

Cilian at a glance

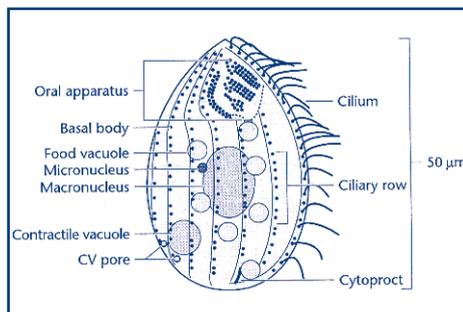
Cilian is an innovative biotechnology company, which uses the singular abilities of Ciliates, a class of eukaryotic micro organisms, for the industrial production of therapeutic proteins. The company's R&D team has more than 10 years of experience in the applied research and development of its expression system based on Ciliates. Founder and CSO of the company, Dr. Marcus Hartmann has spent his scientific career investigating protozoan organisms, particularly Ciliates. Dr. Hartmann is the author of numerous scientific publications, presentations, patents and patent applications, and a pioneer in the area of Ciliate biotechnology.

Since Dr. Hartmann formed the R&D team in 2001, Cilian has developed and achieved a marketable expression technology, brand named CIPEX, which has been optimized for the

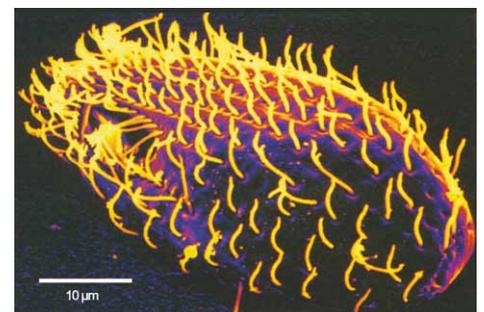
industrial production of complex recombinant drug proteins (biopharmaceuticals). The expression technology is protected by a large patent estate consisting of granted patents and patent applications.

Worldwide Cilian is the only biotech company to apply a system such as CIPEX for corporate partners. Meanwhile the expression system is ready to be utilized for production of a

variety of biological compounds, specifically monoclonal antibodies with improved effector functions as well as viral and protozoan vaccines. Therapeutic targets are the treatment of cancer and respiratory diseases, just to name a few.



Schematic diagram of the Ciliate *Tetrahymena*



Raster electron microscopic image of *Tetrahymena thermophila*

Expression Technology

The Ciliate Performance Expression system (CIPEX system): Efficient, safe and cost effective production of biopharmaceuticals.

Ciliates of the species *Tetrahymena thermophila* are eukaryotic single cell micro organisms, which are characterized by unique cell biology and genetics. The nuclear dimorphism, for instance, results in very high gene doses (45 up to 10.000 copies of a single expression cassette). High AT-content, the non-canonical codon usage and missing evidence of viruses from higher animals have shown that viruses cannot use Ciliates as hosts for proliferation. Thus, the cell biology and the genetics of Ciliates make this micro organism ideally suited for the safe production of biopharmaceuticals.

This was the basis for the development of an innovative heterologous expression system,

which is established now as a proprietary platform by Cilian.

Highlights of the CIPEX system are advantages in the field of process engineering, safety and quality to name but a few.

In consequence the CIPEX system already reached market standard parameters for yield and costs of goods with significant potential for further optimization.

With CIPEX an expression system for the pharma industry is now available, which has proven to be at least as good as currently used expression systems, but is not subject to limitations of some of those systems.

The applicability and transferability of CIPEX to proteins with different characteristics has already been established in feasibility studies.

To date Cilian has demonstrated the successful expression of 14 biopharmaceutical proteins with a space time yield between 50 to 150 mg per liter per day.

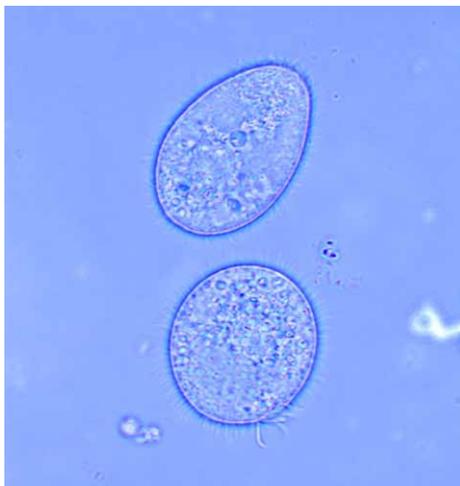
The CIPEX system has been applied to three in-house product developments: a recombinant flu vaccine, a tumor antibody and an enzyme preparation. All three passed proof-of-principle stage.

Characteristics of the Biotechnology of Ciliates

- No contamination issue (free of endogenous infectious agents; genetics and phylogenetic distance to higher animals make viral infection unlikely)
- Functional simplicity (easy to handle apathogenic eukaryotic micro organism; batch, fed batch and continuous cultivation in existing market standard bioreactor equipment possible)
- High gene doses (middle and high copy number expression cassettes allowing for high volumetric productivity)
- Advantageous and consistent oligo-mannose N-glycosylation (no microheterogeneity; no hyperglycosylation; no adverse residues; likely induction of ADCC effector function)
- Ease of scale up (proven 500 liter stir tank and 1500 liter air lift batch fermentation).



Dr. Hartmann explains Ciliate morphology



Light microscopic image of the Ciliate *Tetrahymena*

Parameter	Value
Cell density	2.4 x 10 ⁷ cells/ml (high cell density fermentation)
Max. dry weight	80 g/liter (continuous high cell density fermentation)
Generation time	1.4 - 3 h
Estimated costs medium	2-8 Euro/liter
Tolerated agitation	800 rpm (2 liter scale)
Stable fermentation time	> 1 month (continuous fermentation)
Volumetric productivity (space time yield)	50 - 150 mg/liter/day - extended fed batch (for selected biopharmaceutical proteins)

Benchmark parameters of a typical lab scale fermentation process

Results today

- Expression (proof of principle) of 14 biopharmaceutical proteins.
- Proven absence of endogenous infectious agents like mammalian viruses and mycoplasmas.
- Proof of principle studies completed for:
 - » a recombinant flu vaccine
 - » a tumor antibody
 - » an enzyme preparation against exocrine pancreatic insufficiency

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50 Liter bioreactor in the lab of Cilian