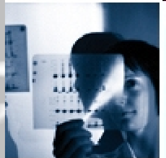
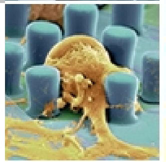
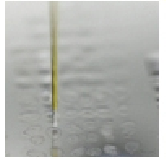
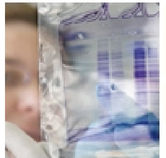
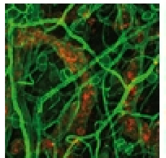
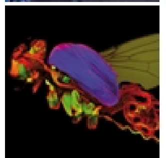
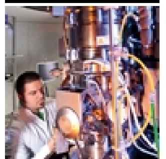
Max Planck Institute of Biochemistry

Research departments

Molecular Structural Biology
Wolfgang Baumeister

Structural Cell Biology Elena Conti

Molecular Medicine Reinhard Fässler

Cellular Biochemistry F.-Ulrich Hartl

Molecular Cell Biology Stefan Jentsch

Proteomics and Signal Transduction
Matthias Mann

Molecular Biology Axel Ullrich

Membrane and Neurophysics
Peter Fromherz

Membrane Biochemistry Dieter Oesterhelt

Structure Research Robert Huber

Structure and Function of Mitochondria
Walter Neupert

Cancer Metastasis Ulf R. Rapp

Research groups

Modeling of Protein complexes Friedrich Förster

Chromosome Organization and Dynamics
Stephan Gruber

Protein Analysis Friedrich Lottspeich

Molecular Immunology and Signaltransduction
Marc Schmidt-Supprian

Maintenance of Genome Stability Zuzana Storchova

Cellular Dynamics and Cell Patterning
Roland Wedlich-Söldner

Molecular Membrane and Organelle Biology
Thomas Wollert

Chaperonin-assisted Protein Folding
Manajit Hayer-Hartl

Intraflagellar Transport Esben Lorentzen

Chromatin Biology Jürg Müller

Muscle Dynamics Frank Schnorrer

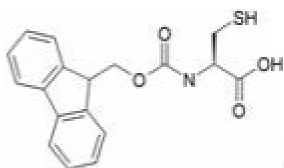
Structural Chronobiology Eva Wolf

NMR Spectroscopy Tad Holak

DNA Replication and Genome Integrity Boris Pfander

Microchemistry Core Facility

Peptide synthesis ❶

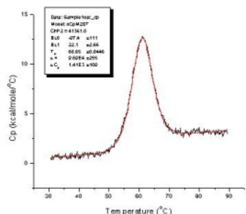


Organic chemistry ❷

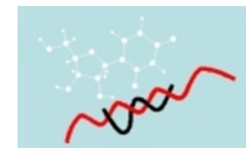
Proteomics ❸

2004 - 2011

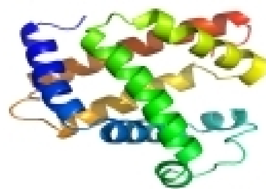
Biophysical methods ❷



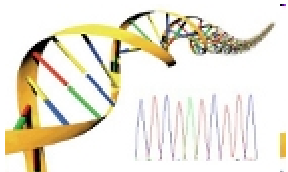
Oligo RNA synthesis ❶



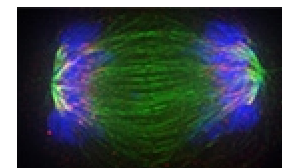
Recombinant protein production ❹



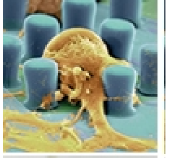
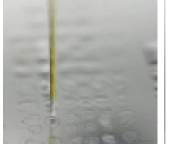
DNA Sequencing ❶

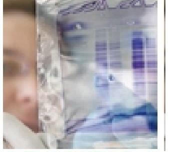
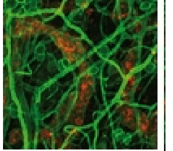


Light Microscopy ❶



Services

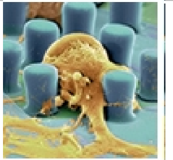
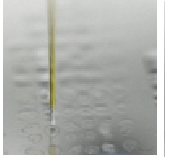
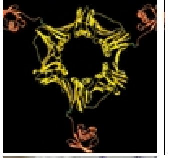
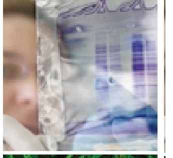
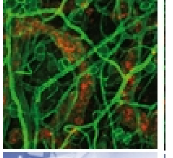



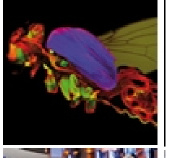
No of constructs / year

100
80
60
40
20
0

biomass production
expression screening
cloning
fermentation projects
insect cells
protein purification
HEK

