IS THREE A CROWD?

AN UPDATE ON NUCLEAR TRANSFER, CLONING AND OTHER EXCITING STORIES

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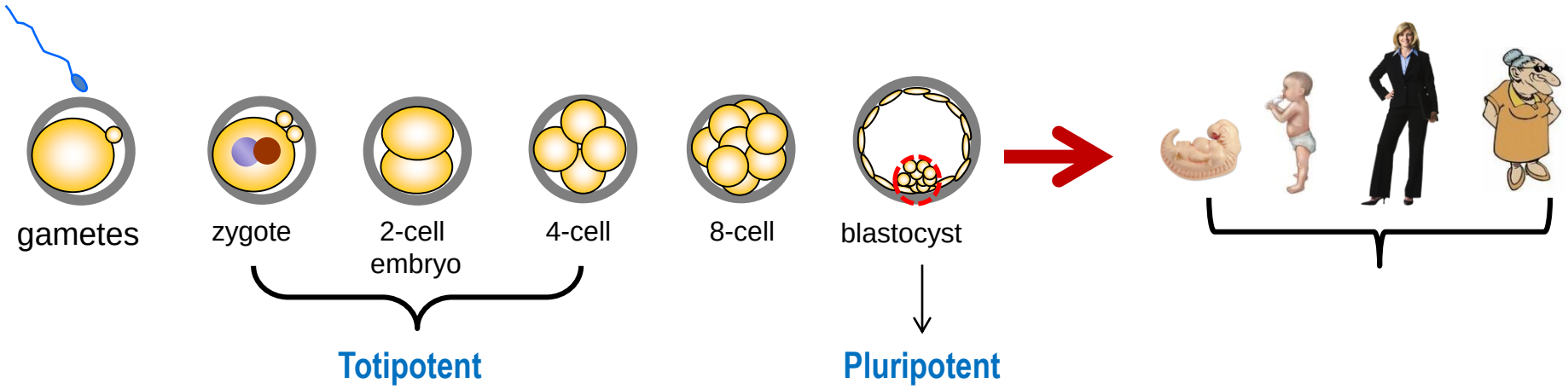
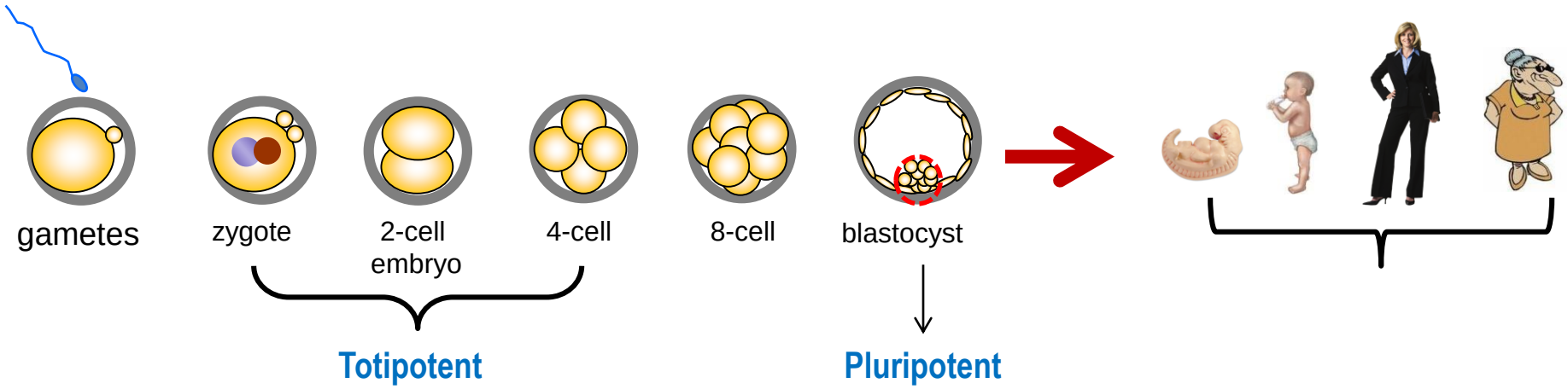
Oregon National Primate Research Center

Cell therapy - in vitro fertilization (IVF)



The American Society of Gene & Cell Therapy defined the cell therapy as **the administration of live whole cells or maturation of a specific cell population in a patient for the treatment of a disease**

Cell therapy - in vitro fertilization (IVF)



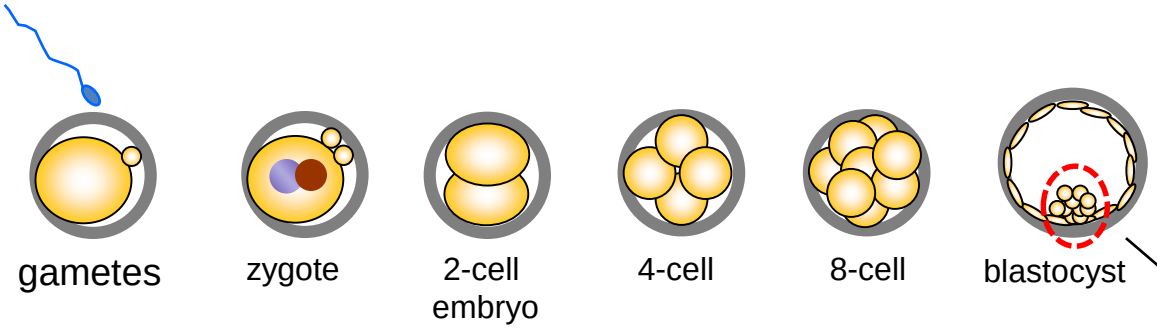
Robert Edwards is awarded the 2010 Nobel Prize for the development of IVF therapy (1978)


Evening News

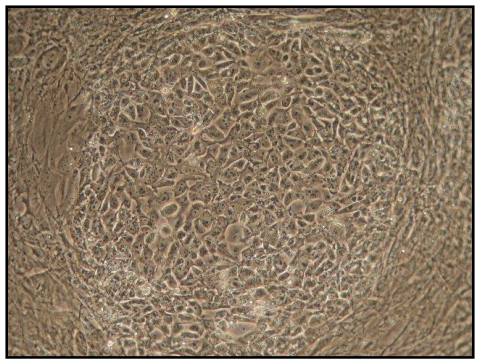
Meet Louise, the world's first test-tube arrival

