0001	VWR
Legionella Growth Supplement	SR 0110C	Oxoid
Magnesium Sulphate Heptahydrat	1.05886.1000	VWR
Maleic Acid	8.17058.1000	VWR
Methanol	1.06009.2500	VWR
Naphtol Blueblack	195243-100G	Sigma-Aldrich
n-Hexane	1.04367.1000	VWR
Petrol Ether (Reag. Ph Eur, Tm 50-70°C)	1.59542.500	VWR
Phorbol-12-Myristate 13-Acetate (PMA)	P-8139	Sigma-Aldrich
Polyethylenglycol (PEG 600)	P-3390	Sigma-Aldrich
Ponceau S	P-7170	Sigma-Alrich
Potassium Acetate	60035	Fluka Biochemika
Potassium Dihydrogen Phosphate	1.04873.1000	VWR
Protease-Inhibitor Mix	39104.02	Serva
Proteose Pepton	ADM C66260	Oxoid
p-Nitrophenyl butyrate (p-NPB)	N-9876	Sigma-Aldrich

Table 3.3 List of used chemicals

Name	Catalog Number	Manufacturer
p-Nitrophenyl phosphate (p-NPP)	N-4645	Sigma-Aldrich
RPMI 1640 Powder	51800-043	Invitrogen
RNase-free Dnase, RQ1	M6101	Promega
Rnase OUT Recombinant Rnase-Inhibitor	10777-019	Invitrogen
Roti-Load 1, (reducing protein loading buffer)	K929.1	Roth
Roti-Load 2, (non-reducing protein loading buffer)	K930.1	Roth
Roti-Nanoquant (protein quantification)	K880.1	Roth
Saponin	S-4521	Sigma-Aldrich
SDS	L-4390	Sigma-Aldrich
Sodium Acetate	S-7653	Sigma-Aldrich
Sodium Azide	1.06688.0100	VWR
Sodium Chloride	1.06404.0500	VWR
Sodium Citrate	S-4641	Sigma-Aldrich
Sodium Tartrate	S-4797-100g	Sigma-Aldrich
Sodium EDTA	E-5134	Sigma-Aldrich
Sodium Hydroxide	1.06469.1000	VWR
Sodium Hypochloride Solution (6-14% active Chlorine)	1.05614.2500	VWR
Sucrose	1.07651.1000	VWR
Tris Base	1.08382.1000	VWR
Tris-HCl	1.08219.1000	VWR
Triton X-100	T-8787	Sigma-Aldrich
Trypan Blue (0.4%)	T-8154	Sigma-Aldrich
Trypsin-EDTA	L11-001	PAA
Tryptone	T-9410	Sigma-Aldrich
Tween20	8.17072.1000	VWR
Yeast Extract	ADM Z45380	Oxoid
β-Mercaptoethanol	115433	VWR
Zinc Chloride	1.08816.0250	VWR

Table 3.4 List of lipids

Name	Abbreviation	Catalog Number	Manufacturer
1,2-Dipalmitoyl-sn-Glycero-3-Phospho-rac-1-Glycerol	DPPG/PG	840455	Avanti
1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine	DPPC/PC	850355	Avanti
1-Palmitoyl-2-Hydroxy-sn-Glycero-3-Phospho-Glycerol	MPLPG/LPG	858122	Avanti
1-Palmitoyl-2-Hydroxy-sn-Glycero-3-Phosphocholine	MPLPC/LPC	855675	Avanti
1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphoethanolamine	DPPE/PE	805705	Avanti
1,2-Dipalmitoyl-sn-Glycerol	1,2-DG	800816	Avanti
1-Monopalmitoyl-rac-Glycerol	1-MPG	M-1640	Sigma-Aldrich
Glyceryl Tripalmitate	TPG	T-5888	Sigma-Aldrich
Palmitic Acid	FFA	P-0500	Sigma-Aldrich
3β-Hydroxy-5-Cholestene (Cholesterol)	Chol	C-3045	Sigma-Aldrich
5-Cholestene-3-Palmitate (Cholesterol Ester)	*CholE*	C-78607	Sigma-Aldrich

3.1.4 Kits

Table 3.5 Overview of used reagents and kits

Name	Catalog Number	Manufacturer
BigDye Terminator V. 3.1 Ready Cycle Sequencing Kit	4337035	Applied Biosystems
E.Z.N.A-Kit (for isolation of genomic DNA)	12-3450-00	Peqlab
Gel Filtration Calibration Kit, Low Molecular Weight	17-0442-01	Amersham Biosciences
Nefa C Kit	994-75409	Wako Chemicals
OneStep RT-PCR Kit	210212	Qiagen
pGEMTeasy Kit	A1360	Promega
Rneasy Mini Kit	74104	Qiagen
QIAprep® Spin Miniprep Kit	27104	Qiagen
QIAQuick® Gel Extraction Kit	28704	Qiagen
Quick Change Site Directed Kit	200519	Stratagene

Table 3.5 Overview of used reagents and kits

Name	Catalog Number	Manufacturer
Quantum Prep PCR Kleen Spin Columns	732-6300	Bio-Rad Laboratories
Quantum Prep Plasmid Midiprep Kit	732-6120	Bio-Rad Laboratories
Silver Staining Kit, Protein	17-1150-01	Amersham Biosciences

3.1.5 Buffers and solutions

Table 3.6 List of buffers and solutions

Name of Buffer	Components	Amount or Concentration
PBS (pH 7.2)	KCl	0.2 g
	KH_2PO4	0.2 g
	Na_2HPO4	1.15 g
	NaCl	8 g
	H_2Obidest ad	1000 ml
TE (pH 8.0)	Tris-HCl	10 mM
	Na_2EDTA	1 mM
Osmotic Shock Buffer	Sucrose	20 % (w/v)
	in TE Buffer	
Protein Purification		
AEX Running Buffer (pH 7.5)	Tris-HCl	20 mM
	Na_2EDTA	5 mM
AEX Elution Buffer (pH 7.5)	Tris-HCl	20 mM
	Na_2EDTA	5 mM
	NaCl	1 M
SDS PAGE Running Buffer (10×) do not adjust pH	Tris Base	30.3 g
	Glycine	144.0 g
	SDS	10.0 g
	H2Obidest ad	1000 ml
Transfer Buffer for Blotting do not adjust pH	Tris Base	3.03 g
	Glycine	14.4 g
	H_2Obidest ad	1000 ml
DNA Electrophoresis		

