Genetically Engineered Mice: Injection/Infection

IBC concerns

Presented at “Institutional Biosafety Committees:
Promoting Optimal Practice Now and in the Future”

June 24-26, 2009

IBC Concerns

- Why does the IBC care?
  - NIH guidelines cover:
    - **Section III-D-4-a**: Experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under **Section III-D-1-a and Section III-D-4-b.**

IBC Concerns

- NIH guidelines state (For Lentiviral delivery vectors):
  - **Section III-D-1-a.** Experiments involving the introduction of recombinant DNA into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

IBC's Goal
A Two Step Process

- Risk Assessment – identifies risk factors associated with the work performed to generate genetically engineered mice.

- Risk Management – manages the risks identified to ensure the safety of all personnel involved in the creation and maintenance of the genetically engineered mice.

What needs to be registered with the IBC?

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  - Genetically engineered rodents created through the use of mouse embryonic stem cells.
  - Genetically engineered rodents created by DNA injection.
  - Genetically engineered rodents created through the use viral vector delivery systems.
  - Genetically engineered rodents that are crossbred to potentially develop a new strain of animal.
  - Experiments involving genetically engineered rodents.

What doesn’t need to be registered with the IBC?

– Purchased genetically engineered mice.

How do we engineer the mice?

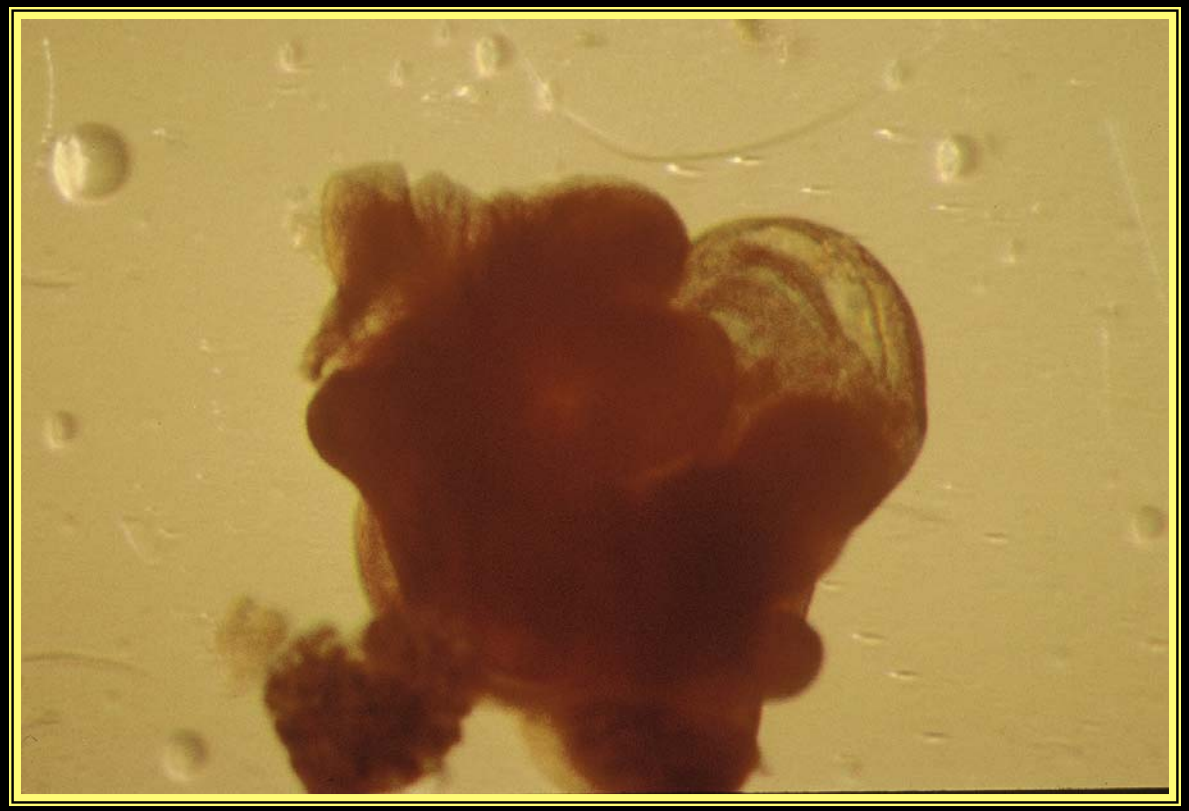
We make transgenic mice by inserting a novel piece of DNA into somatic or germ cells.

We make gene-targeted mice by changing a portion of the mouse genome using mouse embryonic stem cells.

New: We can also make transgenic mice by infecting the embryo with a lentivirus vector containing the modified gene.

