



Cloning Livestock – progress and problems

Ian Lewis, MVSc, PhD

Monash Institute of Medical Research, Monash University

Pacific Biotechnologies Pty Ltd



OUTLINE OF TALK

- **Reasons for cloning livestock**
 - **Hurdles to overcome**
 - **Underlying causes of abnormalities commonly associated with cloning**
 - **An Australian story**



Reasons for cloning livestock

- **improving productivity** – for the farmer and the economy
- **improving the product - consumer**
 - **GM – producing human medicines and vaccines in milk, increasing disease resistance in animals**
 - human and animal health
 - **research into basic cell biology**
 - what makes cells tick

(b) Help to keep farmers efficient



(a) Improved product for consumer



•Consumer interest

The public and consumers decide



- **Public understanding of the pros and cons**
- information

Integrated technology platform for animal production

- **dairy**
- **beef**
- **sheep**

Identify genes of economic importance in production animals



Utilize genetic knowledge to improve products and profitability on Australian farms



non-genetic means

- **management**
- **feeding,**
- **milking practices**



“traditional” breeding methods

- **artificial insemination,**
- **embryo transfer**
- **+/- MAS/GAS**



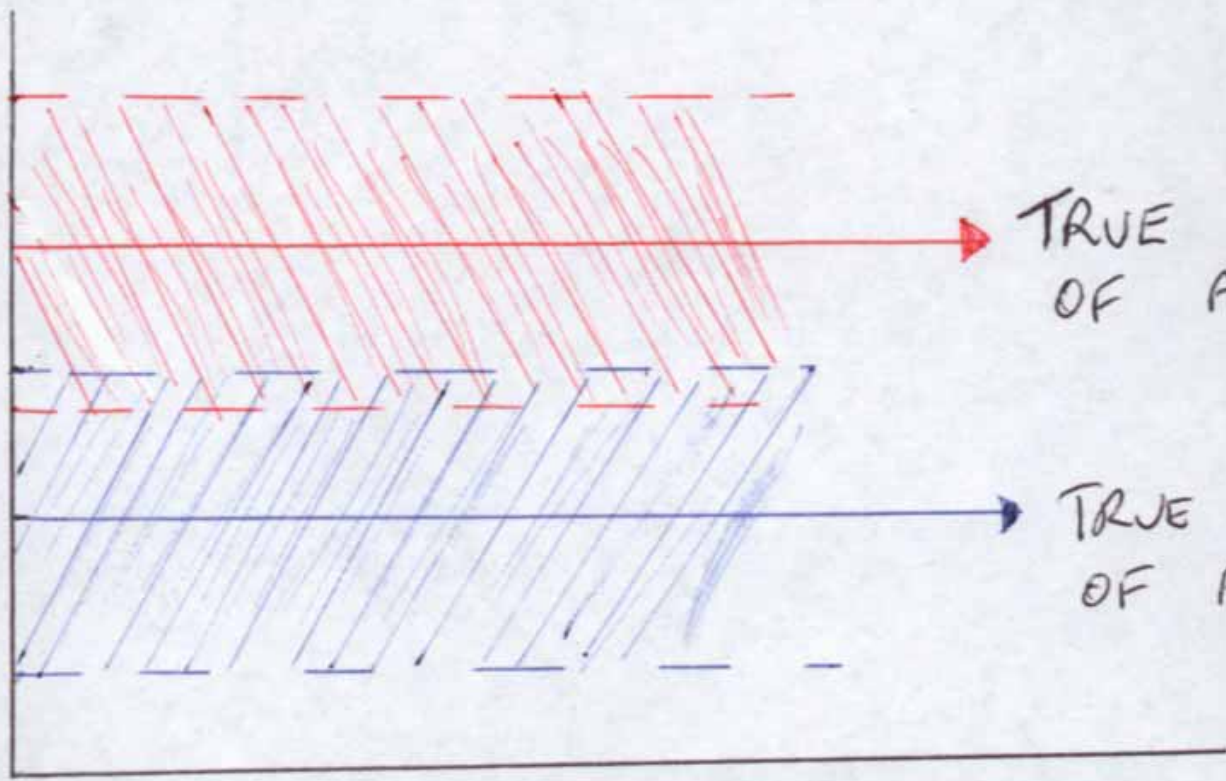
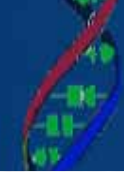
more efficient methods to increase frequency of economically important genes?

- **cloning, +/- GM**
- **+/- MAS/GAS**

- **lactation**
- **meat/wool traits**
- **new “ bioactives”**
- **feed conversion efficiency**
- **fertility**
- **mastitis**
- **disease/parasite resistance**
- **etc**

Why?

- **Successful commercial cloning would have significant (future) implications in agriculture:**
 - **Multiplying animals which are outstanding performers in a particular environment**
 - **dairy cows**
 - **beef and dairy bulls**
- **Many production traits are only 25% heritable or less.**



TRUE GENETIC MERIT
OF ANIMAL A.

TRUE GENETIC MERIT
OF ANIMAL B.





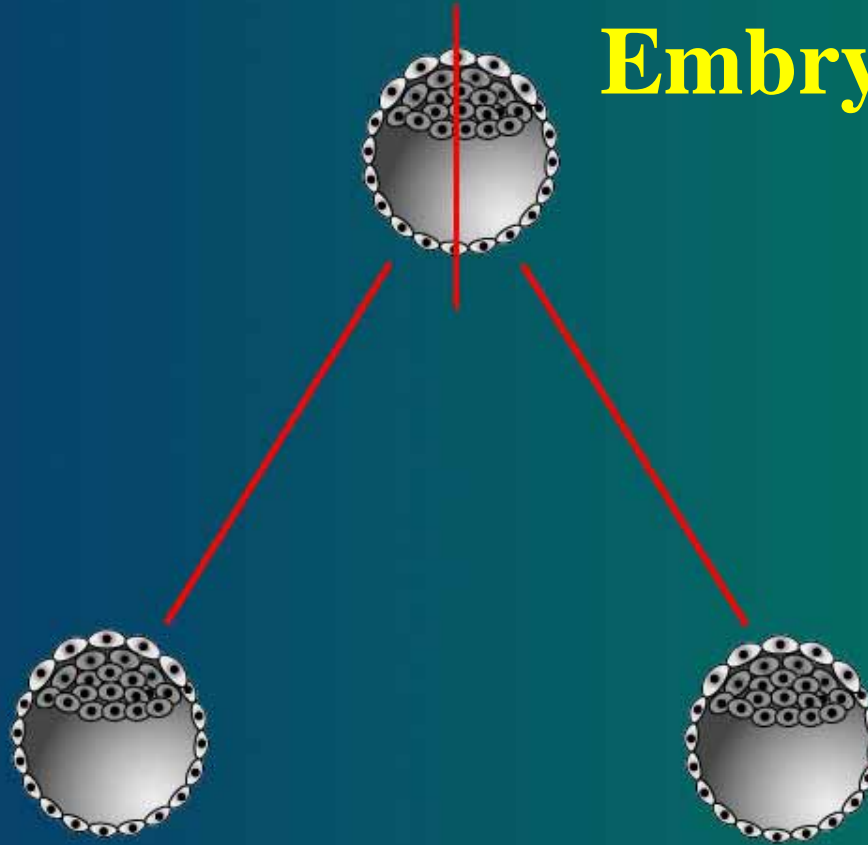
Cloning

– How?



