Somatic cell reprogramming: bioinformatic approaches to establish cell "stemness" and fate

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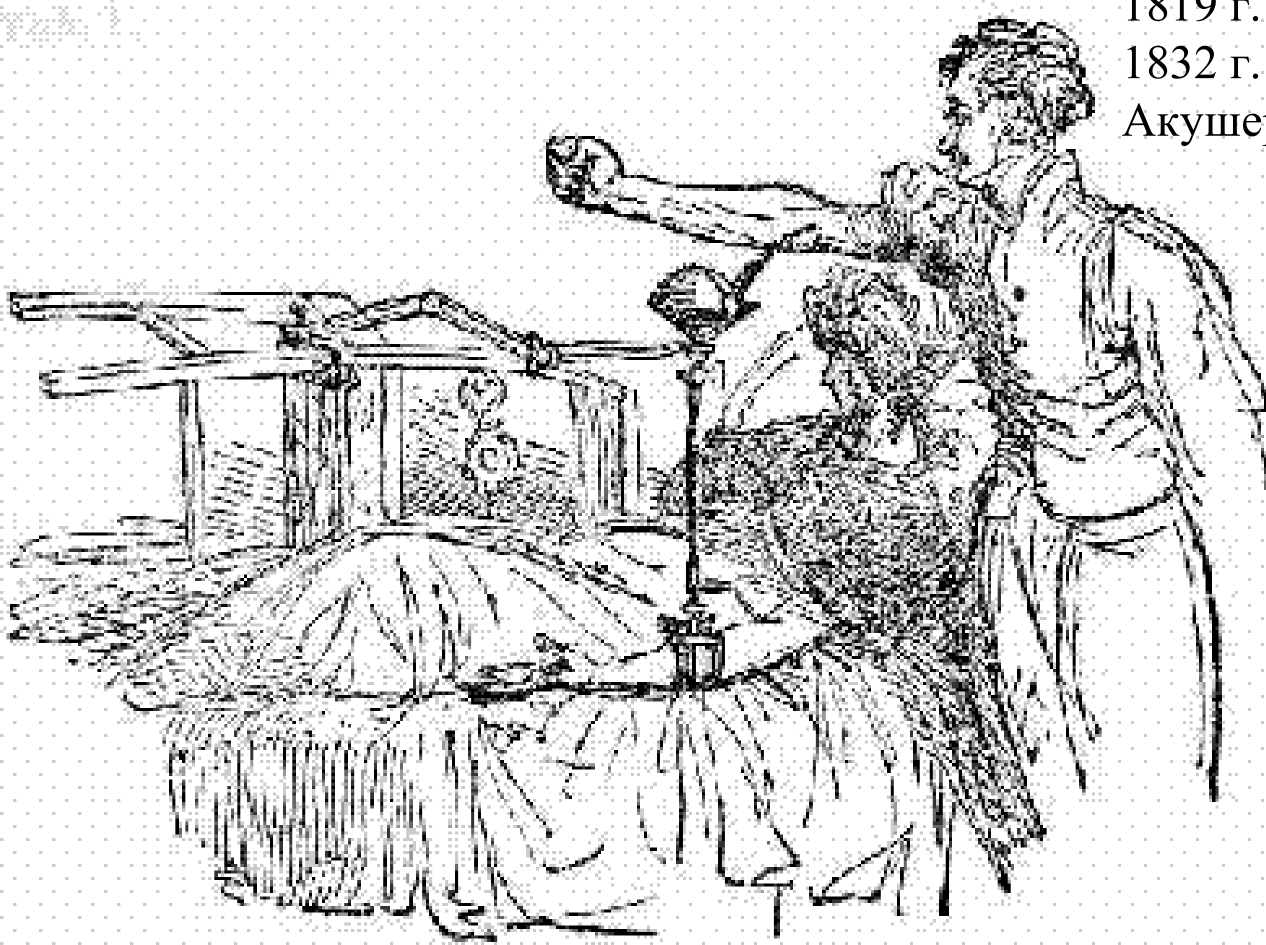
Регенерация тканей- процесс давно известный человечеству

Первое успешное применение клеток

1819 г. Англия, Дж. Бланделл
1832 г. Ст. Петербург
Акушер Андрей Вольф



Первой обоснованной клеточной трансплантацией у человека следует считать переливание АВ0-совместимой крови, впервые выполненное в 1907 году R. Ottenberg.

«Мы должны преуспеть в создании искусственной живой материи, либо найти объяснение почему это невозможно… Поведение наших клеток, как и наше поведение и инстинкты, запрограммировано наследственным аппаратом. Мы подчиняемся законам наших клеток, которые живут двойной жизнью: в мире химии и мире смысла».

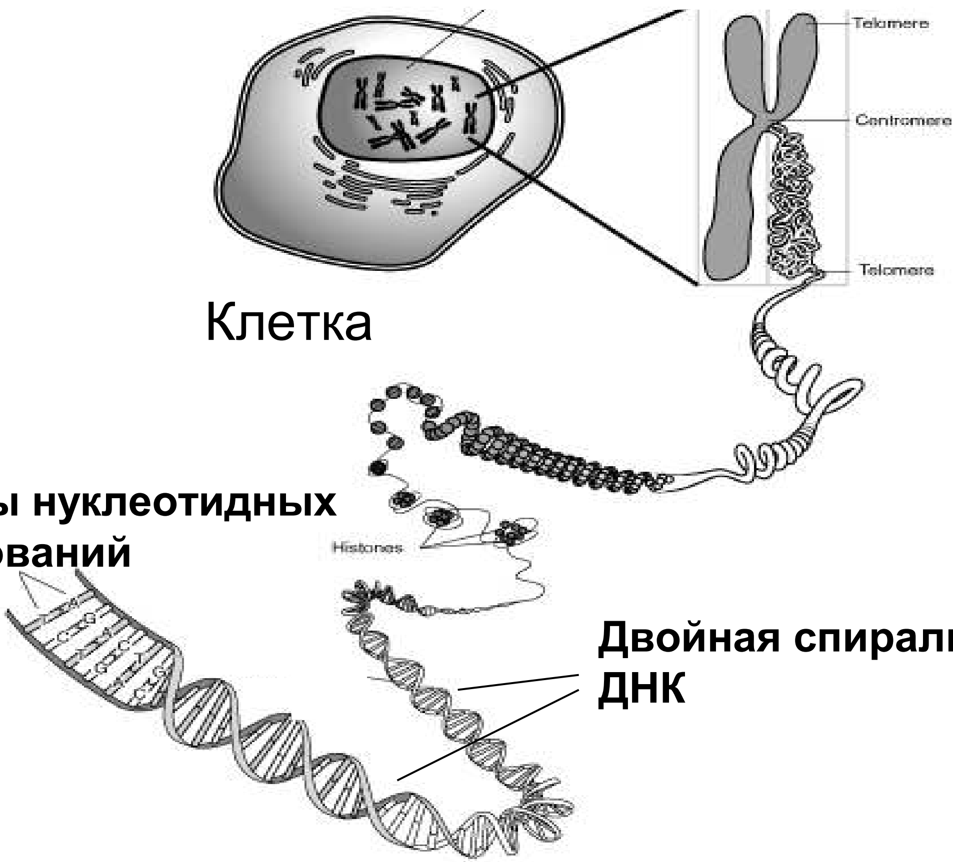
Профессор Росс Гаррисон

Профессор Жак Лёб

Строение клетки

Хромосомы
Хроматиды

Ядро



Вся ДНК клетки- ГЕНОМ,
малая часть ДНК клетки- ГЕН(ы)

Клетка

РЕПЛИКАЦИЯ
-поддержание и наследование
ТРАНСКРИПЦИЯ
-функционирование через ТРАНСЛЯЦИЮ в БЕЛОК

Пары нуклеотидных оснований

Двойная спираль ДНК

Обеспечение целостности организма

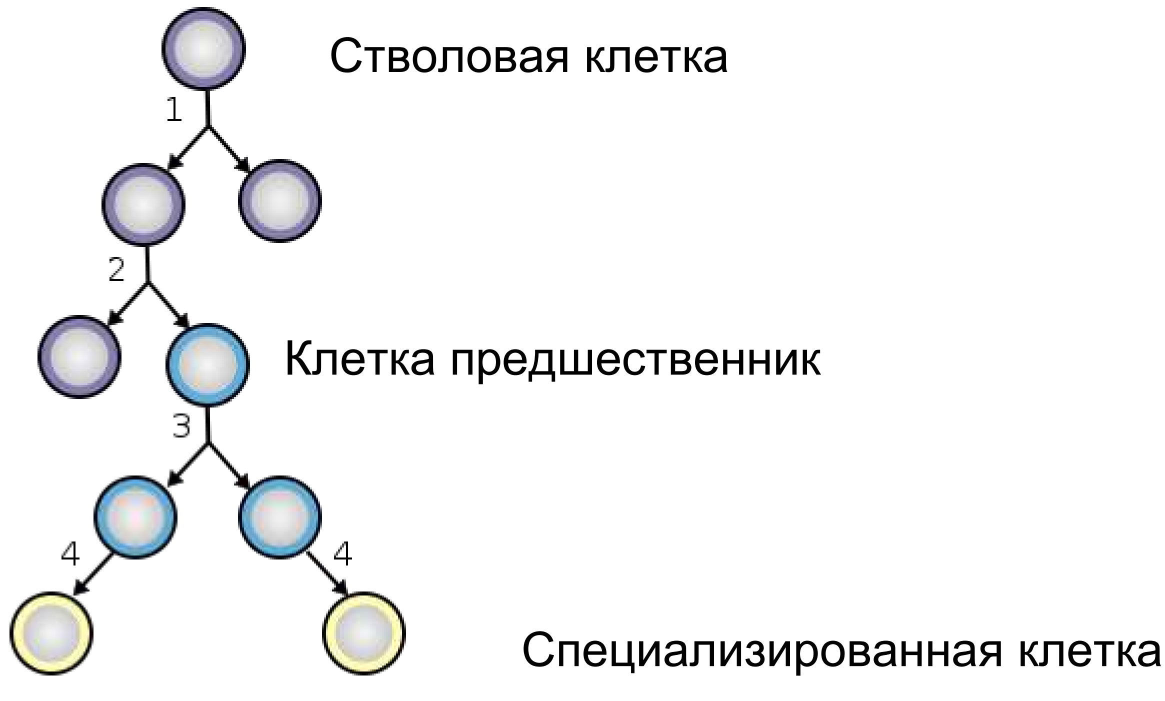
- Большинство клеток организма имеют более короткий жизненный цикл, чем весь организм
- Практически все ткани восстанавливаются в течение жизни
- В течение жизни организма большинство тканей (кожа, кишечник, кроветворная система) продуцируют такое количество клеток, суммарный вес которых во много раз превышает вес организма

Кто первый придумал термин «стволОвая клетка»

Valentin Hacker (1864 -1927)
1892

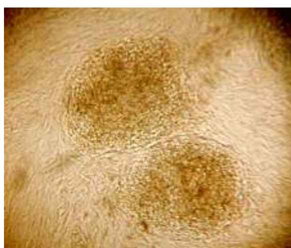
А.А. Максимов
(1874-1928)
1908

Возобновление специализированных клеток происходит за счет стволовых



- Клетки, способные к самообновлению
- Клетки, способные давать начало многим другим типам клеток

Типы стволовых клеток



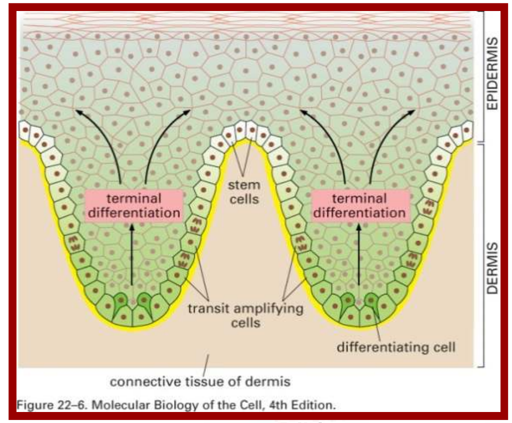
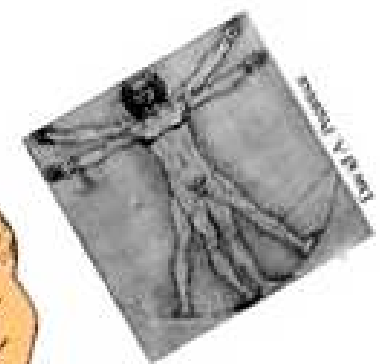


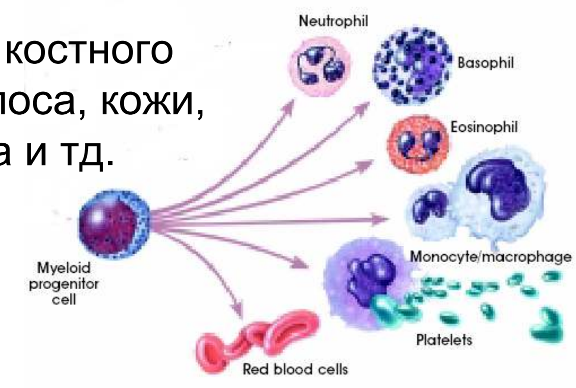
Figure 22–6. Molecular Biology of the Cell, 4th Edition.



