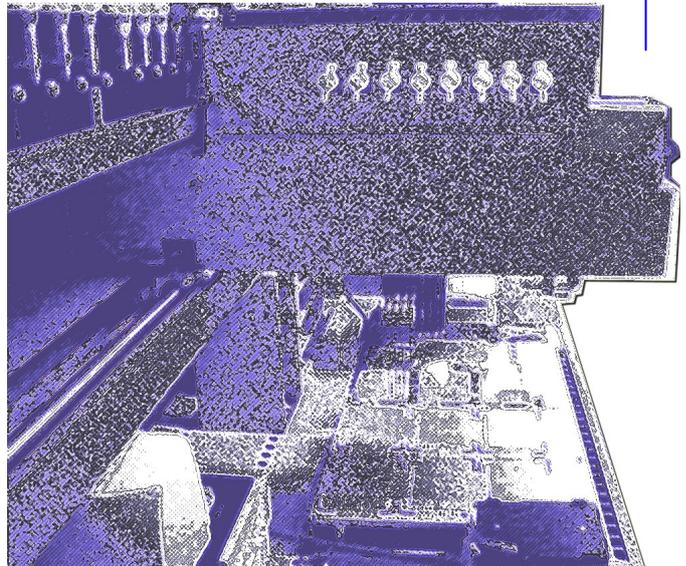
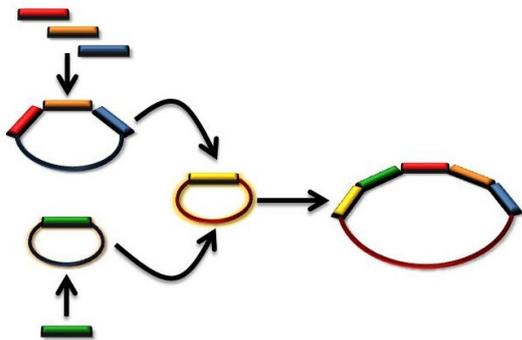
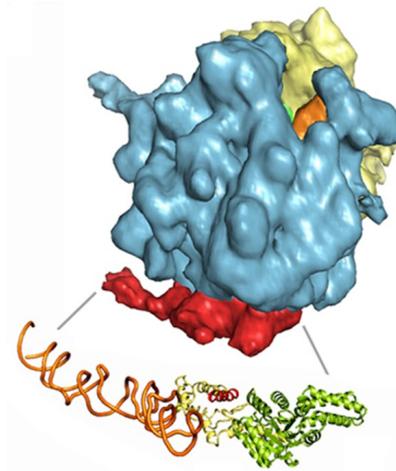


ACEMBL

Leading Platform Technology for
Multi-Protein Complex Production

MULTICOLI

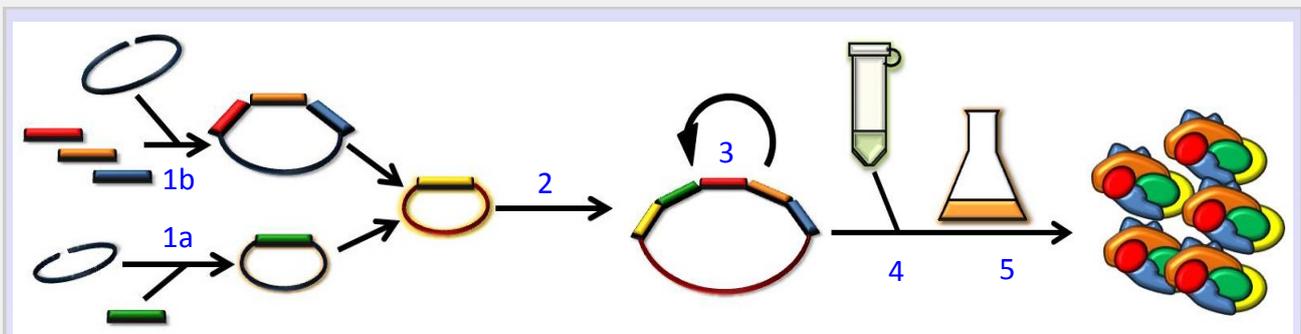
For Protein Expression in *E. Coli*



The ACEMBL *MultiColi* System

An in-depth analysis of the structure^{1,2}, function³ or functional interactions of proteins⁴ or protein complexes⁵ is often complicated or even negated by the fact that some proteins exist only in low amounts in their native cells. This makes heterologous expression of such proteins or the corresponding multi-protein complexes, e.g. for crystallization, indispensable. It becomes especially relevant when these proteins are of interest in bioengineering^{6,7} and bio-remediation⁸, when their role in cellular physiology⁹ needs to be elucidated or when pathways designed for the synthesis of artificial compounds such as polyketides¹⁰ or *in silico* models¹¹ of biochemical pathways require testing or verification.