Services



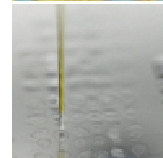
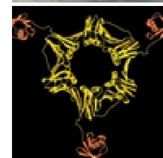
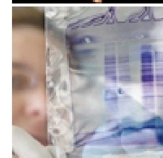
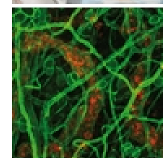
E. coli + recombinant protein
Pseudomonas putida, Ralstonia eutrophica, Streptomyces spec
Corynebacterium glutamicum
S. cerevisiae, S. pombe,
P. pastoris

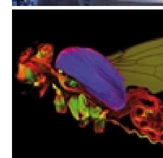
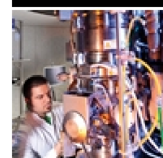
No of constructs / year

100
80
60
40
20
0

biomass production
expression screening
cloning
fermentation projects
insect cells
protein purification
HEK

Services

No of constructs / year

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0

biomass production
expression screening
cloning
fermentation projects
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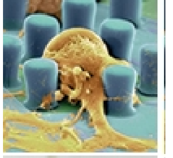
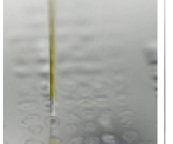
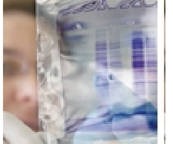
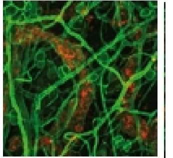
protein purification
HEK

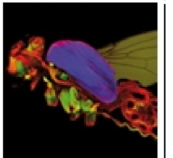
Services











No of constructs / year

100
80
60
40
20
0

biomass production

expression screening

cloning

fermentation projects

insect cells

protein purification

HEK

DNA- , RNA- binding proteins, structural proteins, proteases, chemokines, etc

Bacterial expression - SLIC cloning

turemethods

Harnessing homologous recombination *in vitro* to generate recombinant DNA via SLIC

Mamie Z Li & Stephen J Elledge

NATURE METHODS | VOL.4 NO.3 | MARCH 2007 | 251

Bacterial expression - SLIC cloning

Harnessing homologous recombination *in vitro* to generate recombinant DNA via SLIC

turemethods

Mamie Z Li & Stephen J Elledge

NATURE METHODS | VOL.4 NO.3 | MARCH 2007 | 251

Clontech	In-Fusion	> 95% multi-fragment	18, 80 Eur
GenScript	CloneEZ	> 95% up to 12kb	5, 50 Eur
BioCat	Cold Fusion	> 95% multi-fragment	27, 50 Eur
Invitrogen	GENEART	multi-fragment	17, 05 Eur

Bacterial expression - SLIC cloning

Harnessing homologous recombination *in vitro* to generate recombinant DNA via SLIC

