Extending the Benefits of goGermline™ Chimeras: Expediting time to First Heterozygotes and First Experimental Cohorts

Jonathan Gauntlett, Maree Hagan, Jacqui Watts, Frank Koentgen, and Roger Askew
Ozgene Pty Ltd, Bentley, WA, Australia

Introduction

We investigated the reduction of gene targeting project timelines by comparing time to germline transmission using goGermline™ embryo hosts versus conventional wild-type embryo hosts across over one hundred different embryonic stem (ES) cell clones.

goGermline™ technology

Germline transmission of a genetic modification to chimeric offspring remains a significant challenge adding to uncertain gene targeting project outcomes. The application of goGermline™ technology permits the exclusive transmission of the genome of the injected ES cell by male chimeras[1]. This eliminates the wasteful, costly and time consuming production of F1 mice that have not inherited the genetic modification and reduces project uncertainty.

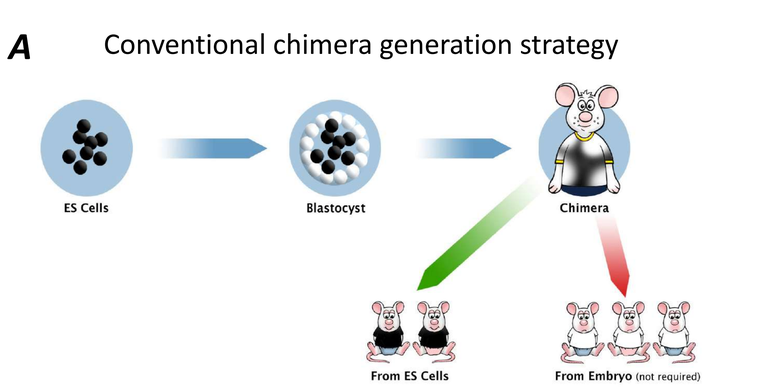
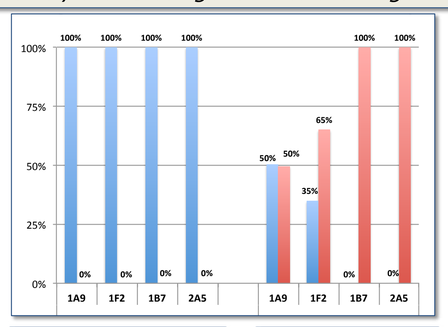


Figure 1. *Outline of chimera F1 outcomes comparing* ***A.*** *wild-type host embryos where F1 offspring can be derived from targeted ES sperm, or host blastocyst derived sperm, or* ***B****. goGermline. This results in no host derived sperm present in chimeras generated, and thus 100% of F1 offspring are derived from the targeted ES cells.*

Comparison of F1 transmission –
conventional chimeras versus goGermline chimeras

Chimeras generated using goGermline embryos transmit exclusively the ES cell genome to the F1 generation



- goGermline & conventional chimeras by goGermline or wt embryo injection respectively
- ES cell transmitted F1
- Host embryo transmitted F1
- Note that rescued transmission for ES cell clones 1B7 and 2A5

Figure 2. *Graph comparing germline transition using wild-type and goGermline blastocysts across 4 targeted ES cell clones. Note rescued transmission for ES cell clones 1B7 and 2A5.*

Methodology

goGermline™ embryos are generated via the conditional KO of the X-linked gene Tsc22d3 which encodes a leucine zipper transcription factor that is essential for spermatogenesis.

The offspring of fertile goGermline™ chimeric males are heterozygous for the modified target gene. In the data presented here, we test the level of benefit of goGermline™ technology by comparing time to GLT from 100 targeted ES cells injected into conventional wt embryos to 156 ES cells injected into goGermline™ embryos.

We also present the use of frozen goGermline™ embryos in an independent laboratory.