Bacterial expression systems are a simple and cheap means for expressing prokaryotic but also some eukaryotic proteins. While multi-protein expression remains a formidable task and generating sufficiently soluble protein complexes at times requires some tweaking, such challenges now become easier with ***MultiColi***, a versatile and convenient Acceptor-Donor vector system for manual or automated recombineering of genes destined for expression in *E. coli*.

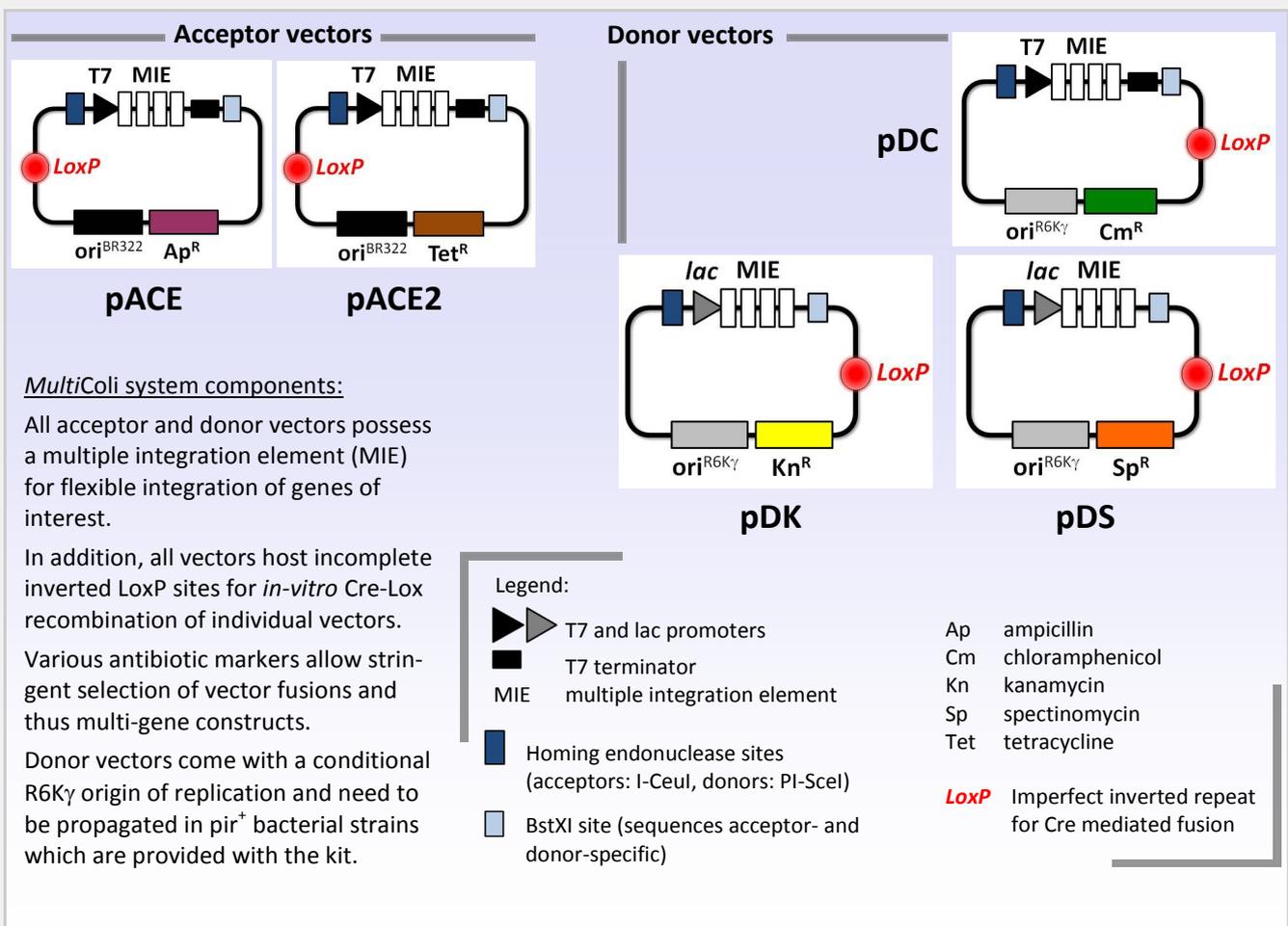


How *MultiColi* works:

