

innovations

from The University of Vermont

TITLE: PRODUCTION OF RECOMBINANT PROTEINS IN
ACANTHAMOEBA CASTELLANII BY STABLE TRANSFECTION

INVENTOR: Erik Bateman

DESCRIPTION: A system for the stable expression of recombinant proteins in the organism *Acanthamoeba castellanii* has been successfully developed. It is the use of this organism for protein production, rather than specific plasmids, for which patent protection is sought. *Acanthamoeba* has previously never been used successfully for this purpose.

Several plasmids designed to express proteins in *Acanthamoeba* were made. These contain the gene for neomycin phosphotransferase (Abbr.: neo^r), an enzyme which confers resistance to the drug neomycin G418. Expression of neo^r is driven by an *Acanthamoeba* promoter previously isolated in the inventor's lab. Other promoters, such as commercially available viral promoters do not work in *Acanthamoeba*. DNA is introduced into *Acanthamoeba* using Superfect (Qiagen). After several days of culture in the presence of neomycin G418, expression of neo^r is evidenced by cell growth. Expression of neo^r was confirmed by Western blotting.

While this simple experiment demonstrates the proof of concept, in order for the system to be useful, a second promoter was introduced into a different part of the basic neo plasmid (above), and used to express green fluorescent protein (GFP). Transfected cells were again grown in the presence of neomycin G418, and after several days, cell growth became apparent. Microscopic analysis showed that GFP is expressed in all cells, and this was confirmed by Western analysis as described above.

A fusion protein containing GFP and *Acanthamoeba* TBP (TATA binding protein) has also been stably expressed in *Acanthamoeba* (not shown).

ADVANTAGES: (see page 2)

PATENT STATUS: Patent Pending

LICENSING STATUS: Worldwide rights available

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ADVANTAGES: The *Acanthamoeba* expression system is presented as an alternative to existing systems such as *E.coli*, yeast, Pichia or insect cells. Each of these has advantages and disadvantages, but some proteins are not well expressed in existing systems, and alternatives such as *Acanthamoeba* are potentially useful. An inducible promoter (CSP21) is available for expression of toxic proteins, and is being tested at time of application.

An additional feature unique to *Acanthamoeba* is that it lacks a cell wall and is therefore very easy to lyse; the first step in producing a recombinant protein. *Acanthamoeba* are simple to grow in shake cultures on a large or small scale using inexpensive media. *Acanthamoeba* can also be cultured to high density in fermentors. Stable cell lines can be induced to form non-growing encapsulated cysts, which can be stored at room temperature for months to years. These cysts are fully viable and retain the genes encoding neo^r and GFP. No other system offers this convenient way to store valuable cell lines.

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