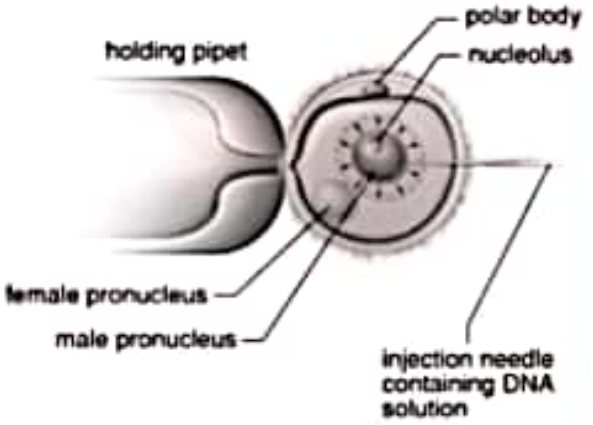
PRODUCTION OF TRANSGENIC ANIMALS – THE METHADOLOGY

- **Step 1 – Construction of a transgene**
  - Transgene made of 3 parts:
    - Pomoter
    - Gene to be expressed
    - Termination sequence

- **Step 2 – Introduction of foreign gene into the animal**
  - Pronuclear microinjection method
  - Embryonic stem cell method.

MICROINJECTION METHOD

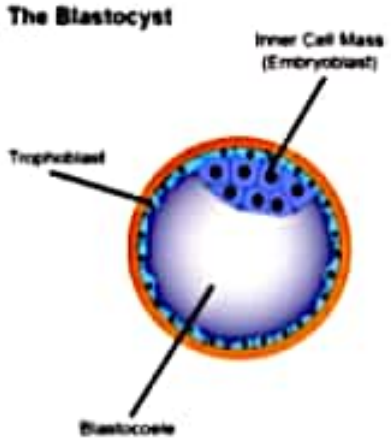
- A female animal is superovulated and eggs are collected.
- The eggs are fertilized in vitro.
- The transgene containing solution is injected into the male pronucleus using a micropipette.



- Eggs with the transgenes are kept overnight in an incubator to develop to a 2 cell stage.
- The eggs are then implanted into the uterus of a pseudo - pregnant female (female which has been mated with a vasectimized male the previous night)

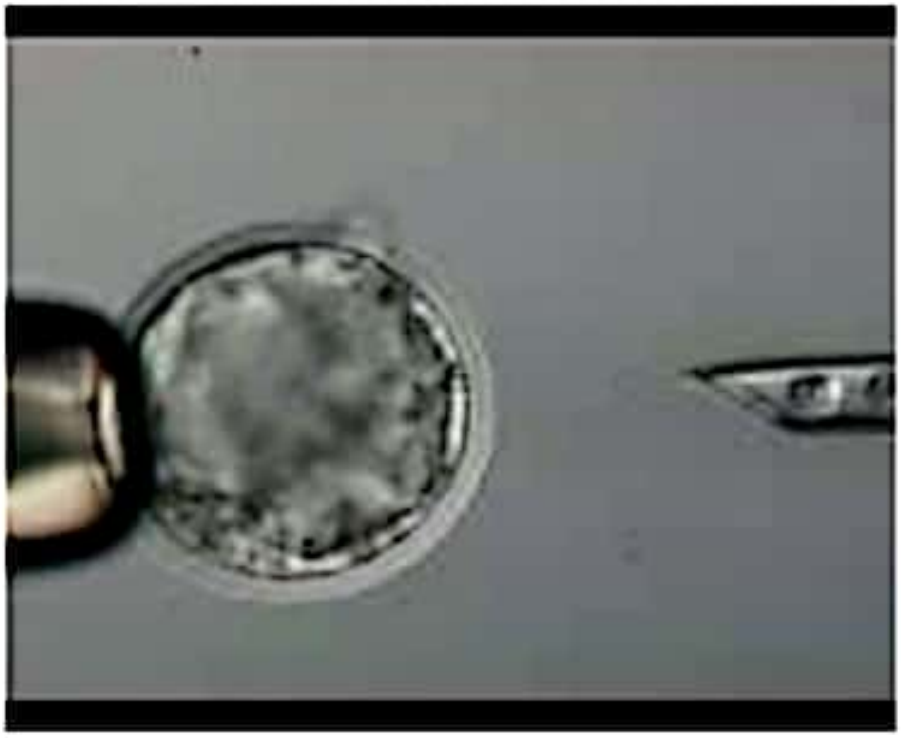
EMBRYONIC STEM CELL METHOD

- Transgenic animals can be created by manipulating embryonic stem cells.
- ES cells are obtained from the inner cell mass of a blastocyst.
- Transgene is incorporated into the ES cell by
  - Microinjection
  - By a retro virus
  - By electroporation
- Transgenic stem cells are grown in vitro.