Making Transgenic Mice

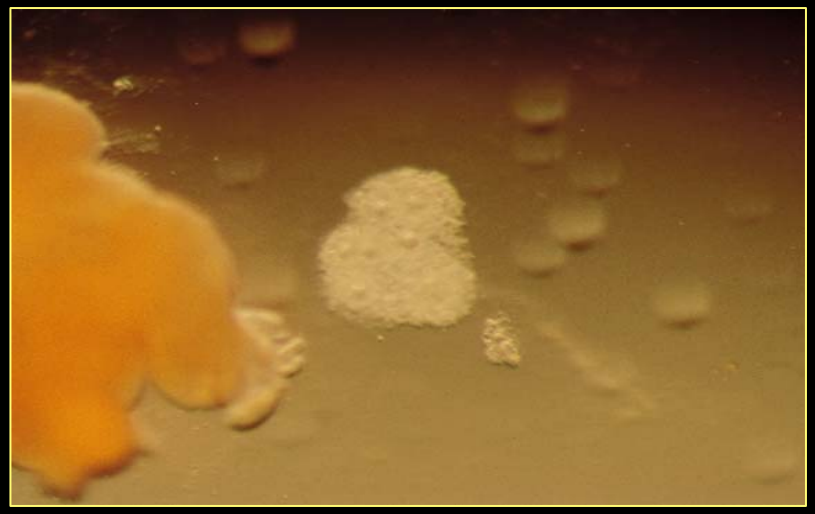
Oviducts are isolated from donor females



Donor females are superovulated using PMSG and HCG, and then mated to stud males. The following day, oviducts are isolated into a growth media spiked with hyaluronidase.

Embryos are Isolated and Washed

Embryos are isolated in HEPES buffered media + hyaluronidase



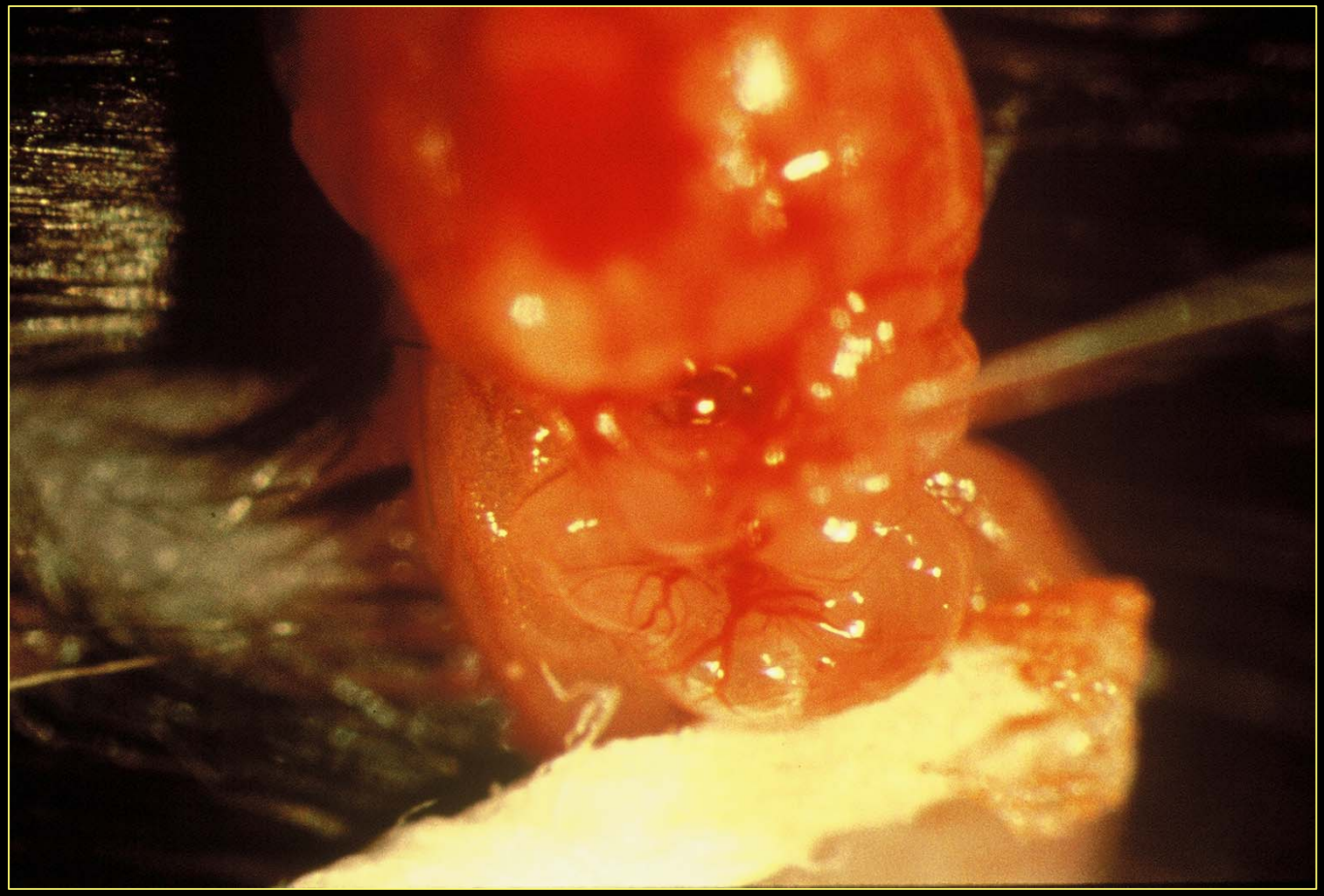
“Clean” embryos are moved to the injection slide using a pulled glass pipette

Embryos are Moved to the Injection Microscope

DNA Injections to Generate Transgenic Mice

Individual embryos are injected with pl amounts of a purified DNA fragment in TE.

