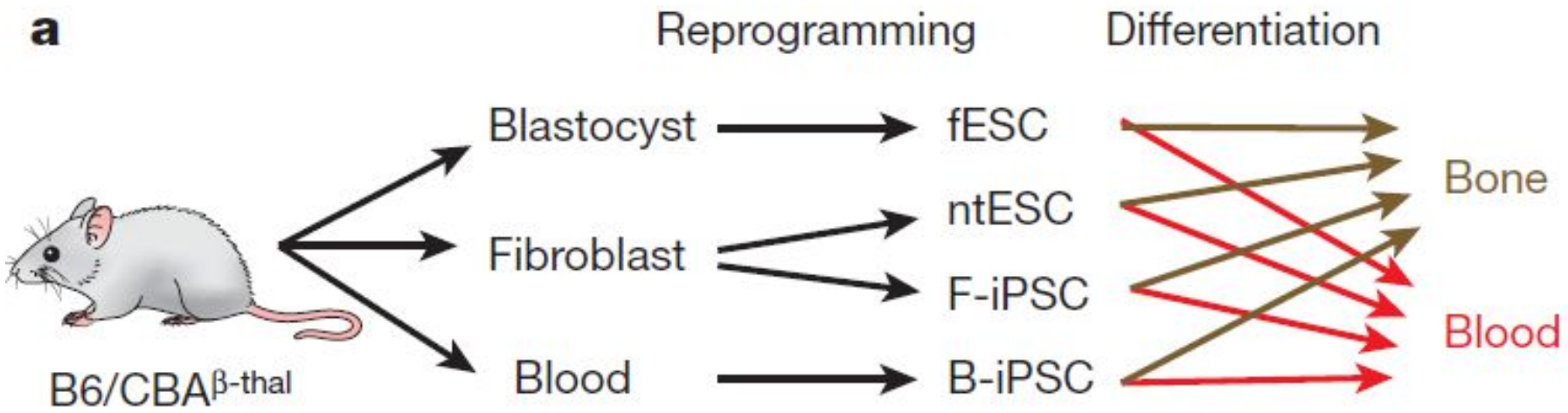
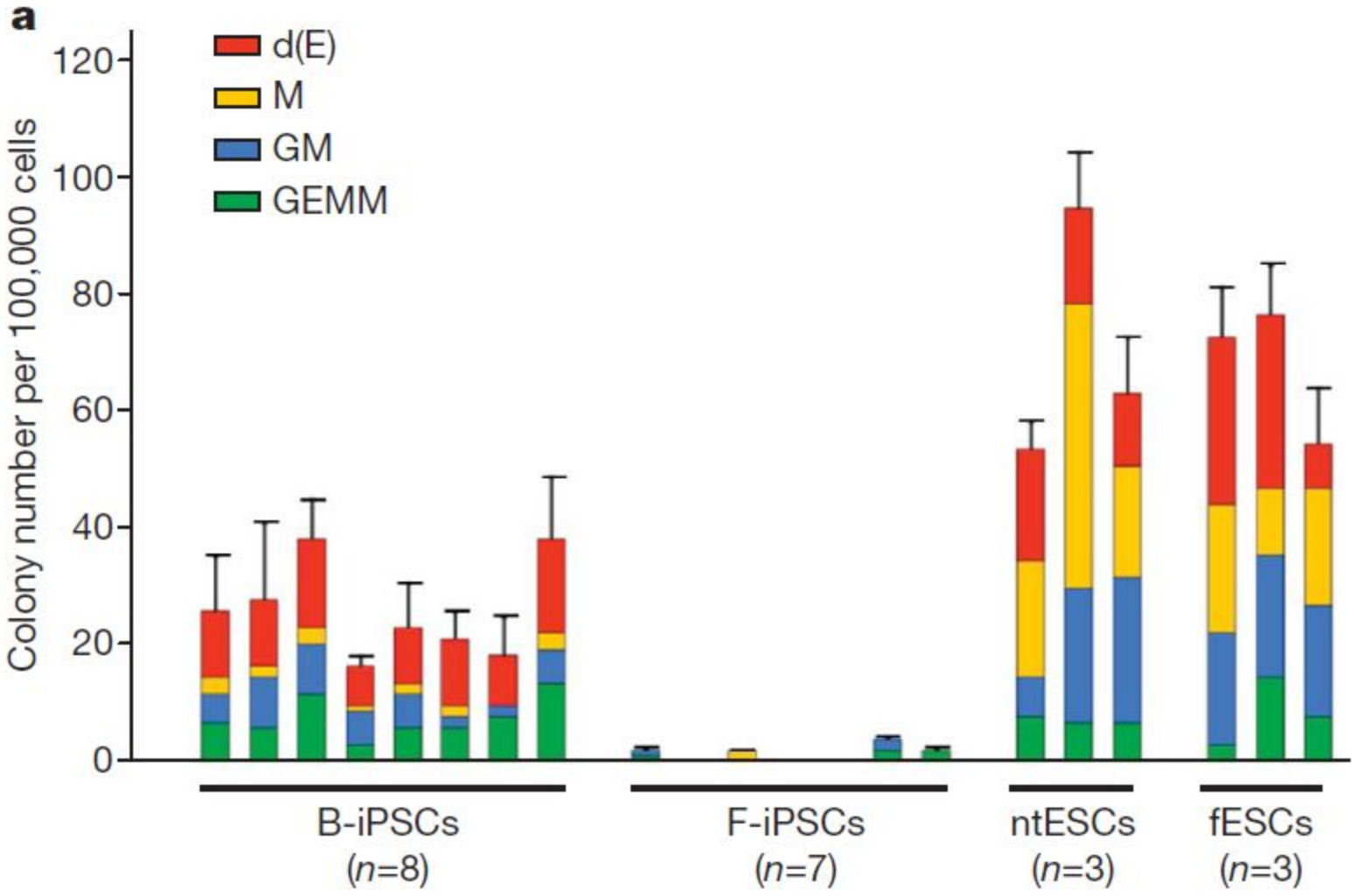
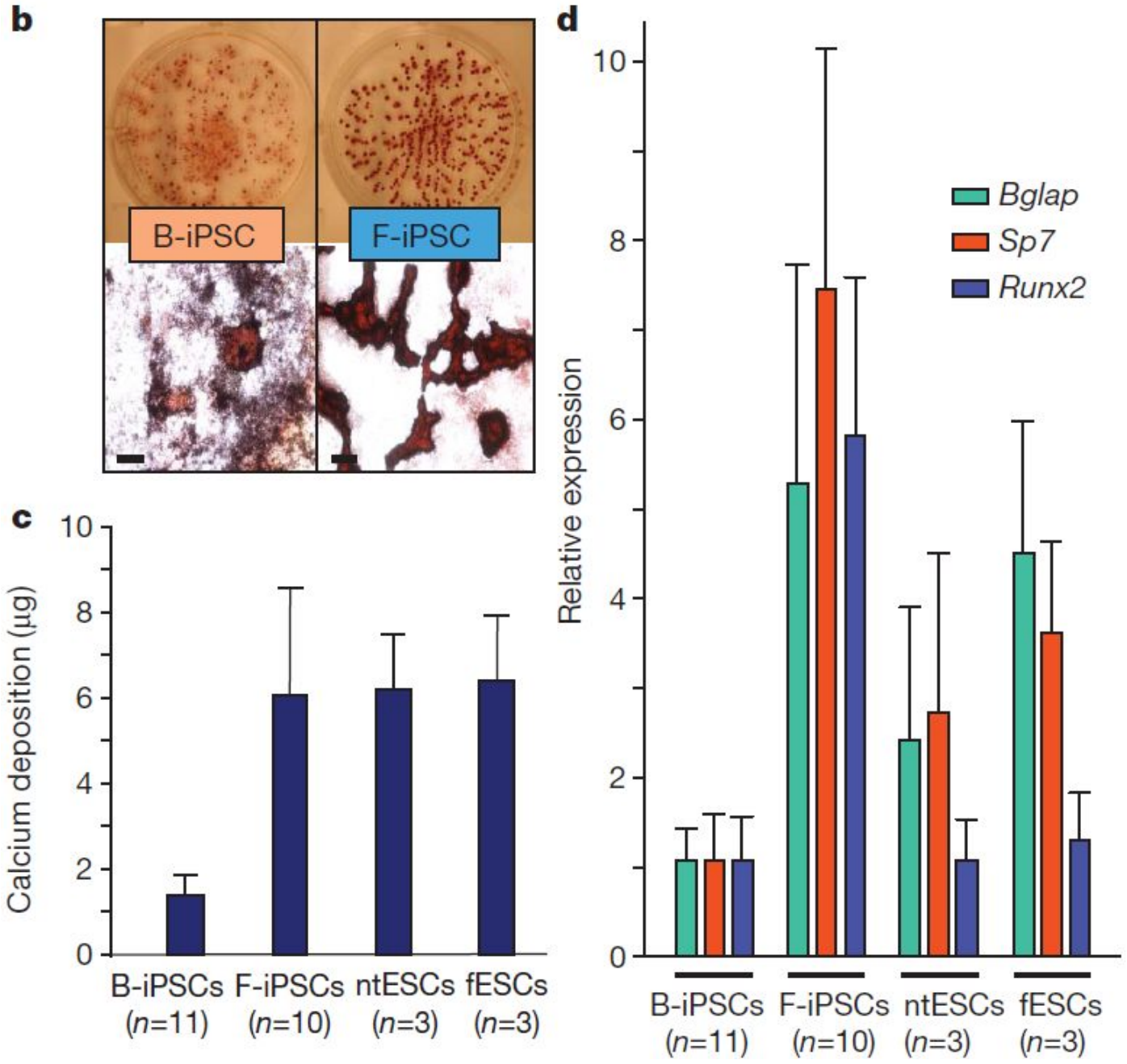
Соматическая память

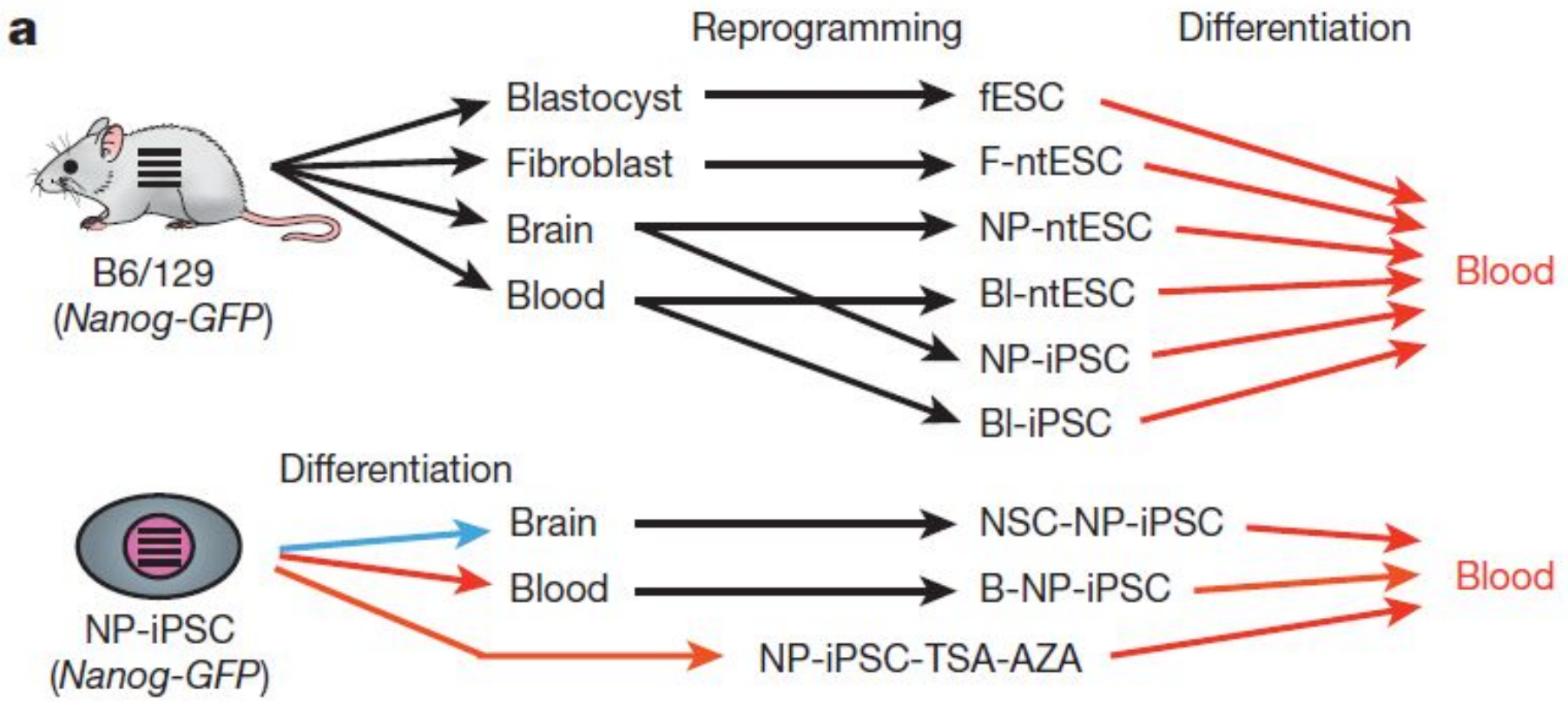


Соматическая память

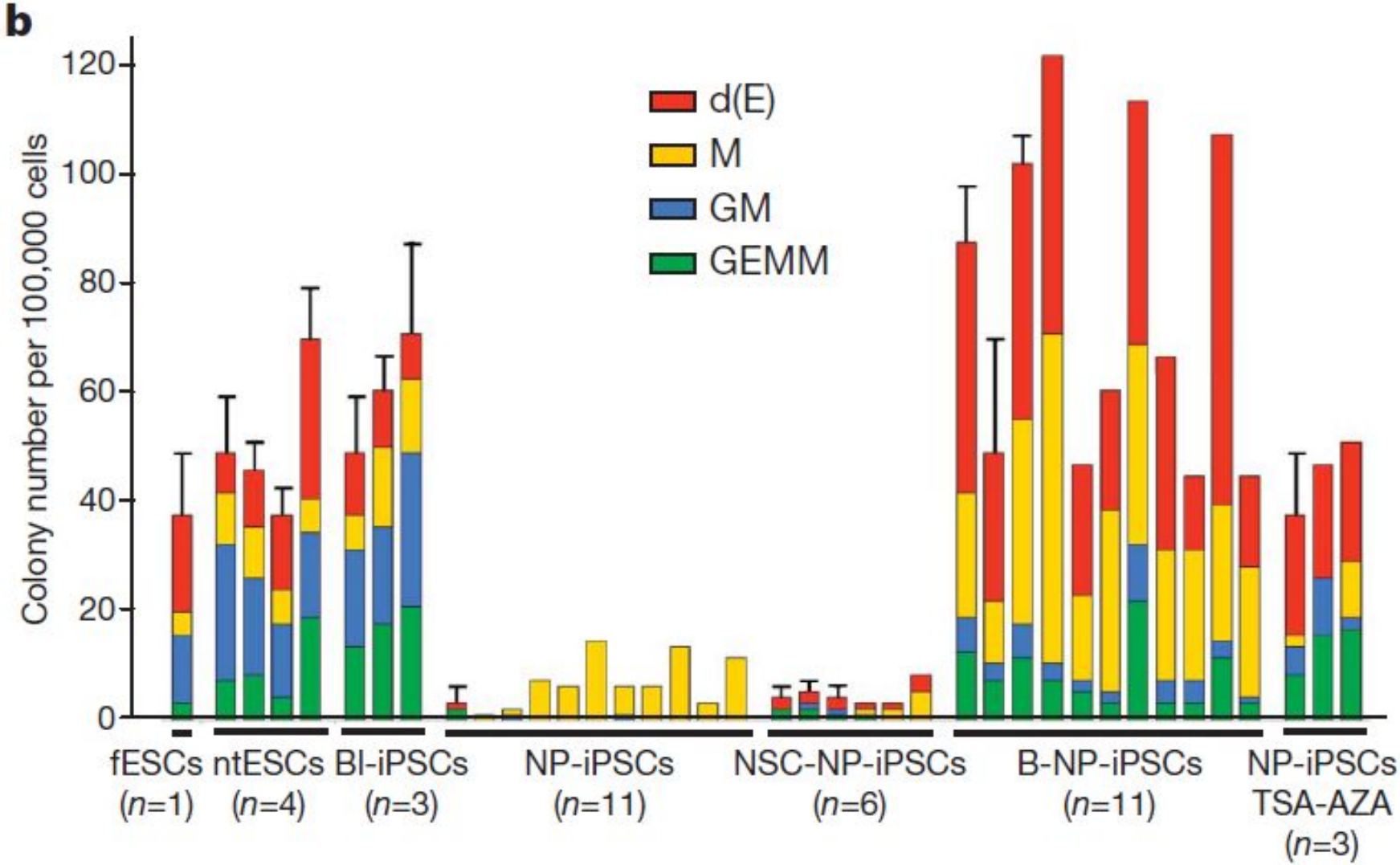



b
B-iPSC
F-iPSC
c
Calcium deposition (μg)
10
8
6
4
2
0
B-iPSCs (n=11)
F-iPSCs (n=10)
ntESCs (n=3)
fESCs (n=3)
d
Relative expression
Bglap
Sp7
Runx2
10
8
6
4
2
0
B-iPSCs (n=11)
F-iPSCs (n=10)
ntESCs (n=3)
fESCs (n=3)