<u>Предшественники</u> дифференцировка в определенные клетки в пределах определенной ткани

<u>Мультипотентные</u> *(много, лат.)* дифференцировка в ткани внутри зародышевых слоев

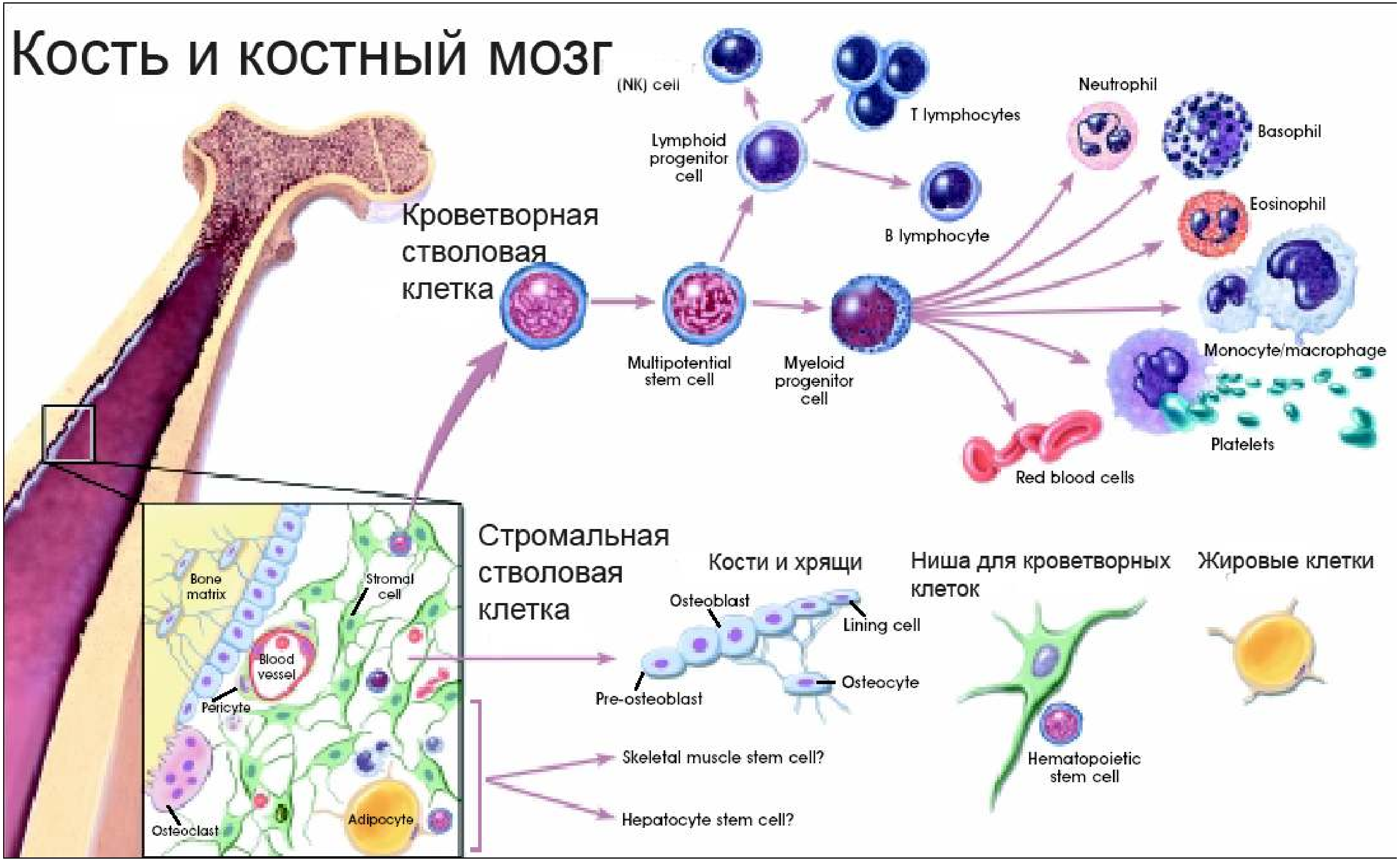
СК крови, костного мозга, волоса, кожи, кишечника и тд.



<u>Плюрипотентные</u> *(много, греч.)* – эмбриональные стволовые клетки

<u>Тотипотентные</u> *(полностью, лат)* - зигота

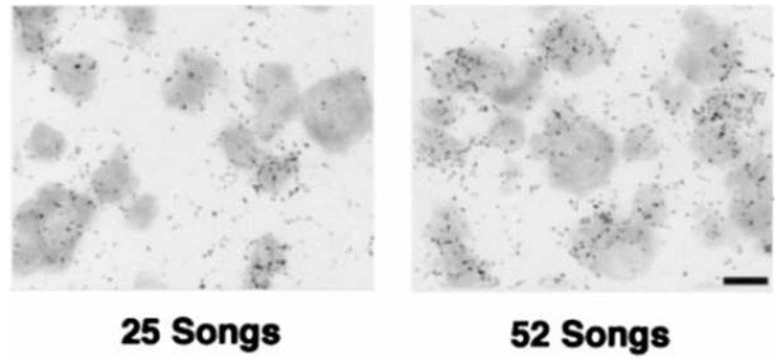
Стволовые клетки костного мозга

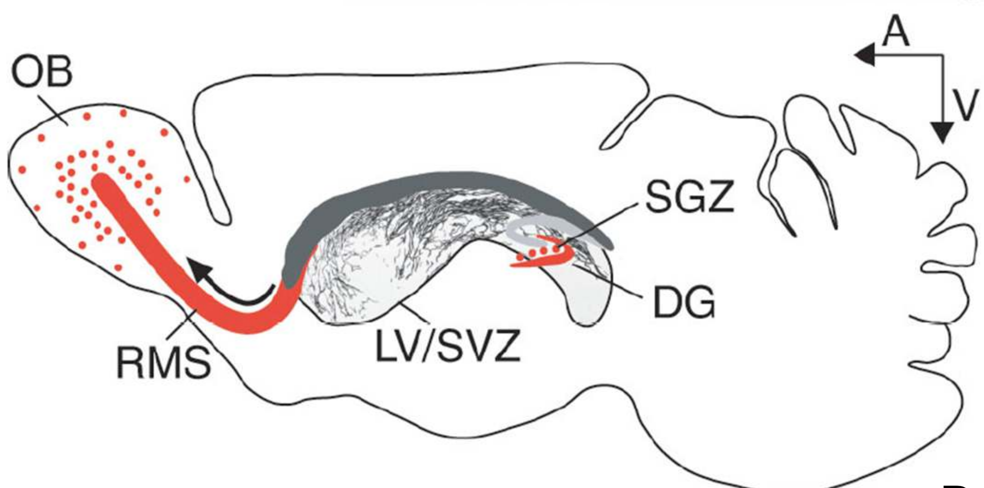


Нервные клетки восстанавливаются!

Altman, J. Are new neurons formed in the brains of adult mammals? Science 135:1127–1128; 1962.