turemethods

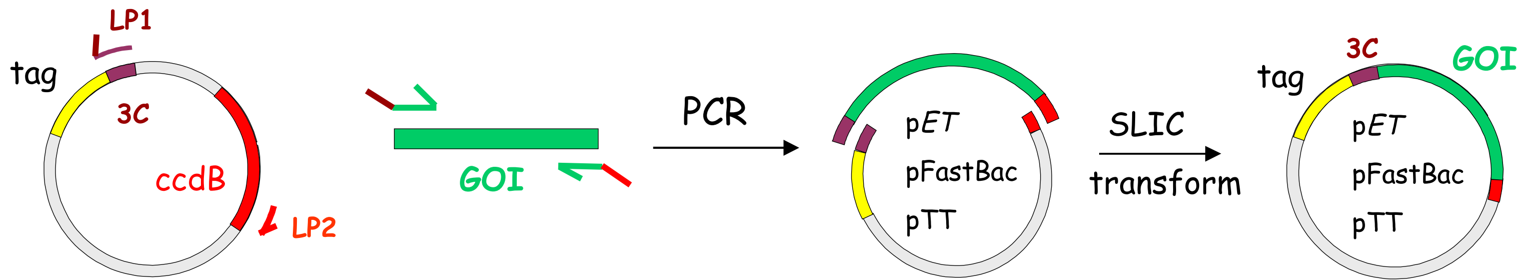
Mamie Z Li & Stephen J Elledge

NATURE METHODS | VOL.4 NO.3 | MARCH 2007 | 251

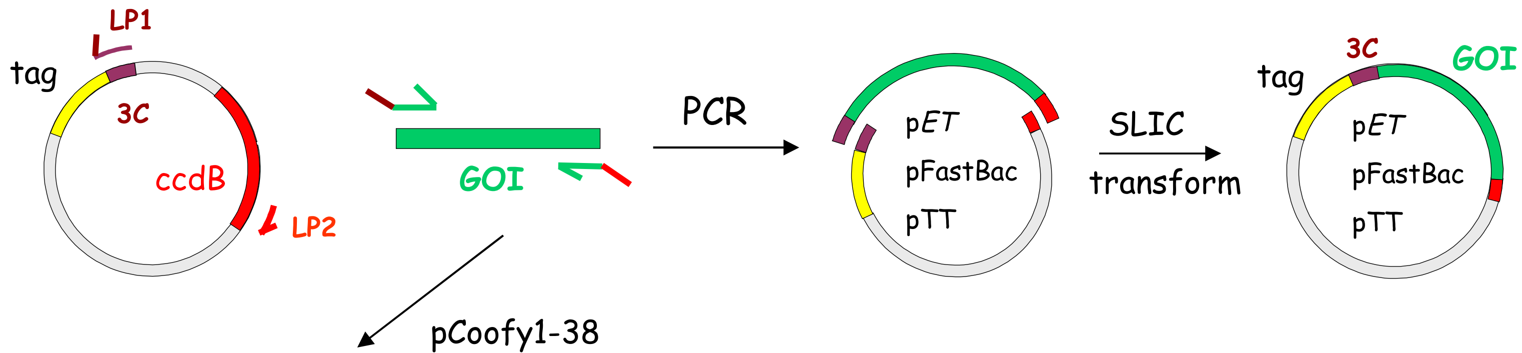
Clontech	In-Fusion	> 95% multi-fragment	18, 80 Eur
GenScript	CloneEZ	> 95% up to 12kb	5, 50 Eur
BioCat	Cold Fusion	> 95% multi-fragment	27, 50 Eur
Invitrogen	GENEART	multi-fragment	17, 05 Eur

- **Versatile**
  sequence and restriction-site independent
- **Fast and Simple**
  no restriction digestion, phosphorylation
  blunt-end polishing
- **Efficient** > 95%
- **Directional**
  insert in desired orientation
  multiple ragment cloning
- **Precise**
  no unwanted base pairs

Design of SLIC expression vectors



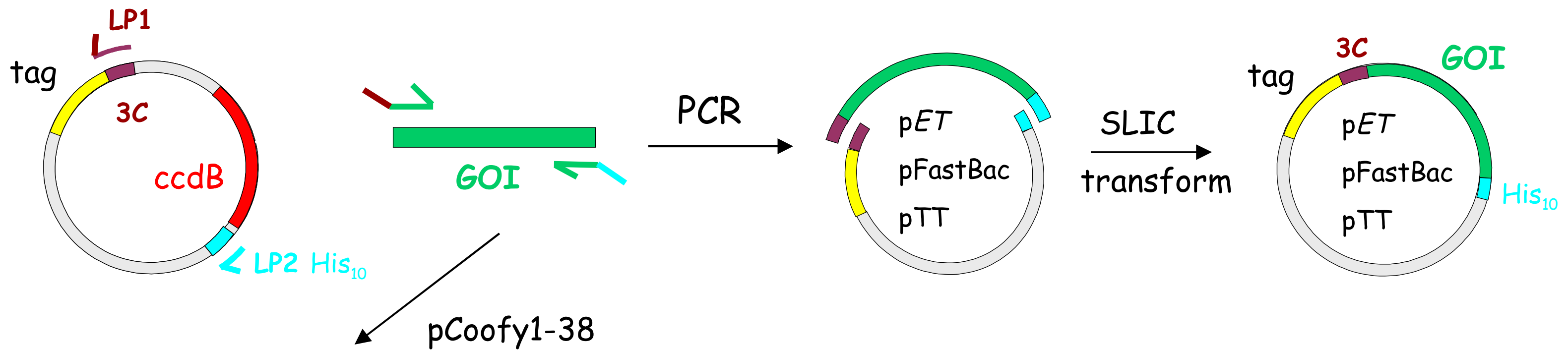
Design of SLIC expression vectors



E. coli

N-tag	double N-tag		N-tag	C-tag
His_6	His_{10}	CBP	CBP	His_{10}
His_{10}	His_{10}	StrepOne	S	His_{10}
S	His_6	Trx	StrepOne	His_{10}
StrepII	His_{10}	Trx	Trx	His_{10}
StrepOne	His_6	GST	MBP	His_{10}
CBP	His_6	MBP	NusA	His_{10}
MBP	His_{10}	NusA	S StrepOne	His_{10}
	S	StrepOne	His_6	StrepOne
	His_6	Sumo1	His_{10}	StrepOne
	His_6	Sumo3		
	His_{10}	Sumo3		