Table 3.6 List of buffers and solutions

Name of Buffer	Components	Amount or Concentration
50×TAE (pH 8.0)	Tris-Base	2.0 M
	Acetic Acid	1.0 M
	Na_2EDTA	0.1 M
DNA Loading Buffer	Bromphenol Blue	0.07 % (w/v)
	Ficoll 400	20 % (w/v)
Southern Blot		
Depurination	HCl	250 mM
Denaturation	NaOH	0.5 M
	NaCl	1.5 M
Neutralization (pH 7.5)	Tris-HCl	0.5 M
	NaCl	1.5 M
20×SSC (pH 7.0), Autoclaved	NaCl	3 M
	Sodium Citrate	300 mM
Equilibration	10×SSC	
Buffer for Blotting	10×SSC	
Low Stringency Buffer	2×SSC	
	SDS	0.1 %
High Stringency Buffer	0.1×SSC	
	SDS	0.1 %
Washing Buffer (pH 7.5)	Maleic Acid	0.1 M
	NaCl	0.15 M
	Tween-20	0.3 % (v/v)
Maleic Acid Buffer (pH 7.5), adjust with NaOH tablets	Maleic Acid	0.1 M
	NaCl	0.15 M
Detection Buffer (pH 9.5)	Tris	0.1 M
	NaCl	0.1 M

Table 3.6 List of buffers and solutions

Name of Buffer	Components	Amount or Concentration
CSPD Working Solution	CSPD 1:100 in Detection Buffer	
Blocking Solution	1 % (w/v) Blocking Reagent (Roche) in Maleic Acid Buffer, Autoclaved	
Antibody Solution	Anti Digoxigenin AP 1:10000 (75 mU/ml) in Blocking Solution	
Pre Hybridization	Dig-Easy-Hyb Granules in H2Obidest (according to the manufacturer's instruction)	
Hybridization	Pre Hybridization Solution with DIG Labeled Probe	

3.1.6 Culture media

Table 3.7 List of used culture media

Legionella pneumophila			
BCYEα agar	27.5 g *Legionella* Agar Base	BYE broth	14.45 g *Legionella* Growth Supplement
	14.45 g *Legionella* Growth Supplement		10 g Yeast Extract
H_2O_{bidest} ad 1000 ml			H_2O_{bidest} ad 1000 ml
BCYE: Buffered Charcoal Yeast Extract			
Suspend *Legionella* agar base in H_2O_{bidest} and autoclave. Let cool to ~50 °C and add sterile *Legionella* growth supplement dissolved in 100 ml H_2O_{bidest}			Dissolve *Legionella* growth supplement together with the yeast extract in H_2O_{bidest} and filter sterilize.
Escherichia coli			
LB Agar	10 g Trypton	LB broth	10 g Tryptone
LB: Luria-Bertani	5 g Yeast Extract		5 g Yeast Extract
	5 g NaCl		5 g NaCl
	15 g Agar		
	H_2O_{bidest} ad 1000 ml		H_2O_{bidest} ad 1000 ml
Suspend all components in H_2O_{bidest} and autoclave.			
Acanthamoeba castellanii			
PYG	2 % (w/v) Proteose Peptone		

Table 3.7 List of used culture media

	0.1 % (w/v) Yeast extract
	40 mM $MgSO_4$ ×7 H_2O
	40 mM $CaCl_2$ × 2 H_2O
	25 mM Na_2HPO_4 × 7 H_2O
	25 mM KH_2PO_4
	0.5 mM $Fe(NH_4)_2(SO_4)_2$ × 6 H_2O
	3 mM Na Citrate
	0.1 M Glucose
PYG: Peptone-Yeast Extract-Glucose	
Suspend all components except the glucose in H_2O_{bidest} and autoclave. Then add filter sterilized glucose solution	
Infection medium for Co-Infection of *A. castellanii* with *L. pneumophila*	
PYG without peptone, yeast extract, and glucose	
U937 macrophages and A549 epithelial cells	
RPMI	RPMI 1640 powder (Invitrogen)
	10 % (v/v) heat inactivated (10 min at 65 °C) FCS
	Prepared according to method D (see Gibco catalog (2004) pages 1-39)
Culture media for elekctrocompetent *E. coli* and for electroporated *E. coli*	
SOB	20 g Tryptone
	5 g Yeast Extract
	0.584 g NaCl
	0.186 g KCl
	H_2O_{bidest} ad 1000 ml (pH 7.0)
Suspend all components in H_2O_{bidest} and autoclave.	
SOC	98 ml SOB medium
	1 ml 2 M Mg^{2+} stock solution
	(20.33 g $MgCl_2$ × H_2O, and 24.65 $MgSO_4$ × $7H_2O$ per 100 ml Mg^{2+} stock solution
	1 ml 2 M glucose stock solution (36.04 g glucose per 100 ml stock solution)
Autoclave the Mg^{2+} stock solution. Filter sterilze the glucose stock solution and add both solutions to SOB.	

3.1.7 Antibiotics

Antibiotic stock solutions were prepared by dissolving the indicated amount of antibiotics in the respective solvent followed by filter-sterilization. The stock solutions were kept in aliquots at -20 °C and were added to the media at the indicated concentrations.

Table 3.8a List of used antibiotics

Antibiotic	Catalog Number	Manufacturer
Ampicillin Sodium Salt	A-9518	Sigma-Aldrich
Chloramphenicol	C-1919/ C-0378	Sigma-Aldrich
Gentamicin Solution 50 mg/ml	15750-037	Gibco
Kanamycin Sulfate	K-1377	Sigma-Aldrich

Table 3.8b Concentrations of used antibiotics and other chemicals

Antibiotic		Stock Solution	Final concentration for *L. pneumophila* [µg/ml]	Final concentration for *E. coli* [µg/ml]
Ampicillin	(Amp)	100 g/l	Not applicable	100
Chloramphenicol	(Cm)	30 g/l EtOH	3-9	30
Gentamicin	(Gm)	50 g/l	10	10
Kanamycin	(Km)	50 g/l	25	50
IPTG		100 mM H_2O_{bidest}	1-5 mM	1 mM
X-gal		10 mg/ml	-	100 µl

3.1.8 Enzymes

3.1.8.1 Restriction endonucleases

All enzymes were purchased from New England Biolabs.