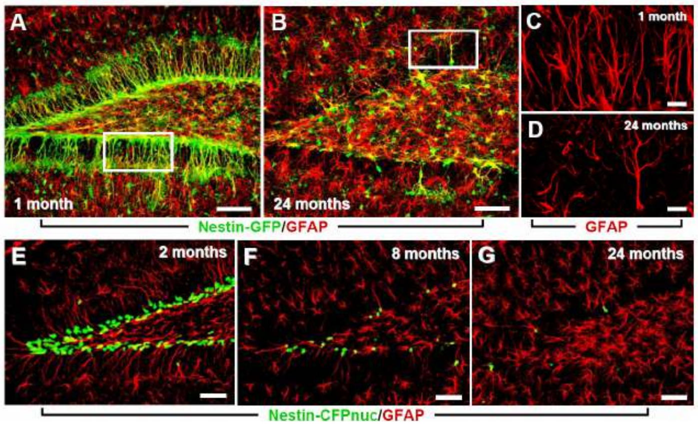
Li et al., 2000

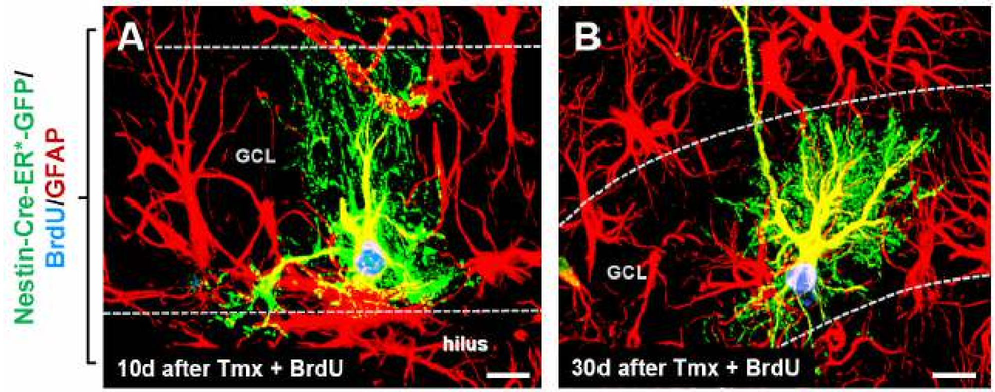


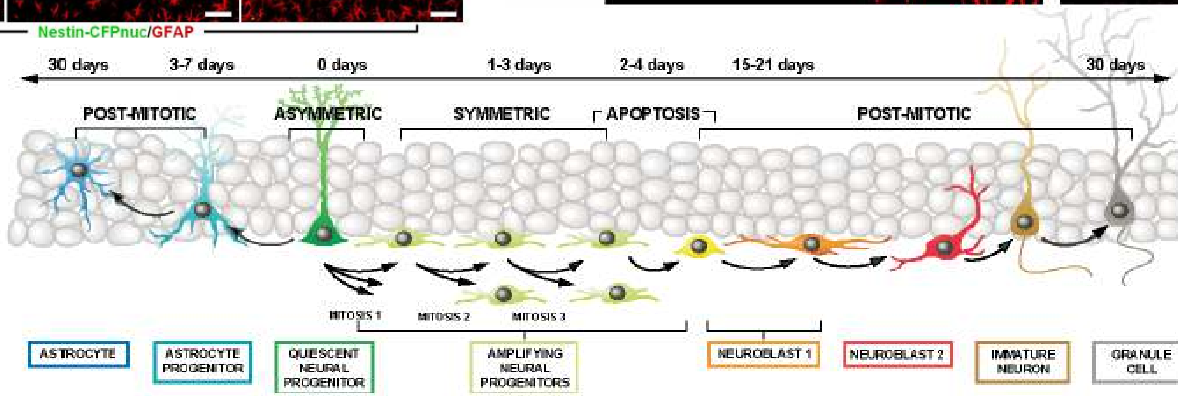


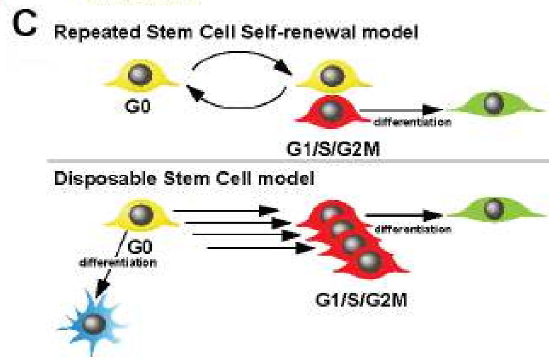
Doetsch F, 2003

Нервные клетки восстанавливаются, но могут и закончиться



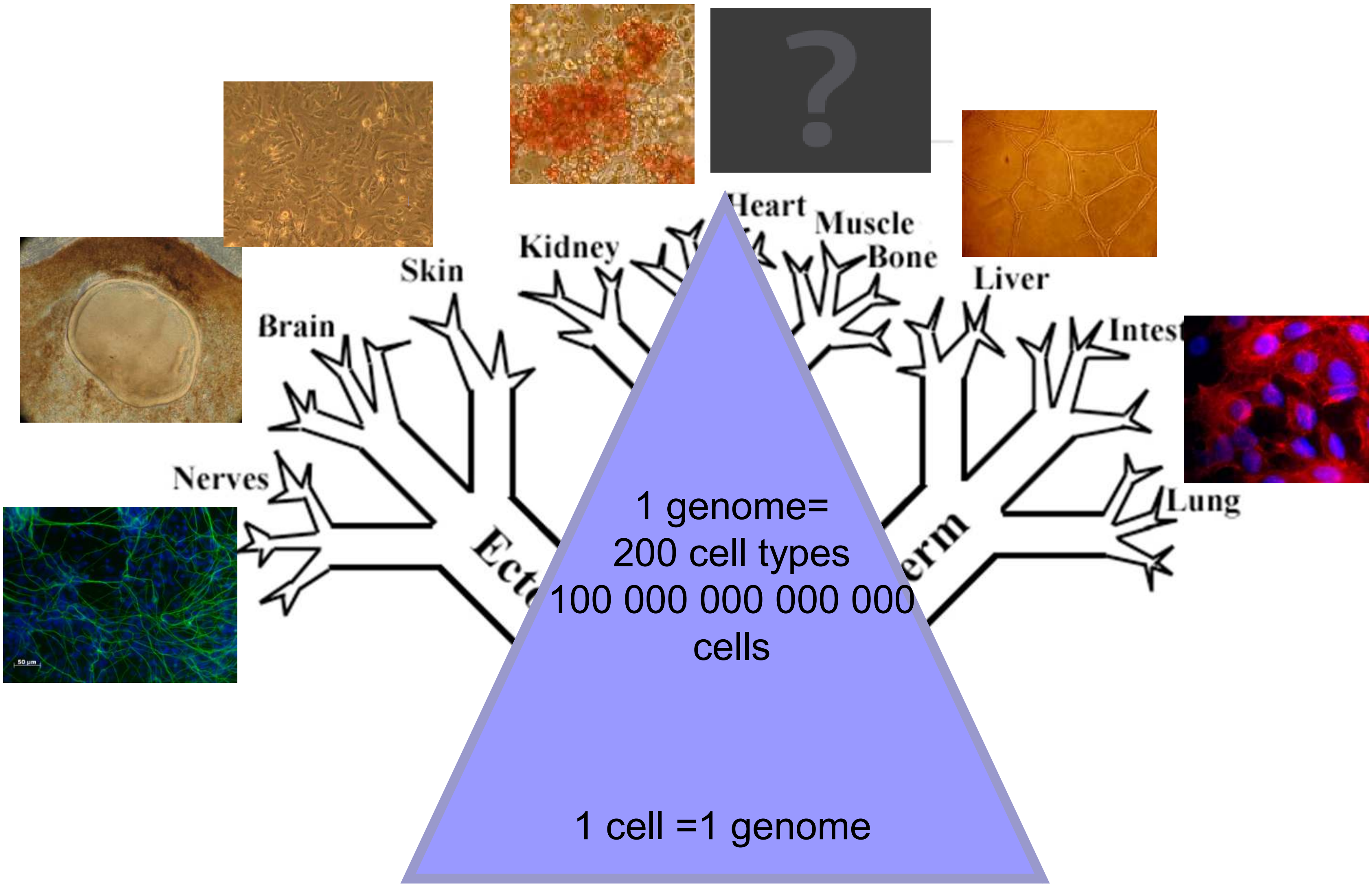




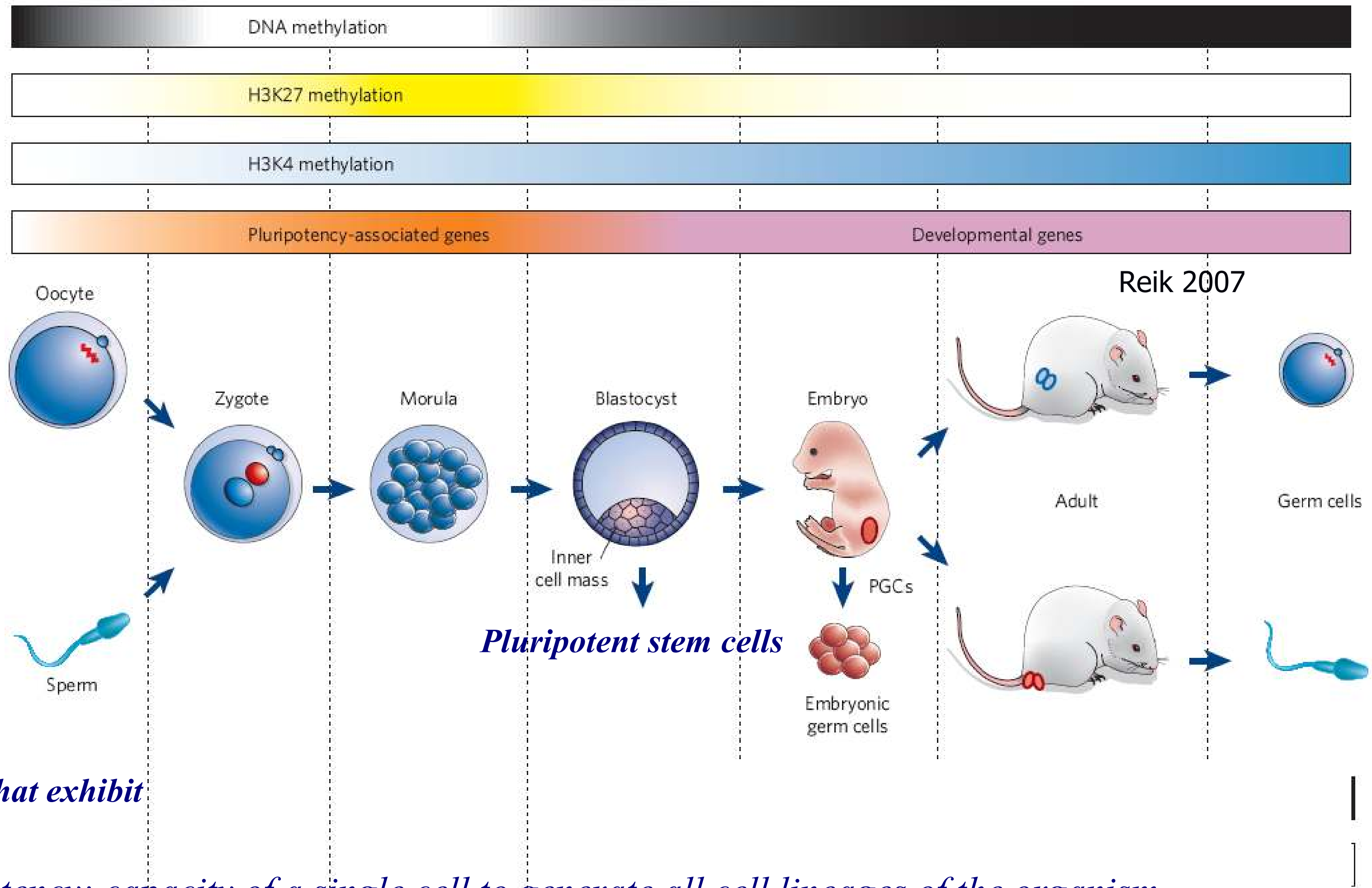


Encinas et al., 2011

Cell specialization program is realized during multicellular organism development via gradual loss of the potential to differentiate into different types of cells.



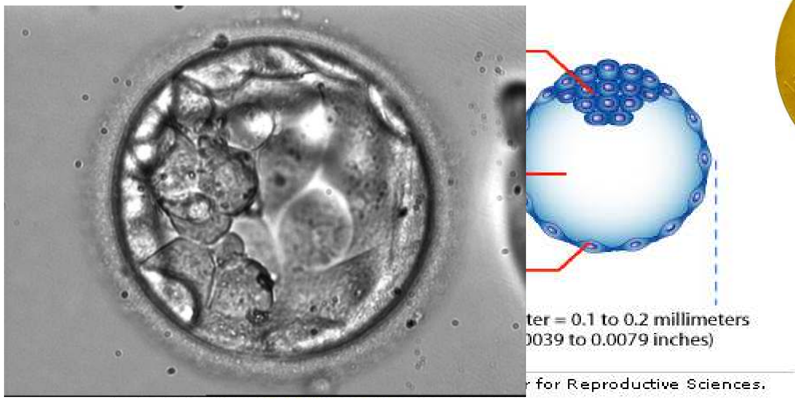
Ontogenetic program is executed on genetic and epigenetic levels



Pluripotency: capacity of a single cell to generate all cell lineages of the organism

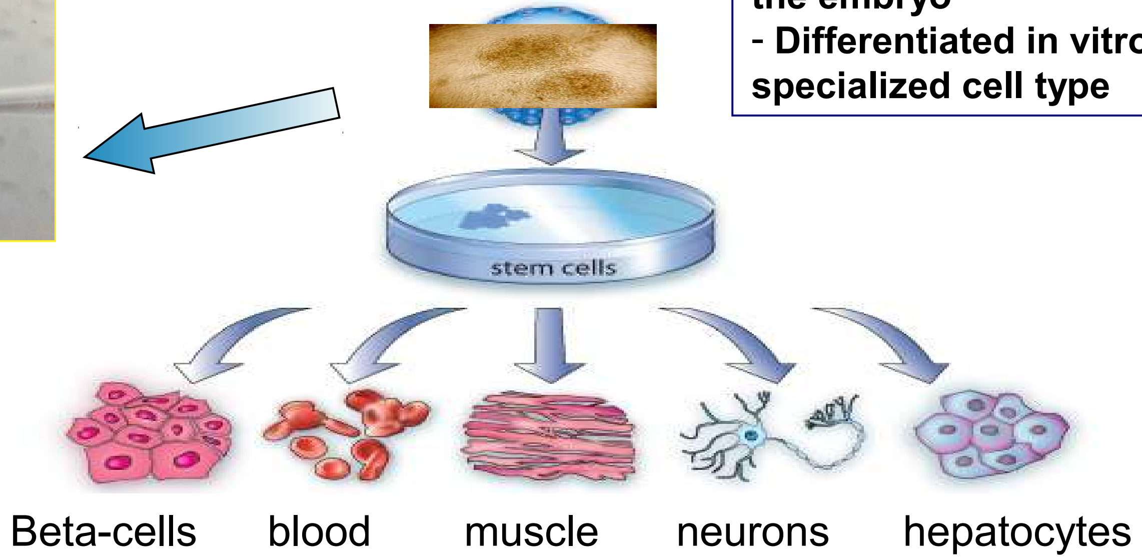
Self renewal: ability of a cell to proliferate in the same state.

Pluripotent stem cells #1 =embryonic stem cells (ESCs)



2007

- Non transformed
- Unlimited divisions in vitro
- Even after genetic manipulations in vitro are able to contribute to the embryo
- Differentiated in vitro in any specialized cell type



Pros and contra human ESC

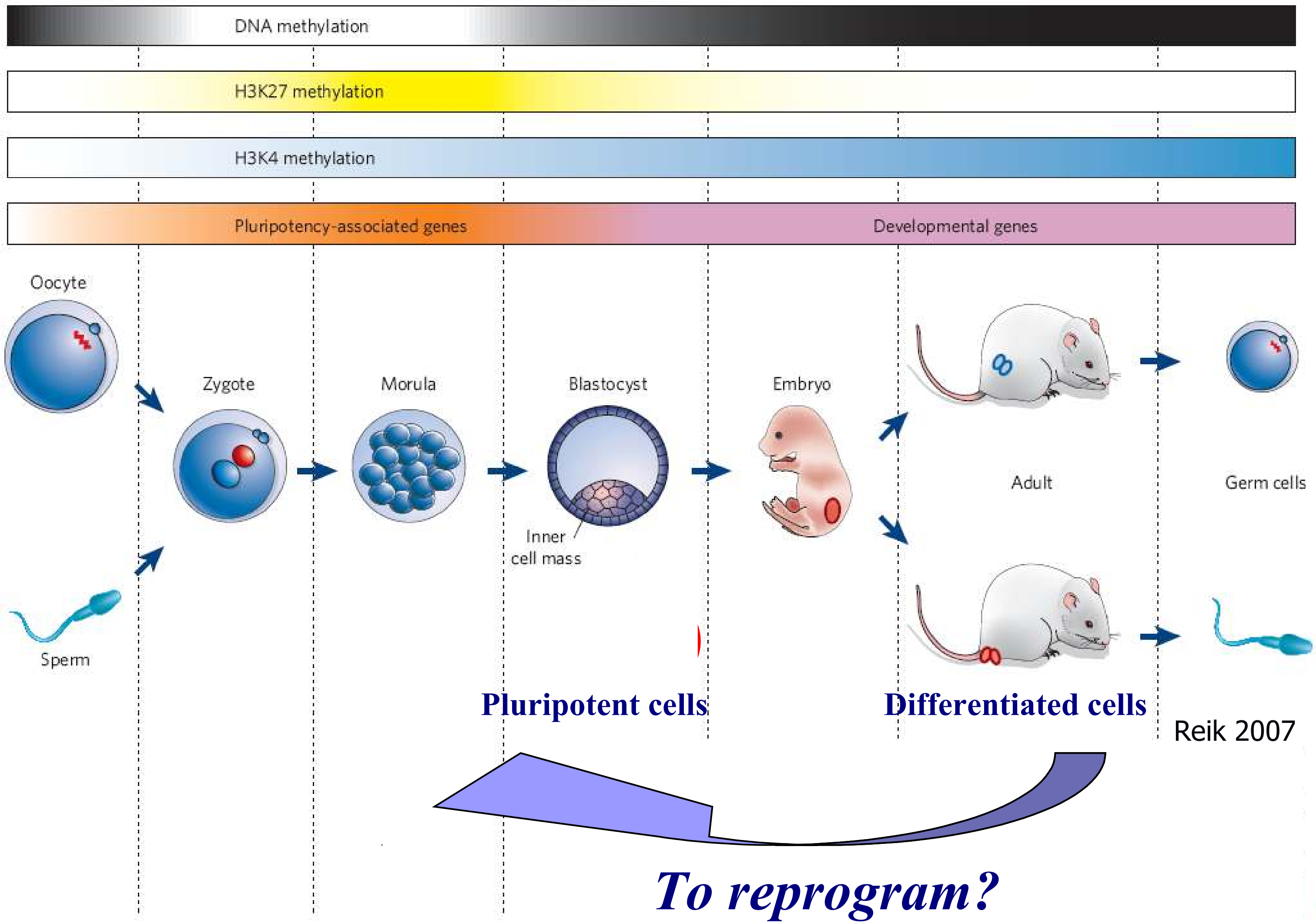
- Natural cells?
- Disease mechanism studies
- Drug screening for toxicity and effectiveness
- Cell therapy

