A Genetic Reporter System for MRI Based on LacZ Expression

N.E. Bengtsson (UF, Program in Stem Cell Biology and Regenerative Medicine); E.W. Scott (UF, Program in Stem Cell Biology and Regenerative Medicine); G.A. Walter (UF, Physiology)

Introduction
Magnetic resonance imaging (MRI) is currently hampered by a lack of genetic reporter systems that can provide molecular specific information in vivo. Few systems exist today and still need to be optimized (1). The commercially available substrate S-Gal™ was originally designed for colorimetric detection of cellular LacZ expression on histological slides. Upon being cleaved by β-galactosidase (β-gal), the enzymatic product of LacZ, S-Gal™ utilizes ferric ammonium citrate (FAC) to produce a non-diffusible, non-toxic, iron containing reaction product (2). This reaction product can be used to detect cellular LacZ expression by MRI.

Experimental
E 12.5 embryos conditionally expressing LacZ under control of sonic hedge hog (Shh) were fixed and stained with S-Gal overnight. Embryos were imaged at 17.6T magnetic field strength using T$_2^*$ weighted 3D gradient echo (GE) scan sequences with parameters: TR=0.5s, TE=5ms, and resolution 30×30×30 µm$^3$. Bone marrow (BM) cells from Rosa26 mice, expressing LacZ ubiquitously, were incubated for 2 h with S-gal and FAC. Following incubation, 0.5×10$^6$ labeled LacZ expressing and labeled wild type BM cells were transplanted into the right and left tibialis anterior (TA) muscles of C57BL6 mice respectively. Subsequently, the animals hind limbs were imaged using T$_2^*$ weighted 3D GE scan sequences at 4.7 and 11.1T magnetic field strength.

Results and Discussion
Following overnight staining, 3D MRI rendering of LacZ expressing and control embryos (A). A clear difference in contrast at the site of LacZ expression and S-Gal staining was observed between control (B) and Shh-LacZ embryos (C). D) Transplanted BM cells expressing LacZ (green) generated a larger volume of negative contrast than control cells (purple) at 4.7T magnetic field strength. E) This volume was observed to be increased with enhanced magnetic field strength (11.1T), thereby indicating greater detection sensitivity of LacZ expressing cells at higher magnetic fields.

Conclusions
The reaction between S-Gal and β-gal (the enzymatic product of LacZ) is specific and the resulting iron containing precipitate can easily be detected by using T$_2^*$ weighted high field strength MRI. Additionally, the contrast enhancing effect of the reaction product is enhanced with increased magnetic fields, thereby enabling easier detection of LacZ expressing cells.

Acknowledgements
This research was supported by the NIH (RO1HL75258; RO1HL78670 and NSF (EEC-0506560). Data were obtained at the AMRIS facility.

References