

2 Materials

2.1 Instruments

Electronic scale	CP2202S-OCE	Sartorius
Water purification	Milli-Q academic	Millipore
	MC1 Analytical AC120S	Sartorius
pH meter	Lab 850	Schott
Photometers	Ultrospec 10	Amersham
	Nanodrop ND-1000	PeQLab
Vortex mixer	7-2020	neoLAB
Rotator	2-1175	neoLAB
Rocking platform	WT16	Biometra
Cell disruption	French press cell FA-031	SIM-AMINCO
	Microfluidizer processor	Microfluidics
Power supply	Power PAC 300	BIO-RAD
PAGE gel chamber	Mini-Protean Tetra Cell	BIO-RAD
DNA gel chamber	HG370/HG330	Savant
Blotting device	TE 70 PWR	Amersham
Gel documentation	LAS-4000	FujiFilm
	UV Table	Biometra
	Transluminator	Roth
	PowerShot A640	Canon
Centrifuges	5417R	Eppendorf
	Avanti J-26 XP	Beckman
	Biofuge 28RS	Heraeus
Incubator	MIR-153	SANYO
Culture shaker	HT	INFORS
Thermal block	Thermomixer 5437	Eppendorf
Thermocycler	PTC-200	MJ Research
Real-time PCR	iQ5 Multicolor	Bio-Rad
Chromatography systems	Äkta explorer	GE Healthcare
	LKB-FRAC-200	GE Healthcare
	Uvicord SII	GE Healthcare
	LCC-501 PLUS	GE Healthcare

Materials

	LKB-REC-102	GE Healthcare
	LKB-Pump P-500	GE Healthcare
	Vision Workstation	Applied Bioscience
Chromatography columns	GSTrap FF column (5mL)	GE Healthcare
	Mono S 4.6/100 PE	Amersham
	Superdex 75 HiLoad 16/60	GE Healthcare
	Superdex 75 HiLoad 26/60	GE Healthcare
	Superdex 75 10/300 GL	GE Healthcare
CD Spectrometer	J720	Jasco
Concentrator	Amicon Ultra	Millipore
Vacuum concentrator	SpeedVac SVC 200	SAVANT
Dialysis	Membrane tubing	Spectra/Por
	Membrane closures	Spectra/Por
Analytical ultracentrifuge	Optima XL-I	Beckman
Static light scattering	Viscotek / Malvern GPC / SEC-system	Viscotek
Crystallization robot	Hydra-Plus-One	Matrix Technologies
	Hydra II	Matrix Technologies
Pipetting robot	Lizzy XXL	Zinsser
Crystallization plate	Crystal quick plate	Greiner Bio-One
	SuperClear pregreased plate	Crystalgen
	22mm siliconized cover slides	Jena Bioscience
Crystal observation	Microscope WILD M3C	Leica
	MZ 75	Leica
Crystal plates storage	Homebase	The Automation Partnership
	Camera COOLPIX 990	Nikon
Nylon loops	CryoLoops	Hampton Research
Pins	CrystalCap Copper	Hampton Research
Home X-ray source	Xcalibur Nova O	Oxford Diffraction

2.2 Chemicals, enzymes, kits

Chemicals

Standard chemicals were purchased from Roth Chemikalien, Sigma-Aldrich or Merck.

Agar, granulated	BD Bioscience
Agarose, electrophoresis grade	Invitrogen
Ammonium persulfate (APS)	Serva
Ampicillin sodium sulfate	Roth
Biotin	Sigma
BugBuster protein extraction reagent	Novagen
Complete protease inhibitor tablets	Roche
Isopropyl-thiogalactoside (IPTG)	AppliChem
Glutathion, reduced (GSH)	Boehringer Mannheim
GSH sepharose 4B	GE Healthcare
Instamed PBS Dulbecco	Biochrom AG
Ni-NTA agarose	Qiagen
Talon superflow beads	Clonetech
N,N,N',N'-tetramethylenediamine (TEMED)	Serva
Overnight Express instant TB medium	Merck
Precision Plus protein dual color standard	Bio-Rad
Poly-vinylidene fluoride (PVDF) transfer membrane	GE Healthcare
Rotiphorese gel 30%	Roth
Seleno-methionine (SeMet)	Acros organics
Sypro Orange	Invitrogen
Thiamin	Sigma
Tryptone	AppliChem
Yeast extract	MP Biomedicals
α -HA affinity matrix	Roche
α -HA antibody (Y11)	Santa Cruz
α -flag antibody (M2)	Sigma
α -mouse Horseradish peroxidase (HRP) conjugate	Jackson Labs