DNA Injections to Generate Transgenic Mice



20-30 injected embryos are implanted into a surrogate mother using standard surgical techniques

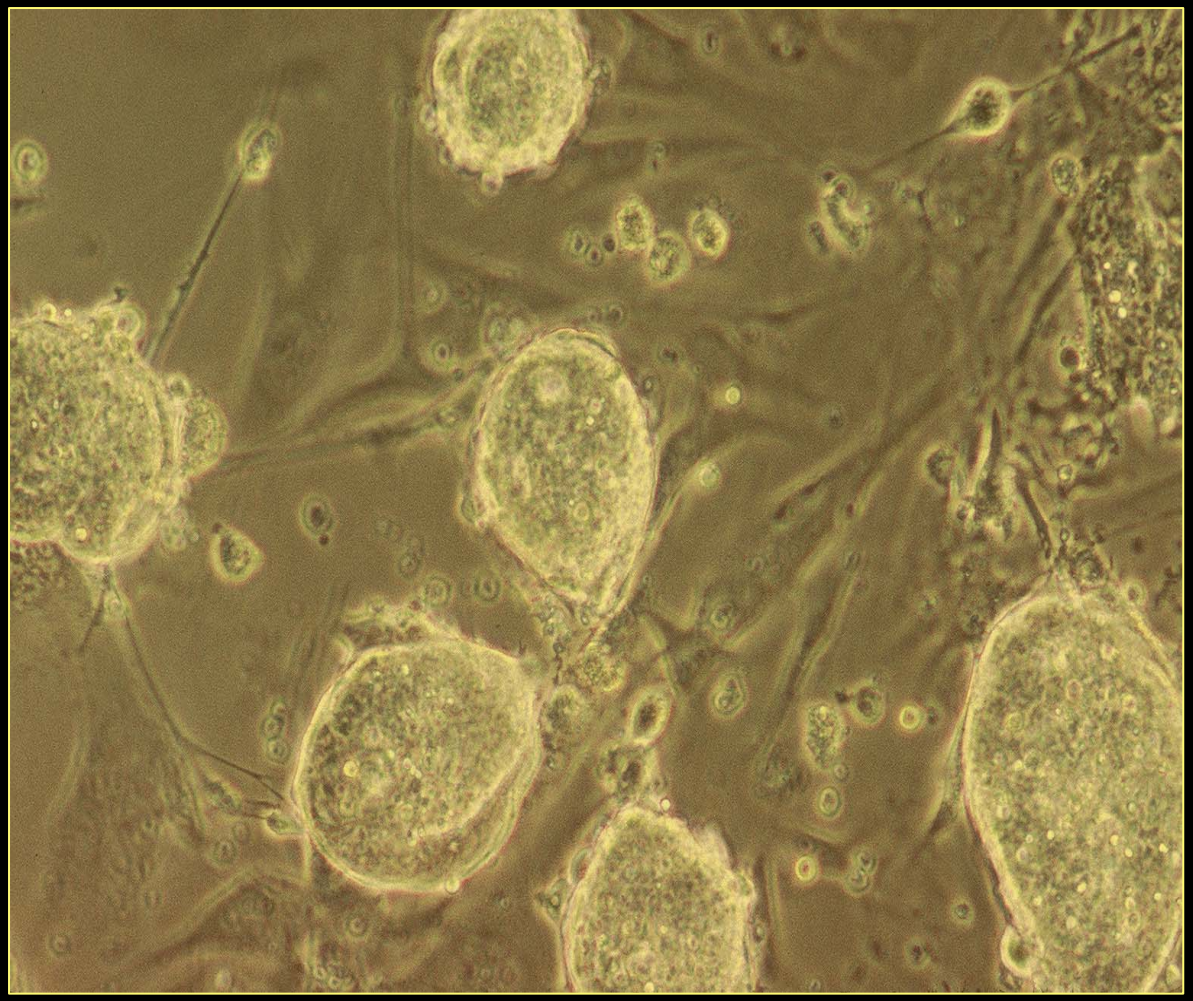
Making Gene-Targeted Mice

Making gene “knock-out/in” mice by homologous recombination in mouse embryonic stem cells.

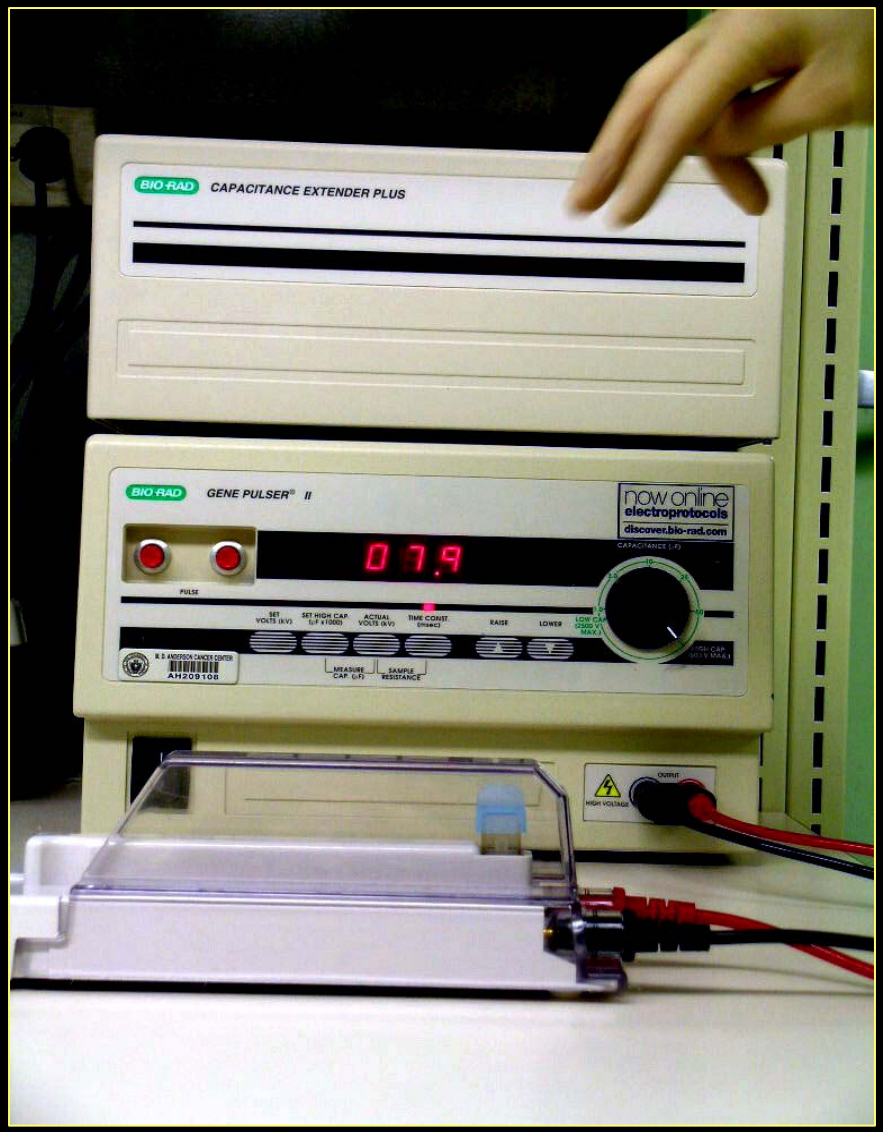
Targeted clones are then injected into mouse blastocysts.

