Poster #10

goGermline™ 2.0: Exclusive Generation of Male goGermline Chimeras Can Double Injection Efficiency

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Abstract
goGermline™ 2.0:
Exclusive Generation of Male goGermline Chimeras Can Double Injection Efficiency

While goGermline (goGermline 1.0) technology already vastly improves the efficiencies of the 3Rs and the timeline for development of gene targeted mouse models, we have further improved these efficiencies with goGermline 2.0 embryos by enabling selective injection of male goGermline embryos so that no female chimeras are produced. For those not familiar with goGermline 1.0, this system enables generation of chimeras in which sperm, and therefore germline transmission (GLT), can only be derived via the injected male embryonic stem (ES) cells. In this situation, the injected ES cells effectively rescue infertility of male goGermline embryos. To achieve this, goGermline 1.0 embryos are engineered to be spermatogenesis deficient. The result is that all fertile male chimeras are germline heterozygotes and exclusively transmit the ES cell genome. This ensures GLT in the first litters of every project and results in overall improvements in the 3Rs, time to GLT, and time to first experiments. However, goGermline 1.0 injection results in production of both male and female chimeras. Since female chimeras cannot transmit the germline of male ES cells, we reasoned that eliminating production of female chimeras was the best opportunity for improving the efficiency of the goGermline system. With this as our goal, we sought to establish a strategy by which only male embryos are harvested and injected. We engineered goGermline 2.0 to distinguish male from female embryos via a fluorescent reporter tag on the X chromosome. Therefore, if only male embryos are injected, only male chimeras will be generated - essentially doubling the current efficiency of chimera production by embryo injection. We will share the detailed strategy, end results and broader implications of this technology on improvements on in the 3Rs, injection time, and vivarium space management.

Introduction

We first developed goGermline 1.0 to optimize to first germline transmission (GLT), the equivalent to project completion. We've since realized the significant 3Rs benefit to using goGermline 1.0 as a result of the greatly reduced production of F1 mice to the first GLT since we see GLT in the first litters for every project.

Then we realized an additional benefit to using goGermline in our our gene targeted mouse model production facility in the next phase of the project – colony expansion. Colony expansion is optimized because all fertile chimeras are also germline heterozygous for the targeted allele. This allows expansion of all new mouse strains starting with fertile chimeric males rather that the F1 generation at least 3 or 4 months after fertile chimeras are identified.

Now we have added an additional level of efficiency for both 3Rs and new model project completion. This next level of efficiency is based on a system that enables the selection of male embryos at the time of embryo isolation. By ES cell injection of only male embryos for chimera production, we can ensure that all chimeras born will be males. Female chimeras cannot be used for the purpose of project completion, because the ES cells are male and, with extremely rare exception, male ES cells do not colonize the female germline to drive production of ES derived eggs.

By "simply" tagging the X chromosome with a fluorescent reporter expressed by the embryo, we can identify female embryos which have inherited the tagged X chromosome from the male parent. This strategy both doubles production of males on average and completely eliminates the natural range of variability of the number of males in a small N - group as demonstrated in this poster.

Summary

- goGermline embryos have been engineered to be spermatogenesis deficient
- Therefore, goGermline chimeras are obligate germline heterozygotes

Results of goGermline 1.0

- statistically half of goGermline embryos are male but male and female embryos are indistinguishable, so a given set of 25 embryos will include a natural probable distribution around the mean of 12.5 males
- We recorded a range in percentage of males born – from 0 to 91% / 11 projects
- Fertile male chimeras are obligate germline heterozygotes so:
- Fewer male chimeras needed to complete a project because 50% of the offspring carry the targeted mutation, so all projects are completed in the first litters
- This also means colony expansion can be started with fertile F0 germline hets rather than F1 germline hets (saving on average 3 to 4 months)
- Complete elimination of uncertainty and variability regarding if and when GLT will occur
- Fewer F1s required to achieve GLT for all targeting projects

