

## ASCC LICENSING PROPOSAL

**NAME:**

SPIN HES Cell Differentiation System

**SUMMARY:**

The SPIN Human Embryonic Stem (HES) Cell Differentiation System is an advantageous method for the differentiation of human ES cells. Unlike alternative differentiation techniques, SPIN results in the reproducible formation of Embryoid Bodies (EBs) of uniform cell size. When used in conjunction with APEL medium (see alternate ASCC proposal), the SPIN system provides a unique, robust method for growth directed human ES cell differentiation.

**INTELLECTUAL PROPERTY:**

The SPIN EB method is the subject of Australian Patent Application No. 2004291559 and US Patent Application Serial Number 10/579,712.

**LICENSING TERMS:**

Type: Exclusive

**DETAILED DESCRIPTION:**

Techniques commonly used to initiate the in vitro differentiation of HES cells include:

1. The embryoid body (EB) approach in which mechanically or enzymatically digested clumps of undifferentiated cells provide the basis for EB formation.
2. Differentiation of HES cells in a monolayer culture
3. Cocultivation of HES cells with a stromal cell layer with lineage inductive properties.

For each technique, there are variables that influence the reproducibility and the precision of differentiation, such as the quality of the starting HES cell population, the presence of feeder cells or extracellular matrices in monolayer cultures and the cell-associated or secreted factors associated with stromal cell cocultivation<sup>1</sup>.

The EB approach is considered to be advantageous relative to alternative approaches as it recapitulates some of the cues and context inherent to in vivo development, rather than merely the differentiation of individual stem cells. Commonly, HES cells are obtained for EB formation by scraping monolayer cultures to release colonies, which results in a heterogenous mixture of cells. This heterogeneity leads to subsequent differentiation that is generally chaotic and disorganised, with wide variability both between and within individual aggregates. Such inconsistency creates significant limitations on the use of human EBs both as a

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<sup>1</sup> Ng E. et al (2008). Nature Protocols 3(5) pg 768

model system in which to study human development and as a source of differentiated cells<sup>2</sup>..

The SPIN EB system overcomes the shortcomings associated with the aforementioned approach to EB generation from HES cells. The underlying principle of this method is that a known number of undifferentiated HES cells are seeded into each well of a 96-well round bottom low attachment plate. This results in the reproducible formation of EBs of uniform size in each well, providing an improved basis for differentiation experiments. A key component of the SPIN system is the use of APEL HESC Differentiation Medium (see separate proposal). In addition to the advantages enabled by its defined, serum free formulation, the incorporation of polyvinylalcohol in APEL medium greatly improves the initial formation of single EBs and overcomes the need for specialised V-bottom microplates.

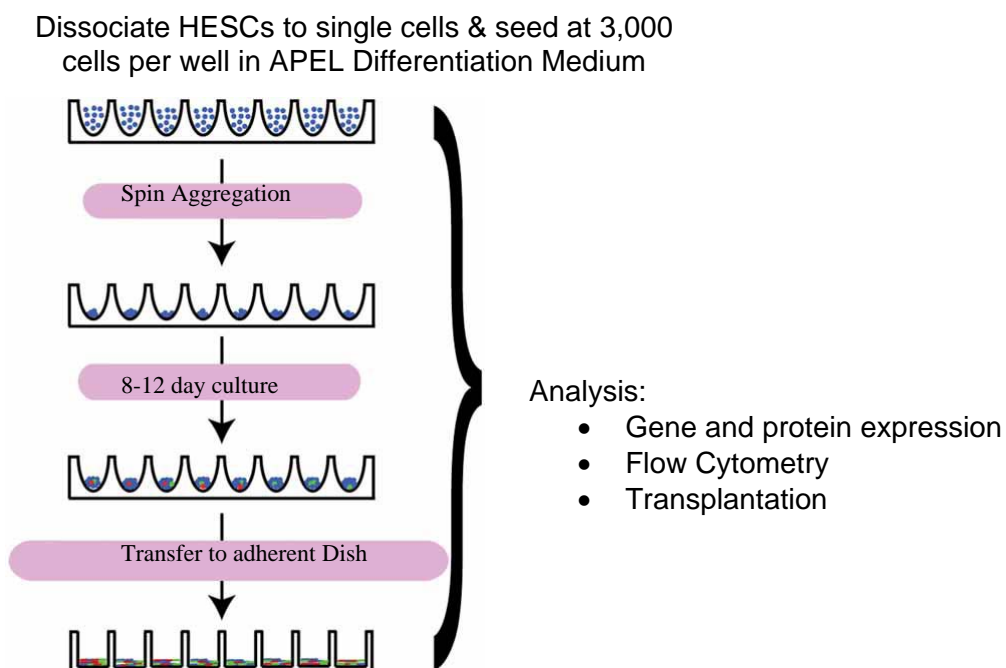
The SPIN differentiation and APEL medium combination has been tested with 6 HES cell lines and been shown to result in multi-lineage differentiation.

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<sup>2</sup> Ungrin M et al (2008). PLoS ONE 3(2) pg e 1565

## METHODOLOGY:

Figure: Summary of SPIN EB Method<sup>1</sup>



For a detailed protocol please refer to Ng E et al (2008).

## COMPETITIVE ADVANTAGES:

1. Permits the improved formation of EBs
2. Results in superior reproducibility of EB formation & differentiation
3. Validated on multiple HESC lines

## PUBLICATIONS:

1. Ng E.S. et al (2008). Nature Protocols Vol. 3(5), pg 768.
2. Ng E.S. et al (2005). Blood 106(5), pg 1601

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