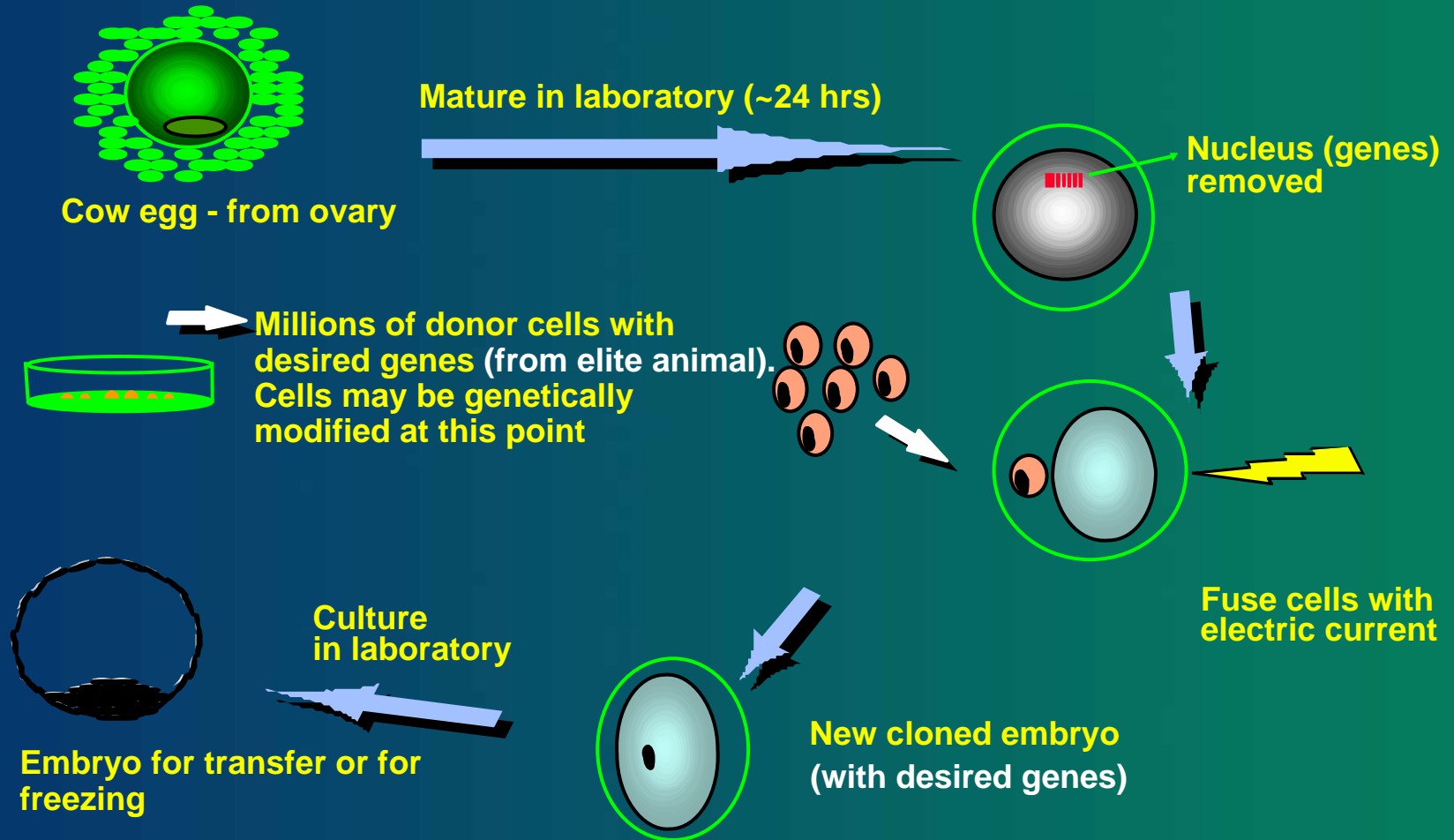
Cloning – how?

Embryo splitting

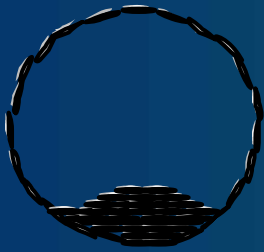


Cloning - how?

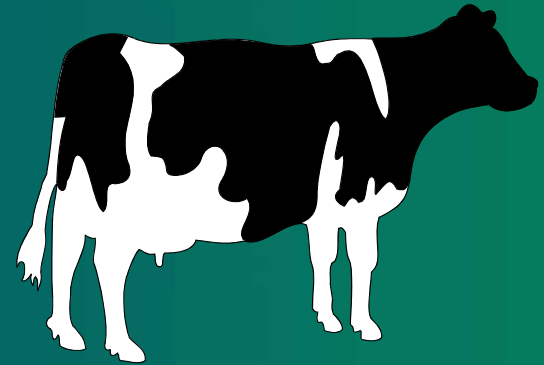
Nuclear transfer



How?

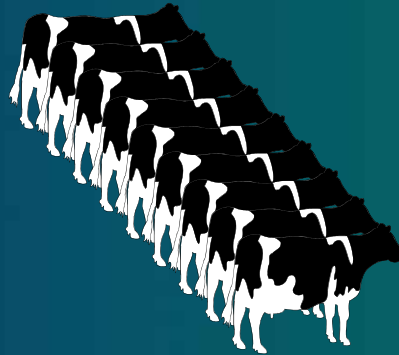


Transfer to surrogate cows



Cloned embryos for:

- immediate transfer or
- freezing for later transfer



Offspring in 9 months

Offspring may be genetically modified

- secrete valuable human proteins in their milk
- have increased disease resistance
- have increased nutraceutical value
eg. extra protein in the milk, improved carcass characteristics

Offspring may be:

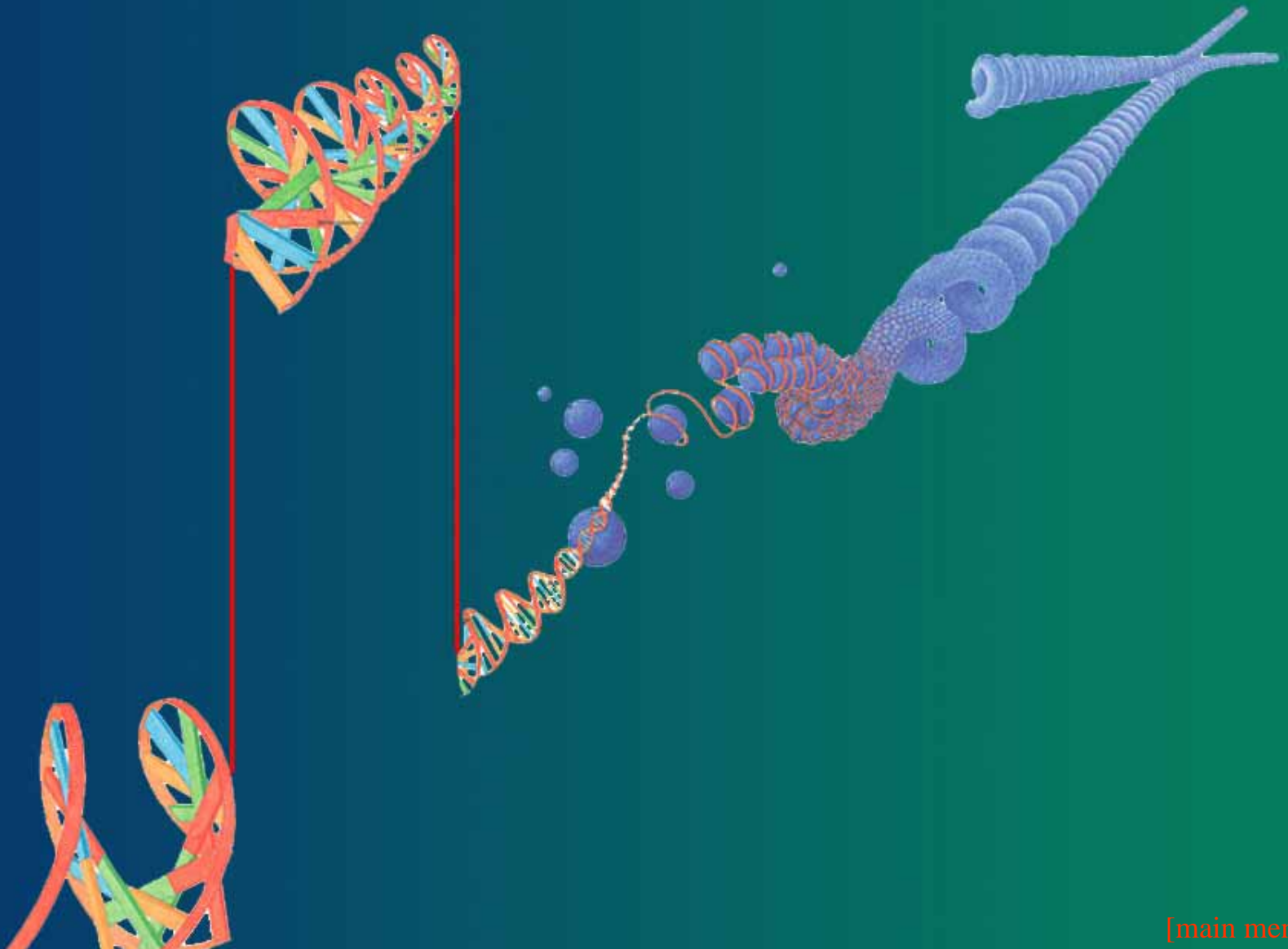
**genetically identical to a proven elite animal
*eg. proven elite bull or cow***



GM (genetic modification/trangensis)

- **Traditionally by pronuclear microinjection**
- **Now routinely done using cloning technology**

Transgenics (GMOs)