SUPERBABE

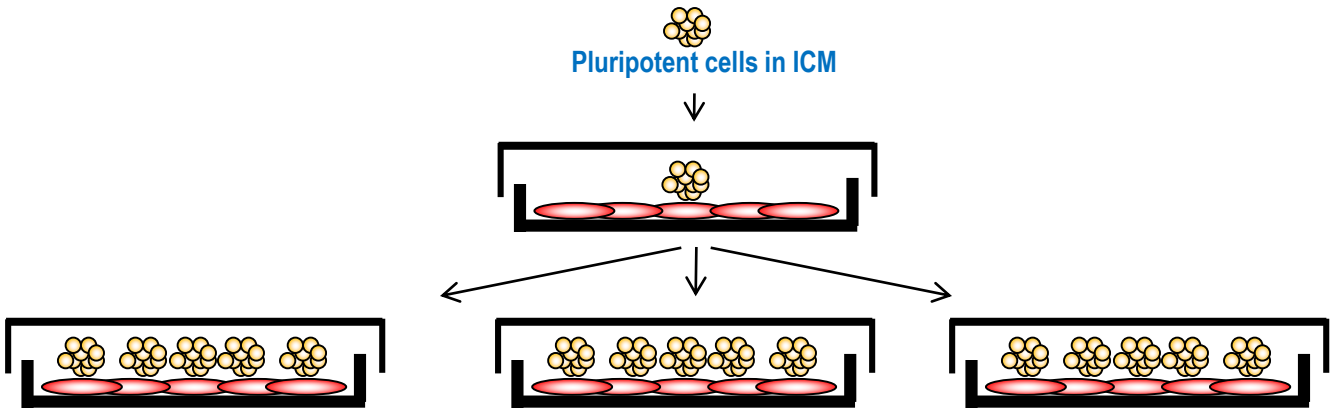
Embryonic Stem Cells



- ✓ self-renewal
- ✓ Immortal



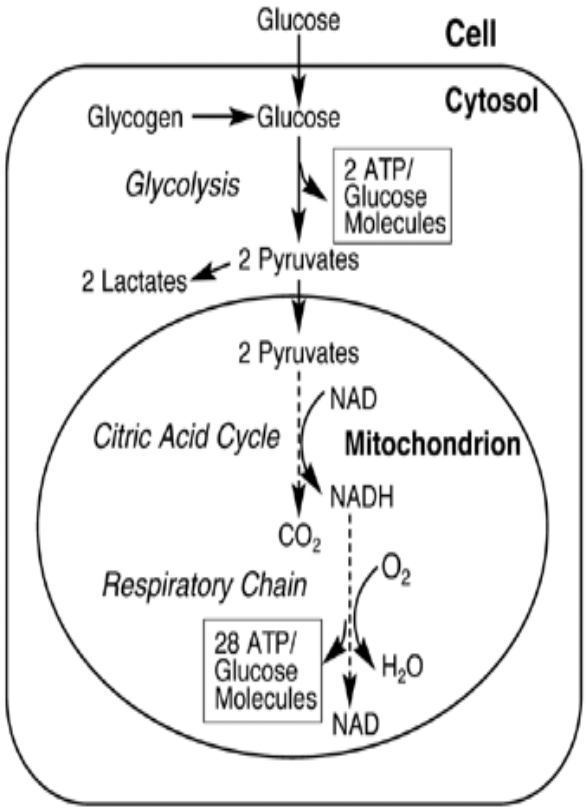
Embryonic Stem (ES) cells

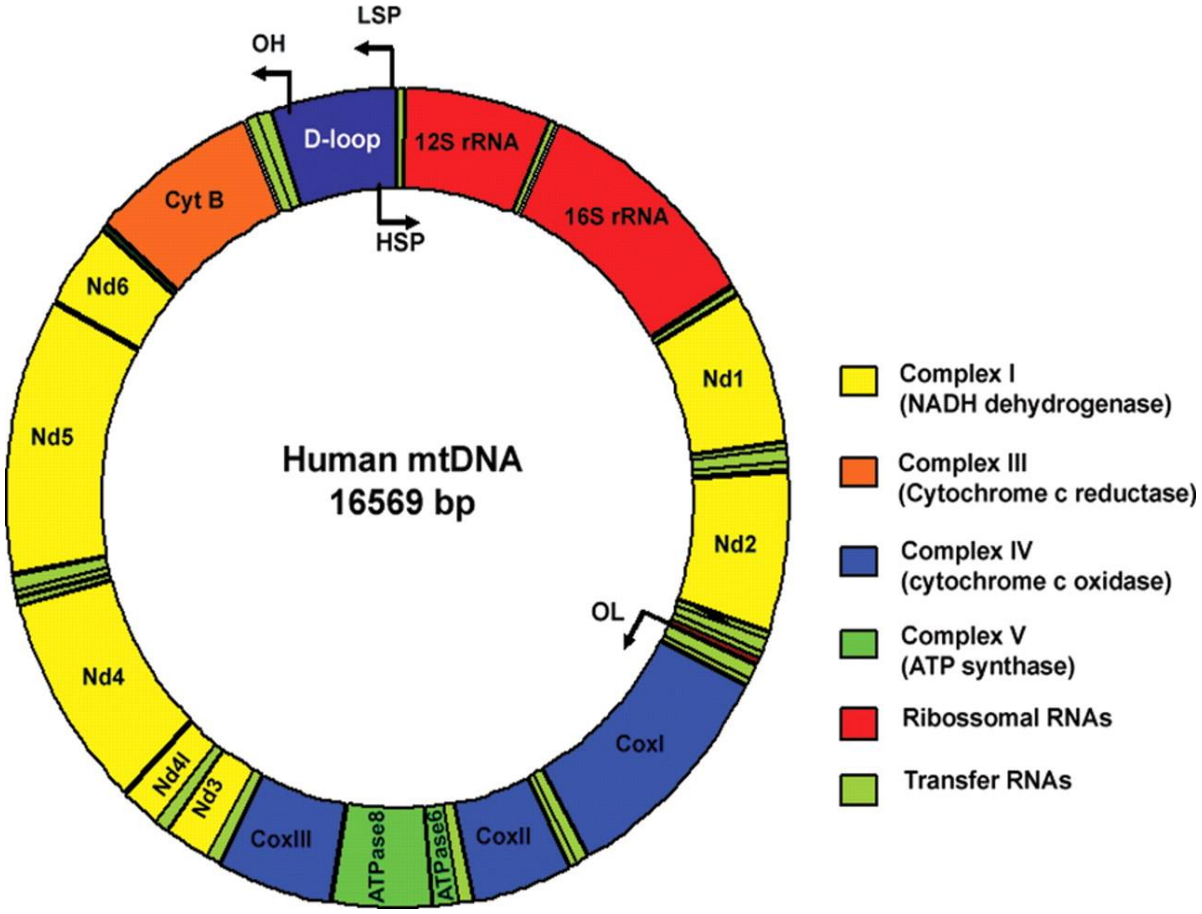


- ✓ Mouse ES cells (Evans and Kaufman, 1981; Martin, 1981)
- ✓ Rhesus macaque ES cells (Thomson et al., 1995)
- ✓ Human ES cells (Thomson et al., 1998)

2007 Nobel Prize: Mario R. Capecchi, Martin Evans And Oliver Smithies

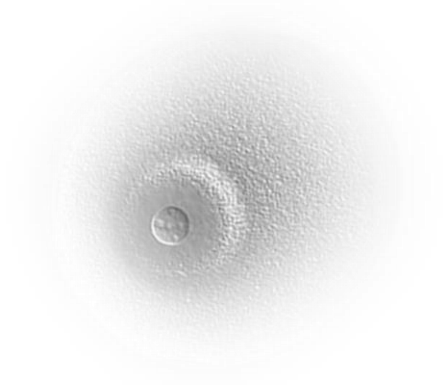
Mitochondrial function and mtDNA



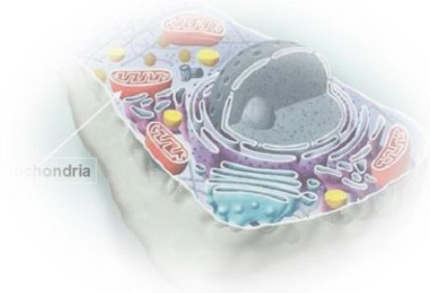


John et al.2010 Human Reproduction

Mitochondrial Genome Fact Sheet



- ✓ mtDNA is maternally inherited - through the egg
- ✓ Clonal inheritance?
- ✓ Cells have thousand copies of mtDNA
- ✓ mtDNA is more prone to mutations (homoplasmy and heteroplasmy)



Diseases caused by mtDNA mutations

- ✓ There are more than 700 known disease-associated mtDNA mutations (mitomap.org)
  - 285 tRNA/rRNA
  - 266 protein coding and control region point mutations;
  - 131 deletions

- ✓ Inherited - **neuropathy, encephalopathy, cardiomyopathy, myopathy, diabetes, metabolic syndromes**

- ✓ Acquired, age related - neurodegenerative diseases, Parkinson, ALS, heart diseases, diabetes, cancer

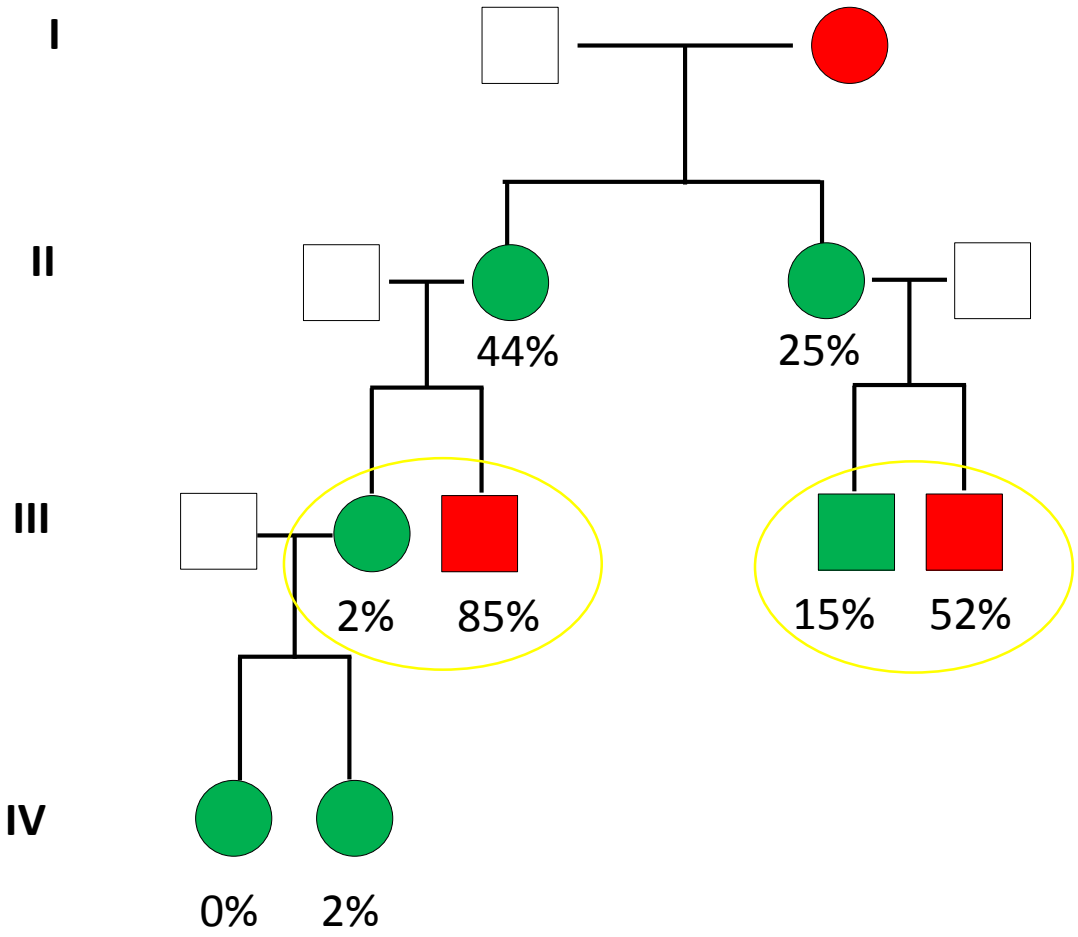
Clinical disorders caused by inherited mutations in mitochondrial DNA

Mitochondrial DNA disorder	Clinical phenotype	mtDNA genotype	Gene	Status	Inheritance
Kearns–Sayre syndrome	Progressive myopathy, ophthalmoplegia, cardiomyopathy	A single, large-scale deletion	Several deleted genes	Heteroplasmic	Usually sporadic
CPEO	Ophthalmoplegia	A single, large-scale deletion	Several deleted genes	Heteroplasmic	Usually sporadic
Pearson syndrome	Pancytopoenia, lactic acidosis	A single, large-scale deletion	Several deleted genes	Heteroplasmic	Usually sporadic
MELAS	Myopathy, encephalopathy lactic acidosis, stroke-like episodes	3243A>G; 3271T>C Individual mutations	*TRNL1* *ND1 and ND5*	Heteroplasmic Heteroplasmic	Maternal Maternal
MERRF	Myoclonic epilepsy, myopathy	8344A>G; 8356T>C	*TRNK*	Heteroplasmic	Maternal
NARP	Neuropathy, ataxia, retinitis pigmentosa	8993T>G	*ATP6*	Heteroplasmic	Maternal
MILS	Progressive brain-stem disorder	8993T>C	*ATP6*	Heteroplasmic	Maternal
MIDD	Diabetes, deafness	3243A>G	*TRNL1*	Heteroplasmic	Maternal
LHON	Optic neuropathy	3460G>A 11778G>A 14484T>C	*ND1* *ND4* *ND6*	Hetero- or homoplasmic Hetero- or homoplasmic Hetero- or homoplasmic	Maternal Maternal Maternal
Myopathy and diabetes	Myopathy, weakness, diabetes	14709T>C	*TRNE*	Hetero- or homoplasmic	Maternal
Sensorineural hearing loss	Deafness	1555A>G Individual mutations	*RNR1* *TRNS1*	Homoplasmic Hetero- or homoplasmic	Maternal Maternal
Exercise intolerance	Fatigue, muscle weakness	Individual mutations	*CYB*	Heteroplasmic	Sporadic
Fatal, infantile encephalopathy; Leigh/Leigh-like syndrome	Encephalopathy, lactic acidosis	10158T>C; 10191T>C	*ND3*	Heteroplasmic	Sporadic

