Preimplantation diagnosis

The preclinical years

Marilyn Monk
Institute of Child Health, University College London

Embryology / microsurgery

Oocytes, polar bodies, embryos and primordial germ cells.

Single cell diagnostic molecular biology

Gene mutation, modification, expression.

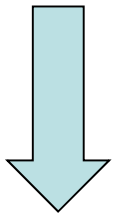
MOLECULAR BIOLOGY OF MICRORGANISMS

1959- 1969 Microbial Genetics

DNA replication and repair in *Escherichis coli*

1970 -1974 Development

Cell signalling and aggregation in *Dictyostelium discoideum*



MOLCULAR BIOLOGY of EMBRYOS

‘Micronise’ from millions of cells to one cell

1974 - Gene expression and its regulation in mammalian development

MOUSE

X Chromosome Inactivation / Imprinting / Methylation

Origin of the germ line

Deprogramming to stem cell

HUMAN

Preimplantation diagnosis

X Chromosome Inactivation/ imprinting / gene expression

Embryo and PGC cDNA libraries

Embryo / cancer genes *ECSA*

Molecular Biology

Bacteria
1959 – 1969

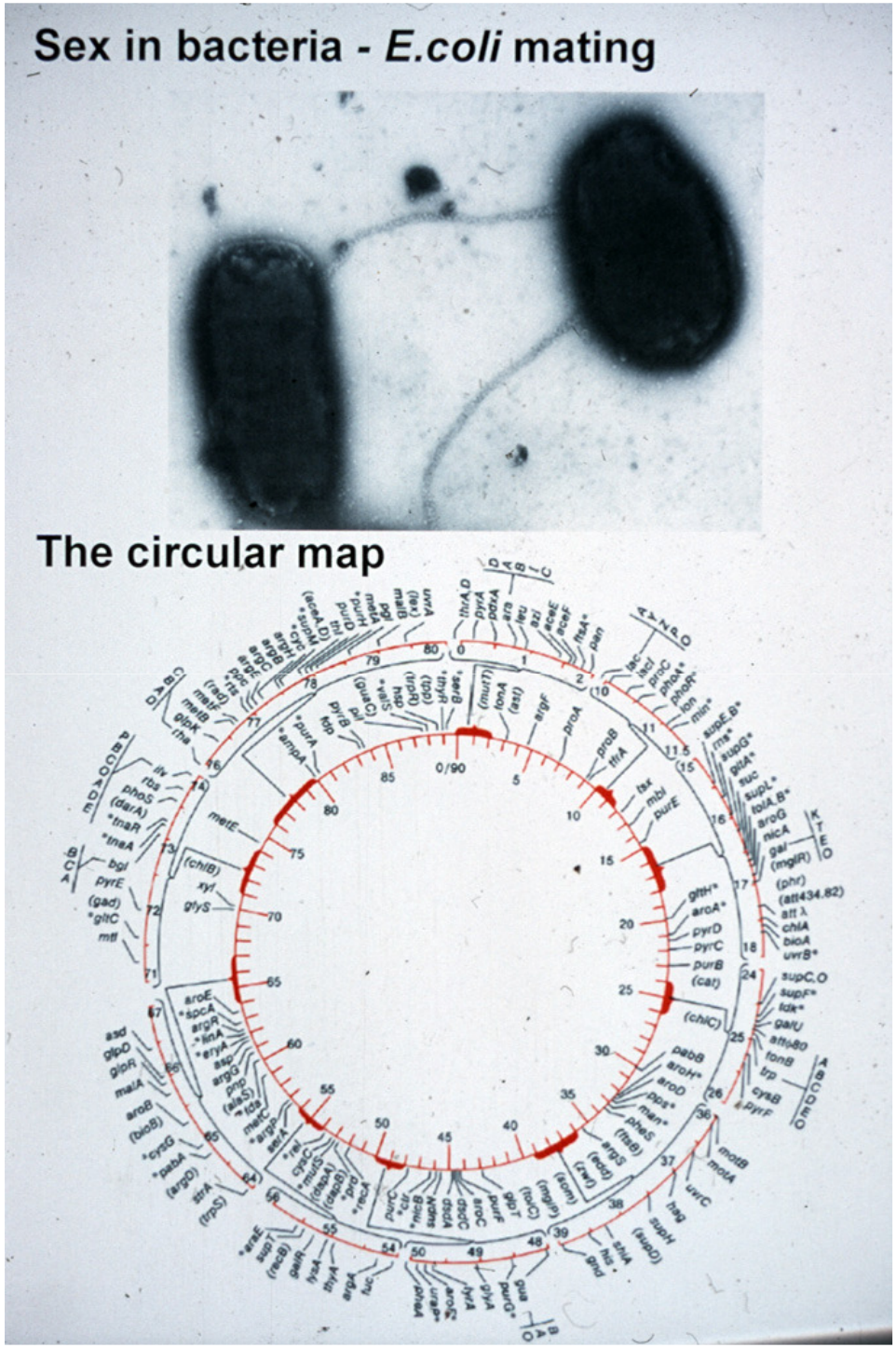
DNA transfer

Mapping

Bacteriophage λ induction

DNA replication

DNA repair



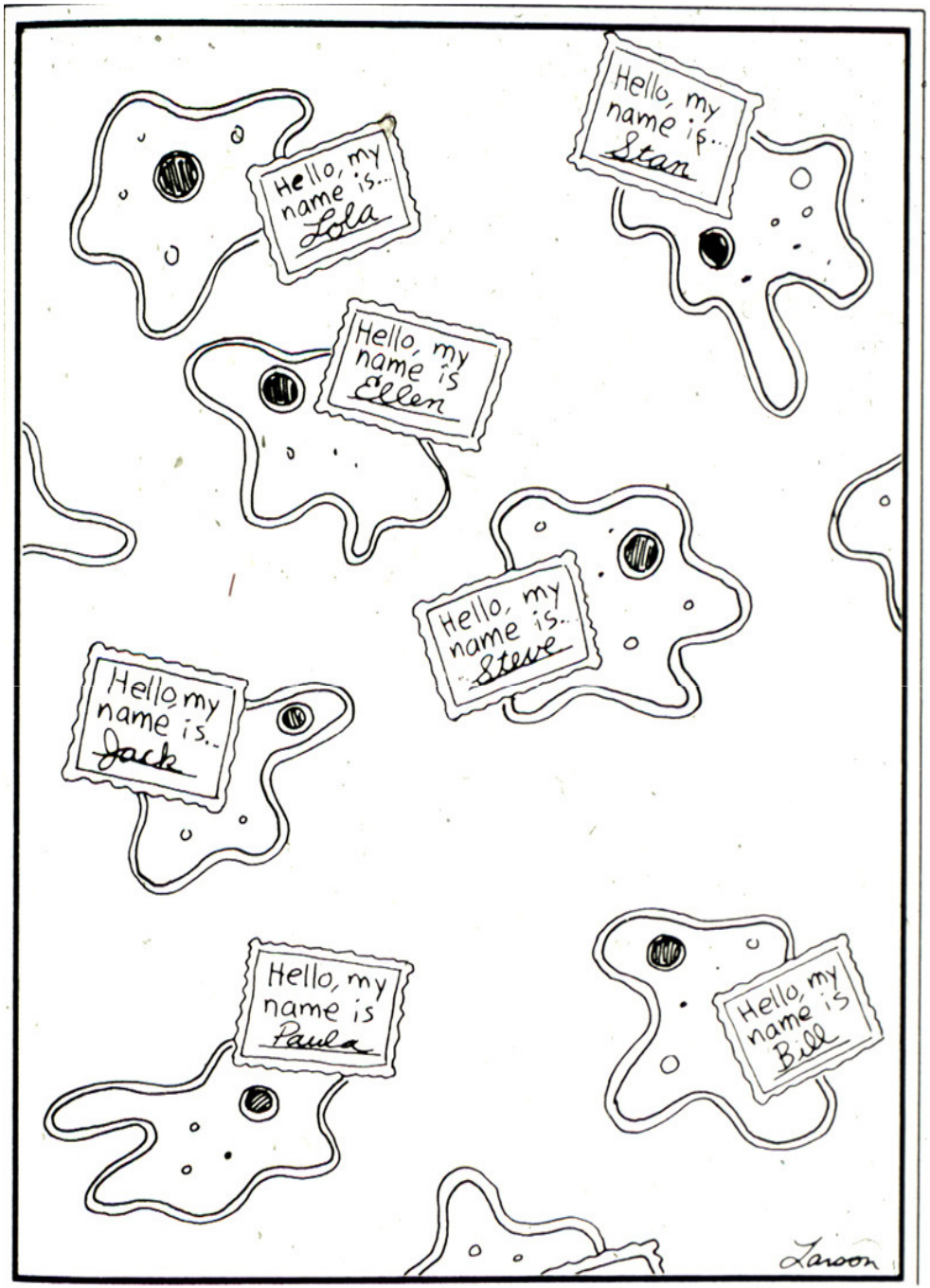
Bored with bacteria

Slime mould amoebae

Dictyostelium discoideum

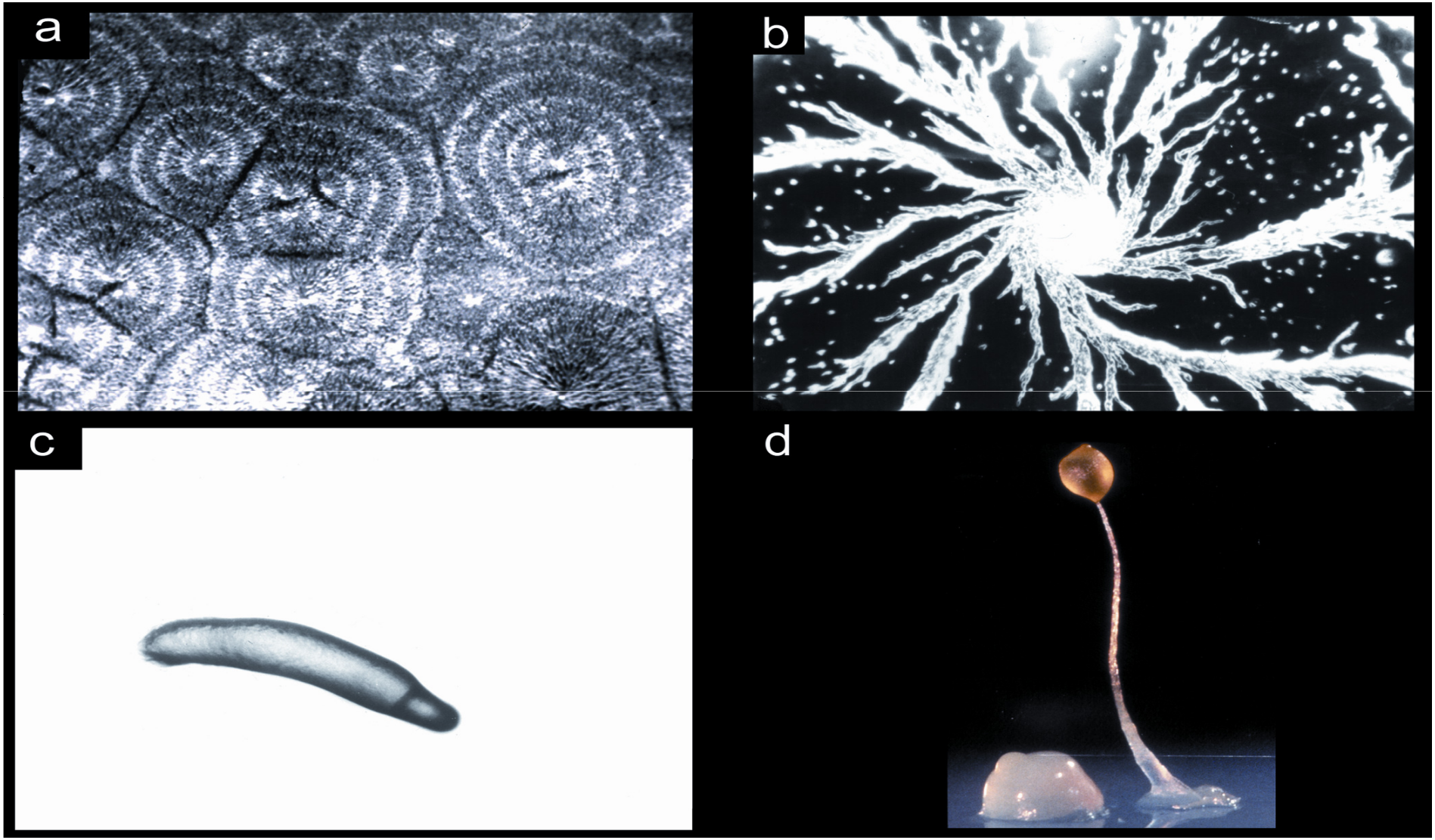
Cell communication

Single cells to multicellular



Amoeba conventions

Parameters of aggregation – cAMP signalling, signal periodicity, velocity - cell refractory period, movement duration & distance

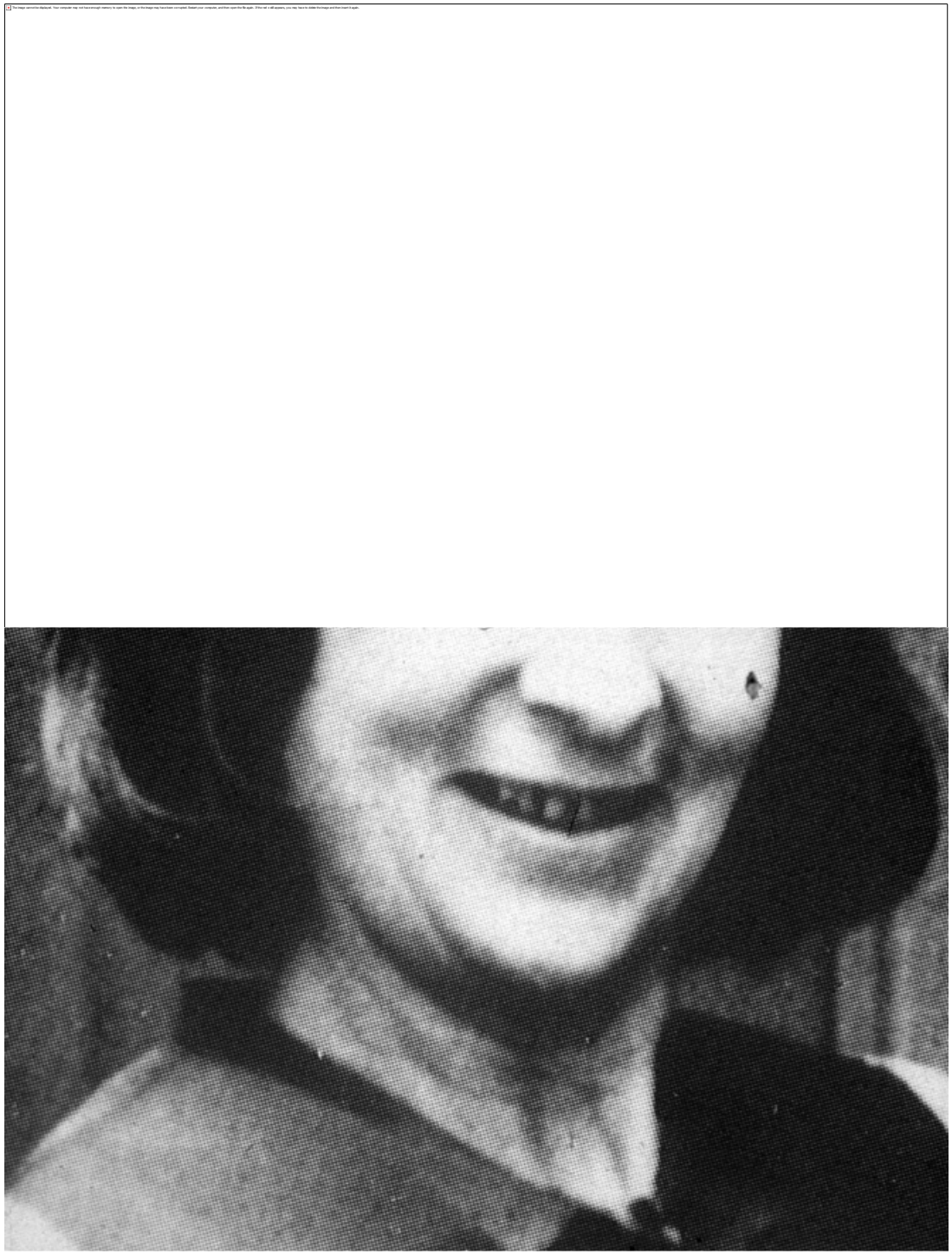


1970 - 1974

Alcantara and Monk, 1974

Anne McLaren

MRC Mammalian Development Unit 1974 - 1992



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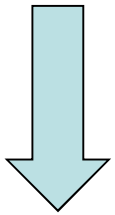

