

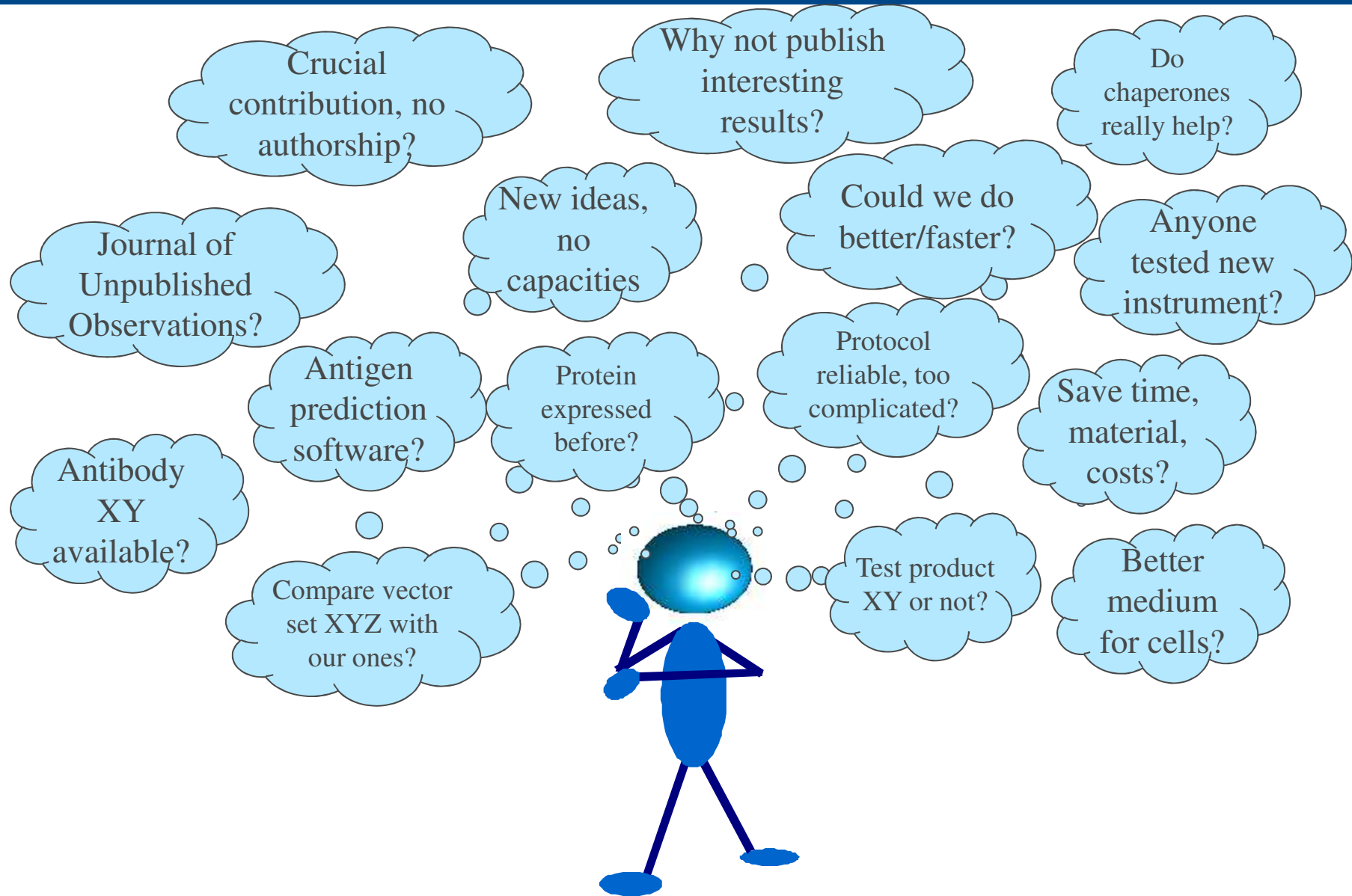


# Protein Production and Purification Partnership in Europe

Initiated in 2010 by Hüseyin Besir (EMBL Heidelberg)