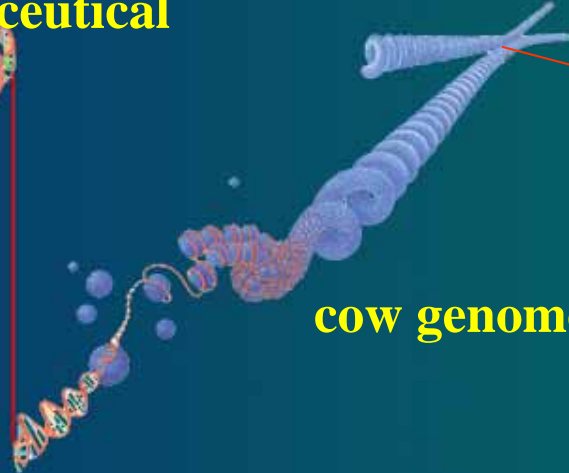


Cloning as a platform for genetic modification

gene for producing human pharmaceutical



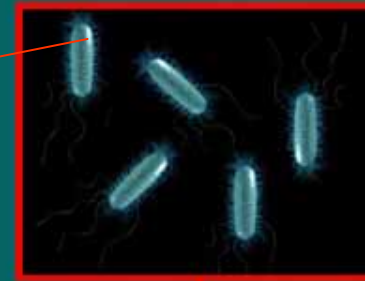
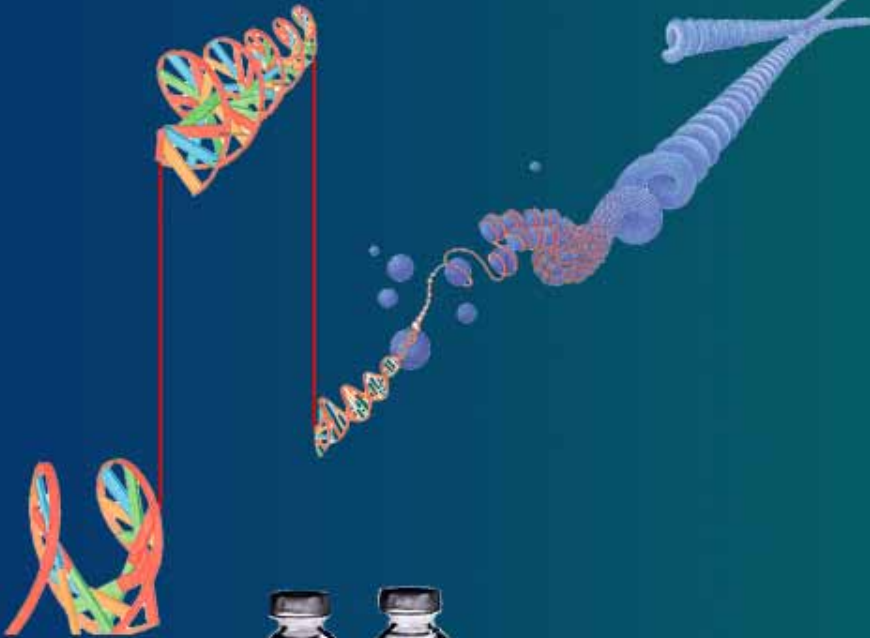
cow genome



human medicine extracted from milk



insulin gene



bacteria
(E.coli)



Insulin for diabetics (now)





Current efficiencies with cloning:

- Since the arrival of Dolly, thousands of apparently healthy and fertile cloned animals derived from somatic cells have been born worldwide.
- **However, the overall efficiency of the technology remains disappointingly low with less than 1% of cloned embryos developing into live, healthy offspring.**

(certain cell lines can give much better outcomes than the average)



Some abnormalities observed from cloning:

- decreased implantation rates
- higher foetal loss to term following implantation;
 - placental abnormalities;
 - hydrallantois;
 - large offspring syndrome (LOS);
 - increased peri-natal morbidity and mortality (bacterial infection, lack of post-natal vigour and lymphoid hypoplasia).



Cloning

- Recently, much of the interest and investment in cloning has waned for a number of reasons.
- **There are significant impediments that must be overcome to realize the potential opportunities that the technology offers.**

*1. Public perception*****

- societal concerns surrounding cloning technologies, animal welfare issues and regulatory requirements

2. Biological - less than 1% of cloned embryos give rise to healthy offspring - unacceptably high percentage of abnormal offspring born.

3. Technical - low efficiency of the technology



1. Societal issues and public perception

- **Demonstrate products from cloned animals are safe to eat**
 - **Japanese study (2004)**
 - **FDA/USDA ruling (due 2006)**
- **Inform public about technologies**
 - **free choice**
- **Solve some of the biological problems**
 - **healthy animals**

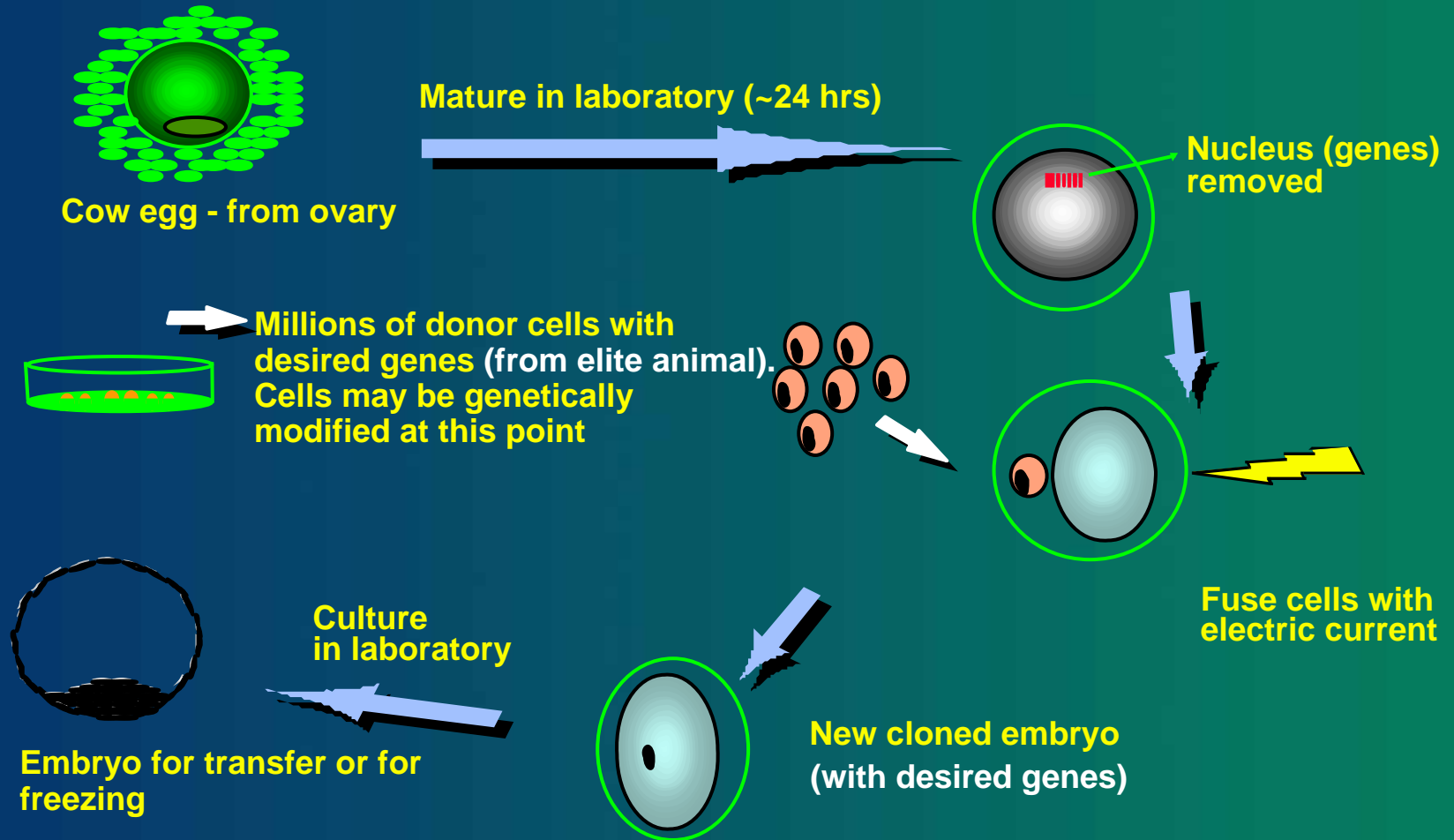




2. Biological problems

Cloning - how?

Nuclear transfer





SEGMENT OF DNA (GENE)



~~transcription~~

Transcription blocked
= gene silenced

RNA



translation

- by **transcription factors**
(genetic mechanisms)