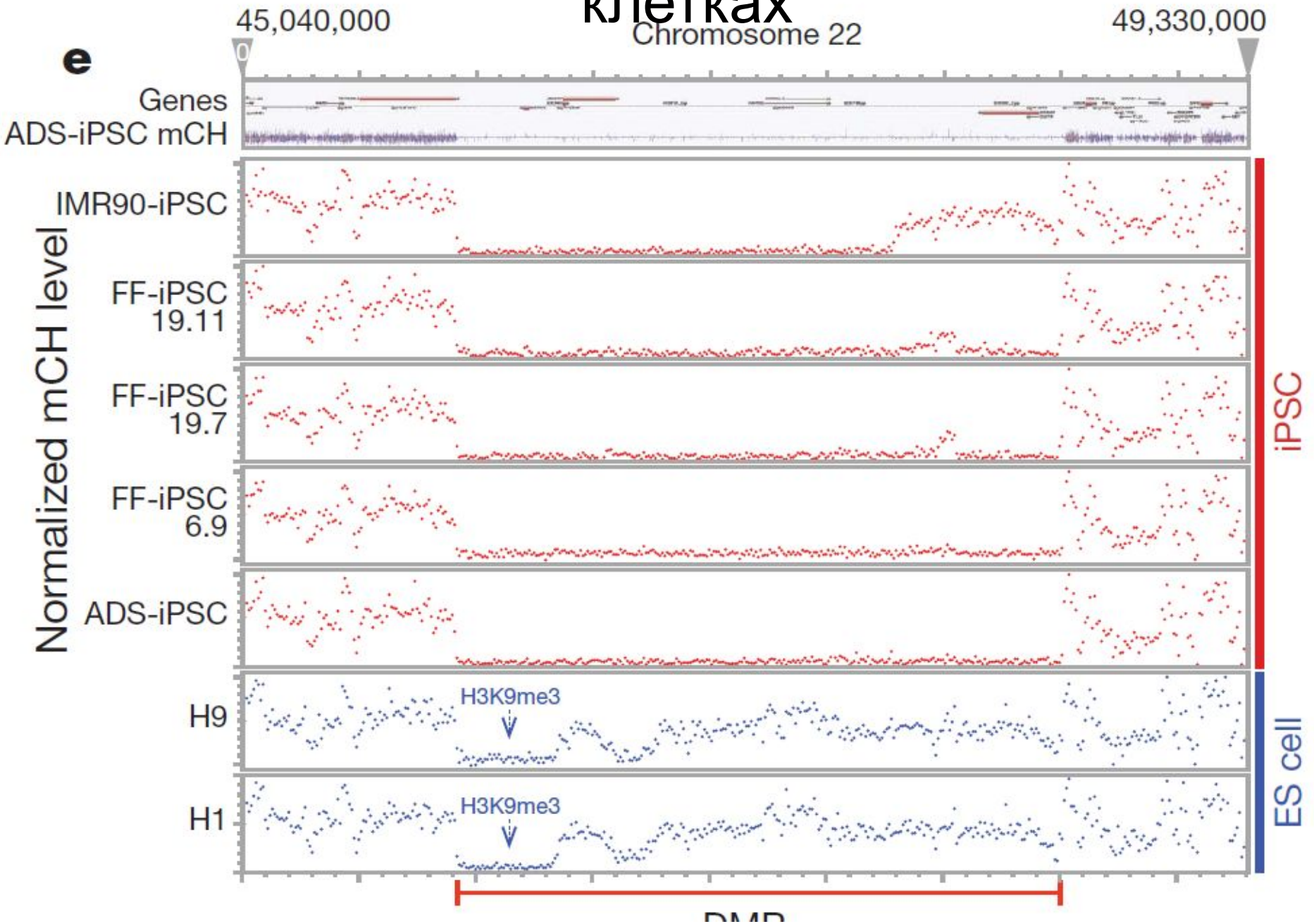
Соматическая память



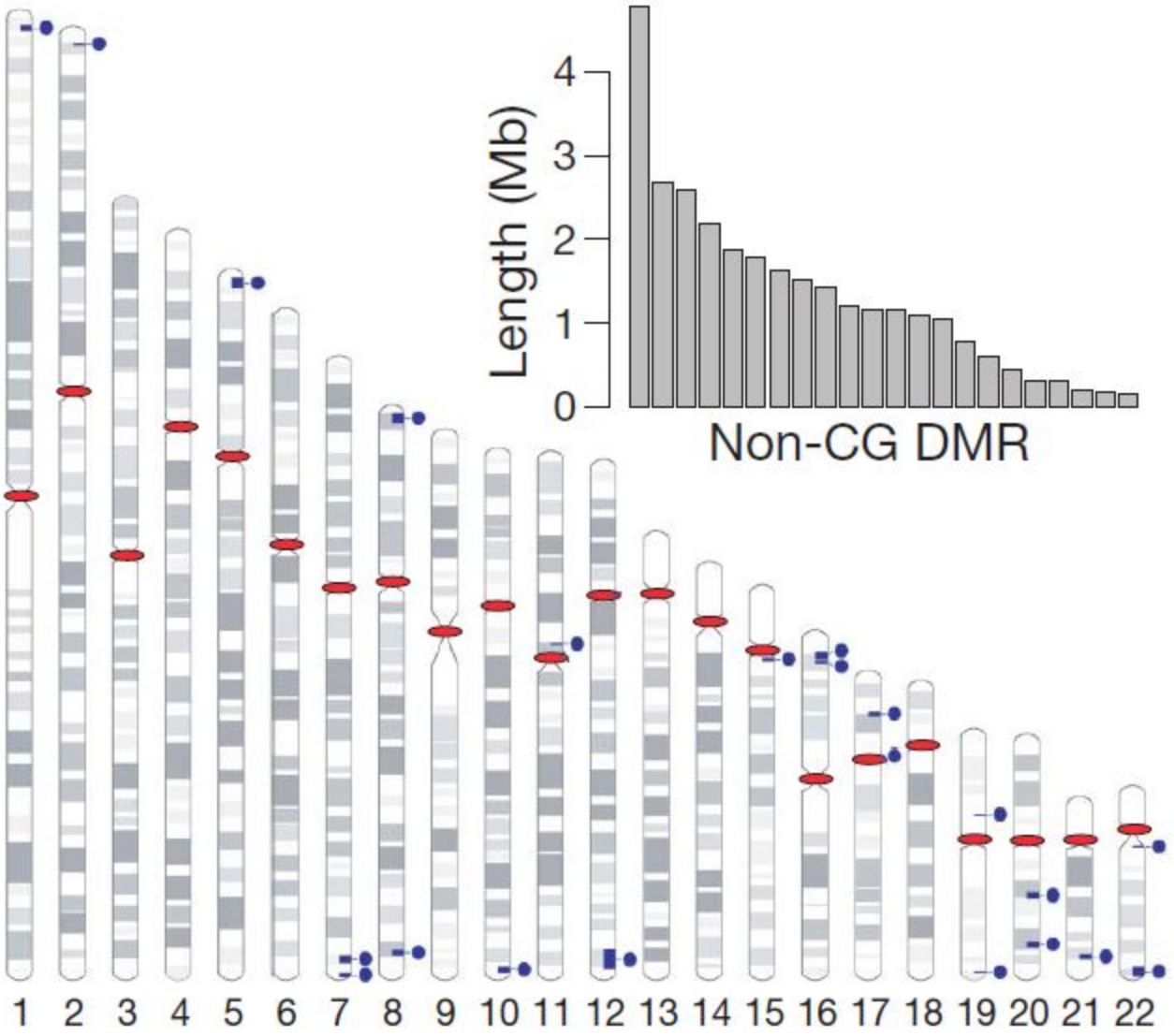
Соматическая память



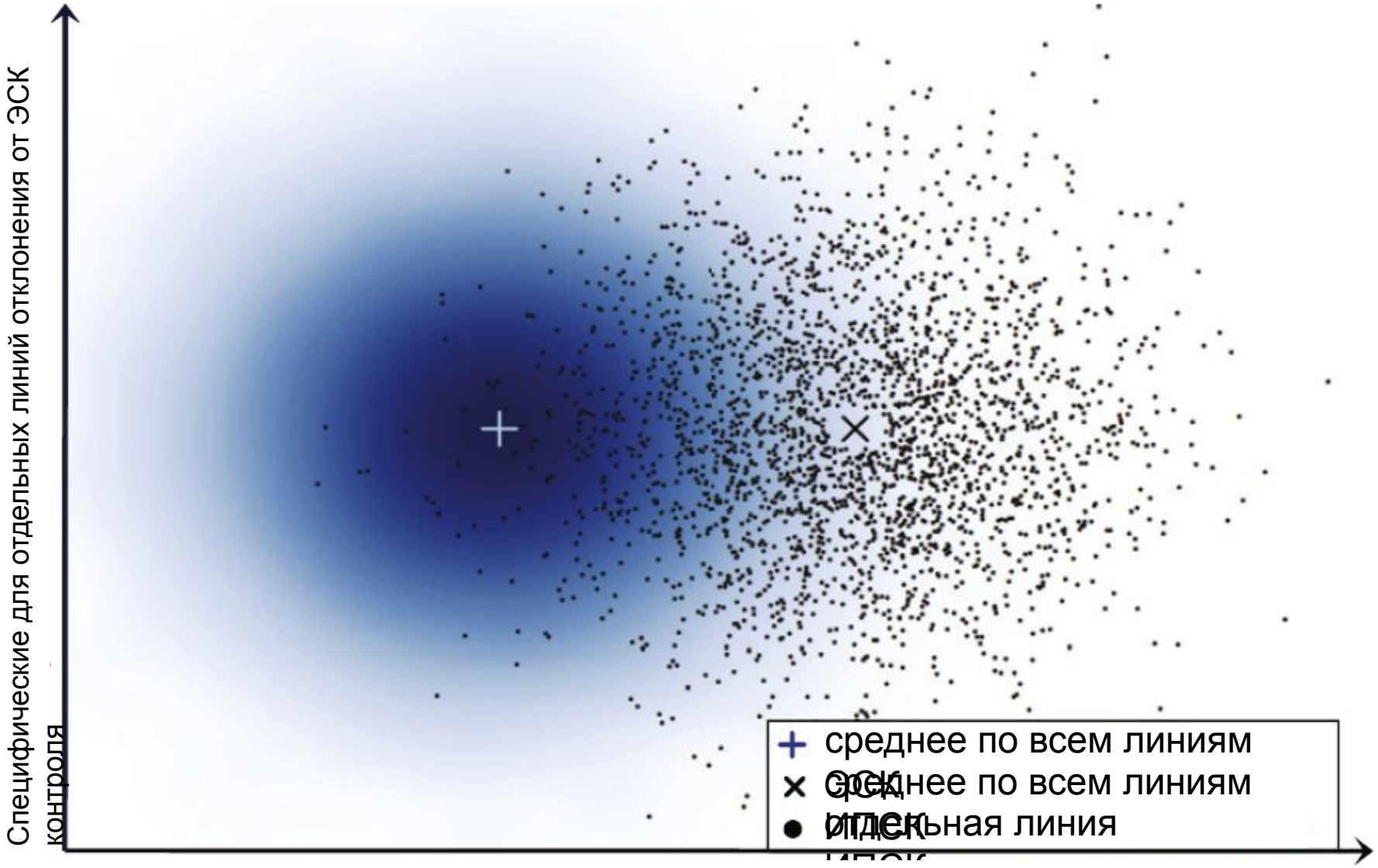
Нарушения метилирования ДНК в iPS клетках



Нарушения метилирования ДНК в iPS клетках



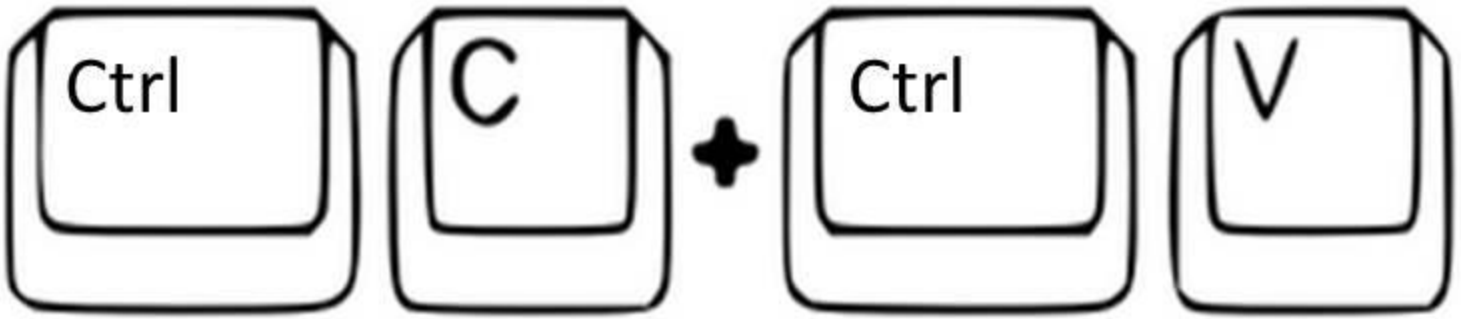
Насколько ИПСК похожи на ЭСК?



Bock et al., 2011 с модификациями

Опишите стратегию лечения пациента от ВИЧ с использованием ИПС клеток.

Нариман Баттулин



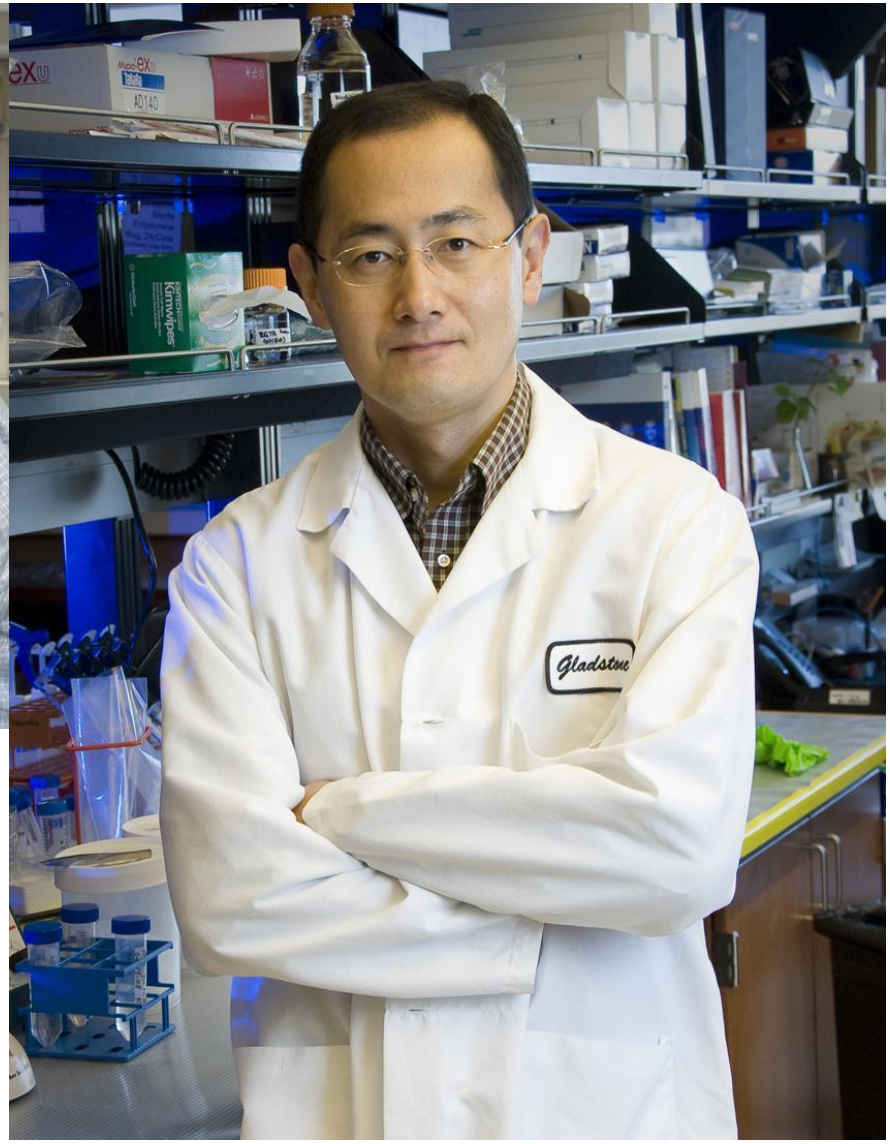
или

все что вы хотели знать о клонировании

индивидуальное развитие

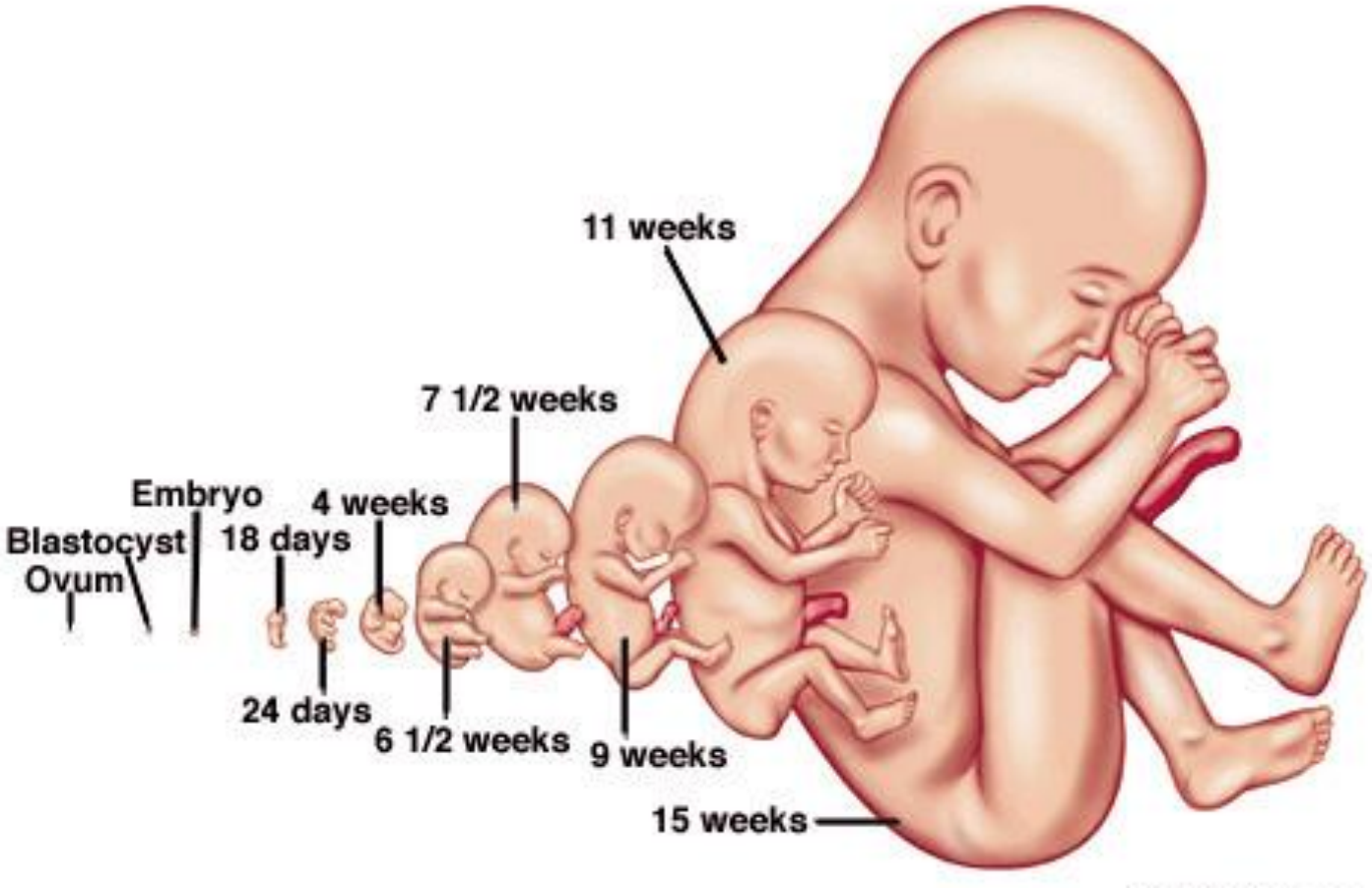
репрограммирование

for the discovery that mature cells can be reprogrammed to become pluripotent

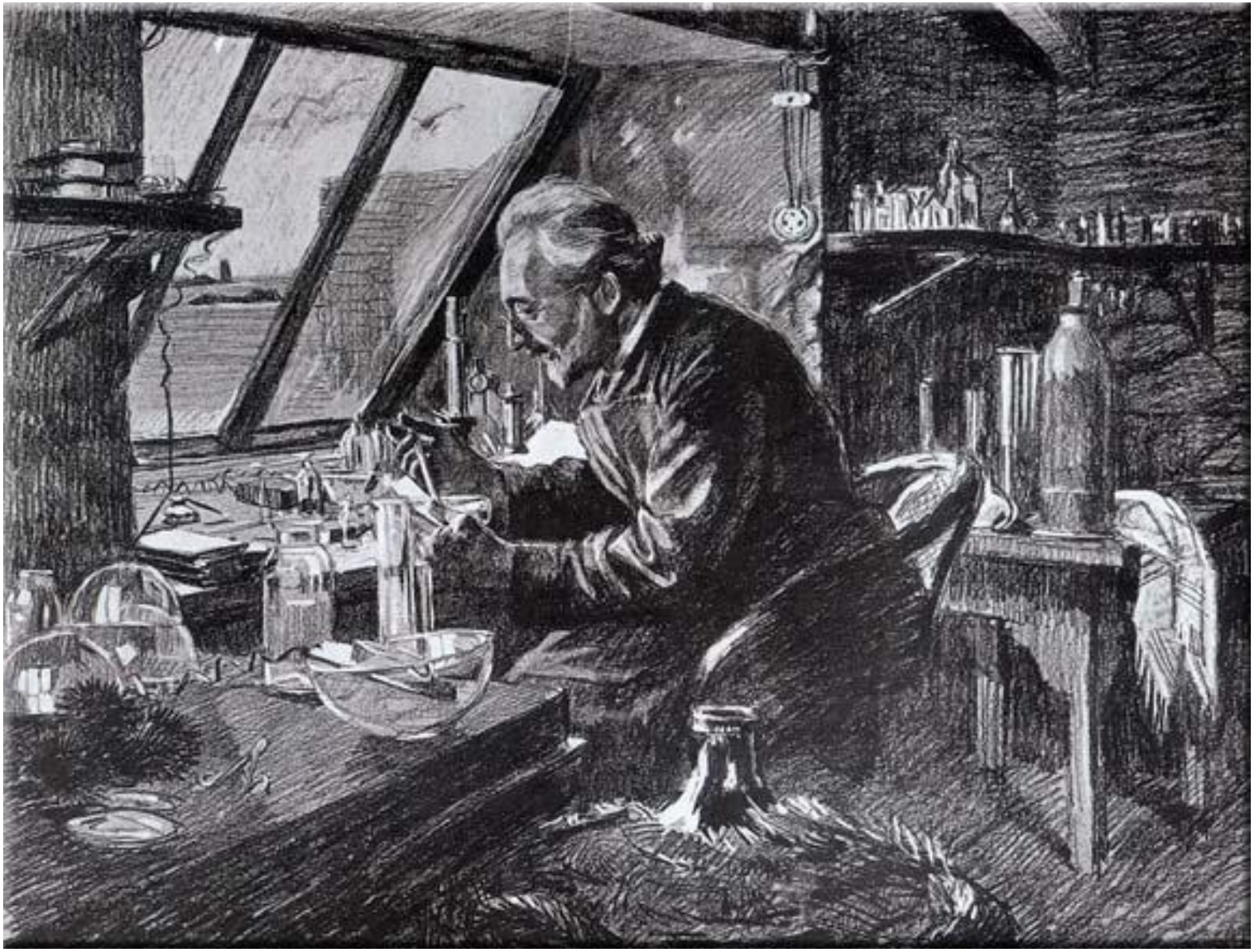


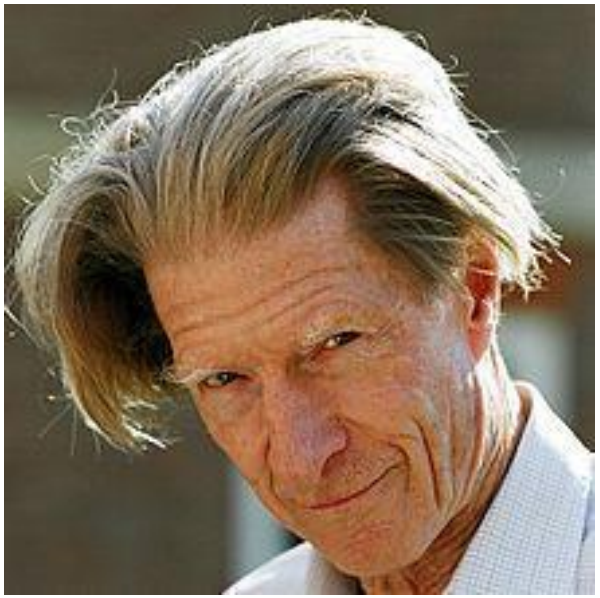
за открытие возможности превращения клеток взрослого организма в эмбриональные

Отличается ли набор генов в различных клетках организма?



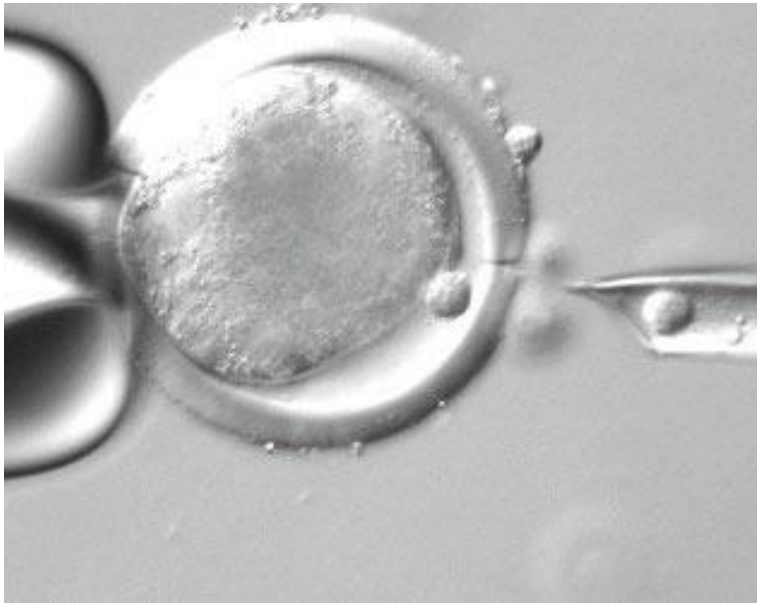
Идею переноса ядра эмбриональной клетки в энуклеированную яйцеклетку высказал Ив Делаж (Yves Delage) в 1895 году





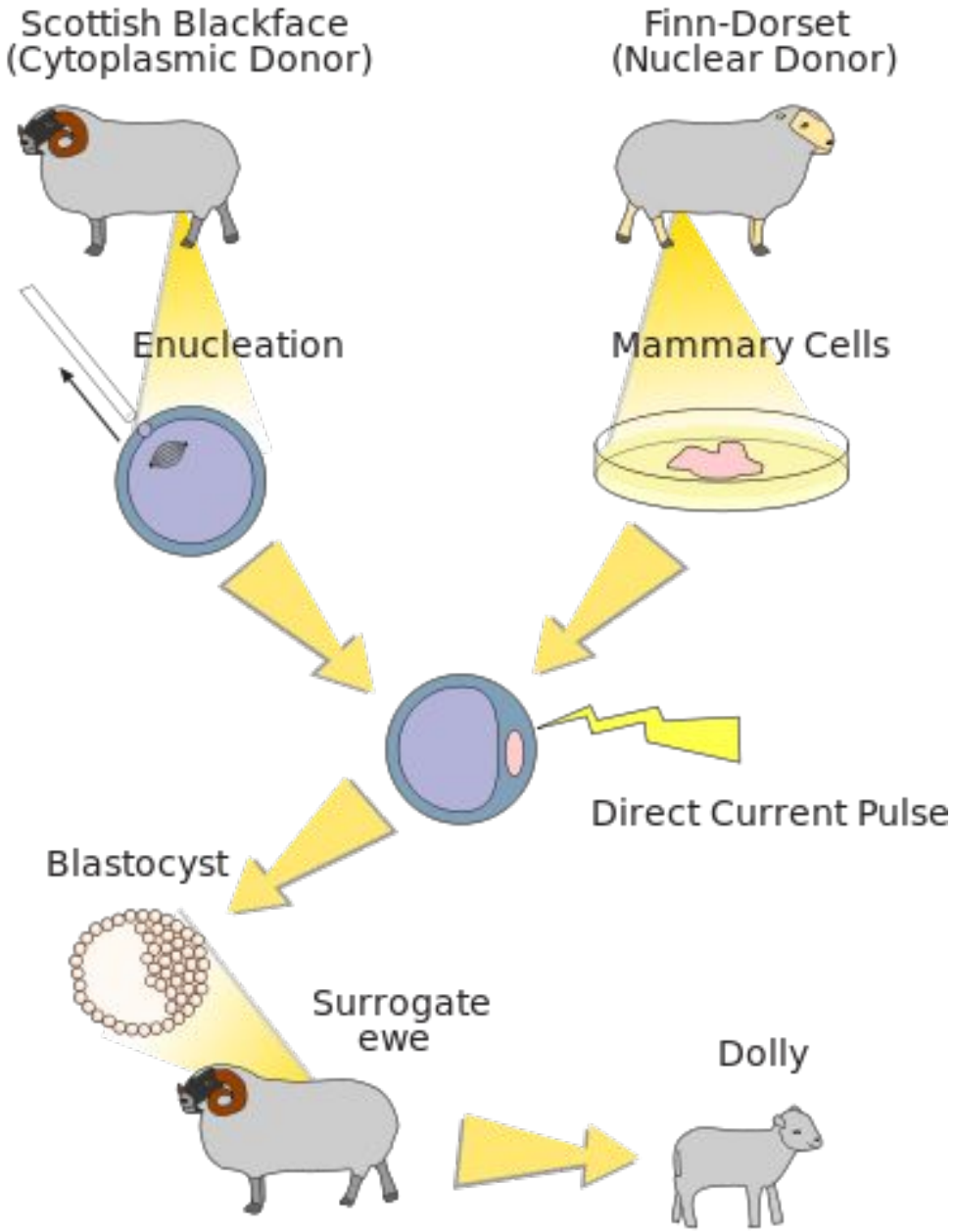
«Идея Джона стать ученым просто смехотворна; если он не может усвоить простых биологических фактов он не сможет стать специалистом; это будет бесполезная трата времени и для него и для его руководителей»