Results of goGermline 2.0

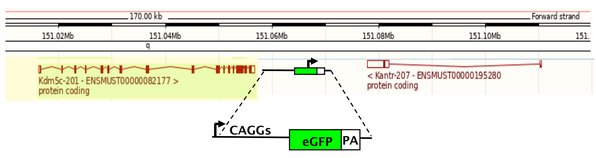
- All goGermline embryos are male so all chimeras are male
- 100% of the variability in the number of male embryos (per25 embryos) removed
- Up to 2x male chimeras born on average per injection session relative to mixed sex

goGermline 2.0 can be used to achieve two types of efficiency goals

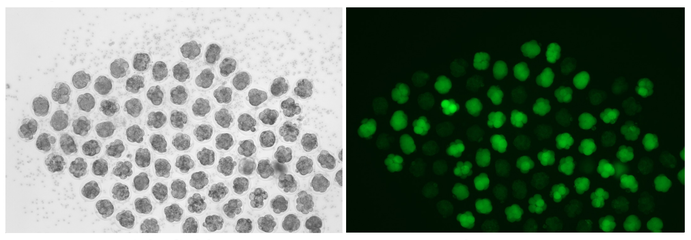
1) Reduce injections by 50% while maintaining same male chimer productivity
   - Save time and downstream resources (cages and husbandry) OR
2) Maintain the same injection schedule and double productivity
   - Make chimeric males less precious

Fig 1 How goGermline 2.0 works – generation, selection and chimeras born

A) X-chromosome is tagged:
with CAGGS-eGFP by targeted insertion between the genes Kdm5c and Kantr



B) Male embryos are isolated from female embryos by fluorescence:
only female embryos inherit X linked eGFP from male parent and fluoresce



Bright field **Fluorescence**

C) Following ES injection transfer :

1) All chimeras are male
2) All these male chimeras are sterile unless infertility is rescued by the injected ES cells
3) All fertile males are germline heterozygous for the targeted allele