1. Clone/assemble a) individual genes or b) multiple genes of interest (GOI) into **acceptor** and/or donor vectors
2. Mix and match your GOIs by (re)combining them into **one** construct
3. Select and amplify your construct
4. Transform *E. coli* with your GOIs-assembly
5. Express your protein or protein complex of choice

MultiColi

- consists of two **acceptor** and three **donor vectors** for manipulation of DNA *in vitro* and in *E. coli*
- vectors possess features that allow you to flexibly insert and recombine your gene(s) of interest into (multi-)gene expression cassettes before assembling your final construct used to transform *E. coli*.
- vectors possess features that enable a fully automated multi-gene construct assembly process; this helps speed up research, e.g. when analyzing the effects of gene variants on crystallization or on the kinetic properties of enzymes
- comes with bacterial strains for the propagation of the donor vectors and donor-donor fusions

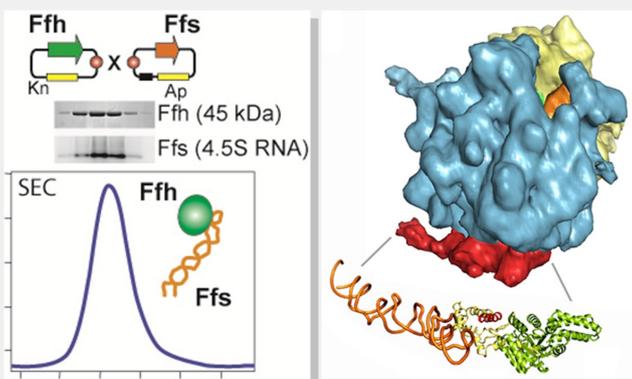


Advantages of *MultiColi*

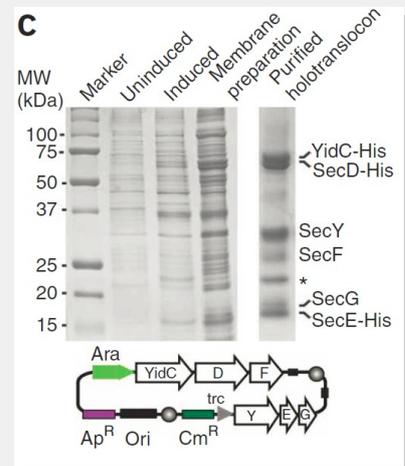
- **express single or multiple proteins** simultaneously
- **assemble multi-gene cassettes** easily and rapidly
- generate and test **gene variants** or **mutants** rapidly and in parallel
- **fuse** single or multigene acceptor and donor plasmids via **Cre recombination**
- improve **complex stability and solubility** through co-expression of partnering proteins
- **automate** your entire multigene expression vector production process for high throughput and scale-up
- **speed up** and quickly **fine-tune/adapt** your gene expression project

What *MultiColi* could do for you

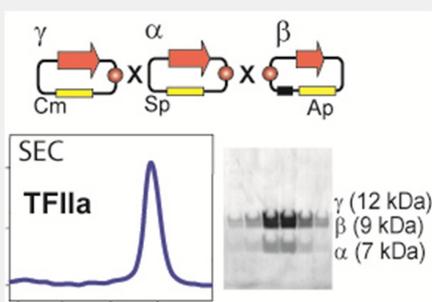
These are only a few examples of proteins and protein complexes that have been expressed using the *MultiColi* expression technology.



Protein-RNA co-expression of SRP (left) and cryo-EM structure of SRP complex bound to the ribosome (right)^A.



Western Blot showing six components of the *E. coli* holotranslocon expressed from fusion of the individual genes into a single vector construct^B.



Production of TFIIA complex (left) and crystal structure of TFIIA (above)^C.

Testimonials from *MultiColi* users

Don't just take our word on it, ask these researchers!