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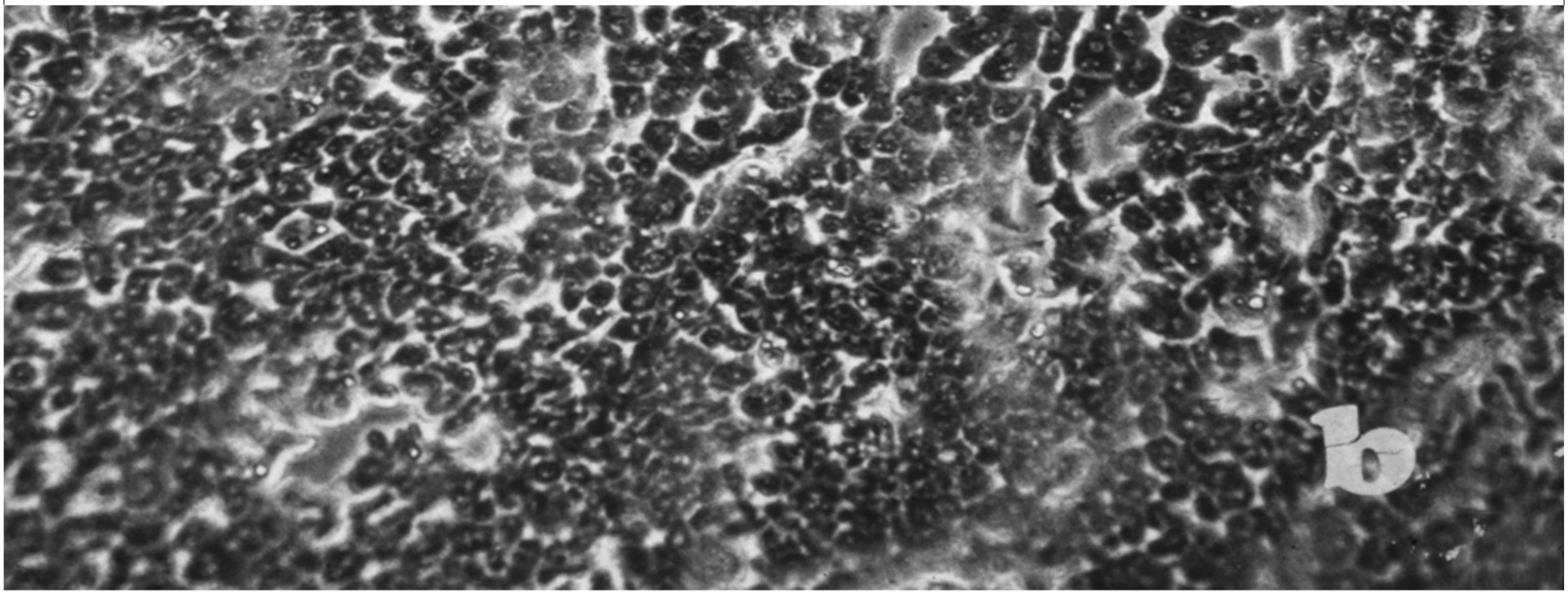
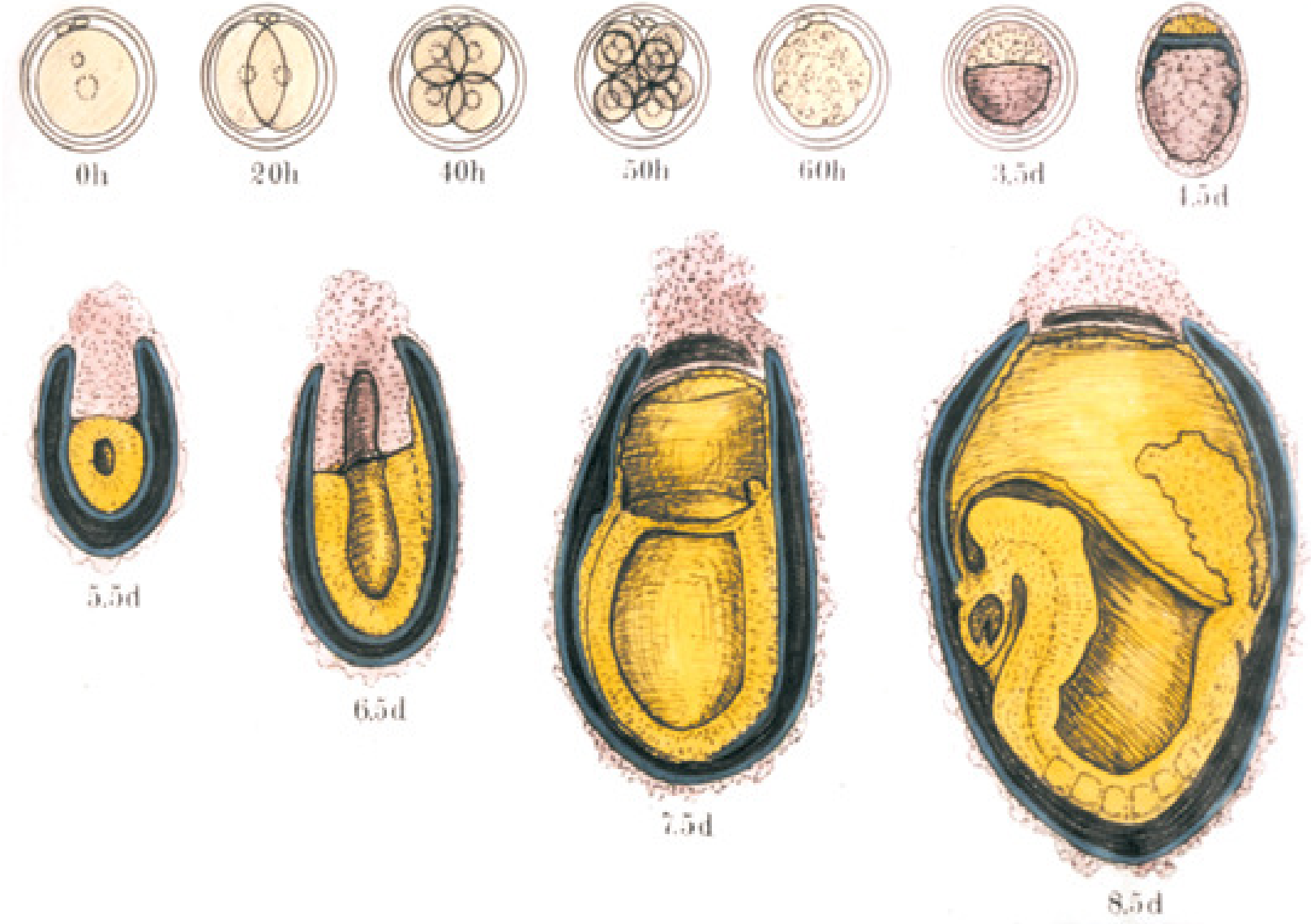


One to a few hundred cells – micro molecular biology



Slide of Rosa Beddington

1974→**Sensitive Single Cell Molecular Assays**

Enzyme activity

2 uL minigels for electrophoresis - LDH

Gene Dosage *Hprt*/*Aprt* double microassay ^{3}H hypoxanthine → ^{3}H IMP
^{14}C adenine → ^{14}C AMP
Ratio X / autosome activities. X^+X^+ 2 : 1 X^+Y , X^+X^+

Gene Specificity *Pgk*-1A & *Pgk*-1B, *G6PD etc*
Distinguish maternal and paternal X-linked gene activity

Single cell PCR - Nested PCR

Gene Mutation, Beta globin, sickle cell, unstable repeats (FRA-X, DM, AR) , etc

Gene Modification Specific CpG methylation of X-linked / Imprinted genes

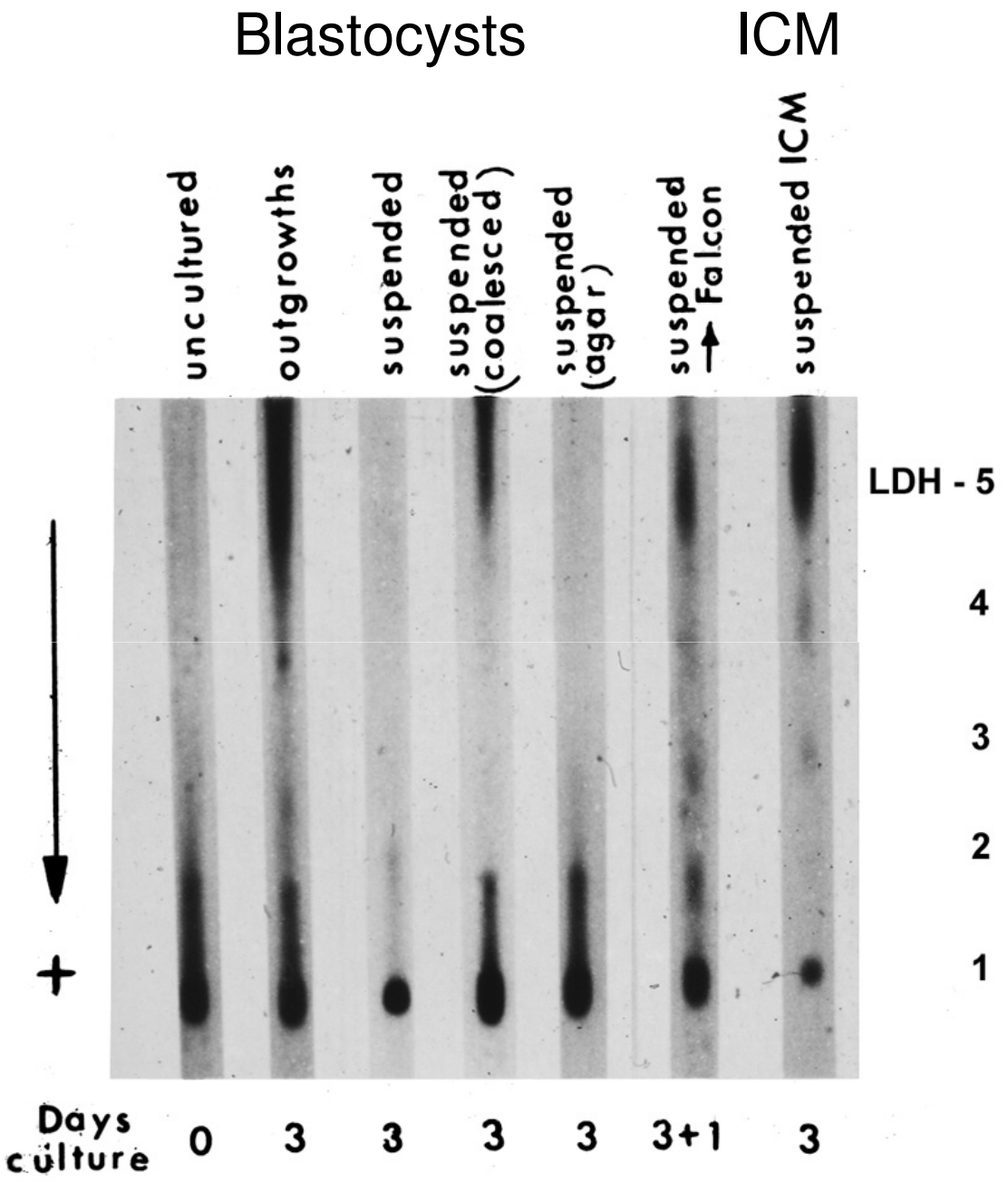
Gene Transcription RT- PCR, allele specific RT-PCR, multiplex RT-PCR

Cell recycling PCR plus FISH

Electrophoresis
of LDH
in single embryos
in 2 uL minigels

Delayed
'implantation'
in vitro

Monk and Petzoldt
Nature 1977



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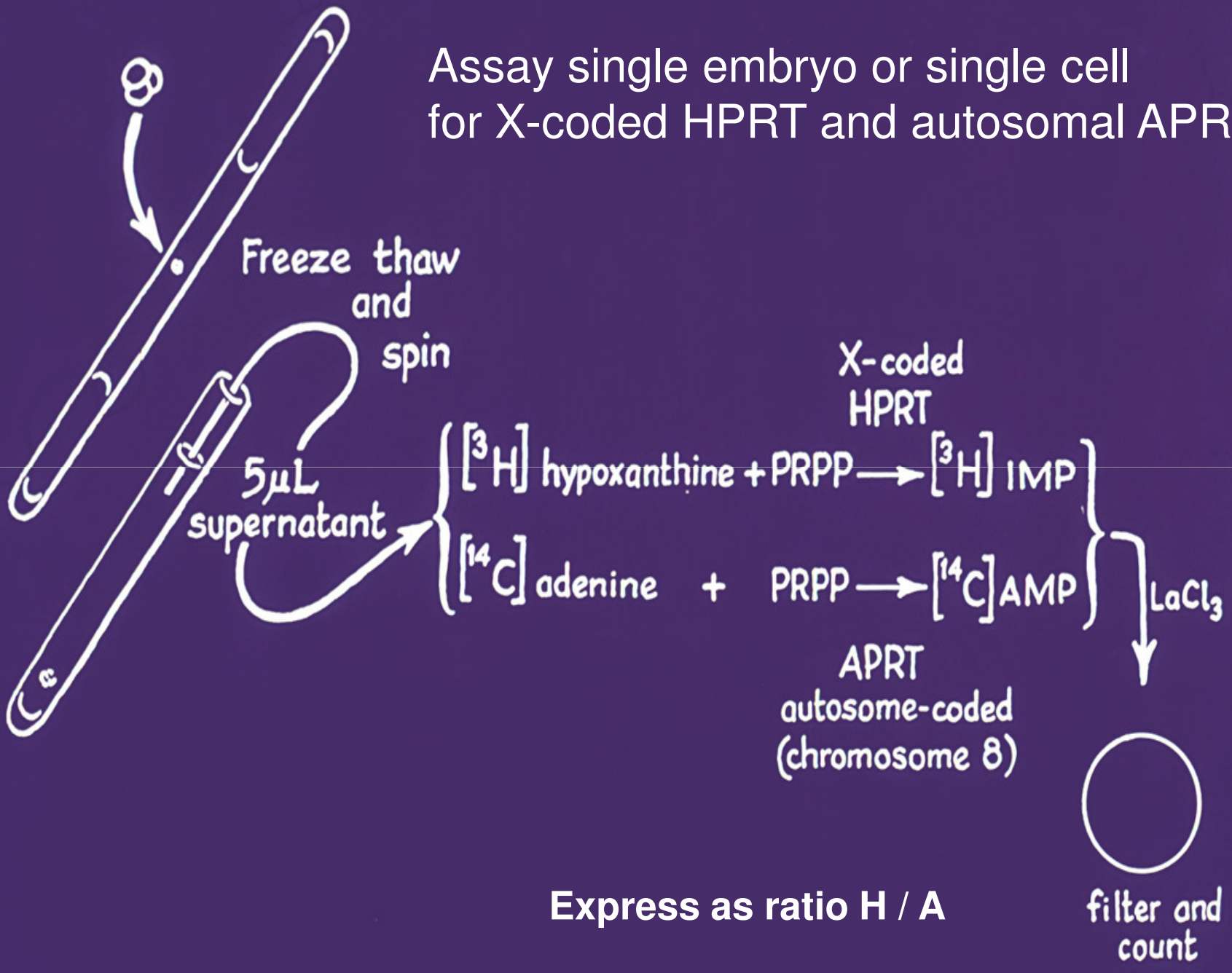
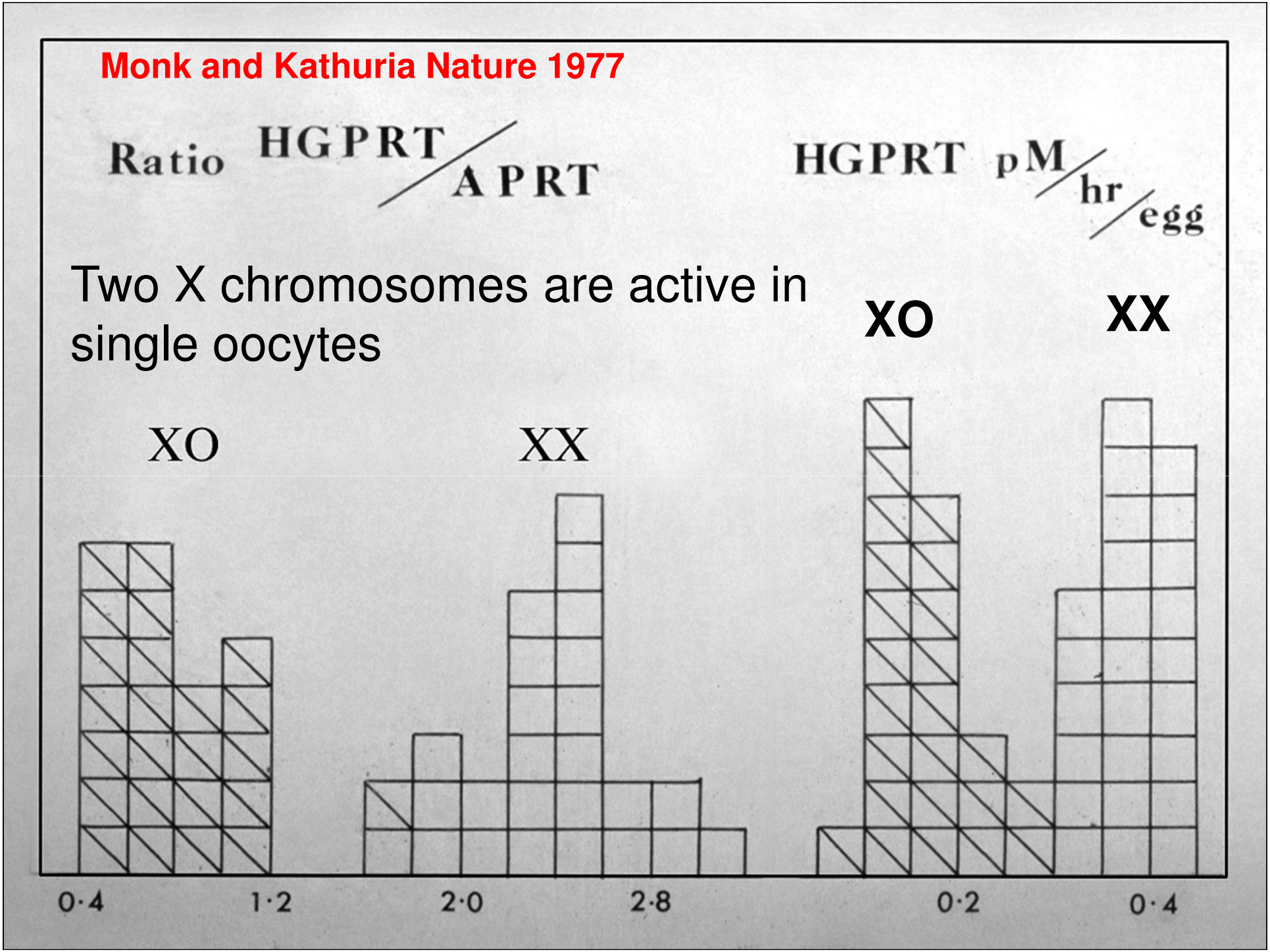
Assay single embryo or single cell
for X-coded HPRT and autosomal APRT