1<sup>st</sup> 'kick-off/brain-storming' meeting in Halle 2011





Copenhagen 2011

Barcelona 2012

Heidelberg 2012

Porto 2013

Vienna 2014

Paris 2014

Dresden 2015

Munich 2015

Heidelberg 2016

Rehovot 2016

Prague 2017

Gent 2018

London 2018





Protein Production and Purification  
Partnership in Europe

## Projects and Benchmarks

Sf21 genome assembly P4EU crowd funded (GeneCore EMBL + VBCF)

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Study: PRJEB12116  
SF21 cell line genome assembly

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### Genetic code expansion for multiprotein complex engineering

Christine Koehler<sup>1</sup>, Paul F Sauter<sup>2</sup>, Mirella Wawryszyn<sup>2</sup>, Gemma Estrada Girona<sup>1</sup>, Kapil Gupta<sup>3</sup>, Jonathan J M Landry<sup>1</sup>, Markus Hsi-Yang Fritz<sup>1</sup>, Ksenija Radic<sup>1</sup>, Jan-Erik Hoffmann<sup>1</sup>, Zhuo A Chen<sup>4</sup>, Juan Zou<sup>4</sup>, Piau Siong Tan<sup>1</sup>, Bence Galik<sup>5</sup>, Sini Junttila<sup>5</sup>, Peggy Stolt-Bergner<sup>5</sup>, Giancarlo Pruneri<sup>6</sup>, Attila Gyenesi<sup>5</sup>, Carsten Schultz<sup>1</sup>, Moritz Bosse Biskup<sup>2</sup>, Hüseyin Besir<sup>1</sup>, Vladimir Benes<sup>1</sup>, Juri Rappsilber<sup>4,7</sup>, Martin Jechlinger<sup>1</sup>, Jan O Korbel<sup>1</sup>, Imre Berger<sup>3,8</sup>, Stefan Braese<sup>2,9</sup> & Edward A Lemke<sup>1</sup>

ones of the most widely used systems  
udy we present an approach using diffe  
sembly contains 4,020 scaffolds of size,



RESEARCH ARTICLE

### Genomic Analysis and Isolation of RNA Polymerase II Dependent Promoters from *Spodoptera frugiperda*

Maren Bleckmann<sup>1</sup>, Markus H.-Y. Fritz<sup>2</sup>, Sabin Bhuj<sup>3</sup>, Michael Jarek<sup>3</sup>, Margitta Schürig<sup>1</sup>, Robert Geffers<sup>3</sup>, Vladimir Benes<sup>2</sup>, Hüseyin Besir<sup>2</sup>, Joop van den Heuvel<sup>1\*</sup>

<sup>1</sup> Department of Structure and Function of Proteins, Research Group Recombinant Protein Expression, Helmholtz Centre for Infection Research, Braunschweig, Germany, <sup>2</sup> Department of Protein Expression and Purification Core Facility & Genomics Core Facility, EMBL Heidelberg, Germany, <sup>3</sup> Department of Genome Analytics, Helmholtz Centre for Infection Research, Braunschweig, Germany

NATURE METHODS | VOL.13 NO.12 | DECEMBER 2016 | 997

Showing results 1 - 6 of 6 results

Study accession	Sample accession	Secondary sample	Experiment accession	Run accession	Tax ID	Scientific name	Instrument model	Library layout	FASTQ files	FASTQ files	Submitted files	Submitted files	NCBI SRA	NCBI SRA file	CRAM Index	CRAM Index
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## Projects and Benchmarks

### Benchmarking the PROSS algorithm-intelligent protein design for improved stability/activity.

pross.weizmann.ac.il/bin/steps

PROSS (Protein Repair One-Step Shop) [required data 12% complete]

Email address  Valid email address to send the reports to. Enter multiple email addresses separated by commas, semicolons or spaces.