}

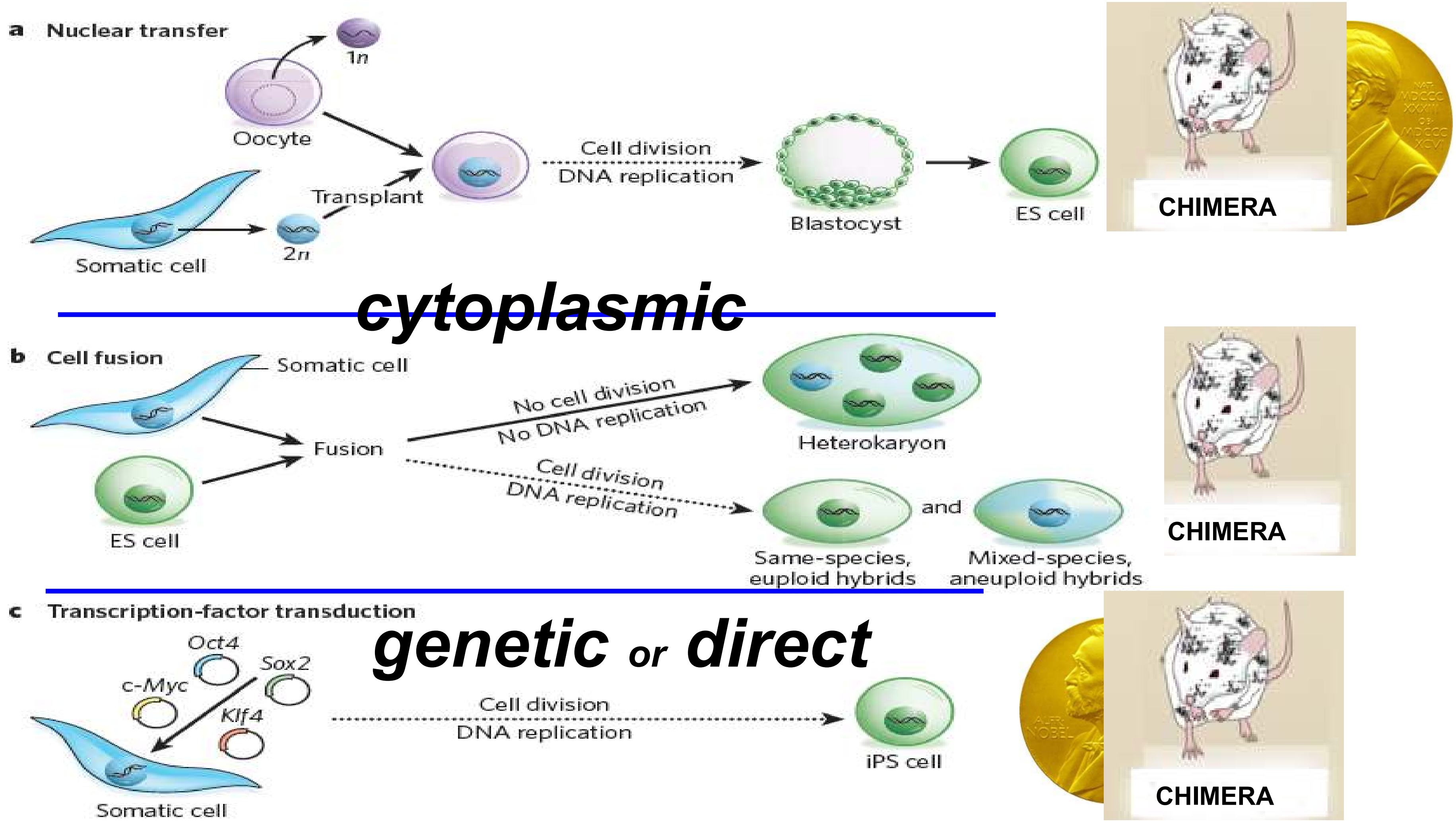
Limited blastocyst availability

Immunological incompatibility with the recipient

Ontogenetic program is executed on genetic and epigenetic levels

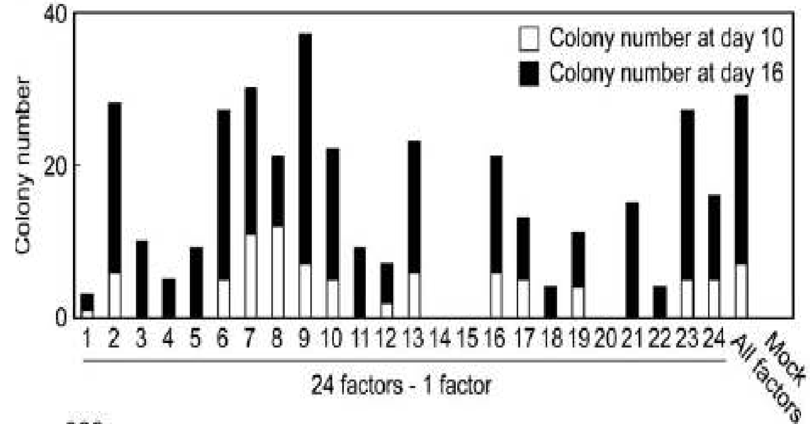


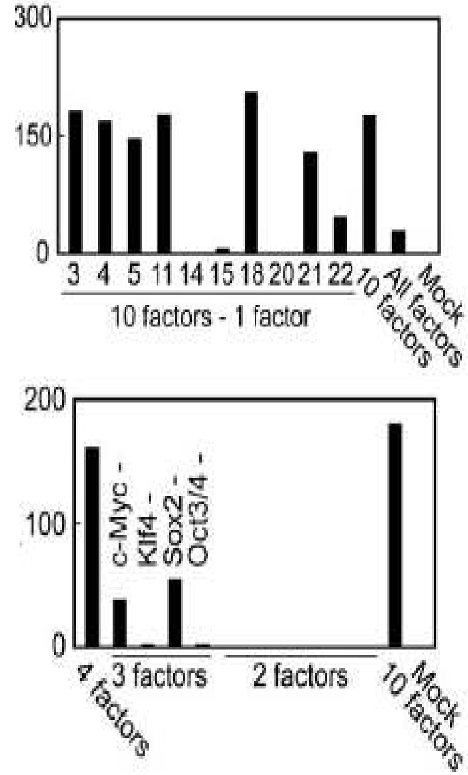
Pluripotent cells from somatic = reprogramming

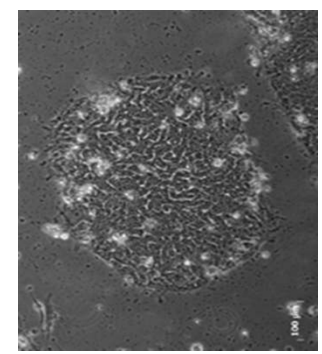


Shinya Yamanaka & Helen M. Blau; Nature|Vol 465|10 June

Yamanaka's "magic cocktail"







In 1986-89 Harold Weintraub with co-workers demonstrated that skin fibroblasts can be converted (reprogrammed) into muscle by MyoD transcription factor overexpression

Genetic reprogramming: induced pluripotent stem cells (iPSC)

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

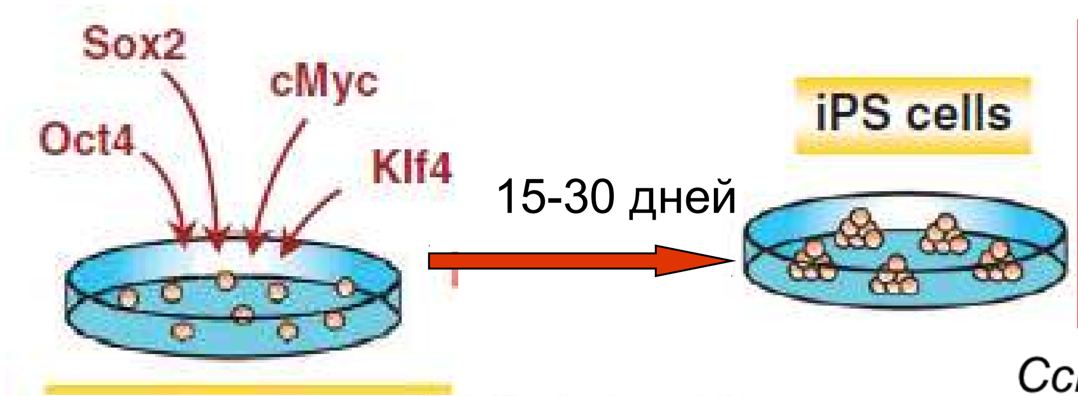
Generation of germline-competent induced pluripotent stem cells

Keisuke Okita[1], Tomoko Ichisaka[1,2] & Shinya Yamanaka[1,2]

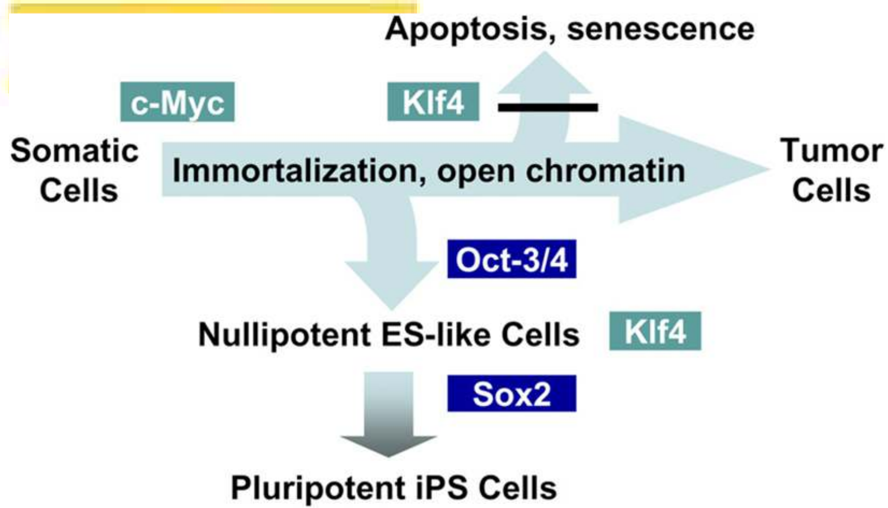
Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
[3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
[4]Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019

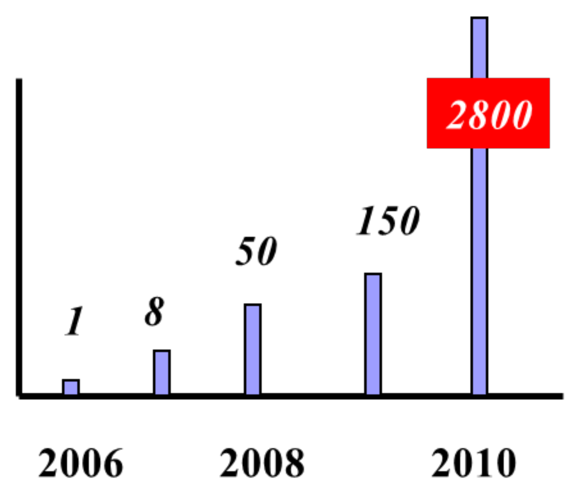
Клетки взрослого организма



Стволовые клетки с индуцированной плюрипотентностью = эмбриональным стволовым клеткам

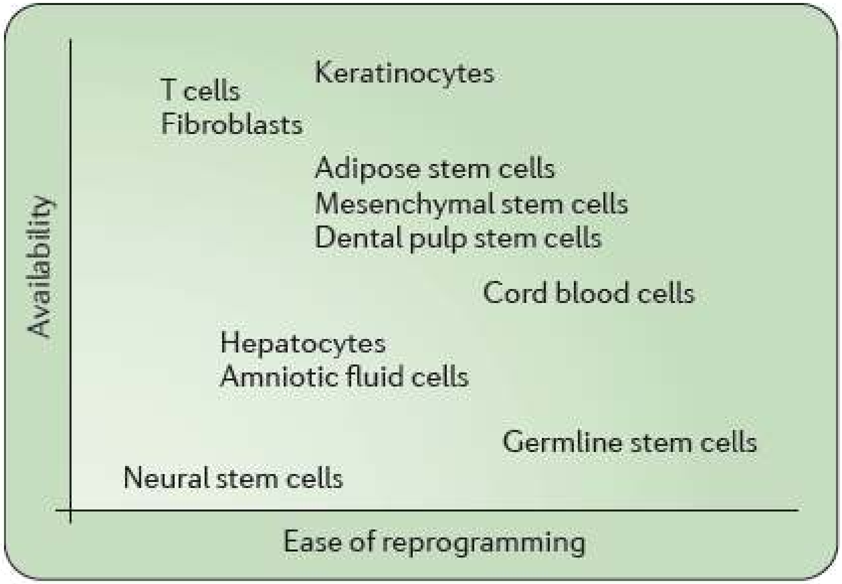


Ссылок в PubMed на iPS



Pluripotency a la carte

Starting cell types



Factors

To express/overexpress

Important for embryonic development:
OCT4, SOX2, NANOG, UTF1, LIN28, SALL4, NR5A2, TBX3, ESSRB, DPPA4

Proliferation and cell cycle:
MYC*, KLF4*, SV40LT*, REM2, MDM2*, cyclin D1*

Epigenetic regulators:
CHD1, PRC2

Others:
vitamin C, hypoxia, E-cadherin, miR-294, TERT*

To repress

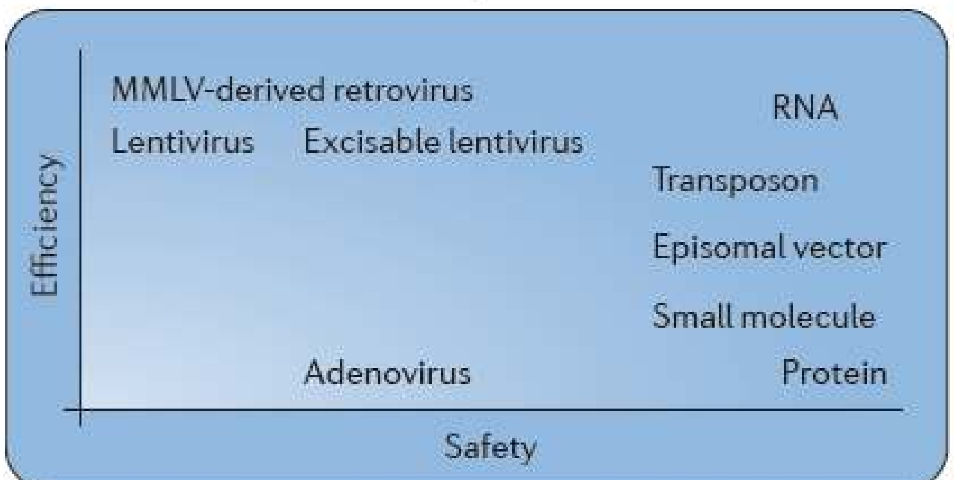
Apoptosis, cell cycle and senescence:
$p16^{INK4A\ddagger}$, $p53^{\ddagger}$, microRNA, p21

Epigenetic regulators:
histone deacetylase, histone demethylase, G9a, DNMT1*

Signalling pathways:
TGFβ, WNT, ERK–MAPK

*Potential oncogene
‡Potential tumour suppressor gene

Delivery modes



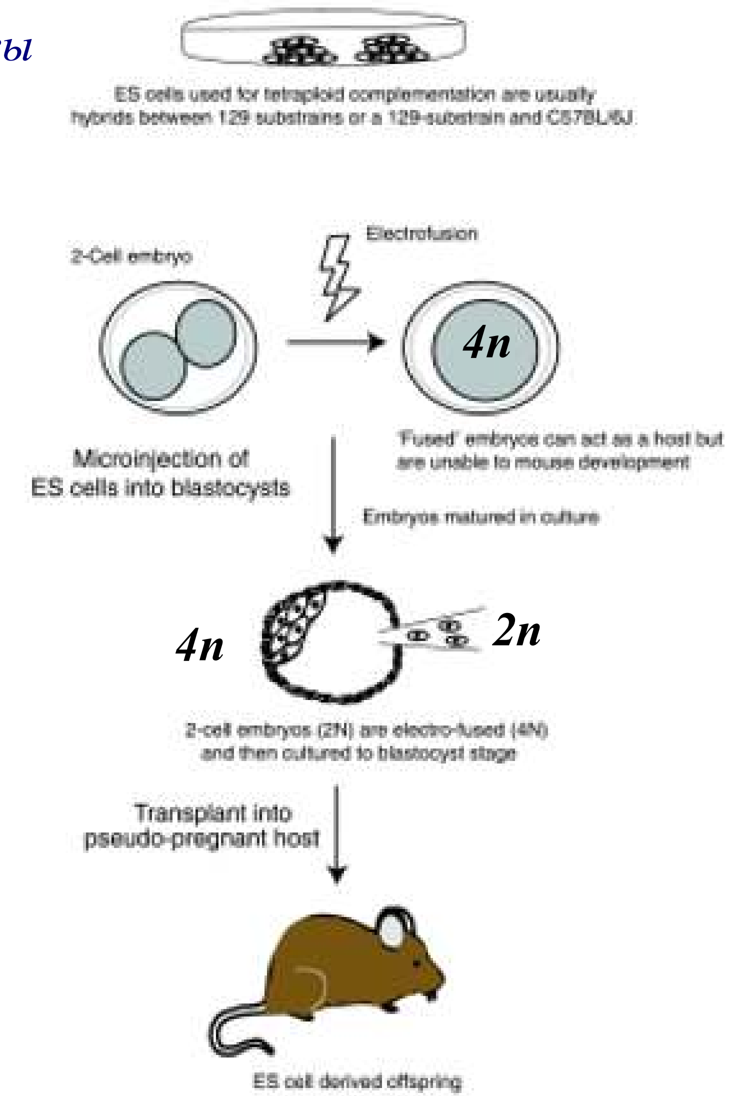
Belmonte et al., 2011. Nature Review Genetics.