Design of SLIC expression vectors



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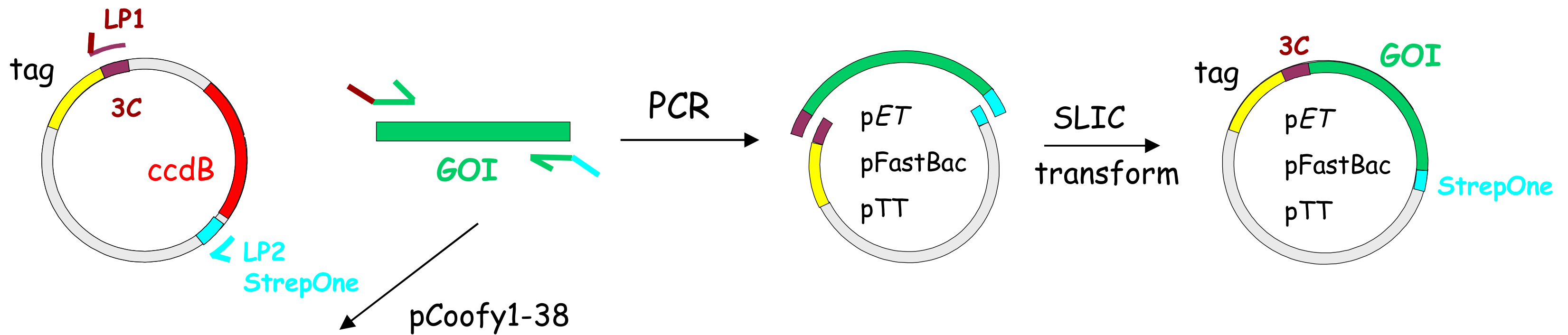
His_{10}	His_{10}	StrepOne	S	His_{10}
S	His_6	Trx	StrepOne	His_{10}
StrepII	His_{10}	Trx	Trx	His_{10}
StrepOne	His_6	GST	MBP	His_{10}
CBP	His_6	MBP	NusA	His_{10}
MBP	His_{10}	NusA	S StrepOne	His_{10}
	S	StrepOne	His_6	StrepOne
	His_6	Sumo1	His_{10}	StrepOne
	His_6	Sumo3		
	His_{10}	Sumo3		

Design of SLIC expression vectors



E. coli

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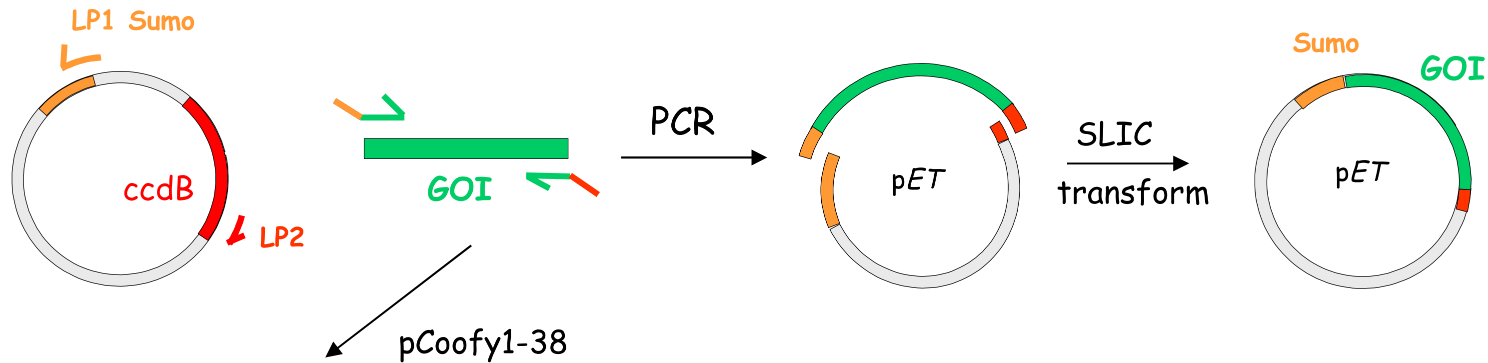
StrepII	His_{10}	Trx	Trx	His_{10}
StrepOne	His_6	GST	MBP	His_{10}
CBP	His_6	MBP	NusA	His_{10}
MBP	His_{10}	NusA	S StrepOne	His_{10}
	S	StrepOne	His_6	StrepOne
	His_6	Sumo1	His_{10}	StrepOne
	His_6	Sumo3		
	His_{10}	Sumo3		