Term Of Use  I accept the Terms and Conditions

Next step | Examples and Frequently Asked Questions

Questions and support  
Algorithm and processing: Adi Goldenzweig adi.goldenzweig@weizmann.ac.il  
Server: Jaime Prilusky jaim.prilusky@weizmann.ac.il  
Overview: Sarel Fleishman sarel.fleishman@weizmann.ac.il

PROSS is free to academic users.  
Commercial users, please email [info.yeda@weizmann.ac.il](mailto:info.yeda@weizmann.ac.il) and [cc.sarel.fleishman@weizmann.ac.il](mailto:cc.sarel.fleishman@weizmann.ac.il)

Please cite:

Goldenzweig, A. et al. Automated Structure- and Sequence-Based Design of Proteins for High Bacterial Expression and Stability. *Mol. Cell* 2016, 63 (2), 337–346.  
PMID: 27425410 PMCID: PMC4961223 DOI: [10.1016/j.molcel.2016.06.012](https://doi.org/10.1016/j.molcel.2016.06.012) PDF HTML

For more about the rationale behind PROSS, checkout our review in *Ann Rev Biochem*:

Goldenzweig, A., Fleishman, S. Principles of Protein Stability and Their Application in Computational Design. *Annu. Rev. Biochem.* 2018.  
PMID: 29401000

More studies using PROSS:

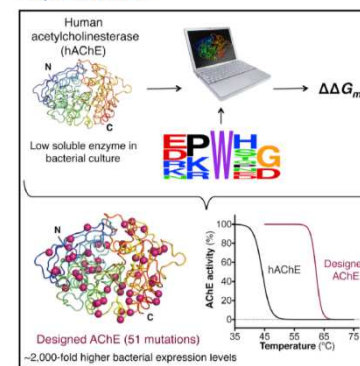
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PMID: 28096331 DOI: [10.1016/j.cell.2017.07.012](https://doi.org/10.1016/j.cell.2017.07.012)
2. Brazzotto, X.; Igert, A.; Guillou, V.; Santoni, G.; Nachon, F. Bacterial Expression of Human Butyrylcholinesterase as a Tool for Nerve Agent Bioscavengers Development. *Molecules* 2017, 22 (11), 1907.  
PMID: 29077024
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PMID: 28159998
5. Goldsmith, M.; Aggarwal, N.; Ashani, Y.; Jubran, H.; Greisen, P. J.; Ovchinnikov, S.; Leader, H.; Baker, D.; Sussman, J. L.; Goldenzweig, A.; et al. Overcoming an Optimization Plateau in the Directed Evolution of Highly Efficient Enzymes. *ACS Synth. Biol.* 2017, 6 (4), 333–345.  
PMID: 28159998
6. Butdun, C. M.; Jean, J. X.; Bedford, M. R.; Howarth, M. SnoopLigase Catalyzes Peptide-Peptide Locking and Enables Solid-Phase Conjugate Isolation. *J. Am. Chem. Soc.* 2018, 140 (8), 3008–3018.  
PMID: 29402082

### Molecular Cell

Technology

### Automated Structure- and Sequence-Based Design of Proteins for High Bacterial Expression and Stability

#### Graphical Abstract



#### Authors

Adi Goldenzweig, Moshe Goldsmith, Shannon E. Hill, ..., Joel L. Sussman, Dan S. Tawfik, Sarel J. Fleishman

#### Correspondence

[dan.tawfik@weizmann.ac.il](mailto:dan.tawfik@weizmann.ac.il) (D.S.T.), [sarel@weizmann.ac.il](mailto:sarel@weizmann.ac.il) (S.J.F.)

#### In Brief

Heterologous expression of proteins and their mutants often results in misfolding and aggregation. Goldenzweig et al. (2016) developed an automated algorithm for protein stabilization requiring minimal experimental testing; for instance, the five tested variants of human acetylcholinesterase showed  $\geq 100$ -fold higher soluble bacterial expression and higher melting temperatures than wild-type.



Protein Production and Purification  
Partnership in Europe

## Projects and Benchmarks

BEVS protein expression benchmarking: Tn7 based BEVs vs. FlashBac vs.  
'custom'-based systems...