PROTEIN

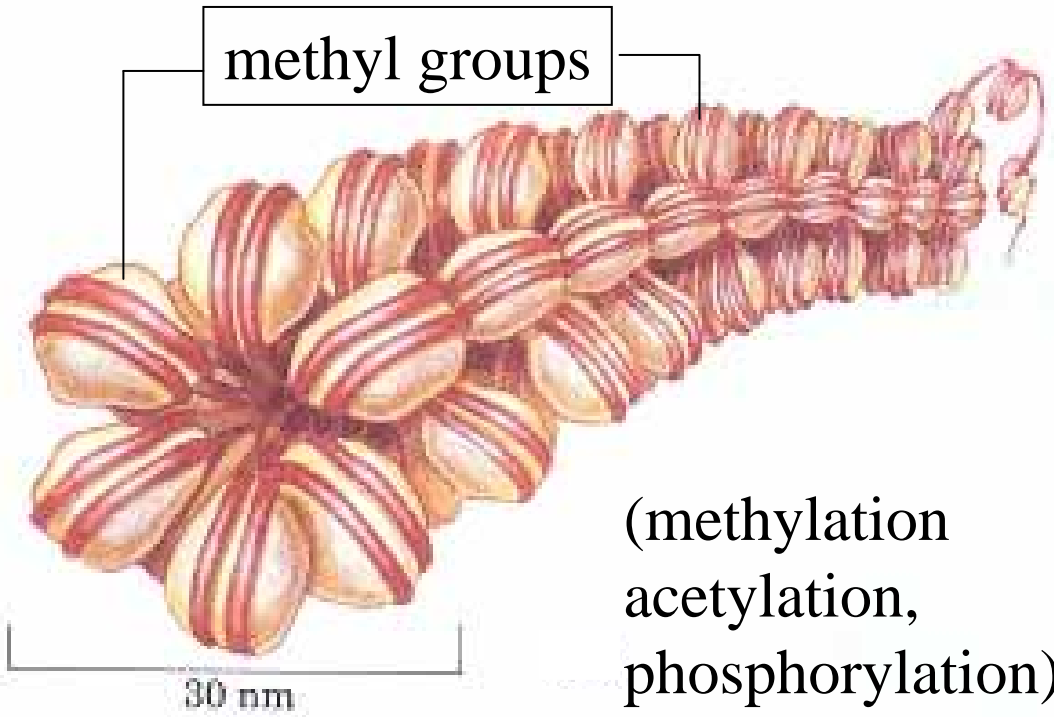
- by epigenetic mechanisms



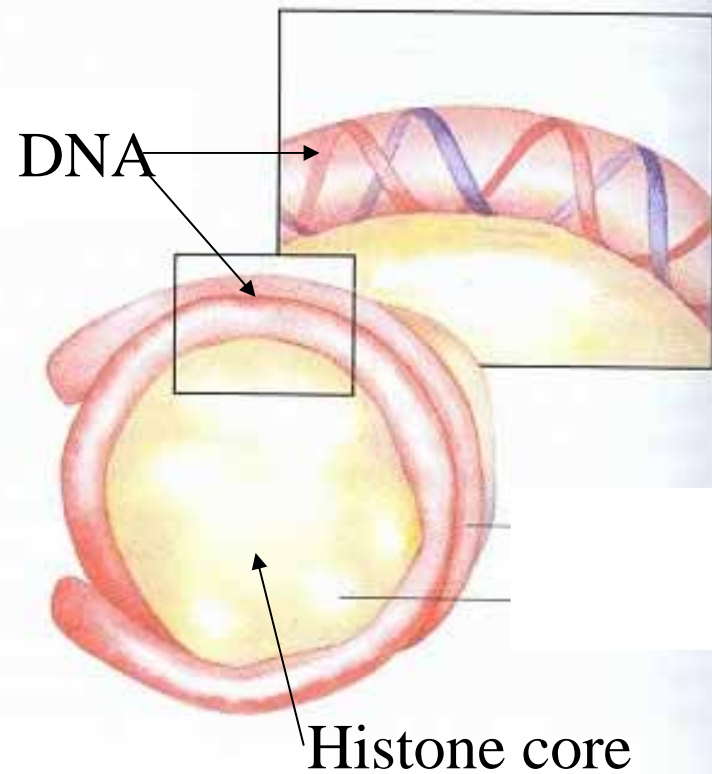
EPIGENETICS

(epi: outside or above)

- the study of **HERITABLE** (*inherited*) changes in gene function, that cannot be explained by changes in DNA structure (sequence)



(methylation
acetylation,
phosphorylation)



DNA: The code for life

cell

chromosome



*Faulty epigenetic reprogramming
responsible for most problems
seen with cloning*

~~A C
T G~~



3. Technical problems

Traditional cloning methods are technically difficult and time consuming



AN AUSTRALIAN STORY



**MONASH INSTITUTE OF REPRODUCTION
AND DEVELOPMENT**

Prof Alan Trounson
Prof Michael Holland
Andrew French
Ian Lewis
Gabor Vajta
Orly Lacham Kaplan
Teija Peura
Rob Daniels
Vanessa Hall
David Pushett
Natasha Korfiatis
Melissa Cooney
Katrina Wilson
Maria Diamente
Natalie Alexopoulos
Mark Lane

**MEAT AND LIVESTOCK
AUSTRALIA**
Dr Len Stevens
(Man.)

DAIRY CRC

Dr Paul Donnelly CEO
Dan Minchin
Michael Holland
Susan Cumming
Anne Salt

**DAIRY RESEARCH AND
DEVELOPMENT CORPORATION
(NOW DAIRY AUSTRALIA)**

Dr Joe Sullivan CEO
Tom Davison
David Conley
John Craven
Rob Morton
David Nation

GENETICS AUSTRALIA CO-OPERATIVE

Mr Bernie Harford CEO
Ian Lewis
Sandy McClintock
Renee Hodgson
Shara Downie
David Nation
Lydia Acciarito
Malinda Egan
Alison Cassar
John Owens
Pat Toohey
Bill Shanahan
Michael Ryan
Peter Coleman
Andrew Harford

**VICTORIAN INSTITUTE
OF ANIMAL SCIENCE**

Dr Chris Langford (Dir.)
Kurt Zuelke
Kevin Nicholas
Sonia Mailer
Rachel O'Dowd

Simplifying the technical aspects of cloning

Traditional cloning methods are:

- **complicated**
- **time-consuming**
- **require expensive equipment**
- **require highly skilled labour**





■ **HAND MADE CLONING**

- simple
- quick
- inexpensive equipment
- no highly skilled labour

Produce equivalent results to traditional methods

“HAND MADE CLONING” PATENT

Lewis IM, Vajta G, Tercirlioglu T (2001)

International Patent. A method of nuclear transfer. PCT/4AU02/00491.





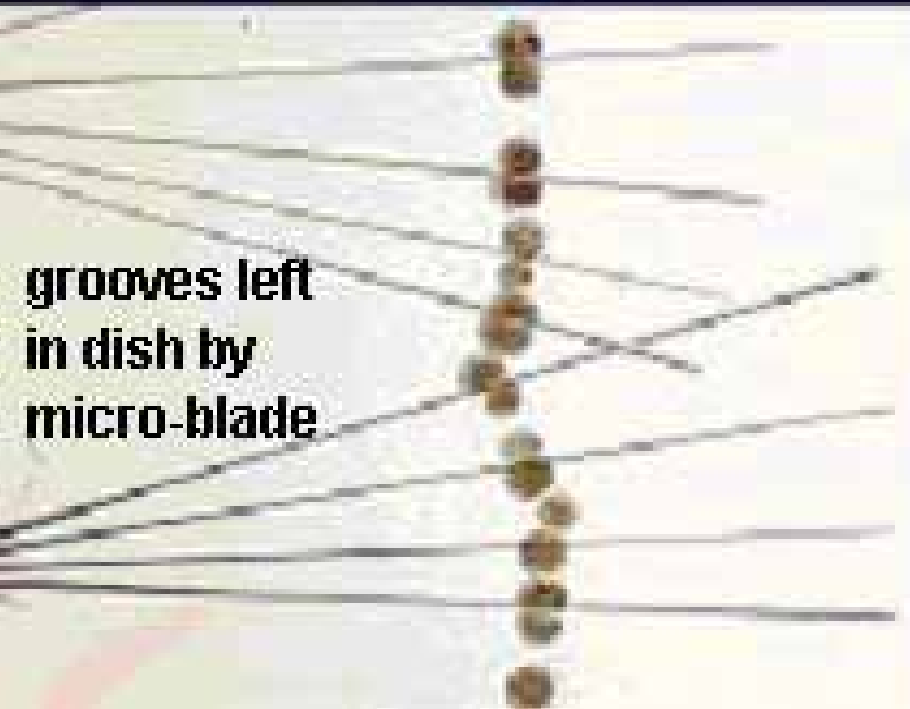
zona-free oocytes



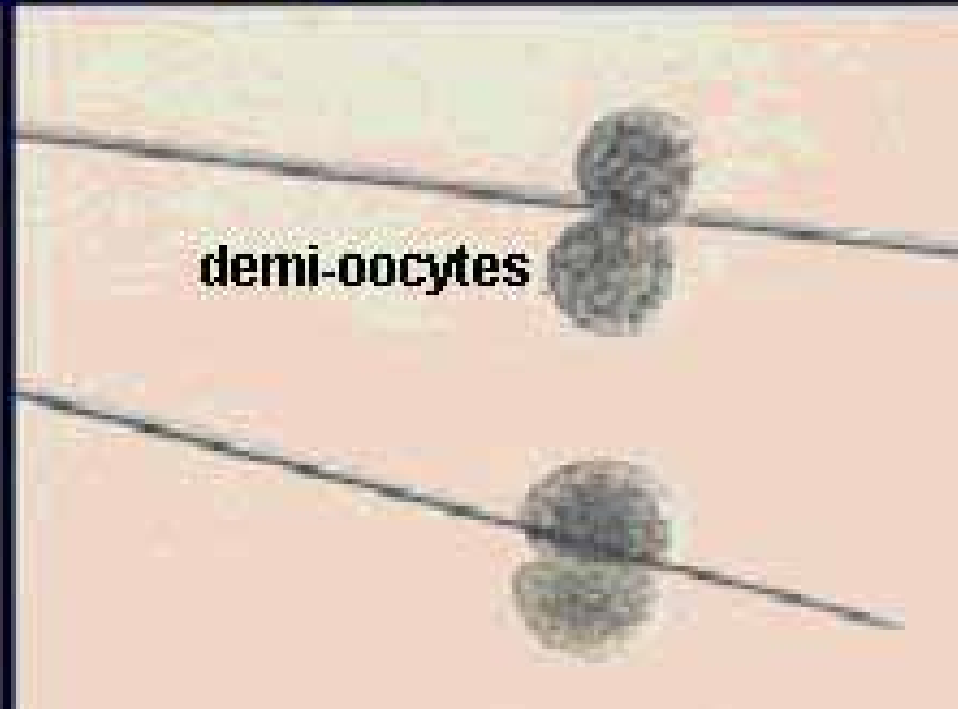
micro-blade



**grooves left
in dish by
micro-blade**



demi-oocytes

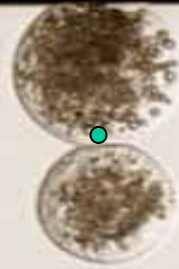


demi-cytoplasm “dipped” in PHA

somatic cells



electrofusion wire



cytoplasm-somatic cell-cytoplasm triplets

electrofusion wire



5 minutes post-fusion



10 minutes post-fusion



The 4 years of clone line testing

Find the best
cows
anywhere in
the world
(including
crossbreds)

