Expression system
Clamydomonas reinhardtii

Introduction
The organism
Molecular biology tools
Parameters of expression
Advantages
References
Contact
Entelechon services
Entelechon Ltd and ASA Spezialenzyme Ltd are proud to present a new state-of-the-art expression system with outstanding new features, giving an exciting new choice to biotechnologists worldwide.

Supported by a grant of the prestigious BioChance programme of the German government, this innovative expression system is an excellent new alternative to the shortcomings of existing systems.

Based on the unicellular green alga *Chlamydomonas reinhardtii*, this expression system offers many vital advantages and a very favourable cost structure.

The following pages describe the expression system in close detail and cover the technical as well as commercial aspects of protein expression in *Chlamydomonas reinhardtii*. 
**The organism**

*Chlamydomonas reinhardtii* is a unicellular alga which has been used as a model organism for several decades. Its generation period is 8 to 24 hours and enables the rapid generation of clones and crossbreeds.

The alga can be grown both heterotrophically and photoautotrophically. Although haploid by default, two mating types are predetermined and can differentiate into gametes which can form diploid zygospores. This enables crossbreeding and easy combination of genetic features.

We have access to cellwall-deficient mutants that synthesize but do not incorporate or polymerize the cellwall protein and thus facilitate the export of transgenic proteins into the medium.

The 100 Mbp genome of *Chlamydomonas* – comprised of 17 chromosomes – has been completely sequenced, with 8-fold coverage. Chlamy, as the alga is affectionately called by the research community, is an excellent model for the research of cell motion, flagellar assembly, and photoreception.

The alga forms colonies on agar plates, and can be easily transformed. We have developed the molecular tools to rapidly and efficiently create transgenic clones for the expression of arbitrary proteins.
Molecular biology tools

All molecular biology tools commonly applied to expression hosts are available for *Chlamydomonas*. Transformation can be performed by simple glass bead agitation or electroporation for increased efficiency. Biolistic transformation of chloroplasts is also possible.

We have developed and optimized a set of efficient resistance markers, such as bleomycin, aminoglycosid-phospho- and -adenine-transferases, acetalactate synthetase, and protoporphyrinogen oxidase.

These are complemented by fluorescence markers such as luciferase and GFP, both of which have been made accessible by codon-optimized synthetic genes.

We have solved the problem of cryoconservation and can now store clones of interest for indefinite amounts of time.

Another valuable tool is a display system: Proteins can be targeted towards the cell membrane where they are displayed to the extracellular medium. This enables the rapid selection of relevant clones, for example in a cell sorting or ELISA setup.

An EST database for *Chlamydomonas* provides over 100,000 ESTs and thus forms an excellent basis for further research on the organism. Moreover, DNA microarrays have been made available which allow for the easy detection of homologous genes.
The fermentation can be performed under a batch-, fed-batch- or flowthrough regime. *Chlamydomonas* can be fermented in simple disposable plastic reactors, and can be grown heterotrophically or photoautotrophically.

Typical yields in the exponential growth phase yields are 230 mg/l of dry biomass.

Typical expression rates, as seen in similar green algae, are 1.4 mg/(l*h) to 8.75 mg/(l*h). The latter has been achieved in *Chlamydomonas* with added acetate, under a 24h light/dark regime.

As a proof of principle, we have successfully expressed a bleomycin/luciferase fusion protein in the nucleus, and have directed the luciferase product alternatively into the cytosol or the extracellular medium.

A bleomycin/GFP fusion protein has been directed into the nucleus, whereas a GFP-APH VIII fusion protein has been directed into the cell membrane. The GFP has been displayed extracellularly, whereas APH VIII was located on the cytosolic side, both linked by a transmembrane domain.
Advantages

The *Chlamydomonas* expression system features a range of important advantages: The alga is non-toxic and non-pathogenic. It can be easily grown and contained.

As carbon source, both CO$_2$ and acetate can be used. This reduces the cost of fermentation and makes contaminations less likely. Simple disposable plastic reactors enable the efficient assembly of large production volumes.

*Chlamydomonas* displays a glycosylation pattern very similar to mammalia. It is thus a valuable alternative for yeasts and other systems which may not provide suitable glycosylation modifications.

We have achieved complete control over the localization of expressed proteins. They can be directed into cell compartments such as mitochondria, chloroplasts, extra-cellular medium or the membrane.

The cells are significantly more biocompatible with an application in a clinical context than *E. coli*. In particular they are free of endotoxin and thus avoid the expensive and tedious endotoxin removal.

Proof of principle - targeting of Renilla luciferase into the extracellular medium.
If you are interested in these services, please contact us at +49 941.9468.360 or info@entelechon.com. Or visit our website at www.entelechon.com where you will find detailed information on all our services.

References

Please find below a list of literature references with regard to *Chlamydomonas reinhardtii* in general as well as to the expression system in particular:


A. Eichler-Stahlberg, M. Fuhrmann, and P. Hegemann (2002) Blick in die Wissenschaft 11, 18-23, “Grüne Fabriken für die Zukunft; Produktion medizinisch relevanter Bioprodukte in Mikroalgen” (“Green factories for the future; production of medically relevant bioproducts in microalgae”)


M. Fuhrmann, W. Oertel and P. Hegemann (1999) Plant Journal 19, 353-361, “A synthetic gene coding for the Green Fluorescent Protein (GFP) is a versatile reporter in Chlamydomonas reinhardtii”


The expression system is still in the BioChance funding stage. This enables us to offer the expression service at very favourable conditions.

The typical steps for a partnership would be the following milestones:

Gene optimization  ➔ transformation  ➔ analysis on a 1L scale  ➔ upscaling  ➔ expression  ➔ purification.

We can provide you with a detailed milestone plan for any or all of these steps. If you are interested in employing the *Chlamydomonas* expression system for the production of your transgenic protein, please contact us at:

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We would be happy to discuss your requirements and expression projects with you.
Entelechon services

In addition to the *Chlamydomonas* expression system, Entelechon offers a wide selection of services:

**Custom gene synthesis**
We have developed a proprietary protocol for the rapid and efficient assembly of large DNA sequence fragments. Entelechon’s gene synthesis service enables you to retrieve any target sequence within the shortest possible timeframe.

**Gene optimization**
Entelechon's proprietary software Leto has been specifically developed to address the problem of gene optimization. An intricate optimization algorithm selects the DNA sequence best suited for maximum expression.

**Bioinformatics**
Building upon our large library of molecular biology tools and routines, we can provide custom bioinformatics services at a very affordable price.

**Molecular biology services**
The highly skilled staff that performs the custom gene synthesis is also available to handle all the molecular biology projects that you intend to outsource. Rely on our efficiently organized lab to achieve outstanding results quickly.

**DNA sequencing**
Our DNA analysis unit is the core of our QC process for custom gene synthesis. The same high quality sequencing service is now available for the analysis of your sequences - no matter if you want to analyze one or many thousand DNA fragments.