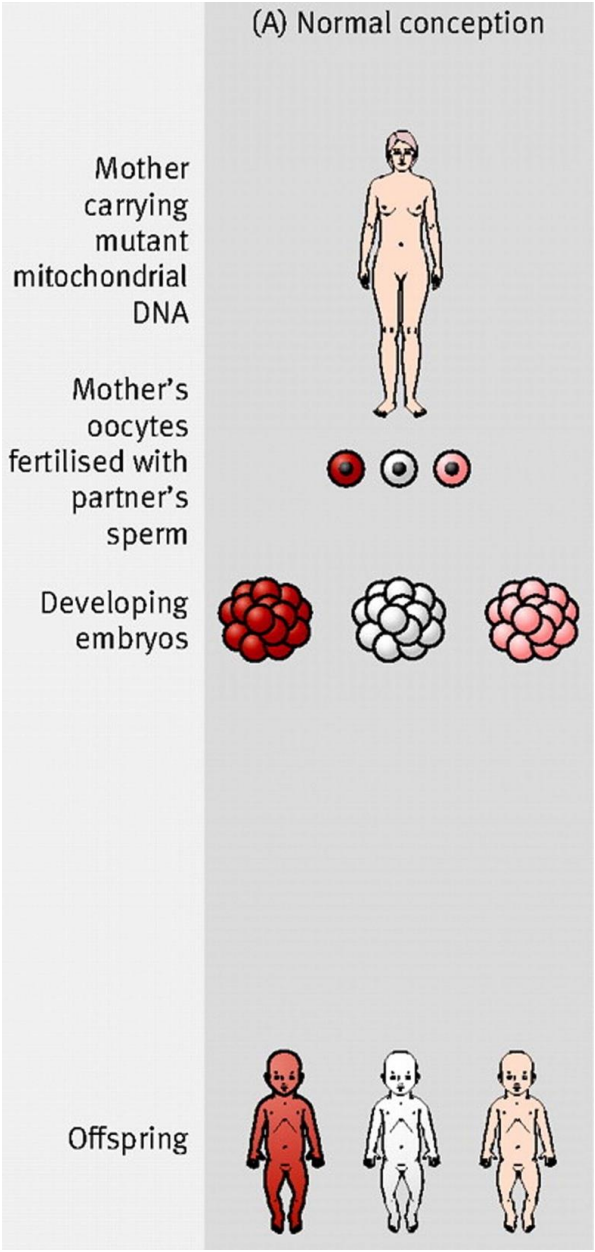
Taylor & Turnbull
Nature Reviews Genetics 2005

Complex nature of mtDNA inheritance

Leber's hereditary optic neuropathy (LHON)

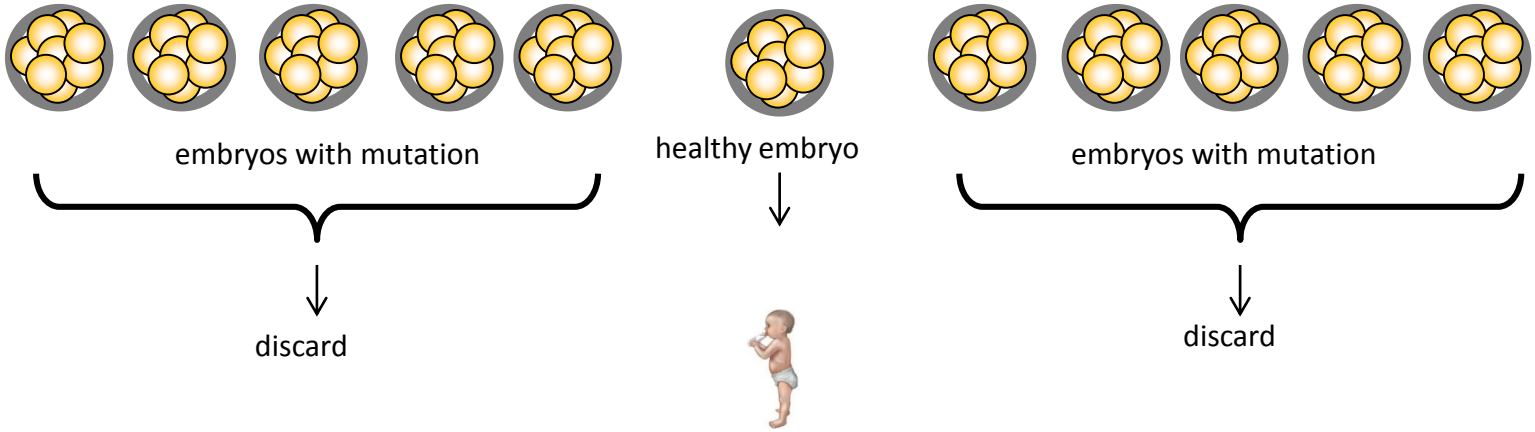


Fixed mode of mtDNA transmission

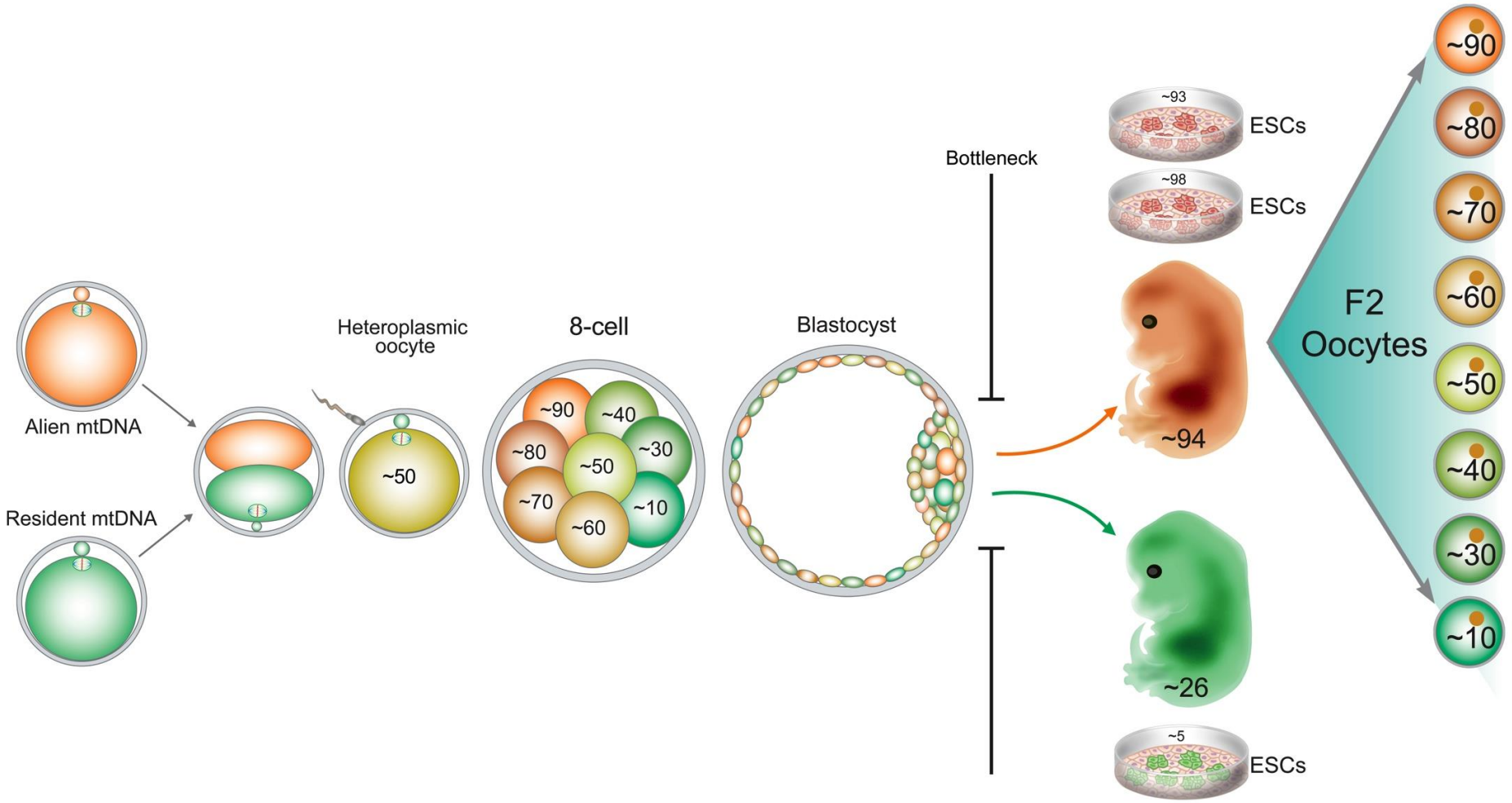


Poulton et al., 2009

Preimplantation Genetic Diagnosis
(also known as embryo screening)