Produce
cloned
embryos
(including
crossbreds)

Distribute
cloned
embryos.
(200 per line
and 100
lines)

Year 0

Year 0

Year 1

Calves are
born

Pick the lines
(including
crossbreds)
which make the
best cloned
females for
extensive use

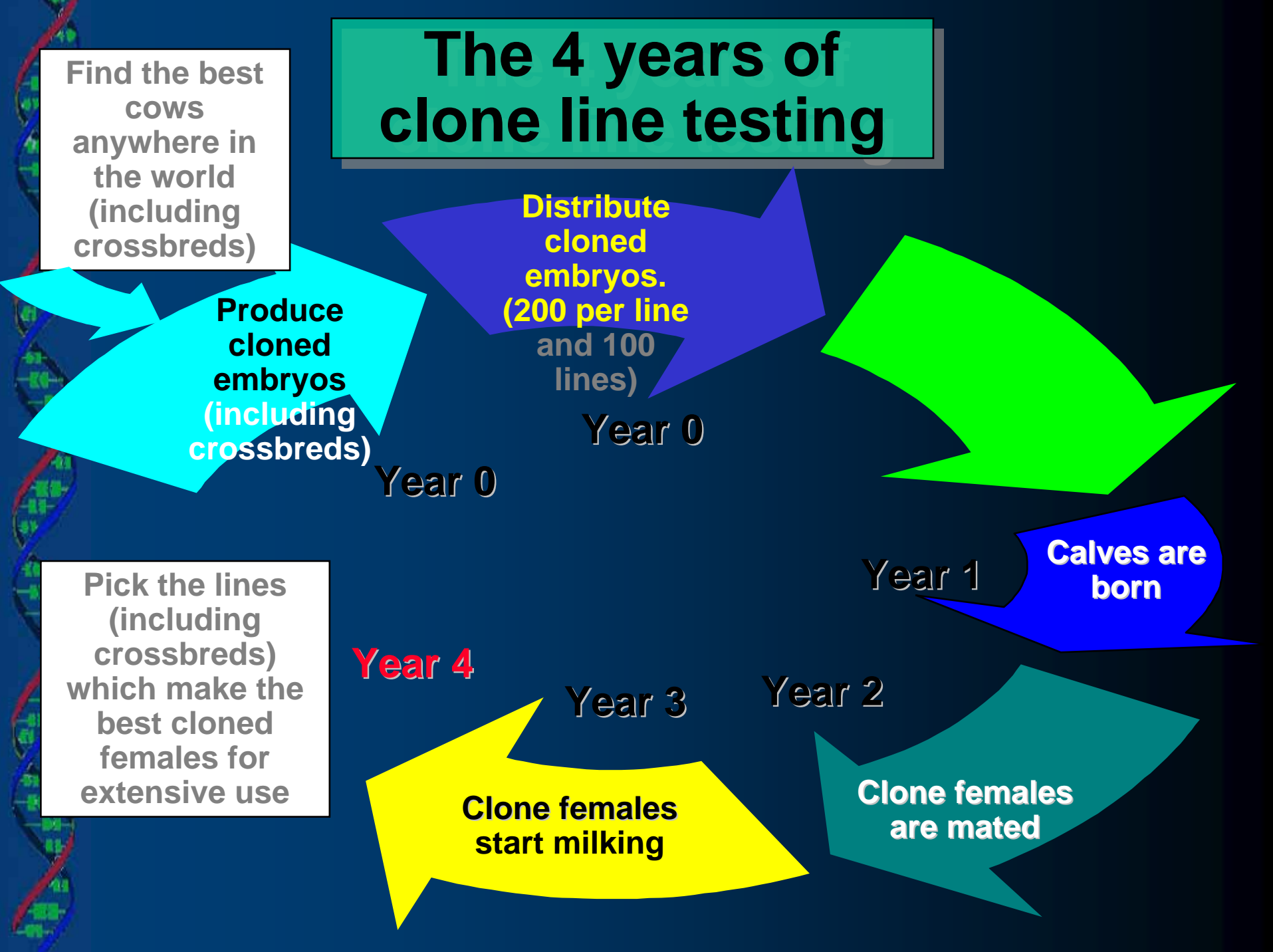
Year 4

Year 3

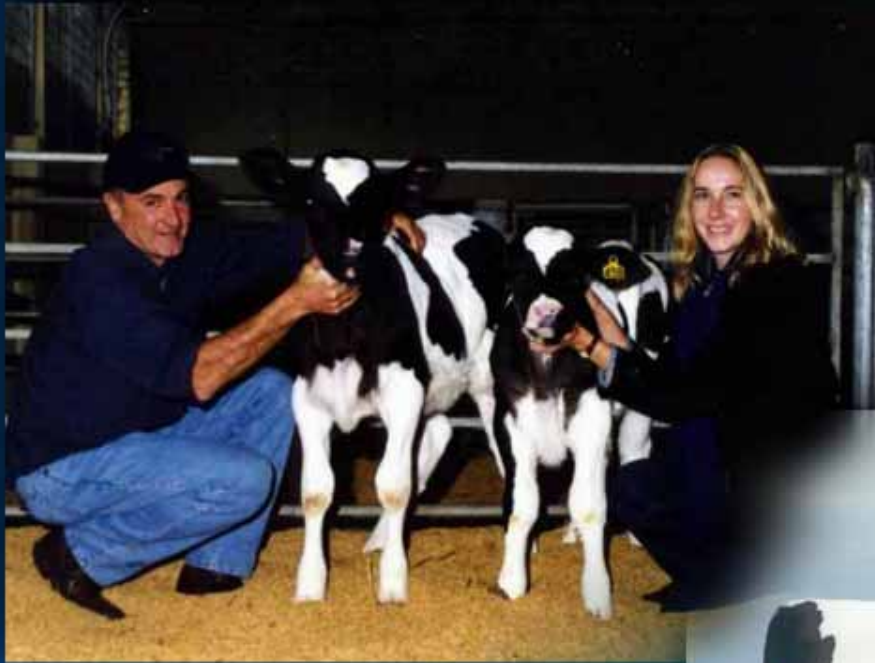
Year 2

Clone females
start milking

Clone females
are mated



Suzi and Mayzi:
Australia's first clones





Rameses



Daughter of
Rameses



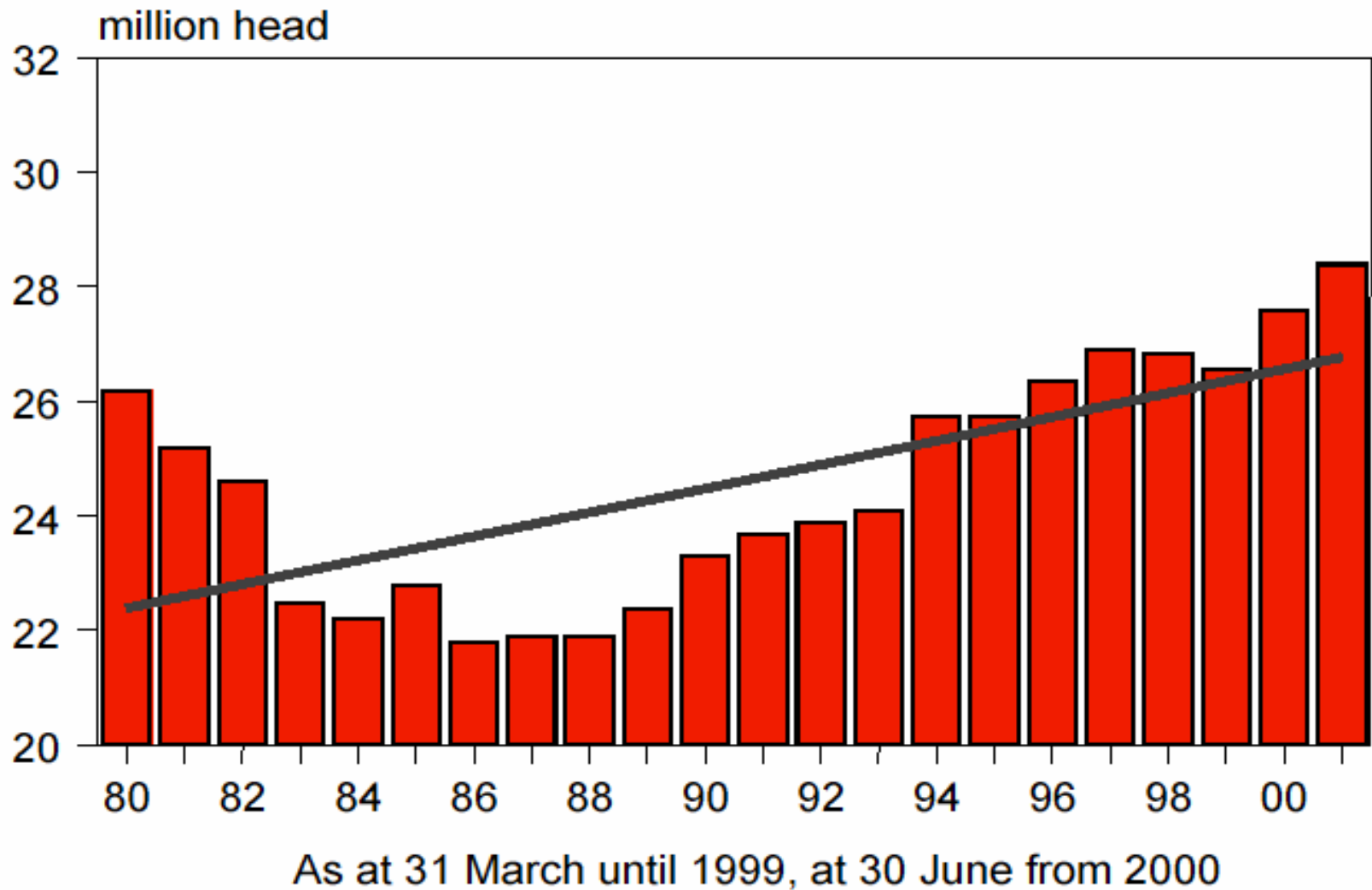
Rameses II



“Extra protein” (GM) cloned calves



Australian cattle herd



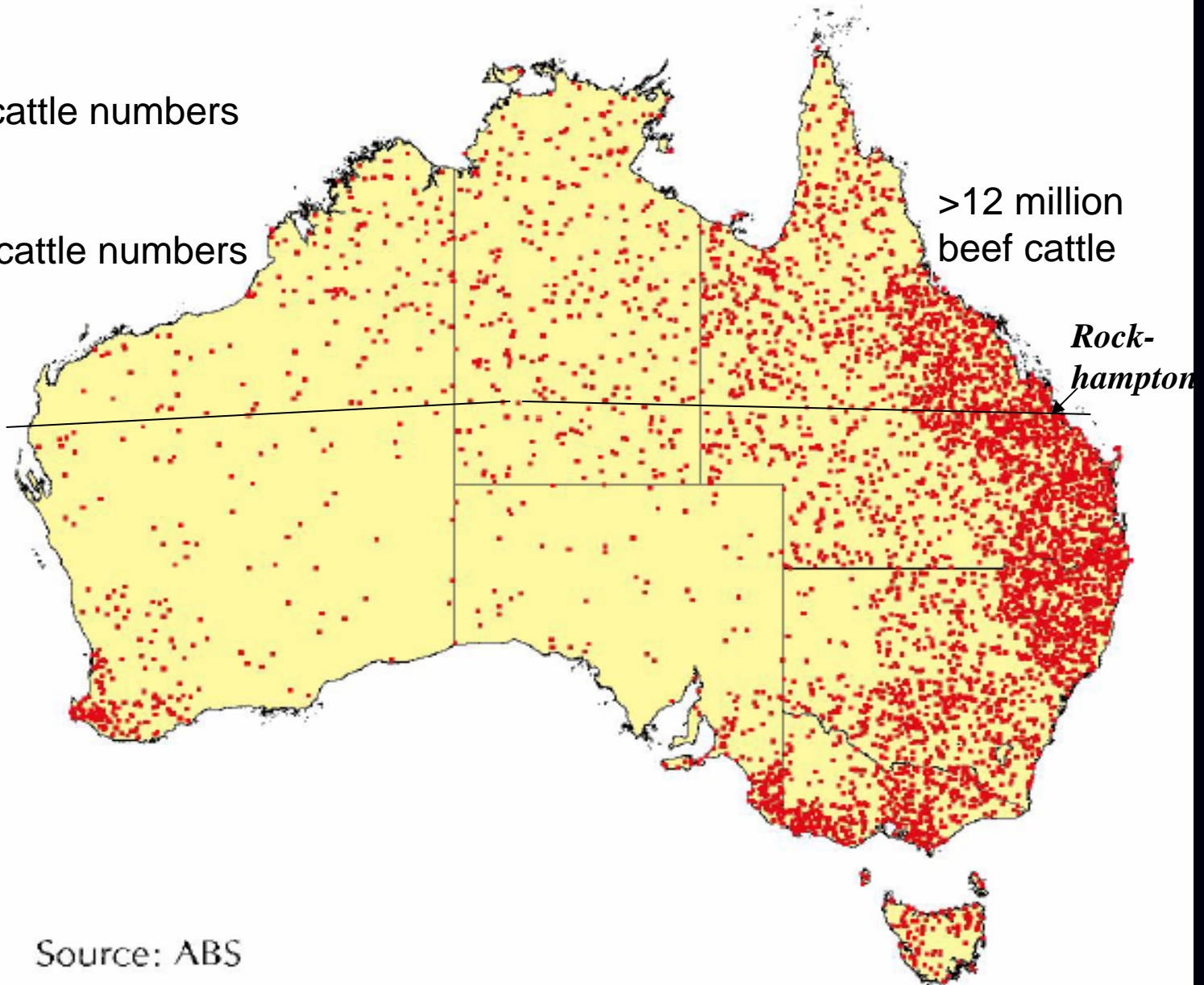
Source: MLA/ABS

Australian Cattle Distribution

Total beef cattle numbers
>25 million

Total dairy cattle numbers
>2 million

**Tropic of
Capricorn**