ES Cell Injections to Generate Gene-Targeted Mice

Embryonic stem cells are grown in culture in DMEM + FBS.



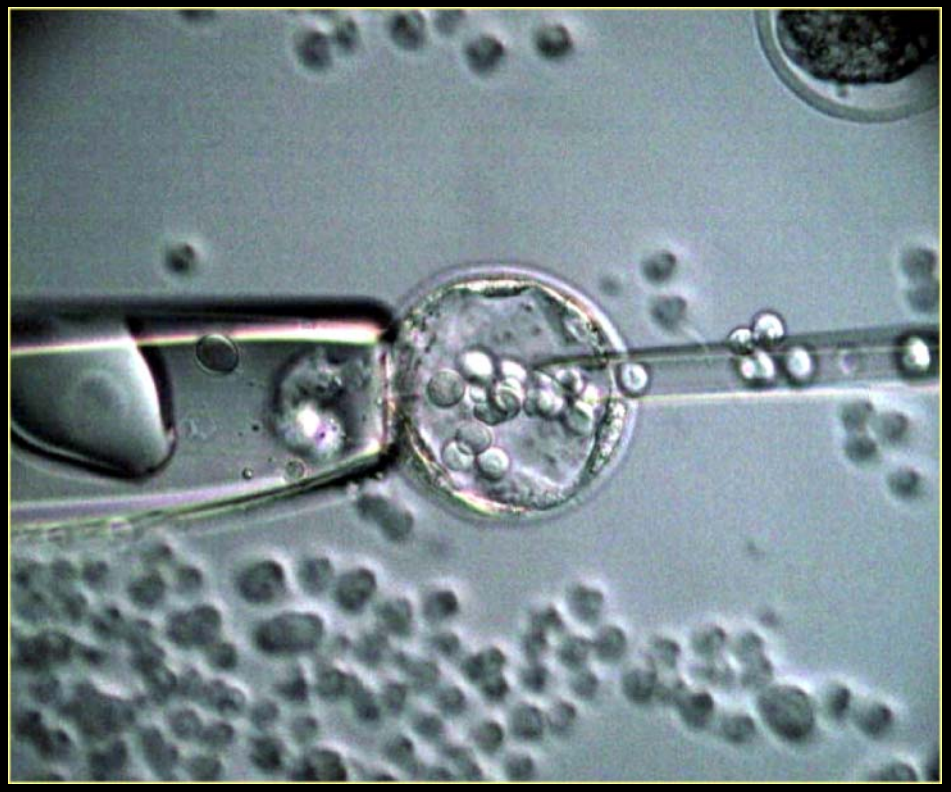
ES Cell Injections to Generate Gene-Targeted Mice