из школьной характеристики, 15 лет




TIME
FICTION BONUS:
"CLONE ON THE RANGE"
BY DOUGLAS COUPLAND
Will There Ever
Be Another You?
A SPECIAL REPORT ON CLONING

Схема эксперимента по получению клонированной овцы

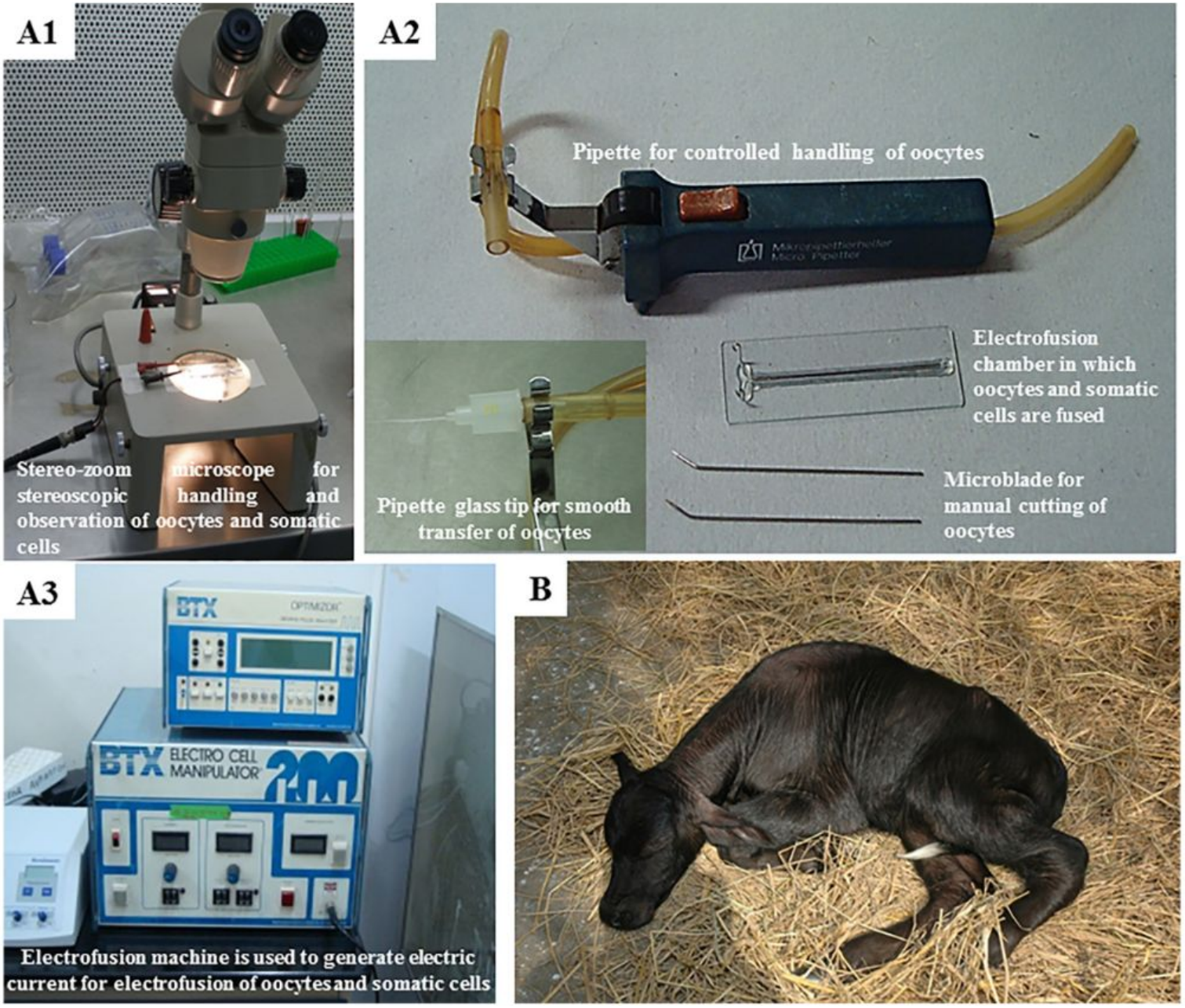


Микроманипулятор

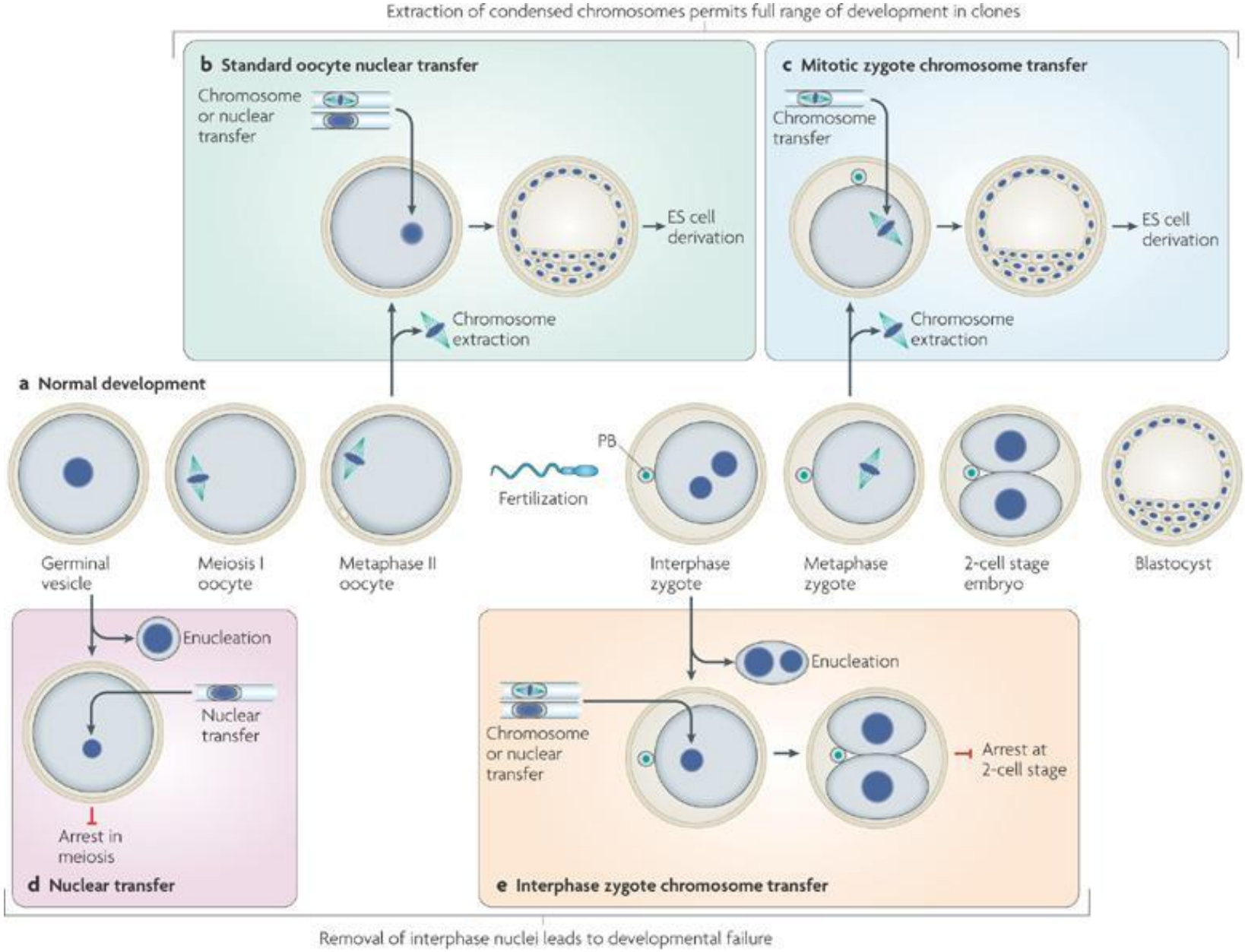

12-12-00 2H
14:19:47
Oocyte Enucleation


12-12-00 2H
14:20:17
Cell Transfer

An update: Reproductive handmade cloning of water buffalo (*Bubalus bubalis*)



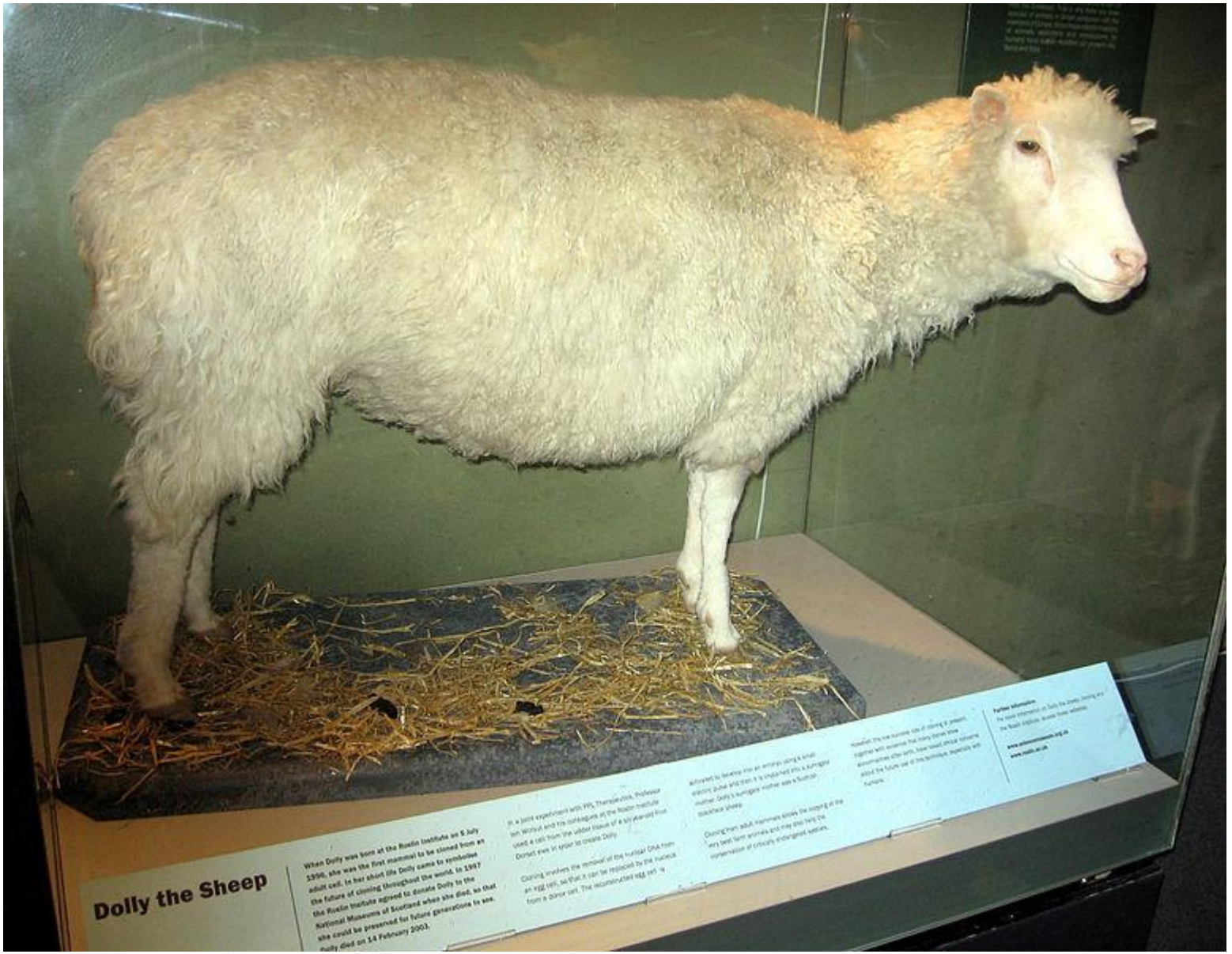
Важна стадия ооцита



Овечка Долли при рождении

Долли жила в комфорте

Овечка Долли, наши дни



Сколько прожила овечка Долли?

12 лет

Table 2. Reported maximum lifespans of cloned animals

Species	Breed	Typical life expectancy of species/breed, years	Reported maximum lifespan of cloned animals, years	Reference
Goat	Dairy goats	15	>15	[Gavin, pers. commun.; 54]
Cattle	Jersey Simmental Fleckvieh	15	11.8 oldest dairy SCNT cow, 2011 14.4 "Lara 8" (euthanized due to project end)	[55] [Brem, unpubl.]
Dog	Afghan hound	10–12	>10	[44]
Sheep	Finn Dorset	<10	9	[53]
Mouse	C57/BL6, DBA/2, 129/Sv	2–3	3	[13]
Cat		15	10 (in 2011)	[56]
Pig	Large, white, Göttingen, Yucatan	15–17	6	[57]

We report here the typical life expectancy as reported (often compared to control animals) in the respective reference; or in [58] (cattle, cat) and [59] (pig). For maximum lifespans, see [18] and references therein.

Aging of Cloned Animals: A Mini-Review Jörg Patrick Burgstaller Gottfried Brem

Table 1. Telomere length of cloned animals (see also Table S2 for details)

Species	Relative telomere length compared to control animals	Studies, n	Cloned animals, n	Cloned animals with normal telomeres, %
Cattle	Normal/longer	5	42	64.6
	Shorter	3	23	
Pig	Normal/longer	3	32	69.6
	Shorter	2	14	
Sheep	Normal/longer	3	6	37.5
	Shorter	3	10	
Goat	Normal/longer	2	8	36.4
	Shorter	3	12	
Mouse	Normal/longer	2	535	100.0
	Shorter	No	No	
Wolf	Normal/longer	No	No	0.0
	Shorter	2	5	
Dog	Normal/longer	2	2	100.0
	Shorter	No	No	

Aging of Cloned Animals: A Mini-Review Jörg Patrick Burgstaller Gottfried Brem

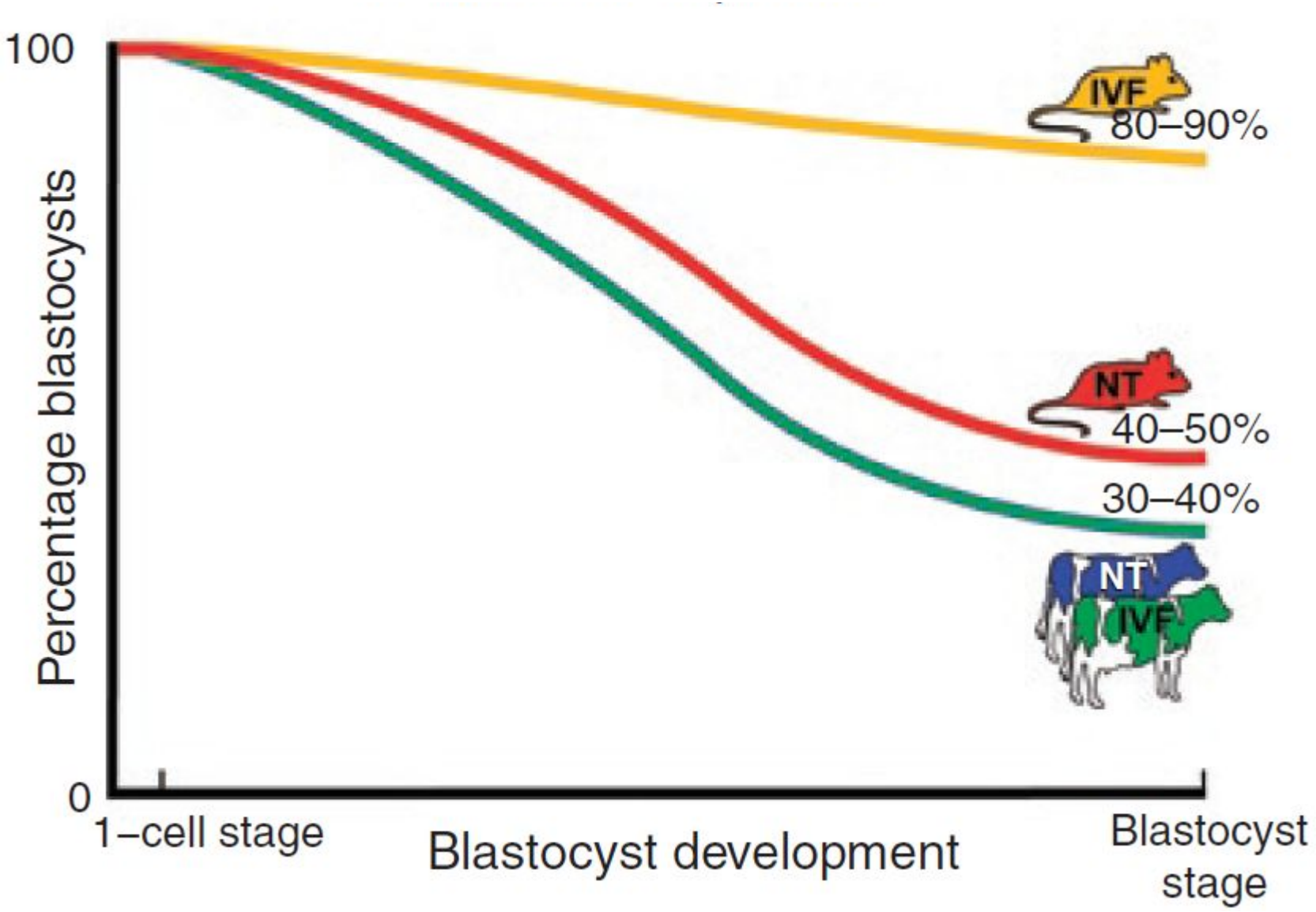
Клонирование очень не эффективно

Долли была единственным родившимся клоном из 277 реконструированных эмбрионов

Даже в наши дни эффективность клонирования 1-5 %

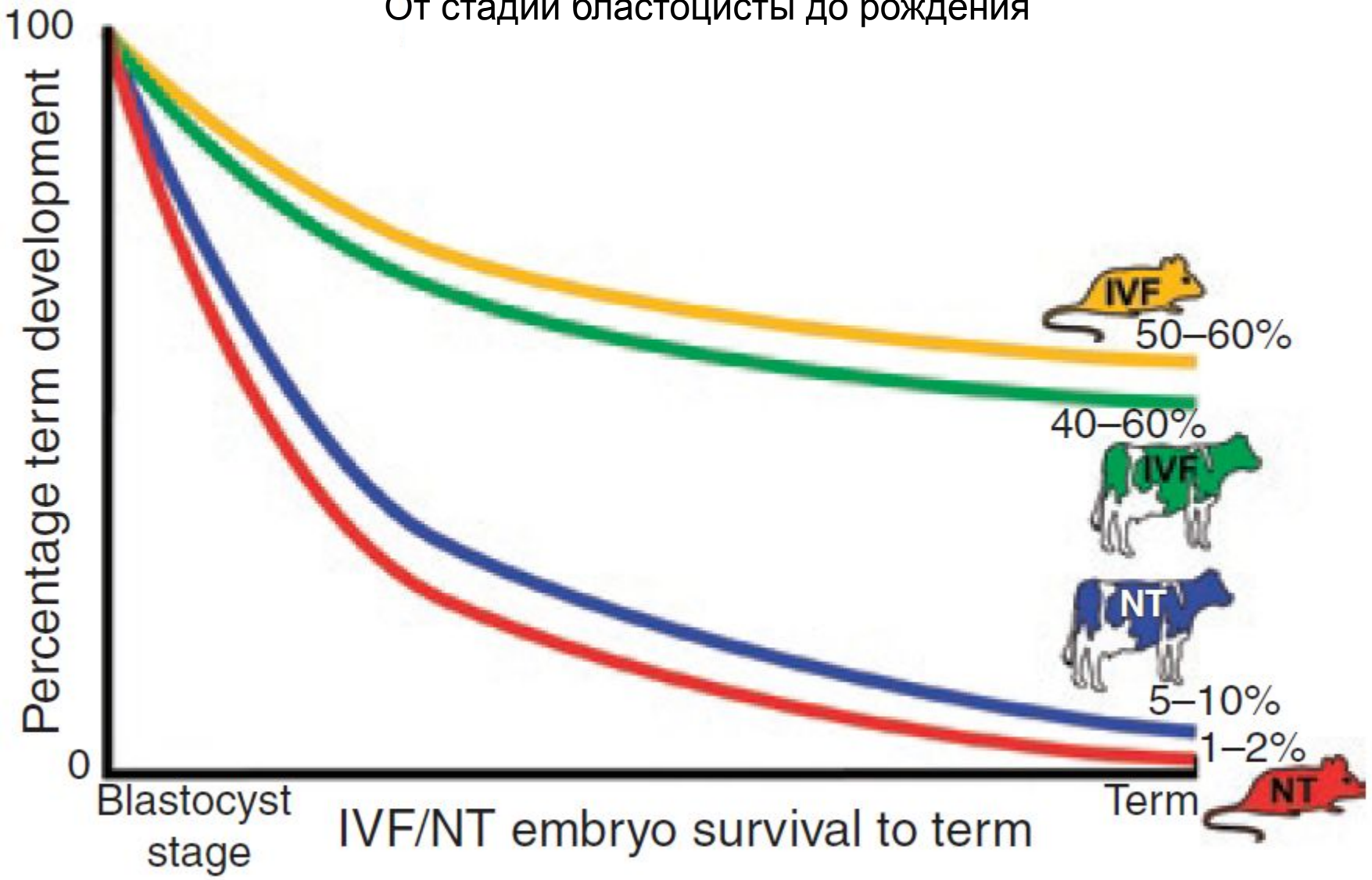
Выживаемость эмбрионов при in vitro манипуляциях

