Australian Government

National Health and Medical Research Council

Research Involving Human Embryos Act 2002

Embryo Research Licensing Committee of the NHMRC

LICENCE

This licence is issued under s.21 of the *Research Involving Human Embryos Act 2002*. This licence authorises the activity specified below, subject to the conditions specified in items 9 and 10 below.

1.	Licence Number:	309729
2.	Licence Holder:	Monash University
3:	Licence Title:	The generation and study of a novel *in-vitro* model of human blastocysts ('iBlastoids').
4.	Date of Issue:	19 October 2022
5.	Licence begins:	19 October 2022
6.	Licence ends:	18 October 2025
7.	Activity authorised by the licence:	This licence authorises the creation and characterisation of iBlastoids, which are human embryos (and human embryo clones) that are generated by reprogramming adult skin cells *in vitro* into a three-dimensional cluster of cells that resembles a blastocyst and has the potential to develop up to the stage at which the primitive streak appears.
8.	Goals of the Activity:	The goals of the licensed activity are to investigate iBlastoids and their role as an *in-vitro* model of early human development, by: • generating and characterising the molecular properties of iBlastoids • studying the molecular drivers for the establishment of iBlastoids • finding alternative methods for the generation of iBlastoids, and • deriving and studying different stem cell types from iBlastoids.
9.	Standard Conditions:	All conditions that are specified in the *Standard Conditions of Licence*.
10.	Special Conditions:	All conditions that are specified in the *Special Conditions for Licence 309729*.

Note: The activity authorised under this licence is subject to the provisions of the *Research Involving Human Embryos Act 2002* and the *Prohibition of Human Cloning for Reproduction Act 2002*. Terms used in this licence which are defined in those Acts carry the same meanings as they do in those Acts.

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Special Conditions for Licence 309729

Licence Number:	309729
Licence Holder:	Monash University
Licence Title:	The generation and study of a novel *in-vitro* model of human blastocysts ('iBlastoids').

The conditions that are specified below are the special conditions that apply to this licence. The *Special Conditions* operate **in addition to** conditions set out in s.24 of the *Research Involving Human Embryos Act 2002* (the statutory conditions) and all conditions identified in the *Standard Conditions of Licence*. The *Special Conditions* prevail where there is an inconsistency between a special condition and a standard condition.

Conditions relating to creation and use of embryos

Condition Number	*Condition*
9101	The licence holder is authorised to create embryos by reprogramming adult skin cells, using the method described in Liu et al., Nature 2021[1] and others as specified in the licence application. The licence holder is permitted minor deviations from the specific published protocols, in order to optimise the technique for this research activity (for example through altered incubation times or addition of different growth factors).
9102	A maximum of 117,010 embryos (assessed as showing basic morphological features of an iBlastoid) may be used for the activity authorised by the licence. Any other structure that is created during this activity that does not show basic morphological features of an iBlastoid must be destroyed immediately. Any structure that is initially assessed as showing morphological features of an iBlastoid but is subsequently found not to be an iBlastoid, must be counted towards the number of embryos used.
9103	Only fibroblast and keratinocyte cells obtained from Monash University staff and students who have given consent to this use of their cells, may be used as the source of cells for the iBlastoid embryos created under this licence.
9104	An outcome must be recorded for every skin sample donated to the research project, irrespective of whether the sample is used in the research project.

[1] Liu, X., Tan, J. P., Schröder, J., Aberkane, A., Ouyang, J. F., Mohenska, M., Lim, S. M., Sun, Y. B. Y., Chen, J., Sun, G., Zhou, Y., Poppe, D., Lister, R., Clark, A. T., Rackham, O. J. L., Zenker, J., & Polo, J. M. (2021). Modelling human blastocysts by reprogramming fibroblasts into iBlastoids. *Nature,* 591(7851), 627-632. https://doi.org/10.1038/s41586-021-03372-y

9105	For the purposes of monitoring the licence holder's compliance with section 14 of the *Prohibition of Human Cloning for Reproduction Act 2002* the licence holder must: (a) record the first day for the period for development of an iBlastoid as the same day the cells commence aggregation; (b) not allow an iBlastoid to develop for more than 14 days, including from the day of commencement provided for in (a), and (c) monitor and record the development of each iBlastoid created, using morphological and/or molecular (gene expression) methods.
9106	The licence holder must immediately cease development of an iBlastoid prior to 14 days if and when the following occur: (a) an iBlastoid reaches a morphological or molecular stage equivalent to a fertilised human embryo at 14 days development; or (b) an iBlastoid is developed in conditions that give rise to the development of the primitive streak, and/or evidence of gastrulation occurs.
9107	For the avoidance of doubt, an iBlastoid created under this licence is a human embryo clone as defined in the *Prohibition of Human Cloning for Reproduction Act 2002*.
9108	The licence holder is authorised to establish up to eighty (80) cloned human embryo derived stem cell lines from the embryos used according to condition 9102.
9109	An embryonic stem cell line derived under this licence is considered to be established and will be counted as one of the cloned embryo derived stem cell lines authorised by Condition 9108 when it meets, or has the potential to meet, the following criteria: • the embryonic stem cell line must possess a stable human diploid karyotype, express immunologically defined markers and genes specific for embryonic stem cells; and • results from initial studies indicate that the cell line is pluripotent and capable of self-renewal.