Enzymes

Restriction endonucleases

*Bam*HI, *Not*I

NEB

Polymerases

Taq, *Pfu*, *Pfu* Plus!, T4 DNA polymerase

Roboklon

T4 DNA ligase

Roboklon

Benzonase nuclease

Novagen

Kits

QIAprep Spin Miniprep Kit

Qiagen

QIAquick PCR purification kit

Qiagen

QIAquick Gel Extraction Kit

Qiagen

ECL Western blotting analysis system

GE Healthcare

Crystallization kits

JBScreen Basic 1-4

Jena Bioscience

Classics II Suite

Qiagen

JCSG Suite

Qiagen

PEGs Suite

Qiagen

2.3 Strains and plasmids

Bacterial and yeast strains

<i>E. coli</i> DH5 α		Invitrogen
<i>E. coli</i> DH5 α -T1R	T1 and T5 phage resistant	Invitrogen
<i>E. coli</i> BL21(DE3)		Novagen
<i>E. coli</i> BL21(DE3)-T1R	T1 and T5 phage resistant	Sigma-Aldrich

Template clones

NIBP	IRALp962C1811Q	RZPD
Ehoc-1	IRCBp5005K242Q5	RZPD

Cell lines

HEK293	Human embryonic kidney fibroblasts transformed with adenovirus genes E1A and E1B	DSZM
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Plasmids

pGex-6P1	<i>E. coli</i> expression vector with ampicillin resistance, <i>tac</i> promoter, N-terminal GST-tag removable with PreScission protease cleavage.	GE Healthcare
pQLink-H	<i>E. coli</i> expression vector with ampicillin resistance, T7/ <i>lac</i> promoter, N-terminal GST-tag removable with TEV protease cleavage, LINK sequences [84].	Konrad Büssow
pTL-HA 1	Vector for cell culture expression, with ampicillin resistance, T7 and SV 40 promoter, N-terminal HA-tag	Ulrich Stelzl
pcDNA3-flag	Vector for cell culture expression, with ampicillin resistance, CMV promoter, N-terminal flag-tag	Daniel Krappmann
pYEX-Bet3p	Bet3p (AA 2-193) cloned on <i>S. cerevisiae</i> expression vector, inserted between <i>Bam</i> HI/ <i>Not</i> I cutting site.	Daniel Kümmel

2.4 Media and buffers

Culture media

LB medium	Tryptone	10 g/l
	Yeast extract	5 g/l
	NaCl	10 g/l
	pH adjusted to 7.4 with NaOH	
LB agar	LB medium	
	Agar	1.5% (w/v)
TB medium	Tryptone	12 g/l
	Yeast extract	24 g/l
	Glycerol	0.4% (v/v)
After autoclaving and cooling down, add sterile KH_2PO_4 to 17 mM, and K_2HPO_4 to 72 mM.		
Overnight Express instant TB medium	Overnight Express instant TB powder	60 g/l
	Glycerol	1% (v/v)
M9 for SeMet labelling (1 l)	Na_2HPO_4	6 g
	KH_2PO_4	3 g
	NH_4Cl	1 g
	NaCl	0.5 g
	MgSO_4 (autoclaved separately)	1 mM
	Glucose (filter-sterile)	0.2% (w/v)
	0.5% (w/v) thiamine (filter-sterile)	100 μl
	4.2 g/l FeSO_4 (filter-sterile)	1 ml
ddH ₂ O (autoclaved)	Make up to 1 l	

Materials

Solid amino acid supplements (for 1 l M9)	L-Lysine	100 mg
	L-Phenylalanine	100 mg
	L-Threonine	100 mg
	L-Isoleucine	50 mg
	L-Leucine	50 mg
	L-Valine	50 mg
	L-Selenomethionine	50 mg