Express as ratio H / A

Monk and Kathuria Nature 1977



Two X chromosomes are active in single oocytes

Postimplantation - 6d

Dosage of HPRT is bimodal

6d epiblast

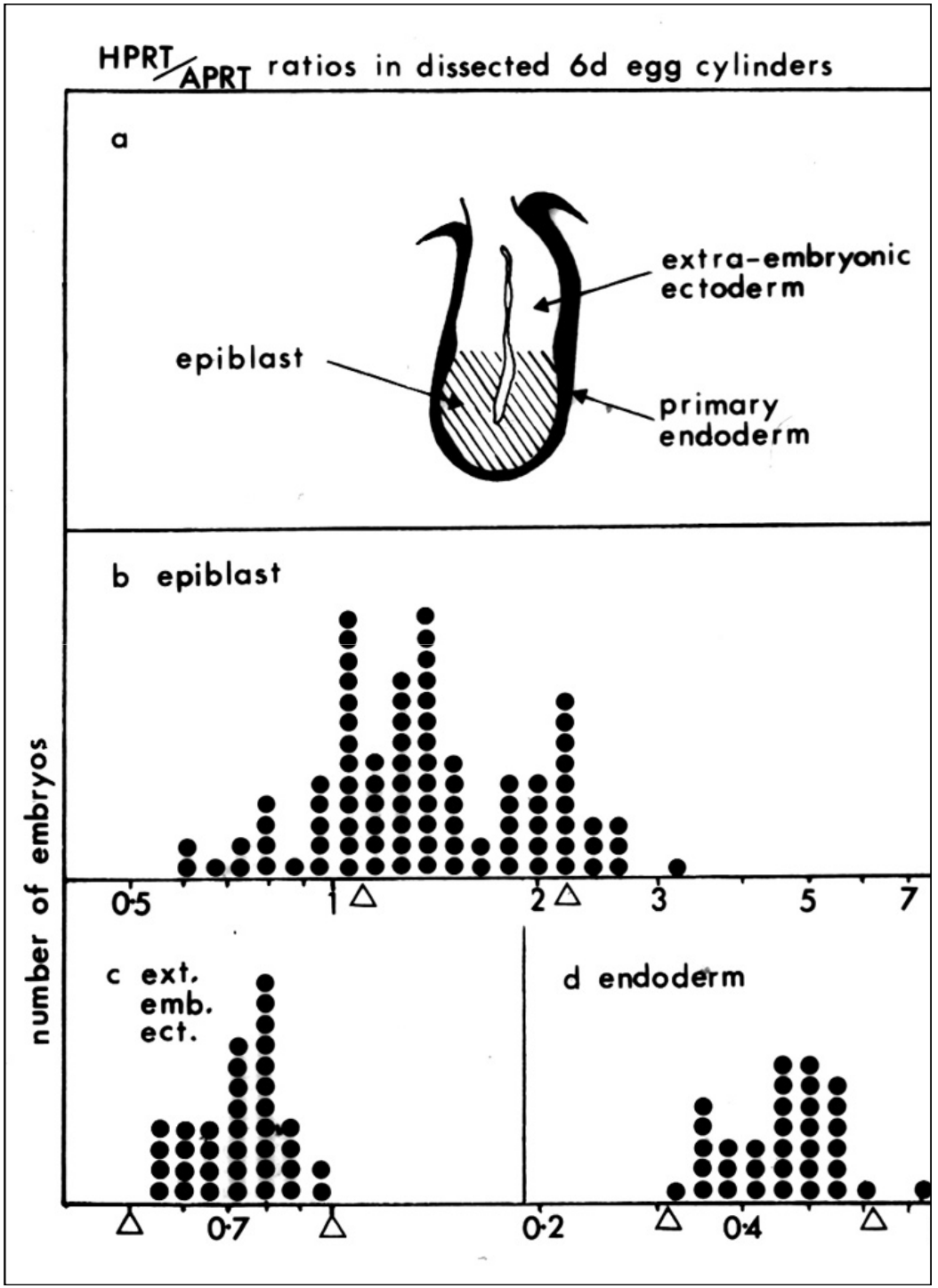
Two X chromosomes active

Dosage HPRT unimodal

6d ex emb ect & ex emb end

One X active

Monk & Harper, Nature 1979



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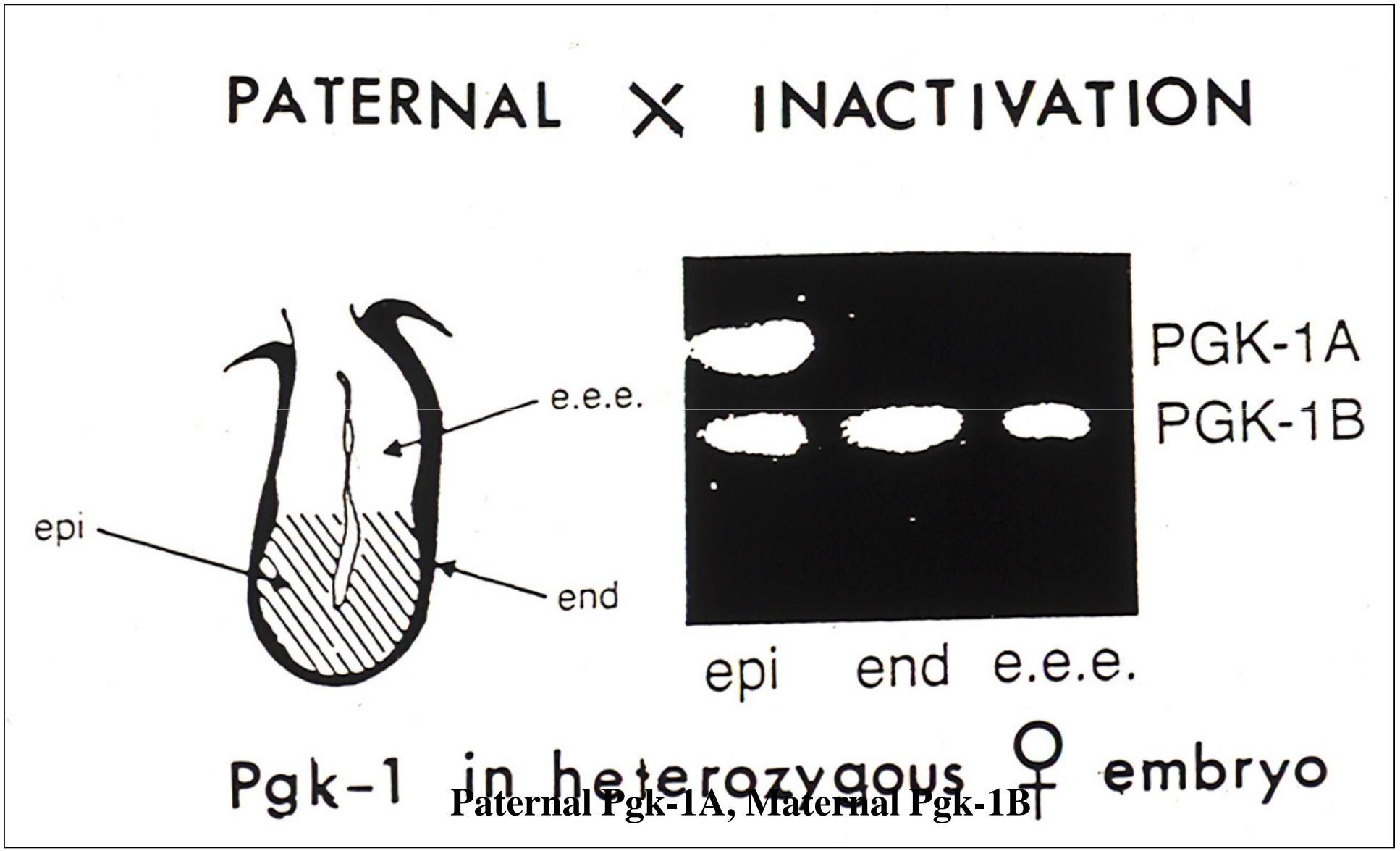
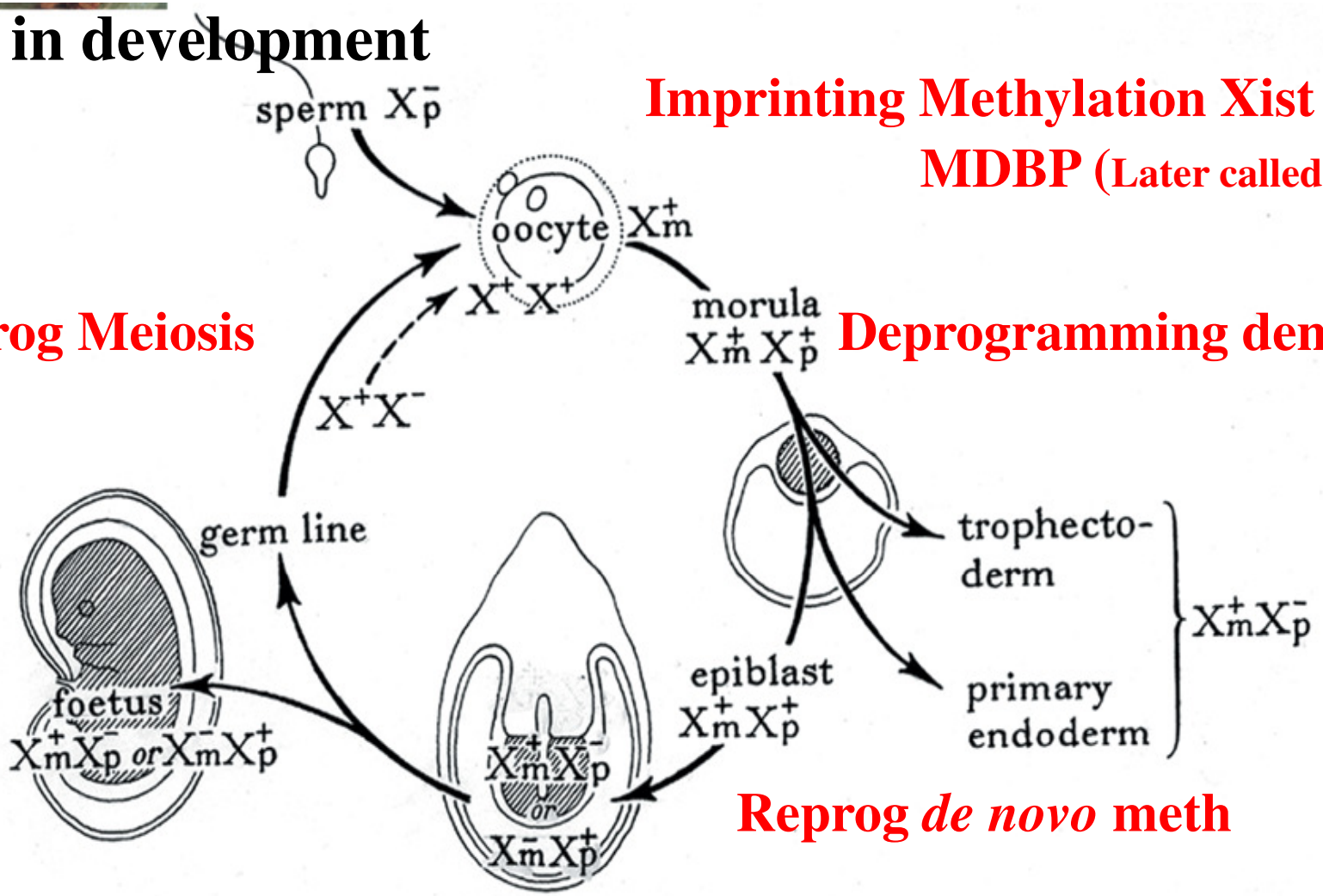
Primary non-random paternal X-inactivation



Paternal Pgk-1A, Maternal Pgk-1B

McMahon, Fosten & Monk, 1981; West & Pappaiannou. 1981

XCI in development



Imprinting Methylation Xist egg

MDBP (Later called Kaiso)

Deprog Meiosis

Deprogramming demeth^n

Reprog *de novo* meth

Late origin of the germ line

Monk et al 1977 - 1983

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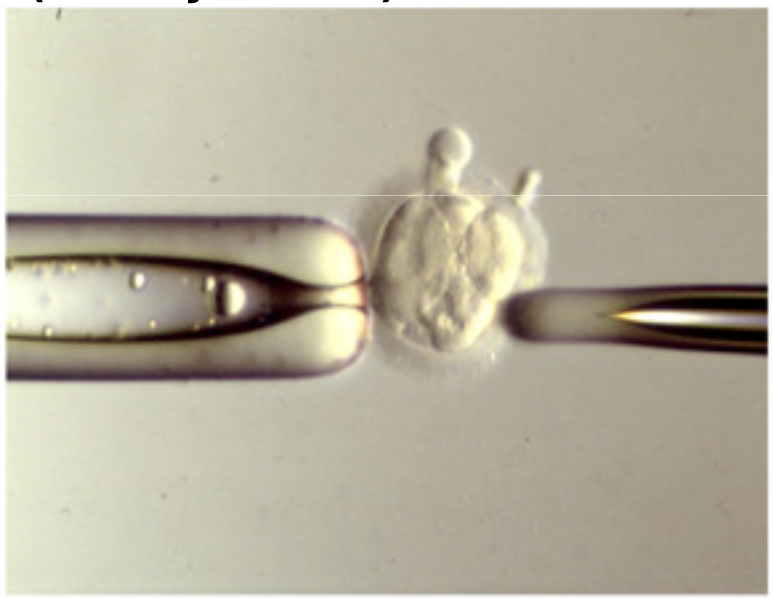
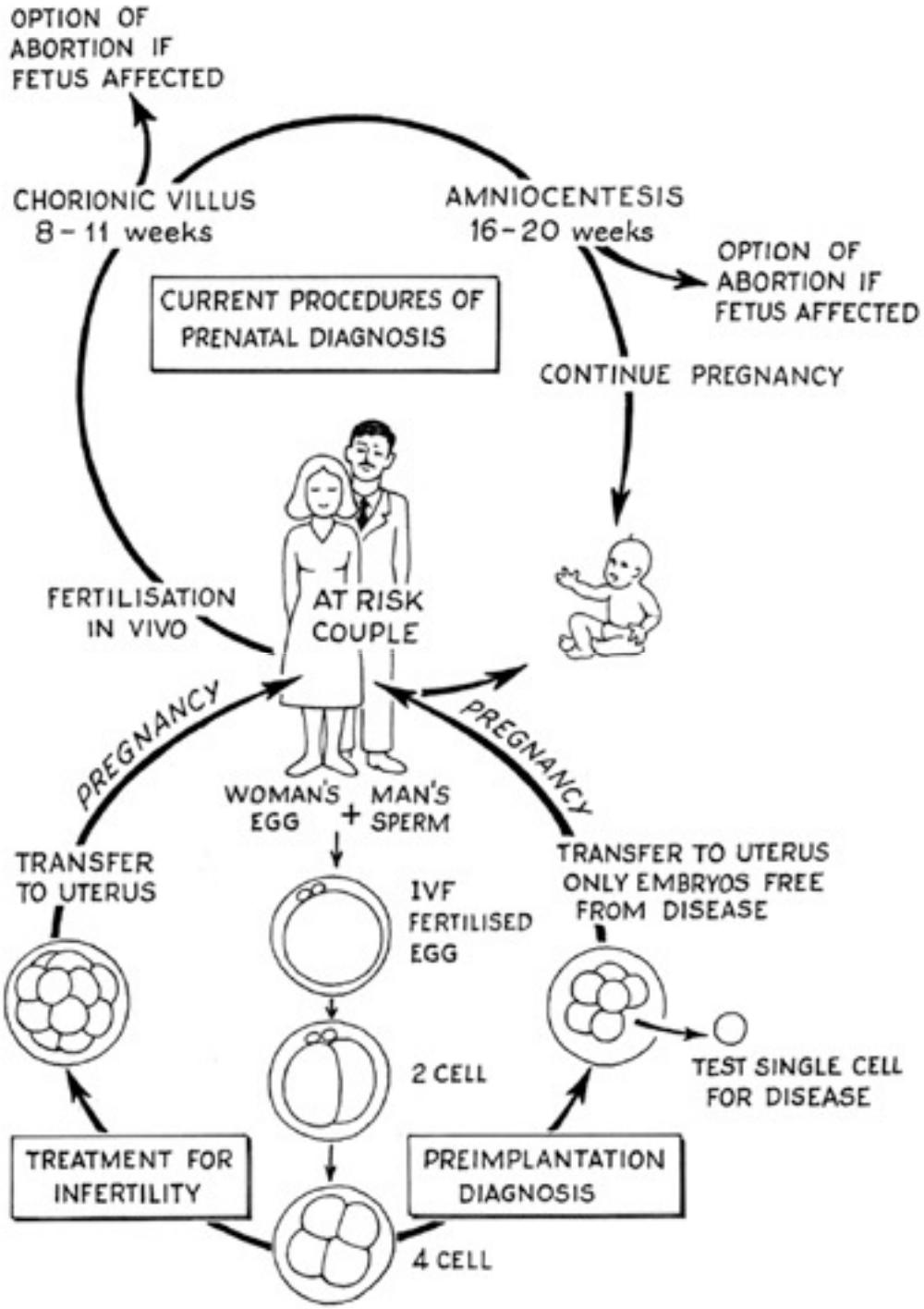
PID ⟶ PGD