Specified Sites

Condition Number	*Condition*
9201	The licence holder must conduct the activity authorised by the licence at the following site: Department of Anatomy and Development Biology and The Australian Regenerative Medicine Institute Level 3 15 Innovation Walk Monash University Clayton Victoria 3800 Monash Micro Imaging Ground level, Building 75 15 Innovation Walk Monash University Clayton Victoria 3800 Biomedical Sciences Level 3, Room 374 Building 76 19 Innovation Walk Monash University Clayton Victoria 3800

9202	The licence holder must hold records (other than donor/patient records) associated with the use authorised by the licence at the following sites: Department of Anatomy and Development Biology and The Australian Regenerative Medicine Institute Level 3 15 Innovation Walk Monash University Clayton Victoria 3800 Monash Micro Imaging Ground level, Building 75 15 Innovation Walk Monash University Clayton Victoria 3800 Biomedical Sciences Level 3, Room 374 Building 76 19 Innovation Walk Monash University Clayton Victoria 3800 Monash Research Office Room 111 Chancellery Building D 26 Sports Walk Wellington Road Monash University Clayton Victoria 3800
9203	The licence holder must securely hold donor (patient) records associated with the skin biopsy component of the licensed activity in a secure facility at the following sites: Department of Anatomy and Development Biology and The Australian Regenerative Medicine Institute Level 3 15 Innovation Walk Monash University Clayton Victoria 3800 Monash University Health Services (Clayton Campus) Ground Floor, Campus Centre, 21 Chancellors Walk Monash University Clayton Victoria 3800
9204	The “use” (as defined in the *Research Involving Human Embryos Act 2002*) of iBlastoids created in accordance with this licence is only to occur at the site listed in 9201. Live iBlastoids must not be transferred from this site to any other location.
9205	The Licence Holder must be aware of, and act in compliance with, relevant legislation that regulates the transfer or export of stem cell lines derived from human embryo clones as it applies to iBlastoids created by activity authorised by this licence (see for example, the permit scheme under *Customs (Prohibited Export) Regulations 1958 (section 8A)*).

Persons authorised to conduct the licensed activity

Condition Number	*Condition*
9301	The Principal Supervisor is responsible for supervision of the activity authorised by the licence.
9302	The Alternative Principal Supervisor is responsible for supervision of the activity authorised by the licence wherever the absence of the Principal Supervisor is documented by the licence holder.
9303	Only Authorised Personnel may conduct the activity authorised by the licence. Authorised Personnel include the Principal Supervisor, Alternative Principal Supervisor and those other persons identified at **Attachment A** to this licence.

Reporting

Condition Number	*Condition*
9401	The licence holder must submit a written report to the Licensing Committee no later than 30 days after the end of each reporting period, that described the activity conducted under the licence in the reporting period. The reporting periods run from 1 March to 31 August and 1 September to 28 February (or 29 February in leap years). For the purposes of monitoring condition 9101 and 9102, the report must include: – information about the efficiency of generating iBlastoids – tracking of actual numbers of iBlastoids used in experiment – the number of cells, other structures and iBlastoids which are destroyed due to not being suitable or required for research. For the purposes of monitoring condition 9105, the report must include: – a description of the relevant research method for each iBlastoid – a summary of the iBlastoid's development including observations and the results of any morphological and molecular (gene expression) testing carried out throughout its chronological development from aggregation, and – any preliminary findings of the activity authorised by the licence. This report is in addition to the requirements of Standard Condition 3001.
9402	The licence holder must within 7 days provide the Licensing Committee a written report on each occasion that the research inadvertently exceeds the morphological stage and/or demonstrates molecular gene expression consistent with exceeding this stage, as described in the application and condition 9106. A written report provided in accordance with this condition must include details on the following matters: (a) a description of the relevant research method (b) a summary of the iBlastoid's development including observations and any testing carried out throughout its chronological development from aggregation (c) the likelihood of reoccurrence and whether any changes are proposed to the research methodology to prevent reoccurrence (d) any preliminary findings on the extent to which the iBlastoid is a model for an early human blastocyst.
9403	When recording an outcome for each skin sample that is donated to the project, as required by Condition 9104, the licence holder is required to use the template specified in Standard Condition 3001.

Conditions relating to proper consent

Condition Number	*Condition*
9501	To obtain proper consent for the activities authorised by the licence, the Licence Holder must use the consent process documented in the application form and supporting attachments, as provided to the Licensing Committee on 26 September 2022 and subsequently approved by the Licensing Committee on 18 October 2022.
9502	For the avoidance of doubt, the requirements of Condition 9501 include use of the Participant Explanatory Statement and Consent Form provided to the Licensing Committee on 26 September 2022 and subsequently approved by the Licensing Committee on 18 October 2022.
9503	A 'cooling off' period of at least 14 days is required between obtaining proper consent and reprogramming of fibroblast or keratinocyte cells in activities authorised under the licence. This is to be documented as part of the consent process.
9504	As specified in the application, Monash University Human Research Ethics Committee will sight consent details for all skin cell samples that grow successfully and provide the notification of consent to the Licensing Committee.

Other conditions

9601	If the licence holder or a report submitted to the Licensing Committee concludes that further creation of embryos within the project will not meet the goals of the activity, the licence holder must cease creation of embryos for the authorised activity.
9602	The licence holder must submit an application to vary the licence if, for any reason, including interim analysis or new research, it requires amendments to the activity authorised by the licence, including change to the experimental paradigm or goals.

Licence 309729: Table of Variations

Date of Variation	Conditions Affected	Description of Changes