It is possible to reconstruct any organism from a single skin cell

Из клетки хвоста каждого можно получить его всего, от хвоста до головы

iPSCs были получены из:

Mouse (*Yamanaka et al.*, 2006)
Humans (*Yamanaka et al.*, 2007)
Rhesus monkey (*Liu et al.*, 2008)
Rats (*Liao et al.*, 2009; *Li et al.*, 2009)
Canine (*Shimada, H. et al*, 2010)
Porcine (*Esteban, M. A. et al.*, 2009)
Marmoset (*Wu, Y. et al.*, 2010)
Rabbit (*Honda, A. et al.*, 2010)
Equine (*Kristina Nagy et al.*, 2011)
Avian (*Lu et al.*, 2011)



Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs

Gabsang Lee[1], Eirini P. Papapetrou[2], Hyesoo Kim[1], Stuart M. Chambers[1], Mark J. Tomishima[1,2,3], Christopher A. Fasano[1], Yosif M. Ganat[1,6], Jayanthi Menon[4], Fumiko Shimizu[4], Agnes Viale[5], Viviane Tabar[2,4], Michel Sadelain[2] & Lorenz Studer[1,2,4]

model pathogenesis and treatment of disease in iPSCs. Familial dysautonomia (FD) is a rare but fatal peripheral neuropathy, caused by a point mutation in the *IKBKAP*[8] gene involved in transcriptional elongation[9]. The disease is characterized by the depletion of autonomic and sensory neurons. The specificity to the peripheral nervous system and the mechanism of neuron loss in FD are poorly understood owing to the lack of an appropriate model system. Here we report the derivation of patient-specific

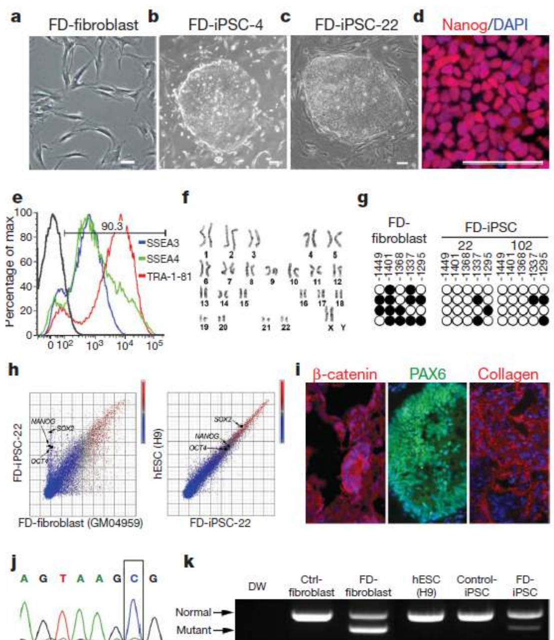


Figure 1 | Establishment of FD-iPSCs from patient fibroblasts. **a–c**, FD patient fibroblasts (**a**) were converted into FD-iPSCs (**b**, **c**) after lentiviral ransduction with OCT4, SOX2, KLF4 and c-MYC. **d**, Nanog protein xpression in FD-iPSC cell line. **e**, Flow-cytometry analysis of FD-iPSCs for luripotent surface markers. **f**, Karyotype analysis of FD-iPSCs. **g**, Bisulphite equencing analysis of the *NANOG* promoter in FD-fibroblast and FD-iPSC lones. **h**, Global gene expression patterns were compared among FD-fibroblast, FD-iPSCs and human ESCs. **i**, Teratoma from FD-iPSCs showed three germ-ayer differentiation as illustrated by the presence of endodermal epithelia xpressing β-catenin, $PAX6^+$ neuroectodermal precursors and mesodermal ollagen$^+$ cells. **j**, Sequencing result showed the 2507+6T > C mutation of *KBKAP* in FD-iPSCs. **k**, Analysis of *IKBKAP* RT–PCR products in messenger RNA derived from normal and FD-specific fibroblasts and pluripotent stem ells. Ctrl, control; DW, distilled water (negative control). Scale bars, 50 µm.

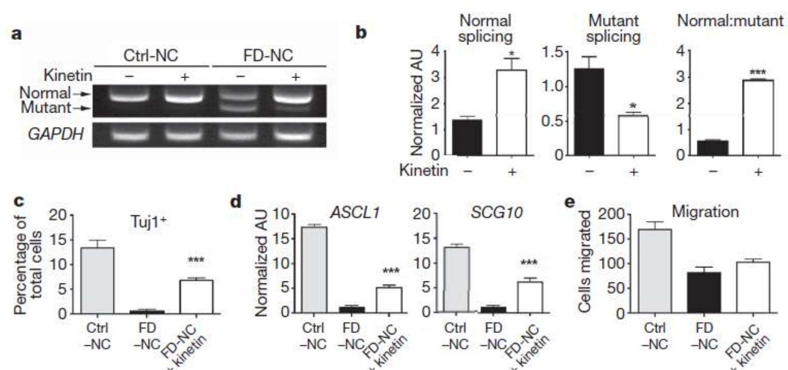
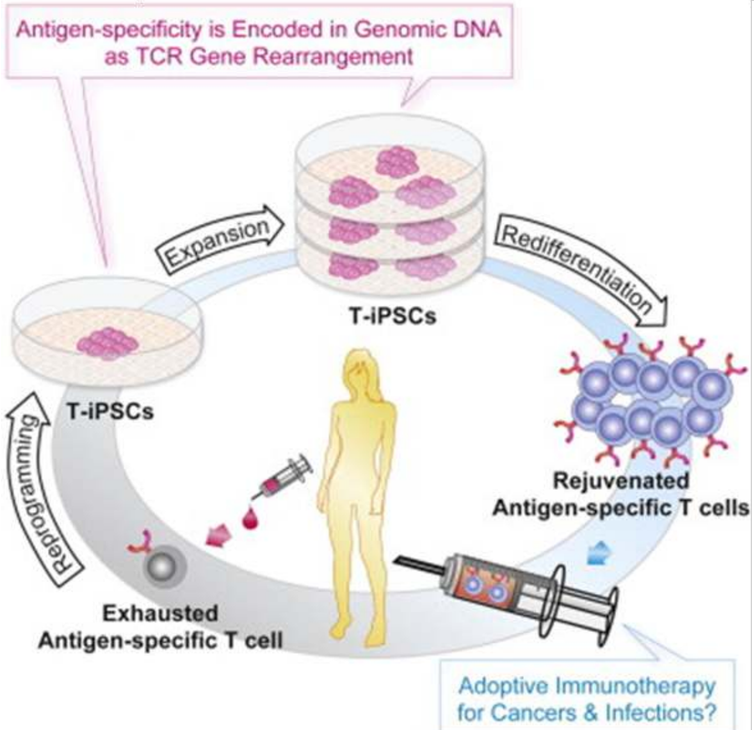


Figure 4 | Validating kinetin as a candidate compound for treating FD-iPSC-derived neural crest cells. **a**, Gel image of RT–PCR products for *IKBKAP* splicing rescued by kinetin treatment in control and FD-iPSC-derived neural crest (NC) cells. **b**, Quantification of band intensity of FD-iPSC-derived neural crest cells normalized by *GAPDH* and the ratio of normal and mutant spliced *IKBKAP* transcripts. $n = 5$; $*P < 0.05$; $***P < 0.001$. **c**, **d**, Tuj1 (**c**) and *ASCL1* and *SCG10* (**d**) expression in neuronal differentiation with kinetin-treated neural crest cells derived from FD-iPSC. $n = 4$; $***P < 0.001$. **e**, Results of kinetin treatment on cell motility (wound-healing assay) in FD-iPSC derived neural crest cells. $n = 4–6$. All values are mean and s.d.

Generation of Rejuvenated Antigen-Specific T Cells by Reprogramming to Pluripotency and Redifferentiation

Cell Stem Cell 12, 114–126, January 3, 2013 ©2013 Elsevier Inc.



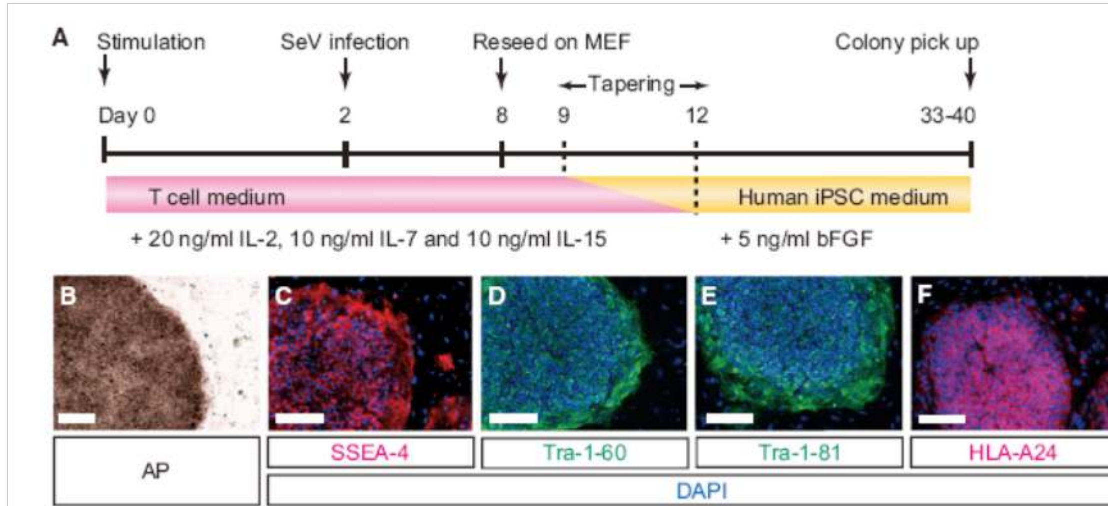
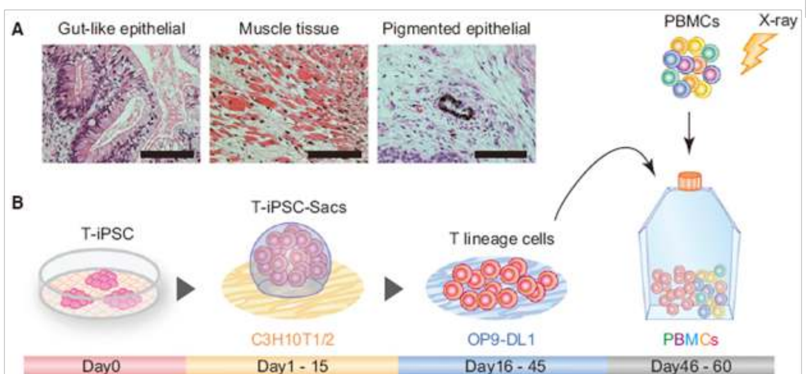
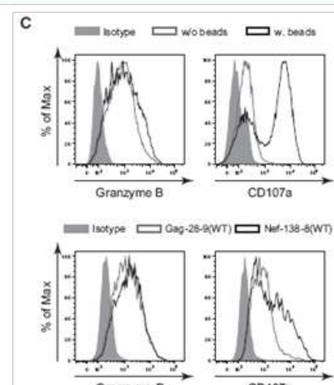
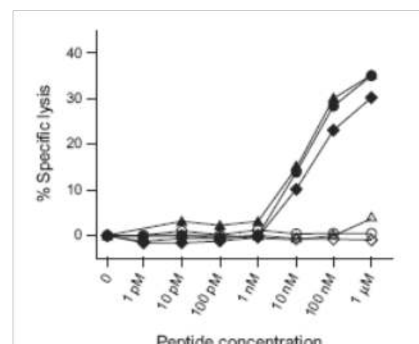


Table 1. Generation of Human T-iPSCs from Various Patient-Derived T Cell Specimens