Biopsy of a blastomere from 8-cell embryo
(Tetsuya Goto)



IVF

HPRT deficiency ⟶ Lesch Nyhan



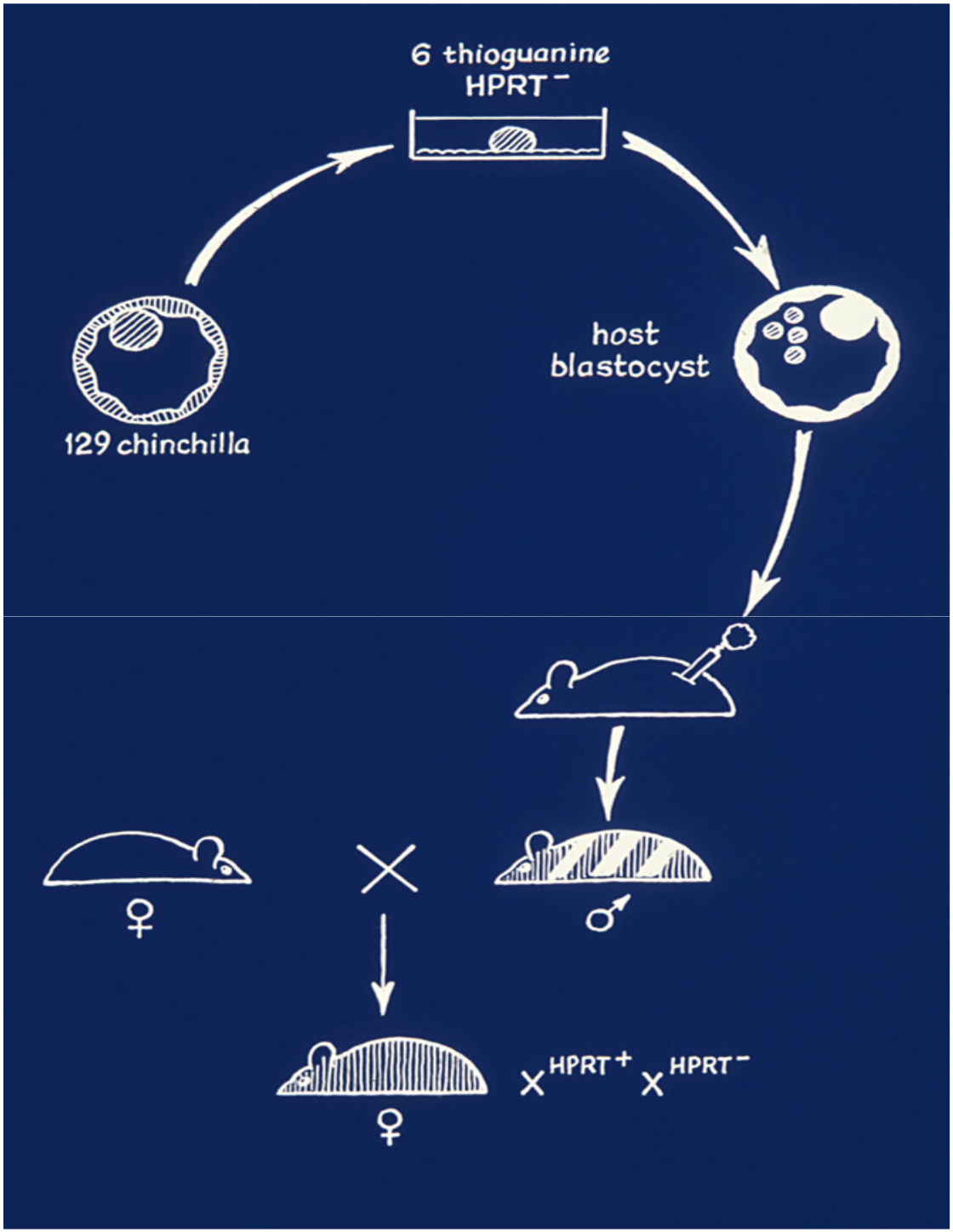
The first genetically engineered mouse

HPRT-deficient
(Lesch-Nyhan)
mouse embryos derived
from germline colonization
by cultured cells

Hooper M, Hardy K,
Handyside A, Hunter S,
and Monk M.

Nature **326,** 292-5 (1987)

Hprt+ / Hprt- female ⟶



Embryos from Hprt+/Hprt- mother
'Lesch Nyhan' mouse model

Diagnosis

of Hprt deficient embryos

by HPRT enzyme activity

in single blastomere

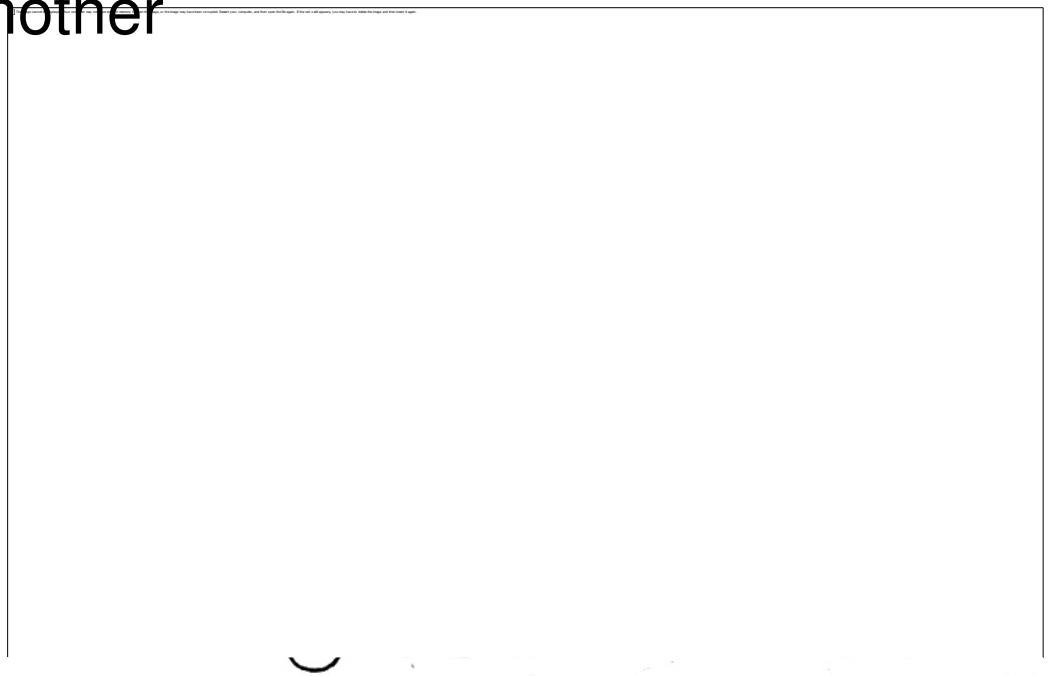
from 8-cell embryo

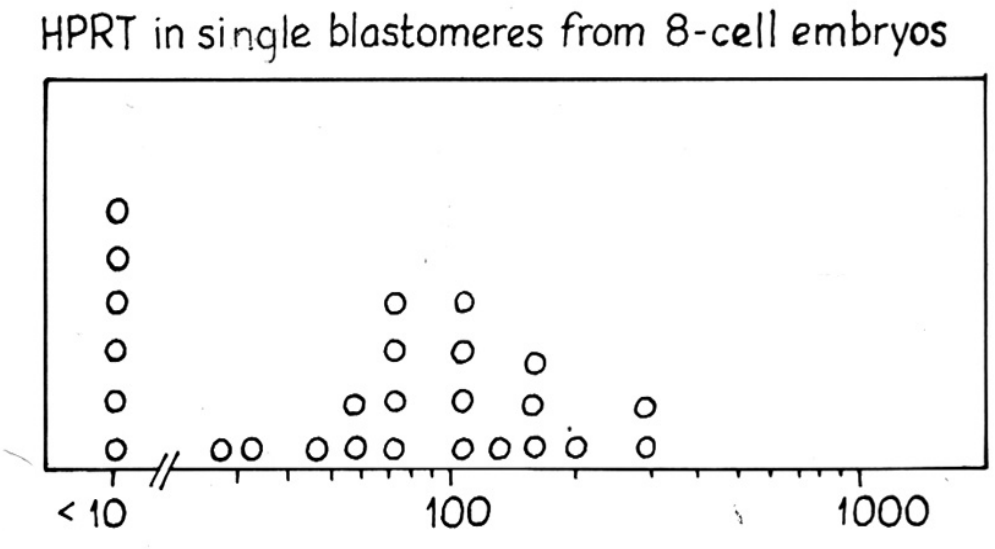
Confirm diagnosis in

transferred biopsied embryos

(A Handyside)

Monk et al, Lancet 1987





Sexing by Hprt dosage

Monk & Handyside 1988

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1984 PCR

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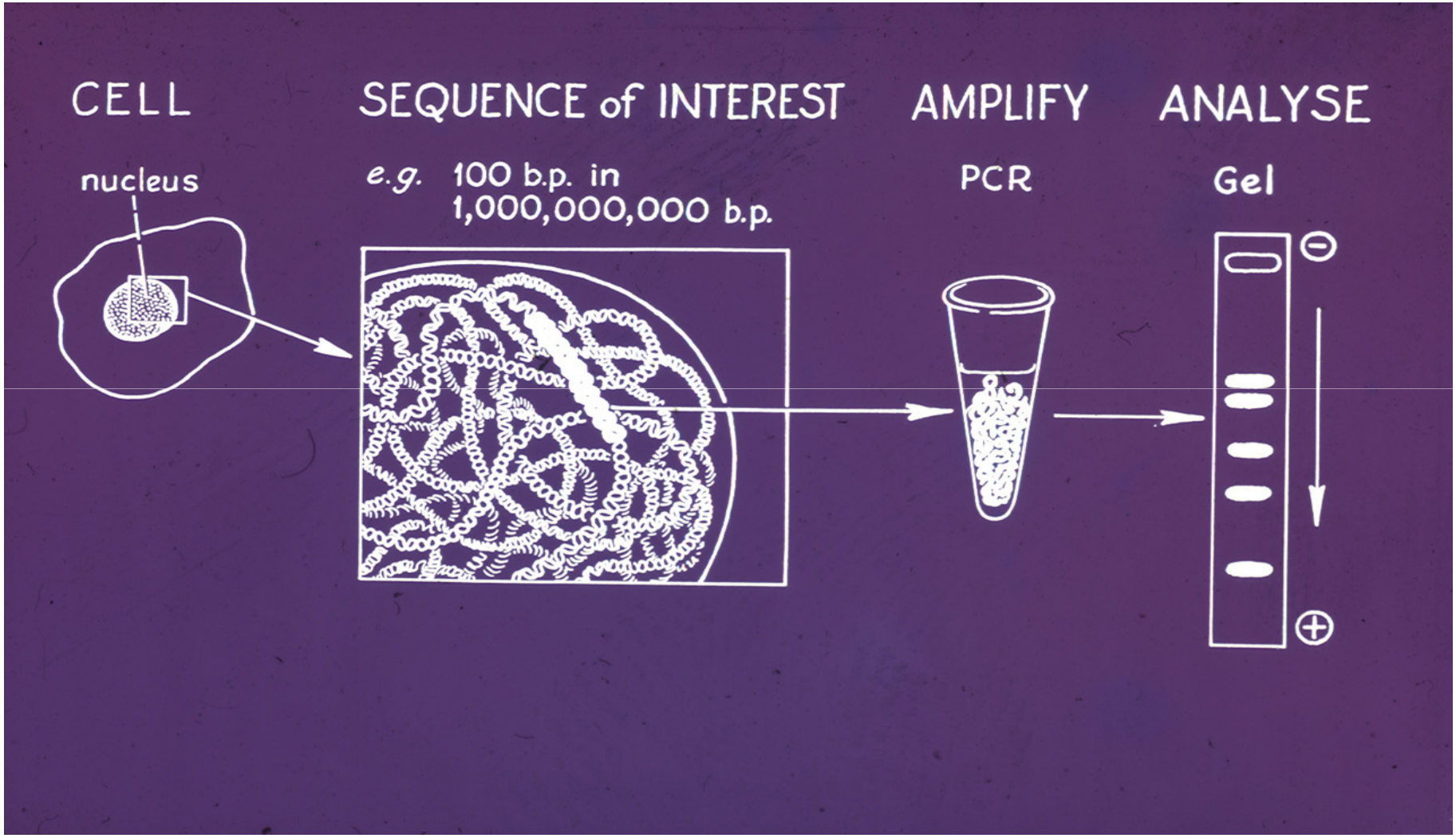
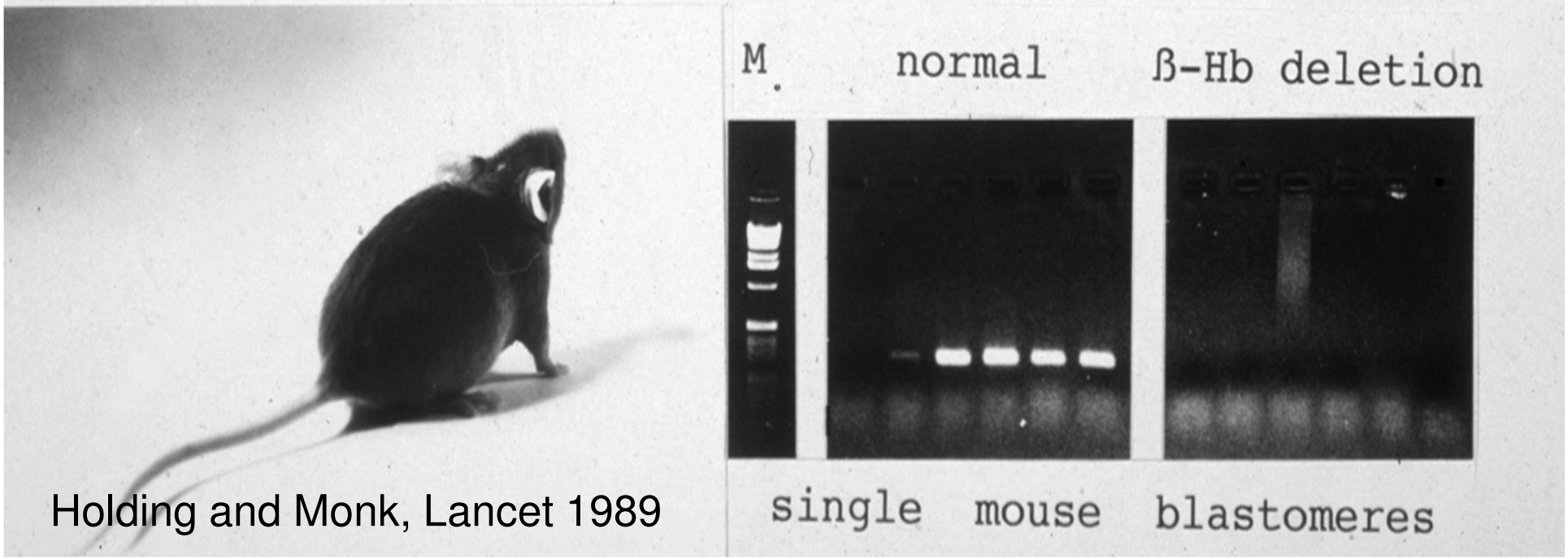
Single Cell PCR



Moving racks of tubes between water baths! – Holding and Monk



Holding and Monk, Lancet 1989

Harper
Fosten
McMahon
Lindsay
Holding
Ao
Grant
Kenealy
Zuccotti
Daniels
Kontogianni
Thornhill
Goto
Adjaye
Lorenzo
Huntriss
Salpekar
Hitchens

Monogenic disease genes

SINGLE CELL ANALYSIS

X - Linked

Duchenne Muscular Dystrophy*
Lesch - Nyhan Disease*
Severe Combined Immunodeficiency*
Fragile X Syndrome*
Kennedy's disease*
X-inactivation specific transcript*
Mono-amine oxidase A*
Sex chromosome-linked repeat sequences*
Haemophilia

Autosomal

Sickle Cell Disease*
Myotonic Dystrophy*
Small nuclear riboprotein polypeptide N*
Antitrypsin Deficiency
Thalassaemia
Cystic Fibrosis
Tay Sachs Disease