Journal of Structural Biology 203 (2018) 71–80



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Journal of Structural Biology

journal homepage: [www.elsevier.com/locate/yjsbi](http://www.elsevier.com/locate/yjsbi)



### Baculovirus-driven protein expression in insect cells: A benchmarking study

Peggy Stolt-Bergner<sup>a</sup>, Christian Benda<sup>b</sup>, Tim Bergbrede<sup>c</sup>, Hüseyin Besir<sup>d</sup>, Patrick H.N. Celie<sup>e</sup>,  
Cindy Chang<sup>f</sup>, David Drechsel<sup>g</sup>, Ariane Fischer<sup>b</sup>, Arie Geerlof<sup>h</sup>, Barbara Giabbai<sup>i</sup>,  
Joop van den Heuvel<sup>j</sup>, Georg Huber<sup>h</sup>, Wolfgang Knecht<sup>k</sup>, Anita Lehner<sup>a</sup>, Regis Lemaitre<sup>g</sup>,  
Kristina Nordén<sup>k</sup>, Gwynn Pardee<sup>l</sup>, Ines Racke<sup>d</sup>, Kim Remans<sup>d</sup>, Astrid Sander<sup>m</sup>, Judith Scholz<sup>n</sup>,  
Magda Stadnik<sup>e</sup>, Paola Storici<sup>i</sup>, Daniel Weinbruch<sup>o</sup>, Isabel Zaror<sup>l</sup>, Linda H.L. Lua<sup>f,\*</sup>,  
Sabine Sunnmann<sup>n,\*</sup>





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## Protein Quality Standard

Concerns about protein quality from academic sources and the need for protein quality standards in academia raised by members as long ago as 2009:

**PepTalk 2009** „*Systems biology needs minimal standards for evaluating Recombinant Protein Functionality*“ A de Marco.

Standards in Genomic Sciences (2011) 5:195-197

DOI:10.4056/sigs.1834511

### **Recombinant protein quality evaluation: proposal for a minimal information standard**

Ashley M. Buckle<sup>1,15</sup>, Mark A. Bate<sup>1</sup>, Steve Androulakis<sup>2</sup>, Mario Cinquanta<sup>3</sup>, Jerome Basquin<sup>4</sup>, Fabien Bonneau<sup>4</sup>, Deb K. Chatterjee<sup>5</sup>, Davide Cittaro<sup>3</sup>, Susanne Gräslund<sup>6</sup>, Alicja Gruszka<sup>7</sup>, Rebecca Page<sup>8</sup>, Sabine Suppmann<sup>9</sup>, Jun X. Wheeler<sup>10</sup>, Deborah Agostini<sup>3</sup>, Mike Taussig<sup>11</sup>, Chris F. Taylor<sup>12</sup>, Stephen P. Bottomley<sup>1</sup>, Antonio Villaverde<sup>13</sup>, Ario de Marco<sup>14,\*</sup>



# Protein Production and Purification Partnership in Europe

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### Protein Quality Standard PQS

There is increasing awareness in the scientific community about the lack of reproducibility and reliability of results published with purified proteins. While protein production is highly regulated and controlled in the pharmaceutical industry by the authorities, there are up to date no guidelines or standards in place in the academic research to guarantee the quality of proteins included in scientific experiments. There is an urgent need to define guidelines for the scientists but also for editors and reviewers of scientific journals and funding agencies for their review processes:

Lebendiker M., Danielli T. and de Marco A. (2014)  
Raynal B., Lenormand P., Baron B., Hoos S. and England P.(2014)

A team of experts in the field of Biophysics (Association of Resources for Biophysical Research in Europe (ARBRE-MOBIEU) and Recombinant Protein Production (Production and Purification Partnership in Europe (P4EU) have developed the following Minimal Protein Quality Standard:

#### Members of the Protein Quality Initiative:

- Bertrand Raynal – Pasteur institute, Paris, France (ARBRE)
- Stefan Knauer – Bayreuth University, Germany (ARBRE)
- Stephan Uebel – Max-Planck Institute for Biochemistry, Munich (ARBRE)
- Rob Meijers – EMBL Hamburg, Germany (ARBRE)
- Nick Berrow – Institute for Research in Biomedicine, Barcelona, Spain (P4EU)
- Kim Remans – EMBL Heidelberg, Germany (P4EU)
- Ario de Marco – University of Nova Gorica, Slovenia (P4EU)
- Mario Lebendiker (coordinator) – Hebrew University of Jerusalem, Israel (P4EU)