От одноклеточной стадии до стадии бластоцисты



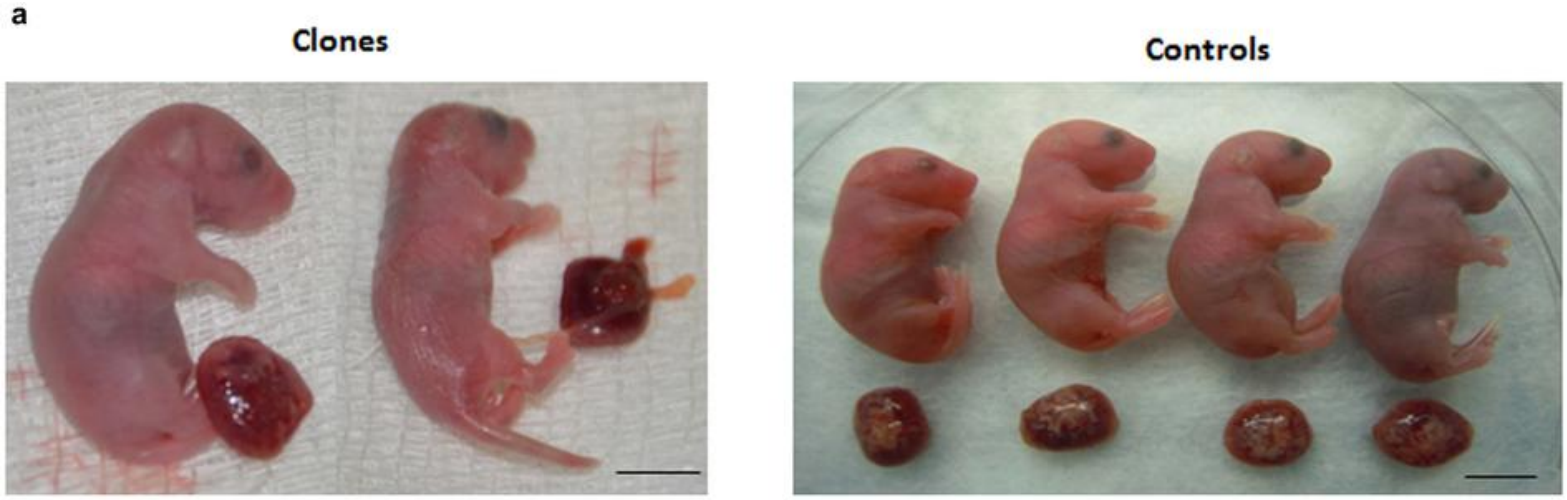
Выживаемость эмбрионов при in vitro манипуляциях

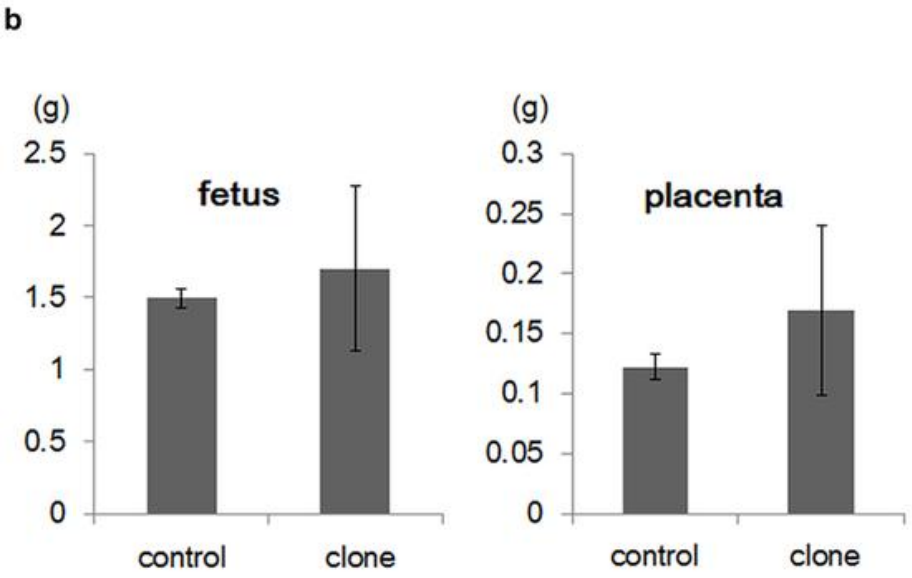
От стадии бластоцисты до рождения



Нарушения развития клонированных животных

увеличенная плацента





Kang et al., 2014

Нарушения развития клонированных животных

кишечная грыжа и аномалии черепа

Journal of Reproduction and Development, Vol. 55, No. 5, 2009

—Full Paper—

Sex-Reversed Somatic Cell Cloning in the Mouse

Kimiko INOUE[1,2], Narumi OGONUKI[1], Kazuyuki MEKADA[1], Atsushi YOSHIKI[1], Takashi SADO[3] and Atsuo OGURA[1,2,4]

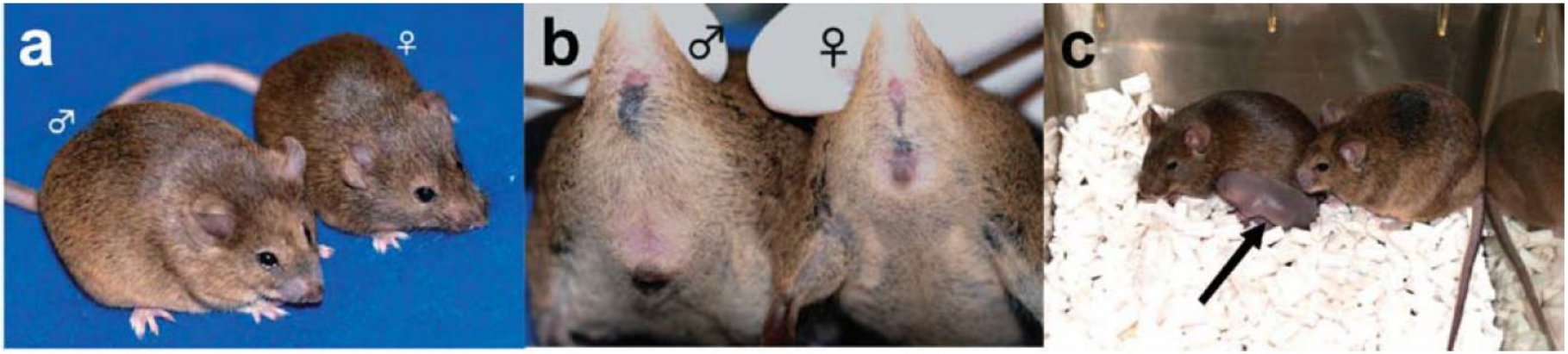


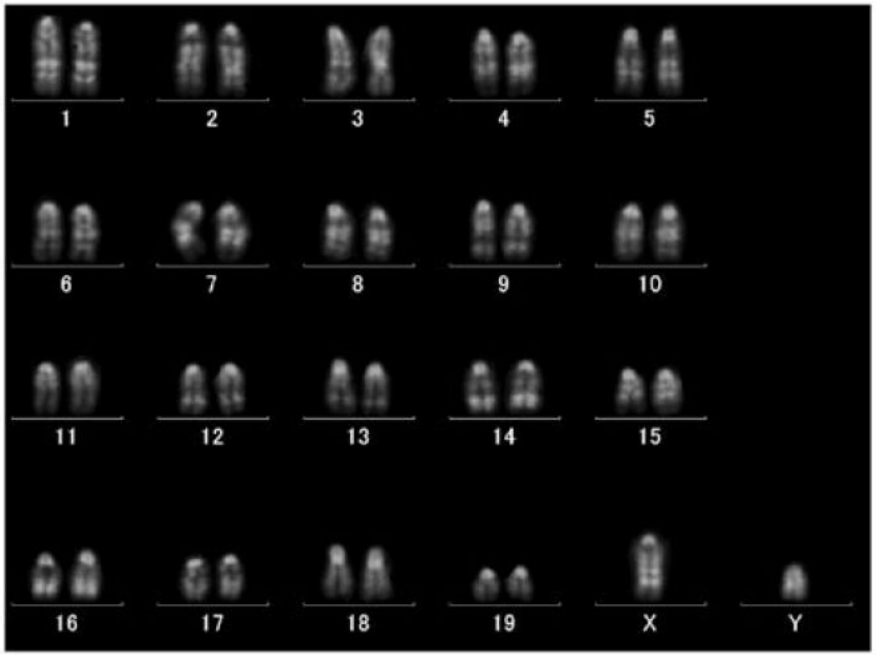
Fig. 1. a, b) Gross appearance (a) and external genitalia (b) of a female mouse and a male mouse cloned from Sertoli cells. c) Offspring (arrow) born from the female cloned mouse. The fertility of the female clone was confirmed by pregnancy and birth of normal offspring.

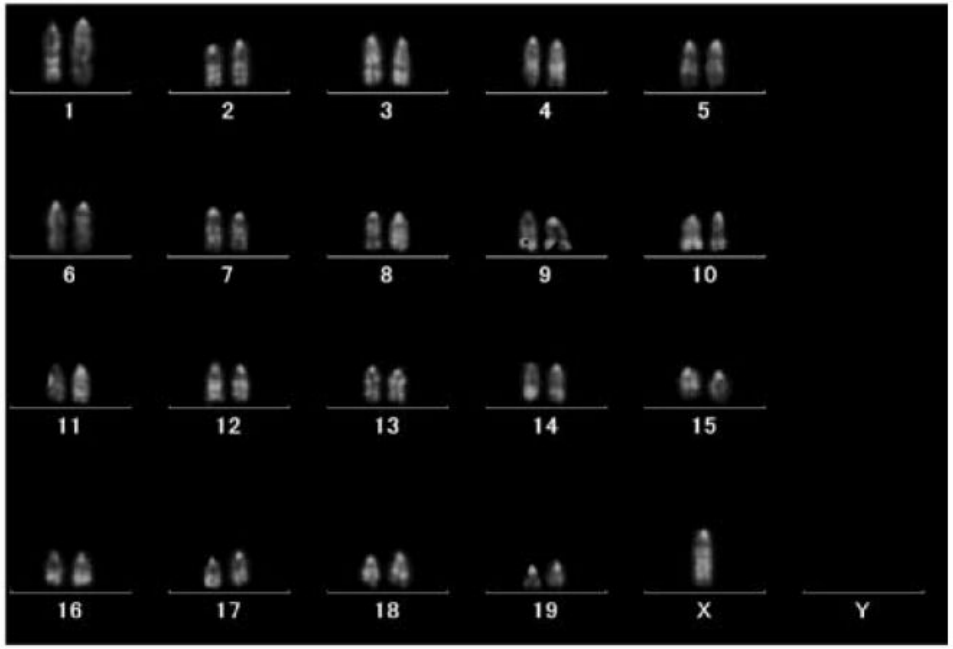
Journal of Reproduction and Development, Vol. 55, No. 5, 2009

—Full Paper—

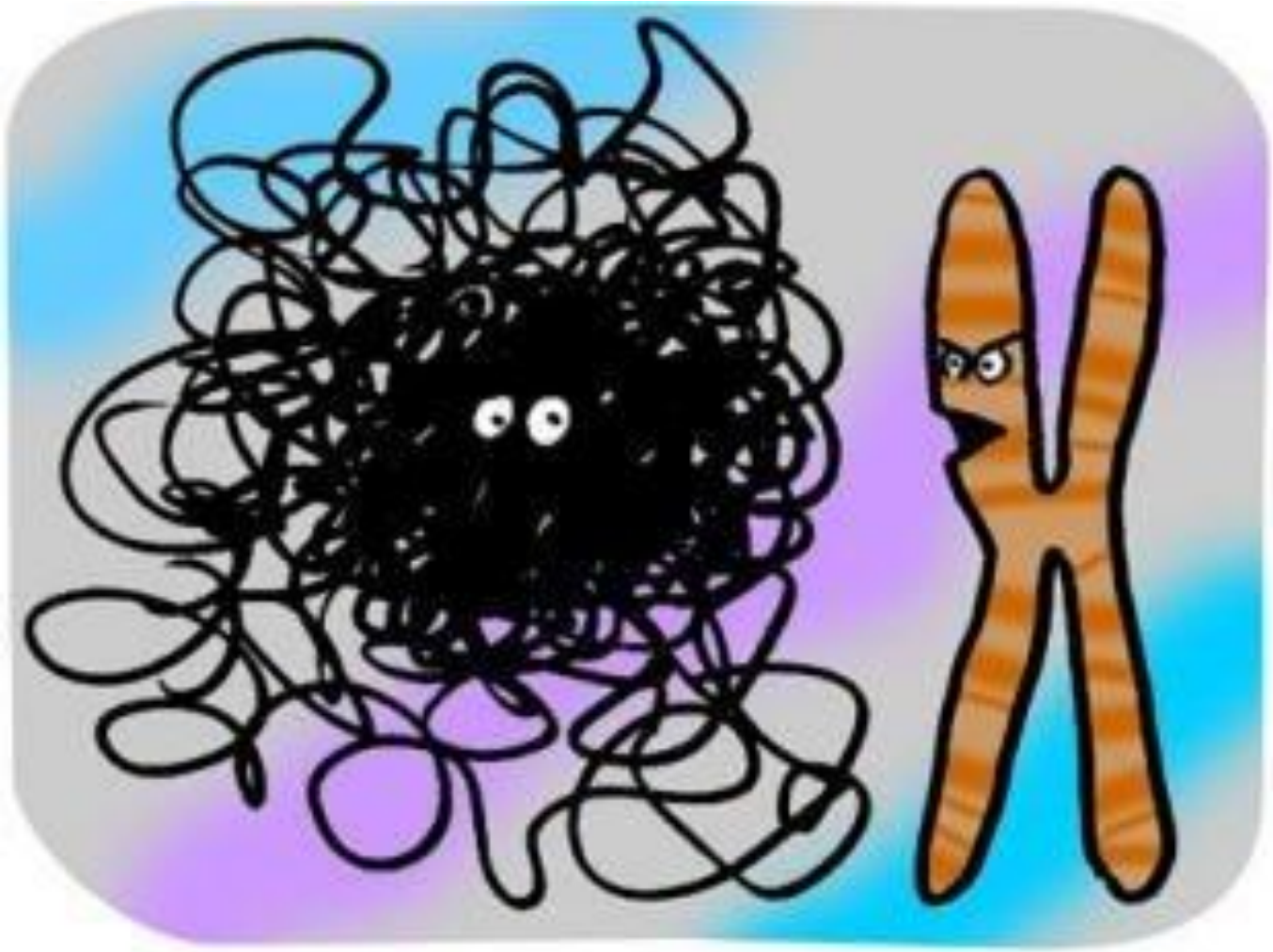
Sex-Reversed Somatic Cell Cloning in the Mouse

Kimiko INOUE[1,2], Narumi OGONUKI[1], Kazuyuki MEKADA[1], Atsushi YOSHIKI[1], Takashi SADO[3] and Atsuo OGURA[1,2,4]

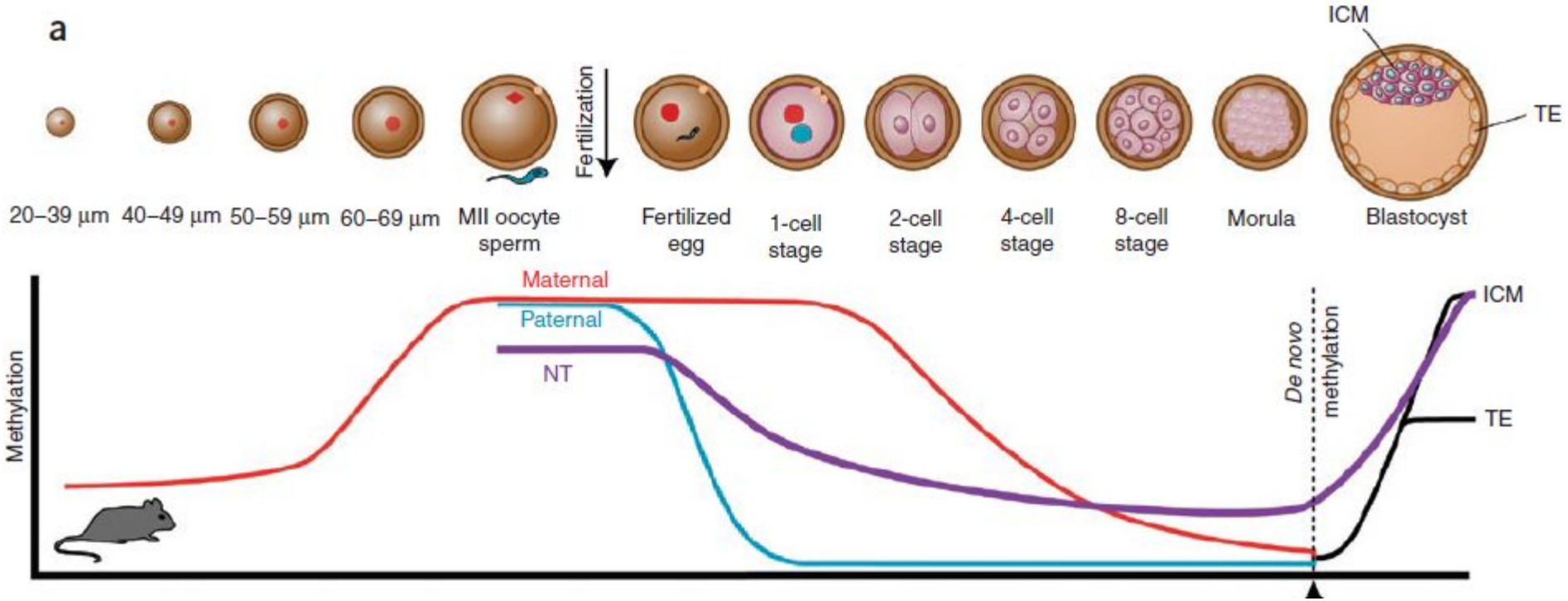




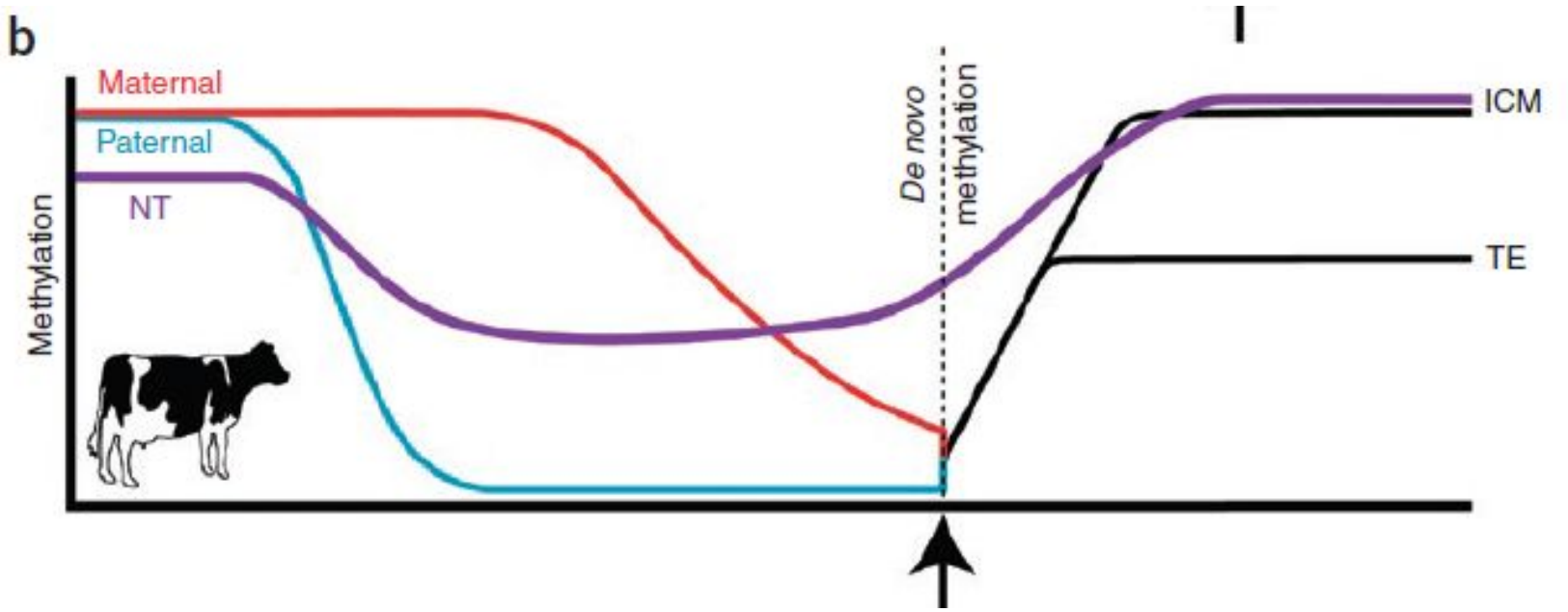
Возможные причины возникновения нарушений при клонировании



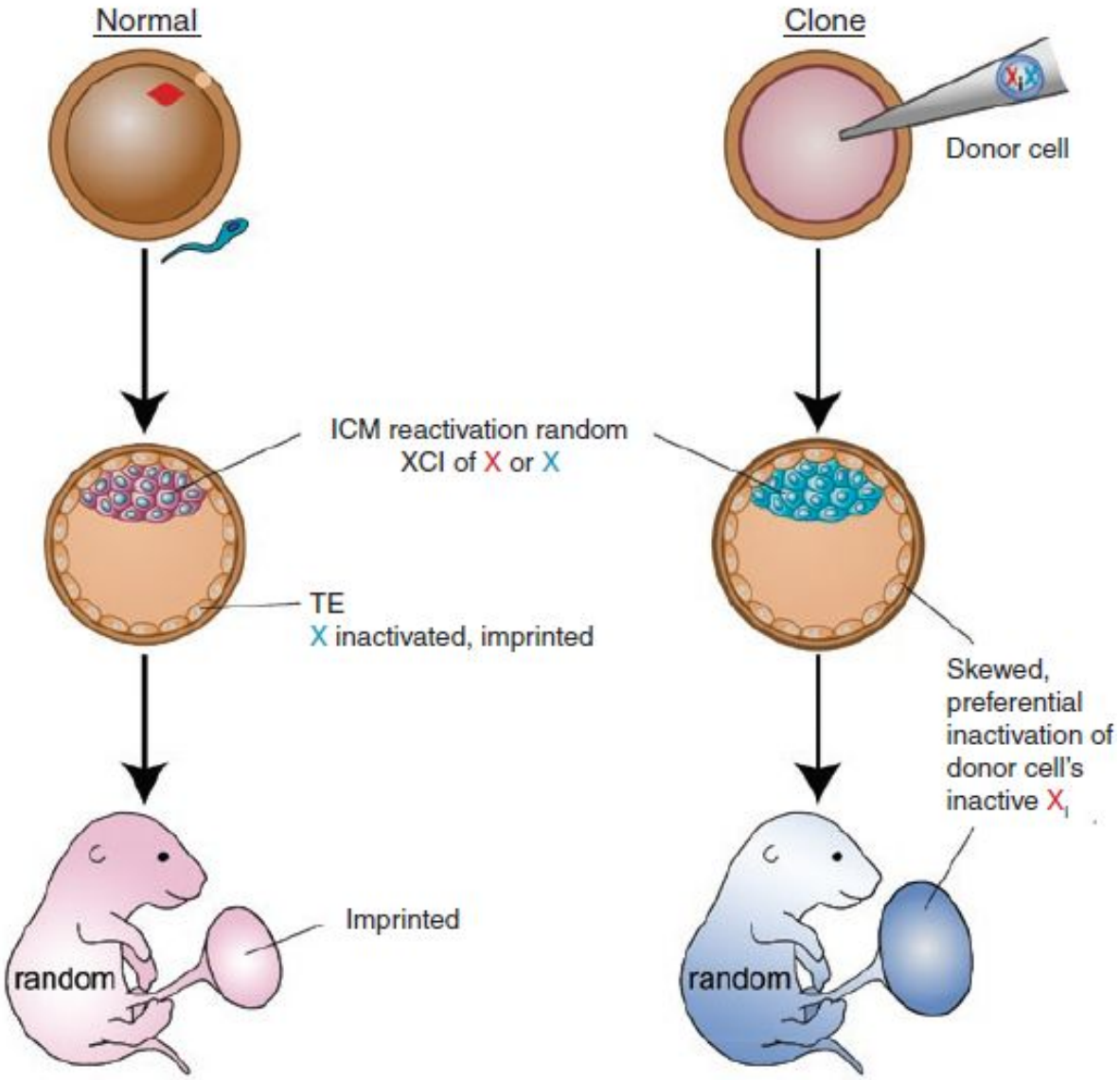
Динамика метилирования ДНК в раннем эмбриональном развитии мыши