Table 3.9 List of used restriction endonucleases

Restriction Enzyme	Catalog Number
*Acc*I	R0161
*Acc*65I	R0599
*Afe*I	V0213
ApaI	R0114
*Ava*I	R0152
*Ava*II	R0153
*Hind*II	R0103
*Hind*III	R0104
*Ava*II	R0153
*EcoR*I	R0101
*EcoR*V	R0195
*Kpn*I	R0142
*Mfe*I	R0589
*Pst*I	R0140
*Sac*I	R0156
*Sac*II	R0157
*Sfo*I	R0606
*Sma*I	R0141
*Spe*I	R0133
*Sph*I	R0182
*Sty*I	R0500

3.1.8.2 Polymerases

Table 3.10 List of used polymerases

NAME	CATALOG NUMBER	MANUFACTURER
PCR Sequencing Mix (Big Dye 3.1)	4337035	Applied Biosystems
Platinum-Taq DNA High Fidelity	11304-011	Invitrogen
Pfu DNA Polymerase, Recombinant	EP0501	MBI Fermentas
RT-PCR Enzyme Mix (Reverse Transkriptase; HotStar *Taq* DNA Polymerase)	210212	Qiagen
Taq DNA Polymerase	M0267	New England Biolabs

3.1.8.3 Further enzymes

Table 3.11 List of other enzymes

Enzyme	Catalog Number	Manufacturer
DNaseI	79254	Qiagen
Lysozyme	L-7651	Sigma-Aldrich
Protein Kinase K	P-2308	Sigma-Aldrich
RNase	R-7003	Sigma-Aldrich
T4 DNA Ligase	M0202	New England Biolabs

3.1.9 Primers

All primers (purification grade: gel filtration) were purchased from TIB-Molbiol (Berlin, Germany) and are based on the sequence found in the *L. pneumophila* Philadelphia-1 database (38). Primers designated gdsl2 refer to *plaC* and primers designated gdsl3 refer to *plaD*.

Table 3.12a Primers used for cloning of the *L. pneumophila plaC* or *plaD* genes

Primer	Used For	Annealing Temperature	Primer Sequence	Size of Amplification Product [bp]
gdsl2_a1_f	amplification of *plaC* with promoter region	52/ 56/ 64.5°C	5′ -TGC TTA AAA ACC GCT CTG GA -3′	1559
gdsl2_b1_r			5′ -TTA ACG GCA TAT TGG GTG AA -3′	
gdsl2_c1_f	amplification of *plaC* without promoter region	56°C	5′ -TTA TGA TCC AAA ACA ACA GG-3′	1383
gdsl2_e1_r			5′ -GAG GAT CAA TTT AGA CAA CTT C -3′	
gdsl2_s2_f	RT-PCR, internal primers	61-62°C	5′ -TGG ATT GAG TAT TTG GCA GAA -3′	461
gdsl2_s2_r			5′ -CAC GTC ACG ATC AGT AGT TTT -3′	
gdsl3_a1_f	amplification of *plaD* with promoter region	62°C	5′ -ACG TCC GAT TTG TAT GAG TG -3′	1942
gdsl3_b1_r			5′ -TTT TGC AAG TGG ATT AGG TGA -3′	
gdsl3_c1_f	amplification of *plaD* without promoter region	53.5°C	5′ -GCAAATATCATGGCCCAAAAA -3′	1551
gdsl3_d1_r			5′ -GAGGGGACGCATTAAAACTTACCAG -3′	
gdsl3_s4_f	RT-PCR, internal primers	62°C	5′ -TAA TCA ACA ACT GCA GAC AAA A -3′	435
gdsl3_s3_r			5′ -AAG CTT CTT TAC CGG TTT ATC G -3′	

Table 3.12b Primers used for sequencing

PRIMER	PRIMER SEQUENCE
gdsl2_s1_f	5′ -GCT TGT CTG ATA ACG GTA ATA -3′
gdsl2_s2_f	5′ -TGG ATT GAG TAT TTG GCA GAA -3′
gdsl2_s3_f	5′ -CTG AAG TAA TGA GCA CCT A -3′
gdsl2_s4_f	5′ -GAA GAT CCG GAG AAA TAT GG -3′
gdsl2_s1_r	5′ -TCA TCC CAG AAT AAA TAG TC -3′
gdsl2_s2_r	5′ -CAC GTC ACG ATC AGT AGT TTT -3′
gdsl2_s3_r	5′ -TCA GGT TCT TGA TTG TTC CC -3′
gdsl2_s4_r	5′ -GAT ATT CCT GCA TCA AGC ACG -3′
gdsl3_s2_f	5′ -CTC CAA AAG GTC GAT TCA CTA A -3′
gdsl3_s3_f	5′ -TTG GGA GGA AAA CGG ACT CAA -3′
gdsl3_s4_f	5′ -TAA TCA ACA ACT GCA GAC AAA A -3′
gdsl3_s5_f	5′ -CAA GCA AAA CAT AGG CAC AAA G -3′
gdsl3_s6_f	5′ -GAC GAC AAG AAG CGA GGA TG -3′
gdsl3_s2_r	5′ -CTT CTT GTC GTC GCC TTG TTT -3′
gdsl3_s3_r	5′ -AAG CTT CTT TAC CGG TTT ATC G -3′
gdsl3_s4_r	5′ -GCC ATA TTG TTC GGG ATT ACT G -3′
gdsl3_s5_r	5′ -CGC CTG ACC ACT CCA CAA C -3′
gdsl3_s6_r	5′ -GCT CTA TCT CAA ATT GCT CTG C -3′

Table 3.13 Primers used for cloning of the *L. pneumophila plaB* gene

Primer	Annealing Temperature	Primer Sequence	Size of Amplification Product [bp]
pla1	56°C	5′-GAATTCAGTATAA AATAATCTAATATC-3′	1495
pla2		5′ –TCTAGATTAATCTA TCTTTTTCCCAGTTG -3′	

These primers were designed and used for cloning of the *plaB* gene by Klaus Heuner (Universität Würzburg) (76).