Fig 2 Summary of wt/gG1.0 and gG2.0 function

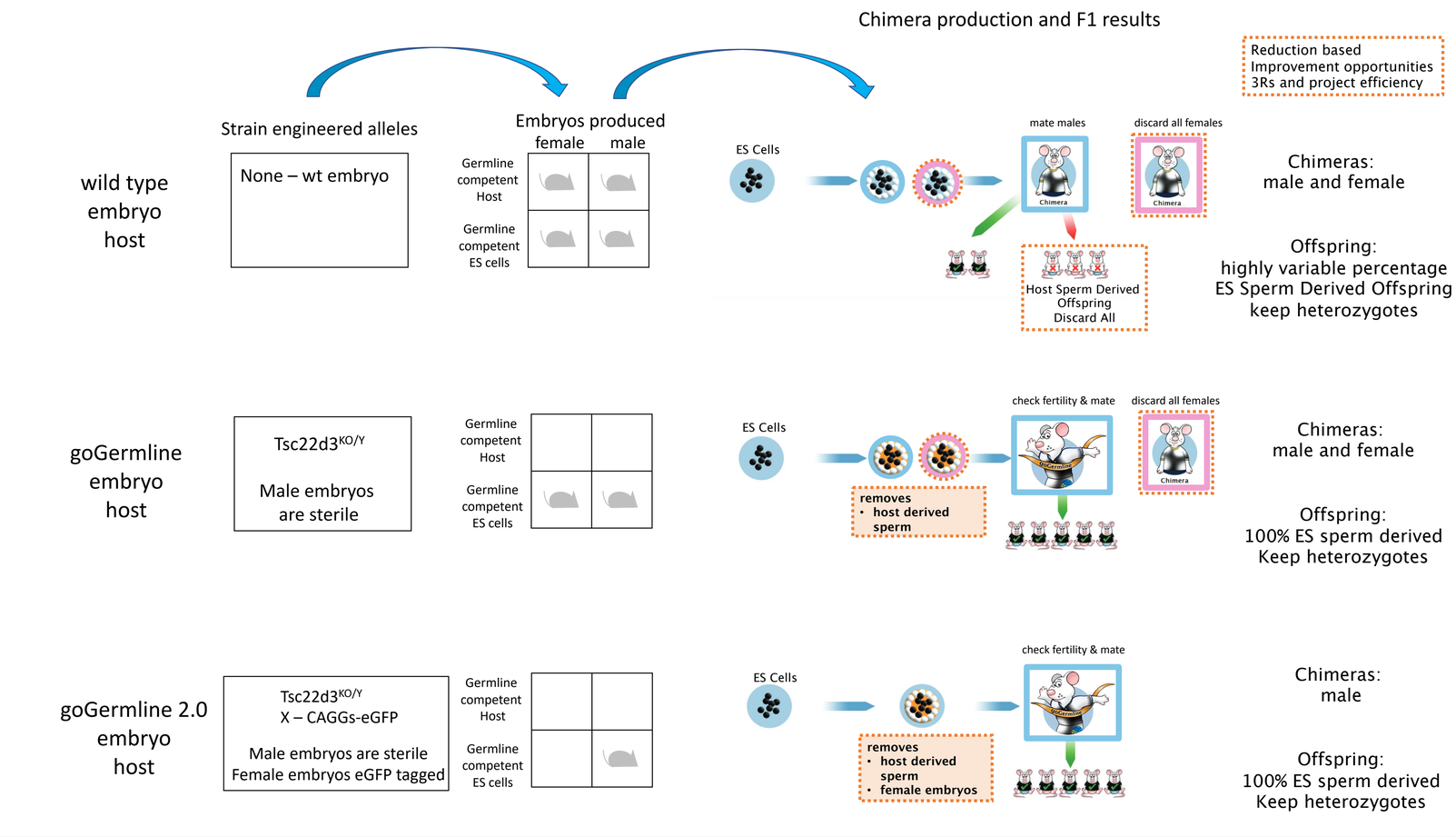
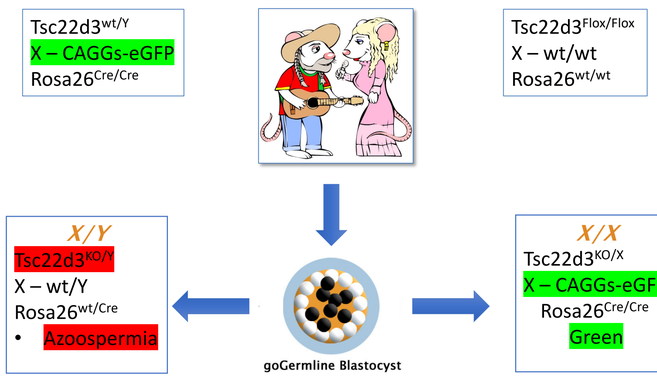


Fig 3 Breeding scheme for generation of goGermline 2.0 embryos



- ❑ **The Tsc22d3 Leucine zipper transcription factor is essential for spermatogenesis**
- ❑ **The Tsc22d3-Flox line is maintained as a homozygous colony**
- ❑ **Tsc22d3-KO (goGermline) embryos are generated for chimera injection by the cross:**
  Tsc22d3Flox/Flox female x Rosa26Cre/Cre male

Koentgen et al, Genesis (2016)

1

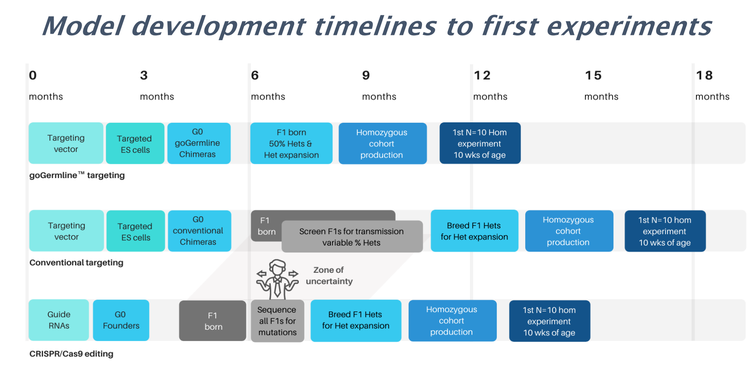
Fig 4 Head-to-head comparison of goGermline 1.0 and goGermline 2.0 – transfer recovery & births