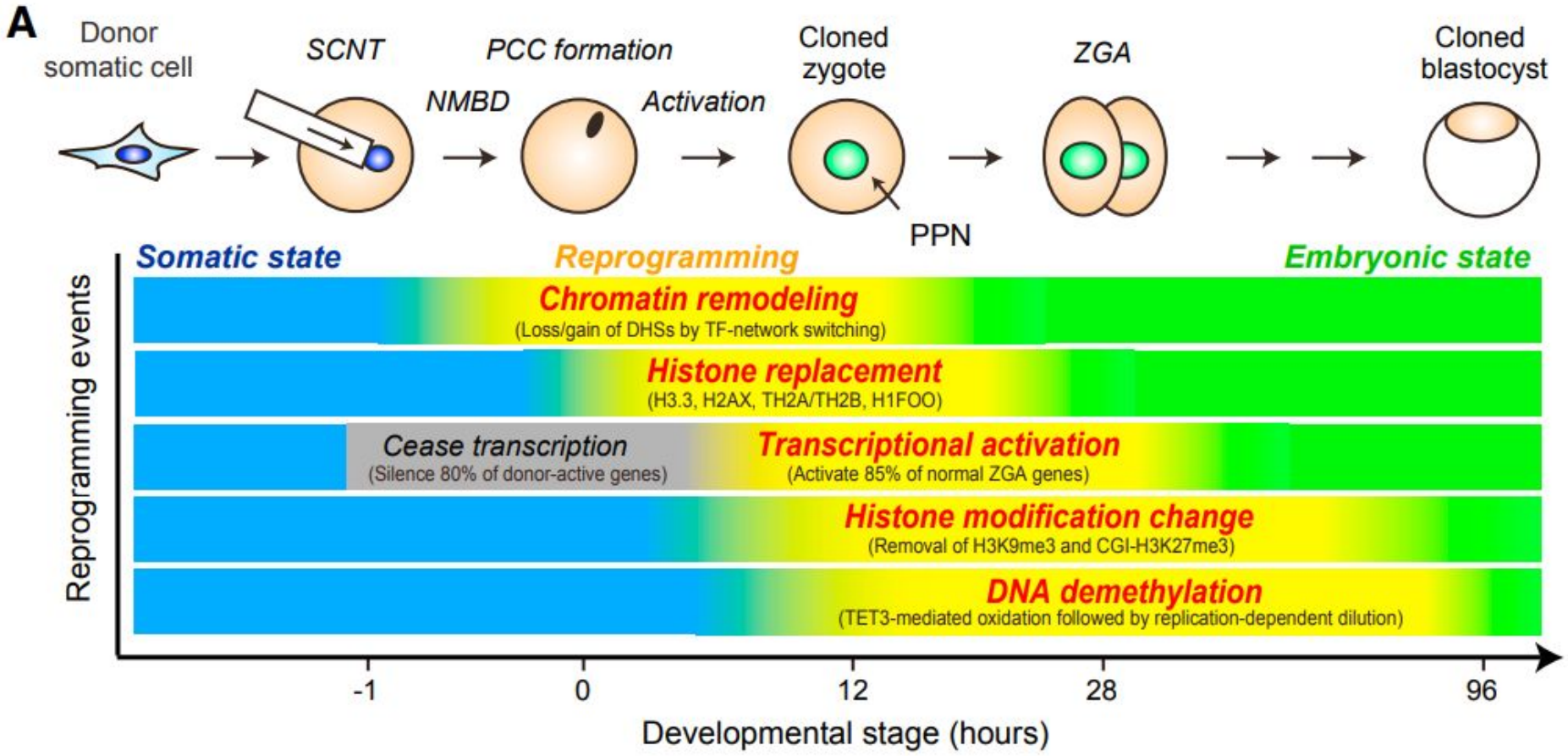


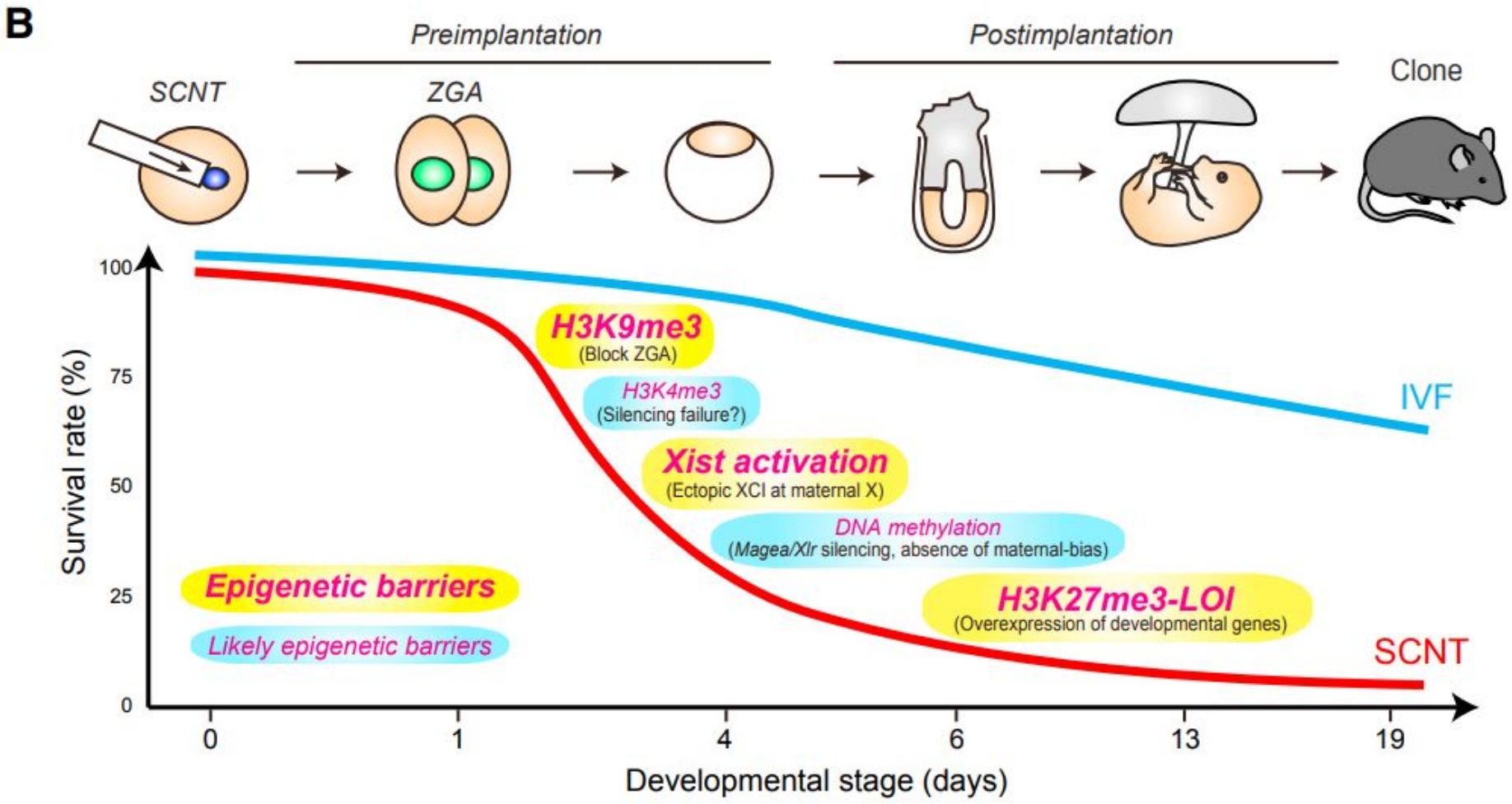
Динамика метилирования ДНК в раннем эмбриональном развитии коров



Одна из возможных причин нарушения развития клонированных животных нарушение инактивации Х-хромосом уклонированных самок




A
Donor somatic cell
SCNT
PCC formation
NMBD
Activation
Cloned zygote
ZGA
Cloned blastocyst
PPN
Somatic state
Reprogramming
Embryonic state
Reprogramming events
Chromatin remodeling
(Loss/gain of DHSs by TF-network switching)
Histone replacement
(H3.3, H2AX, TH2A/TH2B, H1FOO)
Cease transcription
(Silence 80% of donor-active genes)
Transcriptional activation
(Activate 85% of normal ZGA genes)
Histone modification change
(Removal of H3K9me3 and CGI-H3K27me3)
DNA demethylation
(TET3-mediated oxidation followed by replication-dependent dilution)
-1
0
12
28
96
Developmental stage (hours)

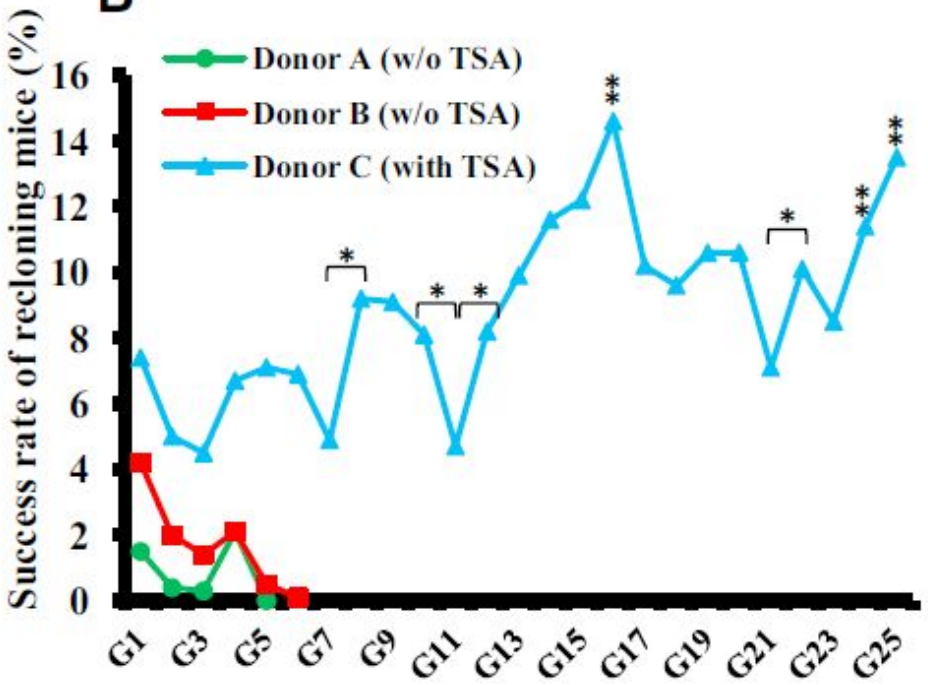

B
Preimplantation
Postimplantation
SCNT
ZGA
Clone
100
75
50
25
0
Survival rate (%)
H3K9me3
(Block ZGA)
H3K4me3
(Silencing failure?)
Xist activation
(Ectopic XCI at maternal X)
DNA methylation
(Magea/Xlr silencing, absence of maternal-bias)
H3K27me3-LOI
(Overexpression of developmental genes)
Epigenetic barriers
Likely epigenetic barriers
IVF
SCNT
0
1
4
6
13
19
Developmental stage (days)

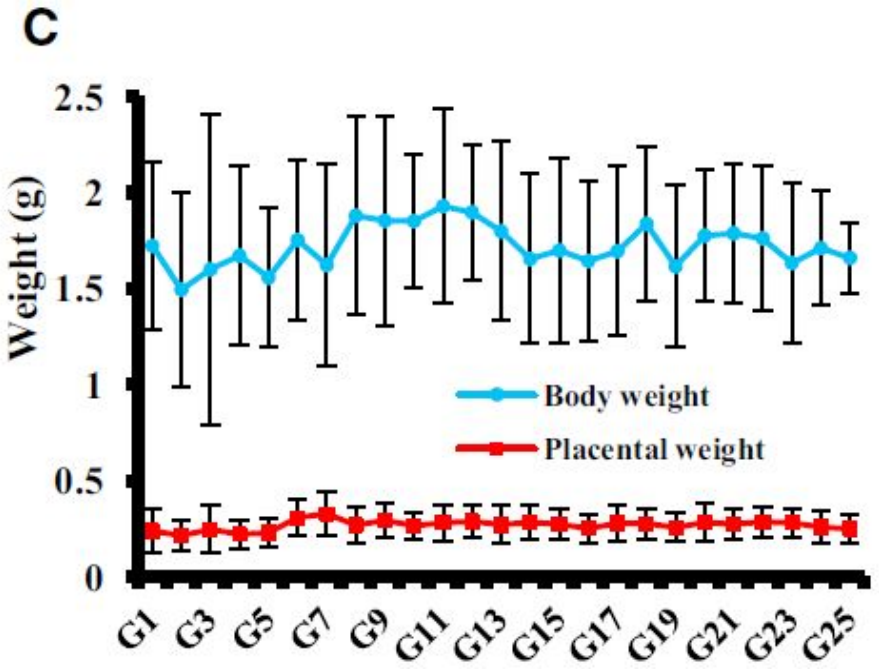
Как узнать происходят ли необратимые изменения при клонировании?

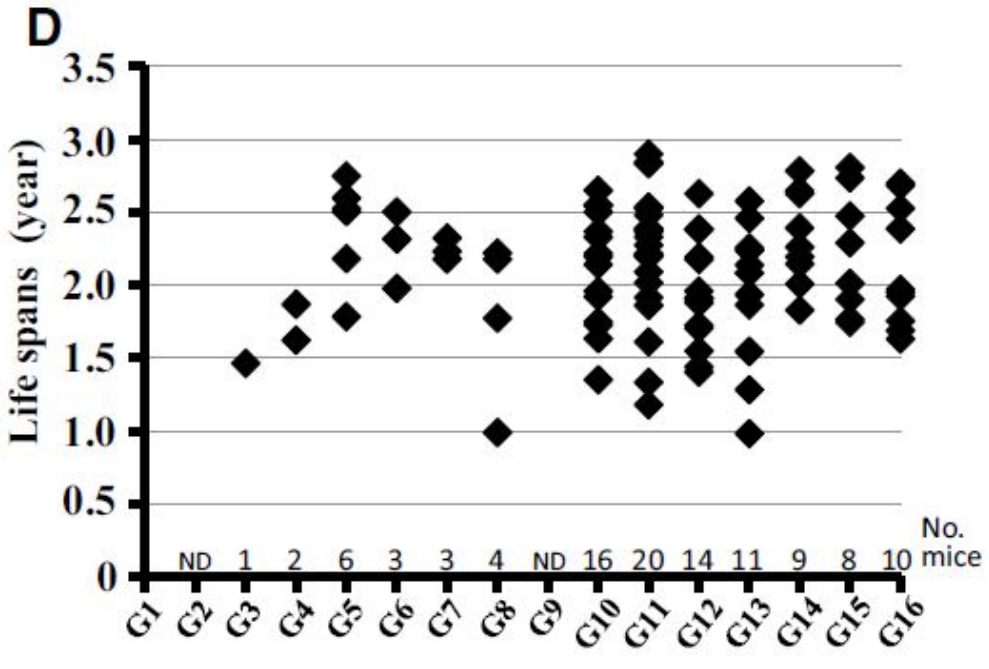
Как долго можно клонировать клонов?

A

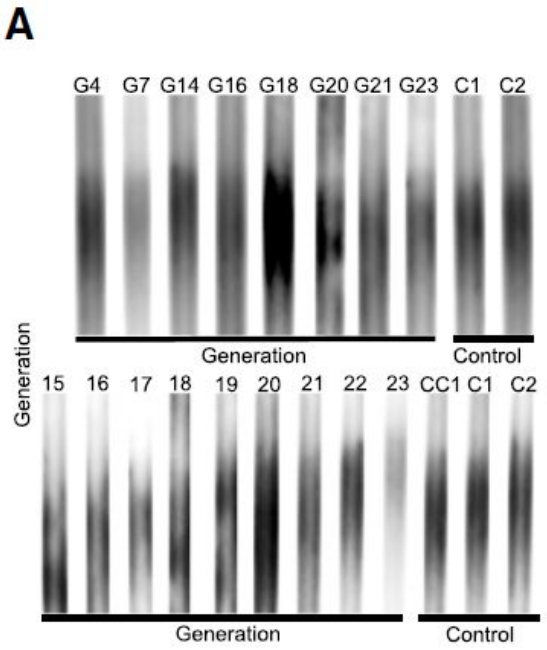
B



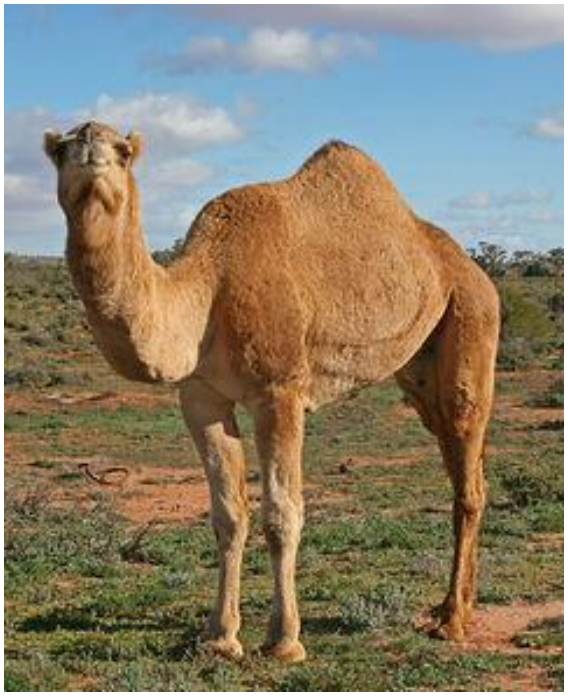




Ни продолжительность жизни ни длинна теломер уклонов не отличались от нормы, но плацента была увеличена



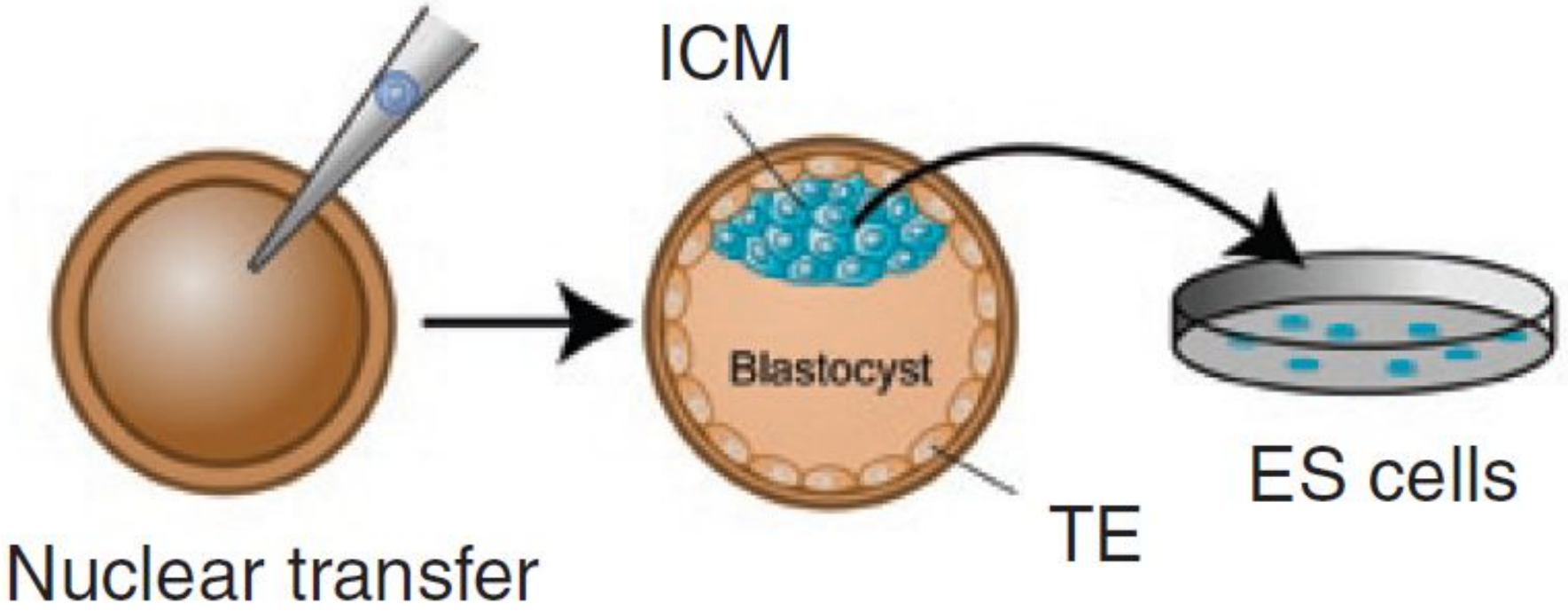
На сегодняшний день клонировано 14 (или чуть более) видов млекопитающих



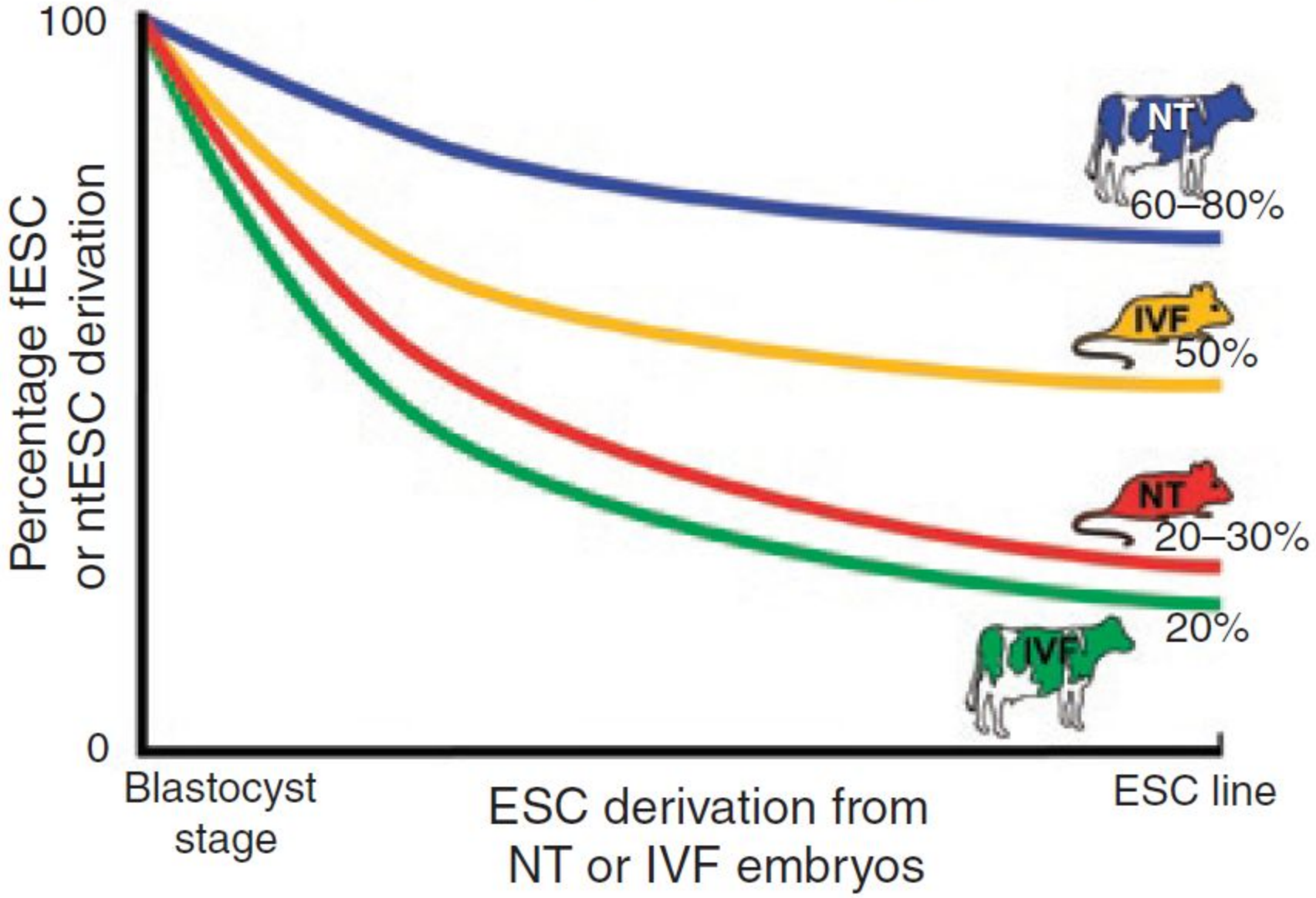

Taeyoung Shin et al.
A cat cloned by nuclear transplantation.
Nature 2002, v.415, 723

