# Progress Towards Development of a Genetically Modified Strain of the Australia Sheep Blowfly *Lucilia cuprina* Suitable for a Sterile Release Program

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Xuelei Li
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**Abstract**

The sterile insect technique (SIT) is concerned with the mass-rearing and release of sterilized insects which mate with "wild-type" females in the field, producing no viable offspring. The aim of this study was to use genetic engineering methods to make a strain of the Australia sheep blowfly *Lucilia cuprina* which is suitable for an area-wide sterile-male release program. The main objectives were: development of an efficient germline transformation system for introducing a target gene into *Lucilia* and development of an inducible female killing system to produce a male only population.

The *piggyBac* and *Minos* transposons were evaluated as transformation vectors for *L. cuprina*. Firstly, *Drosophila melanogaster* was used as a model system to determine if the frequency of both inter-plasmid transposition and germ-line transformation increases with the level of expression of the *piggyBac* transposase. Expression of the *piggyBac* transposase gene was controlled with either the *α1-tubulin*, *hsp83* or *hsp70* promoter, which have strong, intermediate and low constitutive activity respectively. The results show that the frequency of *piggyBac*-mediated germ-line transformation does increase with the level of expression of the transposase. In contrast, there does not appear to be a simple correlation between the level of expression the transposase and the frequency of transposition measured using an inter-plasmid transposition assay. This suggests that this widely used assay may not necessarily predict which is the best "helper" plasmid for germ-line transformation. Secondly, inter-plasmid transposition assays have shown that both *piggyBac* and *Minos* transposases are active in blowfly embryos. Thirdly, *Drosophila* eye color genes and the enhanced green fluorescent protein (*EGFP*) gene were tested as potential markers for identifying transgenic flies. The most promising marker based on transient expression appears to be *EGFP* driven by the Drosophila *polyubiquitin* gene promoter (*pUb-EGFP*). Fourthly, blowfly embryos were coinjected with the *piggyBac* helper driven by the *D. melanogaster*

*hsp70* promoter and the *PUbnlsEGFP* marker gene. Two transgenic *L. cuprina* lines were isolated and characterised by Southern DNA hybridisation analysis and inverse PCR. The transformation frequency was 1.4 to 1.9%. Of the two transformant lines obtained, one had a single copy of the transgene and the other most likely has four copies. This is the first report of germ line transformation of *L. cuprina*.

A tetracycline regulated inducible expression system was adopted to develop a controllable female-killing genetic system based on the *D. melanogaster msl2* gene. One component of the system is the tetracycline dependent transactivator (*tTA*) gene controlled by a constitutive promoter. The other (*tetO-msl2*) is the *msl2* coding region controlled with a promoter bearing seven copies of the tetracycline operator (*tetO*) sequence. Female *D. melanogaster* carrying both a *promoter-tTA* and *tetO-msl2* gene constructs would be predicted to die in the absence of tetracycline due to expression of *msl2*.

In this study several *promoter-tTA* constructs were developed including *WH-arm* which uses the constitutive *armadillo* promoter. Drosophila carring both *WH-arm* and *tetO-lacZ* transgenes were shown by spectrophotometric and histochemical staining assays to express β–galactosidase but only if raised on media that lacked tetracycline. There was a significant decrease in viability of females carrying both *WH-arm* and *tetO-msl2* gene constructs raised on media lacking tetracycline. However lethality was not 100%. Assembly of the MSL complex on female X chromosomes (due to expression of *msl2*) was confirmed by immuno staining of polytene chromosomes with anti-MSL3 antibody. Thus it appears that induction of 100% female lethality will require higher levels of *msl2* expression than obtained with the *WH-arm/tetO-msl2* system for controlling female viability in transgenic *Lucilia*.

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## ABBREVIATIONS

| | |
|---|---|
| *arm* | *armadillo* |
| β | beta |
| Δ | delta |
| λ | lambda |
| bp | base pairs |
| BSA | bovine serum albumin |
| BHI | brain heart infusion |
| *cn* | *cinnabar* gene |
| °C | degrees Celsius |
| cDNA | complementary DNA |
| $ddH_2O$ | double distilled water |
| DNA | deoxyribonucleic acid |
| *D. melanogaster* | *Drosophila melanogaster* |
| dNTPs | dinucleotide triphosphates |
| *E. coli* | *Escherichia coli* |
| EDTA | ethylenediamine tetraacetic acid |
| EGFP | enhanced green fluorescent protein |
| F | female |
| g | gram |
| GFP | green fluorescent protein |
| *hsp* | heat shock protein |
| kb | kilobase pairs |
| mRNA | messenger ribonucleic acid |
| L | liter |
| *L. cuprina* | *Lucilia cuprina* |
| μ | micro |
| M | male |
| M | molar |

| | |
|---|---|
| Mb | million base pairs |
| min | minute |
| *mle* | *maleless* |
| *msl* | *male specific lethal* |
| *mof* | *males absent on the first* |
| nm | nanometer |
| OD | optical density |
| ORF | open reading frame |
| PBS | phosphate-buffered saline |
| PCR | polymerase chain reaction |
| RNase | ribonuclease |
| RNA | ribonucleic acid |
| rpm | revolutions per minute |
| TE | tris-EDTA |
| *tetO* | *tetracycline operator* |
| tetR | tetracycline repressor |
| tTA | tetracycline dependent transactivator |
| tub | tubulin |
| UV | ultra violet |
| U | units |
| UTR | untranslated region |
| V | volts |
| v/v | volume per volume |
| w/v | weight per volume |
| yp | yolk protein |

## LIST OF PUBLICATIONS ARISING FROM THIS THESIS

X. Li, J.C. Heinrich and M. J. Scott (2001) *piggyBac*-mediated transposition in *Drosophila melanogaster*: an evaluation of the use of constitutive promoters to control transposase gene expression. *Insect Molecular Biology* **10(5)**: 447-455.

Jörg C. Heinrich*, Xuelei Li*, Rebecca A. Henry, Neville Haack, Leanne Stringfellow, Allen Heath and Maxwell J. Scott (2002) Germ-line transformation of the Australian sheep blowfly *Lucilia cuprina*. *Insect Molecular Biology* **11(1)**: 1-10.
*These authors contributed equally to this study.