Source: ABS

Potential use of cloned bulls at current efficiencies

Limited use of cloned bulls here?
(already use AI)

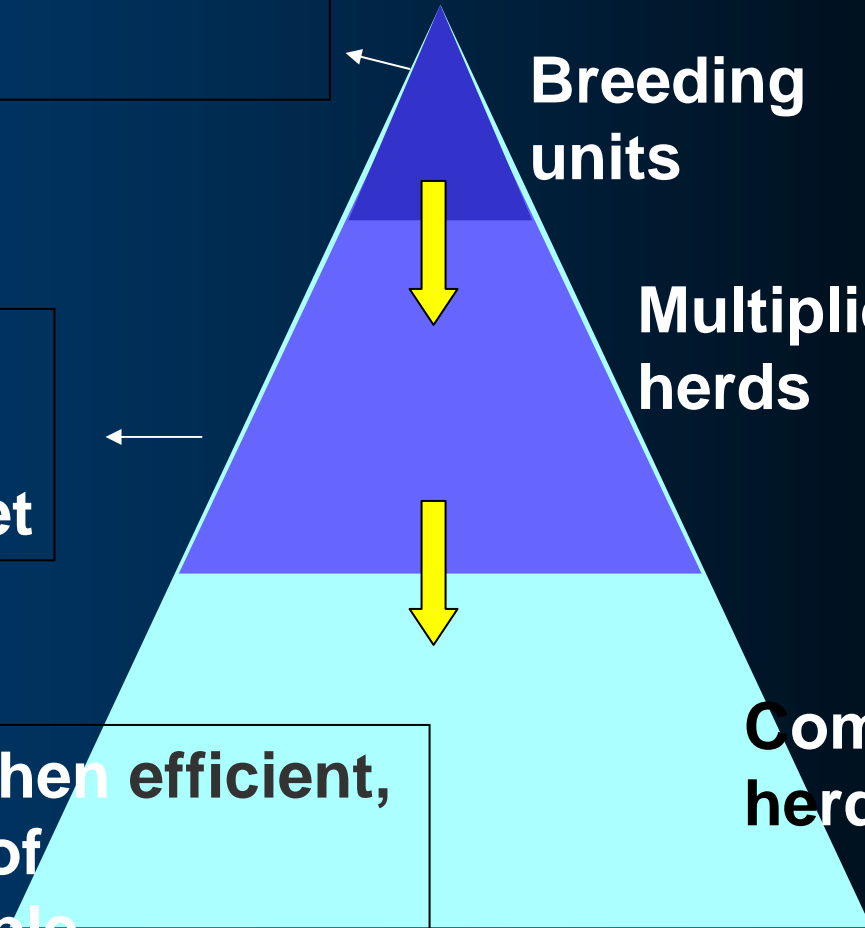
Breeding
units


Good potential here?
- cost effective and
relatively large market


Multiplier
herds

Potential here only when efficient,
low cost production of
cloned bulls is possible

Commercial
herds



- 
- It has been established by our group and others that certain *cell lines* give much better outcomes when used for cloning than others
 - The ability to select those *cell lines* that produce the best outcomes for use in cloning significantly increases efficiencies and reduces costs



- **We have demonstrated that combining selection of particular cell lines that produce “good” cloning outcomes with**

