

# CRT Licensing Opportunity



## Novel Marker of Early ES Cell Differentiation

- First positive cell surface marker, 5T4, for early ES cell differentiation
- Rapid determination of pluripotency and differentiation state of ES cell population in a single, non-destructive assay
- Monoclonal antibodies to mouse and human 5T4 antigen

ENABLING TECHNOLOGY

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### Inventors

Prof. Peter Stern and Dr Chris Ward, Paterson Institute for Cancer Research and Dr Miles Carroll, Oxford BioMedica.

### Application

This marker is a valuable tool for the rapid, non-destructive determination of pluripotency and early differentiation state of ES cell populations. 5T4 phenotyping will be useful for a wide range of techniques such as:

- Screening of culture reagents (including FCS, defined media, feeder cells, growth factors etc.) to establish optimal cell culture conditions for maintaining ES cell pluripotency or induction of particular lineage differentiation;
- Optimising transfer of pluripotent ES cell to improve chimera (germ line) production by ES cell knock out and knock in vehicles and for establishing and maintaining ES cell populations;
- Detecting compounds that induce stem cell differentiation.

Activation of the 5T4 promoter could also be harnessed to induce expression of genes at the beginning of the stem cell differentiation pathway.

### The Technology

Embryonic Stem (ES) cell markers are essential tools for stem cell research and the development of stem cell therapies, including the identification, isolation, culture, propagation and storage of ES cells, monitoring differentiation of ES cells and generation of genetically modified mice. Markers currently used for analysis of ES cell pluripotency such as Oct-4, Rex-1 (transcript markers), SSEA-1 and Forssman (antigen markers) are negatively regulated, with high levels of expression in undifferentiated ES cells that decrease following differentiation. However, these markers are not optimal for accurately determining pluripotency since their levels decrease relatively slowly following the onset of differentiation or the analyses are destructive and require relatively large numbers of cells for RNA extraction.

The inventors have now identified 5T4, a transmembrane glycoprotein, as a novel positive cell surface marker for early ES cell differentiation and a negative indicator of pluripotency (its absence indicating pluripotency), which is conserved in humans and mice. 5T4 is able to rapidly determine both the pluripotency and early differentiation state of an ES cell population in a single, non-destructive assay [1-3]. 5T4 was previously identified by Professor Stern as an oncofoetal antigen, that exhibits restricted expression patterns in adult tissues but is upregulated on many carcinomas [4].

Murine 5T4 (m5T4) is absent on undifferentiated mouse ES cells and rapidly upregulated upon differentiation induced by removal of LIF from the growth medium, including cells derived from all three germ layers, as evidenced by FACS, western and RT-PCR. m5T4 antigen expression on ES cells is unaffected by

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extended passage, cloning or growth on gelatine-treated plates, allowing differentiation analysis for a wide range of ES cell applications. Absence of m5T4 has been shown to be a more accurate and sensitive indicator of ES cell pluripotency than SSEA-1, with SSEA-1+ve/5T4+ve ES cells showing significantly decreased chimera forming efficiency than SSEA-1+ve/5T4-ve ES cells [1].

Human 5T4 (h5T4) expression has also been shown to be negatively associated with both pluripotent ES cells that express Oct-4 (Figure 1) and optimised culture conditions of germ cell tumour derived embryonal carcinoma cells. Furthermore, loss of Oct-4 from pluripotent ES cells is accompanied by upregulation of h5T4 expression on the differentiating cell populations (Figure 2) [2, 3]. Studies with human ES cells are ongoing.

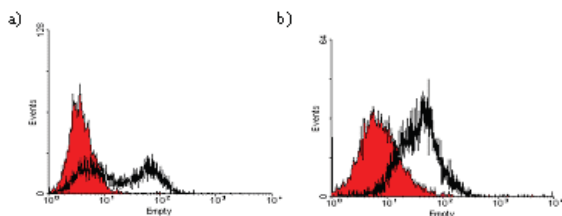


Figure 1. FACS analysis of 5T4 expression of cells from (a) "undifferentiated" hES cell colonies and (b) differentiated hES cells grown on fibronectin coated plates and no PEFs.

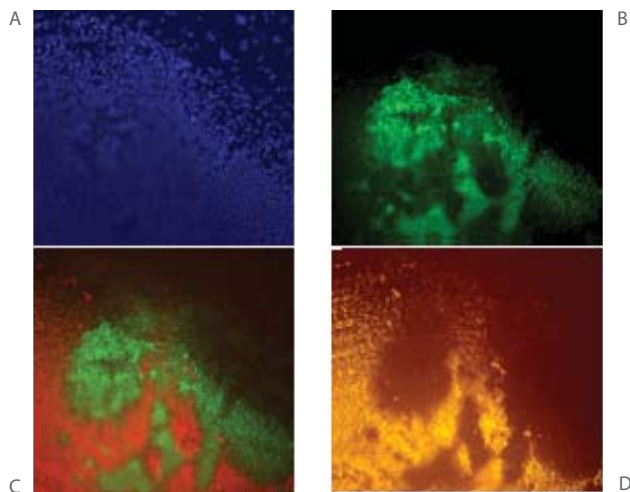


Figure 2. Dual 5T4/Oct-4 staining of hES colonies showing 5T4 expression is mutually exclusive with Oct-4: (a) Dapi, (b) 5T4 stained using Alexafluor 488, (c) 5T4 and OCT-4, (d) OCT-4 stained using Alexafluor 546.

## Intellectual Property

Patent applications on 5T4 as an ES cell marker in Europe, US, Canada and Australia. Anti-m5T4 and anti-h5T4 monoclonal antibodies are also available for use in stem cell applications.

## Commercial Opportunity

This technology is available for licensing on a non-exclusive or field-exclusive basis.

## References

1. Ward CM, Barrow K, Woods AM, Stern PL (2004). The 5T4 oncofoetal antigen is an early differentiation marker of mouse ES cells and its absence is a useful means to assess pluripotency. *J. Cell. Science.* 116(22):4533-4542.
2. Patent application WO2004/005926.
3. Ward CM, Eastham AM, Stern PL (2006). Cell Surface 5T4 antigen is transiently upregulated during early human embryonic stem cell differentiation: Effect of 5T4 phenotype on neural lineage-formation. *Exp Cell Res* 312(10):1713-1726.
4. King KW, Sheppard FC, Westwater C, Stern PL, Myers KA. (1998). Organisation of the mouse and human 5T4 oncofoetal leucine-rich glycoprotein genes and expression in foetal and adult murine tissues. *Biochim. Biophys. Acta.* 1445(3):257-70.

Contact: Martyn Bottomley [mbottomley@cancertechnology.com](mailto:mbottomley@cancertechnology.com)

Ph: +44 (0)203 469 6300