Introduction and strategy
This work serves as an assessment on the quality of services provided to research groups to generate CRISPR/cas9 genome edited mice. To minimize the influence of variables generated by genotyping and for easy estimation of knockout efficiency, mutagenesis was generated in Tyrosinase (Tyr) on chromosome 7 which is responsible to black coat color and eye pigmentation. A well characterized and efficient CRISPR/cas9 knock out strategy was adopted from Chen et al. (2016) to ensure the assessment outcome is closely associated with the service performance to be assessed. Lastly, to reduce the involvement of more sensile animals (the 3Rs principles) and to speed up the assessment process, E12.5 embryos, instead of postnatal pups, developed from manipulated zygotes were harvested to obtain the assessment outcome. The assessment outcome was quantified by the proportion of embryos losing retinal pigmentation, presumably as the results of detrimental mutations in Tyr. (ref: PMID: 27151215)

Date of Microinjection: 9th March 2017

Materials
Host strain: C57BL/6J
Cas9 mRNA: 100ng/ul working (GeneArt™ CRISPR nuclease mRNA, Invitrogen)
gRNA: 100ng/ul working (Synthesized and purified with GeneArt™ Precision gRNA Synthesis Kit, Invitrogen)

Results:
No. of zygotes injected: 112
No. of surviving post-injected zygotes and surgically transferred to surrogates: 55 (in 3 surrogates)
Total no. of E12.5 embryos harvested: 21
No. of embryos losing retinal pigmentation: 18 (see Fig. 1 & 2)

Conclusion:
The functional knockout efficiency from this microinjection experiment is 86% (18/21)