C57BL/6NTac double and single albino mutant mice generation for efficient germline transmission of Chimera

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Introduction

Ideally host blastocysts for C57BL/6 ES cells should be co-isogenic and chimeras preferably identifiable by coat color. The most common strains used are C57BL/6 albino and BALB/c. BALB/c mice however, respond poorly to superovulation and result in low blastocyst yields, forcing the use of an increased number of female blastocyst donors. Available albino C57BL/6 strains have been obtained due to spontaneous mutations in the tyrosinase locus. These provide a suitable alternative but due to the recessive nature of the *Tyr*^{c} mutation, coat-color detection of the ES-cell contribution in germline transmitted G1 offspring requires breeding of chimeras to albino C57BL/6. More importantly, a pure genetic C57BL/6 substrain background may only be maintained by subsequent backcrossing. Furthermore the mutated *Tyr*^{c} allele will be inherited by the progeny if not actively screened against and removed from the colony.

To address this we decided to generate a double albino mutant that carries a dominant Agouti allele (*A*$^{tm1.1Arte}$) in addition of a tyrosinase recessive allele (*Tyr*^{tm1Arte}) in the C57BL/6NTac genetic background.

When this C57BL/6NTac-*A*$^{tm1.1Arte}$ *Tyr*^{tm1Arte} double mutant is used as host blastocyst with C57BL/6 ES cells, the resulting black/albino chimeras can be mated directly with C57BL/6NTac mice and allow recognition of germline transmission in the G1 offspring just by coat color.

We recognize that here are some instances in which the scientist might prefer not to have present the agouti allele and just have the single tyrosinase mutation, for this we have bred C57BL/6NTac mice that contain the single *Tyr*^{tm1Arte} mutation.

Materials and Methods

In this study, we have combined a loss-of-function point mutation at the tyrosinase locus (Fig. 1) with a reversion of the non-agouti locus to a dominant *agouti* (*A*) allele (Fig. 2) in the C57BL/6N substrain genetic background. Homozygous C57BL/6NTac-*A*$^{tm1.1Arte}$ *Tyr*^{tm1Arte} double mutated albino mice are used as blastocyst donors for C57BL/6NTac ES cell injections (Fig 3). Resulting black/albino chimeras can be mated to strain C57BL/6NTac and still allow recognition of germline transmission in G1 offspring by coat color.

Fig 1: Inactivation of the Tyrosinase gene

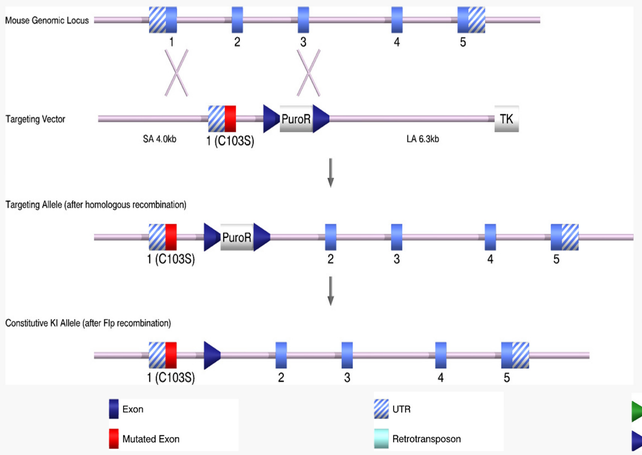

Fig 2: Reversion of the non-agouti allele

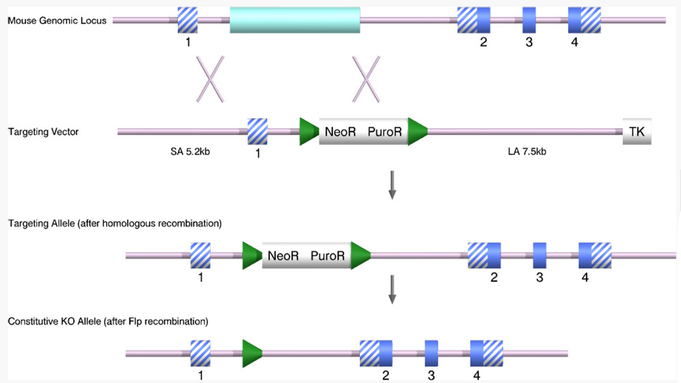

Table 1 Germline Transmission of Single and Double Albino Mutants

Chimera Production	BALB/c	Albino	Albino++
Nomenclature	BALB/c	C57BL/6NTac-*Tyrc* ^{tm1Arte}	C57BL/6-*A* $^{tm1.1Arte}$ *Tyrc* ^{tm1Arte}
Genotype	Tyr: c/c A:wt/wt	Tyr: ko/ko A: wt/wt	Tyr: ko/ko A: ki/ki
SOV efficiency	Poor	Equivalent to C57BL/6NTac	Equivalent to C57BL/6NTac
Germline Transmission	YES	YES	YES

Fig 3: Use of double mutant albino as embryo donors