Клонирование человека

Терапевтическое клонирование – клонирование с целью получения линии ЭС клеток (ntES cells)



Эффективность терапевтического клонирования

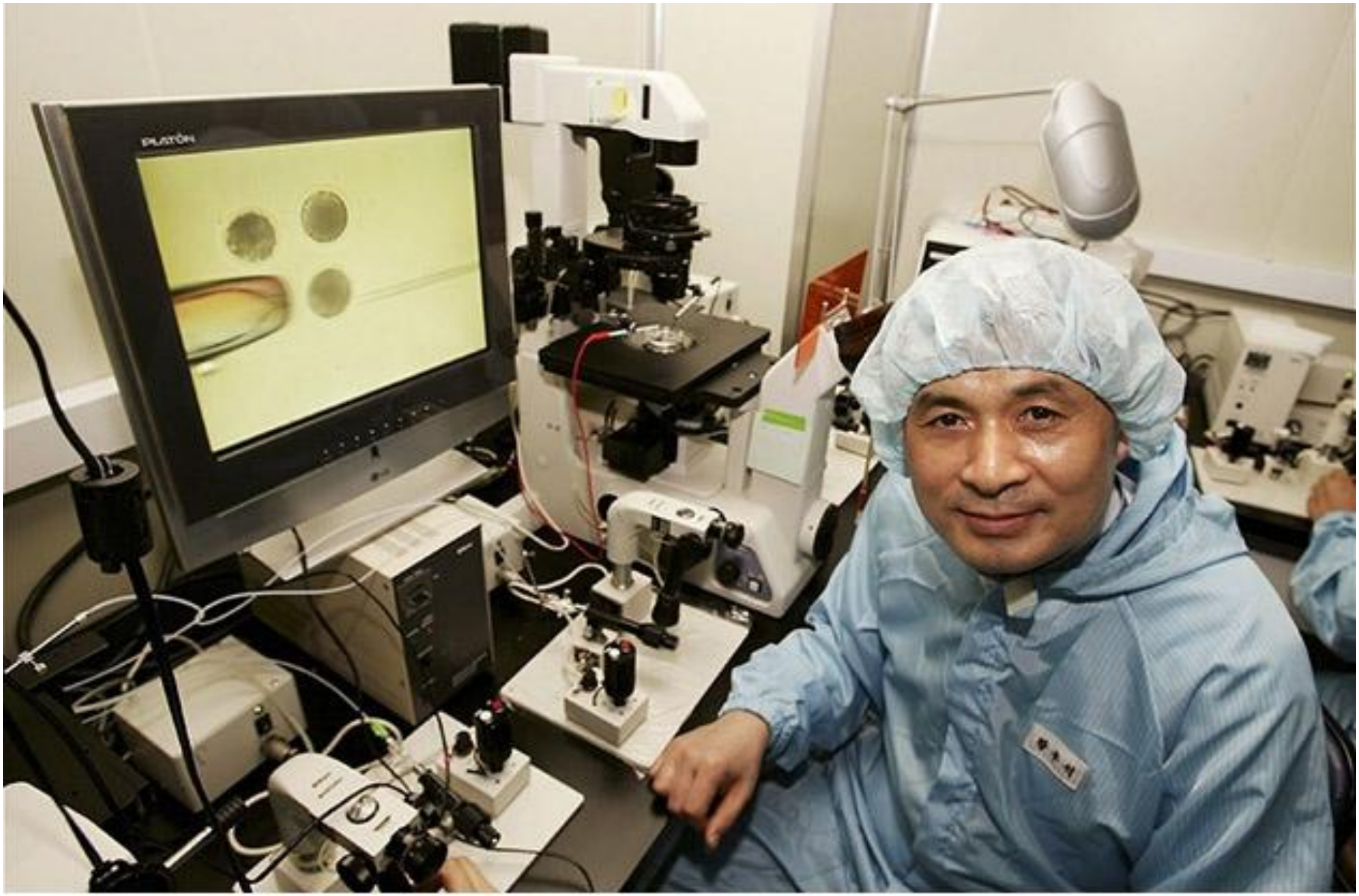


Терапевтическое клонированиие – идеально подходит для лечения наследственных митохондриальных заболеваний

Проблемы технологии терапевтического клонирования

1. До сих пор не было опубликовано ни одного успешного эксперимента по получению ntES клеток человека
2. Источник ооцитов (где их брать?)
3. С какого момента эмбрион является человеком?
4. Насколько стабильны линии ntES клеток

Woo Suk Hwang в 2004 и 2005 годах сообщил о успешном терапевтическом клонировании человека



Расследование показало, что результаты были сфальсифицированы!

Шухрат Миталипов впервые осуществил терапевтическое клонирование человека

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,[4] 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,*]
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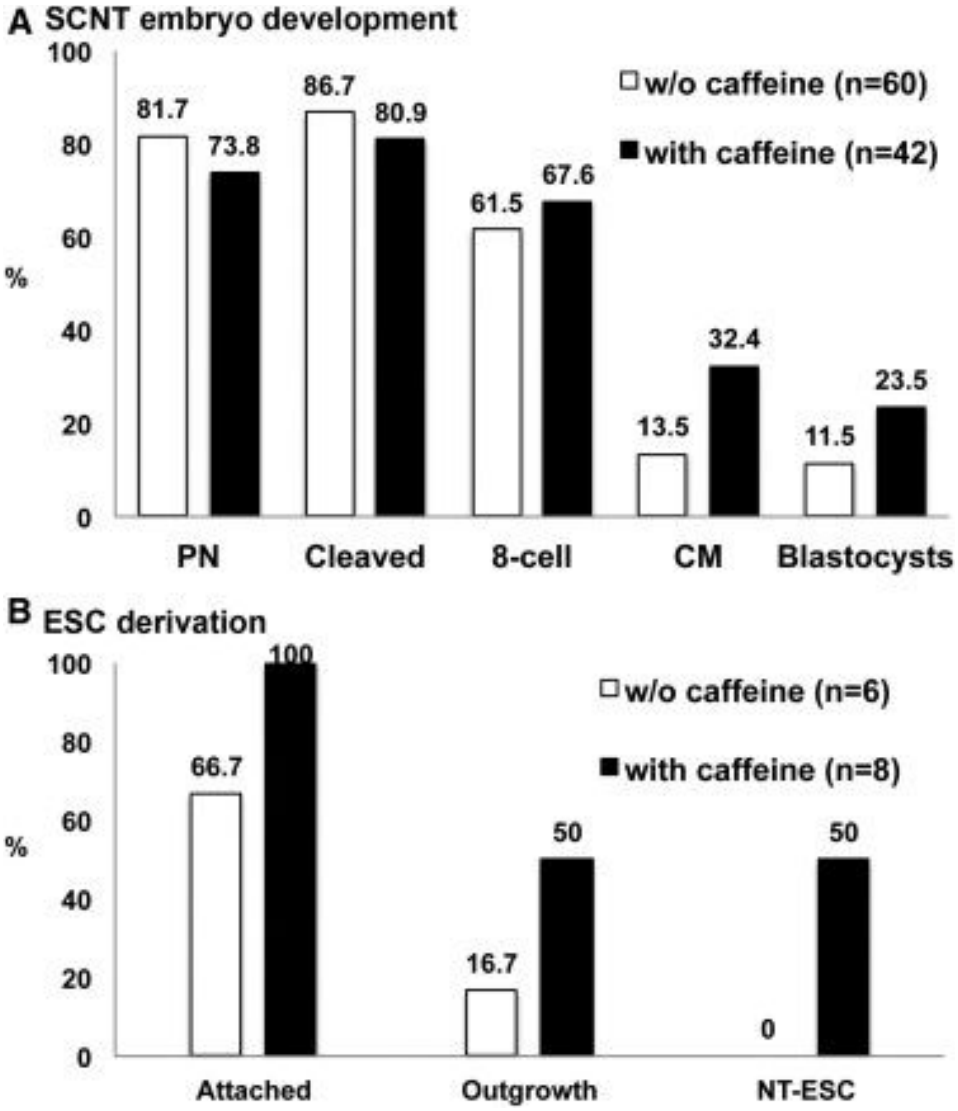
http://dx.doi.org/10.1016/j.cell.2013.05.006

Кофе и клонирование



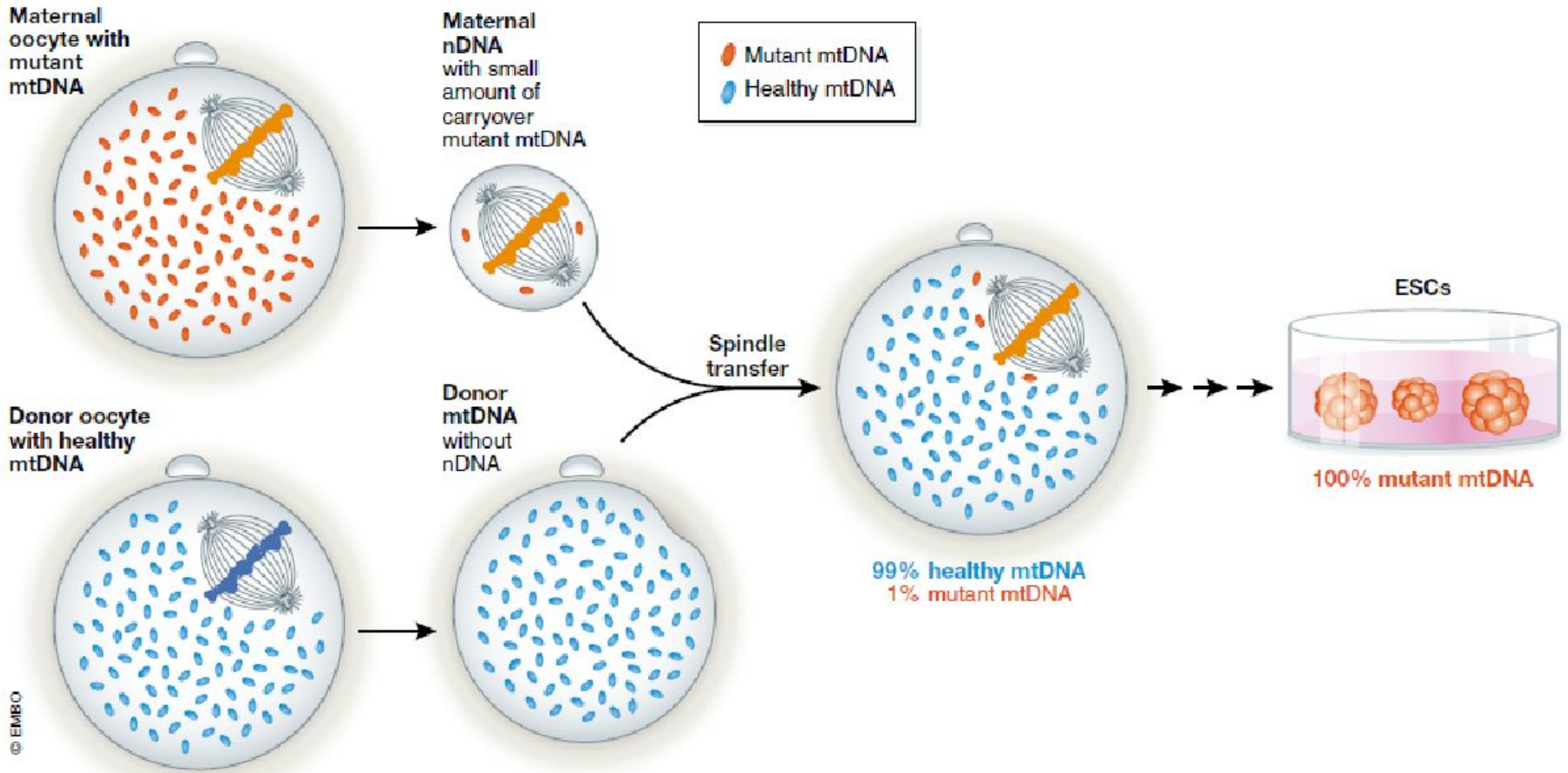


Figure 1. Mitochondrial replacement in human oocytes by spindle transfer and subsequent reversal to the maternal mtDNA in ESCs.
Mitochondrial replacement by spindle transfer isolates and transfers a meiotic spindle apparatus with the chromosomes from an unfertilized maternal oocyte into a donor oocyte cytoplasm containing healthy mtDNA. Blue and orange dots depict normal and mutant mtDNA, respectively. Replacement is not absolute and results in a small (less than 1%) carryover of mutant mtDNA from the maternal oocyte. After fertilization, preimplantation embryo development, or expanded culture of ESCs, the proportion of carryover mtDNA may increase resulting in complete reversal to the mutant maternal mtDNA.

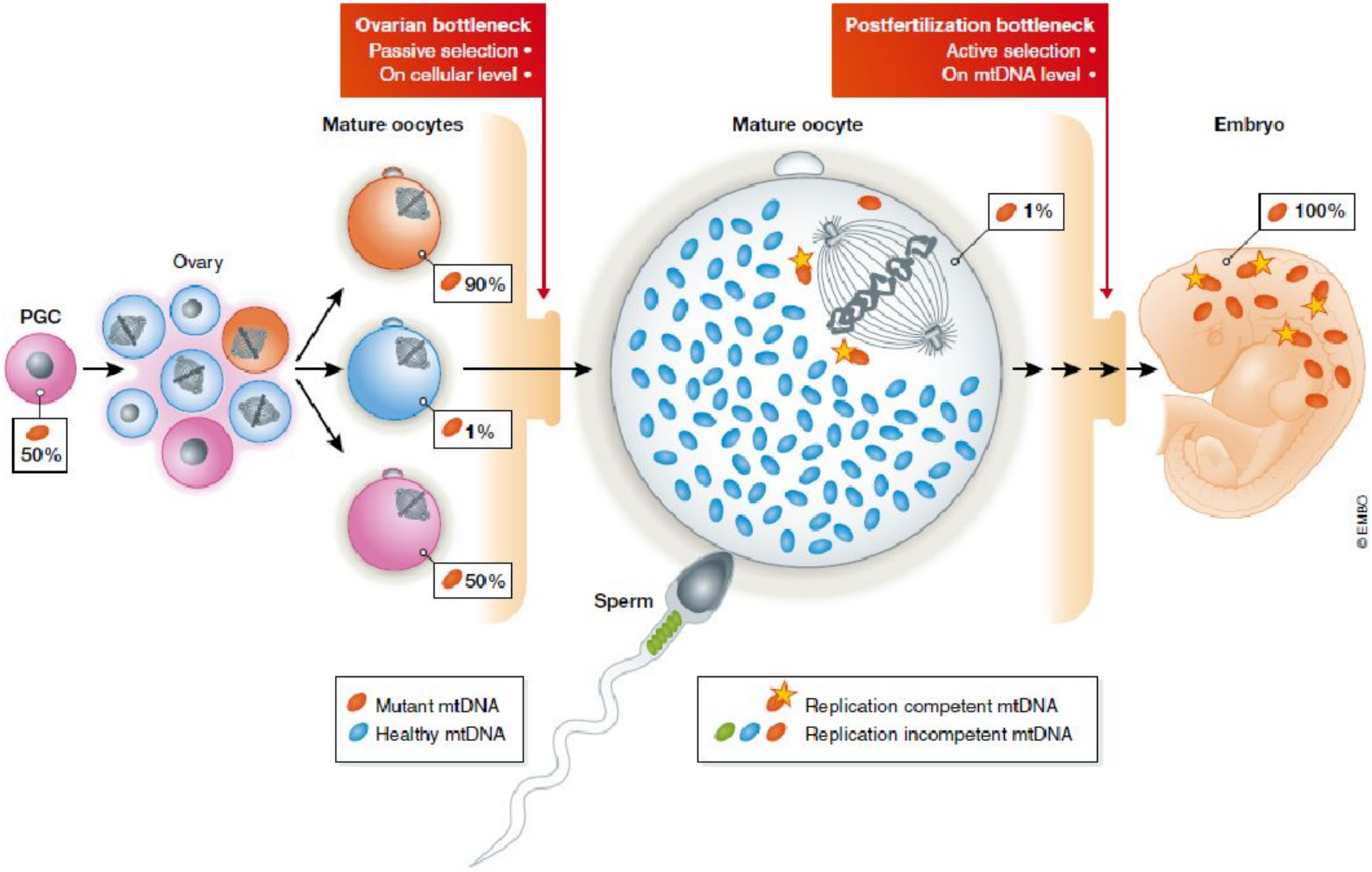
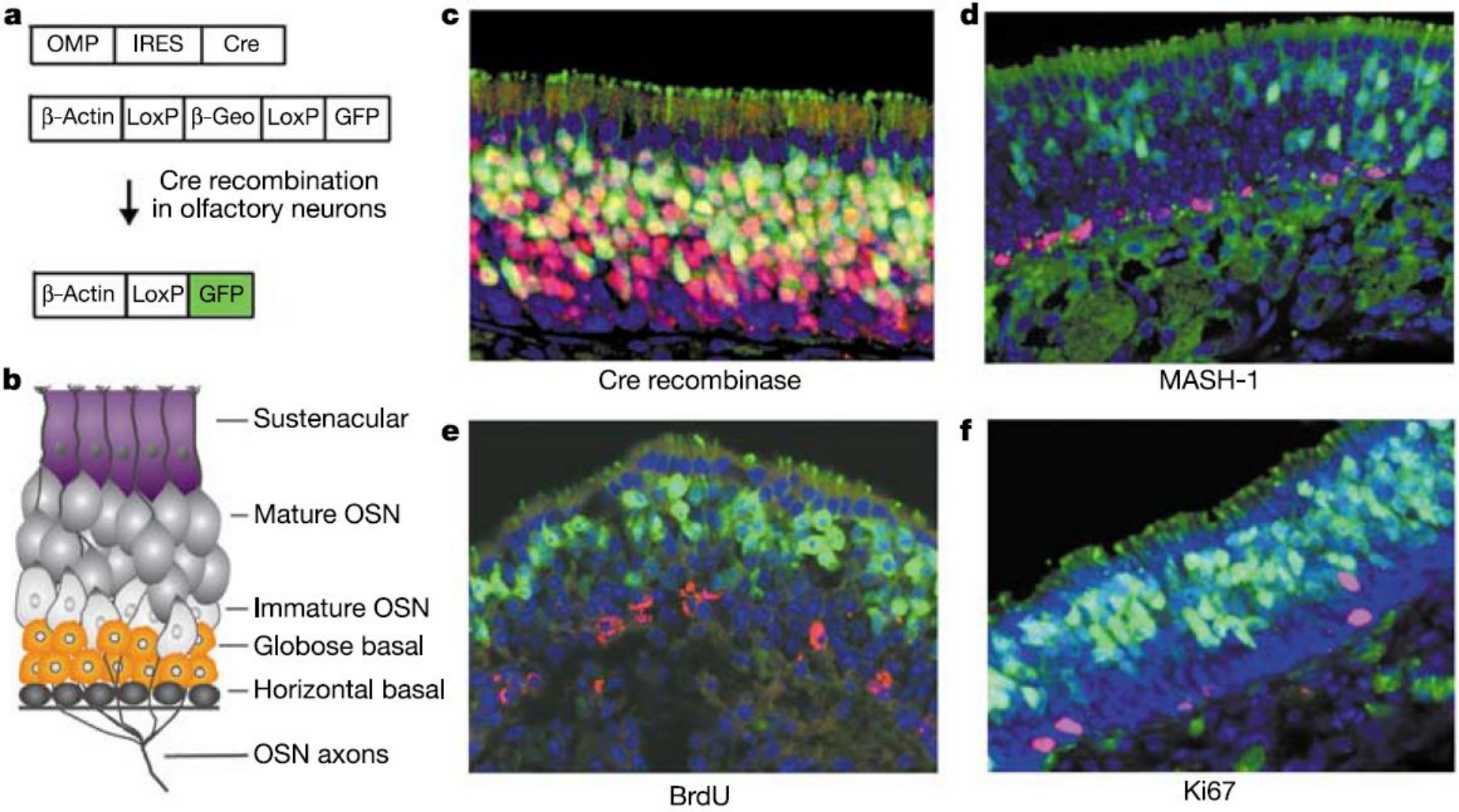


Figure 2. Model of the post-fertilization mtDNA bottleneck.