Design of SLIC expression vectors



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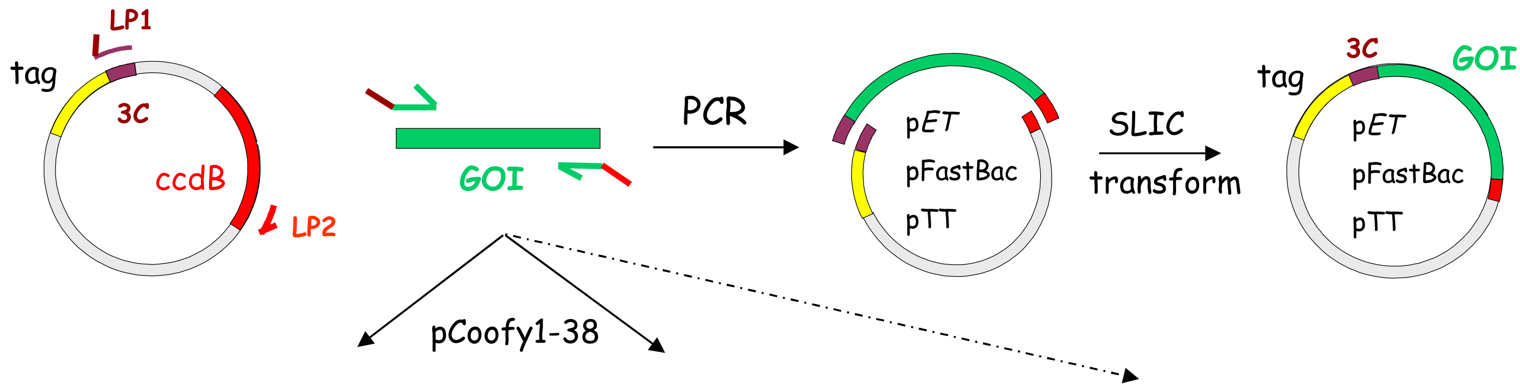
StrepOne	His_6	GST	MBP	His_{10}
CBP	His_6	MBP	NusA	His_{10}
MBP	His_{10}	NusA	S StrepOne	His_{10}
	S	StrepOne	His_6	StrepOne
	His_6	**Sumo1**	His_{10}	StrepOne
	His_6	**Sumo3**		
	His_{10}	**Sumo3**		

Design of SLIC expression vectors



E. coli

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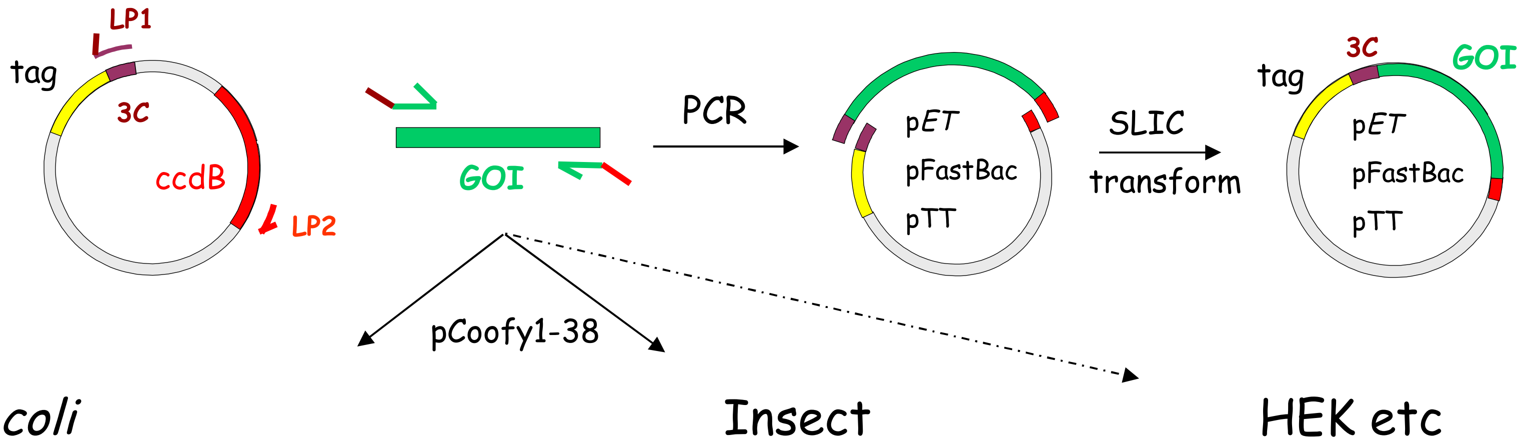
His$_{10}$	His$_{10}$	StrepOne	S	His$_{10}$
S	His$_6$	Trx	StrepOne	His$_{10}$
StrepII	His$_{10}$	Trx	Trx	His$_{10}$
StrepOne	His$_6$	GST	MBP	His$_{10}$
CBP	His$_6$	MBP	NusA	His$_{10}$
MBP	His$_{10}$	NusA	S StrepOne	His$_{10}$
	S	StrepOne	His$_6$	StrepOne
	His$_6$	Sumo1	His$_{10}$	StrepOne
	His$_6$	Sumo3		
	His$_{10}$	Sumo3		

Insect

transient *pIEX*	N-His$_6$
Baculovirus *pFastBac*	N-His$_6$
	N-His$_6$-GST
	N-His$_6$-MBP

