

## Technology Offer

### DEPI – direct epitope identification of known antigens and DANI – direct antigen identification of unknown antigens

Reference Number: TO 01-00649



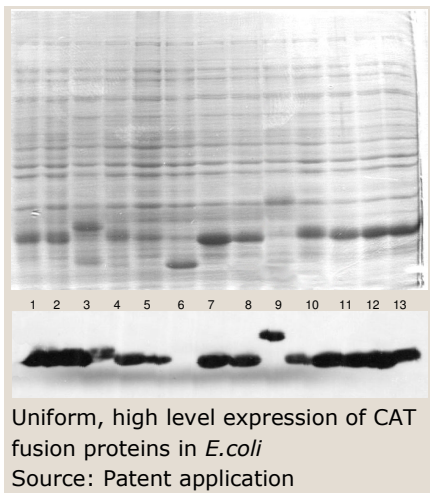
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### Challenge

CD4<sup>+</sup> T helper (T<sub>H</sub>) cells play a central role in initiating and maintaining adaptive immune responses against viruses and tumors. Identification of antigens and epitopes recognized by T<sub>H</sub> cells is critical for characterizing, understanding and modulating antigen-specific immune responses with regard to the development of vaccines and other forms of immunotherapy. For the identification of MHC class II-restricted antigens, two methods are currently used: the biochemical fractionation of proteins from cells expressing the antigen combined with mass spectrometric sequencing of highly purified fractions containing the antigen and genetic approaches based on testing of T cells with pools of cDNA libraries expressed in prokaryotic or eukaryotic cells. However, many antigens will remain undetected, and only a small proportion of eukaryotic genes are expressed at sufficiently high levels in bacteria to allow for screening of large cDNA libraries.

### Technology

A simple and reliable procedure for identification of T<sub>H</sub> cell antigens and epitopes was developed. To define T<sub>H</sub> cell epitopes within known antigens, peptides were fused to GFP and expressed at high levels in bacteria. Identification of unknown antigens, however, is not feasible with this method because of the much larger number of colonies that have to be screened. Therefore, short antigenic fragments created by digestion of whole cDNA libraries with frequently cutting restriction enzymes are randomly ligated to chloramphenicol acetyltransferase (CAT) in a bacterial expression vector. Bacteria expressing antigen-CAT fusion proteins are then fed directly to MHC class II<sup>+</sup> APCs and probed with antigen-specific T<sub>H</sub> cells. By selection with chloramphenicol, bacteria expressing the antigen "in frame" are selected and a uniform expression of the different fragments is achieved.



### Commercial Benefit and Opportunity

The novel technologies offer several advantages:

- identification of epitopes even within proteins which are difficult to express in bacteria
- assessing the role of individual amino acids for MHC binding or T cell recognition
- identification of T<sub>H</sub> cell antigens in complex cDNA libraries
- identification of T<sub>H</sub> cell antigens and directly mapping of T<sub>H</sub> cell epitopes
- analysis of large antigens and antigens toxic for bacteria or APCs

The technology is available for (non)-exclusive licensing. Parties interested in collaborative research and development are highly welcomed.

### Developmental Status

Using the DEPI assay, specific MHC class II epitopes for EBNA3A could be identified. Using the DANI approach, two antigens (BALF4 and BNRF1) recognized by Epstein-Barr virus (EBV) specific T<sub>H</sub> cells were identified for proof of principle.

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## **Patent Situation**

Patent applications are pending in EP (1880015), CA (2605933) and US.

## **Relevant Publication**

Milosevic et al. (2005), J. Immunol. Methods 306, 28-39; Milosevic et al. (2006), J. Virol. 80, 10357-10364.