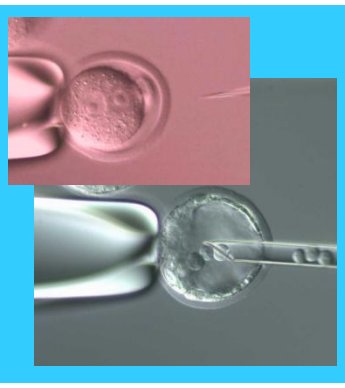
Transgenics and the 3Rs
What's it all about?

What are GA animals?

Animals that have had:

- their heritable DNA intentionally altered,
- or who carry a genetic mutation recognised as harmful,
- or the progeny of such animals.



How are GA animals created?

The most common methods involve adding DNA by microinjection into 1-cell stage embryos, or manipulation of ES cells to add or remove genes before they are injected into early embryos to create chimeras.

Replacement

- **Before** creating a novel GA animal review the scientific need and check that it does not already exist.

Use resources such as:

- FESA (www.har.mrc.ac.uk/mousebook)
- IMSR (www.informatics.jax.org/imsr/index.jsp)
- FIMRe (www.fimre.org)
- EuMMCR (www.eummcr.org)

Reduction

- Optimise the design, preparation, integration and expression of the transgene to minimise the overall number of animals involved in producing the GA line by:

Microinjection

- basing transgenes on genomic rather than cDNA sequences, wherever possible;
- linearising DNA constructs and removing plasmid sequences prior to microinjection;
- using only high-quality DNA preparations free from chemical contaminants and debris;
- including intronic sequences in the transgene to avoid integration site effects;

ES cell manipulation

- using constructs isogenic to the strain from which the ES cells are derived;
- characterising ES cells before injecting;
- using early passage ES cells, cultured under conditions optimal for the maintenance of pluripotency;
- using host blastocysts from inbred strains to maximise the likelihood of achieving germline transmission.

Refinement

- Consider using **Conditional** and **Reporter** strains, or **targeted expression** to help regulate harmful effects arising from undesirable transgene expression.
- **Only** female mice weighing more than 13g should be used for **superovulation** or **mating**.
- Animals selected as **embryo recipients** should have both good reproductive performance and maternal behaviour.
- **Embryo recipients** should weigh 20-30g to ensure pregnancy can be efficiently supported.
- Bilateral **embryo transfer** should involve only **one** incision.
- **Vasectomies** should be carried out via a small incision in the scrotal sac.
- Mice undergoing surgical procedures such as **embryo transfer**, or **vasectomy** should always be provided with adequate peri-operative **analgesia** and care.
- Use **aseptic surgical technique** for embryo transfer, including clipping the hair and cleansing the skin with a surgical scrub.
- If the GA animals experience **no adverse effects**, they should be bred and maintained as **homozygous**.
- If breeding **heterozygous** animals is unavoidable, **use surplus mice** for other scientific purposes, such as tissue banking or cadavers for surgical training.
- **Novel GA mice** should be archived as frozen embryos, or sperm as part of the creation process.

The 3Rs (Replacement, Reduction, Refinement) are essential principles of humane experimental technique.

- Always be alert to **3Rs** opportunities.
- Include the 3Rs in **all** your publications, presentations and posters (the 3Ps).

This poster has been produced following an RSPCA/LASA/IAT meeting hosted by NIMR in London on the 4th February 2009.

Transgenics and the 3Rs
Good practice and care

GA animals & A(SP)A 1986

Under A(SP)A 1986 it is presumed that any genetic alteration may have the effect of causing pain, suffering, distress or lasting harm in the individuals or their offspring.

Project licence authority is needed for the creation, maintenance and breeding of GA animals. Halfway through gestation/ incubation a mammal or bird becomes protected. Fish & amphibians are protected once they are capable of independent feeding.

Good Practice

- When **breeding animals** for use by others ensure there are good lines of communication and regularly review the **scientific need** for each GA line (encourage archiving).
- Keep **accurate** breeding records, look out for developmental or birth defects, and reductions in fertility or libido, so that appropriate action can be taken.
- Use **non-invasive** methods of identification wherever possible and **remember** if genotyping is not required then one animal can be left unmarked in each cage.
- Regularly review your method of tissue sampling for **genotyping. Always** use the **least invasive** and keep the amount of tissue taken to a **minimum**.
- Do not use **ear notching** in mice younger than 2 weeks old.
- Avoid **tail biopsy.** If unavoidable, the most humane age to perform it in mice is 3-4 weeks old, however appropriate anaesthesia and analgesia **should always** be given.
- Use **welfare assessment** sheets to help assess each GA strain's requirements, starting at birth and continuing through the animal's **entire life span**.
- Ensure **information** regarding the nature of the phenotype and any **specialist care** required is accessible to **all** relevant staff and a copy stays with the animals if **transported** to a different establishment.
- Avoid the **transport** of live mice. Fresh or frozen gametes, or embryos, should be used wherever possible.

Care and Welfare

GA animals are all different, some phenotypes will be obvious whilst others will not. Be alert for **unexpected** or subtle phenotypes and look out for inherent welfare issues.

They may have different:

- susceptibilities to disease
- humidity and temperature requirements
- light sensitivities
- hearing ranges
- enrichment requirements
- nutritional needs
- nesting and bedding requirements
- growth rates

and more…..

The same strain on a **different background** might show a completely **different phenotype**.

Think '*outside the box*' and give your animals the best welfare!

Remember...

- **Know your animals, look and listen for anything unusual.**
- **Look at all aspects of your animals lives for ways to reduce potential suffering and improve welfare.**
- **Keep up to date with good practice, don't assume current practice is best practice.**

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