HEK etc

Design of SLIC expression vectors



N-tag	double N-tag		N-tag	C-tag
His_6	His_{10}	CBP	CBP	His_{10}
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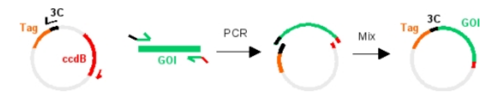
MBP	His_{10}	NusA	S StrepOne	His_{10}
	S	StrepOne	His_6	StrepOne
	His_6	Sumo1	His_{10}	StrepOne
	His_6	Sumo3		
	His_{10}	Sumo3		

Primer Design Tool

- Biophysical Methods
- DNA Sequencing
- Peptide Synthesis Service
- Light Microscopy
- Recombinant Protein Production
  - News
  - Materials: Plasmids, Strains, Enzymes
  - Cloning
  - Expression Screening
  - Expression in E. coli
  - Expression in Insect cells
  - Expression in Leishmania tarentolae
  - Expression in Mammalian cells
  - Expression in Pichia pastoris
  - Fermentation
  - Protein Purification
  - Status Report
  - List and Prices
  - Order Form
  - How to find us
- Custom OligoRNA Synthesis Service
- Proteomics / Mass

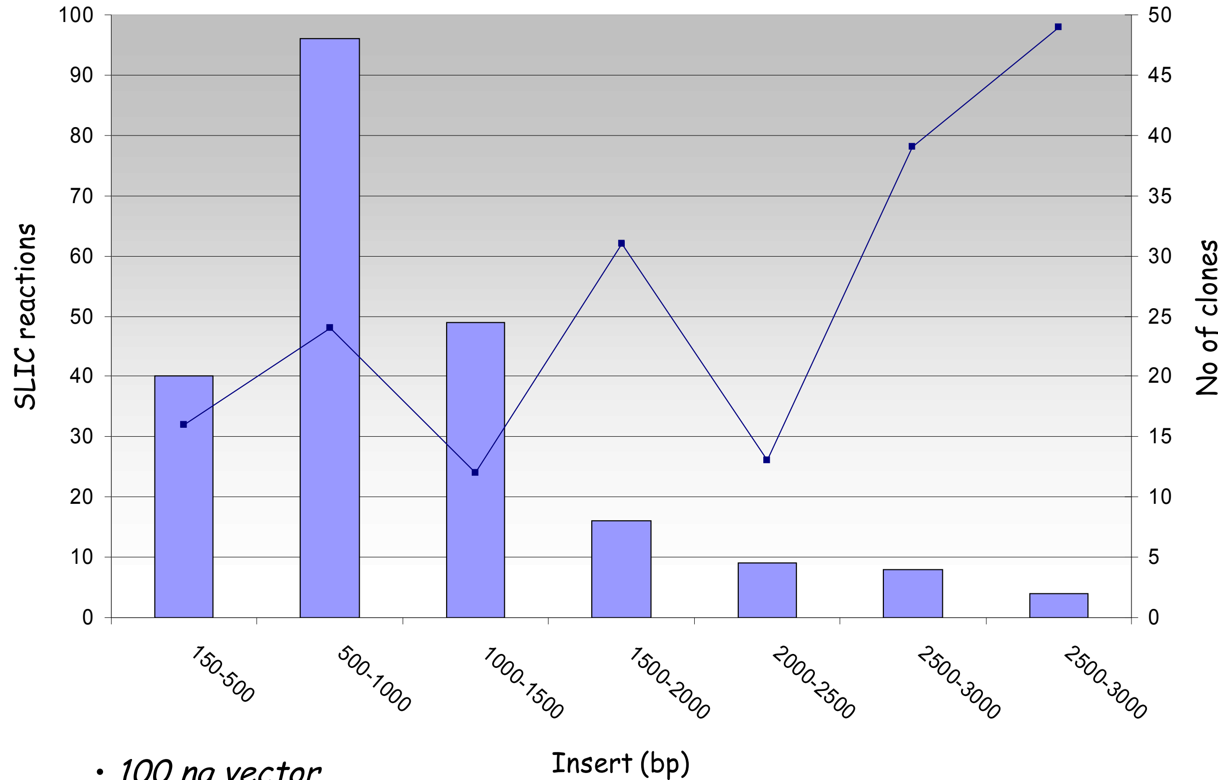


Primer Design and Cloning Order
All SLIC expression vectors currently available at the Core Facility are listed in the Primer Design the sequence of your gene of interest, PCR primers as well as internal sequencing primers cloning into all vectors selected from the list.
The tool can be used in two modes:
Design: primer design and display of DNA and protein parameters for all recombinant constructs s
Order: primer design and display of all DNA and protein parameters for all recombinant construct:
Your order of the selected constructs will be directed to the Core Facility. You need to order the by the tool, PCR amplify – please note, that a high fidelity polymerase, e.g. PhusionTM is recomn and deliver the PCR reaction to the Core Facility (Judith Scholz).