Table 3.14a Primers used for cloning of *L. pneumophila* 130b *unk1*

Primer	Used For	Annealing Temperature	Primer Sequence	Size of Amplification Product [bp]
unk1_a1_f	amplification of *unk1* with promoter region	56°C	5′ -gTCCCATCCgATTAgAACA-3′	1008
unk1_b1_r			5′ –AGCGCACATTAGAAAGCATA C-3′	
unk1_c1_f	RT-PCR, internal primers	56°C	5′ -CAC TAT AGC AAT GAA CAG GAA-3′	557
unk1_d1_r			5′ -TCG AAA AAG GGT AGA ATC ATA-3′	
unk1_m_f	site-directed mutagenesis	56°C	5′-GTCAGCGGTAATCTTACAGGATT GGACGCTCA GTGGAACGAAA AC-3′	
unk1_m_r			5′-ATCATCACAGCTCAGTTCATGT GCGCGGAACC CCTATTTG-3′	

Table 3.14b Primers used for sequencing of *unk1*

Primer	Primer Sequence
unk1_s1_f	5′ -ATC CTA TCT GAA AGA ATA TAA-3′
unk1_s2_f	5′ -TCT AAC GAT CCT GAT ATG ATT-3′
unk1_s3_r	5′ -TAT CGC GTG CTG TTC CGG ATC-3′
unk1_s4_r	5′ -GCC ATT TGA CTA AGA TTA GGC-3′

Table 3.15a Primers used for cloning of *L. pneumophila* Philadelphia-1 *lvrE*

Primer	Used For	Annealing Tempera-ture	Primer Sequence	Size of Amplification Product [bp]
lvrE_a1_f	amplification of *lvrE* with promoter region from Philadelphia-1→	56°C	5′-CACTCGAACTATAA ACCACAC-3′	1049

Table 3.15a Primers used for cloning of *L. pneumophila* Philadelphia-1 *lvrE*

Primer	Used For	Annealing Tempera-ture	Primer Sequence	Size of Amplification Product [bp]
lvrE_b1_r			5′ –AACCTAAAATATCAA CAAAAG-3′	
lvrE_c1_f			5′ –CGCCAGTAAAAGCA GAACAAA-3′	1254
lvrE_d1_r			5′ –CTTAGCTCATATAGA AAATC-3′	
lvrE_e1_f	RT-PCR, internal primers	56°C	5′-CAGGCAAATGACAAG GAACT-3′	556
lvrE_f1_r			5′-CATAGGCTGAAGGCAA ATACC-3′	
lvrE_mua1f	site-directed mutagenesis	56°C	5′-AAACCGACCTCAATAACAA TGAAGGGATACAG ACGCTCAGTGGAACGAA-3′	
lvrE-mub1r			5′-CAGCGCGGGCAT TGGTTACTTTATGTGCGCGG AACCCCTATT-3′	

Table 3.15b Primers used for sequencing of *lvrE*

Primer	Primer Sequence
lvrE_s1_f	5′ -GAGGCGTTATACAGTAAAATTGGT-3′
lvrE_s2_f	5′ -GGGCATTATCTTCTGGGTCGAT-3′
lvrE_s3_f	5′ -CCTTTGCCGCACTTCTG-3′
lvrE_s4_f	5′ -CCGGACTATTATTGGAGTTCGACT-3′
lvrE_s1_r	5′ - TAACATGCCCCAAAGTACTAGCTT -3′
lvrE_s2_r	5′ - CCTGATTGGTTGTCTATGGTTTG -3′
lvrE_s3_r	5′ - CCCCGTTATATTCTTCGCCAATG -3′
lvrE_s4_r	5′ - ATATGAGCCCAAAGTTCCTTGTCA -3′

Table 3.16a Primers used for cloning of *L. pneumophila* 130b *aas* with its putative promoter region

Primer	Annealing Temperature	Primer Sequence	Size of Amplification Product [bp]
aas_a1_f	56°C	5′ -TACAggAATTAAggACTCg-3′	2418
aas_b1_r		5′ -GGGCTAAAATAAGGGCTAATACTA-3′	

Table 3.16b Primers used for sequencing of aas

Primer	Primer Sequence
aas_s_a1_f	5′ -GGCTTCTATAATTGATGTGT-3′
aas_s_a2_f	5′ -GAAGGGGCGGAGCACAGT-3′
aas_s_a3_f	5′-AAACGGCTGTAGGCGAAACT-3′
aas_s_a4_f	5′-CCGTTTTCTTGGTGACA-3′
aas_s_a5_f	5′-ATCAAACAAGGGCTACTATT-3′
aas_s_a6_f	5′-GTTGGCATGATACGGGAGAT-3′
aas_s_a7_f	5′-GCCAAATTCCTGTCCTG-3′
aas_s_b1_r	5′ -AATTTGCTCCCCCTTTCTT-3′
aas_s_b2_r	5′ -GCACCATAGCCTTCATAAAT-3′
aas_s_b3_r	5′-TGGCATGACTAAGAGCAACC-3′
aas_s_b4-r	5′-CATGGCAGGTATTCGTCTAT-3′
aas_sb5_r	5′-TCCGTATTCGATGTTTAT-3′
aas_b6_r	5′-TTGGCAATAATGACAGAATG-3′

