# Performance Assessment on RNA Microinjection for CRISPR/cas9 Genome Editing (Transgenic Service)

**Joanne Tam & Siva Tsang, March2017**

***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 CRSPR/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 March2017

***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) |

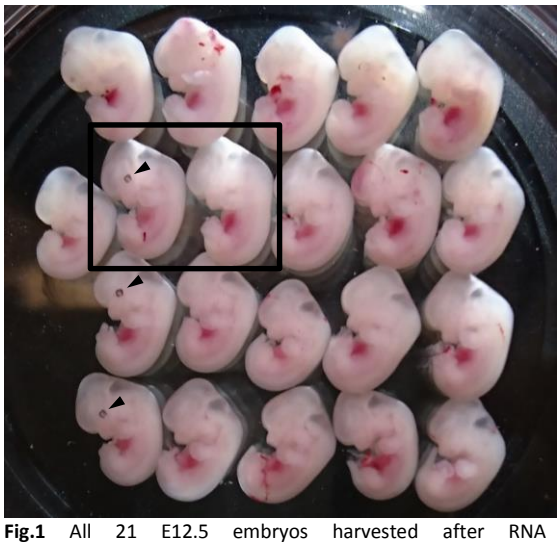

**Fig.1** All 21 E12.5 embryos harvested after RNA microinjection. Normal retinal pigmentation (indicated by arrowheads) is observed in 3 embryos only. The rest of the 18 embryos lost retinal pigmentation.

**Fig.2** A pair of embryos magnified from the inset in Fig.1. The right embryo shows the loss of pigmentation in its eye (open arrowhead), presumably as the result of *Tyr* mutation. Whereas, the left embryo shows normal retinal pigmentation (close arrowhead).

**Conclusion:**

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