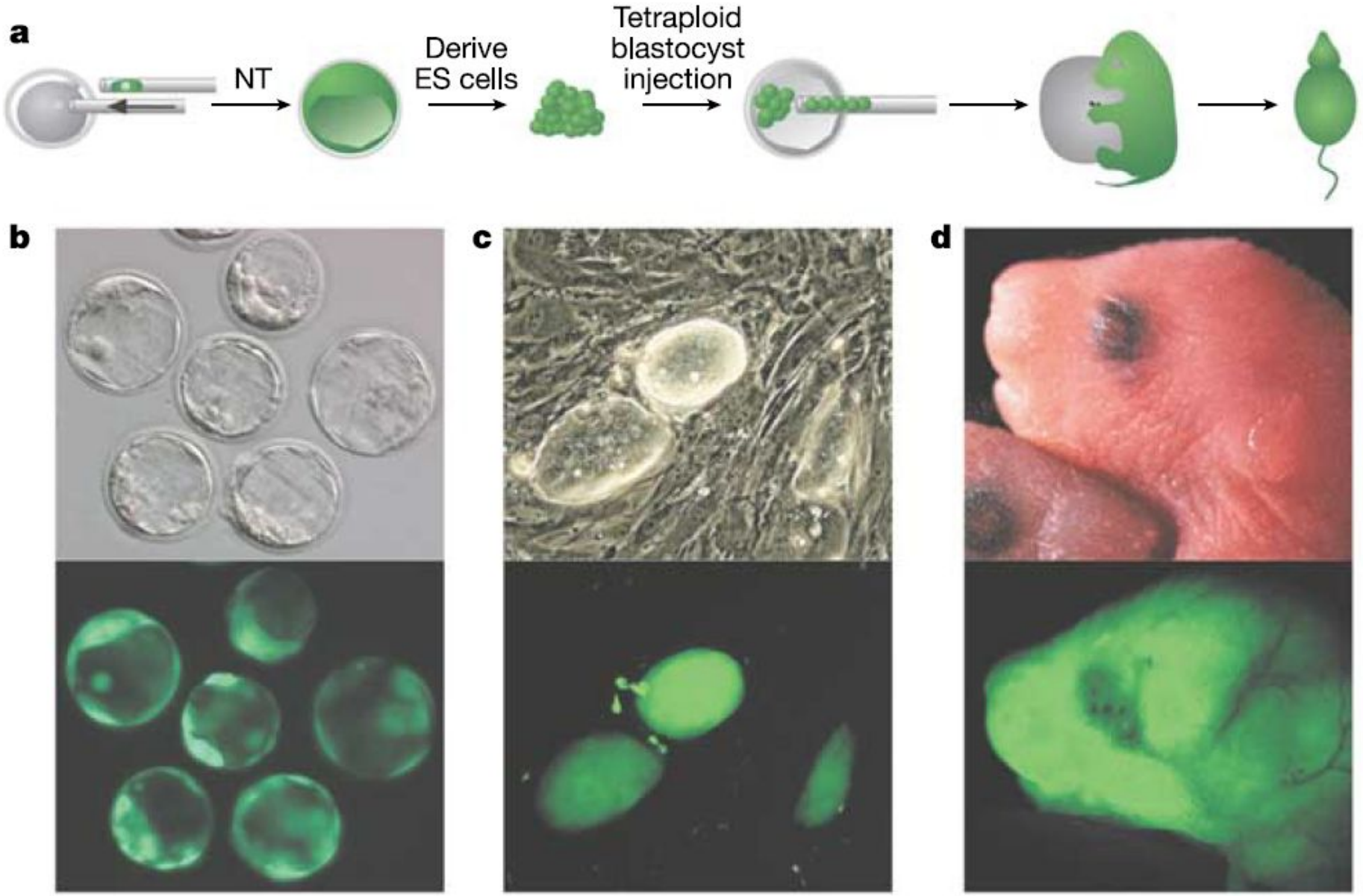
During the first ovarian bottleneck, individual mature oocytes may acquire various heteroplasmy levels of mtDNA mutation. These mutation levels in oocytes may change again after fertilization during subsequent embryonic development resulting in a dramatic increase in mutant mtDNA levels in offspring. This rapid shift in mtDNA heteroplasmy is likely due to preferential replication of a small selected population of mtDNAs in mature oocytes. The vast majority of mtDNA molecules (99%) in an oocyte are not replicated and will be lost during subsequent embryonic development. Similarly, paternal, sperm mtDNA introduced during fertilization will be passively lost due to lack of replication. Replication-competent mtDNA (1%) is likely marked epigenetically by the time of oocyte maturation and co-localized within perinuclear compartment in human oocytes. PGC: primordial germ cell. Blue, orange, and green dots represent normal, mutant, and sperm mtDNA, respectively. Star-marked red dots depict replication-competent mtDNA.

Клонирование мышей из терминально дифференцированных клеток – сенсорных обонятельных нейронов

2 000 000 клеток, 1500 генов рецепторов



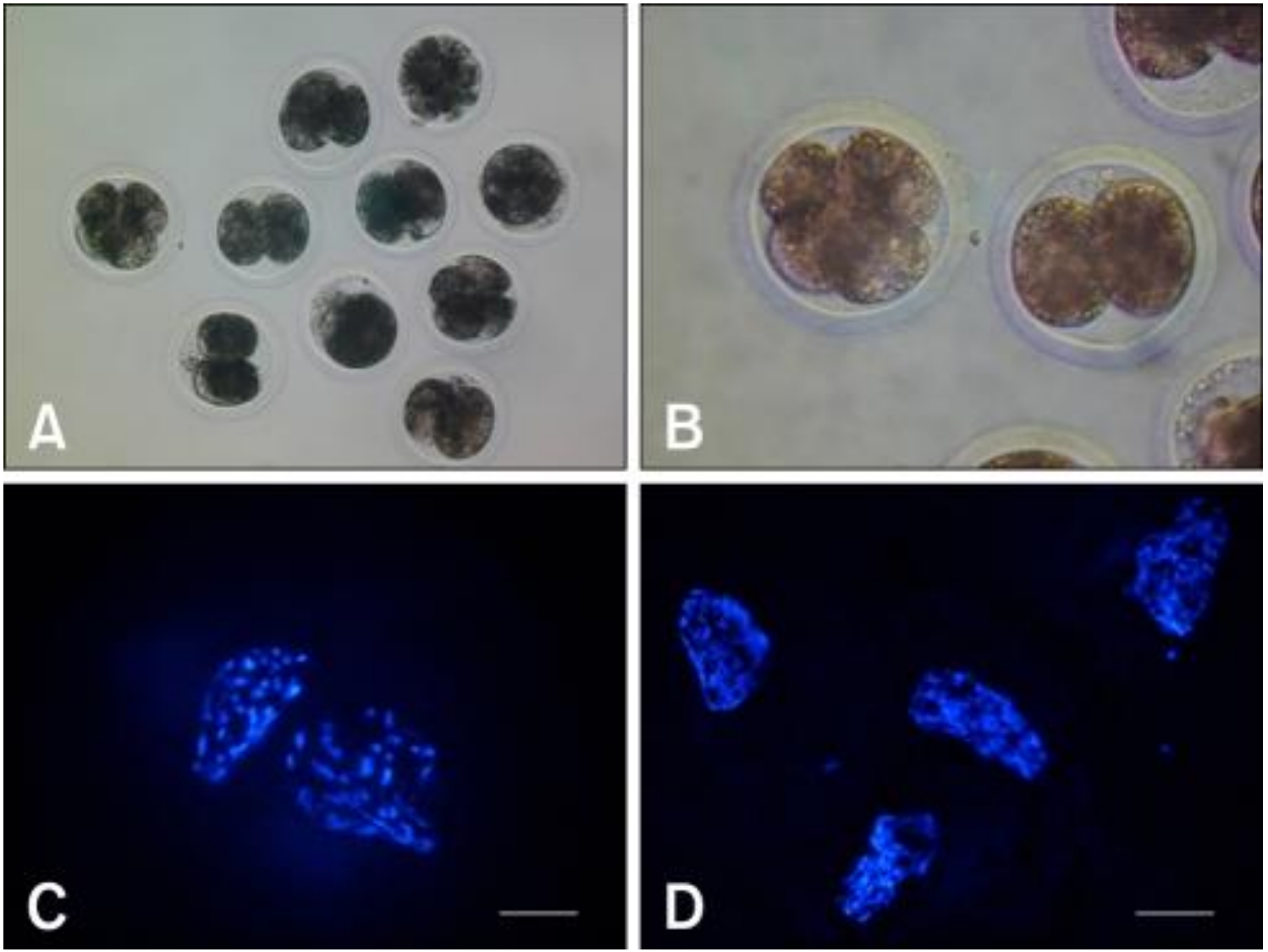
Клонирование мышей из терминально дифференцированных клеток – сенсорных обонятельных нейронов



Клонирование сейвала

Клонирование сейвала

Ядра фибробластов кита инъецировали в энуклеированные ооциты свиньи

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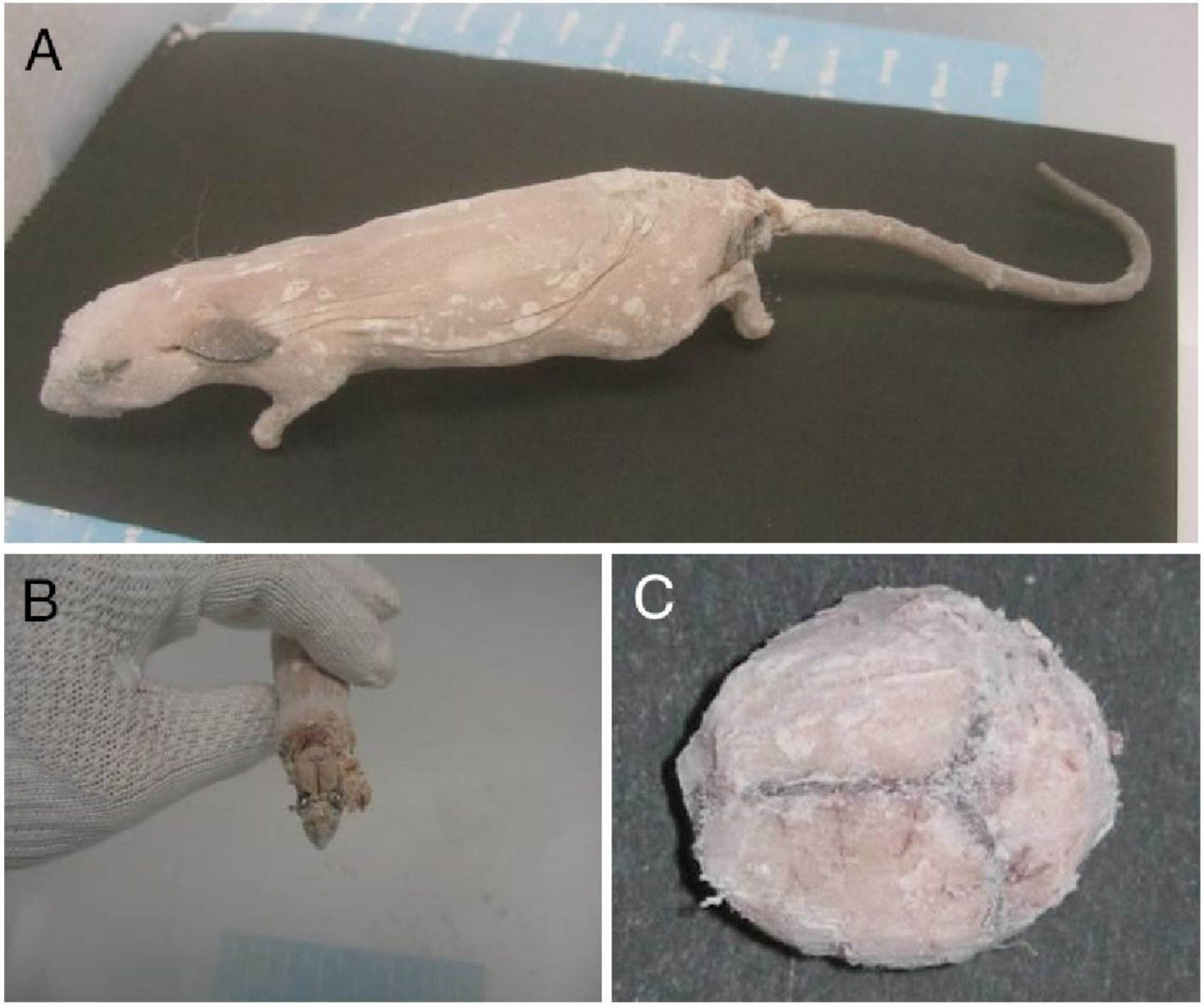
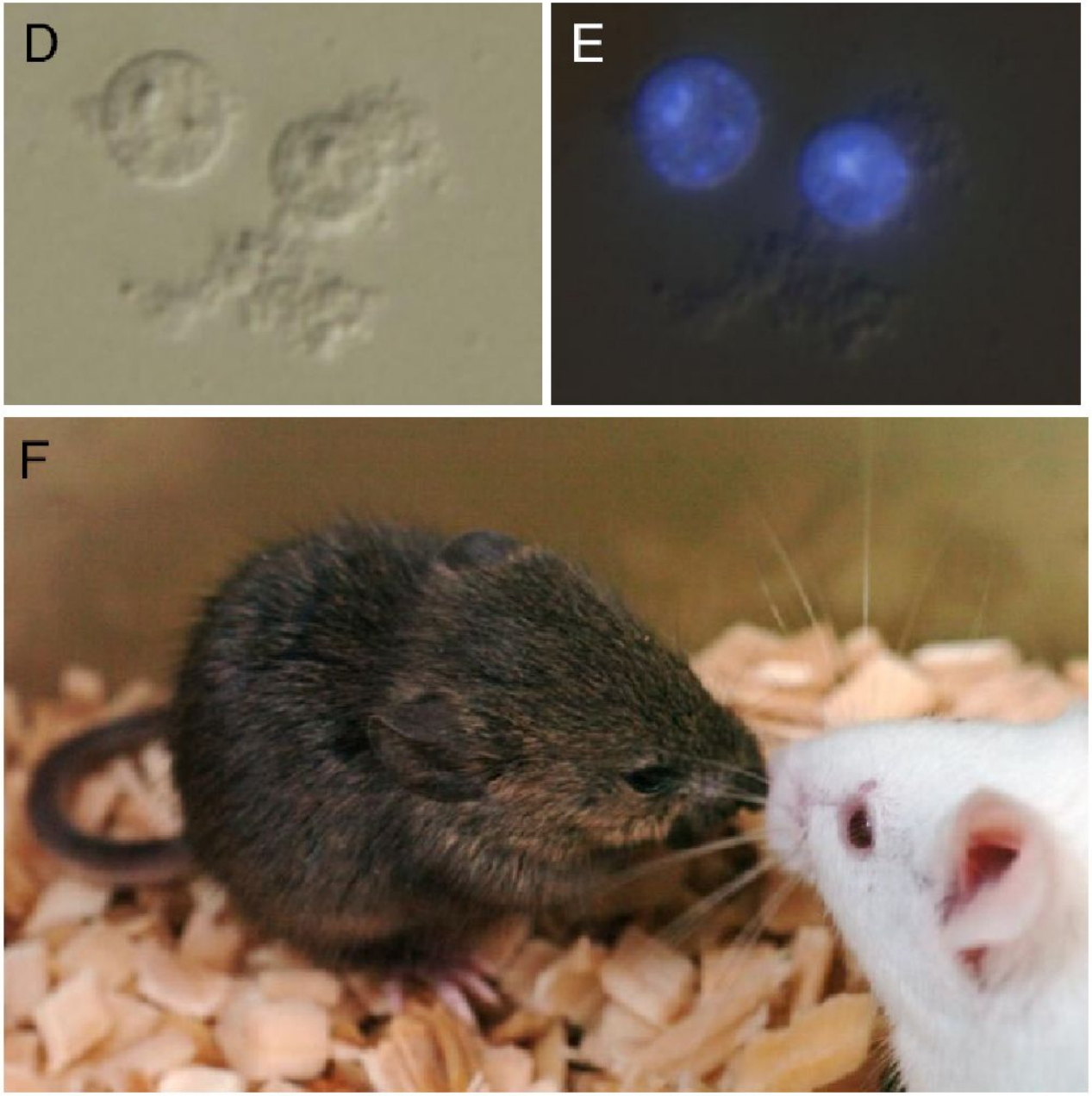
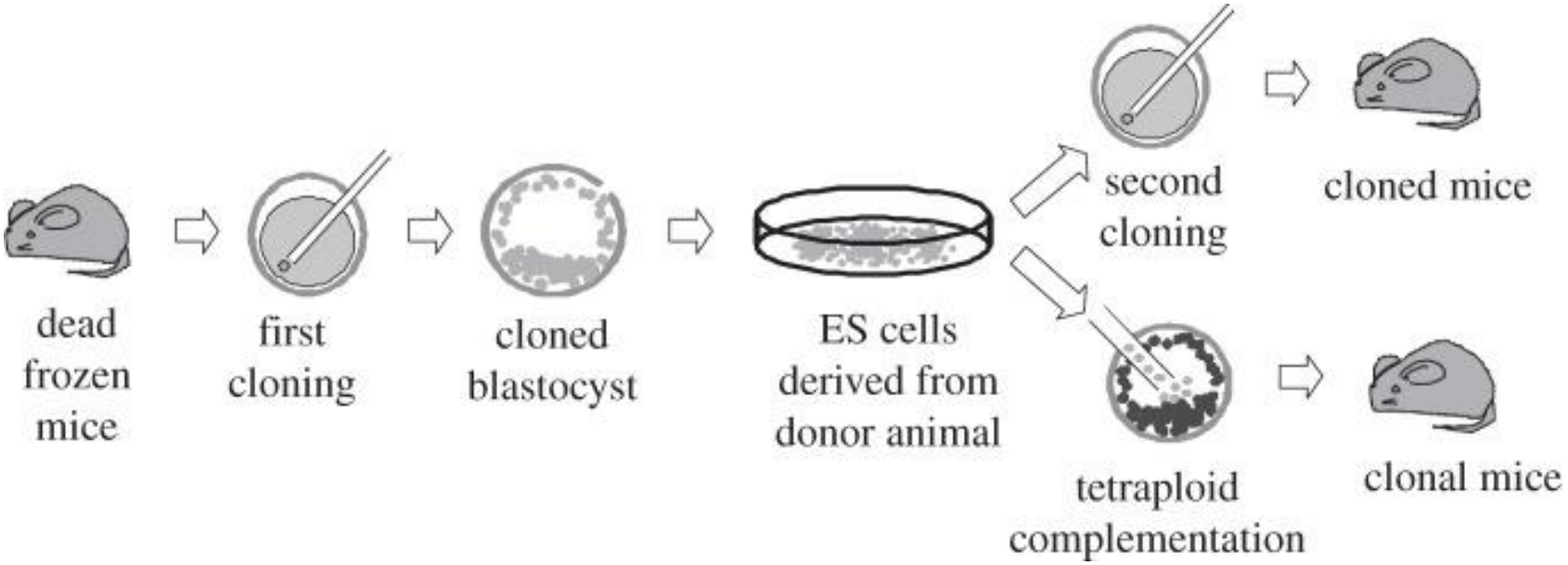
Успешное клонирование мыши 16 лет (!) пролежавшей в морозильнике



Успешное клонирование мыши 16 лет (!) пролежавшей в морозильнике




dead
frozen
mice
first
cloning
cloned
blastocyst
ES cells
derived from
donor animal
second
cloning
cloned mice
tetraploid
complementation
clonal mice

«Воскрешение» Ясуфуку

Ясуфуку – папа 40000 телят. 30% животных этой породы его прямые потомки

Мраморная говядина

«Воскрешение» Ясуфуку

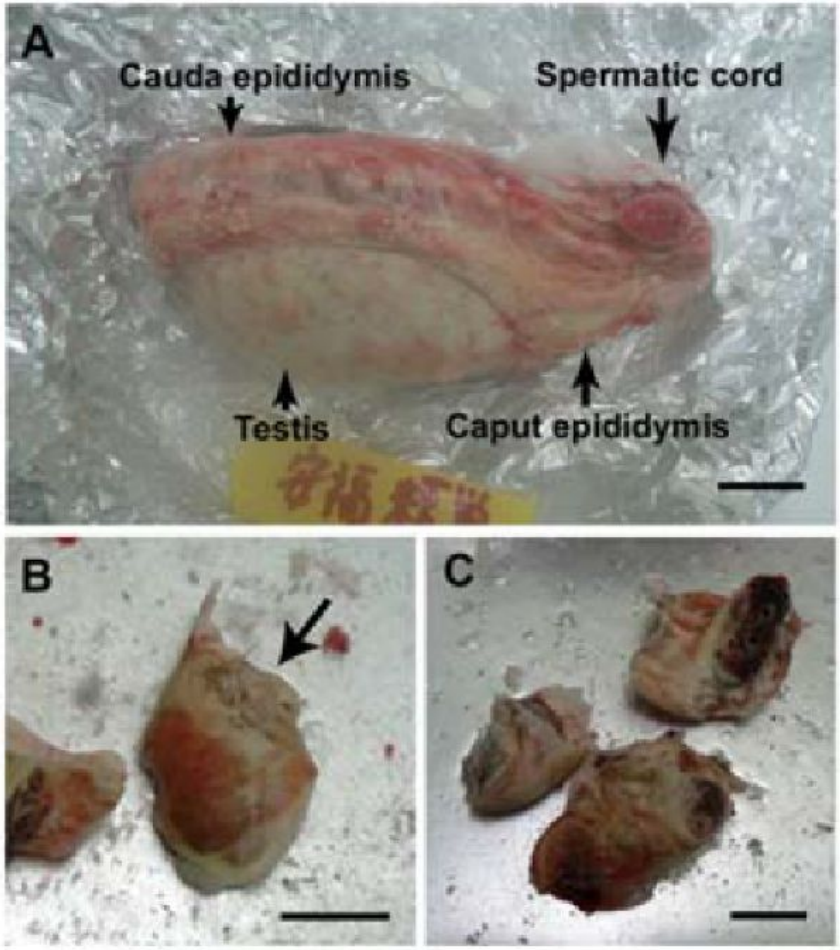


Figure 1. One of Yasufuku's testicles frozen for 13 years. The testicle was stored in a −80°C freezer for 10 years and then transferred to liquid nitrogen for 3 years. **(A)** Yasufuku's frozen testicle. **(B)** Part of the caput epididymis (arrow). **(C)** Spermatic cords that had been cut into three pieces. Scale bars represent 2 cm.

doi:10.1371/journal.pone.0004142.g001

Ясуфуки 2.0

Один из клонов погиб вскоре после рождения от проблем с легкими

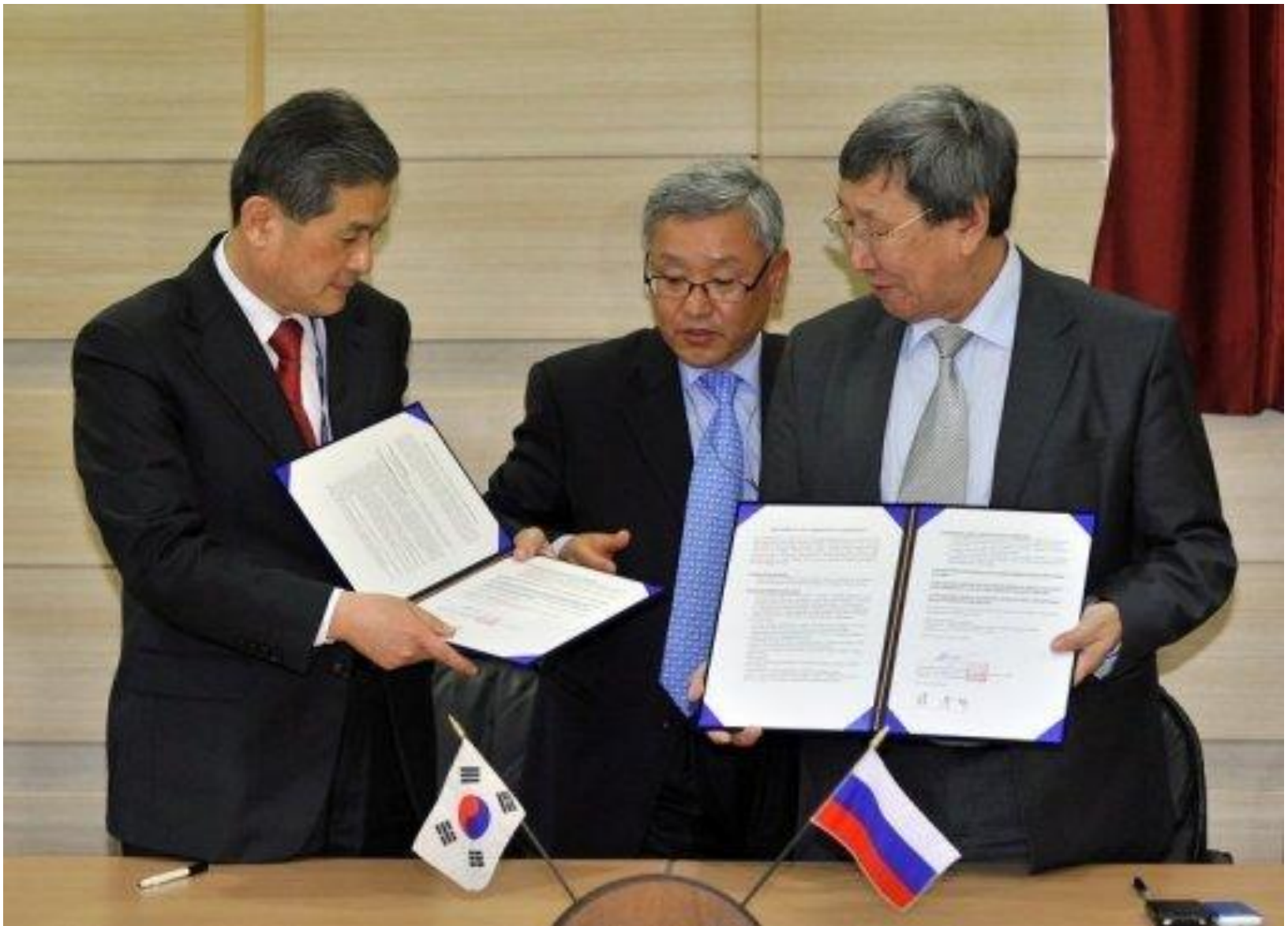
LENTA.RU
Наука и техника
1 СЕНТЯБРЯ 2014, ПОНЕДЕЛЬНИК, 16:06

