



Recombinant protein expression using protozoan organisms

The Challenge: One of the main drawbacks for developing vaccines and new drugs against some parasites has been the difficulty in obtaining large amounts of the necessary immunogens and putative drug targets using heterologous expression systems, due to either a lack of expression or expression as insoluble inclusion bodies. Moreover, a general problem that has not yet been resolved for most heterologous systems is the production of large proteins; there are few targets with lengths greater than 800 amino acids. Specially, this is true for organisms of the phylum Apicomplexa, which are responsible for serious illnesses characterized by high morbidity and mortality. Some of them are in the list of bioterrorism agents since the infection is transmitted orally and the pathogenic oocysts remain viable for long periods in soil and water. Examples of dangerous protozoan parasites include *Plasmodium falciparum* (malaria), *Toxoplasma* (infects immuno-compromised individuals), *Cryptosporidium* and *Cyclospora* (water and food-borne illnesses). Therefore, there is a need for the development of alternative heterologous system for production of Apicomplexa proteins as a fundamental tool for protein structure- function studies, antigen production, screening and profiling of candidate drugs, and understanding their mechanisms of action.

UMBI Solution: UMBI investigators have developed a novel *Perkinsus* heterologous expression system that can be useful not only for the production of recombinant proteins of medical and veterinary relevance but also for large proteins of interest. This expression system increases the number of genes that can be targeted for protein structure-function studies, production of antigens and *in vitro* drug screening.

Commercial Applications:

- A novel protein expression system to produce recombinant proteins in large quantity from protozoan parasites, e.g. *Plasmodium*, *Toxoplasma*, and *Cryptosporidium*, and large proteins in general.
- Research tool for protein function/structure studies, antigen production, screening and profiling of candidate drugs, and understanding their mechanism of action.

Advantages:

- Single-celled eukaryotic organism closely related to protozoan parasites, extensive genomic studies.
- Overcomes limitations of prokaryotic (e.g. *Escherichia coli*) or eukaryotic (yeast, mammalian) vector systems for production of protozoan proteins.
- Non-pathogenic to humans.

- Scalable growth in a full-defined cell free medium.

Stage of Development: Reduced to practice

Patent Status: Pending PCT patent application

Licensing Potential: UMBI is seeking exclusive or non-exclusive licensees to this technology. The UMBI Inventor would welcome the opportunity to collaborate with any licensee to further refine the invention or extend its capabilities.

Lead Inventor & UMBI Reference: Robledo, 07-05; 07-006

Relevant Publications:

1. Fernández-Robledo JA, Lin Z, Vasta GR. 2008. Transfection of the protozoan parasite *Perkinsus marinus*. Mol Biochem Parasitol. 157(1):44-53.
2. Robledo JA, Courville P, Cellier MF, Vasta GR. 2004. Gene organization and expression of the divalent cation transporter Nramp in the protistan parasite *Perkinsus marinus*. J Parasitol. 90(5): 1004-14.
3. Gauthier, J.D., Feig, B. and Vasta, G.R. 1995. Effect of fetal bovine serum glycoproteins on the *in vitro* proliferation of the oyster parasite *Perkinsus marinus*: Development of a fully defined medium. J. Eukaryot Microbiol. 42: 307-13.
4. Gauthier, J.D. and Vasta, G.R. 1995. *In vitro* culture of the eastern oyster parasite *Perkinsus marinus*: Optimization of the methodology. J. Invertebr Pathol. 66: 156-68.

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