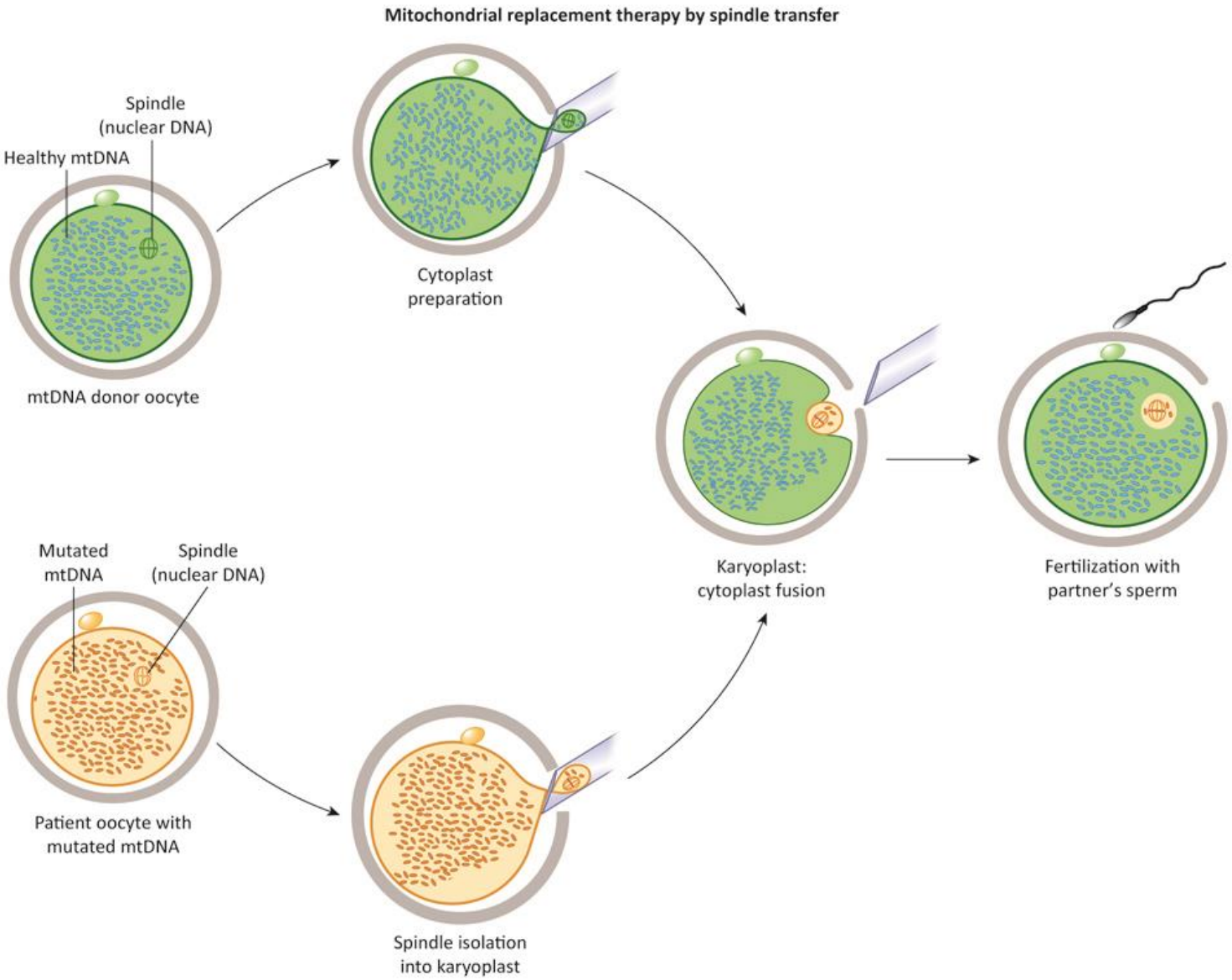


Mitochondrial genome segregation and bottleneck

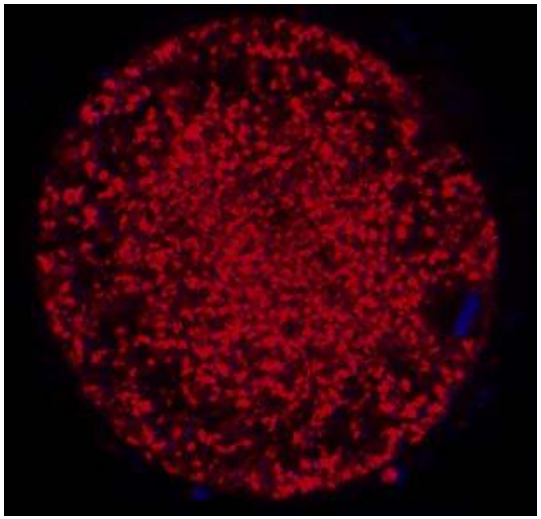


Lee et al., Cell Reports 2012

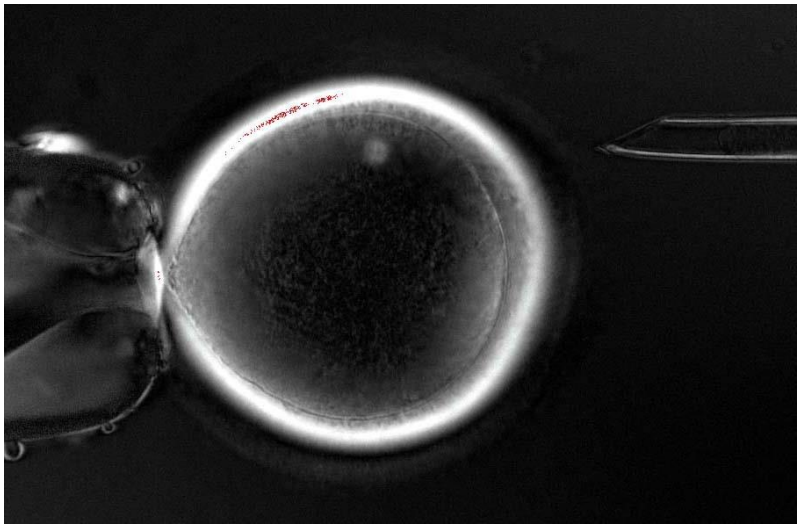
Mitochondrial Replacement Therapy
to prevent germline transmission of mtDNA mutations



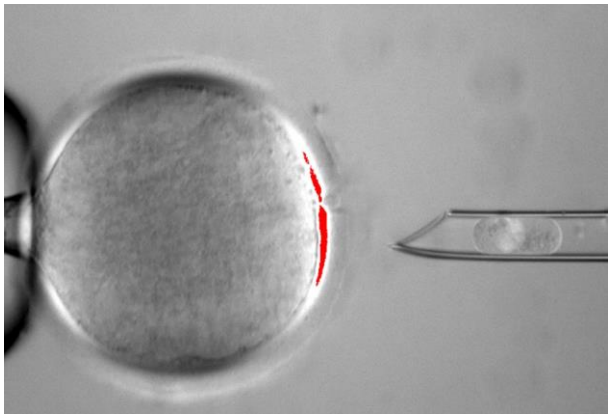
Mitochondrial gene replacement in oocytes



Distribution of mitochondria in mature oocytes



Spindle imaging



Spindle removal

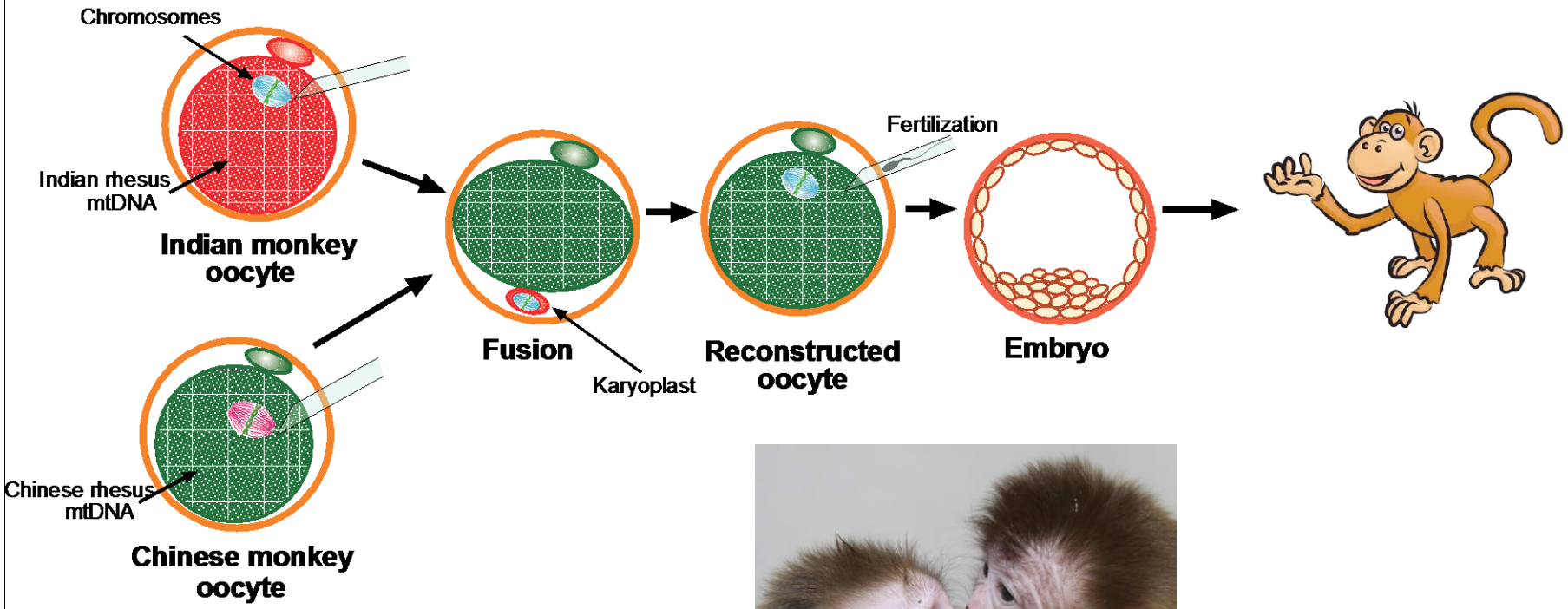
Separated chromosomes (nuclear DNA) and mitochondrial DNA

Vol 461|17 September 2009|doi:10.1038/nature08368 nature

ARTICLES

Mitochondrial gene replacement in primate offspring and embryonic stem cells

Masahito Tachibana[1], Michelle Sparman[1], Hathaitip Sritanaudomchai[1], Hong Ma[1], Lisa Clepper[1], Joy Woodward[1], Ying Li[1], Cathy Ramsey[1], Olena Kolotushkina[1] & Shoukhrat Mitalipov[1,2,3]