*At Molecular Embryology Unit, ICH

INHERITED GENETIC DISEASE

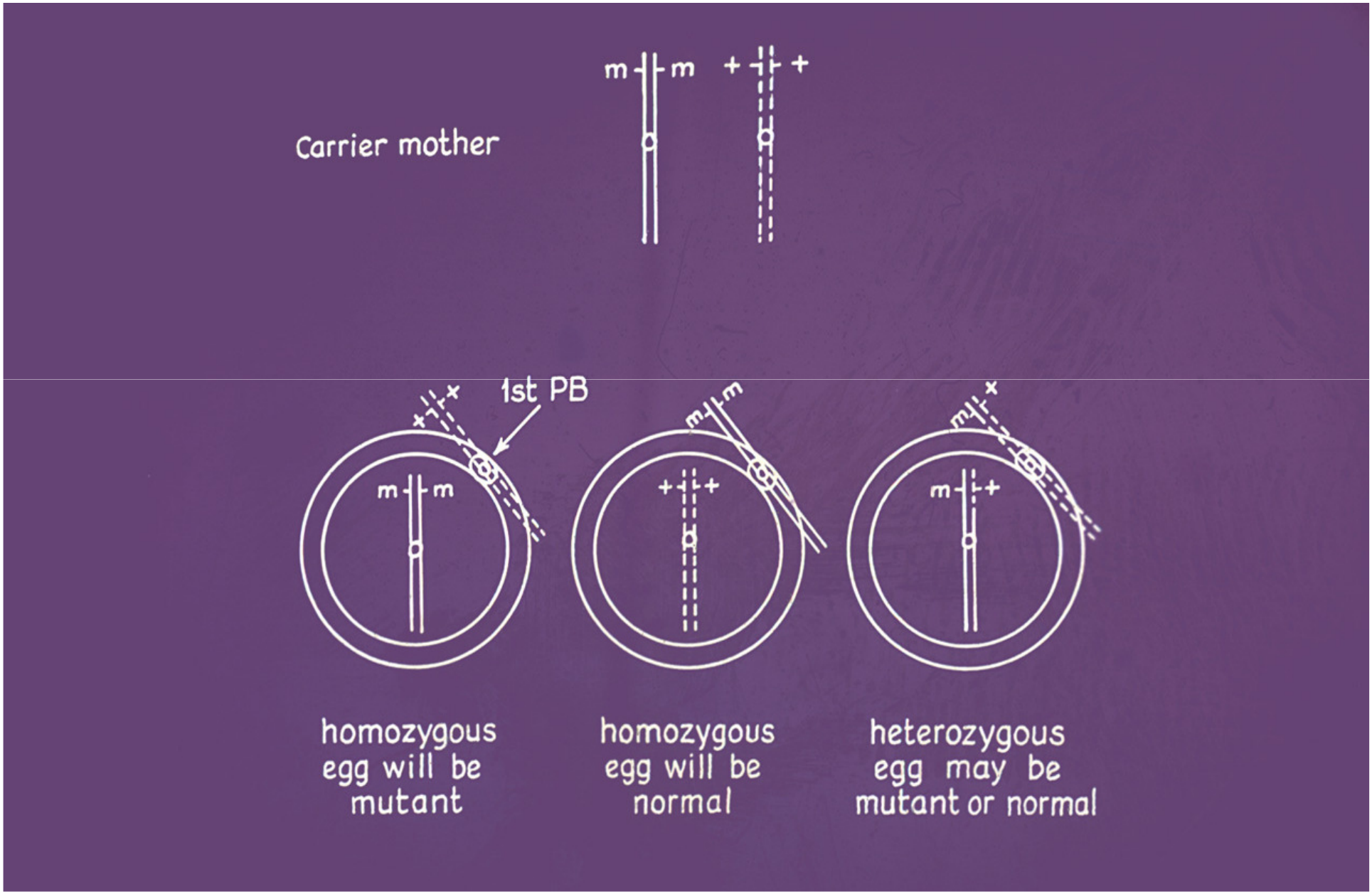
OOCYTE DIAGNOSIS

Biopsy of first polar body

Fertilise only eggs without defect

Avoids interference with embryos

Diagnosis of egg by examination of polar body



Diagnosis of sickle cell in human polar body

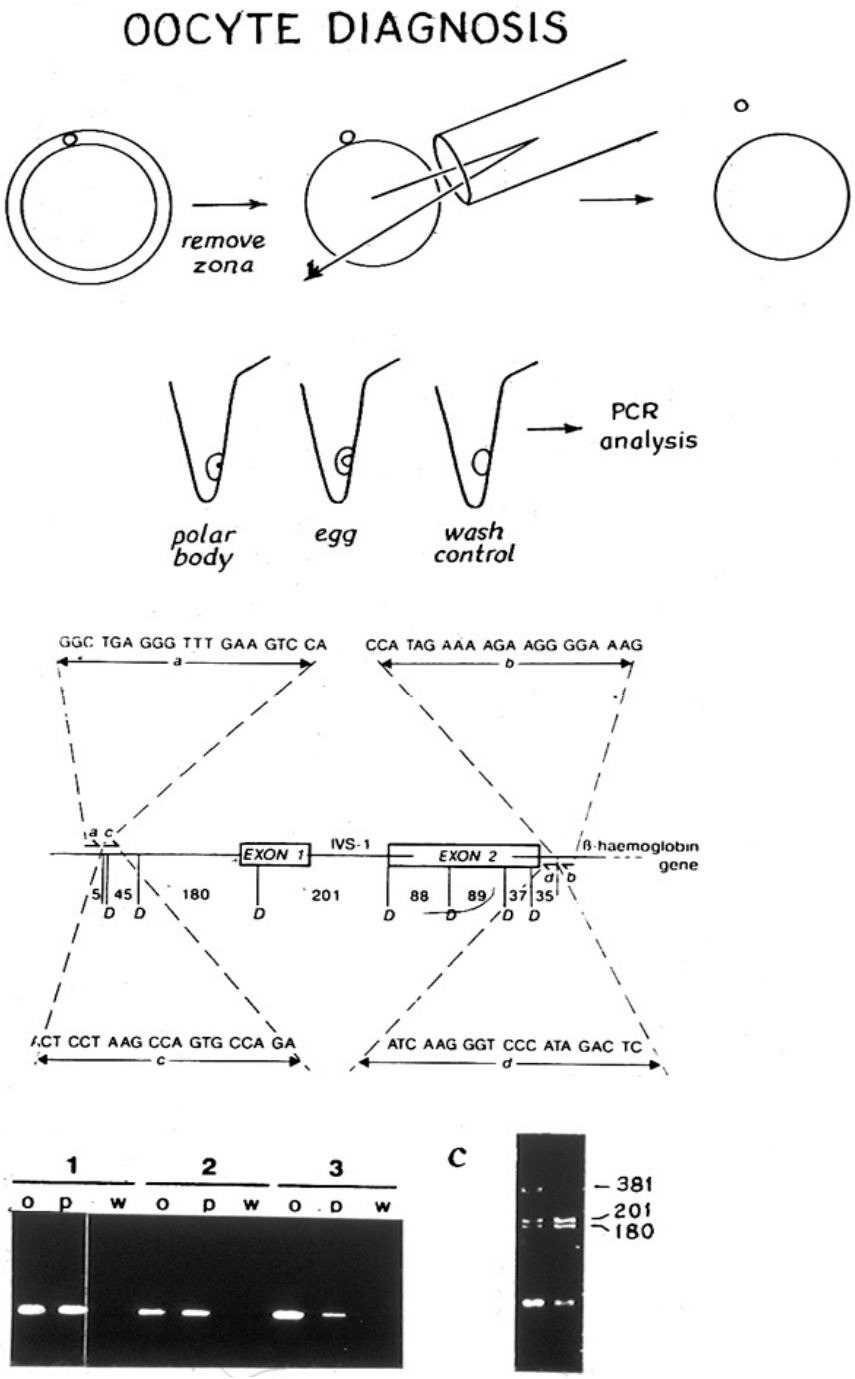
Separate polar body and egg
(Peter Braude's lab)

Nested PCR
amplification
of beta haemoglobin sequence
over sickle cell mutation

in

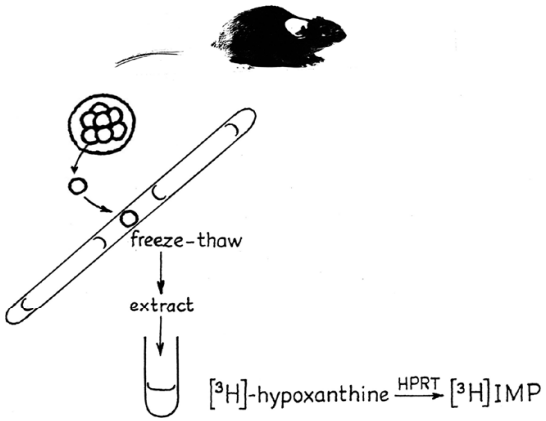
Individual human oocytes
and polar bodies.

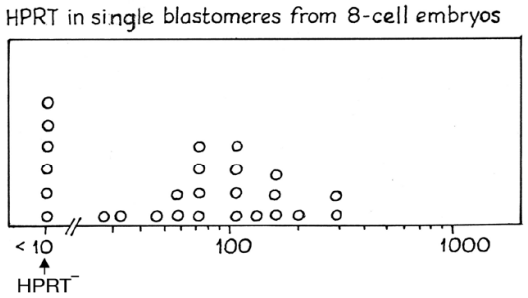
Monk M and Holding C.
Lancet **335,** 985-8 (1990)



Early Models for Preimplantation Diagnosis Summary

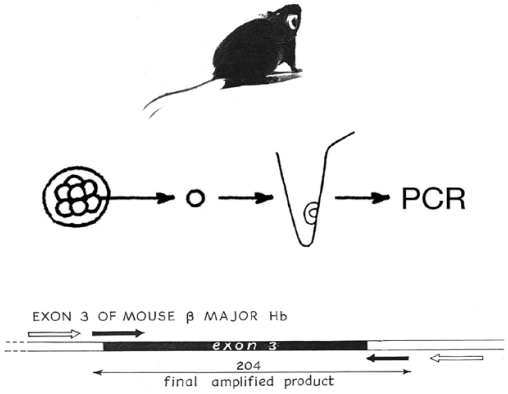
a. Lesch-Nyhan $X^{HPRT+}X^{HPRT-}$





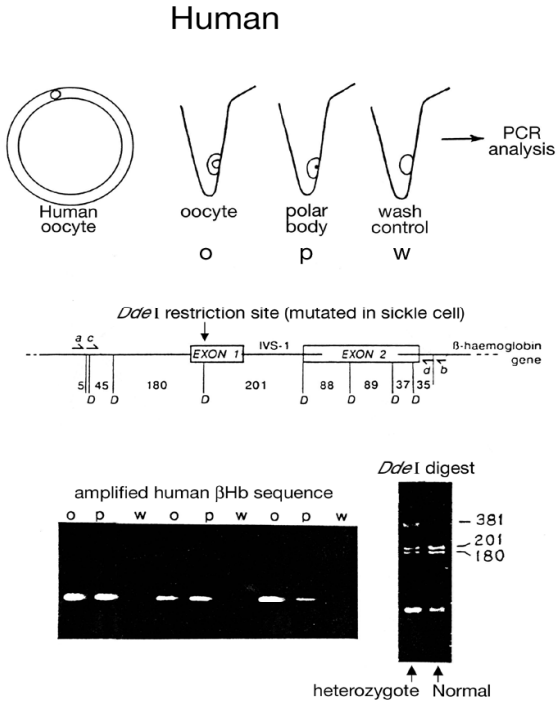
Monk et al, 1987

b. Thalassaemia $\beta Hb^{+} / \beta Hb^{\nabla}$



Holding & Monk, 1989

c. Sickle cell anaemia



Monk & Holding, 1990

QUALITY CONTROL

Efficiency of amplification in single cell

Efficiency of detection of both alleles

Extent of contamination

"Fingerprint" origin of cell

Monk, Kenealy and Mohadjerani 1993

Cell Recycling

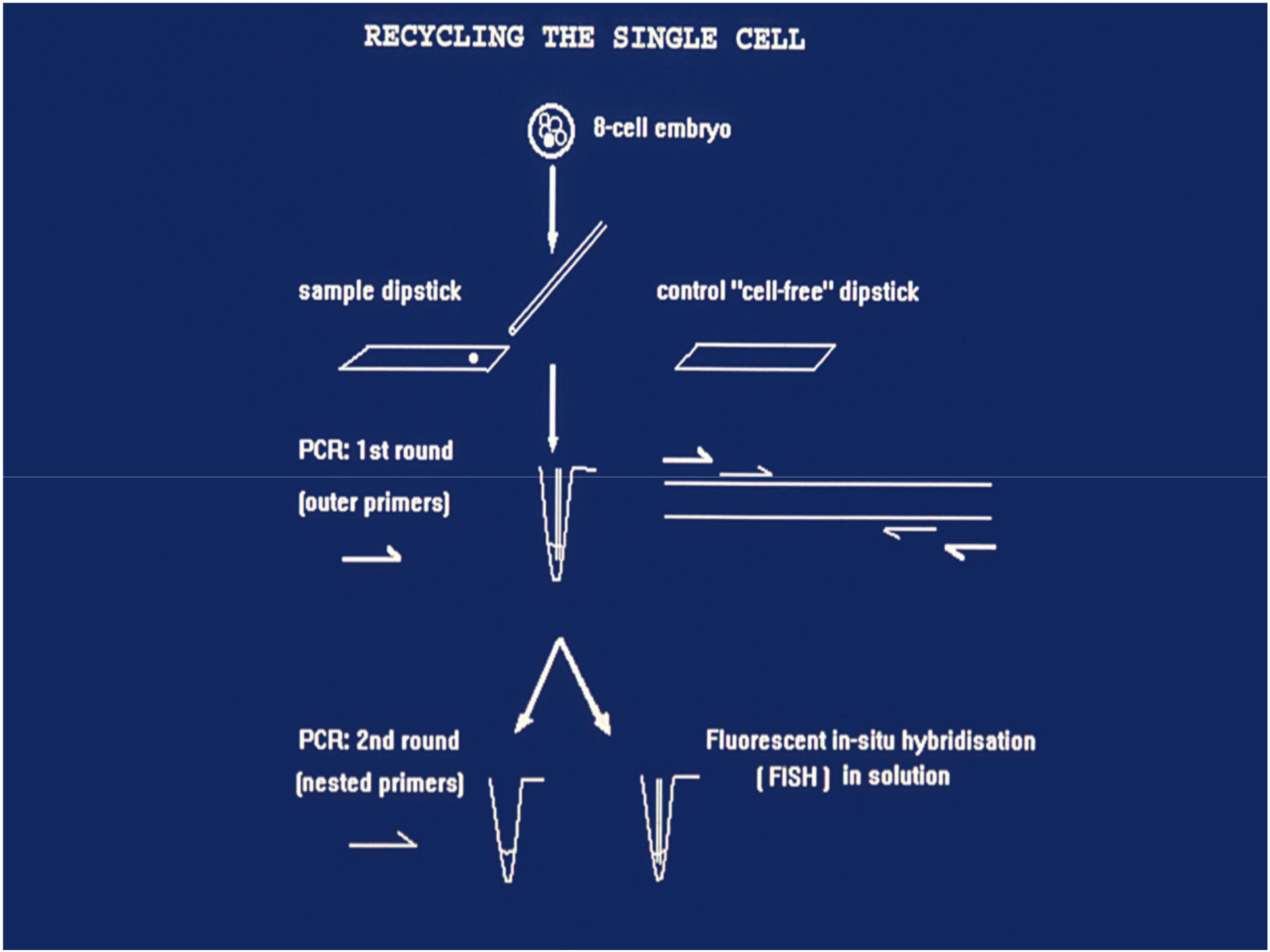
A method to analyse both chromosomes

and the DNA of specific gene sequences

in the same single cell fixed to glass.

