Large scale production of GM mice for research

Karolinska Center for Transgene Technologies (KCTT)
Comparative Medicine, Karolinska Institutet

Content

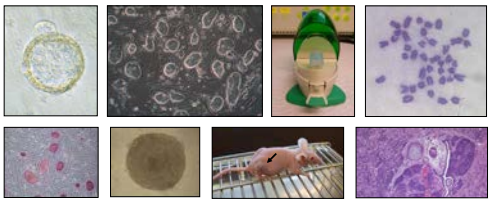
- KCTT intro
- Transgenic mice
- Gene targeted mice
  →Knockouts/knockins
- Aspects on GM in mice
  →Strain background effects – modifier genes
  →Genetic redundancy
  →Multiple alleles

Karolinska Center for Transgene Technologies (KCTT) – facts & figures

- Established:
  → Year 2000 by merging of two existing KI facilities
- Location:
  → Two sites: KI Campus Solna and KI Campus Huddinge
- No. of staff:
  → 12
- Yearly production:
  → No. of new Tg/KO:
    - 30-40 strains
  → No. of re-derivations:
    - 100-120 strains
  → No. of cryopreservations:
    - 80-100 strains

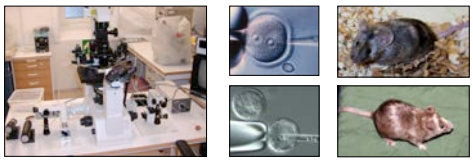
Embryonic stem cell services

- Gene targeting of mouse ESC
- Derivation and characterization of new mouse ESC lines



Microinjection services

- Microinjection of DNA into fertilized eggs for the production of transgenic mice
- Microinjection of ESCs into blastocysts/morulas for the production of chimeric mice



Cryopreservation and rederivation services

- Cryopreservation of mouse strains
  → Embryos or sperm
  → In Vitro Fertilization (IVF)
- Mouse strain recovery and rederivation services
  → From frozen embryos or sperm
  → Pathogen decontamination into SPF conditions
- Partner of the European Mouse Mutant Archive (EMMA)

EMMA

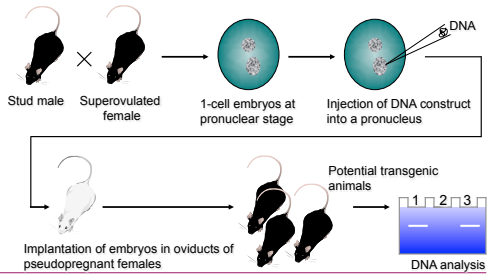
Different ways of modifying the mouse genome

- Phenotype-driven (forward genetics)
  - → Chemical
    - ENU
    - Chlorambucil
  - → Radiation
    - X-ray
- Gene-driven (reverse genetics)
  - → Insertional mutagenesis
    - Gene trapping
    - Transposon
  - → Transgenic technologies
  - → Gene targeting technologies
  - → Targeted genome editing

Production of

TRANSGENIC MICE

Production of transgenic mice by pronuclear microinjection



Microinjection of mouse zygotes

Video by J. Wilbertz

Transgenic construct and integration aspects

- Type of construct: plasmid/BAC/PAC/YAC
- Choice of promoter (ubiquitous/tissue-specific/regulatable)
- Random, single-site integration → hemizygous
- Multiple copies (concatamers)
- Position-dependent effects
- Insertional mutagenesis of endogenous gene
- Delayed integration → genetic mosaicism
- Instability of concatamers
- Co-transfer of two unlinked DNA constructs

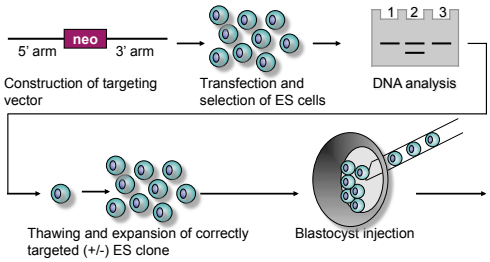
Transgenic mice: Different types of studies

- Gain-of-function studies
  - → Wild-type vs mutated gene
  - → Gene from same or different species
  - → Homologous vs heterologous promoter
- Rescue/complementation studies
- Promoter analysis studies
  - → Linking regulatory region to reporter genes (e.g. lacZ, GFP)
- Loss-of-function studies
  - → Toxin gene (e.g. diphteria toxin)
  - → Ribozyme
  - → Dominant-negative gene
  - → RNAi (knock-downs)

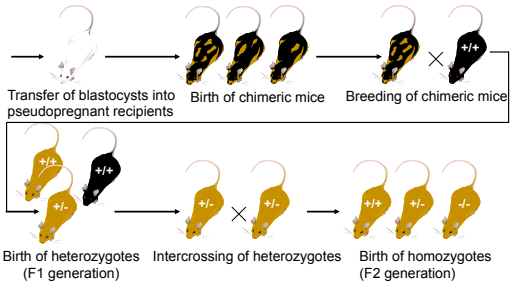
Production of

KNOCKOUT/KNOCKIN MICE

Production of knockout mice by gene targeting in ES cells (I)



Production of knockout mice by gene targeting in ES cells (II)



Injection of ES cells into blastocysts

Video by J. Wilbertz

Breeding for germline transmission (GLT)

Gene targeted mice: Different types of studies

- Loss-of-function studies (knockouts)
  - →Conventional vs conditional (tissue-specific KO)
- Gain-of-function studies (knockins)
  - →E.g. human disease mutation
  - →Recombinase genes (Cre or Flp)
  - →Reporter genes (e.g. lacZ, GFP)

Average production numbers in the creation of a new GM mouse strain

Parameter	Tg	KO/KI
Embryo donors	20	20
Foster mothers	6-8	4-6
Injected embryos	60-100	40-60
No. of live offspring	30-50	20-30
No. of Tg$^+$/chimeric	3-5	5-15
No. of Tg founders/GLT	2-4	3-6

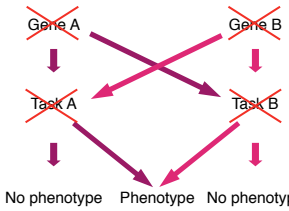
Some general issues on what we have learned from

STUDIES OF GM MICE

Genetic background effects on GM mouse phenotype

GM strain	Background strain: Phenotype	Reference
Keratin 8 KO	**C57BL/6 × 129/Sv:** ~E12 † (liver defects) **FVB/N:** Colorectal hyperplasia	Baribault et al., Genes Dev, 1994, 8:2964-73
EGFR KO	**129/Sv:** Mid-gestation † (placental defects) **CF-1:** Peri-implantation † (ICM degeneration) **CD1:** † at ~3w (multi-organ defects)	Threadgill et al., Science, 1995, 269:230-234
TGFβ1 KO	**C57BL/6JOla × NIH/Ola:** ~50% E10.5 † (defective yolk sac vasculopoiesis/ hematopoiesis); ~50% † at weaning (autoimmune disease) **129/Sv × CF-1:** ~50% pre-implantation †, ~50% † at weaning (autoimmune disease)	Kallapur et al., Mol Reprod Dev, 1999, 52:341-9.

Gene redundancy revealed in knockout experiments



Example: MyoD gene family; Weintraub (1993) Cell 75:1241-1244

Creation of mice with multiple GM alleles

- By breeding
- By deriving zygotes or ES cells from GM mice
- By using the CRISPR/Cas9 system