Blastocyst Genotyping - Refining Cryopreservation Quality Control

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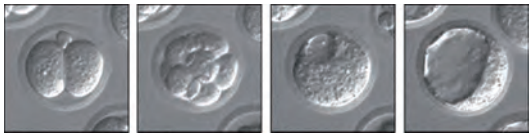
Introduction

Cryopreservation has become an integral part of large scale mouse production and phenotyping programmes such as the Mouse Genetics Project (MGP) at the Wellcome Trust Sanger Institute (WTSI). As a collaborator of the International Mouse Phenotyping Consortium (IMPC) WTSI creates over 200 protein coding knockout mouse lines a year. Cryopreservation safeguards these valuable colonies whilst meeting our ethical aim of reducing animal usage and removing complete colonies from the shelf. To ensure ease of access for the wider research community, cryopreserved material from unique mutant lines is distributed to sustainable repositories such as the European Mutant Mouse Archive (EMMA) and the Knockout Mouse Project Repository (KOMP). EMMA is a non-profit repository, spread over several nodes throughout Europe, that receive, archive and distribute medically relevant mouse models. WTSI deposit over 250 unique mutant mouse lines each year as part of the IMPC. Prior to distributing cryopreserved material, it must first be established that the correct line has been frozen, and that it can be recovered. This is confirmed by a thorough quality control (QC) process.

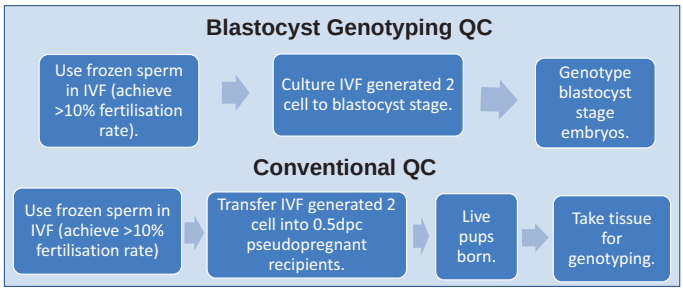
Historically, attaining live born pups from cryopreserved material has been the benchmark level of recovery required to pass QC. From these live pups, tissue samples can be taken to confirm the zygosity. This process unequivocally demonstrates that a line is viable and has been successfully cryopreserved. Unfortunately however, this process is quite costly in terms of the number of mice that are required as IVF donors, embryo transfer recipients and QC litters. This process also requires two types of regulated procedures.



Genotyping Blastocysts

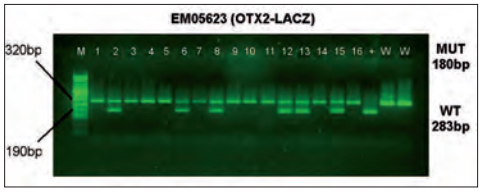
Genotyping blastocyst stage IVF generated embryos instead of live born pups offers a quicker, more ethical and cheaper solution than the traditional method of QC. This process removes the requirement for embryo transfers and subsequent litters all together. The number of IVF donors can also be reduced because genotyping blastocysts gives more material to genotype, not less. Over 90% of 2 cell embryos develop to blastocyst whilst only 30% of transferred embryos are recovered as live pups. This significantly decreases the likelihood of requiring more than one IVF. Combined, these changes significantly decreasing the total number of mice required to confirm QC.

There are however some draw backs to using this method for some mutant lines. Potential problems could arise with multiple mutants or challenging PCRs (none so far – not relevant for IKMC lines), there is also no gaurantee as to the viability of any given line. We now have a weight of data showing these two points are not relevant for IKMC strains. Additionally, due to the low number of cells present in blastocyst stage embryos, it is essential to use good negtive controls.



Protocol Development

Working collaboratively as part of the Infrafrontier R & D WP(5) WTSI, CNR-Monterotondo, Institut Clinique de la Souris (ICS), TAAM-CDTA, Biocenter Oulu, and Centro Nacional de Biotecnologia (CNB-CSIC) created several protocols specifically to genotype blastocyst stage embryos. All contributing nodes developed fully working protocols, the percentage of successfully genotyped blastocysts varied between them from 89-98%. In light of the successfulness of blastocyst genotyping protocols, Infrafrontier now accept blastocyst genotyping as a primary form of QC in replacement of genotyping live born pups for EUCOMM/KOMP KO first alleles.