FISH plus PCR

Holding, Thornhill & Monk 1994, 1996

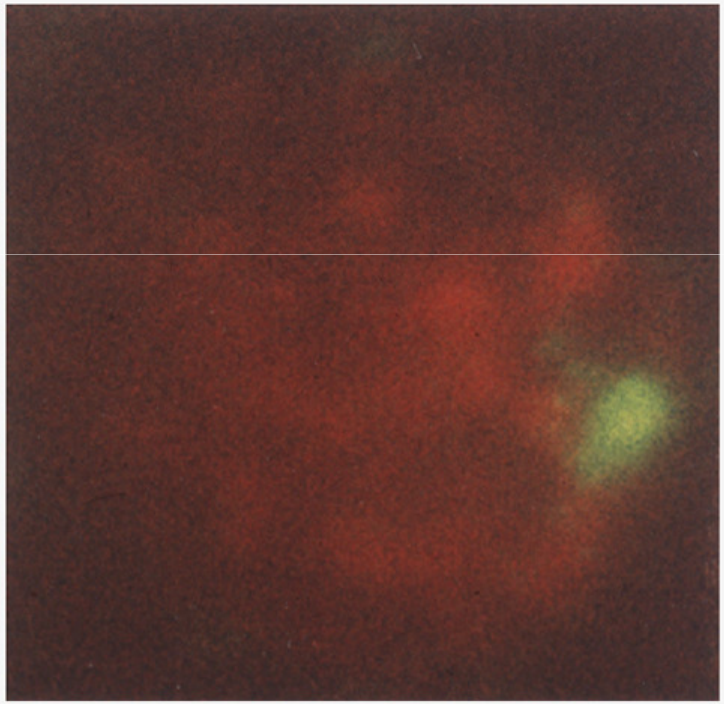

RECYCLING THE SINGLE CELL
8-cell embryo
sample dipstick
control "cell-free" dipstick
PCR: 1st round
(outer primers)
PCR: 2nd round
(nested primers)
Fluorescent in-situ hybridisation
(FISH) in solution

CELL RECYCLING

chromosomes and genes in a single cell

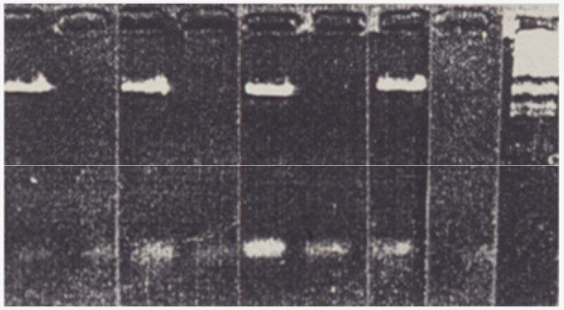
SINGLE BLASTOMERE

Y-CHROMOSOME BY FISH



B-GLOBIN SEQUENCE BY PCR

BL C BL C BL C BL C M



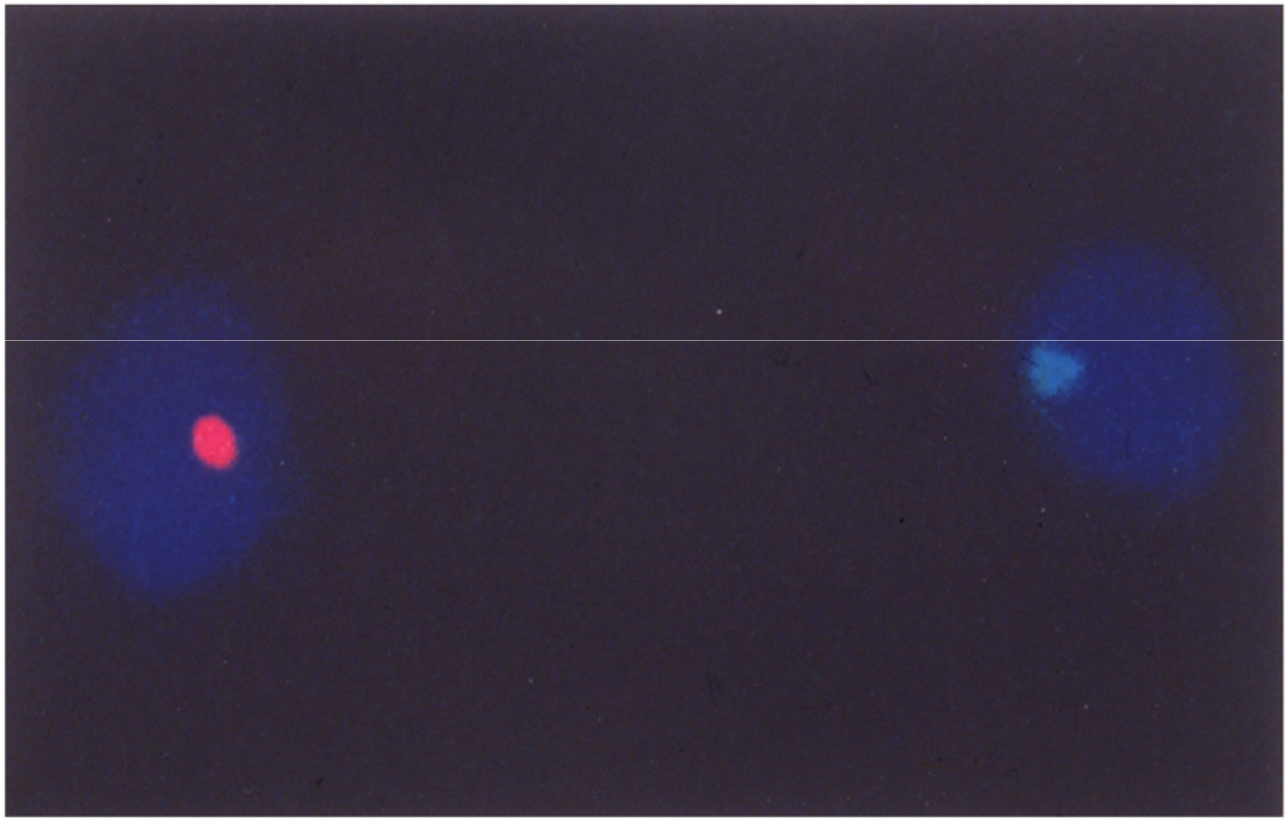
Results for 4 individual blastomeres fixed to glass

Thornhill et al, 1993

Also sexing and DMD, Thornhill and Monk 1996

X OR Y CHROMOSOME IN SINGLE HUMAN SPERM

49.0% X **49.2% Y**



Thornhilll, 1993

Gene expression and its regulation in embryos and germ cells

- **Direct analysis (one or two genes per embryo)**

Expression of known genes

Expression of imprinted genes

Epigenetic mechanisms of regulation of expression – CpG methylation

- **Embryo and PGC amplified cDNA (unlimited analysis)**

Comparison of expression of many genes within and between embryos

Identification of stage specific and treatment specific genes

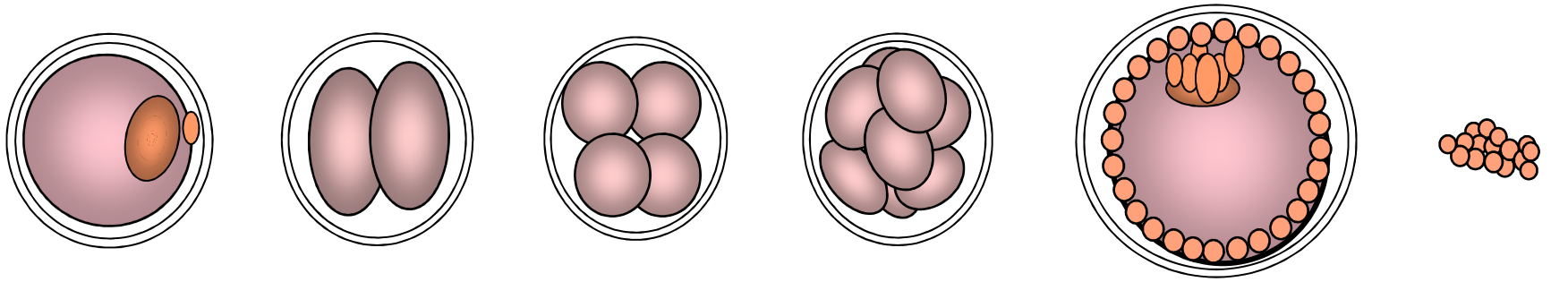
(GV, MI, MII, embryos and PGCs +/- S/O +/- IVM Marker genes)

Isolation of novel genes - oocyte-, embryo-, PGC-specific expressed genes

Isolation of embryo / cancer genes

cDNA libraries from single embryos

a limitless resource of all the expressed genes in



egg 2 cell 4 cell 8 cell blastocyst PGCs

Human eggs and embryos, surplus to IVF requirement and donated for research.

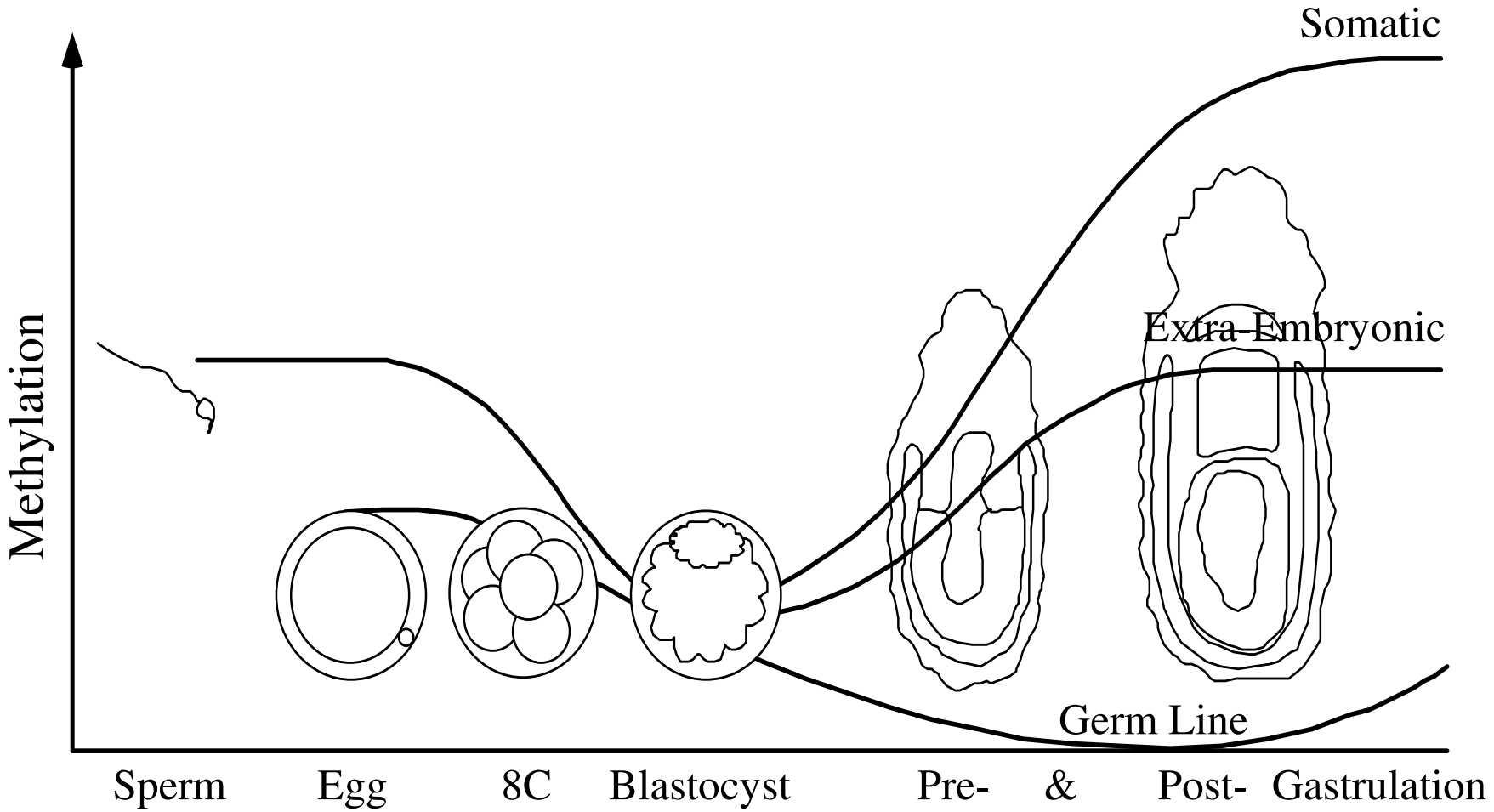
Human fetal primordial germ cells, EC and ES cells

Lysed to release mRNA which is copied and amplified as cDNA

cDNA is cloned into vector for libraries

cDNA screened for known and novel embryonic genes

Demethylation deprogramming and reprogramming in development



Monk, Boubelik & Lehnert 1987

Genes specifically expressed in human embryonic cells?

Initiation de-programming to proliferative stem cell

De-programming erasure gametic epigenetic programmes

Maintenance undifferentiated (archetypal) stem cell state

Immortality Embryo stem cells removed from

developmental constraints grow indefinitely

ICM ➲ES PGC ➲EG

Invasiveness e.g.,trophectoderm, PGC migration (*OLF-R?)*

Expression expected to be specific to embryos and germ cells

Human embryonic stem cells

Globally demethylated,

Self renewing and grow indefinitely *in vitro*

Undifferentiated and pluripotent

Invasive

Cancer cells share these properties

Isolate human embryonic and primordial germ cell genes which are not expressed in somatic cells.

Test their expression on cancer cells

New cancer genes? New cancer vaccines?

Isolation and identification
of oocyte-, embryo- and PGC-specific expressed genes
also expressed in cancers

Differential display

DNA sequence Design primers

Confirm stage specific expression (or treatment specific)

Confirm not expressed in range of somatic tissues

Test expression on range of cancer cDNAs

Database analysis Map Identify pseudogenes

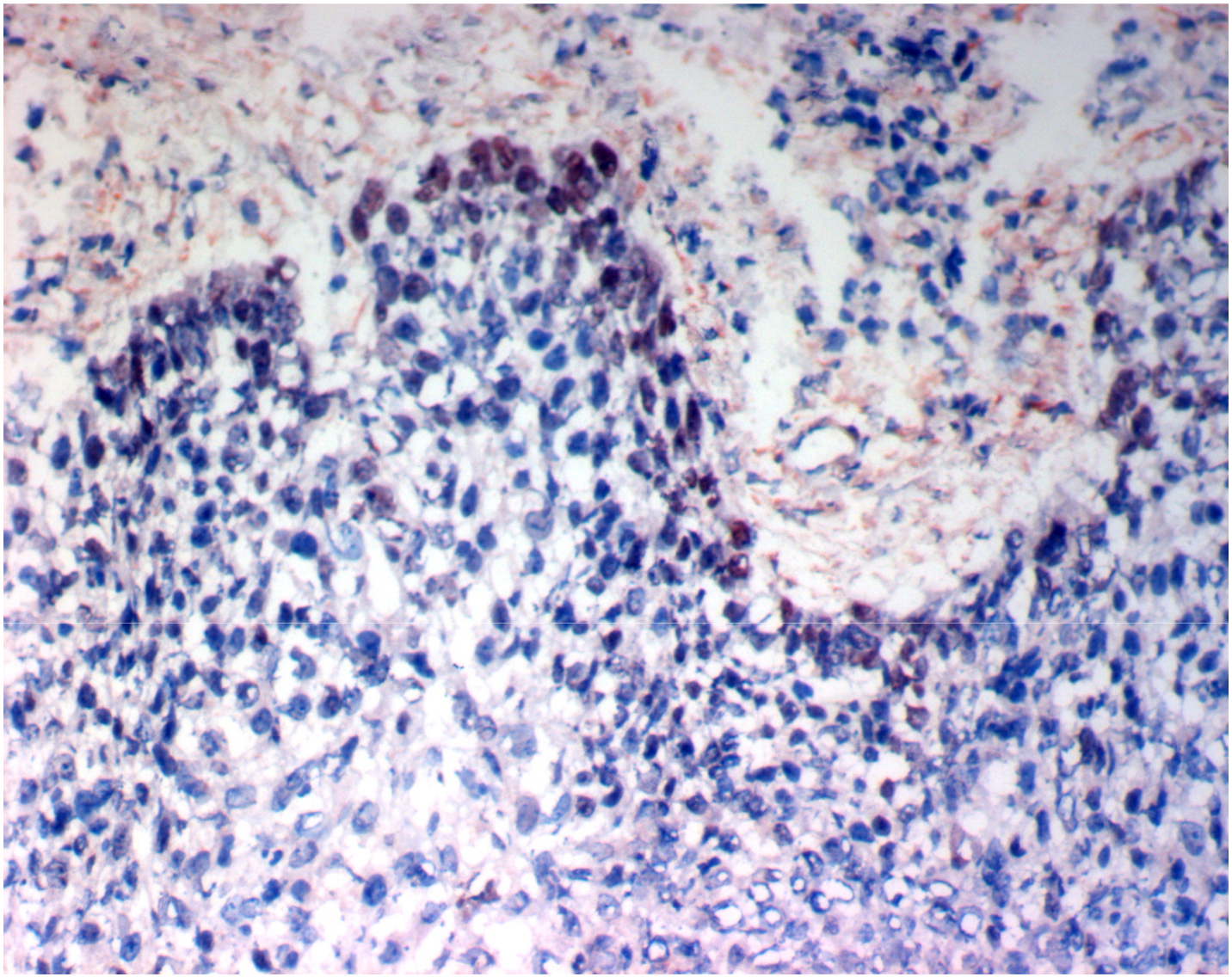
Extend sequences, identify gene exon/intron structure

Northern blot for protein

Immuno-histochemistry on cancer sections

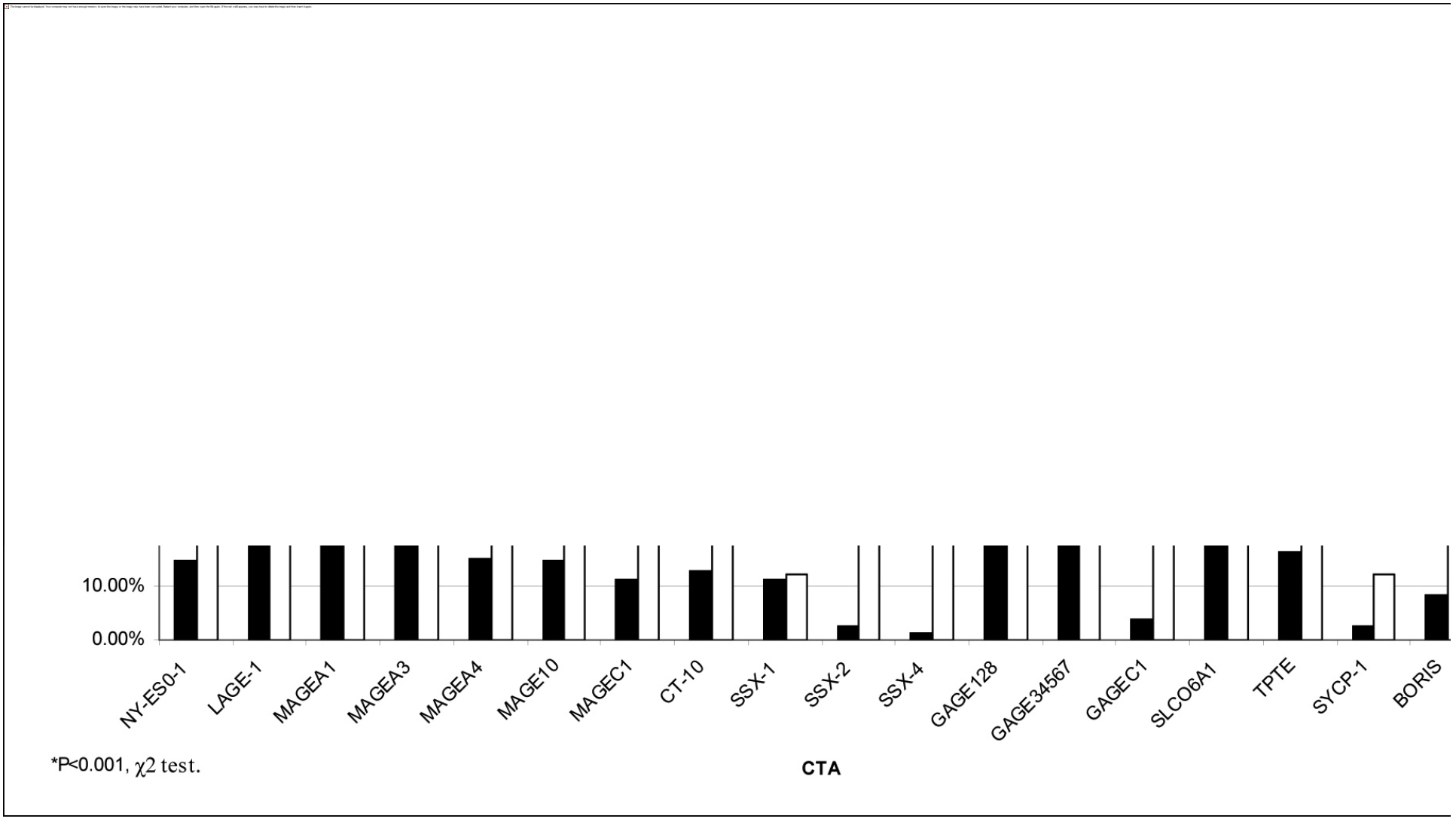
Co-expression with other CT genes

Presence of antibody in patient serum



ECSA stains basally located subpopulation of cells in squamous cell NSCLC …putative cancer stem cells?