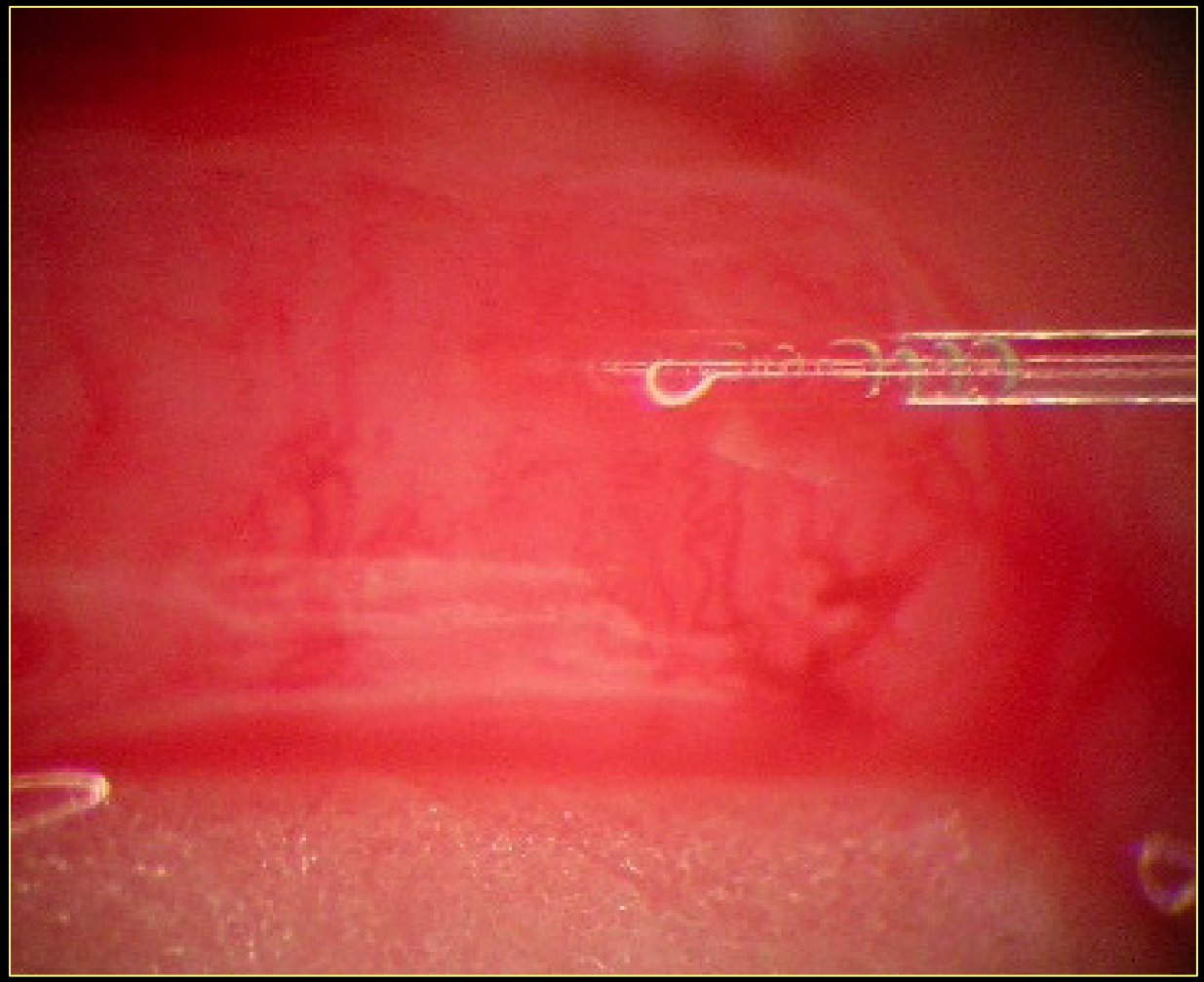
Cells are treated with trypsin to get a single cell suspension. This is mixed with DNA and then electroporated. Cells are plated on selective media.

ES Cell Injections to Generate Gene-Targeted Mice

Cells identified by the investigator as homologous recombinants are injected into blastocyst stage embryos.



ES Cell Injections to Generate Gene-Targeted Mice



10-15 injected embryos are implanted into a surrogate mother using standard surgical techniques

Generating Transgenic Animals using Lentivirus

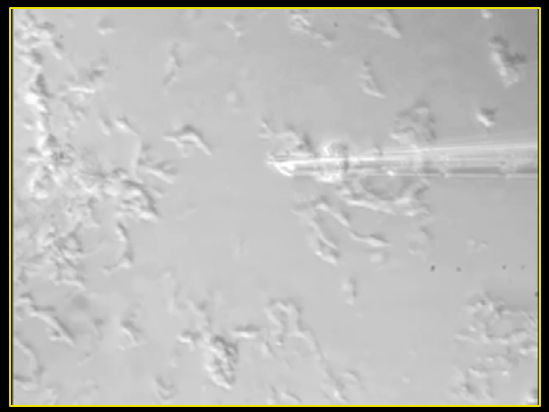
Retroviruses
ssRNA viruses
Stable integration
Generally host-restricted:
- HIV-1 = human
- FIV = felines
- SIV = non-human primates

Generating Transgenic Animals using Lentivirus

A 4-5 µl slurry of viral particles and cellular debris is auto-pipetted onto a glass slide and covered with oil.