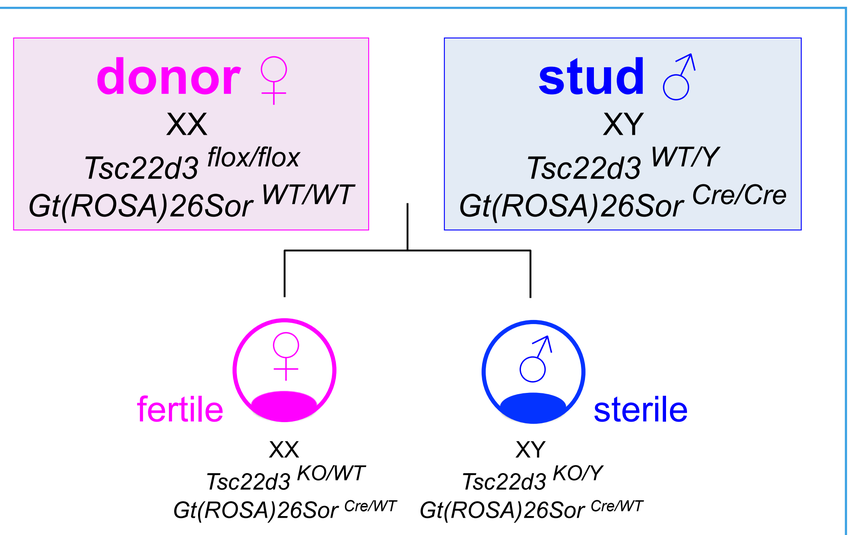
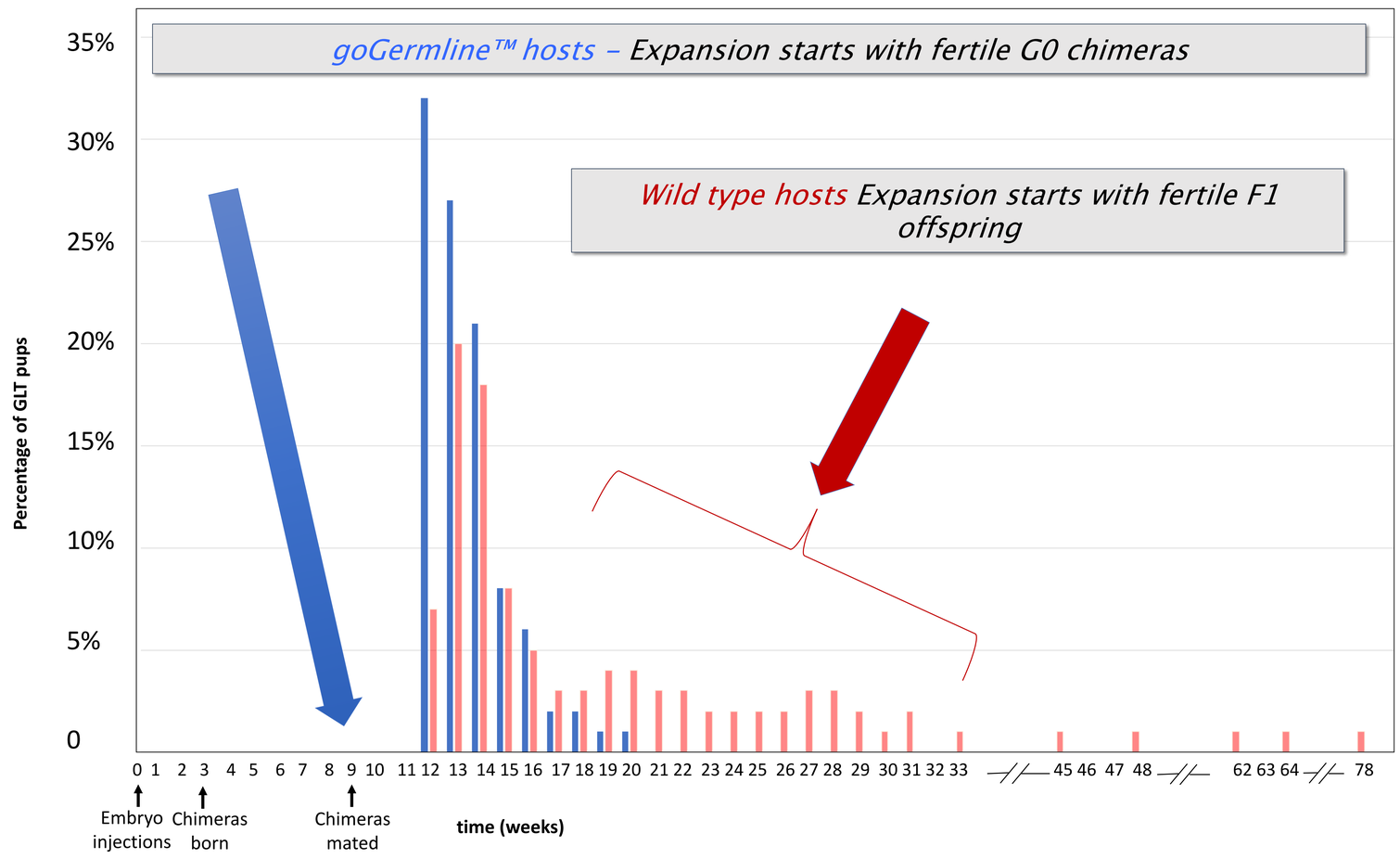