Table 3.17 other primers

Primer	Used For	Annealing Temperature	Primer Sequence	Size of Amplification Product [bp]
pGEMT_a1_f	test for the presence of an insert in the pGEMTez vector	64.5°C	5′ -CCA GTC ACG ACG TTG TAA AA -3′	—
pGEMT_b1_r			5′ -TTC CGG CTC GTA TGT TGT GT -3′	
pBCKS_a1_f	test for the presence of an insert in the pBCKS vector	62°C	5′ -GGT TTT CCC AGT CAC GA -3′	—
pBCKS_b1_r			5′ -CGC GCA ATT AAC CCT CAC -3′	
pMMB_a1_f	test for the presence of an insert in the pMMB2002 vector	54°C	5′-AAT TAA TCA TCG GCT CGT ATA ATG-3′	—
pMMB_b1_r			5′-CTC TCA TCC GCC AAA ACA G-3′	
GyrRTb_f	positive control in RT-PCR (binds to *L. pneumophila* gyrase gene)	61.5°C	5′-AAT CCC ACT GCA GCA AAA TC-3′	123
GyrRTb_r			5′-TGG TAA ACC GGC AAT ATC CA -3′	
RTgyr_a1_f	positive control in RT-PCR (binds to *L. pneumophila* gyrase gene)→ larger amplification product	56 °C	5′-CACATATGGCCGGCTTTAGAG-3′	469
RT_gyr_b1_r			5′-TCGCGCTTGTTTTGCTGAG-3′	
K12gyr_a1_f	positive control in RT-PCR (binds to *E. coli* gyrase gene)	64°C/68°C	5′ -acggtcgtcgcgcggtattgaa -3′	391
K12gyr_b1_r			5′ -AACGCCGCGATGATGTCTTT -3′	
plaA_d1_f	espression analysis of Corby *plaA* by RT-PCR	52-60°C	5′-CTGGCTTCACAGACGCAACC-3′	1151
plaA_e1_r			5′-GCATCATCCAGCTTCTTGTC-3′	
kan_a1_f	amplification of Km^R from pET28b	62°C	5′ -GAC GCT CAG TGG AAC GAA AAC -3′	978

Table 3.17 other primers

Primer	Used For	Annealing Temperature	Primer Sequence	Size of Amplification Product [bp]
kan_b1_r			5′ -ATG TGC GCG GAA CCC CTA TT -3′	
lipB_GmR_f	amplification of Gm^R from pVA15-1 (10)	64°C/68°C	5′ -TAAAAATTCATTTCGTTACC -3′	1024
lipB_GmR_r			5′ - CCTTACAATAATGCCACCAA-3′	
patA_a1_f	amplification of Philadelphia-1 *patA* with putative promoter region	52°C	5′-tacatgcttatagtggttcg-3′	2193
patA_b1_r			5′-AAAGGTTTGGCAGGATAGT-3′	
unk2_a1_f	amplification of 130b *unk2* with putative promoter region	56°C	5′-AATGGGTTTTGCTGGAAGTGA-3′	2539
unk2_b1_r			5′-ATAATAAGGCGACAGGGTTTG-3′	
hbpB_a1_f	amplification of 130b *hbpB* with putative promoter region	56°C	5′-AATAAGGCCAGTTTGTTTTC-3′	627
hbpB_b1_r			5′-CAGCATTTTTAAGAGGATTTC-3′	
patJ_a1_f	amplification of 130b *patJ* with putative promoter region	56°C	5′ACATTTTCAGTAGTTCAAGGTG-3′	1587
patJ_b1_r			5′AATAAGGGTACGCGTTCATCAT-3′	

3.1.10 Plasmids

Table 3.18 List of vectors for cloning

Name	Catalog Number	Manufacturer
pET-28b	69865-3	Stratagene
pGEMTez	A1360	Promega
pBCKS+	212217	Stratagene
pMMB2002	not applicable	(164)
pVA15-1 = pGEMTez+*lipB* ::Gm^R	not applicable	(10)
pLM845= pLAW344+rpoS	not applicable	(83)
pUC18	SD0051	Fermentas

Table 3.19 Overview of *L. pneumophila* 130b *plaA*, *plaC* and *L. pneumophila* 130b or Corby *plaD* plasmid constructs

Name	Construct	Size of insert [kbp]	Antibiotic selection marker	Primers used for amplification or cloning strategy
pAF7(75)	pGEMT+*plaA*::Km^R	2.129	Amp^R, Km^R	plaA_d1_f+plaA_e1_r
pAF10	pGEMTez+*plaC**#	1.559	Amp^R	gdsl2_a1_f+gdsl2_b1_r
pBH1	pGEMTez+*plaC*	1.383	Amp^R	gdsl2_c1_f+gdsl2_e1_r
pMY2	pBCKS+*plaC*	1.383	Cm^R	derived from pBH1 with *Spe*I&*Sac*II
pBH5	pGEMTez+*plaC*::Km^R*#	2.375	Amp^R+Km^R	derived from pAF10 with *Hind*III
pMY3	pMMB2002+*plaC*	1.383	Cm^R	derived from pMY2 with *Sac*I&*Kpn*I
pMY4	pGEMTez+*plaC*#	1.559	Amp^R	gdsl2_a1_f+gdsl2_b1_r
pMY5	pBCKS+plaC#	1.559	Cm^R	derived from pMY4 with *Spe*I&*Sac*II
pMY6	pMMB2002+*plaC*#	1.559	Cm^R	derived from pMY5 with *Sac*I&*Kpn*I
pMY7	pMMB2002+*plaC*#	1.559	Cm^R	gdsl2_a1_f+gdsl2_b1_r, amplified with *Pfu* and introduced into *Sma*I lineraized pMMB2002
pSB2	pGEMTez+$plaD_{130b}$#	1.942	Amp^R	gdsl3_a1_f+gdsl3_b1_r
pBH3	pBCKS+$plaD_{130b}$#	1.942	Cm^R	derived from pSB2 with *Sac*I&*Apa*I
pBH4	pBCKS+$plaD_{130b}$::Km^R#	2.920	Cm^R+Km^R	derived from pBH3 with *Afe*I
pER3	pGEMTez+$plaD_{Corby}$	1.551	Amp^R	gdsl3_c1_f+gdsl3_d1_r
pER4	pBCKS+$plaD_{Corby}$	1.551	Cm^R	derived from pER3 with *Acc*I&*Apa*I

-*the cloned *plaC* sequence contains a point mutation at base pair position 30 where guanine is mutated to adenine. #contains putative promoter region

Table 3.20 Overview of *L. pneumophila* Corby *plaB*, *L. pneumophila* Philadelphia-1 *patA*, *and L. pneumophila* 130b *patJ* plasmid constructs