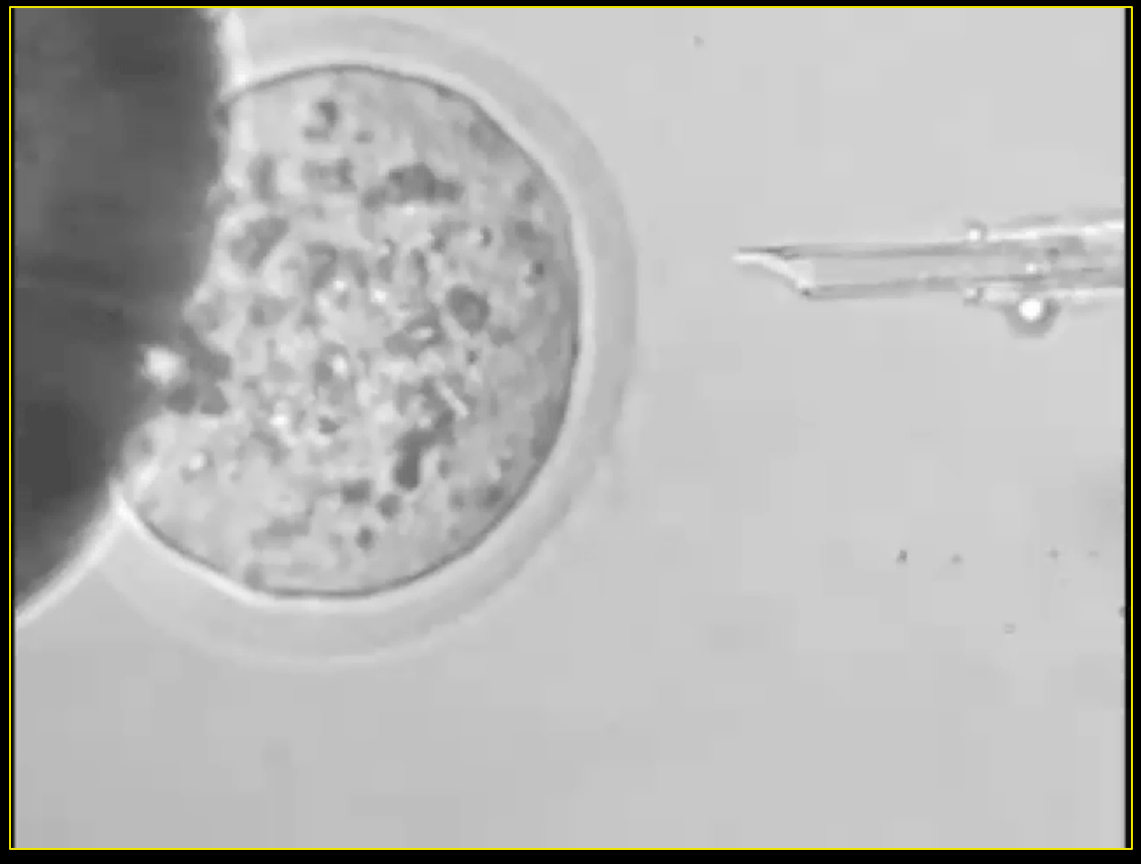
The half-life of a lentivirus at room temperature is quite short--most of the virus will be inactive after 6 hours at room temperature.

The injection needle is loaded with approx. 3 µl of the solution using gentle negative pressure from a hydraulic device.

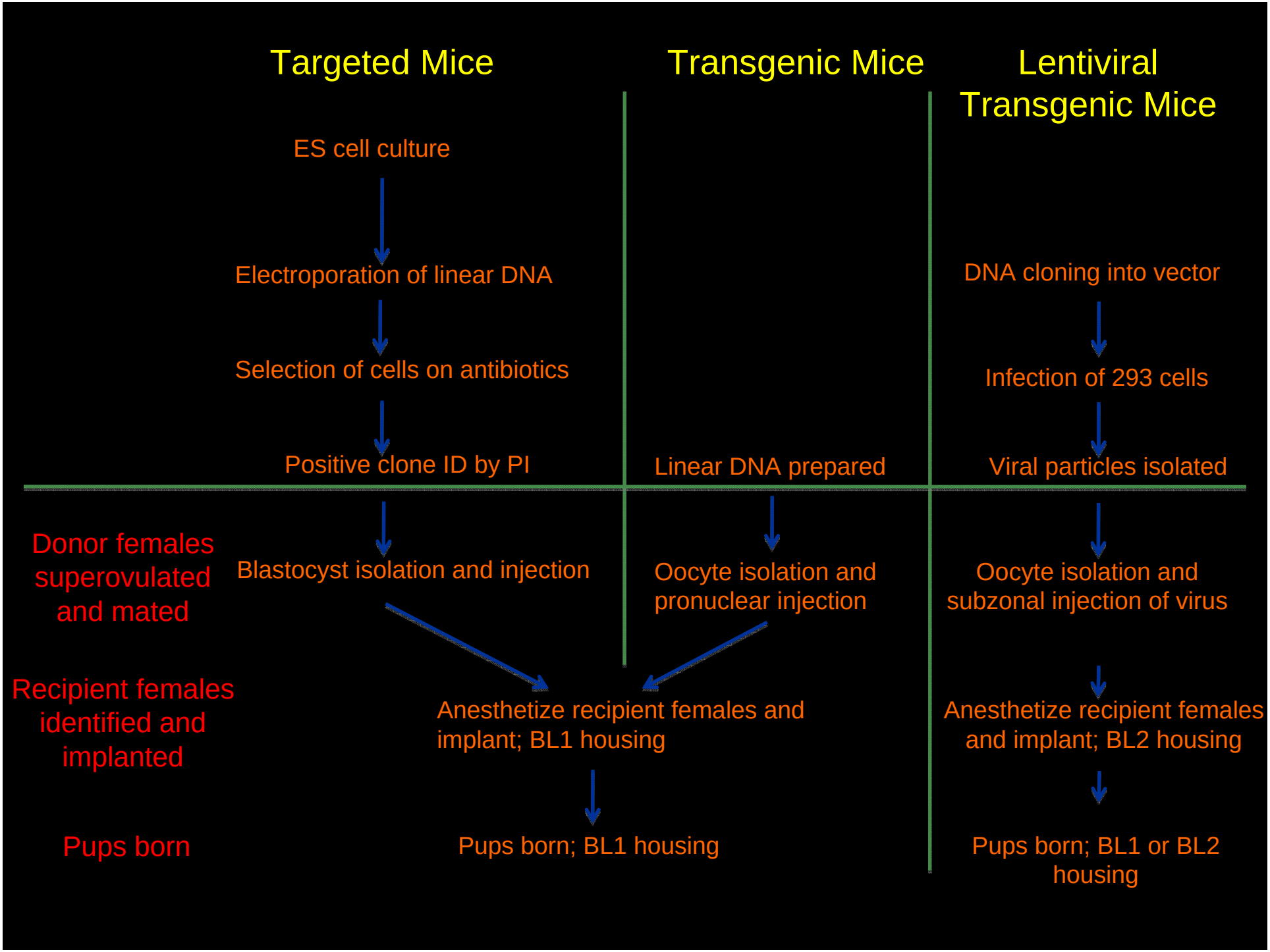


The embryos are contained in a droplet of media under oil and are injected at 400X magnification.