Figure 3. *Generation of goGermline (Tsc22d3-KO) male embryos*

Results

Figure 4. Expansion of heterozygous colonies –
goGermline™ chimeras expanded at least 1 generation earlier



- Fertile chimeras from wt host injections are germline chimeric
- Fertile chimeras from goGermline ™ host injections are germline heterozygotes and can be expanded

- 88% of the projects using goGermline™ reached GLT in the first month (first litters) of births (weeks 12-15)
- 56% of the projects using wt embryos reached GLT in the first month (first litters) of births (weeks 12-15)
- 1 month later (weeks 16-20), GLT was achieved for the balance (12%) of projects using goGermline™
- 4 months later, GLT was achieved for an additional 40% of the projects using wt embryos (weeks 16-31)
- The remainder (5%) of the wt projects reached GLT between 5 and 12 months later

These results were replicated using frozen goGermline™ 8 cell embryos compared to fresh wt embryos in an independent laboratory.

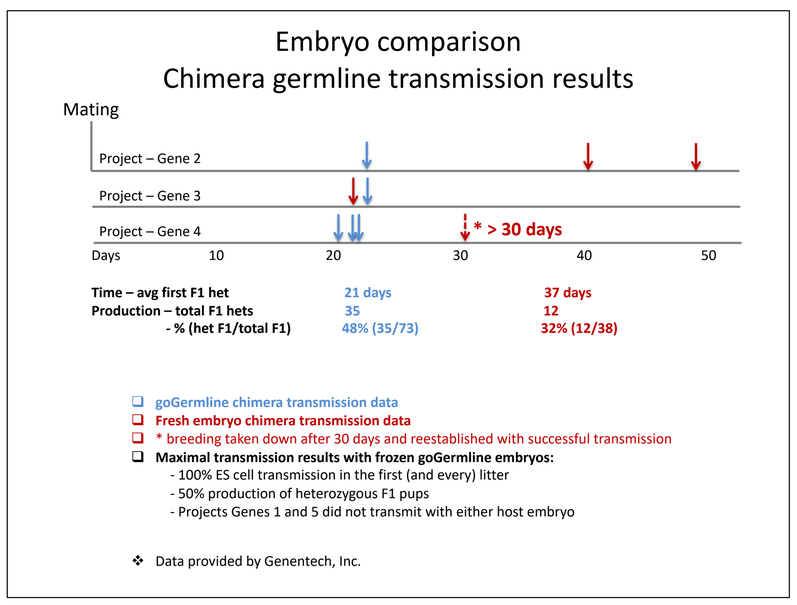


Figure 5*. Germline transmission of frozen goGermline vs fresh wt embryos. Data provided by Genetech, Inc.*

Discussion

Use of goGermline™ results in a significant positive 3R's impact by dramatically reducing the number of F1 offspring generated to reach GLT and project completion.

Since fertile chimeric males are germline heterozygous, they are equivalent, in regard to colony development, to their F1 heterozygous offspring. Therefore, early breeding colonies can be developed by direct expansion breeding of chimeric founders rather than the conventional strategy of waiting another generation (3 months) before expansion breeding of F1 heterozygous offspring.

The benefit of goGermline™ embryos for project generation is further expanded with the observed reduction in time to GLT.

Reference:

1. Genesis. 2016 Jun;54(6):326-33. doi: 10.1002/dvg.22938. Epub 2016 May 18. **Exclusive transmission of the embryonic stem cell-derived genome through the mouse germline.**
Koentgen F, Lin J, Katidou M, Chang I, Khan M, Watts J, Mombaerts P.

goGermline by Ozgene – winner of the inaugural ISTT 3Rs prize
www.ozgene.com/goGermline