### Projects and Benchmarks

PROSS Benchmarking

Insect and HEX benchmarking

SP21 genome assembly

### Courses

Hydrodynamic and thermodynamic analysis of biological macromolecules and their interactions: multi-method approaches and global data analysis, Prague, Czech Republic September 23–28, 2018

### Upcoming P4EU Meetings

13th P4EU Meetings Ghent, Belgium, July 4th – 5th 2018

14th P4EU Meetings London, UK, 3rd-4th December 2018

### Upcoming Conferences

CTLS 2018, July 1-4th, Ghent, Belgium

Proteins and Peptides Conference, 23-26 July 2018, Université de Genève

Minimal information to provide in publications
Minimal quality control parameters that should be tested on protein sample
Extended quality control parameters

The full guidelines are also available as a single pdf document [here](#) (downloads document).

The proposed guidelines are intended to lay the groundwork for the standardization and reproducibility of data. The goals of this document are to disseminate operative guidelines in our laboratories through the existing networks, to raise awareness amongst colleagues and collaborators and to encourage the whole scientific community to implement these guidelines in publications, e.g. as part of the supplementary information. The implementation of protein production and QC data will allow greater transparency to readers and enable efficient reproducibility in other



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### Protein Quality Standard PQS

Protein production and purification in the pharmaceutical industry is highly regulated and controlled by the authorities, there are up to date no guidelines or standards in place in the academic research to guarantee the quality of protein included in scientific experiments. There are no guidelines or standards in place in the academic research to guarantee the quality of protein included in scientific experiments. There are no guidelines or standards in place in the academic research to guarantee the quality of protein included in scientific experiments.

Lebendiker M., Danieli T. and de Marco A. (2014)  
Raynal B., Lenormand P., Baron B., Hoos S. and England P.(2014)

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Minimal information for reporting protein production and purification (MIRAP) initiative  
Bertrand Raynal – Pasteur Institute, Paris, France (ARBRE)  
Stefan Knauer – Bayreuth University, Germany (ARBRE)  
Stephan Uebel – Max-Planck Institute for Biochemistry, Munich, Germany (ARBRE)  
Ario de Marco – University of Nova Gorica, Slovenia (P4EU)  
Mario Lebendiker (coordinator) – Hebrew University of Jerusalem, Israel (P4EU)

Minimal information for reporting protein production and purification (MIRAP) initiative
Minimal quality control parameters that should be used for protein sample
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### Projects and Benchmarks

PROSS  
Benchmarking  
Protein production and purification benchmarking

Protein production and purification benchmarking  
thermodynamic analysis of biological macromolecules and their interactions: multi-method approaches and global data  
Prague, Czech Republic  
23-28, 2018

Upcoming P4EU Meetings  
4th - 5th 2018

14th P4EU Meeting,  
London, UK, 3rd-4th  
December 2018

### Upcoming Conférences

CTLS 2018, July  
1-4th, Ghent,  
Belgium  
July 2018, Université  
de Genève

**Implemented over one year  
Data collected-ca. 150 samples  
Correlation between levels of  
quality control and  
performance in downstream  
experiments.  
Currently re-  
writing/submitting as Nature  
review...**



Protein Production and Purification  
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## Courses

December 2013 Practical Course on Mammalian Protein expression, Helmholtz Center, Braunschweig

November 2017 Advanced Methods in Protein Purification, MPIB Martinsried

May/June 2018 Expert training in MultiBac expression,, Harwell Research Complex, Oxford



Small numbers of students, intensive hands-on practical experience



# Protein Production and Purification Partnership in Europe

<https://p4eu.org/>

- New homepage launched August 2017
- >125 members from ca.90 labs CFs/PIs/Pharmas/Biotech)
- Tens of thousands of views
- Job advertisement, members publications
- Public and confidential communication forum

