

GAPTrap

A safe harbour that allows targeted and stable integration of a transgene

The opportunity

The capacity for Human Pluripotent Stem Cells (hPSCs) to generate all cell types of the human body represents a valuable tool for the study of human development and disease, as well as a source for cell therapies. However, the genetic modification of hPSCs has proved difficult due to issues such as low transfection efficiency, gene silencing, and down regulation of transgenes in differentiated progeny.

Safe harbours permit stable transgene expression in PSCs and their differentiated progeny. Currently, targeted transgene integration into safe harbours both disrupts the endogenous allele and requires the co-integration of an exogenous promoter to drive transgene expression. Disruption of endogenous genes can have deleterious effects on PSCs and their differentiated progeny. The integration of promoters can cause off-target effects, such as uncontrolled activation of nearby genes and transgene silencing.

We have identified a unique safe harbour within a constitutively expressed, well-characterised gene ("GAPTrap").

The technology

The advantage of **GAPTrap** is that the endogenous promoter drives transgene expression, therefore permitting high, stable transgene expression whilst maintaining unaltered endogenous gene expression. Further, transgene expression can be effectively modulated, facilitating the generation of conditional hPSC lines for tracking differentiation of PSCs and studying gene function.

Demonstrated expression with a range of transgenes

GAPTrap has proved amenable to numerous conformations with a suite of gene tags and reporters in hPSCs. For example, transgenes encoding Luciferase, Green Fluorescent Protein, Blue Fluorescent Protein can be integrated, as well as selectable markers Mygro, Meo and MURO.

Importantly, data to date has demonstrated that transgene expression is highly reproducible in numerous cell lines. As shown in the figure below, the fluorescence intensity of reporter protein expression in GAPTrap reporter induced PSC (iPSC) lines overlaps in three separate cell lines, compared to untagged iPSCs.

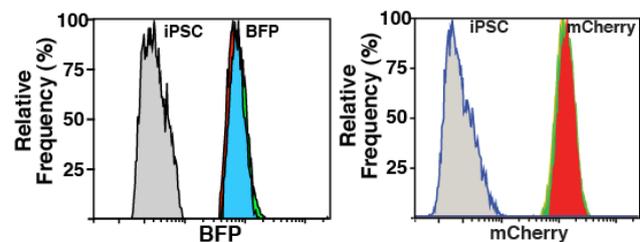


Figure 1 Fluorescence intensity of Blue Fluorescent Protein (BFP) and mCherry cell lines (n=3) compared to untagged iPSCs.

Transgene expression persists when modified hPSCs are transplanted into mice.

GAPTrap can be used to integrate the Luciferase gene. Modified cells can be transplanted into rodent models and the grafts can be identified in live animals, in real time (see below).

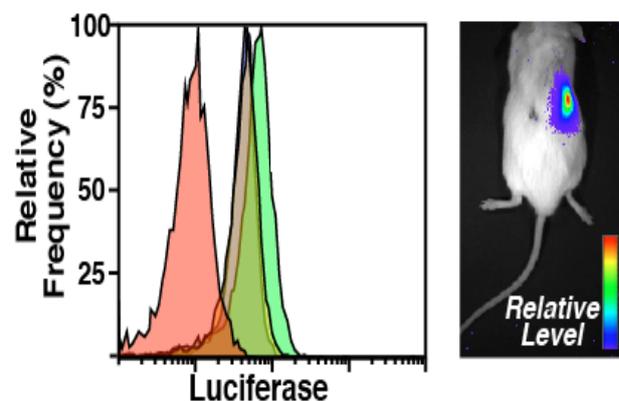


Figure 2 Fluorescence intensity of GAPTrap-Luciferase modified hESCs (n=3, green, beige and blue, all overlapping) and wild type hESCs (orange). Luciferase expressing cells transplanted and later imaged in a live mouse (right panel).

Transgene expression persists in differentiated hPSC progeny.

Teratoma assays have confirmed that **GAPTrap** transgene expression persists in a wide range of differentiated cell types, showing that the transgene is not being silenced upon differentiation of modified pluripotent cell lines.

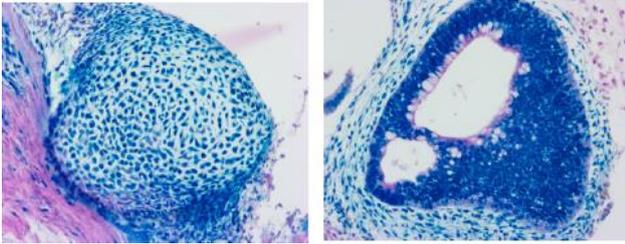


Figure 3 Teratoma formation in vivo from transplanted GAPTrap-LacZ-iPSCs. LacZ expression is maintained in differentiated cell types such as cartilage and epithelium.

Inducible transgene expression

GAPTrap is also amenable to the generation of inducible cell lines, such that upon the addition of a drug or hormone, transgene proteins are activated.

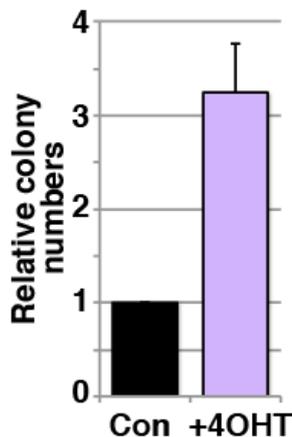


Figure 4 Blood Cell Colony formation assay with differentiated inducible GAPTrap-cMYC hPSCs. The induction of cMYC by the addition of a drug (+4OHT) results in an increase in colony numbers compared to the control.

Applications

GAPTrap has the potential to be a highly effective tool for transgene expression in medical research.

With further development, the technology may also have potential in clinical applications.

Opportunity for partnership

The Murdoch Children's Research Institute is seeking a **licensee** for the technology as a **research tool**.

MCRI are also seeking partners interested in co-investment to develop the technology for clinical applications.

Intellectual Property

This technology is the subject of an International (PCT) application (PCT/AU2015/000682) in the name of the Murdoch Children's Research Institute. Further, there are national phase filings in Australia; Canada; Europe; Japan; and the United States.

Key publications

Kao et al., GAPTrap: A Simple Expression System for Pluripotent Stem Cells and Their Derivatives, Stem Cell Reports (2016)

<http://dx.doi.org/10.1016/j.stemcr.2016.07.015>