Molecular Biology Buffers

TAE buffer	Tris-HCl	40 mM
	Sodium acetate	5 mM
	EDTA	1 mM
	pH adjusted to 8.0 with acetic acid.	
5 x DNA sample buffer	Bromophenol blue	0.25% (w/v)
	Glycerol	30% (v/v)
DNA molecular weight marker	100 bp DNA ladder, or 1 kb DNA ladder	0.5 µg/µl
	DNA sample buffer	1x
TFB 1 buffer	RbCl	100 mM
	MnCl ₂	50 mM
	KOAc, pH 5	30 mM
	CaCl ₂	10 mM
	Glycerol	15% (v/v)
TFB 2 buffer	RbCl	10 mM
	CaCl ₂	15 mM
	MOPS, pH 6.8	10 mM
	Glycerol	15% (v/v)

Protein purification

Cell disruption buffer	BugBuster reagent	25 ml
	Complete protease inhibitor	1 tablet
	Benzonase	10 μ l
	MgCl ₂	2 mM
PBS buffer	Instamed PBS Dulbecco	9.55 g/l
His-lysis buffer (30 ml)	NaH ₂ PO ₄ , pH 8	50 mM
	NaCl	500 mM
	Imidazole	20 mM
	Complete protease inhibitor	1 tablet
	Benzonase	5 μ l
His-wash buffer	NaH ₂ PO ₄ , pH 8	50 mM
	NaCl	500 mM
	Imidazole	50 mM
His-elution buffer	NaH ₂ PO ₄ , pH 8	50 mM
	NaCl	500 mM
	Imidazole	250 mM
His-dialysis buffer	Tris-HCl, pH 8	50 mM
	NaCl	150 mM
	DTT (1,4-dithiothreitol)	2 mM
	Glycerol	5% (v/v)
GST-lysis buffer (60 ml)	β ME (β -mercaptoethanol)	5 mM
	PMSF	1 mM
	In PBS buffer	
GST-elution buffer	Tris-HCl, pH 8	100 mM
	GSH, reduced	20 mM

Materials

GST-dialysis buffer	Tris-HCl, pH 8	50 mM
	NaCl	150 mM
	βME	5 mM
Mono S buffer A	MES, pH 6	20 mM
Mono S buffer B	MES, pH 6	20 mM
	NaCl	1 M
Tca17 protein buffer	HEPES, pH 7	20 mM
	NaCl	300 mM
	DTT	2 mM
	Glycerol	5% (v/v)
Ehoc-1 protein buffer	Tris-HCl, pH 8	20 mM
	NaCl	500 mM
	βME	5 mM
	Glycerol	5% (v/v)
SeMetTca17 protein buffer	HEPES, pH 7	20 mM
	NaCl	300 mM
	DTT	5 mM
	EDTA	1 mM
	Glycerol	5% (v/v)
SLS buffer	HEPES, pH 7	20 mM
	NaCl	300 mM
	βME	5 mM
SLS-2 buffer	HEPES, pH 7	20 mM
	NaCl	300 mM
Low-salt buffer	Tris-HCl, pH 8	20 mM
	NaCl	50 mM

SDS-PAGE

Electrophoresis buffer	Tris	25 mM
	Glycine	190 mM
	SDS	0.1% (w/v)
Stacking gel buffer	Tris	0.5 M
	pH adjusted to 6.8 with 1 M HCl	
Resolving gel buffer	Tris	1.5 M
	pH adjusted to 8.8 with 1 M HCl	
SDS stock solution	SDS (sodium dodecyl sulfate)	10% (w/v)
APS stock solution	APS (ammonium persulfate)	10% (w/v)
Stacking gel	Rotiphorese gel 30%	1 ml
	Stacking gel buffer	1.875 ml
	Millipore H ₂ O	4.6 ml
	10% SDS	75 µl
	10% APS	37.5 µl
	TEMED	10 µl
15% Resolving gel	Rotiphorese gel 30%	7.5 ml
	Resolving gel buffer	3.75 ml
	Millipore H ₂ O	3.5 ml
	10% SDS	150 µl
	10% APS	75 µl
	TEMED	17.5 µl
2 x SDS sample buffer	pH 6.8 Tris-HCl	50 mM
	DTT	100 mM
	SDS	2% (w/v)
	Bromophenol blue	0.1% (w/v)
	Glycerol	10% (v/v)