The screenshot shows the P4EU website homepage. At the top, there is a navigation bar with links for Home, About Us, News, Join Us, Protein Quality Standard PQS, Jobs, Contact, and Archives. Below the navigation bar, the P4EU logo and name are displayed. The main content area is titled "Welcome to the P4EU Network" and contains a "Login" section with fields for Username or Email and Password, a "Lost Password?" link, and a "Remember me" checkbox. To the right of the login section, there is a "Projects and Benchmarks" section with links to "PROSS Benchmarking", "Insect and HEK benchmarking", and "Sf21 genome assembly". Below this, there is a "Courses" section with a link to "Hydrodynamic and thermodynamic analysis of biological macromolecules and their interactions: multi-method approaches and global data analyses, Prague, Czech Republic September 23-28, 2018". At the bottom, there is an "Upcoming P4EU Meetings" section with links to "13th P4EU Meeting, Ghent, Belgium, July 4th - 5th 2018" and "14th P4EU Meeting, London UK, 1st-4th".



# Protein Production and Purification Partnership in Europe

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## Wiki

### [P4EU Wiki](#)

- > [Molecular Biology](#)
- > [Protein Purification](#)
- > [Protein Quality control](#)
- > [Protein – Protein Interaction](#)
- > [Expression Hosts](#)

Welcome to the P4EU Wiki. This is an interactive protocol database with a “comment” function to add corrections, suggestions, improvements etc. You find three different types of entries:

[+](#) Textbook protocols

[+](#) “Expert User” Protocols

[+](#) “Author” Protocols

### Review Process

The submitted protocols will undergo a review process. Members of the editorial board will distribute the protocols to the respective experts within the P4EU network for review. Members of the editorial board:

- > [Patrick Celie](#), NKI Protein Facility, Netherlands Cancer Institute (NKI)
- > [Mario Lebendiker](#), Protein Purification Facility, Wolfson Centre for Applied Structural Biology, Jerusalem
- > [Frederico Silva](#), Protein Production and Purification Unit, IBMC Porto



# Protein Production and Purification Partnership in Europe

Author	Posts	
	August 11, 2017 at 13:04	#820
	<a href="#">EDIT</a>   <a href="#">CLOSE</a>   <a href="#">STICK (TO FRONT)</a>   <a href="#">MERGE</a>   <a href="#">TRASH</a>   <a href="#">SPAM</a>   <a href="#">REPLY</a>	



Sabine  
Suppmann  
Moderator

Dear Colleagues  
welco  
My fir  
can yo  
Thank  
Best  
Sabin

August 11, 2017 at 13:14

[EDIT](#) | [MOVE](#) | [SPLIT](#) | [TRASH](#) | [SPAM](#) | [REPLY](#) #821



Andrea Vala  
Participant

Hi Sabine,

We bought the following strain for a similar purpose (purification of a protein without endotoxin) But the project ended up being

August 11, 2017 at 13:25

[EDIT](#) | [MOVE](#) | [SPLIT](#) | [TRASH](#) | [SPAM](#) | [REPLY](#) #822



Kim Remans  
Participant

Hi Sabine,

We had to do some endotoxin-free purifications for an immunology group last year. We “decontaminated” the Akta with NaOH and Triton-X114 and used fresh columns. We measured the LPS content after each step (Ni-NTA, tag removal, second Ni-NTA and ion exchange) and after the HiTranO virtually no LPS remained (0.5

...all in the time you have got as far as ‘choose option 3 for...’ on official tech support lines!!



## Protein Production and Purification Partnership in Europe



### Minimal criteria for P4EU membership are:

In your function as head or staff member of a Core Facility / Research group you provide expertise, services, training, or access to equipment for recombinant protein production for your local scientific community AND/OR have a strong interest in methodological research in the subject of recombinant protein production.

Active participation in network activities, including attendance of, and presentations at P4EU meetings, contributions to expert protocols on the P4EU Wiki page, participation in projects or joint grant applications as well as workshops and/or staff exchanges.

Please complete the following form as thoroughly as possible, **ensuring that your e-mail address is entered correctly**. After submission your application will be approved and you will shortly receive your P4EU login details, and temporary password, by mail. Also remember to check your Spam/Junk box for this mail. Please note: information about instrumentation and methodology will be password protected. This information is fundamental to search for specific expertise and advice within the network.

save

Personal information	Institution	Equipment
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Your e-mail address*		
I accept the terms and conditions*		