Monk & Holding, 1991 John, Fortunato, Cebon and Monk

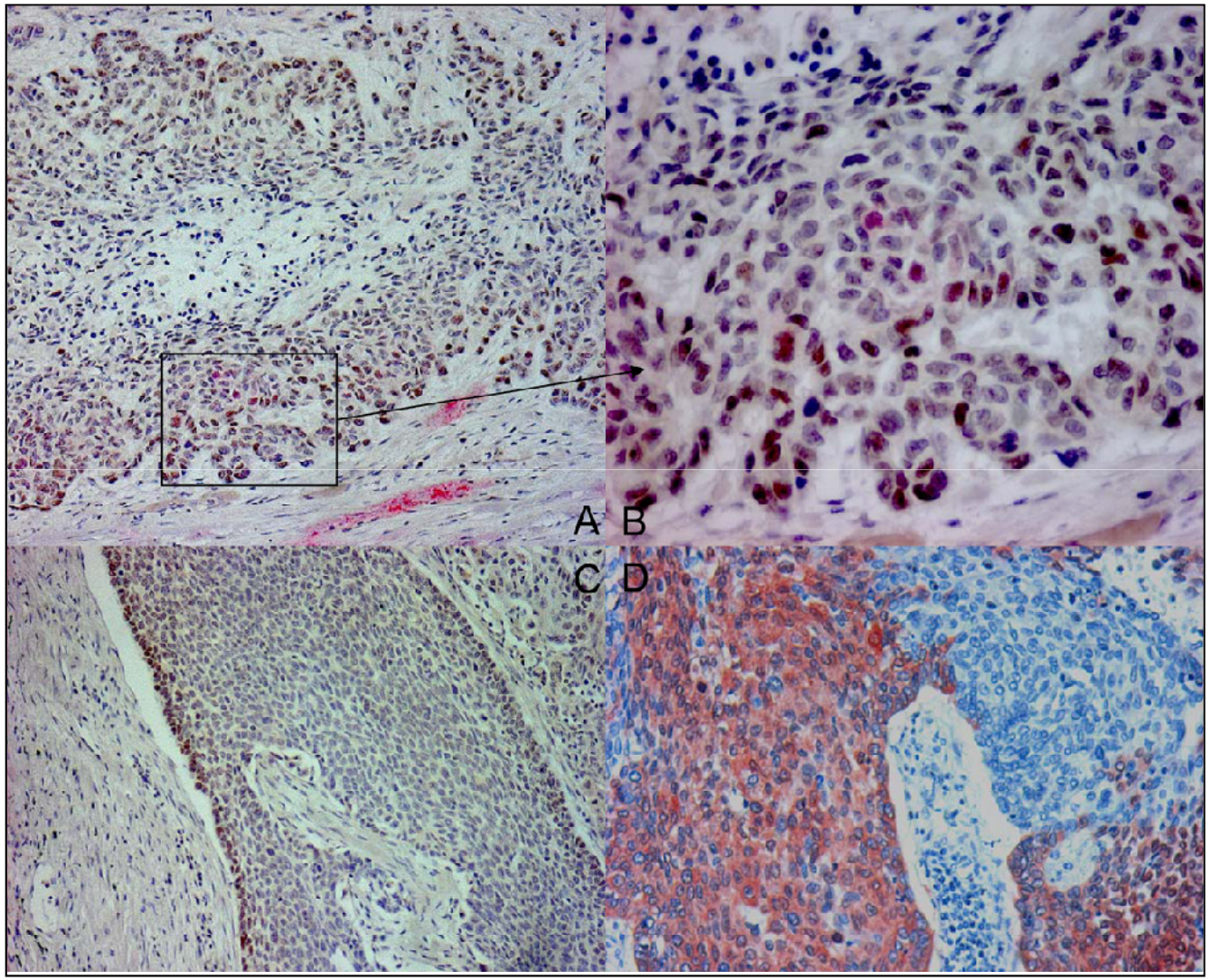


Enrichment for CTAs in *ECS*A positive tumours

Caballero, Monk, Cebon,

ECSA is co-expressed with CT antigens in stem cells in squamous cell NSCLC

ECSA & NY-ESO-1



ECSA & NY-ESO-1

MAGE-C1

NY-ESO-1 and MAGE-C1 stain many more cells in the tumour

John et al, 2008

ECSA is an immunogenic cancer stem cell gene

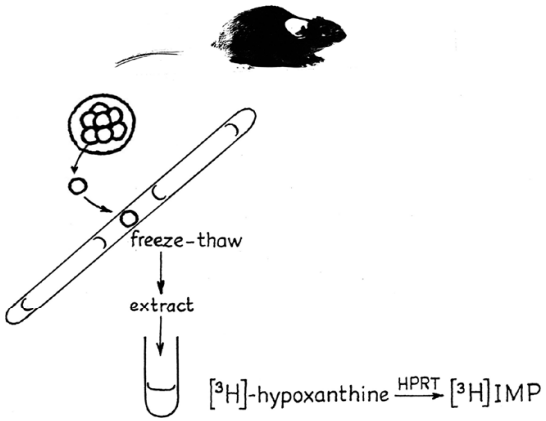
- Minority of cells, basally located, in lung cancer.
- Wide range of cancers - lung, colon, lymphoma, melanoma
- Many CT genes are co-expressed with *ECSA* but they also extend into other derivative cells (*NY-ESO*-1, *MAGE*-C1)
- **Caution** - *In vitro* may not reflect *in vivo*!

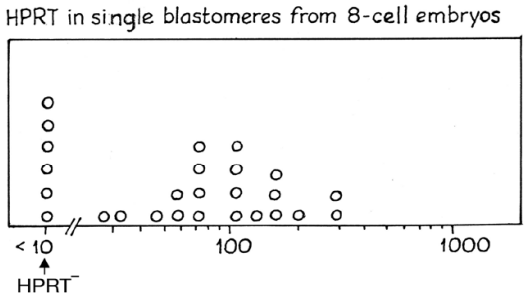
  Passaged cancer cell lines and ES cells accumulate changes in gene expression e.g., *OCT*4 in Clontech passaged cancers & cancer cell lines and *BORIS* in ES cells

  (Monk & Holding, 2001; Monk, Hitchens & Hawes, 2008).

Early Models for Preimplantation Diagnosis Summary

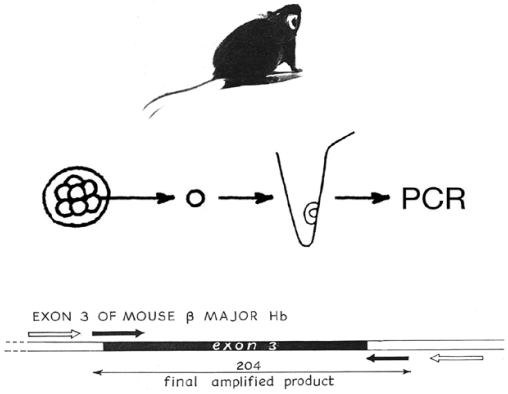
a. Lesch-Nyhan $X^{HPRT+}X^{HPRT-}$





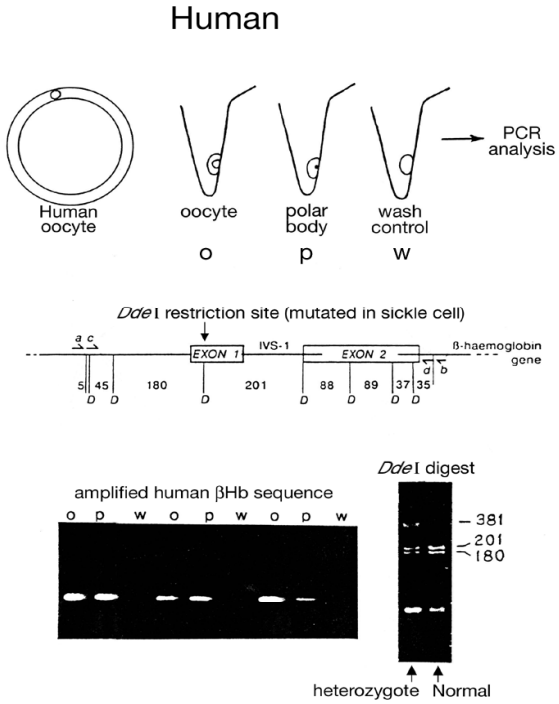
Monk et al, 1987

b. Thalassaemia $\beta Hb^{+} / \beta Hb^{\nabla}$



Holding & Monk, 1989

c. Sickle cell anaemia



Monk & Holding, 1990

ACKNOWLEDGEMENTS

Mammalian Development Unit

Anne McLaren
Mary Harper
Mandy Fosten
Asangla Ao

Susan Lindsay
Andy McMahon
Mark Grant
Michael Boubelik

Maurizio Zuccotti
Cathy Holding

Institute Child Health London

Roland Levinsky
Marcus Pembrey
Rob Daniels
Alan Thornhill
Elena Kontogianni
John Huntriss
Tetsuya Goto
James Adjaye
Roberto Lorenzi

Maurizio Zuccotti
Cathy Holding
Megan Hitchins

Kings College Hospital

Virginia Bolton

Hammersmith Hospital

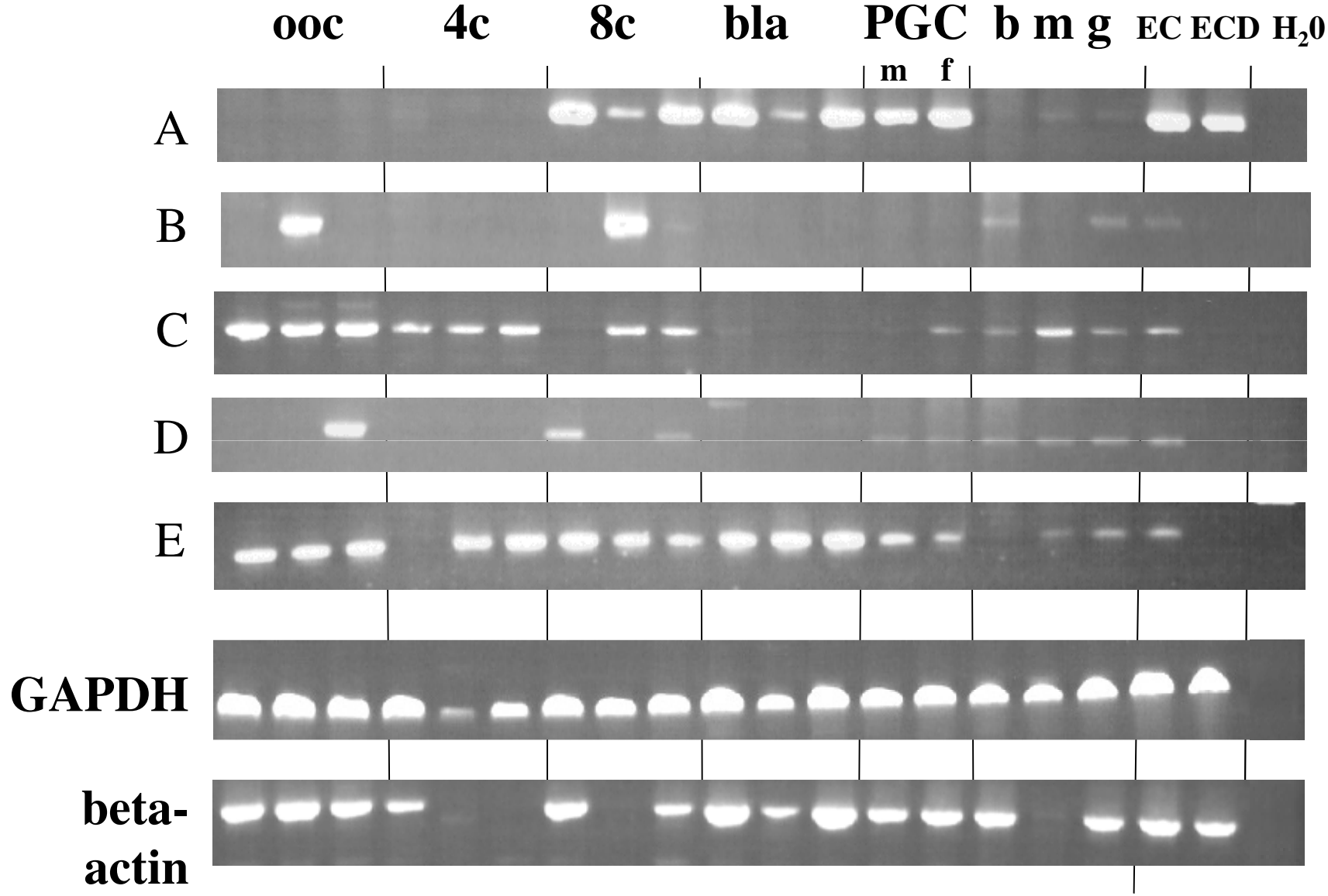
Alan Handyside

Ludwig Institute of Cancer Research

Melbourne

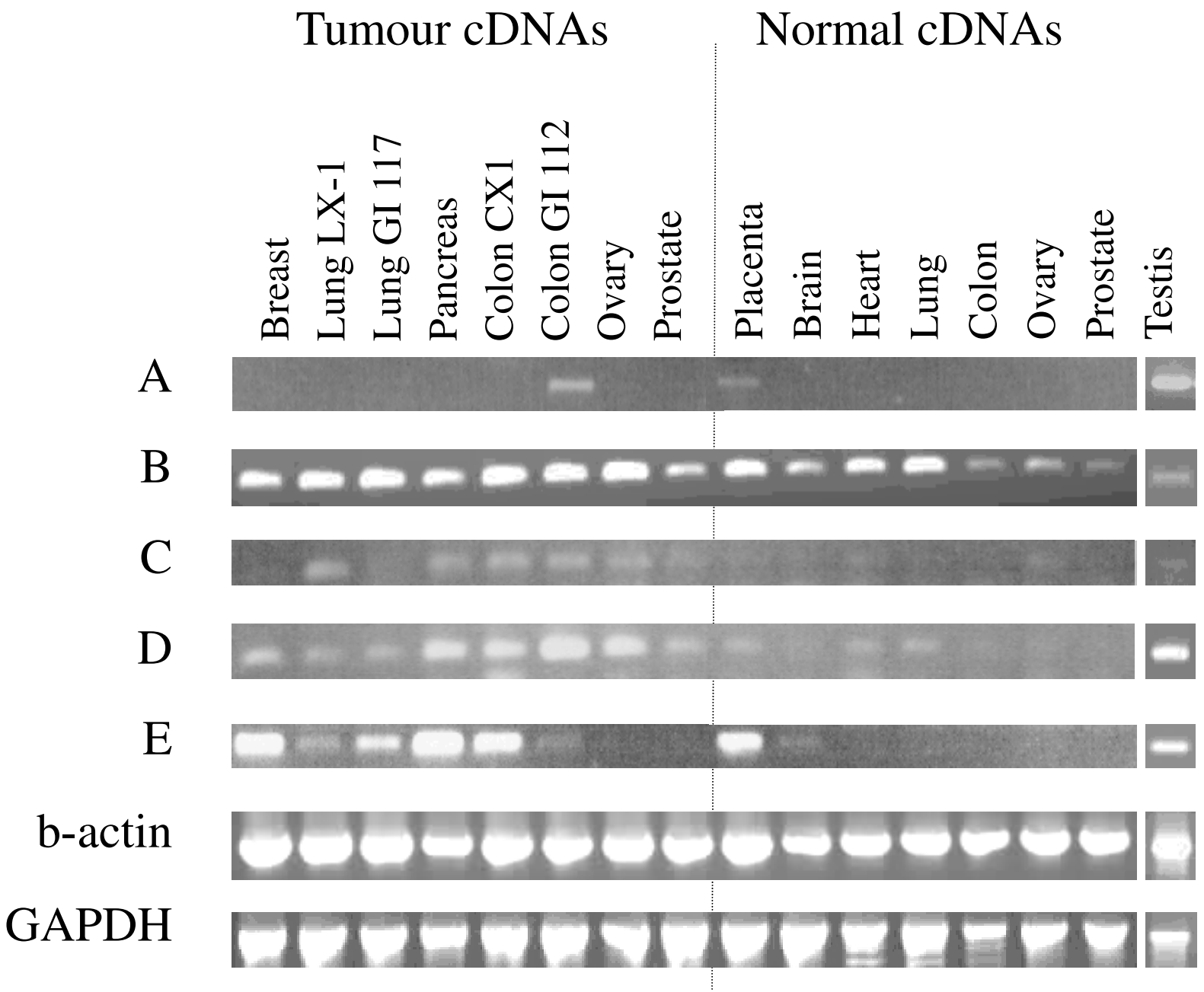
Tom John
Suzanne Svobodova
Jonathan Cebon

Confirmation of specificity of expression of human embryonic genes



Monk & Holding, 2001

Expression of human embryonic genes in human tumours



Profiles of expression of *ECSA*, *OCT4*, *BORIS and NANOG* in embryos and cultured ES cells

- ***ECSA***

Embryo-specific gene, putative cancer stem cell gene, expressed ir
high proportion of lung, liver and colon cancers.