Name	Construct	Size of insert [kbp]	Antibiotic selection marker	Primers used for amplification or cloning strategy
pKH190	pUC18+*plaB*	1495	Cm^R	pla1+pla2
pKH192	pBCKS+*plaB*	1495	Cm^R	derived from pKH190
pKH194	pUC18+*plaB*::Km^R	2473	Cm^R+Km^R	derived from pKH190 with *Sac*II
pKH195	pBOC20 *plaB*::Km^R	not known	Cm^R+Km^R	derived from pKH194
pPA1	pGEMTez+*patA*$^{\#}$	2.193	Amp^R	patA_a1_f+patA_b1_r
pPA3	pBCKS+*patA*$^{\#}$	2.193	Cm^R	derived from pPA1 with *Spe*I&*Sac*II
pSB13	pMMB2002+*patA*$^{\#}$	2.193	Cm^R	derived from pPA3 with *Hind*III&*Sac*I
pPA5	pGEMTez+*patA*::$Km^{R\#}$	3.171	Cm^R+Km^R	derived from pPA1 with *Sfo*I
pSB3	pGEMTez+$patJ_{130b}$$^{\#}$	1.587	Amp^R	patJ_a1_f+patJ_b1_r

- $^{\#}$contains putative promoter region

-pKH190, pkH192, pKH194, and pKH195 were kindly provided by Klaus Keuner (University of Würzburg) (76).

Table 3.21 Overview of *L. pneumophila* 130b *unk1*, *unk2, hbpB*, *aas* as well as *L. pneumophila* Philadelphia-1 *lvrE* plasmid constructs

Name	Construct	Size of Insert [kbp]	Antibiotic selection marker	Primers used for amplification or cloning strategy
pSB1	pGEMTez+*unk1*$^{\#}$	1.008	Amp^R	unk1_a1_f+unk1_b1_r
pSB4	pBCKS+*unk1*$^{\#}$	1.008	Cm^R	derived from pSB1 with *Spe*I&*Sac*II
pSB11	pBCKS+*unk1*::$Km^{R\#}$	1.986	Cm^R+Km^R	derived from pSB4 with unk1_m_f+unk1_m_r
pSB14	pMMB2002+*unk1*$^{\#}$	1.008	Cm^R	derived from pSB4 with *Sac*I&*Hind*III
pSB6	pGEMTez+*unk2*$^{\#}$	2.539	Amp^R	unk2_a1_f+unk2_b1_r
pSB7	pGEMTez+*hbpB*$^{\#}$	0.627	Amp^R	hbpB_a1_f+hbpB_b1_r
pSB5	pGEMTez+*aas*$^{\#}$	2.418	Amp^R	aas_a1_f+aas_b1_r
pSB16	pGEMTez+*aas*::$Km^{R\#}$	3.396	Amp^R+Km^R	derived from pSB5 with *Afe*I
pSB9	pGEMTez+*aas*::$Gm^{R\#}$	3.442	Amp^R+Gm^R	derived from pSB5 with *Afe*I
pSB17	pBCKS+*aas*$^{\#}$	2.418	Cm^R	derived from pSB5 with *Apa*I&*Pst*I
pSB8	pGEMTez+*lvrE*$^{\#}$	1.049	Amp^R	lvrE_a1_f+lvrE_b1_r
pSB10	pGEMTez+Δ*lvrE*::$Km^{R\#}$	2.003	Amp^R+Km^R	derived from pSB8 with lvrE_m_f+lvrE_m_r
pSB12	pBCKS+*lvrE*$^{\#}$	1.049	Cm^R	Derived from pSB8 with *Apa*I&*Sac*I
pSB15*	pMMB2002+*lvrE*$^{\#}$	1.049	Cm^R	lvrE_a1_f+lvrE_b1_r amplified with *Pfu* and introduced into *Sma*I lineraized pMMB2002
pSB18	pMMB2002+*lvrE*$^{\#}$	1.049	Cm^R	derived from pSB8 with *Sac*I&*Sph*I

Table 3.21 Overview of *L. pneumophila* 130b *unk1*, *unk2, hbpB*, *aas* as well as *L. pneumophila* Philadelphila-1 *lvrE* plasmid constructs

Name	Construct	Size of Insert [kbp]	Antibiotic selection marker	Primers used for amplification or cloning strategy
pSB19	pLM854+*lvrE*[#]	1.049	Cm^R	lvrE_a1_f+lvrE_b1_r (with *pfu*)
pMMBNlvrE	pMMB2002N+*lvrE*, vector contains additional NdeI site before its start codon	0.795	Cm^R	Received from Emmy DeBuck, Leuven, Belgium

- [#]contains putative promoter region

-* restriction analysis revealed that pSB15 lacks the *lvrE* insert

3.1.11 Strains

Table 3.22 Overview of wild type strains

Name	Catalog Number	Origin
Escherichia coli DH5α	18258-012	Invitrogen
Legionella pneumophila Serogroup 1 130b	BAA-74	Wadsworth, ATCC
Legionella pneumophila Serogroup 1 Philadelphia-1	33152	ATCC
Legionella pneumophila Serogroup 1 Corby	not applicable	(107)
Acanthamoeba castellanii	30234	ATCC
U937 monocytes	CRL-1593.2	ATCC

Table 3.23 Overview of recombinant strains (for details on constructs see Tables 3.19-3.21)