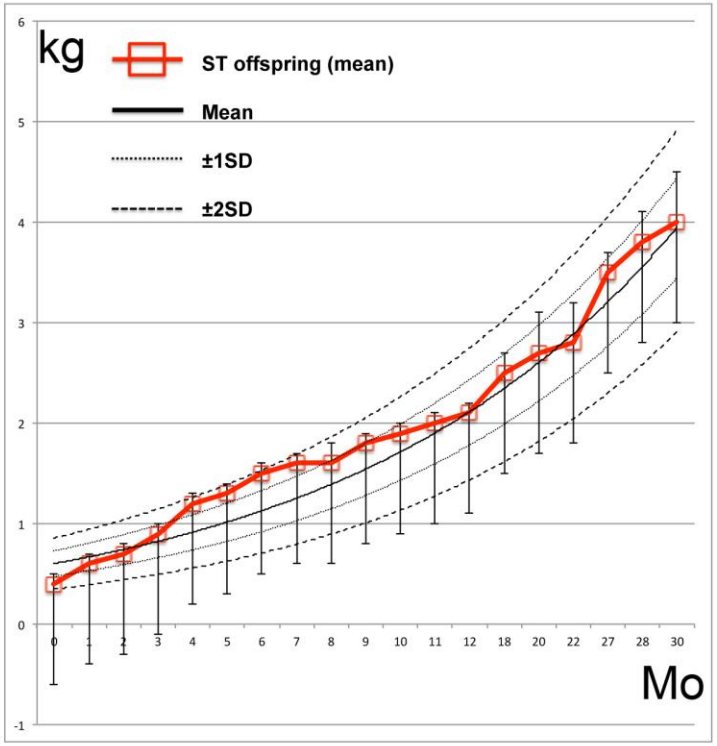


Tachibana et al., Nature, 2009

Mito & Tracker

A

B



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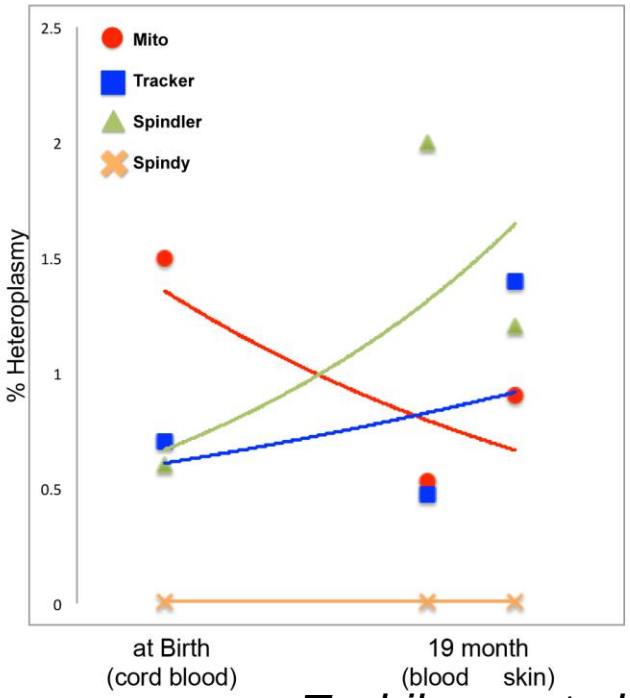


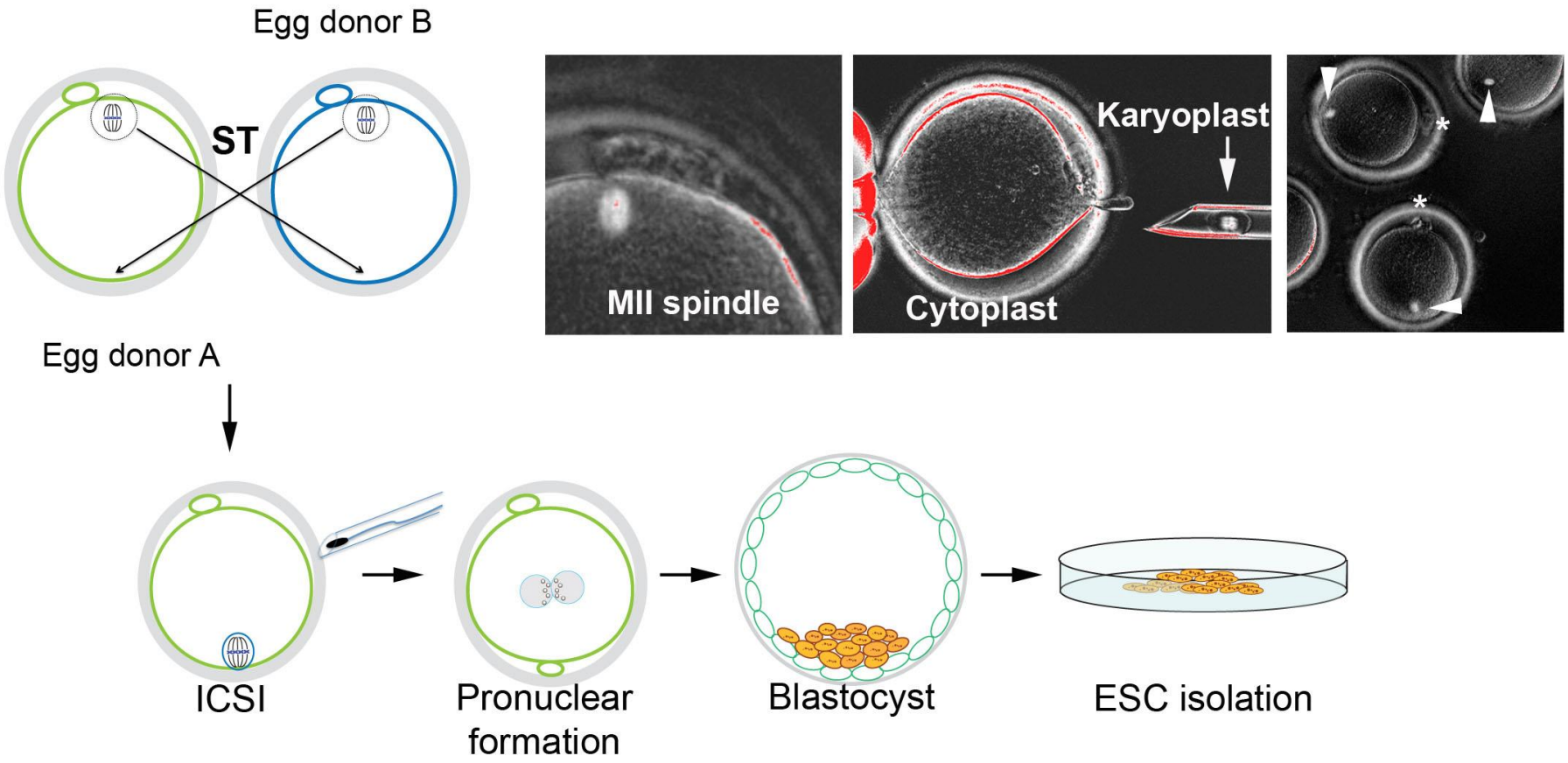
Tachibana et al., Nature, 2013

ARTICLE

doi:10.1038/nature11647

Towards germline gene therapy of inherited mitochondrial diseases

Masahito Tachibana[1], Paula Amato[2], Michelle Sparman[1], Joy Woodward[1], Dario Melguizo Sanchis[1], Hong Ma[1], Nuria Marti Gutierrez[1], Rebecca Tippner-Hedges[1], Eunju Kang[1], Hyo-Sang Lee[1], Cathy Ramsey[1], Keith Masterson[2], David Battaglia[2], David Lee[2], Diana Wu[2], Jeffrey Jensen[1,3], Phillip Patton[2], Sumita Gokhale[4], Richard Stouffer[1,2] & Shoukhrat Mitalipov[1,2]



Tachibana et al., Nature, 2013

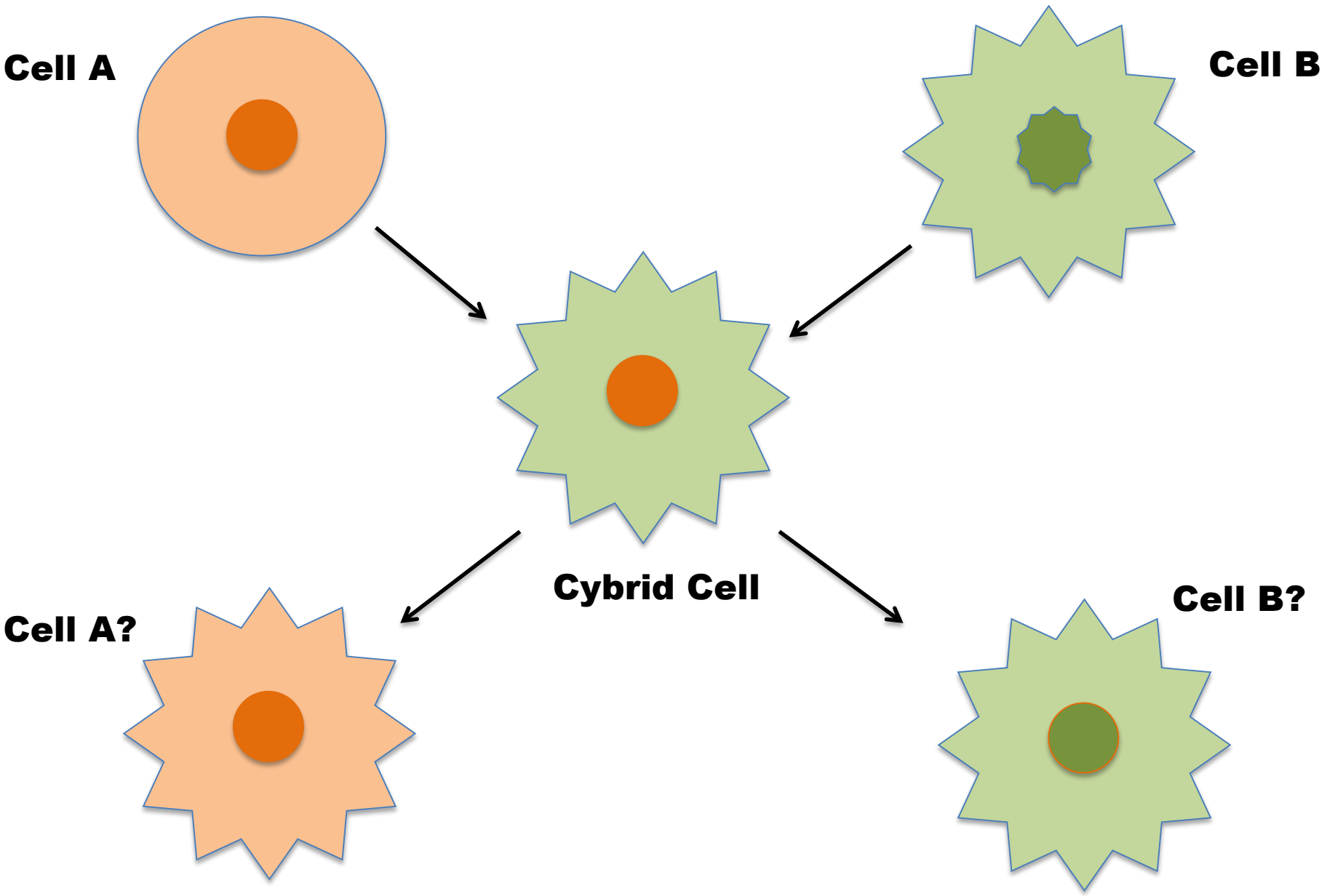
MRT Highlights

- ✓ Use of mt genome from donor egg (not recombinant)
- ✓ Applicable to any mtDNA mutation type
- ✓ Replacement of entire deficient cytoplasm in patient oocytes
- ✓ Preclinical animal studies demonstrate safety and efficacy
- ✓ Approved in UK for clinical applications