- **the simplified “hand made cloning” technologies described, together with other novel procedures such as automated oocyte harvesting developed by the group over 12 years**

- **Healthy cloned animals can be generated for \$5,000 to \$10,000 each** (*taking all “failures” into account*)



- This is approaching the levels of efficiencies required for commercial cloning of bulls for the northern Australian beef herd

- *However, societal and animal welfare issues remain significant limitations*



THANKS FOR LISTENING

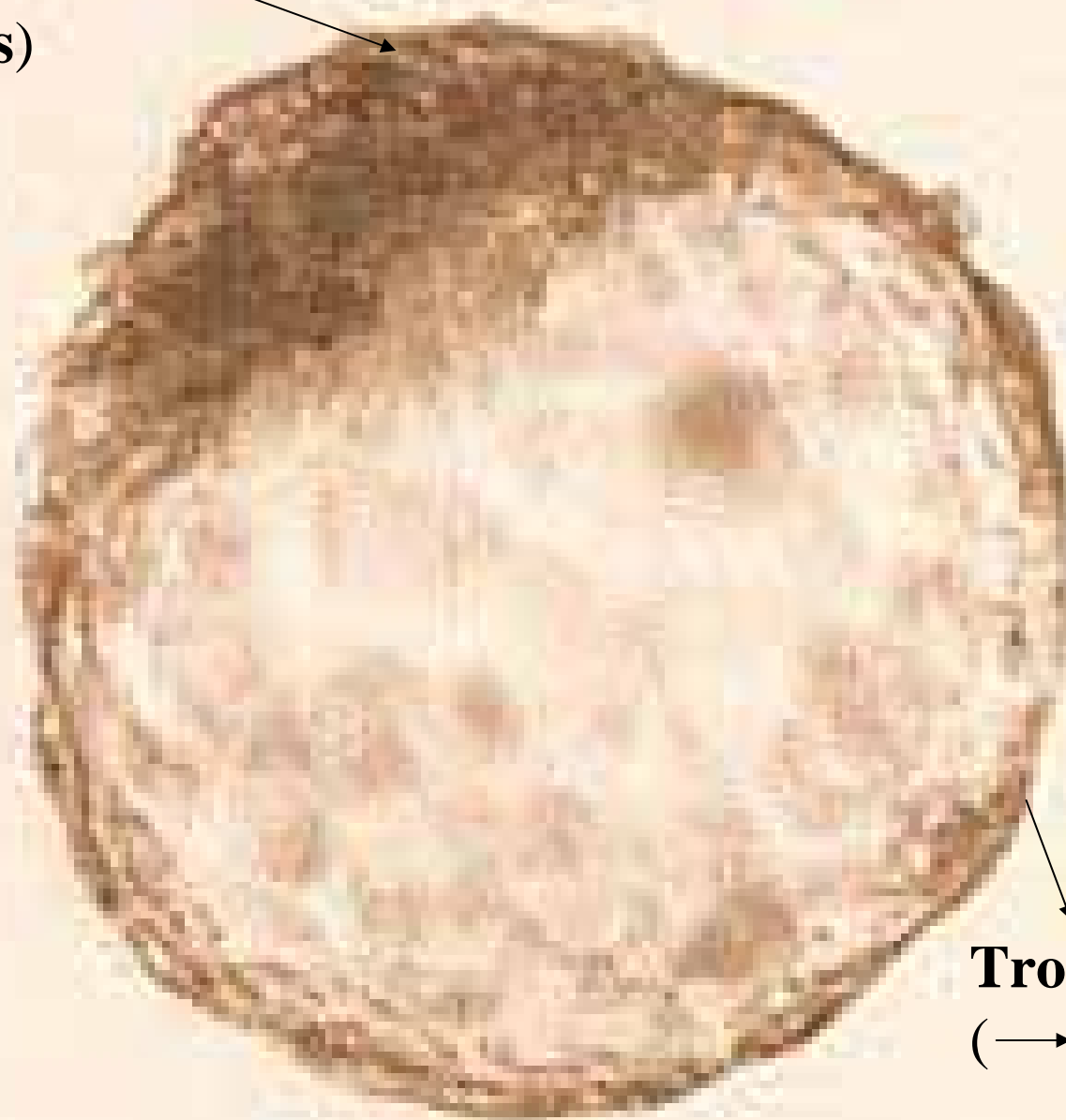




Therapeutic cloning

Inner cell mass cells

(→ foetus)

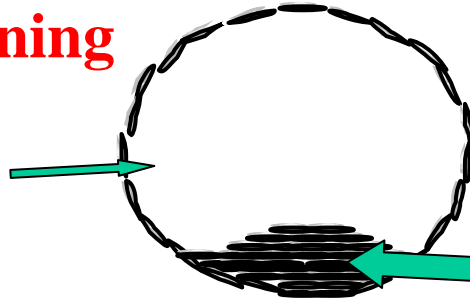


Trophoblast cells

(→ placenta)

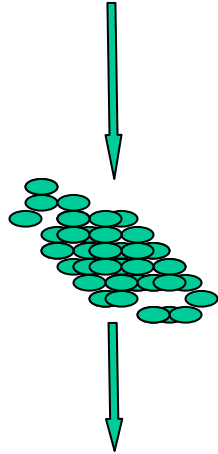
Therapeutic cloning

Trophectoderm
(placenta)



Inner cell mass cells (foetus)

ES cells
(totipotent)



- cultured under appropriate conditions can give rise to embryonic stem (ES) cells (mice and primates only)

Embryonic stem cells cultured under appropriate conditions can, theoretically, give rise to all cell and tissue types

Pancreatic cells

Mature nerve cells

Heart cells



Summary

- Advanced breeding technologies such as *IVF and cloning*, aim to improve on traditional animal breeding technologies (including AI) that have been used to successfully increase production efficiencies through genetic improvement.
- **There is still some way to go before these newer technologies are efficient enough to achieve these aims**
- Over the coming years, the practical use of technologies such as IVF and cloning will increase as there is increased understanding of:
 - the genome and how genes interact with each other and with the environment,
 - marker assisted selection (**MAS**) and gene assisted selection (**GAS**)

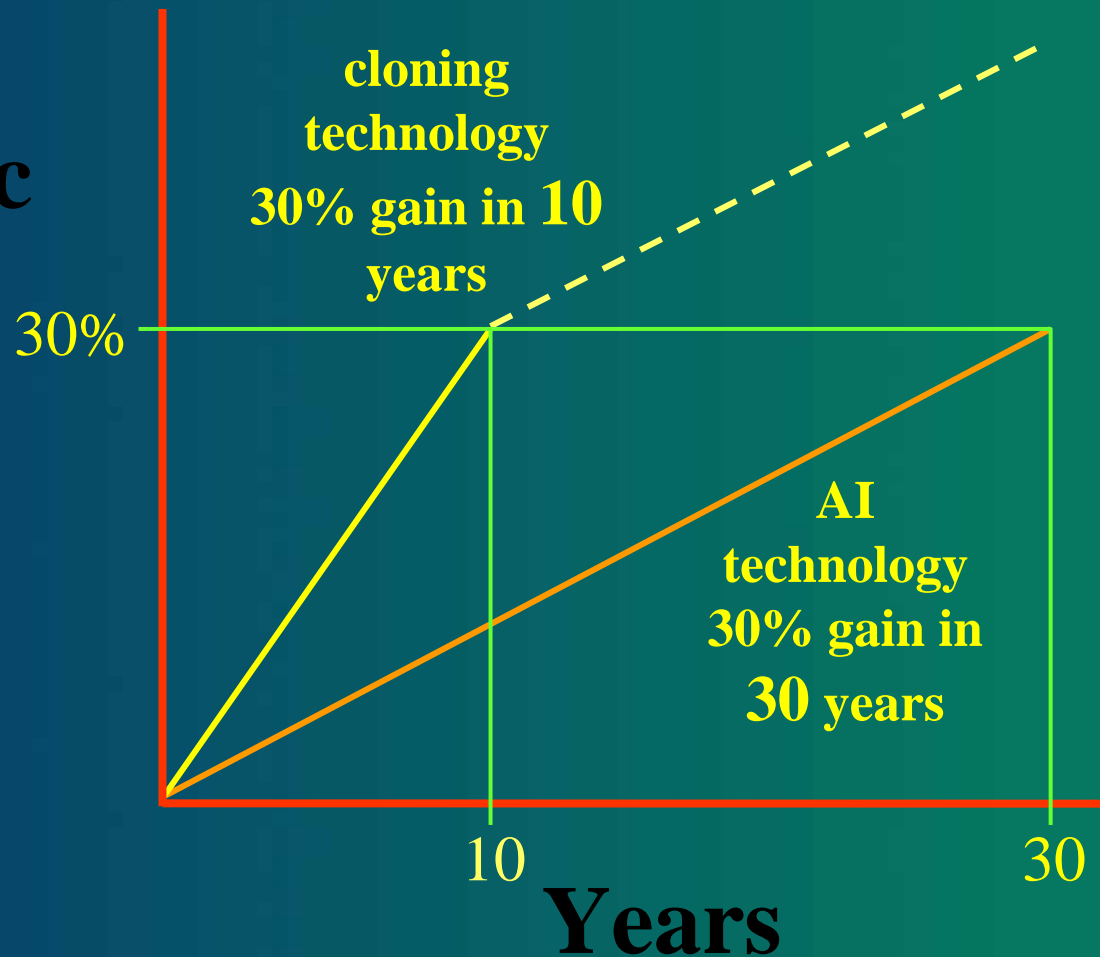
Why Clone Cattle?