Donor *L. pneumophila* strain	Acceptor	Vector	ORF	Construct	Recombinant Strain	Clone
Escherichia coli						
Sg1 130b	DH5α	pGEMTez	*plaA*	(pAF7)	DH5α (pAF7), (75)	
Sg1 130b	DH5α	pGEMTez	*plaC*$^{\#}$	(pAF10)	DH5α (pAF10)	
Sg1 130b	DH5α	pGEMTez	*plaC* without promotor	(pBH1)	DH5α (pBH1)	1, 2, 7, 8
Sg1 130b	DH5α	pBCKS	*plaC*::Km$^{R\#}$	(pBH5)	DH5α (pBH5)	1
Sg1 130b	DH5α	pBCKS	*plaC* without promotor	(pMY2)	DH5α (pMY2)	1,2,5
Sg1 130b	DH5α	pMMB2002	*plaC* without promotor	(pMY3)	DH5α (pMY3)	6,8
Sg1 130b	DH5α	pGEMTez	*plaC*$^{\#}$	(pMY4)	DH5α (pMY4)	1
Sg1 130b	DH5α	pBCKS	*plaC*$^{\#}$	(pMY5)	DH5α (pMY5	1,2
Sg1 130b	DH5α	pMMB2002	*plaC*$^{\#}$	(pMY6)	DH5α (pMY6)	1,2,3
Sg1 130b	DH5α	pGEMTez	*plaD*$^{\#}$	(pSB2)	DH5α (pSB2)	1-8
Sg1 130b	DH5α	pBCKS	*plaD*$^{\#}$	(pBH3)	DH5α (pBH3	1-8
Sg1 130b	DH5α	pBCKS	*plaD*::Km$^{R\#}$	(pBH4)	DH5α (pBH4)	1, 2, 3, 4, 7
Sg1 Corby	DH5α	pGEMTez	*plaD* without promotor	(pER3)	DH5α (pER3)	A1, B6, A4
Sg1 Corby	DH5α	pBCKS	*plaD* without promotor	(pER4)	DH5α (pER4)	A4, A6, B2, B7
Sg1 Corby	DH5α	pUC18	*plaB*	(pKH190)	DH5α (pKH190) from Klaus Heuner	
Sg1 Corby	DH5α	pBCKS	*plaB*	(pKH192)	DH5α (pKH192) from Klaus Heuner	
Sg1 Corby	DH5α	pUC18	*plaB*	(pKH194)	DH5α (pKH194)	from K. Heun-er
Sg1 130b	DH5α	pGEMTez	*patJ*$^{\#}$	(pSB3)	DH5α(pSB3)	1, 2, 7, 8
Sg1 Philadelphia-1	DH5α	pGEMTez	*patA*$^{\#}$	(pPA1)	DH5α(pPA1)	1-6
Sg1 Philadelphia-1	DH5α	pBCKS	*patA*$^{\#}$	(pPA3)	DH5α(pPA3)	1-3
Sg1 Philadelphia-1	DH5α	pBCKS	*patA*$^{\#}$	(pSB13)	DH5α(pSB13)	1-4

Table 3.23 Overview of recombinant strains (for details on constructs see Tables 3.19-3.21)

Donor *L. pneumophila* strain	Acceptor	Vector	ORF	Construct	Recombinant Strain	Clone
Sg1 130b	DH5α	pGEMTez	*unk1*#	(pSB1)	DH5α(pSB1)	1-4
Sg1 130b	DH5α	pBCKS	*unk1*#	(pSB4)	DH5α(pSB4)	1-4, 6, 7
Sg1 130b	DH5α	pBCKS	*unk1*::Km^{R}#	(pSB11)	DH5α(pSB11)	6.1, 6.2
Sg1 130b	DH5α	pMMB2002	*unk1*#	(pSB14)	DH5α (pSB14)	1, 5
Sg1 130b	DH5α	pGEMTez	*unk2*#	(pSB6)	DH5α(pSB6)	2, 3, 6, 8
Sg1 130b	DH5α	pGEMTez	*hbpB*#	(pSB7)	DH5α(pSB7)	2, 5, 6, 8
Sg1 130b	DH5α	pGEMTez	*aas*#	(pSB5)	DH5α (pSB5)	3-5, 7
Sg1 130b	DH5α	pGEMTez	*aas*::Km^{R}#	(pSB16)	DH5α (pSB16)	2
Sg1 130b	DH5α	pGEMTez	*aas*::Gm^{R}#	(pSB9)	DH5α (pSB9)	
Sg1 130b	DH5α	pBCKS	*aas*#	(pSB17)	DH5α (pSB17)	1-3
Sg1 Philadelphia-1	DH5α	pGEMTez	*lvrE*#	(pSB8)	DH5α (pSB8)	1-5
Sg1 Philadelphia-1	DH5α	pGEMTez	Δ*lvrE*::Km^{R}#	(pSB10)	DH5α (pSB10)	7, 8
Sg1 Philadelphia-1	DH5α	pBCKS	*lvrE*#	(pSB12)	DH5α (pSB12)	1, 3
Sg1 Philadelphia-1	DH5α	pMMB2002	*lvrE*#	(pSB18)	DH5α (pSB18)	1, 2, 3
Sg1 Philadelphia-1	DH5α	plm845	*lvrE*#	(pSB19)	DH5α (pSB19)	2, 8, 12, 16
Sg1 Philadelphia-1	DH5α	pMMBN-lvrE	*lvrE* without promotor	(pMMBNlvrE) from Emmy DeBuck (Belgien)	DH5α (pMMBNlvrE)	1-4
Legionella pneumophila						
-	*L. pneumophila* 130b	-	Km^{R}	-	*plaC*::Km^{R} 130b	8, A5, B2,C4
-	*plaC*::Km^{R} 130b cl. A5 (=plaC5)	pMMB2002	-	-	*plaC*::Km^{R} 130b (pMMB2002) = plaC5 (pMMB2002)	1,2
Sg1 130b	*plaC*::Km^{R} 130b cl. A5 (=plaC5)	pMMB2002	*plaC*#	pMY7	*plaC*::Km^{R} 130b (pMY7) = plaC5 (pMY7), directly electroporated into *L. pneumophila* plaC5 mutant.	1,7
-	*L. pneumophila* Corby	-	Km^{R}	-	*plaD*::Km^{R} Corby	A4, C2, D8

Table 3.23 Overview of recombinant strains (for details on constructs see Tables 3.19-3.21)