The solution of viral particles is injected into the space between the zona pellucida and the oocyte cell membrane.



The Results


Targeted Mice
Transgenic Mice
Lentiviral Transgenic Mice
ES cell culture
Electroporation of linear DNA
Selection of cells on antibiotics
Positive clone ID by PI
DNA cloning into vector
Infection of 293 cells
Viral particles isolated
Linear DNA prepared
Donor females superovulated and mated
Blastocyst isolation and injection
Oocyte isolation and pronuclear injection
Oocyte isolation and subzonal injection of virus
Recipient females identified and implanted
Anesthetize recipient females and implant; BL1 housing
Anesthetize recipient females and implant; BL2 housing
Pups born
Pups born; BL1 housing
Pups born; BL1 or BL2 housing

Commonalities

Preparation of Donor Females

- Superovulation using IP injections of the donor females with pregnant mare's serum and human chorionic gonadotropin

[Concerns are needle sticks causing localized immune response (in the case of PMS) or a possible infection (in the case of HCG) and the possibility of animal bites.]

Commonalities

Injection of DNA, cells or virus

- Use of pulled glass injection pipettes (can often be purchased “ready to go”

- Filling the injection needles

[Concern is needle sticks causing a localized immune response in the case of DNA or cells, or the possibility of an infection in the case of lentivirus.]

Commonalities

Implanting the Injected embryos

- Use of pulled glass transfer pipettes for implantation of embryos

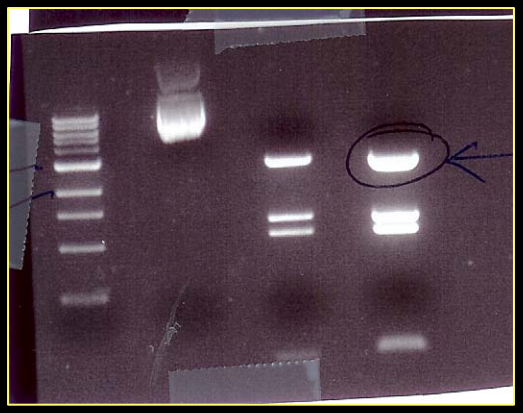
- Anesthesia of the animals
  - injectable anesthesia
  - inhaled anesthesia

[Concern is breakage of the glass pipette, possible needle sticks with injectable anesthesia and having proper scavenging systems with inhaled anesthesia.]

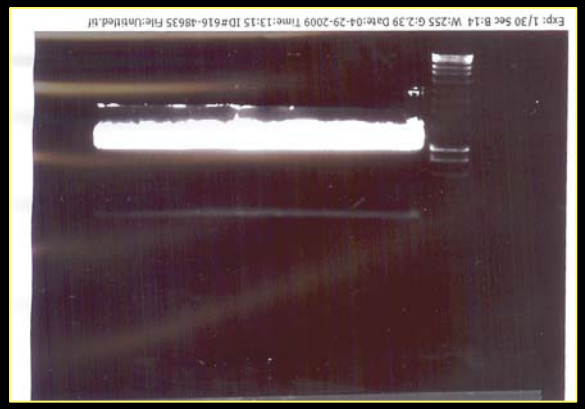
Differences

DNA preparation for pronuclear injection:

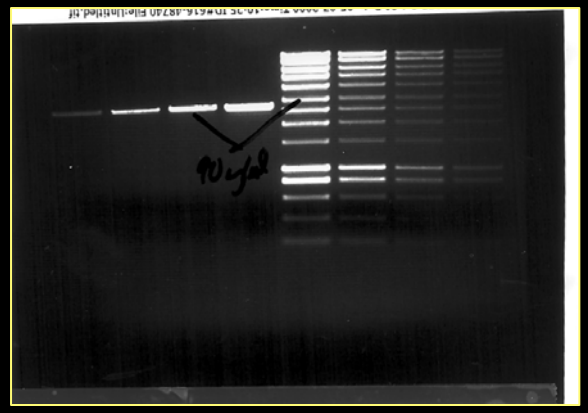
Uses rDNA, but in a non-hazardous state.



DNA digest from PI



DNA prep gel



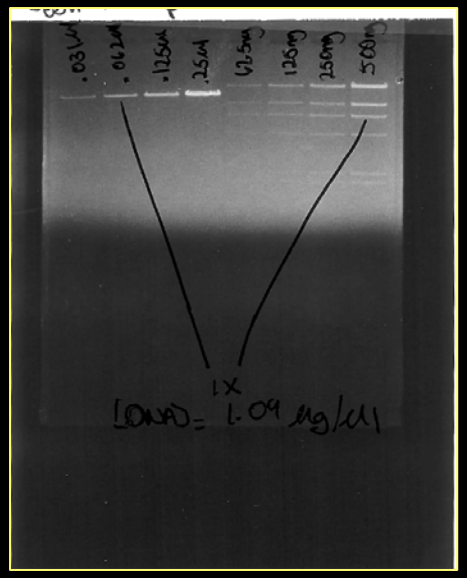
DNA Quantitation

The DNA is prepared for injection as a linear fragment (in most cases).
Neither bacterial DNA nor the plasmid backbone are maintained.