PCR mix:

dNTPS (2mM)	3ul
PCR BUFFER 10x	3ul
PRIMER 1	(0.5pm/ul)
PRIMER 2	(0.5pm/ul)
PRIMER 3	(0.5pm/ul)
H2O	
TAQ POLYMERASE (5U/ul)	0.3
DNA	5ul
TOTAL	30ul

PCR program:

1) 95° C x 2'00"
2) 95° C x 0'30"
3) 64° C x 0'30"
4) 72° C x 1'30"
5) step 2 to step 5 x 50 repeats
6) 72° C x 10'00"
7) 4° C forever
8) End

F.Scavizzi, CNR Monterotondo

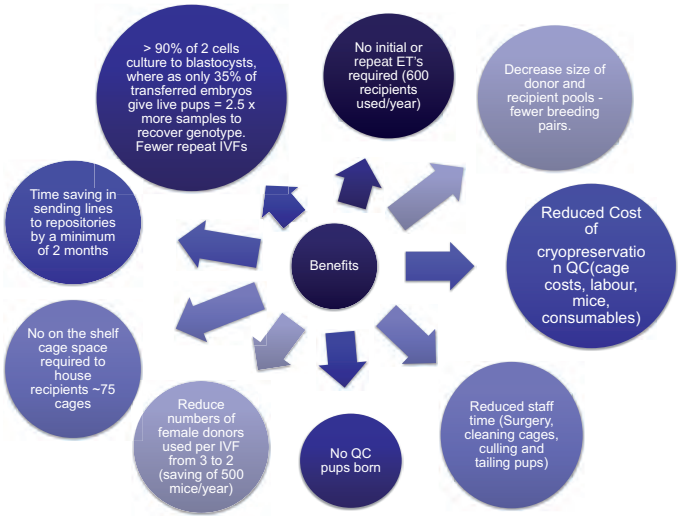
Animal Welfare

Over 263 unique mouse lines have been deposited by WTSI at EMMA/Infrafrontier nodes in the past year, as part of the IMPC. To generate the QC passes for these lines via the conventional QC method it would require 2884 mice and 1261 regulated procedures. Compared to just 413 mice if the same number of lines had passed QC via blastocyst genotyping. These figures include IVF donor females, embryo transfer recipients and their subsequent litters, but do not include any reduction in the size of core colonies expected from using blastocyst genotyping QC.

QC protocol	Number of Lines Archived	Number of Donors	Number of Embryo Transfer Recipients	Number of Live Mice Born	Total
Conventional	263	930	331	1623	2884
Blastocyst Genotyping	263	350	0	0	413

We would also see significant decreases in the number of regulated procedures required.

Regulated Procedure	Reduction in regulated procedures
Superovulation	580
Surgical Embryo Transfer	~331



Conclusions

The IMPC is still in its early stages and over 15, 000 mutant mouse lines are yet to be deposited. If more repositories and depositors accept blastocyst genotyping confirmation of a lines zygosity as a method of QC, the reduction in the total number of mice required for all lines to be deposited over the whole project could be reduced by over 100,000.

Refining the level of QC required for repository distribution represents a positive advance in the refinement of archiving mutant mouse lines, not only at WTSI but for all EMMA/Infrafrontier contributors. Genotyping blastocysts reduces the number of embryo transfer recipients, subsequent litters and the number of IVF donors required, which in turn results in a reduction in the size of core colonies. The number of surgical procedures required is also dramatically reduced, further helping to fulfil our ethical commitment to the 3Rs.

Whilst genotyping blastocysts doesn't fulfil the sometimes desirable recovery of offspring, we believe it represents a compromise that still allows EUCOMM/KOMP Knock-out first alleles to be deposited reliably and safely whilst making significant gains in other respects.

Acknowledgements

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