- **Selective animal breeding**
 - animals that are more productive and healthy
 - improved production efficiency.
- **Natural mating.**
- **Artificial insemination/embryo transfer/IVF**
- *Cloning*

Why Clone Cattle?

% Genetic Gain



Why Clone Cattle?



- Current AI technology is 50 years old.
- Only get half the genetic package.

The 4 years of clone line testing

Find the best
cows
anywhere in
the world
(including
crossbreds)

Produce
cloned
embryos
(including
crossbreds)

Distribute
cloned
embryos.
(200 per line
and 100
lines)

Year 0

Year 0

Year 1

Calves are
born

Pick the lines
(including
crossbreds)
which make the
best cloned
females for
extensive use

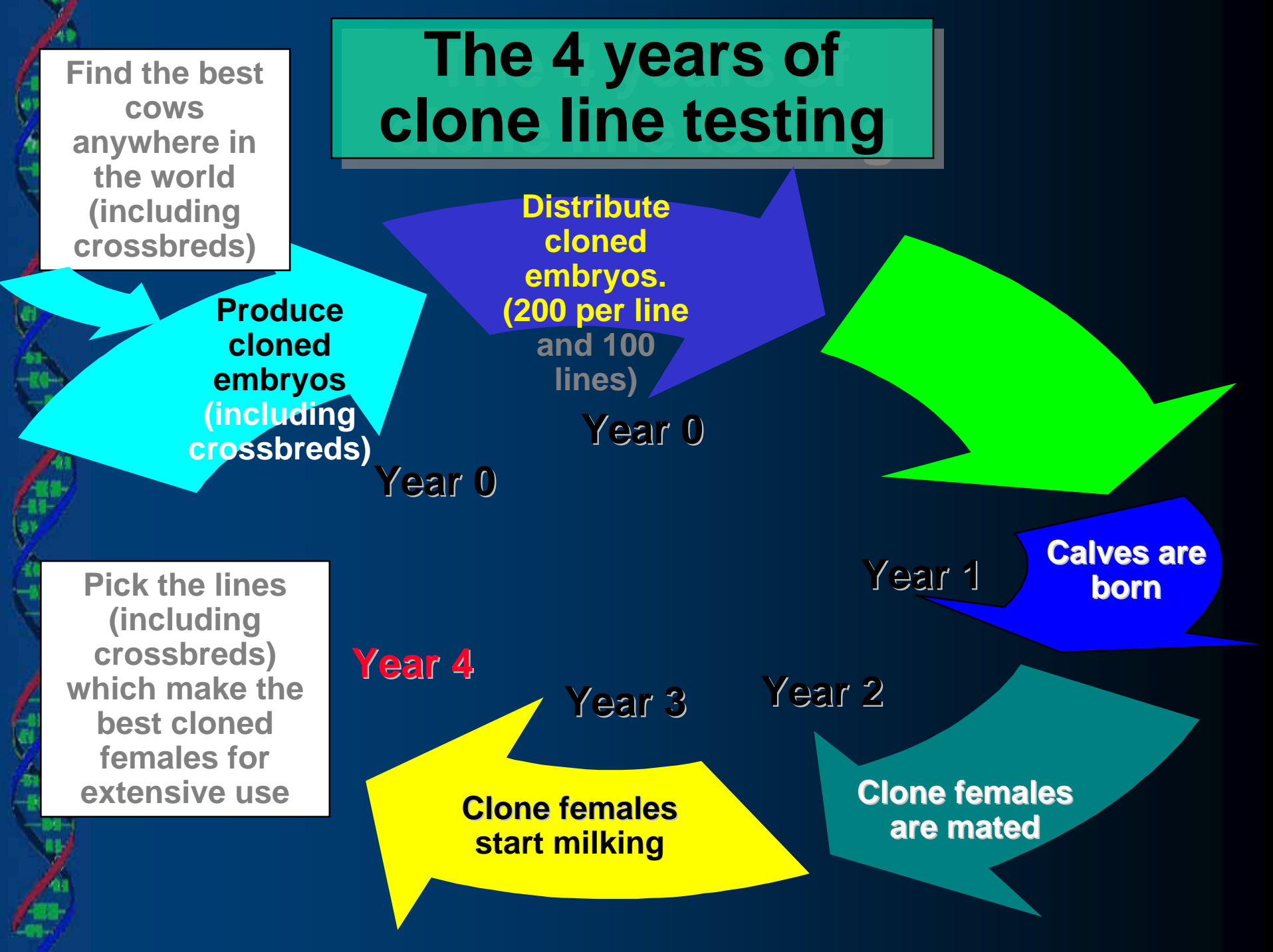
Year 4

Year 3

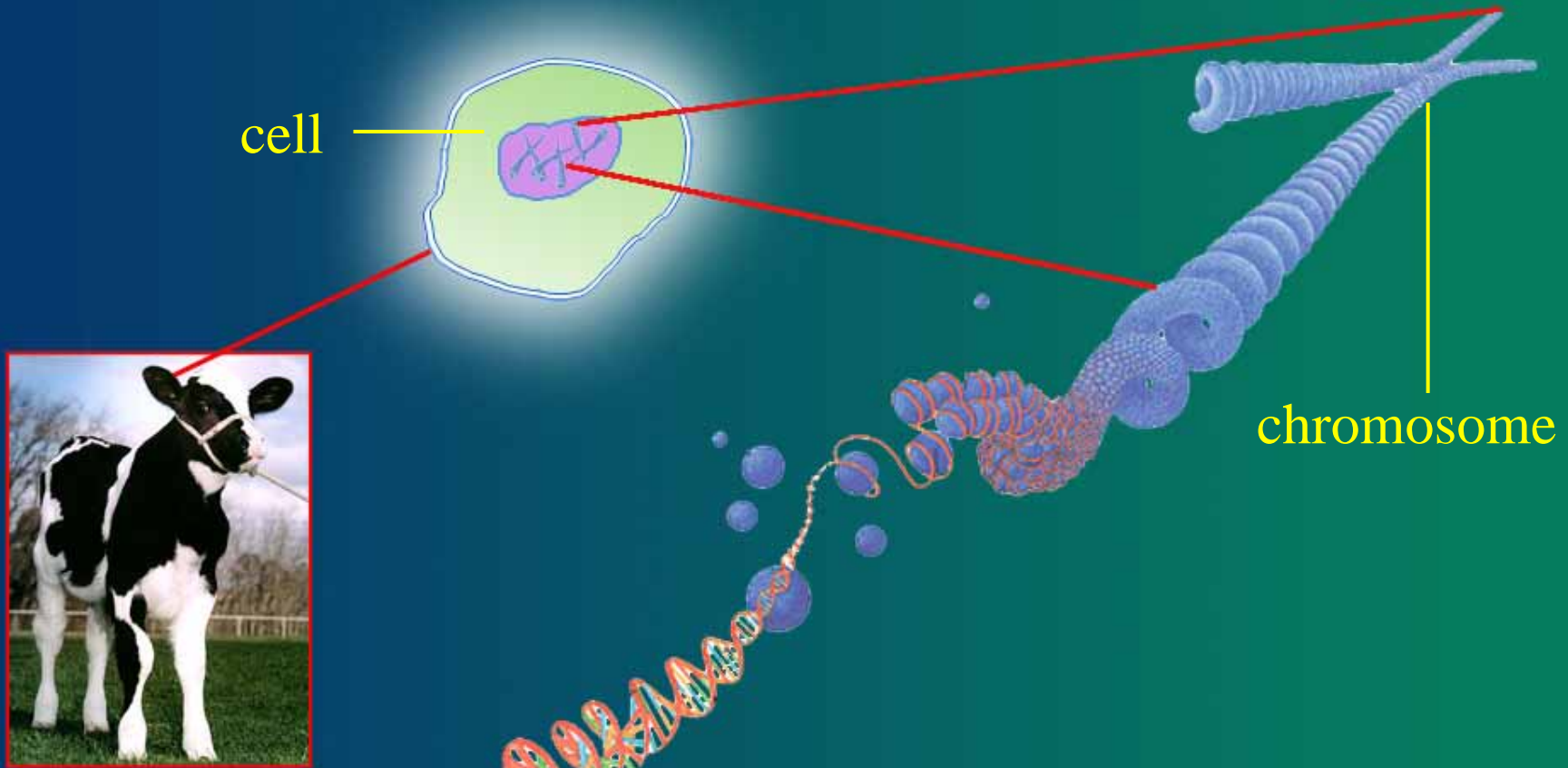
Year 2

Clone females
start milking

Clone females
are mated



DNA: The code for life



~~A C
T G~~



The addition of methyl groups to DNA
is normally associated with the silencing
(switching off) of genes

DNA methylation is the major epigenetic system
in mammals and is essential for normal
development.

(Acetylation and phosphorylation also play a role)