

Regenerative Medicine Minnesota

Progress Report

Grant Title: Development of Inducible Immunodeficient Swine

Grant Number: RMM-2017-BB-01

Requester: Daniel F. Carlson, PhD

Project Timeline: 5/30/17 – 5/29/18

Brief description of project: The long-term goal of the project was to produce inducible RAG2/IL2-R γ immunodeficient (regRG-KO) founder animals at Recombinetics with intermediate goal to design, build and test regRG-KO component constructs in vitro before combining them to single cells and cloning by somatic cell nuclear transfer. We specialize in genome engineering of pigs as models of human disease with an emphasis on the development of innovative solutions for the unmet need of human organs and tissues for preclinical testing, and ultimately, transplantation into patients. Immunodeficiency of the animal is very advantageous to the humanization of pig organ or tissue processes.

Where did this project take place? All plasmid construction, cell editing, and validation took place at Recombinetics' Laboratory space is located at 1246 University Avenue West, St. Paul, MN 55104. Somatic cell nuclear transfer (cloning) of RAG2/IL2R γ -KO pig cells took place at CRI in Mt. Horeb, WI.

People impacted by project and where they are from: Recombinetics collaborates with a variety of excellent researchers in Minnesota (Table 2). Establishment of immunodeficient swine in our collaborative or independent research programs has been discussed with over half of these investigators. This grant has provided initial stage resources for production of regRG-KO animals. As this project continues using Recombinetics internal funding and perspective grant applications, the resulting founder animals will be of beneficial use to many of these researchers.

University of Minnesota	Position	Mayo Clinic	Position
Peter Igarashi	Nesbitt Chair and Head of the Department of Medicine	Timothy J. Nelson	Dir. Todd and Karen Wanek Family Program.
David A. Largaespada	Asst. Dir. Masonic Cancer Center: Basic	Scott Nyberg	Artificial Liver and Liver Transplantation Laboratory
Walter C. Low	Professor	Joseph Lillegard	Children's Pediatric Surgery-Research @ Mayo Clinic
Jakub Tolar	Dir. Stem Cell Institute		
James Dutton	Assistant Professor		
Clifford Steer	Professor		
Tim O'Brien	Professor		
Anne Parr	Assistant Professor		
Maxim Cheeran	Associate Professor		

What was the outcome of the project? (Did the project work the way you expected it to? What were the successes? What were the failures? How did it impact regenerative medicine in Minnesota?)

The project currently ongoing using Recombinetics internal funding. To date, we have successfully generated and sequenced verified 2/3s of the projected 18kb inducible transgene.

Some unanticipated delays were due to repetitive sequences in the chosen promoters (specifically DAZL). This has impeded our ability to construct the entire cassette, but individual components to express IL2R γ and Rag2 have been successfully developed. We successfully generated porcine RAG2/IL2R γ KO primary cell lines from established porcine fibroblast lines and used them for cloning by somatic cell nuclear transfer (three transfers) with the goal of generation of embryonic cell lines that will ultimately be the recipient of the final vector assembled from the three component plasmids. During embryo development, cells are regenerated and “reset” allowing them to be used for additional gene-editing procedures.

Overall, we have achieved ~75% of our anticipated goal at the beginning of the grant period, and we are happy that Recombinetics has decided to continue the project on internal funds. We feel this method of producing SCID or any other animals with failure to thrive genotypes will have value in variety of ways as a platform technology.

Please list any of the following that have resulted from your Regenerative Medicine Minnesota grant funding:

- **Publications and/or manuscripts submitted for publication**
 - None as of yet related to our regRG-KO project
- **Disclosures/patents**
 - “Inducible Disease Models Methods of Making them and use in Tissue Complementation”
 - US: 16/101,295
 - Int: PCT/US2018/046346
- **Other grant applications and/or awards**
 - None as of yet related to our regRG-KO project

Responsible Spending:

Please let us know how you spent the money. Any unspent funds must be returned.