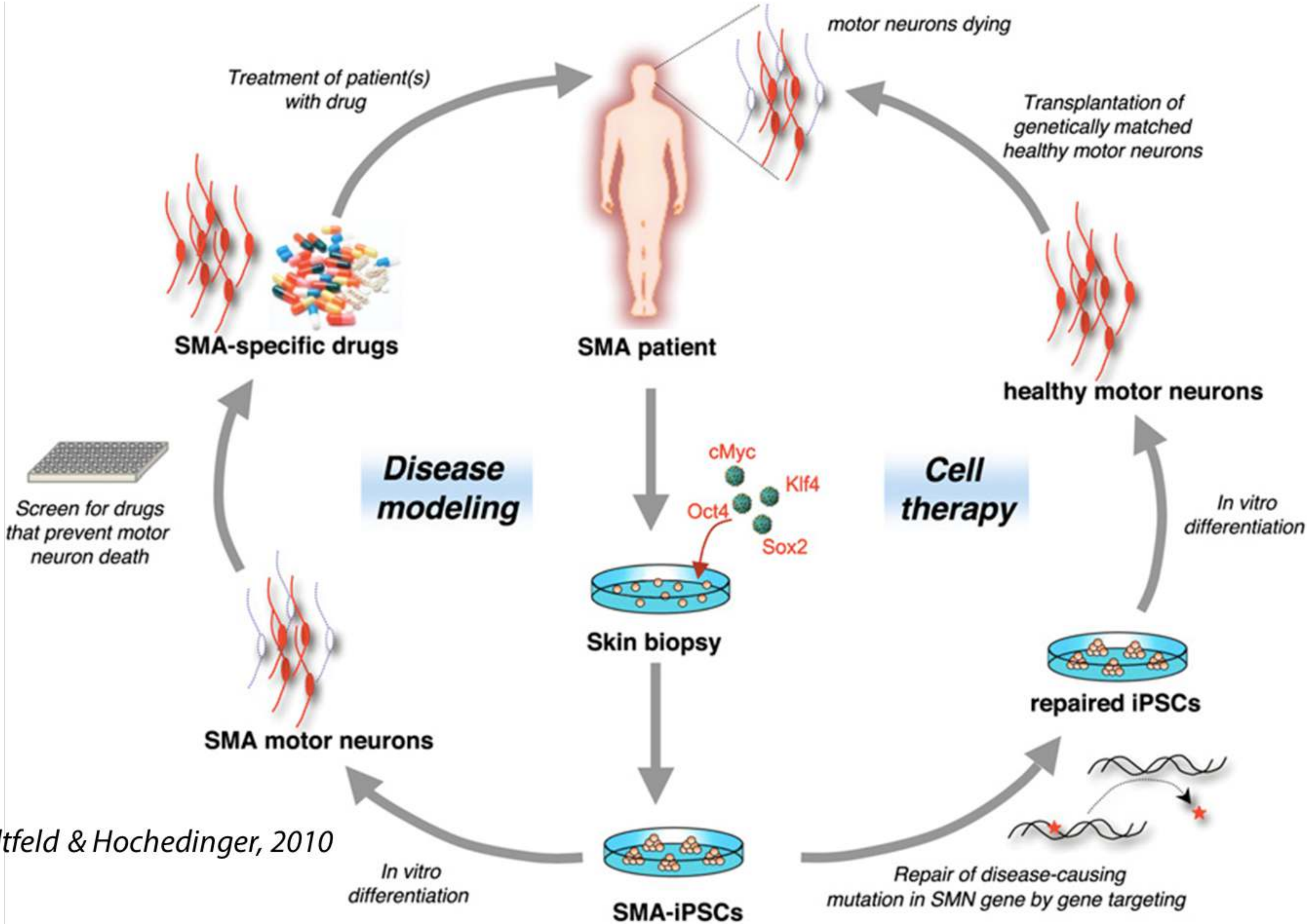
Antigen	T Cell Source	Initial Cell Number	No. of ESC-like Colonies	No. of Colonies Picked up for Establishing T-iPSC Clones	Date (MM/YYYY)
HIV-1 Nef	monoclonal T cell clone	4×10^5	7	7	05/2011
CMV pp65	polyclonal tetramer-sorted cells	~5,000	15	15	07/2011
GAD	monoclonal T cell clone	1×10^6	>100	not picked up	08/2012
		5×10^5	>100	19	08/2012
α-GalCer	FACS-sorted Vα24⁺ cells	1×10^6	>100	not picked up	08/2012
		5×10^5	>100	7	08/2012







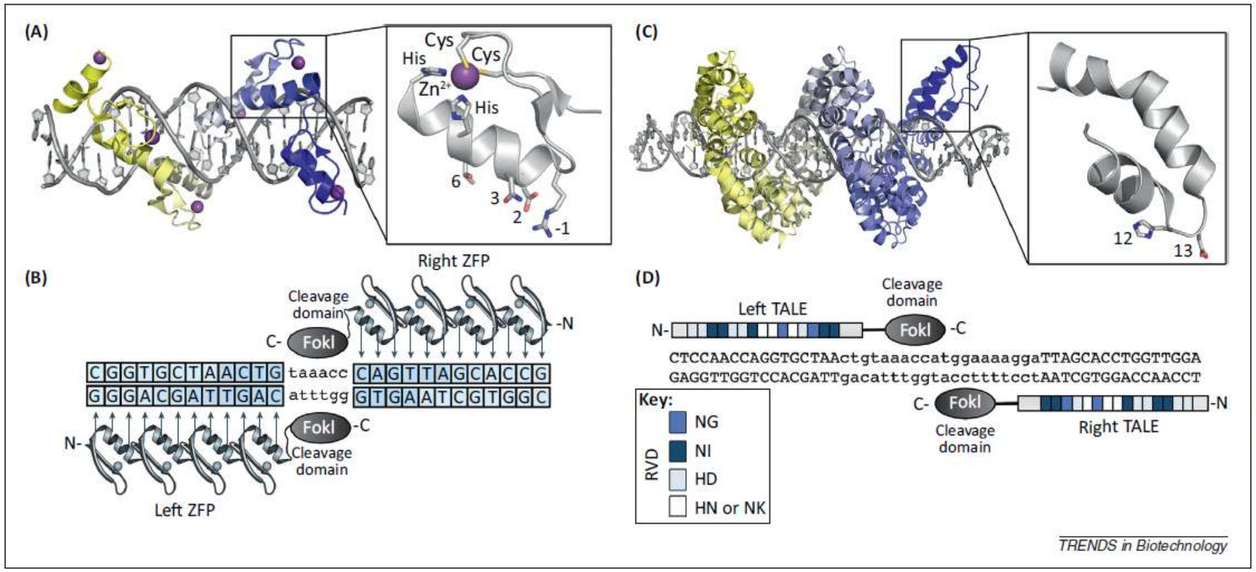
iPSC promises

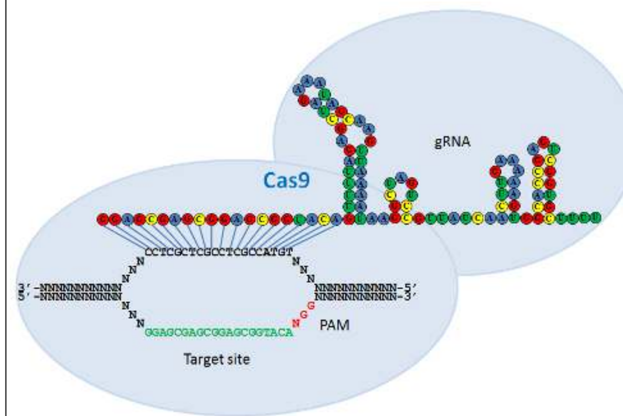


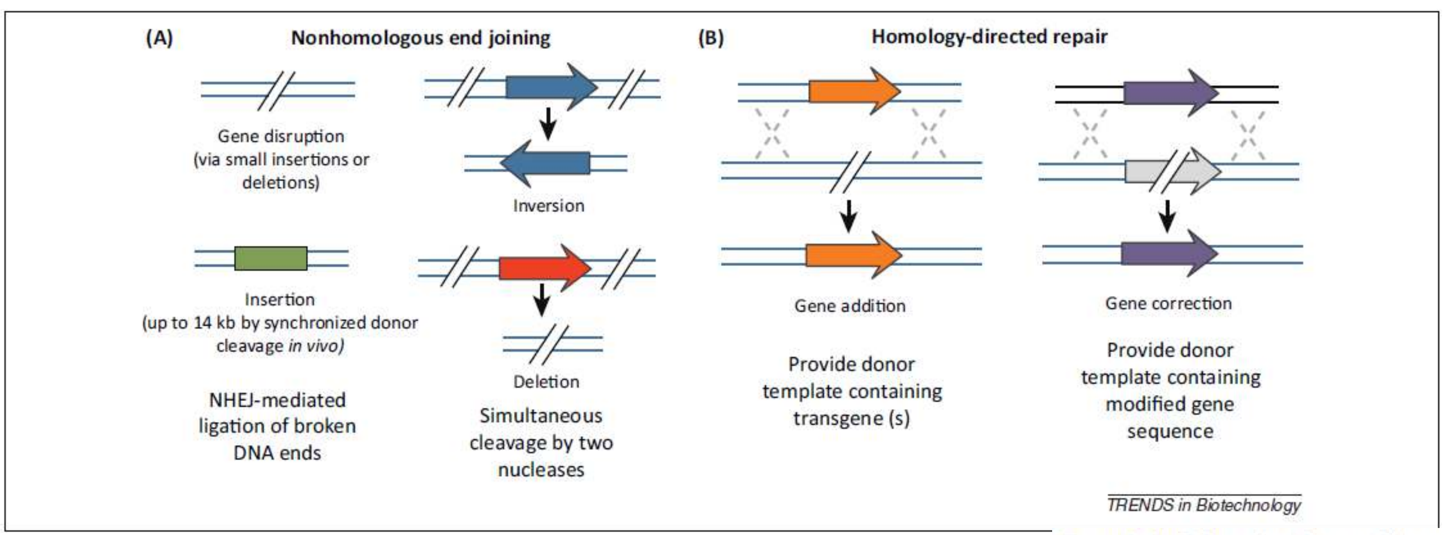
Stadtfeld & Hochedinger, 2010

Genome editing

Zinc Finger Nucleases (ZFN) TALEN CRISPR/Cas9







Trends in Biotechnology July 2013, Vol. 31, No. 7

Type of modification	Organism	Genes	Nucleases	Refs
Gene disruption	Human	*CCR5*	ZFN	[65,91,92]
			TALEN	[25,52]
			CRISPR/Cas	[101]
	Human	TCR (T cell receptor)	ZFN	[94,95]
	Zebrafish	*gol* (Golden), *ntl* (No tail), *kra*	ZFN	[66,68]
	Pig	*GGTA1* (α1, 3-galactosyltransferase)	ZFN	[77]
		LDLR (LDL receptor)	TALEN	[76]
	Bovine	*ACAN12*, *p65*	TALEN	[76]
	Human	*EMX1*, *PVALB*	CRISPR/Cas	[102]
	Rat	*IgM*, *Rab38*	ZFN	[70]
	Arabidopsis	*ADH1*, *TT4*	ZFN	[81]
	C. elegans	*ben-1*, *rex-1*, *sdc-2*	ZFN/TALEN	[78]
	Hamster	*DHFR*	ZFN	[37]
	Drosophila	*yellow*	ZFN	[72]
	Rice	*OsSWEET14*	TALEN	[84]
Gene addition	Human	*OCT4*, *PITX3*	ZFN/TALEN	[45,46]
	Human	*CCR5*	ZFN	[97]
	Human	*F9* (Coagulation Factor IX)	ZFN	[86]
	Mouse	*Rosa26*	ZFN	[57]
	Human	AAVS1	ZFN	[45,96,97]
			TALEN	[46]
			CRISPR/Cas	[103]
	Human	*VEGF-A*	ZFN	[17]
	Zebrafish	*th* (tyrosine hydroxylase), *fam46c*, *smad5*	TALEN	[80]
	Maize	*IPK1*	ZFN	[82]
Gene correction	Human	*IL2RG*	ZFN	[44,85]
		A1AT (α_1-antitrypsin)	ZFN	[89]
		HBB (β-globin)	ZFN	[87,88]
		SNCA (α-synuclein)	ZFN	[90]
	Tobacco	*SuRA*, *SurRB* (acetolactate synthase)	ZFN	[83]
	Drosophila	*yellow*	ZFN	[71]

Cell. 2011 July 22; 146(2): 318–331. doi:10.1016/j.cell.2011.06.019.

Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations

REGENERATIVE MEDICINE

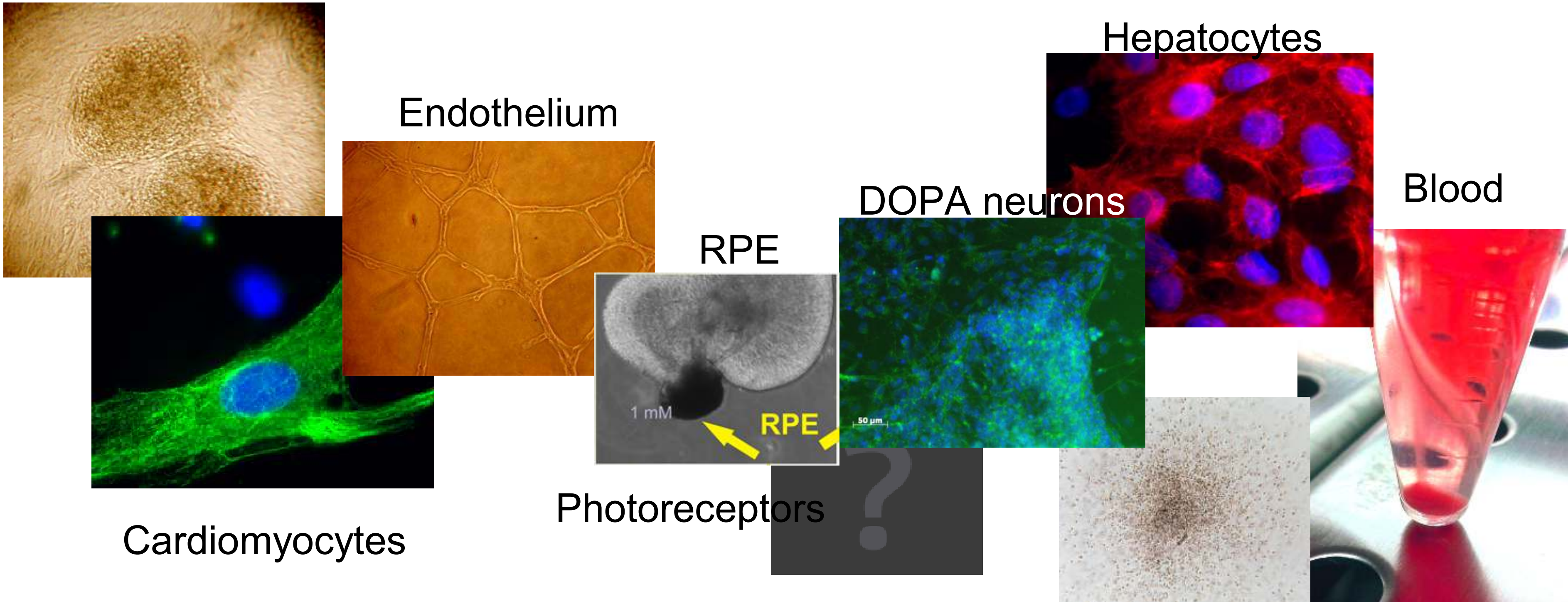
In Situ Genetic Correction of the Sickle Cell Anemia Mutation in Human Induced Pluripotent Stem Cells Using Engineered Zinc Finger Nucleases