Differences

DNA preparation for gene targeting:

Uses linearized rDNA.



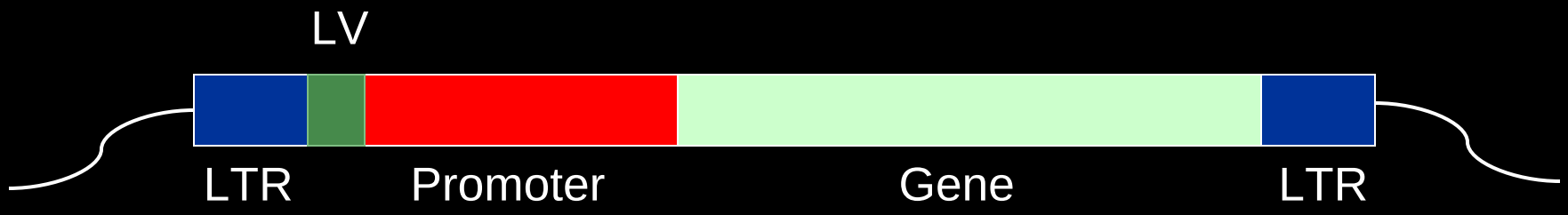
DNA Quantitation

The DNA is prepared for injection as a linear fragment (in most cases).
Bacterial DNA is not present. The plasmid backbone is present, but linear.

Differences

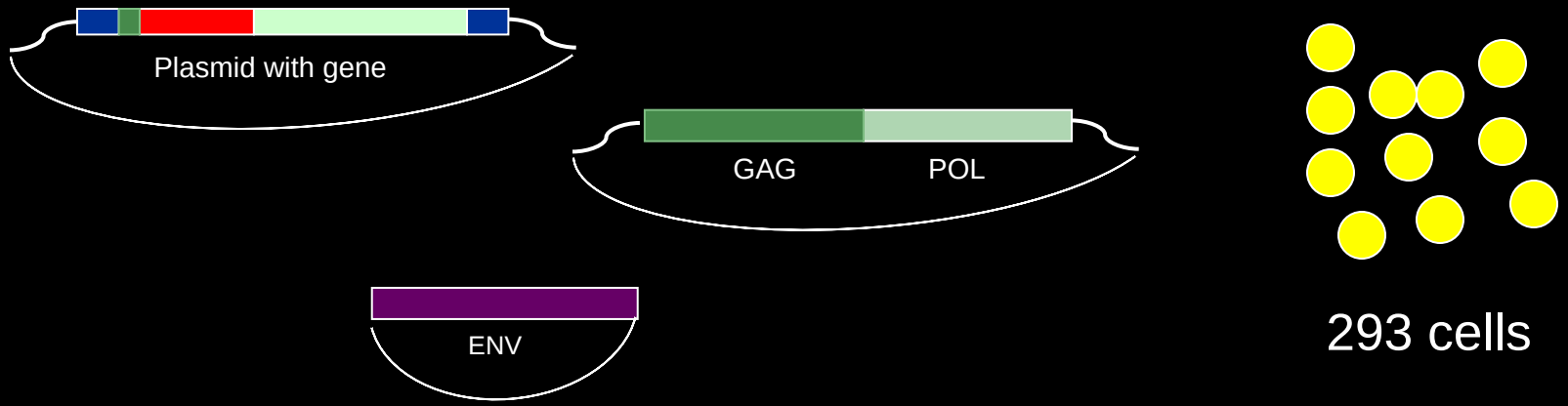
Preparation of the Lentivirus:

The DNA is prepared in the lab by putting a promoter-gene cassette into a lentiviral vector flanked by LTR sequences. This is grown for storage in a plasmid vector in bacterial cells.



Differences

The plasmid is transfected into 293 cells (HEK) along with the VSVg plasmid (to provide the envelope protein) and a plasmid containing the *gag and pol* genes. This allows for viral growth.



Differences

The viral supernatent is collected and centrifuged to remove cellular debris.

The virus is titered by determining infection of 293 cells in response to viral dilutions. (Titer should be 0.5-5 x 10^6 pfu/µl)

[Due to the possibility of viral reconstitution by recombination, BL2 conditions should be used at all times when handling lentiviruses. The IBC should be notified prior to use of these viruses.]

Differences

Injection of the Lentivirus:

To minimize the possibility of aerosols, the virus solution should be kept under oil when out in the open (ie: when filling needles and on the injection slide).

Decontaminate the injection slide carefully or dispose of it in a biohazard container after use.

[Due to the possibility of viral reconstitution by recombination, BL2 conditions should be used at all times when handling lentiviruses. The IBC should be notified prior to use of these viruses.]

Pronuclear and Blastocyst Injections:

BL-1 Containment Procedures

Resulting animals considered non-hazardous

Standard housing conditions are used.

PPE Requirements set based upon the desired microbiological status of the resulting mice and/or for allergen containment.

Differences

Animals made using lentiviruses should be treated as possible biohazards due to the possibility of viral recombination.

[Due to the possibility of viral reconstitution and subsequent shedding by the animals, BL2 conditions should be used at all times when handling lentiviral transgenic animals. The IBC should be notified prior to use of these viruses.]

Lentiviral Precautions

References

For Pronuclear Injections and Blastocyst Injections

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