Donor *L. pneumophila* strain	Acceptor	Vector	ORF	Construct	Recombinant Strain	Clone
-	*L. pneumophila* Corby	-	Km^R	-	*plaA*::Km^R Corby	A3, B5, B7, C8, D1
-	*L. pneumophila* Corby	-	Km^R	-	*plaC*::Km^R Corby	A1-A5
-	*L. pneumophila* Corby	-	Km^R	-	*plaB*::Km^R Corby	1, 60
-	*L. pneumophila* 130b	-	Km^R	-	*plaB*::Km^R 130b	
Sg1 Corby	*L. pneumophila* Corby	pBCKS	*plaB*	pKH192	Corby (pKH192)	
-	*L. pneumophila plaB*::Km^R Corby cl. 60(=plaB60)	pBCKS	-	-	plaB60 (pBCKS)	
Sg1 Corby	*L. pneumophila plaB*::Km^R Corby cl. 60(=plaB60)	pBCKS	*plaB*	pKH192	plaB60 (pKH192)	
Sg1 Philadelphia-1	*L. pneumophila* 130b	pBCKS	*patA*	pPA3	130b (pPA3)	
-	*L. pneumophila* 130b	-	Km^R	-	*patA*::Km^R 130b	A4, A5
Sg1 Philadelphia-1	*patA*::Km^R 130b	pBCKS	*patA*	pPA3	*patA*::Km^R 130b cl 4 (pPA3)	
Sg1 Philadelphia-1	*patA*::Km^R 130b	pMMB2002	*patA*	pSB13	*patA*::Km^R 130b cl 4 (pSB13)	
Sg1 130b	*L. pneumophila* 130b	pBCKS	*unk1*	pSB4	130b (pSB4)	
-	*L. pneumophila* 130b	-	Km^R	-	*Δunk1*::Km^R 130b	1, 2, 2A (=3)
Sg1 130b	*Δunk1*::Km^R 130b cl.1	pMMB2002	*unk1*	pSB14	*Δunk1*::Km^R 130b cl.1 (pSB14)	2-4, 8, 1A-3A
Sg1 130b	*Δunk1*::Km^R 130b cl.2	pMMB2002	*unk1*	pSB14	*Δunk1*::Km^R 130b cl.2 (pSB14)	1, 5

Table 3.23 Overview of recombinant strains (for details on constructs see Tables 3.19-3.21)

Donor *L. pneumophila* strain	Acceptor	Vector	ORF	Construct	Recombinant Strain	Clone
Sg1 Philadelphia-1	*L. pneumophila* Philadelphia-1	pMMB2002	*lvrE*	pSB18	Philadelphia-1 (pSB18)	
-	*L. pneumophila* 130b	-	Km^R	-	*ΔlvrE*::Km^R 130b	1.1, 2.1, 1A, 2A, 4C
-	*L. pneumophila* Philadelphia-1	-	Km^R	-	*ΔlvrE*::Km^R Philadelphia	16, 19, 22, 23
Sg1 Philadelphia-1	*ΔlvrE*::Km^R Philadelphia cl.19	pMMB2002	*lvrE*	pSB18	*ΔlvrE*::Km^R Philadelphia cl.19 (pSB18)	
Sg1 Philadelphia-1	(lvrE::KmR Philadelphia cl.19	pMMBN	lvrE	pMMBNlvrE	(lvrE::KmR Philadelphia cl.19 (pMMBNlvrE)	
Sg1 Philadelphia-1	*ΔlvrE*::Km^R 130b cl. 1.1	pMMB2002	*lvrE*	pSB18	*ΔlvrE*::Km^R 130b cl. 1.1 (pSB18)	1-8
Sg1 Philadelphia-1	*ΔlvrE*::Km^R 130b cl. 1A	pMMBN	*lvrE*	pMMBNlvrE	(lvrE::KmR 130b cl. 1A (pMMBNlvrE)	
Sg1 Philadelphia-1	(lvrE::KmR 130b cl. 2A	pMMBN	lvrE	pMMBNlvrE	(lvrE::KmR 130b cl. 2A (pMMBNlvrE)	
Sg1 Philadelphia-1	*ΔlvrE*::Km^R 130b cl. 4C	pMMBN	*lvrE*	pMMBNlvrE	*ΔlvrE*::Km^R 130b cl. 4C (pMMBNlvrE)	
-	*L. pneumophila* 130b	-	Km^R	-	*aas*::Km^R 130b	9a, 13a, 16b
-	*plaB*::Km^R 130b	-	Gm^R	-	*plaB*::Km^R/*aas:*:Gm^R 130b	2D/7B
-	*L. pneumophila* 130b	-	Km^R	-	*plaA*::Km^R 130b	(75)
-	*L. pneumophila* 130b	-	Km^R	-	*proA*::Km^R 130b	(131)
-	*L. pneumophila* 130b	-	Km^R	-	*lspDE*::Km^R 130b	(163)

- [#]contains putative promoter region

3.1.12 DNA standards

Table 3.24 Overview of DNA mass standards for gel electrophoresis

100 bp DNA mass standard		1 kb DNA mass standard		DNA *Längenstandard* II (DIG labelled)
Size [bp]	DNA conc. [ng/µl]	Size [bp]	DNA conc. [ng/µl]	Size [bp]
1517	45	10002	42	23130
1200	35	8001	42	9416
1000	95	6001	50	6557
900	27	5001	42	4361
800	24	4001	33	2322
700	21	3001	125	2027
600	18	2000	48	564
517	97	1500	36	125
500	97	1000	42	
400	38	500	42	
300	29	The 100 bp (catalog number N3231L) and the 1kb (catalog number N3232L) standards were purchased from New England Biolabs and the DIG labelled DNA *Längenstandard* II (catalog number 1218590) was purchased from Roche.		
200	25			
100	48			

Table 3.25 Overview of protein mass standard for electrophoresis

Prestained SDS-PAGE standards, low range	Precision Plus Protein Standards, unstained
Molecular weight [kDa]	Molecular weight [kDa]
113	250
91	150
49.9	100
35.1	75
28.4	50
20.8	37
Both, the prestained, low range (catalog number 161-0305) and the unstained protein standard (catalog number 161-0363) were purchased from Bio-Rad Laboratories.	25
	20
	15
	10

3.1.13 Software

Table 3.26 Overview of web-based and local software for analysis of DNA sequences and protein sequences

Software	
NCBI Blast:	http://www.ncbi.nlm.nih.gov/BLAST/
Legionella Genome Projekt Website:	http://genome3.cpmc.columbia.edu/~legion/
SignalP	http://www.cbs.dtu.dk/services/SignalP/
DNA Star Inc.	EditSeq, PrimerSelect, SeqMan, MapDraw, MegAlign
Pedant Website	http://pedant.gsf.de/
PSORTb version 2.0	http://psort.org/
BPROM	www.softberry.com
MaliP	www.softberry.com
Quantity One	Bio-Rad Laboratories
Software for Conony Counting	Acolyte
Magellan V5.03	Tecan
FPLC Software	PrimeView&PrimeView Evaluation