Materials

2 x non-reducing SDS sample buffer	pH 6.8 Tris-HCl	50 mM
	SDS	2% (w/v)
	Bromophenol blue	0.1% (w/v)
	Glycerol	10% (v/v)

Coomassie staining buffer	Coomassie R-250	0.1% (w/v)
	Ethanol	50% (v/v)
	Acetic acid	10% (v/v)

Destain solution	Ethanol	40% (v/v)
	Acetic acid	10% (v/v)

Silver staining

Fixation solution	50% (v/v) Acetone	60 ml
	50% (v/v) Trichloroacetic acid	1.5 ml
	37% Formaldehyde	25 μ l

Reducing solution	10% (w/v) $\text{Na}_2\text{S}_2\text{O}_3$	100 μ l
	ddH ₂ O	60 ml

Silver stain solution	20% (w/v) AgNO_3	800 μ l
	37% Formaldehyde	600 μ l
	10% (w/v) $\text{Na}_2\text{S}_2\text{O}_3$	25 μ l
	ddH ₂ O	60 ml

Development solution	Na_2CO_3	1.2 g
	37% Formaldehyde	25 μ l
	10% (w/v) $\text{Na}_2\text{S}_2\text{O}_3$	25 μ l
	ddH ₂ O	60 ml

Immuno-precipitation and pull-down assays

CoIP buffer	HEPES, pH 7.5	50 mM
	NaCl	150 mM
	NP-40	0.2% (v/v)
	glycerol	1 mM
	DTT	1 mM
Binding buffer 1	HEPES, pH 7.5	20 mM
	NaCl	150 mM
Elution buffer 1	GSH, reduced	15 mM
	HEPES, pH 7.5	20 mM
	NaCl	150 mM
Binding buffer 2	HEPES, pH 7.5	20 mM
	NaCl	150 mM
	Imidazole	50 mM
Elution buffer 2	HEPES, pH 7.5	20 mM
	NaCl	150 mM
	Imidazole	250 mM

Western blot and immunodetection

Semi-dry transfer buffer	Tris	25 mM
	Glycine	150 mM
	Methanol	10% (v/v)
	should be pH 8.3 without adjusting	
PBST buffer	PBS buffer	1 x
	Tween-20	0.1% (v/v)

2.5 Synthetic oligonucleotides

Synthetic oligonucleotides were ordered from Eurofins MWG Operon.

2.5.1 Gene-specific primers

Primer	sequences (5'→3')	restriction site
Tca17_f	A TTA <u>GGA TCC</u> ATG AGC TTG AGG CCT TGC	<i>Bam</i> HI
Tca17_r	AGT <u>TGC GGC CGC</u> TAA TAA TTC AAC TTC AGT TGA AAT AAA CAA	<i>Not</i> I
Trs33p_f	A TTA <u>GGA TCC</u> ACA CAT AGT AAT AAT GTA GGA CAT CCC C	<i>Bam</i> HI
Trs33p_r	AGT <u>TGC GGC CGC</u> TAA CTG CGG CAT TGT GAC TTG	<i>Not</i> I
Ehoc1_f16	T AGA TTA <u>GGA TCC</u> GAG AAC AAG CCC ATC GTC AC	<i>Bam</i> HI
Ehoc1_r211	AAT GTT AGT <u>TGC GGC CGC</u> TAA CTT CTC CCT CAA GGT TCT CAT G	<i>Not</i> I

2.5.2 Vector-specific primers

Primer	sequences (5'→3')	vectors
pGEX fwd	CCA AAA TCG GAT CTG GTT C	pGEX
pGEX rev	CAG AGG TTT TCA CCG TCA TCA	pGEX
pQTEV3U	TAT AAA AAT AGG CGT ATC ACG AGG	pQLink-H
pQTEV3L	CCA GTG ATT TTT TTC TCC ATT TT	pQLink-H