The *MultiColi* technology is the first fully automatable system for protein complex production in *E.coli*. We believe it will set new standards for multi-protein expression in this versatile laboratory host organism.

Dr. Imre Berger, Group Leader, Structural Complexomics, EMBL

Press releases

„Getting a grip on complexes“: www.embl-em.de/downloads/9/press03may09.pdf

„Komplexe in den Griff bekommen“: www.idw-online.de/pages/de/news312976 (in German)

„Automatable pipeline“: www.labnews.co.uk/laboratory_products.php

„Tipp 133: Automatisch Komplexe kriegen—Expression von Multiproteinkomplexen“: www.laborjournal.de/rubric/tricks/tricks/trick133.lasso (in German)

Relevant publications

Bieniossek C, Nie Y, Frey D, et al. (2009). Automated unrestricted multigene recombineering for multiprotein complex production. *Nature Methods* 6: 447-450.

Nie Y, Bieniossek C, Frey D, et al. (2009). ACEMBLING multigene expression vectors by recombineering. *Nature Protocols*, doi: 10.1038/nprot.2009.104

Nie Y, Viola C, Bieniossek C, et al. (2009). Getting a grip on complexes. *Current Genomics* 10: 558-72.



Group leader Dr. Imre Berger and research technician Maxime Chaillet present the liquid handling station they use to implement a fully automated process for protein production in *E.coli*^D.

Cited references

1) Gupta et al., 2009, *PlosOne* 4: e8028 | 2) Airola et al., 2010, *Structure* 18: 436ff. | 3) Shafqat et al, 2010, *J Mol Biol* 398: 497ff. | 4) Kohanski and Collins, 2008, *Cell* 133: 947f. | 5) Schaffitzel et al., 2006, *Nature* 444: 503ff. | 6) Patnaik, 2008, *Biotechnol Progress* 24: 38ff. | 7) Kind et al., 2010, *Metab Eng* Apr 8, epub ahead of print | 8) Cao et al., 2009, *Appl Microbiol Biotechnol* 85:207ff. | 9) Umejiwgo et al., 2008, *Chem Biol* 15: 70ff. | 10) Zhan, 2009, *Curr Top Med Chem* 9: 1958ff. | 11) Finley et al., 2010, *BMC Systems Biol* 4:7 | 12) Bieniossek et al., 2009, *Nat Meth* 6: 447ff.

Image references

A) Images courtesy of Dr. Christiane Schaffitzel, EMBL Grenoble | B) from Bieniossek et al., 2009, *Nat Meth* 6: 447ff. (with kind permission from Dr. I. Berger) | C) Image based on RCSB protein data base entry 1NH2 (Bleichenbacher et al., 2003), doi: 10.2210/pdb1nh2/pdb | D) Image with kind permission from Dr. I. Berger

Put your research in 5th gear!

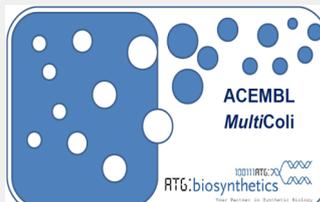
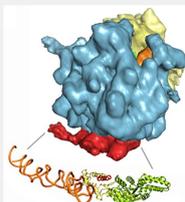
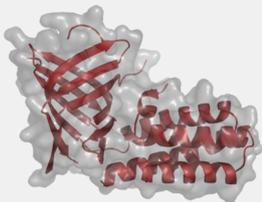
IF

- speed and performance matter in your research
 - you want an easy-to-handle system
- you want maximum flexibility in mixing and matching genes
 - you want first-class expression results
- you intend to automatize and scale-up your entire protein expression processes

THEN

the *MultiColi* System is what you need.

Get the expression results you are looking for!



Order online, via fax or telephone or have a company representative call you back for more information and to discuss our additional products and services, e.g.

- our gene synthesis service
- our proprietary codon and gene expression optimization service (*evoMAG*)
- optimizing gene assemblies for cloning (*evoMAG*)
- conceptualizing and planning complex projects

For more details on additional services please inquire about our separate brochures or go to our webpage.

ATG:biosynthetics GmbH

 Weberstraße 40
79249 Merzhausen ▪ Germany
 +49 (0)761 8889424
 +49 (0)761 8889425
 www.atg-biosynthetics.com
info@atg-biosynthetics.com

VAT-ID/Umsatzsteuer-Ident-Nr.
DE 217 329 965