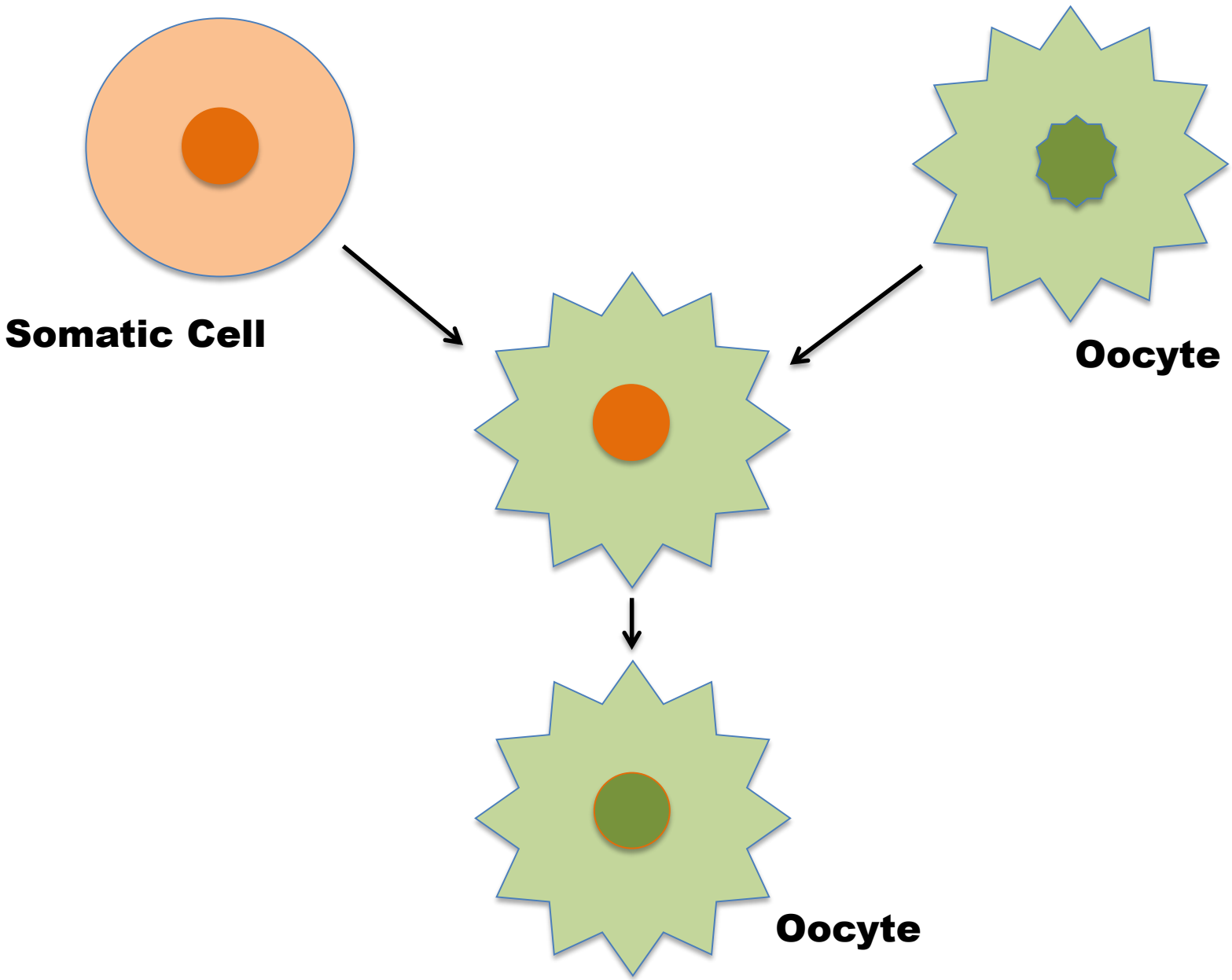
Mail Online

Three-parent IVF

Concept of reprogramming by cytoplasm



Concept of reprogramming by cytoplasm



Reproductive Cloning

2012 Nobel Prize: **John B. Gurdon and Shinya Yamanaka**

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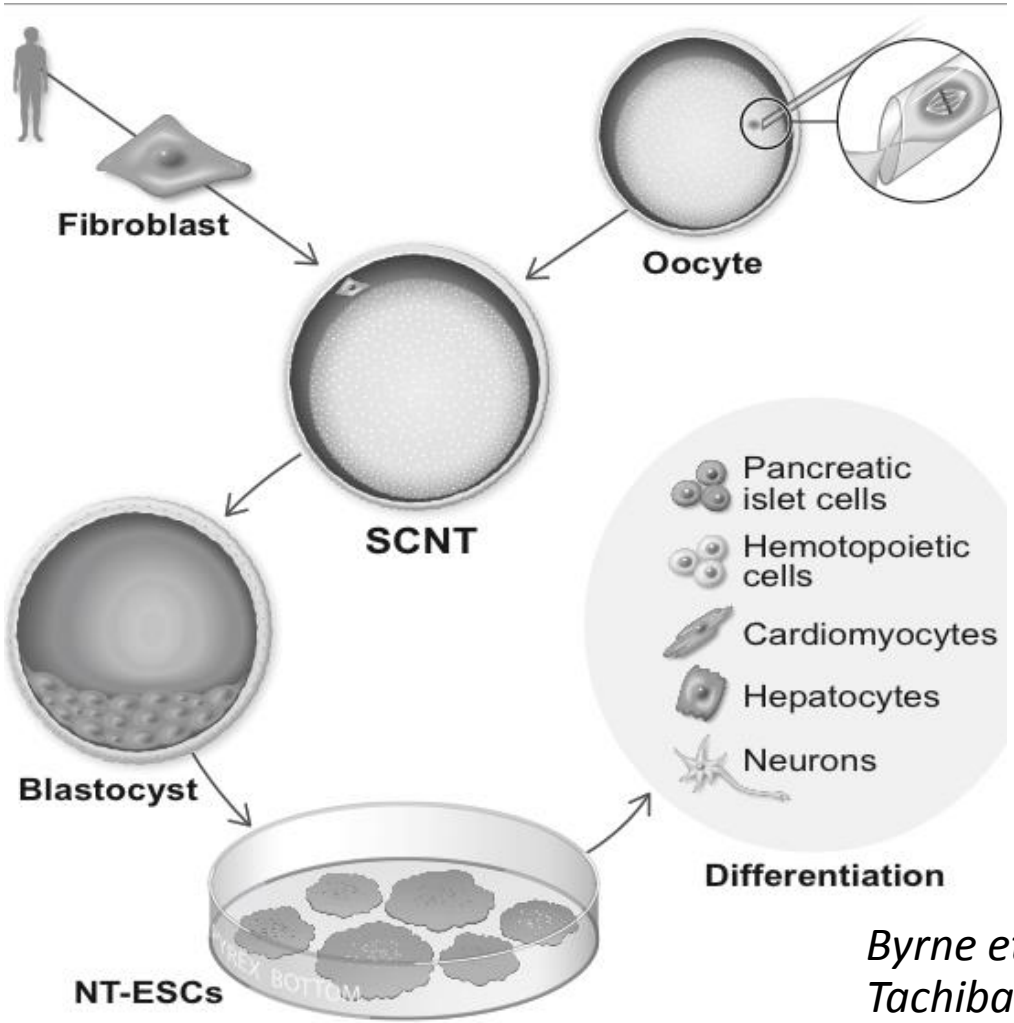
Cell

Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer

Masahito Tachibana,[1] Paula Amato,[2] Michelle Sparman,[1] Nuria Marti Gutierrez,[1] Rebecca Tippner-Hedges,[1] Hong Ma,[1] Eunju Kang,[1] Alimujiang Fulati,[1] Hyo-Sang Lee,[1,6] Hathaitip Sritanaudomchai,[3] Keith Masterson,[2] Janine Larson,[2] Deborah Eaton,[2] Karen Sadler-Fredd,[2] David Battaglia,[2] David Lee,[2] Diana Wu,[2] Jeffrey Jensen,[1,4] Phillip Patton,[2] Sumita Gokhale,[5] Richard L. Stouffer,[1,2] Don Wolf,[1] and Shoukhrat Mitalipov[1,2,*]