Russian human pluripotent stem cell bank from healthy and diseased individuals

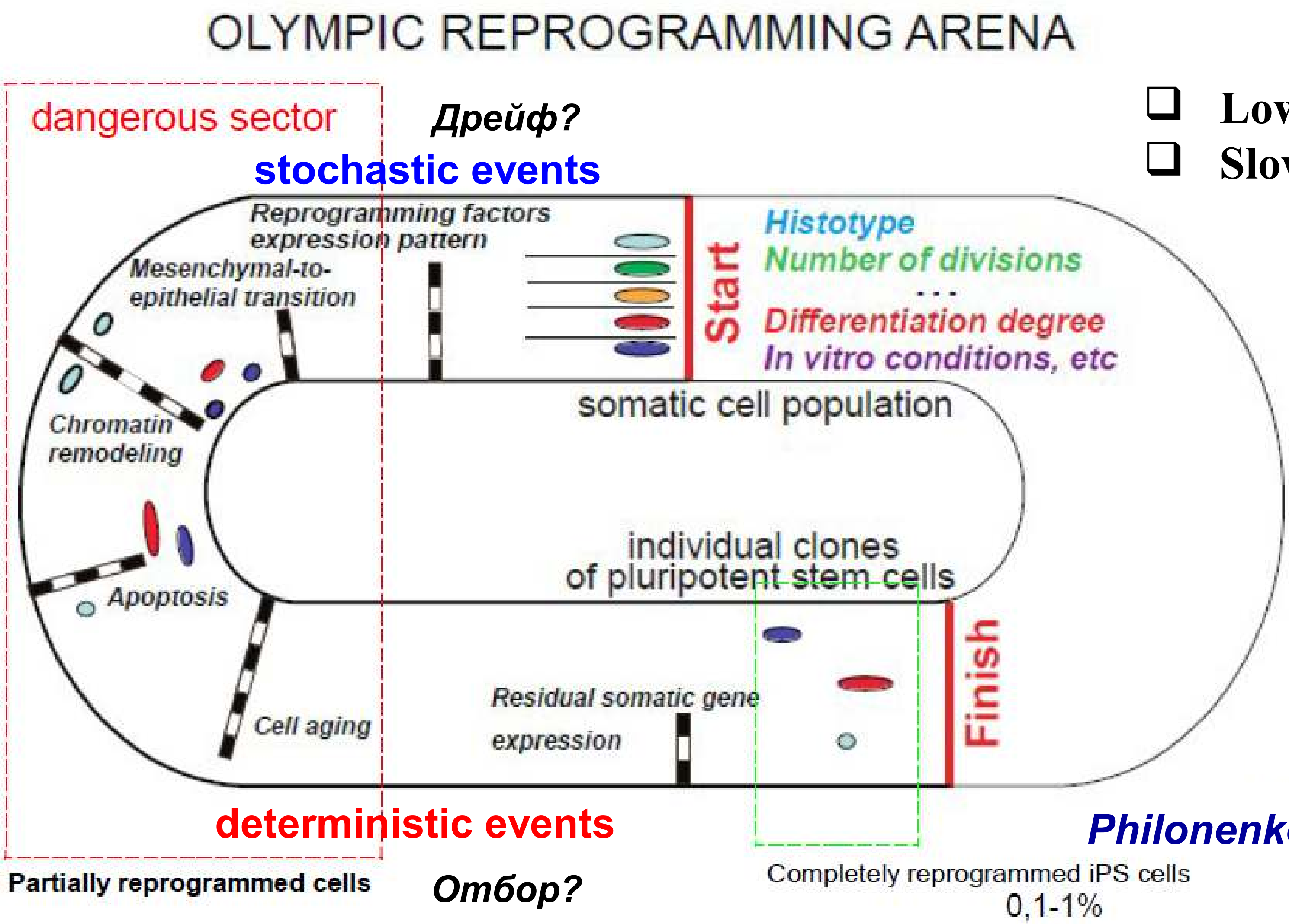
iPSC from healthy donors (3), Parkinson disease patients (3), Huntington disease patients (3), Alzheimer patients (4), shizofrenia patients (3), Statgardt maculodystrophy patients (3), amyothrophic lateral sclerosis patients (3), disferlinopathy patients (2), etc.

More than 200 human iPSC lines

6 human ESC lines (Russia), 5 human ESC lines (NIH)



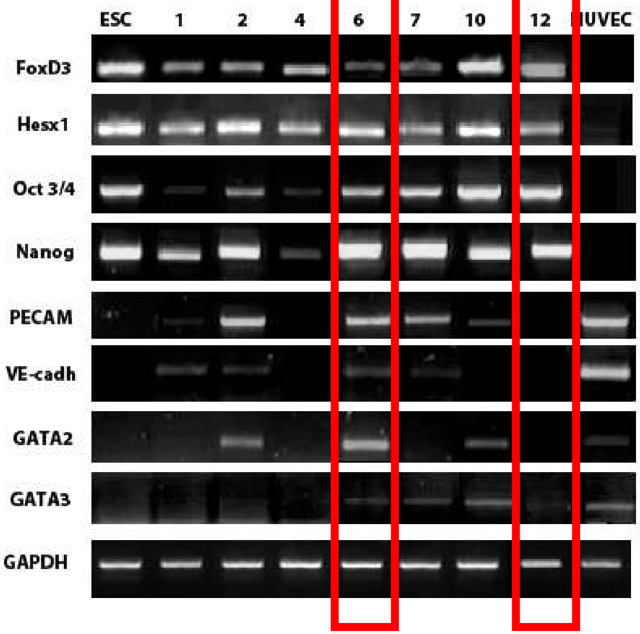
- Low Efficiency
- Slow Kinetics



Philonenko et al, 2011

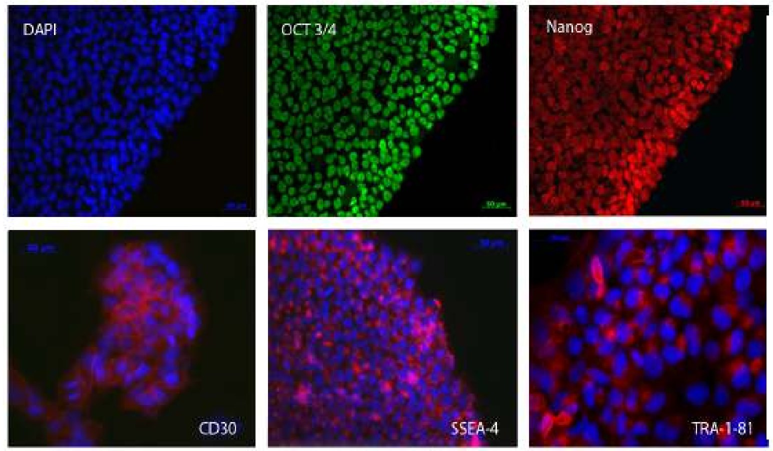
Human iPSCs characterization

RT-PCR

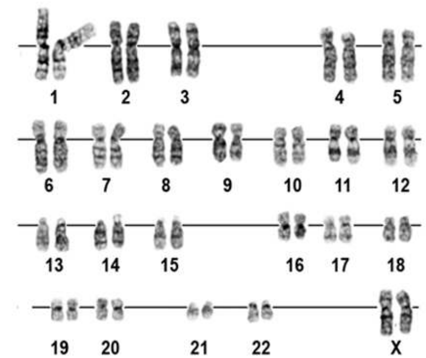


Transgene silencing

Immunohistochemical markers



Karyotype and STR analysis

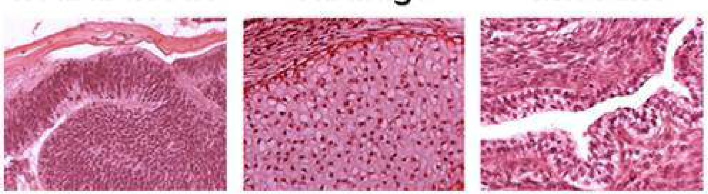


In vivo differentiation

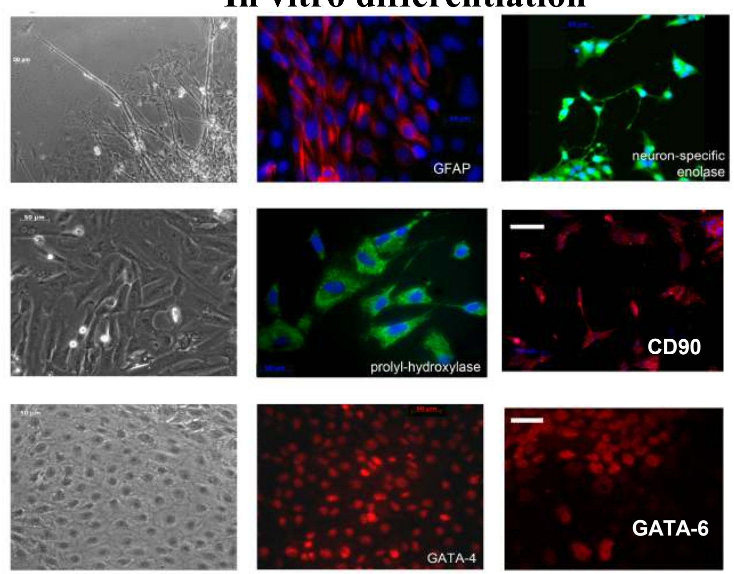
neural tissue

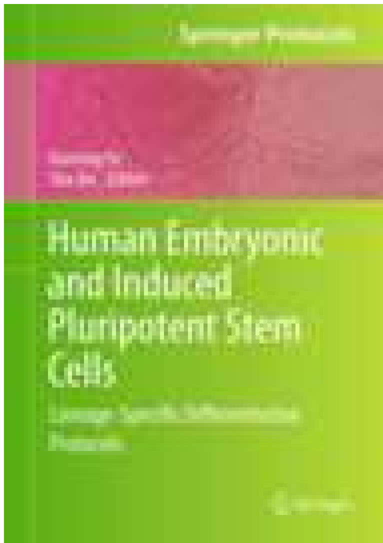
cartilage

intestine



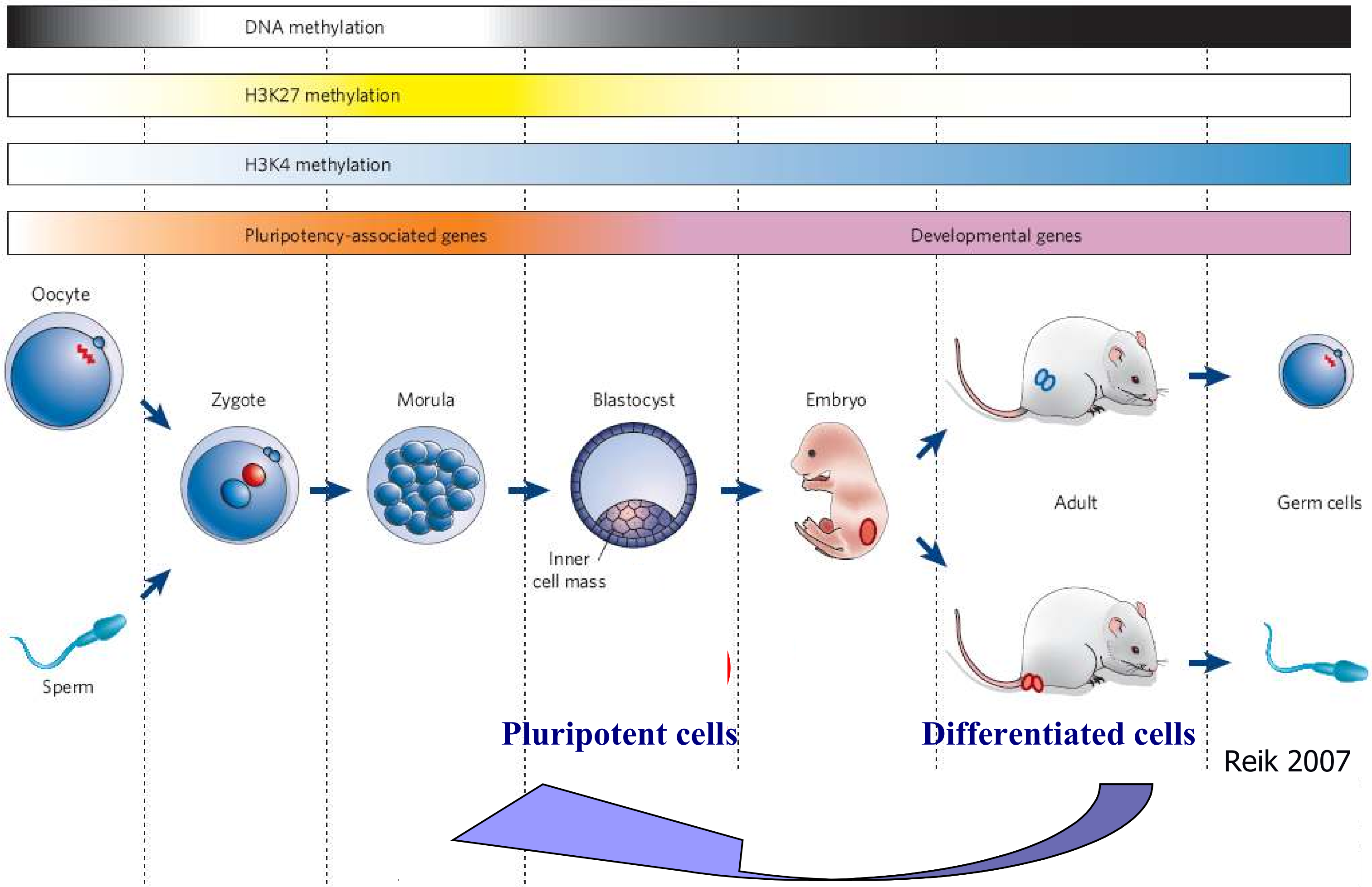
In vitro differentiation





Shutova et al, 2009
Lagarkova et al, 2010

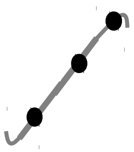
Ontogenetic program is executed on genetic and epigenetic levels



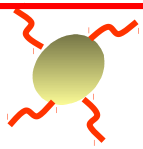
Reik 2007

To reprogram?