Monk and Holding, 2001

- ***BORIS***

Brother of regulator of imprinted sites (brother of *CTCF*)
Expressed only in primary spermatocytes in testis (when *CTCF* off)
Candidate gene for deprogramming in spermatogenesis
CT gene

Loukinov et al., 2002

- ***OCT4*** and *NANOG*

Standard well-known embryonic genes

Different developmental profiles of expression

of *OCT*4, *BORIS*, *ECS*-A

in human -

Gonads – testis and ovary

Oocytes

Preimplantation embryos

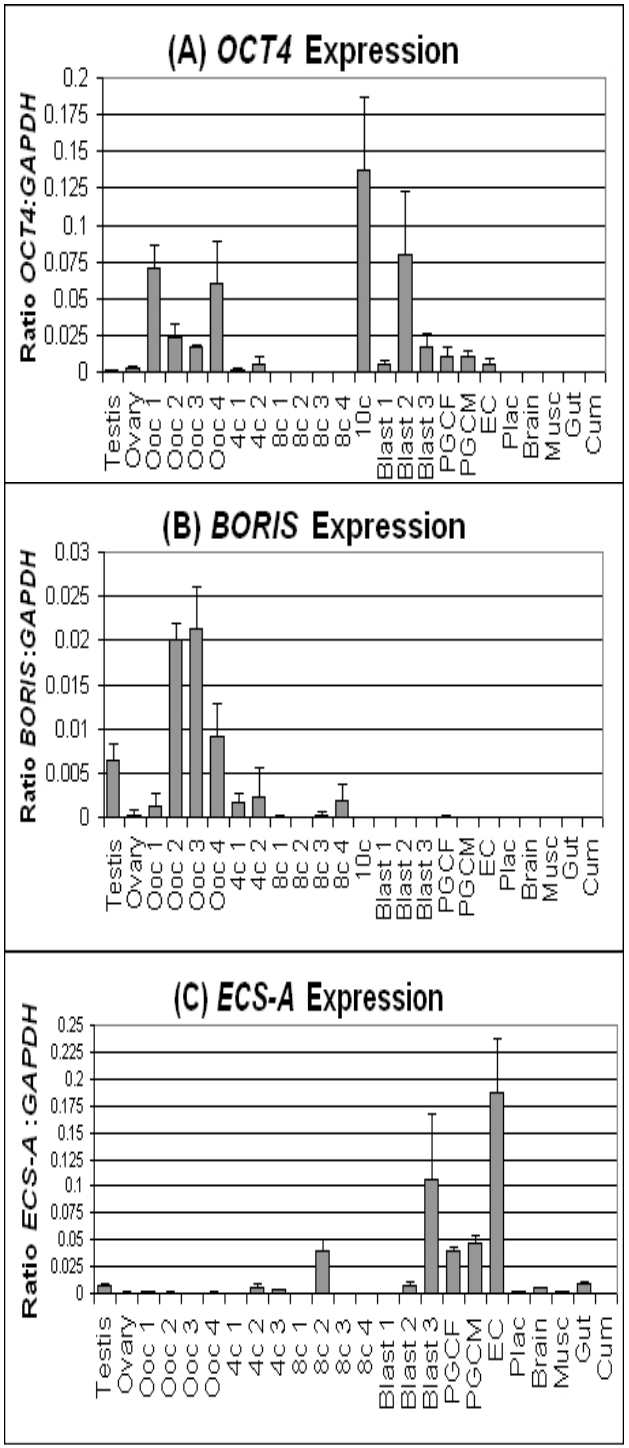
Primordial germ cells

Placenta

Fetal brain muscle and gut

Cumulus cells

Monk& Hitchins, 2006

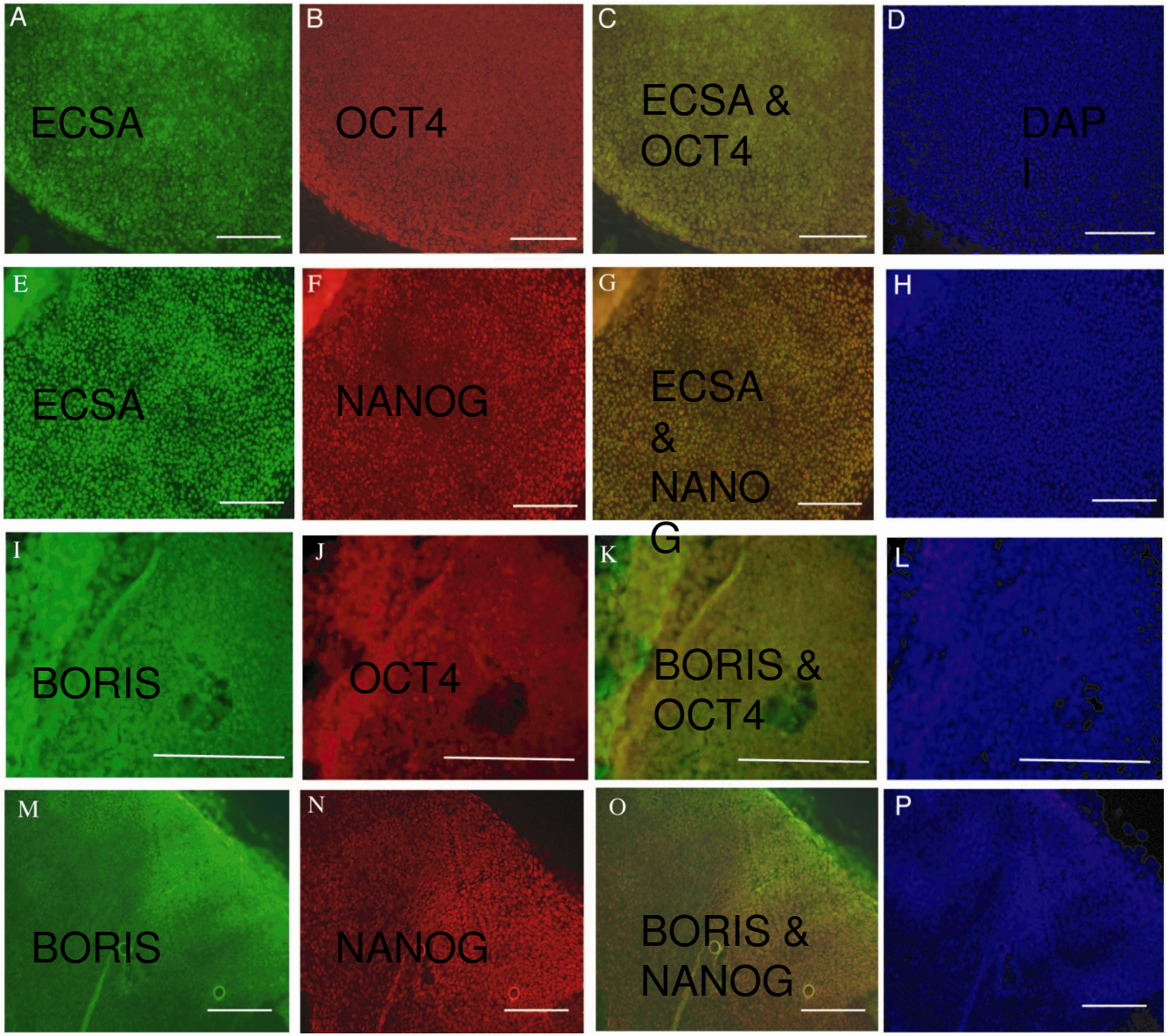


OCT*4/*GAPDH

BORIS*/*GAPDH

ECS*-A/*GAPDH

Co-expression ECSA, BORIS, OCT4 and NANOG in human ES cells



Monk, Hitchins & Hawes, 2008

INHERITED GENETIC DISEASE

For couples at risk: 1 in 4 offspring affected

PRENATAL DIAGNOSIS

Amniocentesis ~16
Chorionic villus ~11
} weeks pregnancy

If fetus affected – abortion offered

INHERITED GENETIC DISEASE

PRE-IMPLANTATION DIAGNOSIS

Biopsy of single blastomere
Biopsy of 5 trophectoderm cells
} <1 week after fertilisation

Replace only embryo without defect to mother. Abortion is therefore avoided.

PRE-IMPLANTATION BIOPSY AND DIAGNOSIS

Sexing rabbit embryos by sex chromatin in trophoblast sample.

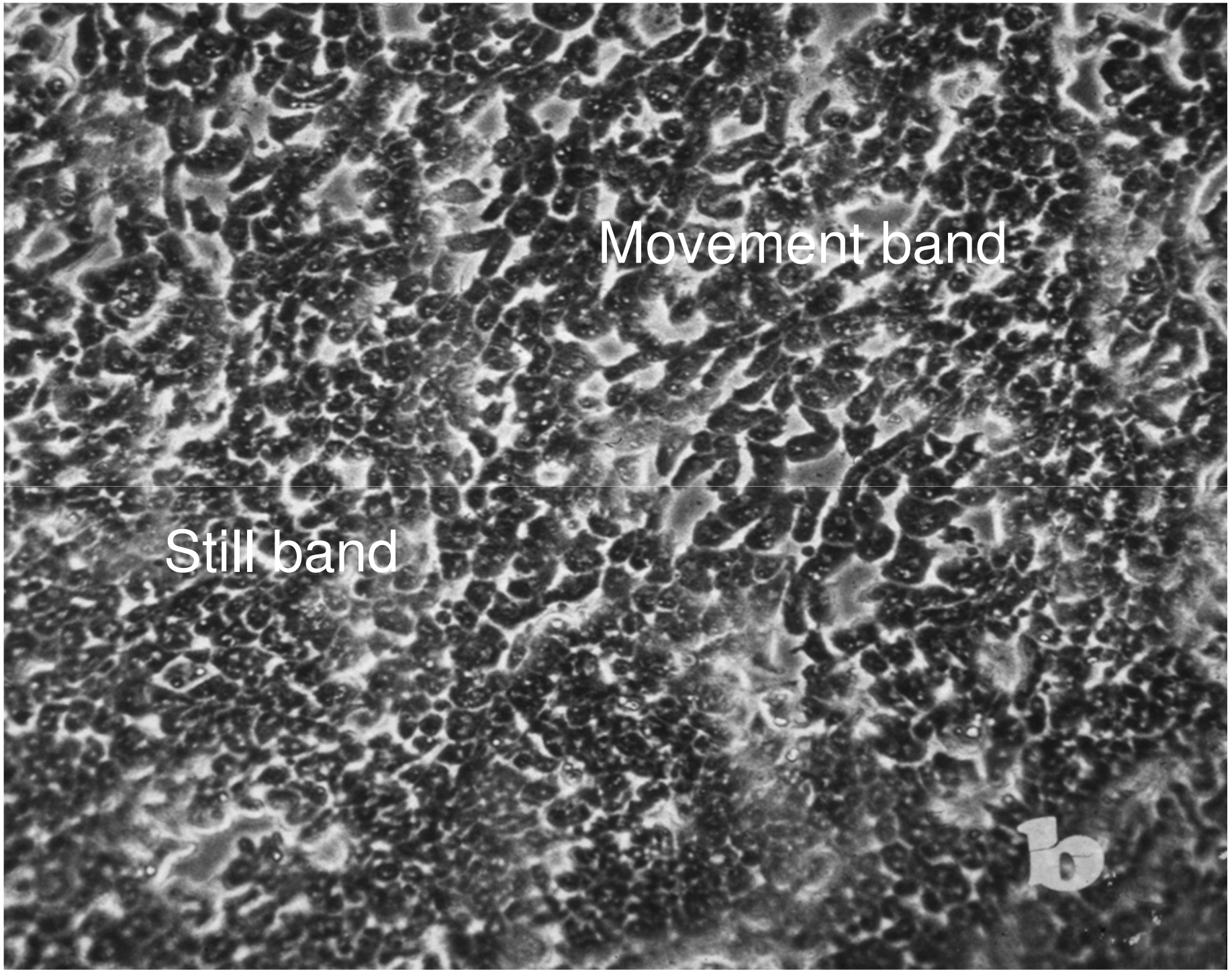
(*Gardner & Edwards*, 1968)

Embryo-splitting at 2-cell and 4-cell stage to produce monozygotic twins or quadruplets in cows and sheep.

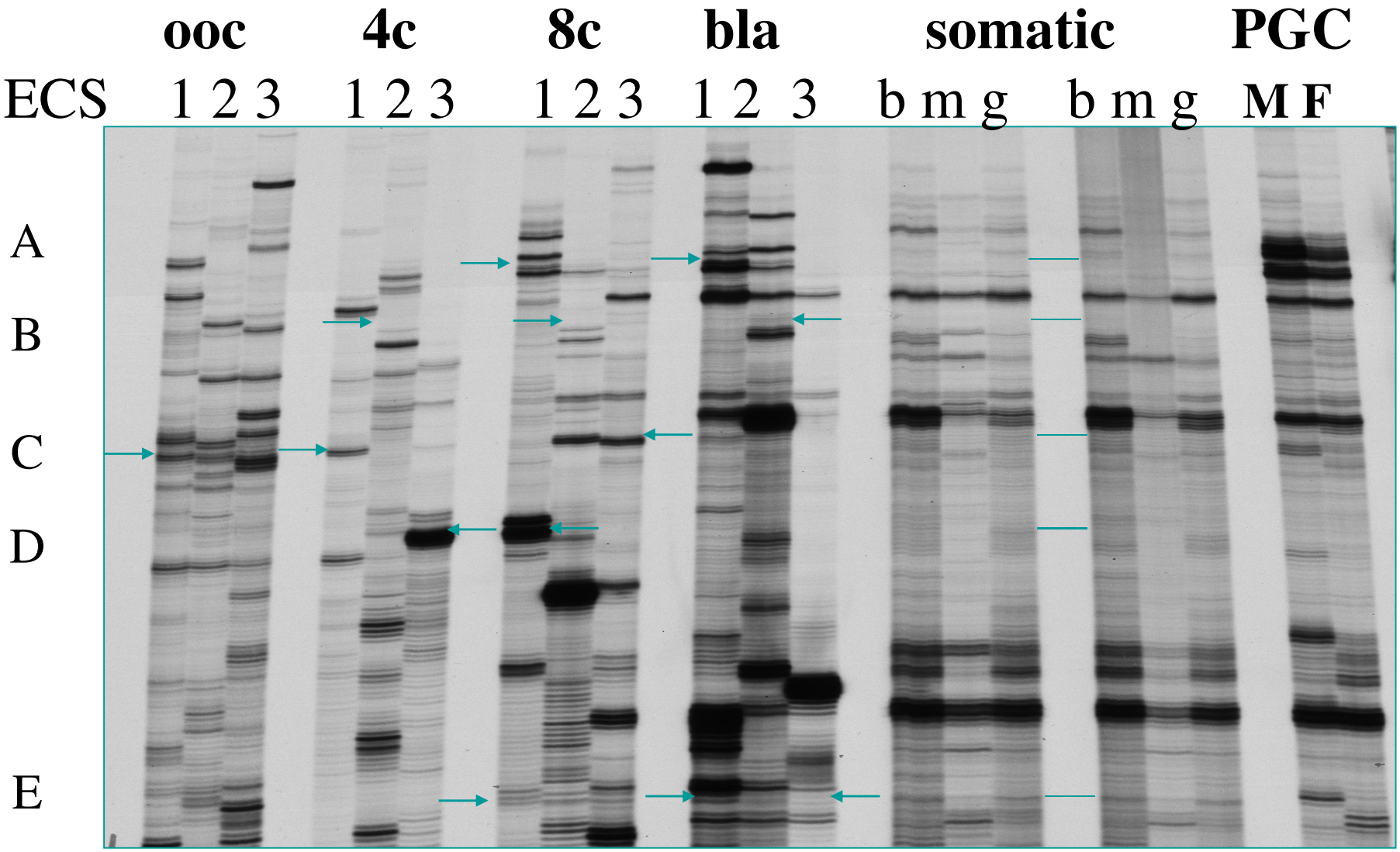
(*Fehilly & Willadsen*, 1986)

Diagnosis of sex or genetic disease in a single cell from the 8-cell stage or a few trophoblast cells from the blastocyst.


Slime mould amoebae
Dictyostelium discoideum
1970 -1974


Movement band
Still band
10

Differential display identifies human embryo-specific expressed genes



Monk & Holding, 2001