Byrne et al., Nature, 2007
Tachibana et al., Cell, 2013

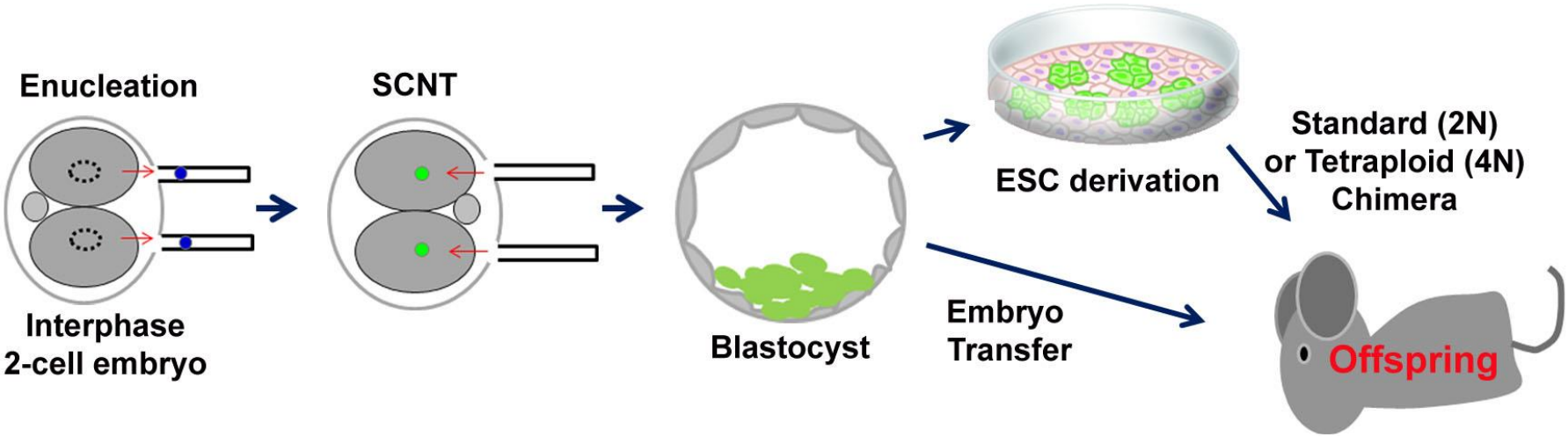
Somatic Cell Nuclear Transfer (SCNT) – reprogramming by factors in oocyte cytoplasm

- ✓ Ability of oocyte cytoplasm to reprogram is universal across mammalian species

- ✓ Factors and mechanisms of SCNT – based reprogramming are unknown

- ✓ Oocyte may not be the only cell capable of reprogramming

SCNT into enucleated 2-cell interphase embryos

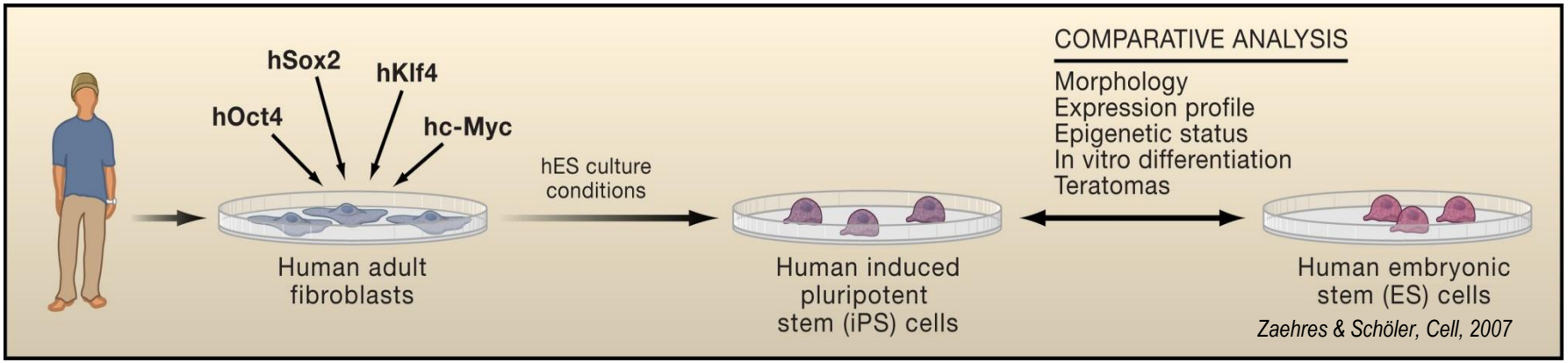


Kang et al., Nature 2014

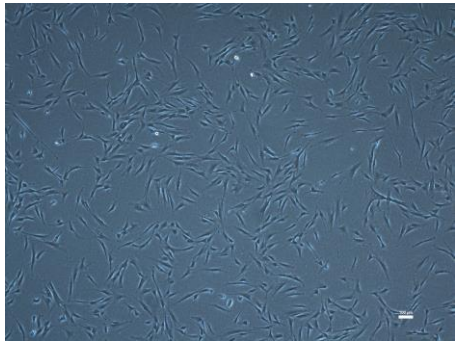
Direct reprogramming

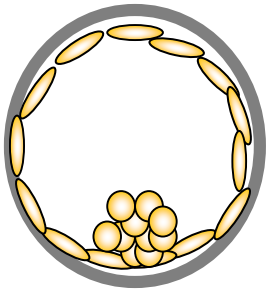
Introduction and overexpression of several genes transforms somatic cells to pluripotent iPS cells

Gene combinations: - OCT4, SOX2, KLF4 and c-MYC (*Yamanaka*)
OCT4, SOX2, NANOG and LIN28 (*Thomson*)