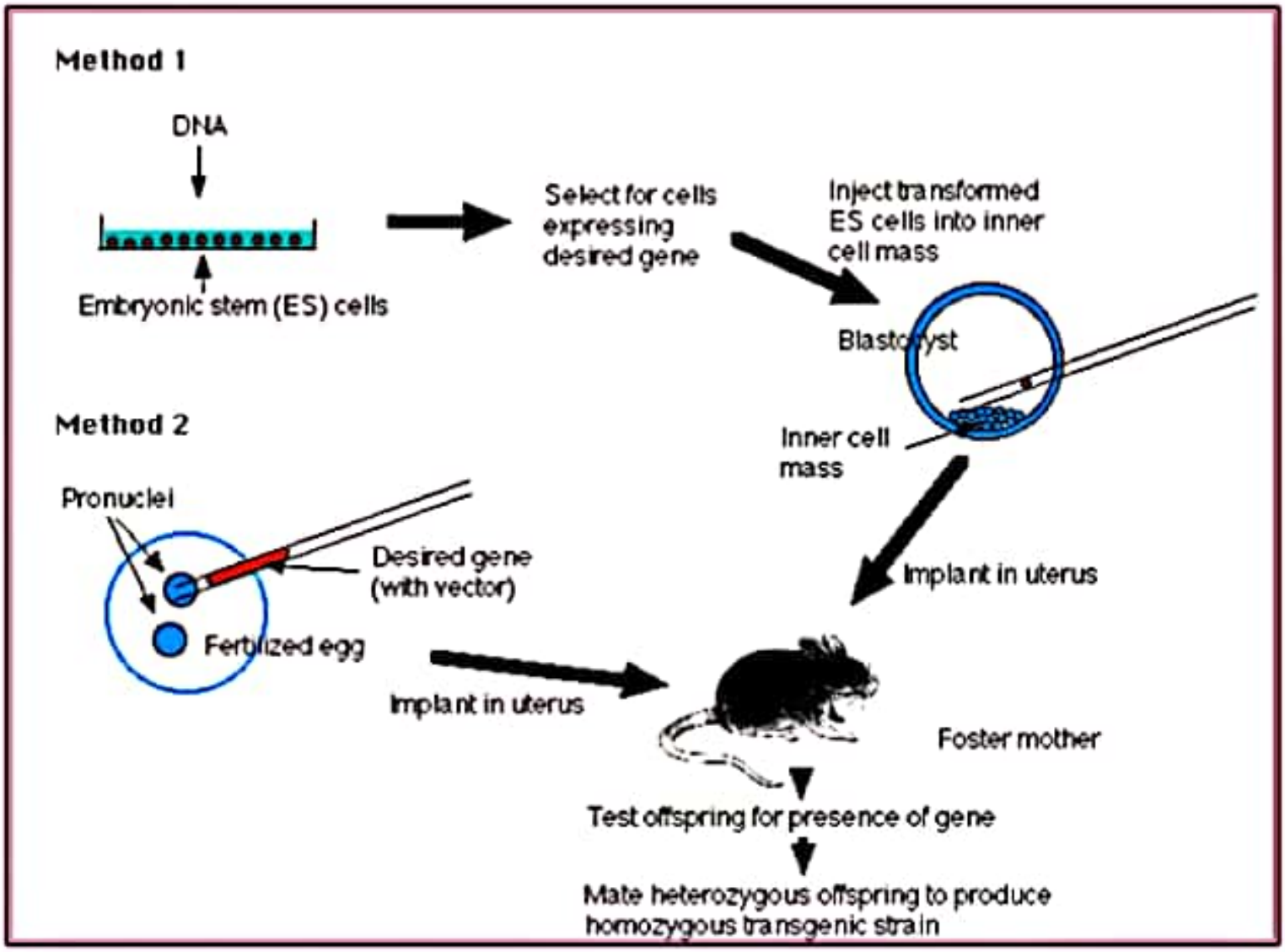


- Then they are inserted into a blastocyst and implanted into a host's uterus to grow normally.

BLASTOCYST MICROINJECTION



- **Step 3: Screening for transgenic positives**
  - Transgenic progenies are screened by PCR to examine the site of incorporation of the gene
  - Some transgenes may not be expressed if integrated into a transcriptionally inactive site.
- **Step 4: Further animal breeding is done to obtain maximal expression.**
  - Heterozygous offsprings are mated to form homozygous strains.



PROBLEMS

- Multiple insertion – too much proteins
- Insertion into an essential gene – lethality
- Insertion into a gene leading to gene silencing
- Insertion into a different area can affect the gene regulation