Date of injetion	Projects in head to head comparison	Number of embryos Injected and transferred	Number of pups born	Percent transfer recovery	Number of male pups	Percent transferred males born	Percent of pups that are male
Germline 2.0							
8-Jun-22	Project 1	24	9	38%	9	38%	100%
10-Jun-22	Project 2	12	12	100%	12	100%	100%
16-Jun-22	Project 3	24	16	67%	16	67%	100%
1-Jul-22	Project 4	12	2	17%	2	17%	100%
8-Jul-22	Project 5	12	9	75%	9	75%	100%
15-Jul-22	Project 6	24	9	38%	9	38%	100%
20-Jul-22	Project 7	24	6	25%	6	25%	100%
3-Aug-22	Project 8	12	2	17%	2	17%	100%
17-Aug-22	Project 9	24	7	29%	7	29%	100%
				45%		45%	100%
Germline 1.0							
21-Jun-22	Project 1	24	15	63%	7	29%	47%
22-Jun-22	Project 2	24	11	46%	10	42%	91%
23-Jun-22	Project 3	24	22	92%	13	54%	59%
24-Jun-22	Project 4	24	9	38%	4	17%	44%
24-Jun-22	Project 5	24	15	63%	8	33%	53%
28-Jun-22	Project 6	24	14	58%	6	25%	43%
28-Jun-22	Project 7	24	17	71%	8	33%	47%
29-Jun-22	Project 8	24	14	58%	7	29%	50%
29-Jun-22	Project 9	24	17	71%	7	29%	41%
30-Jun-22	Project 10	24	10	42%	7	29%	70%
30-Jun-22	Project 11	24	8	33%	0	0%	0%
				58%		29%	50%

Percent range of male pups Born **100%**

Percent range of male pups Born **0 – 91%**

Fig5



References

2016 - Koentgen et al. genesis. 2016 Jun;54(6)
2017 - Hai Zhou et al. genesis. 2019 Jun; 57(6)
2017 – Winner of Inaugural ISTT 3Rs prize
2020 – Patented in AU & NZ
2021 – Patented in AT, BE, DE, DK, EU, FI, FR, GB, HU, IE, IT, JP, NL, NO, PT, SG, SE, TR
2022 – Patented in HK & KP - patent pending US

Xist-dependent imprinted X inactivation and the early developmental consequences of its failure.
Maud Borensztein, Laurène Syx, Katia Ancelin, Patricia Diabangouaya, Christel Picard, Tao Liu, Jun-Bin Liang, Ivaylo Vassilev, Rafael Galupa, Nicolas Servant, Emmanuel Barillot, Azim Surani, Chong-Jian Chen, Edith Heard
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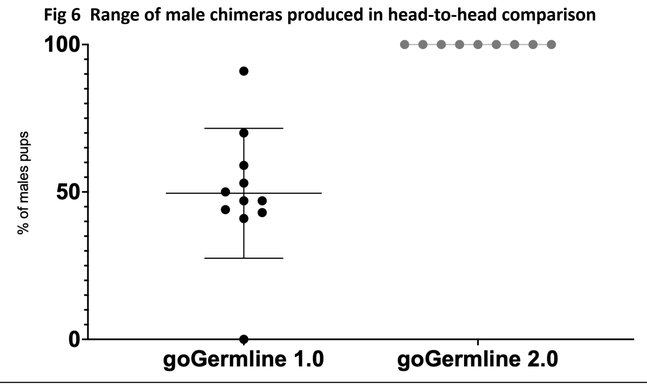
Fig 6 Range of male chimeras produced in head-to-head comparison



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