Comparative analysis of pluripotent stem cell types





In Vitro Fertilization

Somatic Cell Nuclear Transfer
NT-ESCs

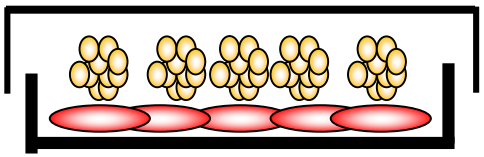
4 NT-ESC lines

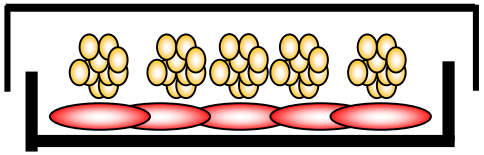
Direct reprogramming:
iPSCs

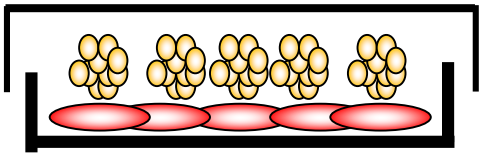
4 iPS cell lines

IVF-ESCs

2 hESO cell lines

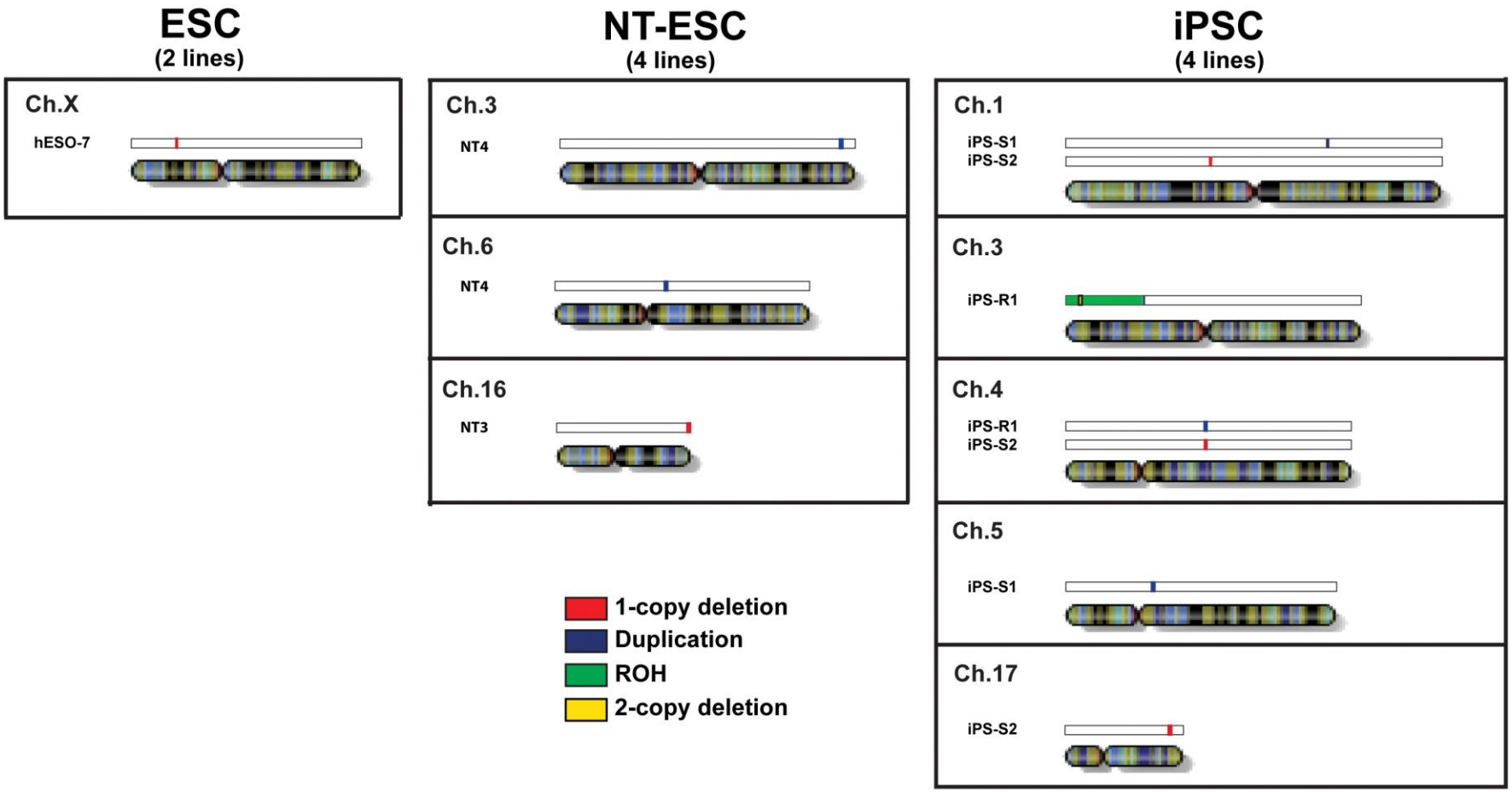






Ma et al., Nature, 2014

Copy Number Variations



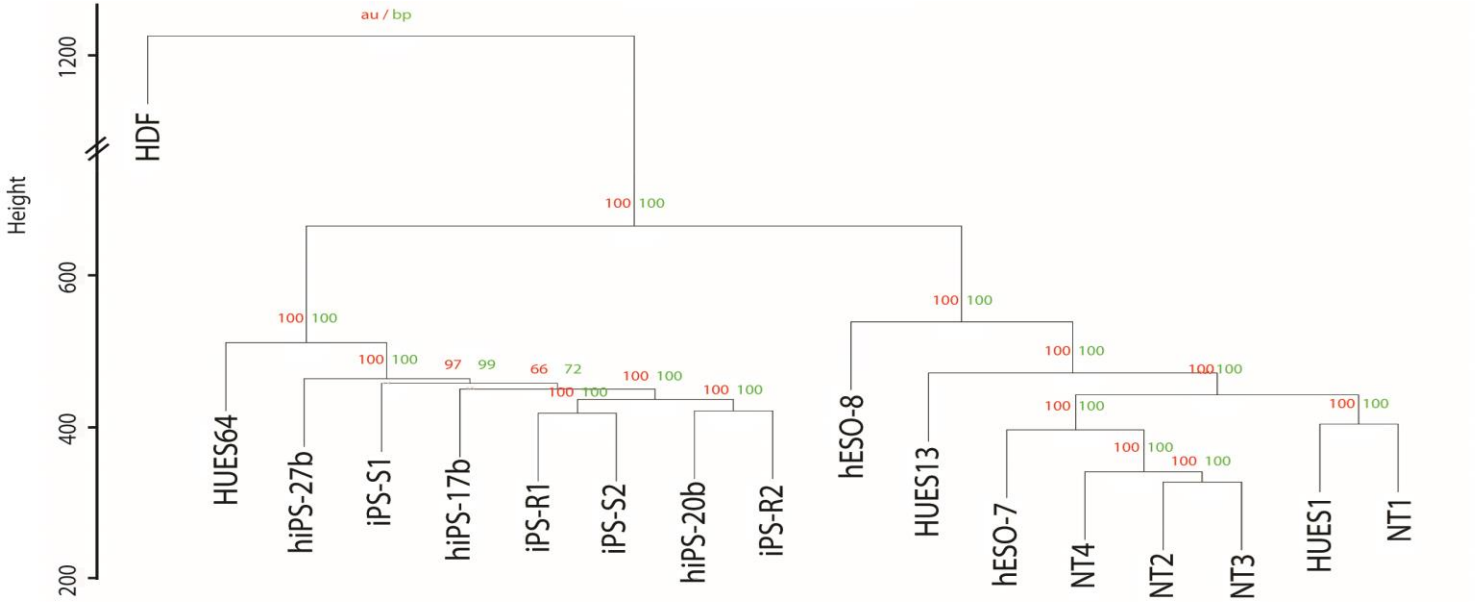
Ma et al., Nature, 2014

Copy Number Variations

Stem cell type	Duplication	1 copy deletion	2 copy deletion	ROH	Total	# cell lines	#CNVs/cell line
iPSCs-Retro	2		1	1	4	2	2
iPSCs-Sendai	2	3			5	2	2.5
iPSCs-Total	4	3	1	1	9	4	2.25
NT-ESCs	2	1			3	4	0.75
IVF-ESCs		1			1	2	0.5

Ma et al., Nature, 2014

DNA Methylation

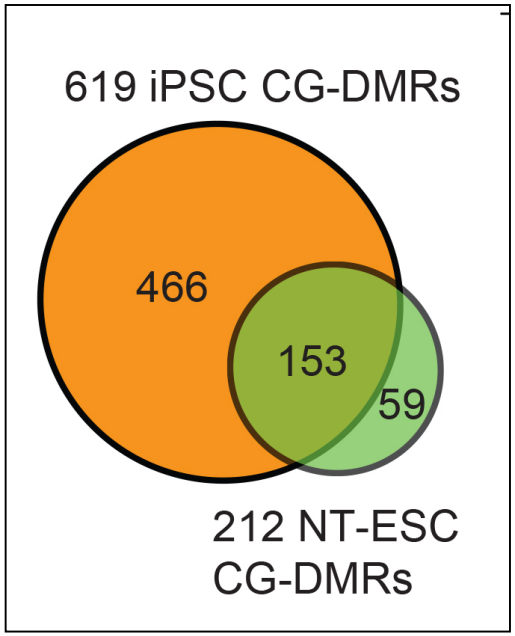


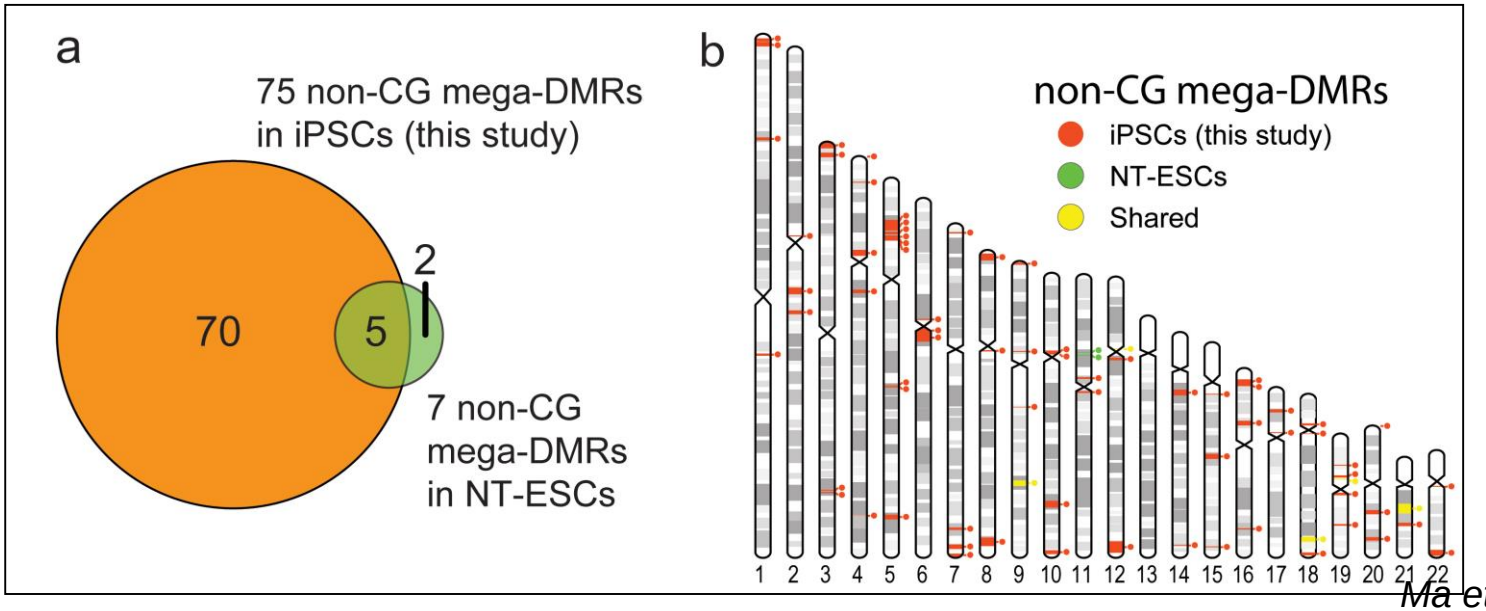
Somatic cell methylation memory in reprogrammed cells

	Differentially Methylated Probes different from IVF-ESCs	DMPs shared with HDF
iPSCs	6478	780
NT-ESCs	110	87
Shared	91	74

Ma et al., Nature, 2014

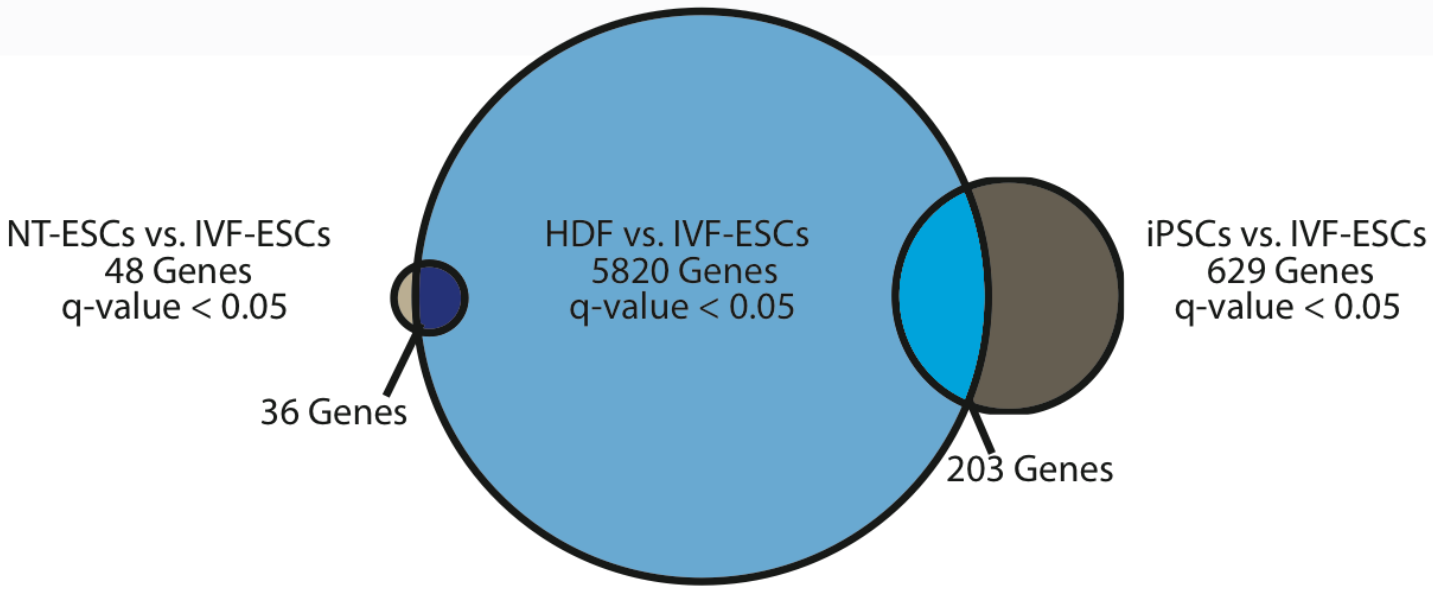
Aberrant CG and non-CG methylation





Ma et al., Nature, 2014

Transcriptional memory in NT-ESCs and iPSCs



Ma et al., Nature, 2014

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