LOGIN
Login:
Password:
Order | Design

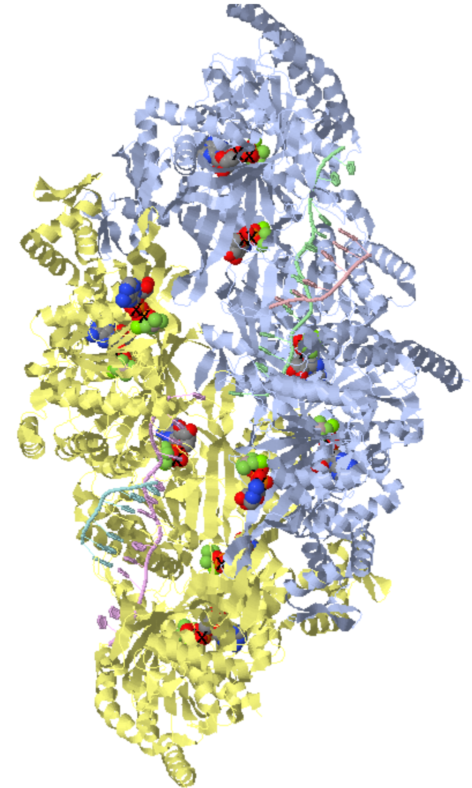
Chose **"Order"** if you want the MPI Core Facility to clone your Gene of Interest.
Chose **"Design"** if you want to do the cloning yourself. Additional information will be shown.

SLIC statistics



- *100 ng vector*
- *vector : insert 1:3*
- *RecA 2ng*
- *RecA buffer*
- *30 min 37°C*
- *transform into chemocompetent Omnimax cells*

- **fast and simple**
- **efficient**
- **no background**
- **but: take care of ccdB**



Chen *et al* ,Nature 2008

Bacterial expression with pCoofy's

Bacterial eGFP expression with pCoofy's

90
80
70
60
50
40
30
20
10
0
GFP in total cellular protein (%)
4000
3500
3000
2500
2000
1500
1000
500
0
GFP fluorescence (FU)
His6
His10
CBP
StrepI
S-Tag
His6-Gst
His6-Sumo3
TrxA
His6-MBP
NusA

Upscale HCD fermentation

2ml

- Rosetta, BL21-AI, Shuffle
- autoinduction / YG
- 18, 24, 30, 37°C
- oD_{600} = 2-40
- ~ 50-100 μg, tag removal
- BioAnalyzer + LCMS

Upscale HCD fermentation

2ml

- Rosetta, BL21-AI, Shuffle
- autoinduction / YG
- 18, 24, 30, 37°C
- oD_{600} = 2-40
- ~ 50-100 µg, tag removal
- BioAnalyzer + LCMS

1 L

oD_{600} = 20-40 / 40-60 g cells

Upscale HCD fermentation

2ml

- Rosetta, BL21-AI, Shuffle
- autoinduction / YG
- 18, 24, 30, 37°C
- oD_{600} = 2-40
- ~ 50-100 µg, tag removal
- BioAnalyzer + LCMS

1 L

oD_{600} = 20-40 / 40-60 g cells

10 L

oD_{600} = 20-40 / 400-600 g cells

Upscale HCD fermentation

2ml

- Rosetta, BL21-AI, Shuffle
- autoinduction / YG
- 18, 24, 30, 37°C
- oD_{600} = 2-40
- ~ 50-100 µg, tag removal
- BioAnalyzer + LCMS

1 L

oD_{600} = 20-40 / 40-60 g cells

10 L

oD_{600} = 20-40 / 400-600 g cells

- best protocol 24°C autoinduction*
- 18°C expression YG / IPTG batch
- MBP tagged proteins LB / Glucose fed-batch (acid)

* F.W.Studier (2005) *Protein Expression and Purification* 41 207-234

Selenomethionine incorporation

Target protein	BAR protein, 4 methionines
B834 ($meth^-$)	Novagen OnEx2 125 μg/ml SeMeth 12g cells / L - poor expression
B834 ($meth^-$)	Studier MM + 10μg/ml Meth IPTG + 125 μg/ml SeMeth 24g cells / L - high expression
Rosetta ($meth^+$)	Studier MM IPTG + 125 μg/ml SeMeth 40g cells / L - high expression

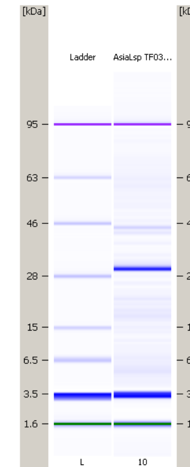
Selenomethionine incorporation

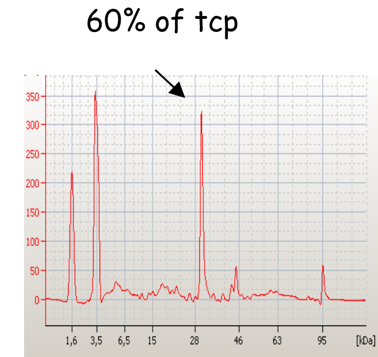
Target protein — BAR protein, 4 methionines