12:57, 1 сентября 2014

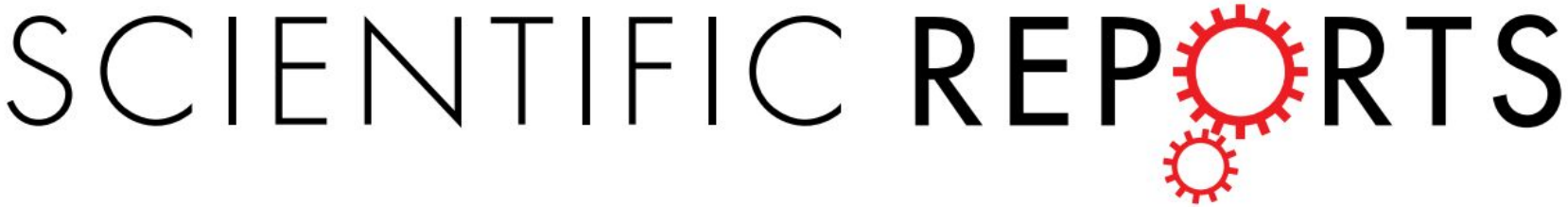
Путин заинтересовался возможностью клонирования мамонтов

Фото: s-vfu.ru

«South Korean scientist Hwang Woo-Suk and Vasily Vasiliev, vice director of North-Eastern Federal University of Russia's Sakha Republic, exchange agreements during a signing ceremony on joint research at Hwang's office in Seoul. The research collaboration agreement will help Russian and S.Korean scientists to recreate a woolly mammoth which last walked the earth some 10,000 years ago.»



www.nature.com/scientificreports



OPEN

Signs of biological activities of 28,000-year-old mammoth nuclei in mouse oocytes visualized by live-cell imaging

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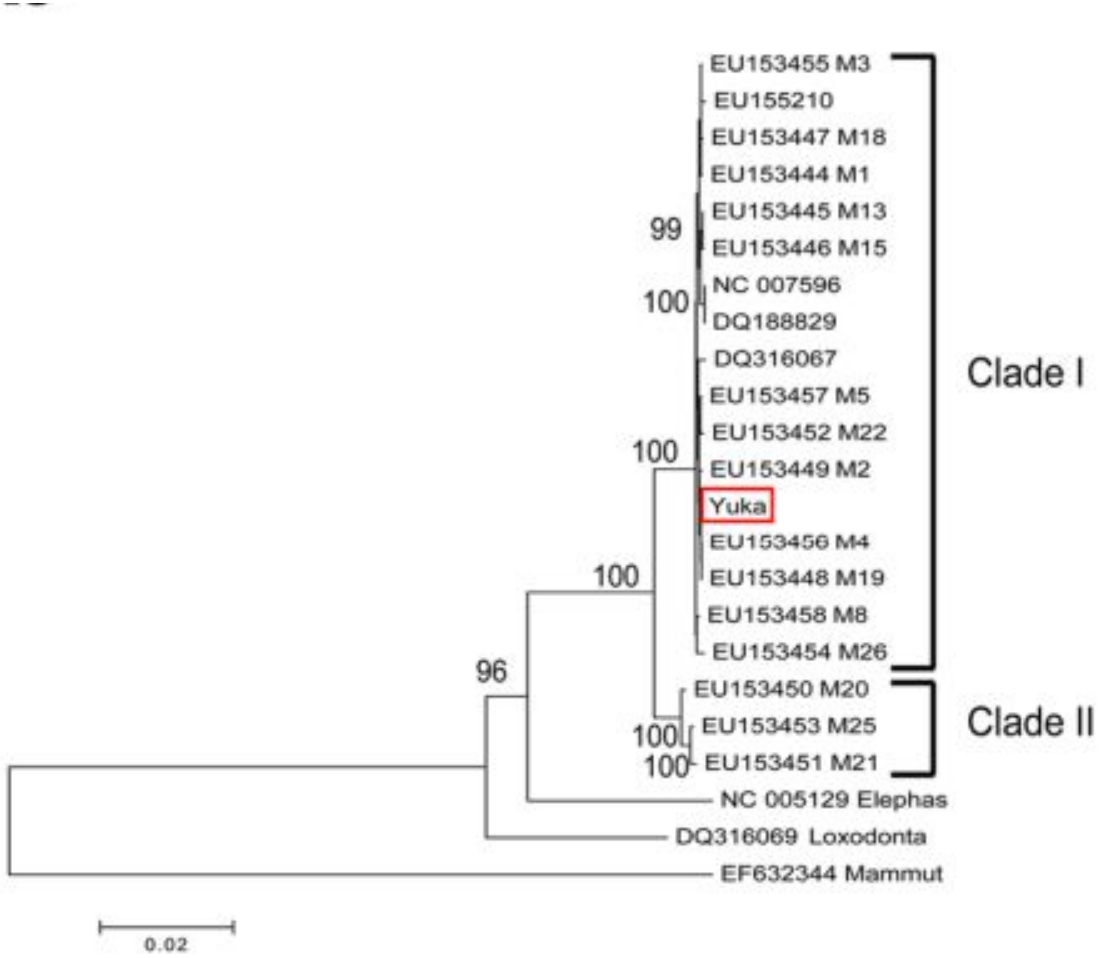
Accepted: 19 February 2019
Published online: 11 March 2019

Kazuo Yamagata[1], Kouhei Nagai[1], Hiroshi Miyamoto[1], Masayuki Anzai[2], Hiromi Kato[2], Kei Miyamoto[1], Satoshi Kurosaka[2], Rika Azuma[1], Igor I. Kolodeznikov[3], Albert V. Protopopov[3], Valerii V. Plotnikov[3], Hisato Kobayashi[4], Ryouka Kawahara-Miki[4], Tomohiro Kono[4,5], Masao Uchida[6], Yasuyuki Shibata[6], Tetsuya Handa[7], Hiroshi Kimura[7], Yoshihiko Hosoi[1], Tasuku Mitani[1], Kazuya Matsumoto[1] & Akira Iritani[2]


EU153455 M3
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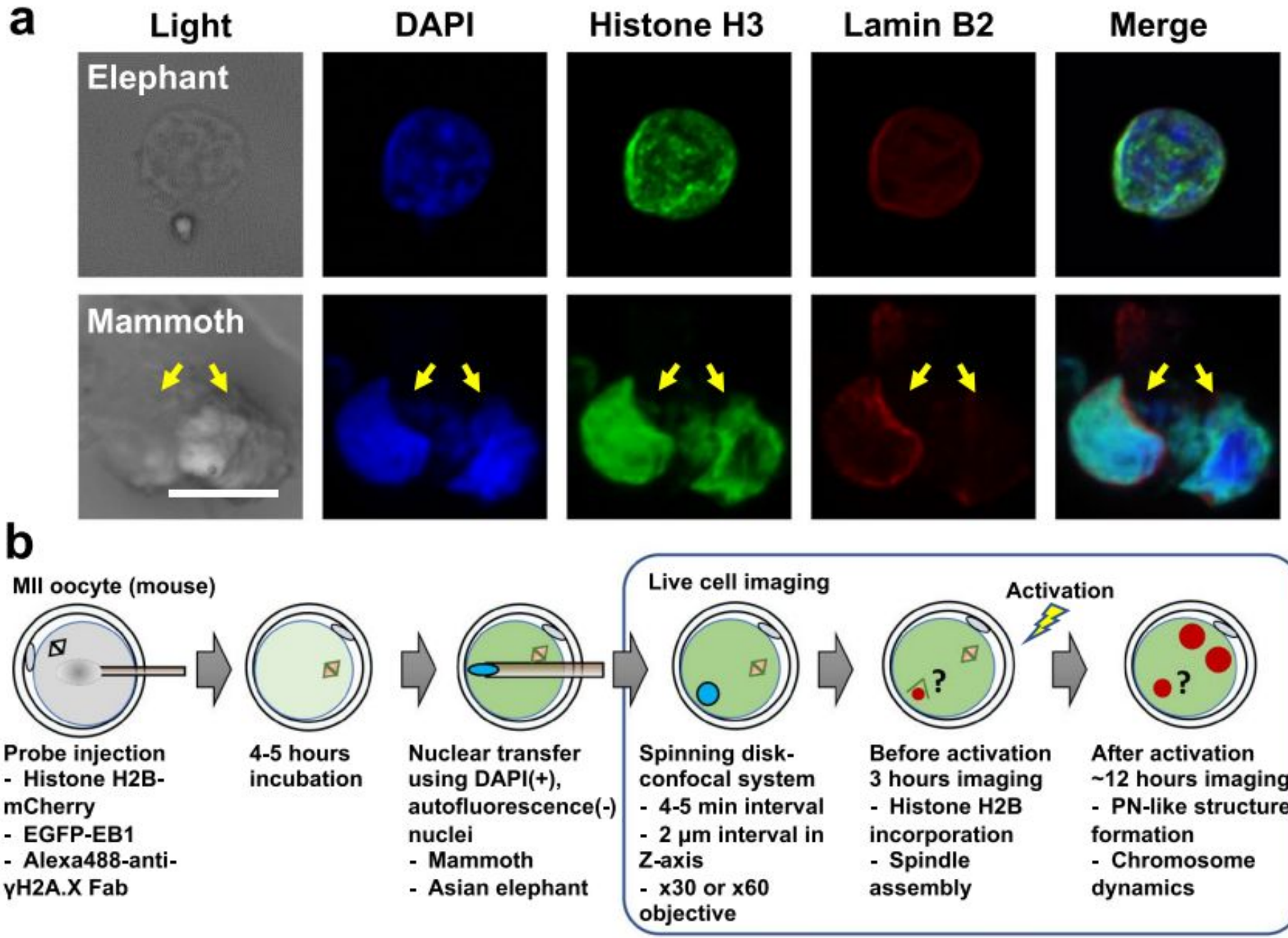
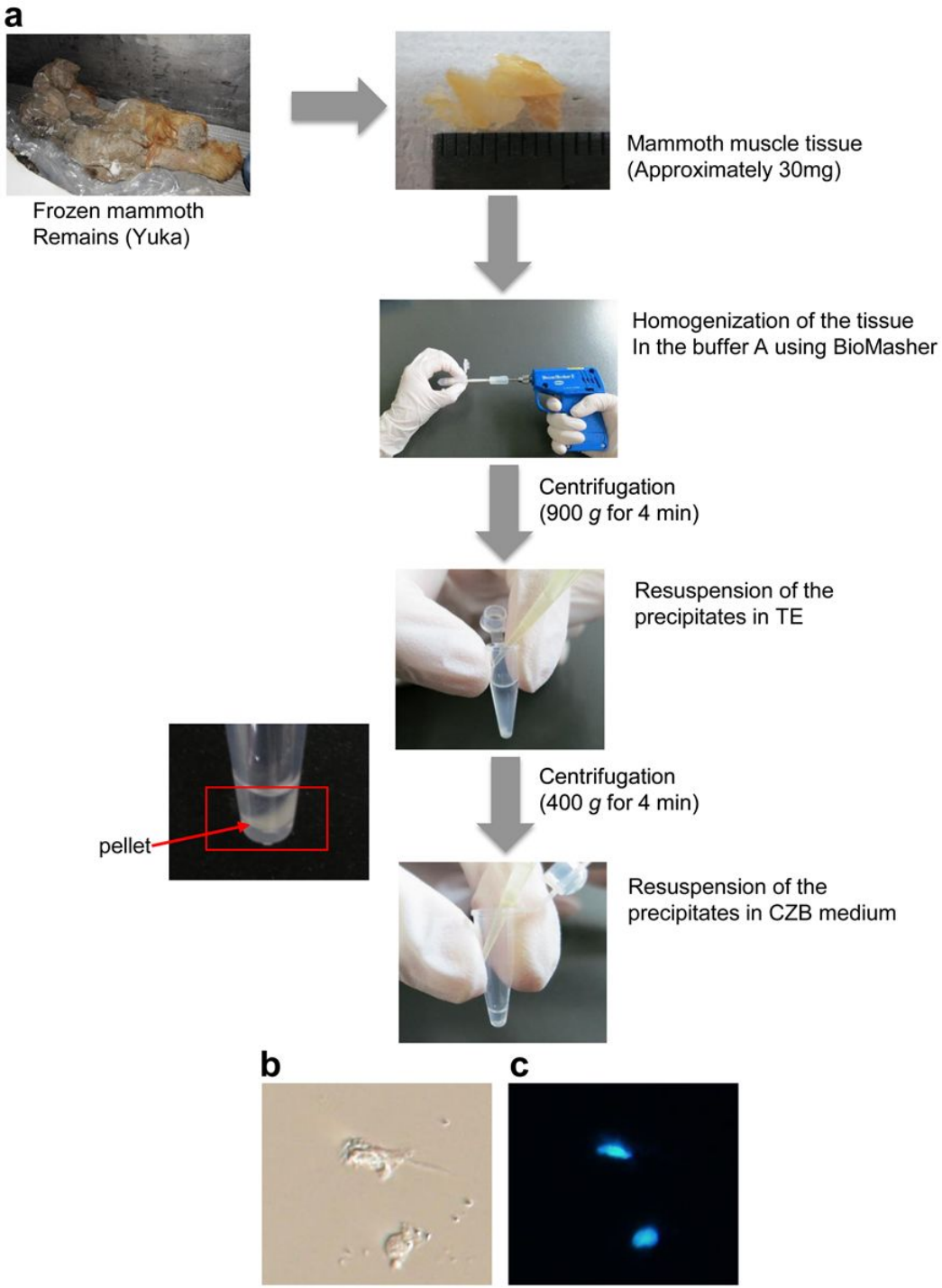
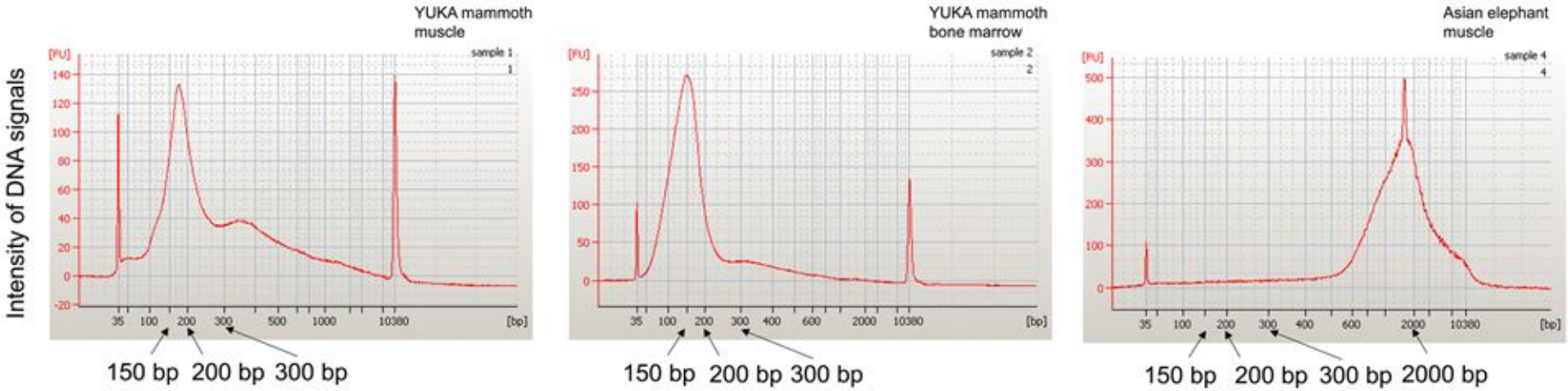
EU153457 M5
EU153452 M22
EU153449 M2
Yuka
EU153456 M4
EU153448 M19
EU153458 M8
EU153454 M26
EU153450 M20
EU153453 M25
EU153451 M21
NC 005129 Elephas
DQ316069 Loxodonta
EF632344 Mammut
99
100
100
100
96
100
100
Clade I
Clade II
0.02

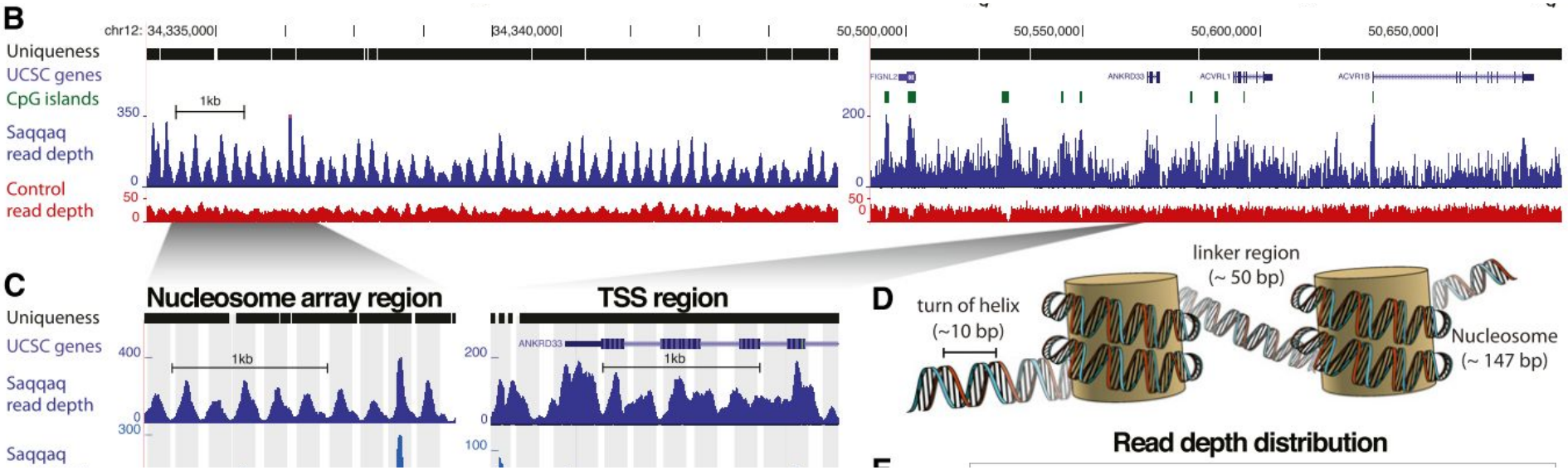

a
Light
DAPI
Histone H3
Lamin B2
Merge
Elephant
Mammoth
b
MII oocyte (mouse)
Probe injection
- Histone H2B-mCherry
- EGFP-EB1
- Alexa488-anti-γH2A.X Fab
4-5 hours incubation
Nuclear transfer using DAPI(+), autofluorescence(-) nuclei
- Mammoth
- Asian elephant
Live cell imaging
Spinning disk-confocal system
- 4-5 min interval
- 2 μm interval in Z-axis
- x30 or x60 objective
Activation
Before activation 3 hours imaging
- Histone H2B incorporation
- Spindle assembly
After activation ~12 hours imaging
- PN-like structure formation
- Chromosome dynamics


a
Frozen mammoth
Remains (Yuka)
Mammoth muscle tissue
(Approximately 30mg)
Homogenization of the tissue
In the buffer A using BioMasher
Centrifugation
(900 g for 4 min)
Resuspension of the
precipitates in TE
pellet
Centrifugation
(400 g for 4 min)
Resuspension of the
precipitates in CZB medium
b
c


Intensity of DNA signals
YUKA mammoth muscle
sample 1
1
[FU]
140
120
100
80
60
40
20
0
-20
35
100
200
300
500
1000
10380
[bp]
150 bp
200 bp
300 bp
YUKA mammoth bone marrow
sample 2
2
[FU]
250
200
150
100
50
0
35
100
200
300
400
600
2000
10380
[bp]
150 bp
200 bp
300 bp
Asian elephant muscle
sample 4
4
[FU]
500
400
300
200
100
0
35
100
200
300
400
600
2000
10380
[bp]
150 bp
200 bp
300 bp
2000 bp

Genome-wide nucleosome map and cytosine methylation levels of an ancient human genome

Jakob Skou Pedersen,[1,14,15] Eivind Valen,[2,3,14] Amhed M. Vargas Velazquez,[4] Brian J. Parker,[3] Morten Rasmussen,[4,5] Stinus Lindgreen,[3,4,6] Berit Lilje,[3] Desmond J. Tobin,[7] Theresa K. Kelly,[8] Søren Vang,[1] Robin Andersson,[3] Peter A. Jones,[8] Cindi A. Hoover,[9] Alexei Tikhonov,[10,11] Egor Prokhortchouk,[12,13] Edward M. Rubin,[9] Albin Sandelin,[3] M. Thomas P. Gilbert,[4] Anders Krogh,[3,4] Eske Willerslev,[4] and Ludovic Orlando[4,15]



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Dog Clone

The Dog Cloning Company

This site is dedicated to Wolfie and Bubble. Their love changed my life forever

If your Pet Has just died please Click here now before it's too late!

Hello, my name is Peter and I have cloned both of my dogs, Wolfie and Bubble. I created this website in honor of them and for those of you who, like myself, need accurate information about dog cloning. The cost to clone a dog is not cheap and I know you may have many concerns about the entire cloning process, which company can really clone your dog, and which company is just a middle man. I do not make any money for this site but I do charge companies to advertise their cell banking services. I then donate 100% of it to an animal charity of my choice. Why do I do this? Because I love animals with all my heart.

EN 11:11 03.09.2014

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- Clone Updates
- You are in Grief
- My Story
- Am I Happy
- Links
- News
- FAQ
- Contact

Dog Clone

Your Dog has just Died and you want to save his cells for Cloning

Important! Please read below and act now!

If your dog has just passed then you must act quickly as in right now! There is a 5 day window to successfully extract good quality cells from your beloved friend before the cells begin to deteriorate.

This means that once your dog has passed the body will begin to quickly deteriorate and your chances of extracting quality cells diminishes with every passing day.

Warning!!!

Do not place your dog in the Freezer. If you do there will be zero chance of cloning your dog. You must wrap your dogs entire body with wet bathing towels and place it in the Refridgerator to keep it cool. Do this first and then call us right away. Time is of the essence!

You must act now to preserve the cells of your dog. Cloning cannot be done with just DNA, Blood, Skin, Hair, Teeth, Etc. Cloning can only be done with live tissue taken from your dog immediately after death. After your dog dies, the deteriation of your dogs live tissue will begin. After 5 days it is too late. There will be no way to clone your dog. You'll need to call us right away and we'll will send you a biopsy kit. Give us a call at 608-345-1321 and we'll will ship- a biopsy kit directly to your veterinarian complete with instructions for the doctor to follow. The Biopsy kit is a Styrofoam box with ice packs and vials to store your pets tissues or biopsy samples when shipping back to us. The vet will extract quality tissue and cells from your dogs body. The Vet will than place your pets biopsy samples in the container and ship it back via next day air to our storage facility. Once there we will will culture the cells from the sample tissue to ensure that the cells are viable. The cells are then frozen and stored in a liquid

EN 11:11 03.09.2014