B834 (meth$^-$) — Novagen OnEx2 125 µg/ml SeMeth
12g cells / L - poor expression

B834 (meth$^-$) — Studier MM + 10µg/ml Meth
IPTG + 125 µg/ml SeMeth
24g cells / L - high expression

Rosetta (meth$^+$) — Studier MM
IPTG + 125 µg/ml SeMeth
40g cells / L - high expression





Rosetta + SeMeth,
total lysate Techfors

Selenomethionine incorporation

Target protein	BAR protein, 4 methionines
B834 (meth-)	Novagen OnEx2 125 µg/ml SeMeth 12g cells / L - poor expression
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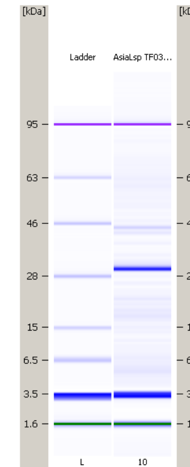
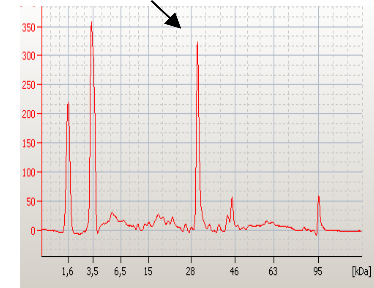
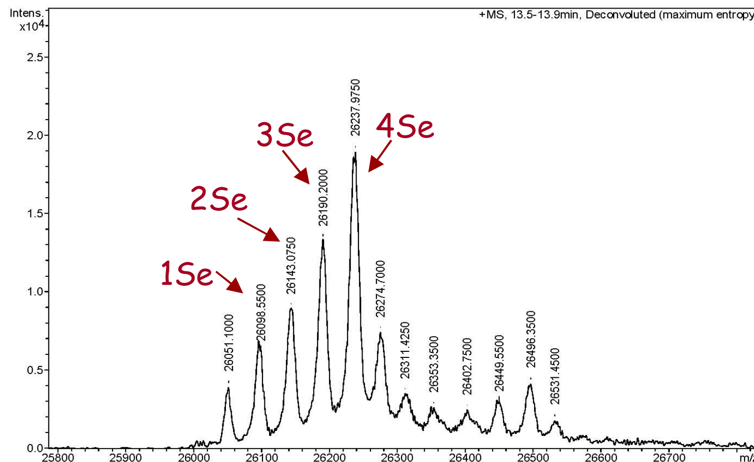


60% of tcp

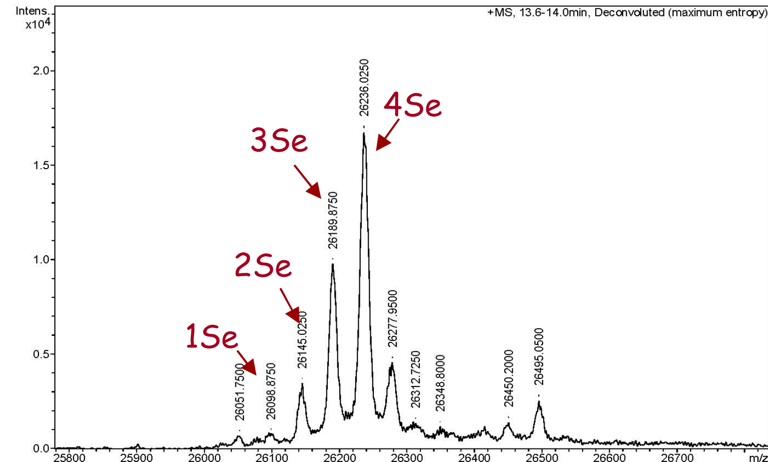


Rosetta + SeMeth,
total lysate Techfors

Rosetta



B834



N. Ziolkowska, T.Walther, submitted in *Nature Structural Biology*

Outlook “Large Scale failure”

SLIC shifted problems to protein purification

soluble aggregates, degradation, precipitation
proteins solubilized by tags often not correctly folded!

- reliable monitoring of protein quality at **small scale** (Sypro Orange, Enzo Stain not sufficient) **before** production
- protocols to prevent aggregation
- small scale in different hosts >> quality >> production in the proper host

Journal of Structural Biology 172 (2010) 14–20

Widening the bottleneck: Increasing success in protein expression and purification ☆

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Acknowledgements

Ario de Marco

IFOM-IEO

Hüseyin Besir

EMBL Heidelberg

Sandra Thuns, Tim Bergbrede

Kirill Alexandrov

MPI of Molecular Physiology

Stephan Uebel

Lissy Weyher

Evi Stieger

Claudia Franke

Judith Scholz