Epigenetic regulation of gene expression

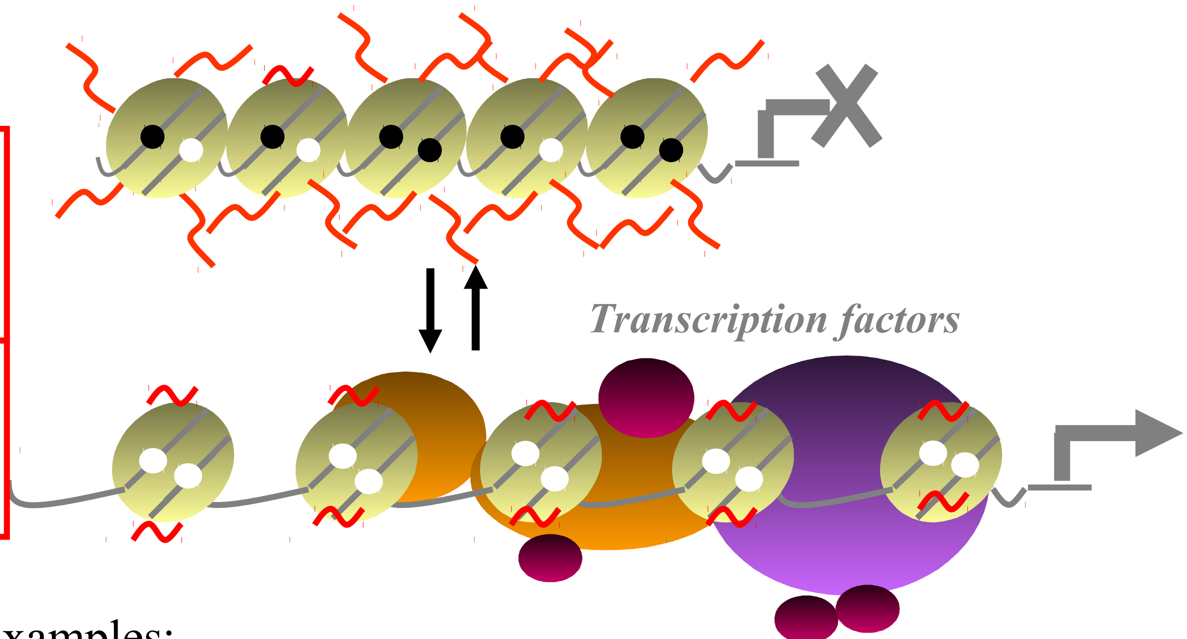


CpG DNA methylation 5meC



Histons modifications

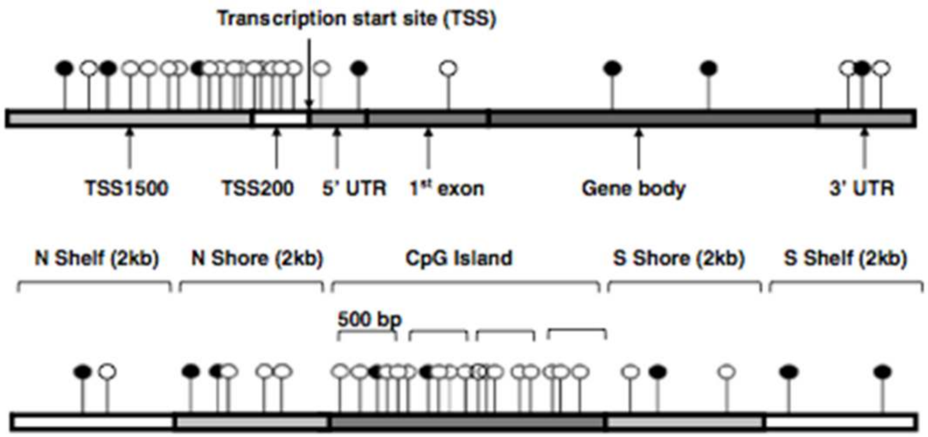
Non coding RNA

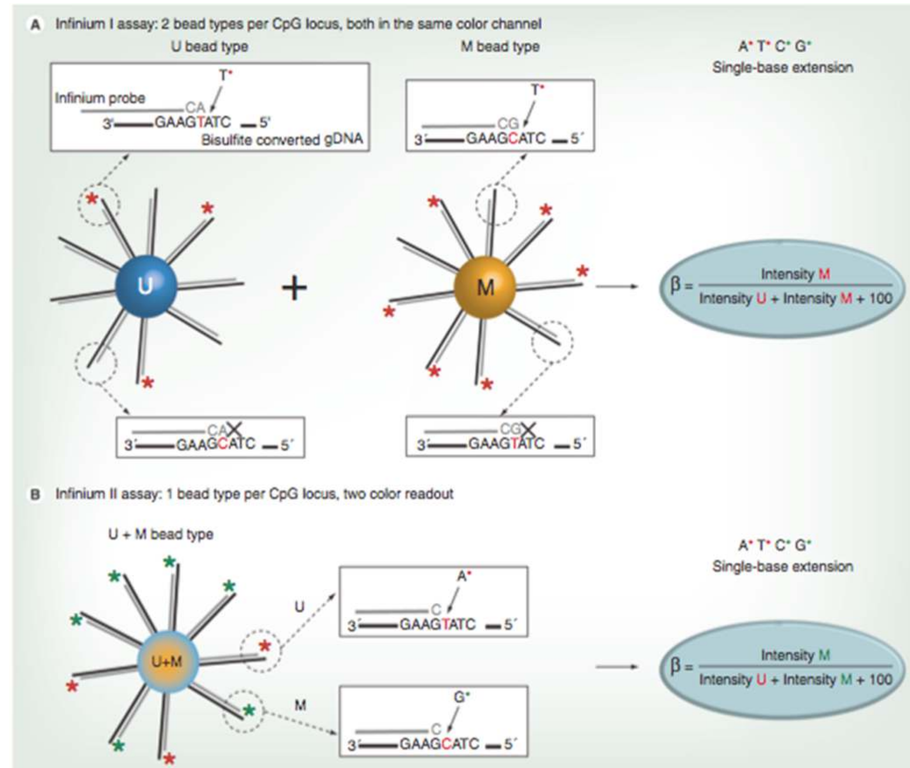


Examples:

- Developmental and tissue specific gene expression
- X chromosome inactivation in female cells
- Transformation
- Aging

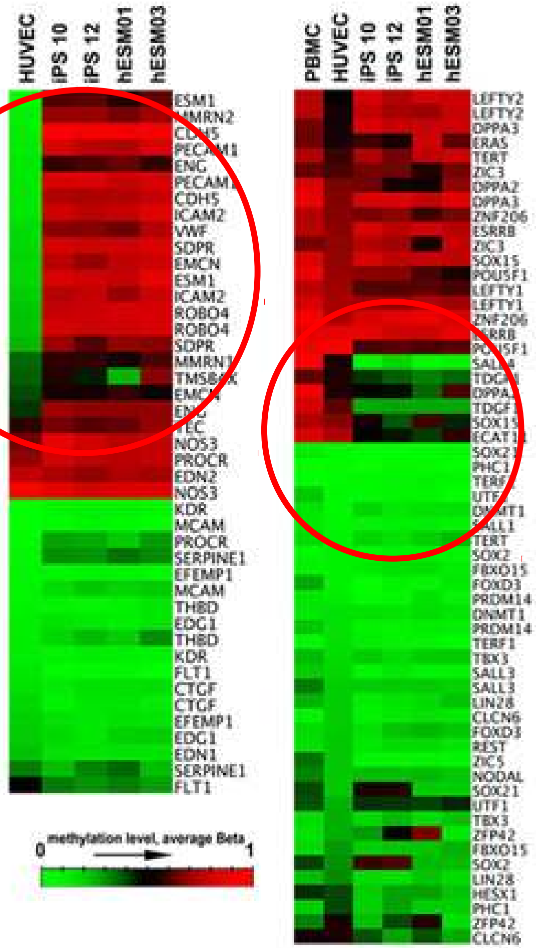
Illumina Infinium HumanMethylation bead chip 27K and 450K



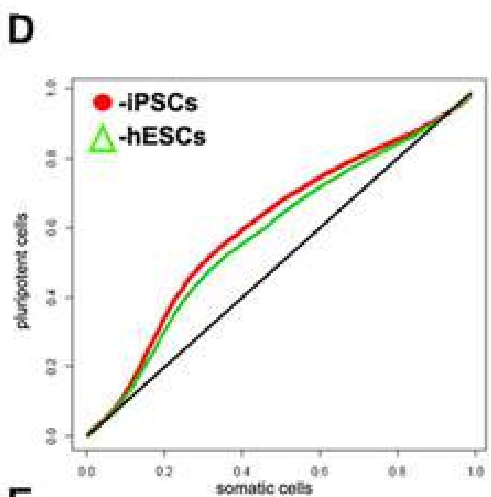


Methylation changes in promoter regions of 14400 genes

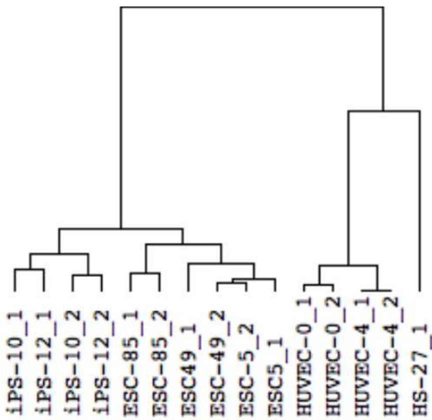
HeatMap метилирования регуляторных районов 25 наиболее известных эндотелиальных генов и 33 генов, связанных с плюрипотентностью.

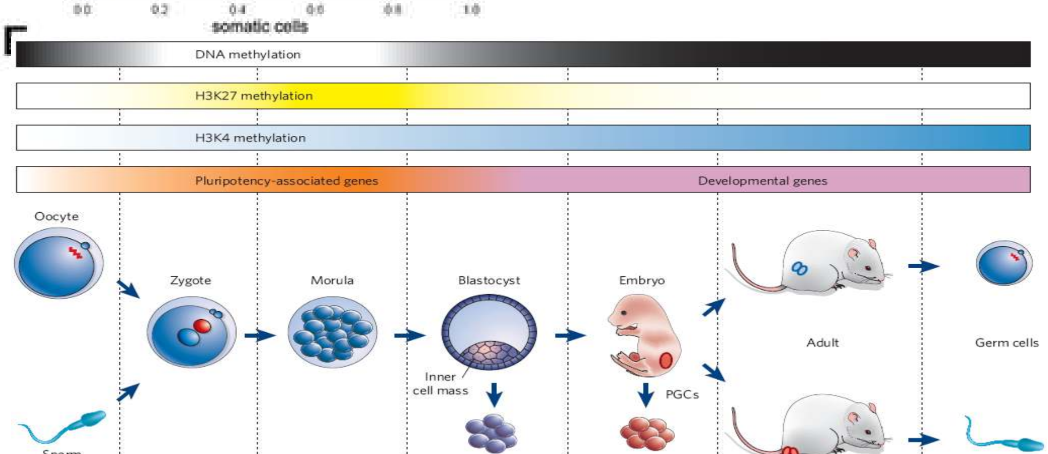


Lagarkova_Fig3



Hierarchical sample clustering (unsupervised).

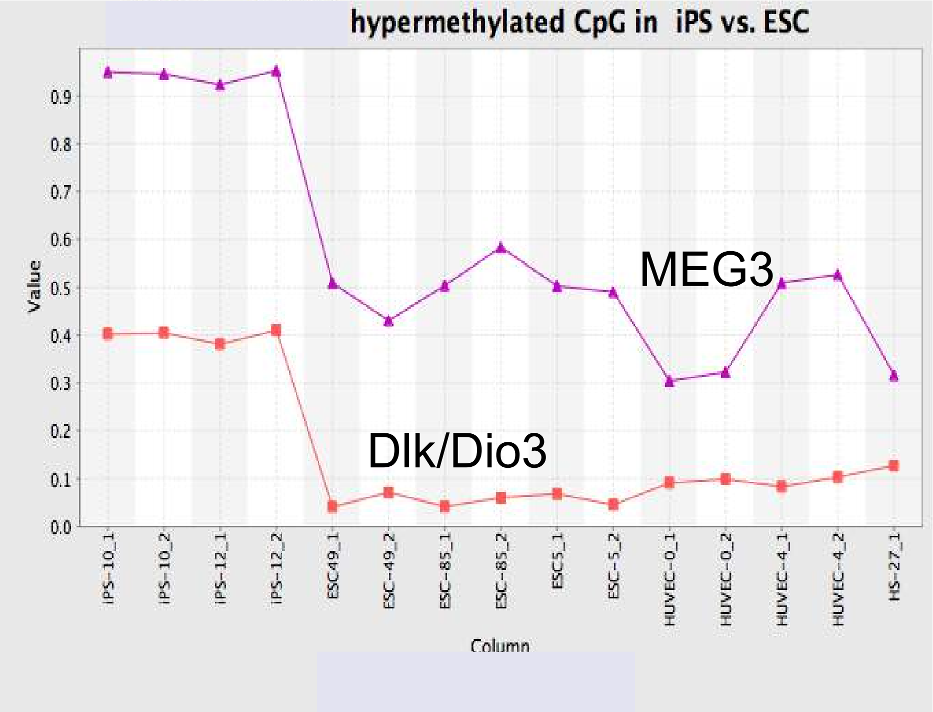


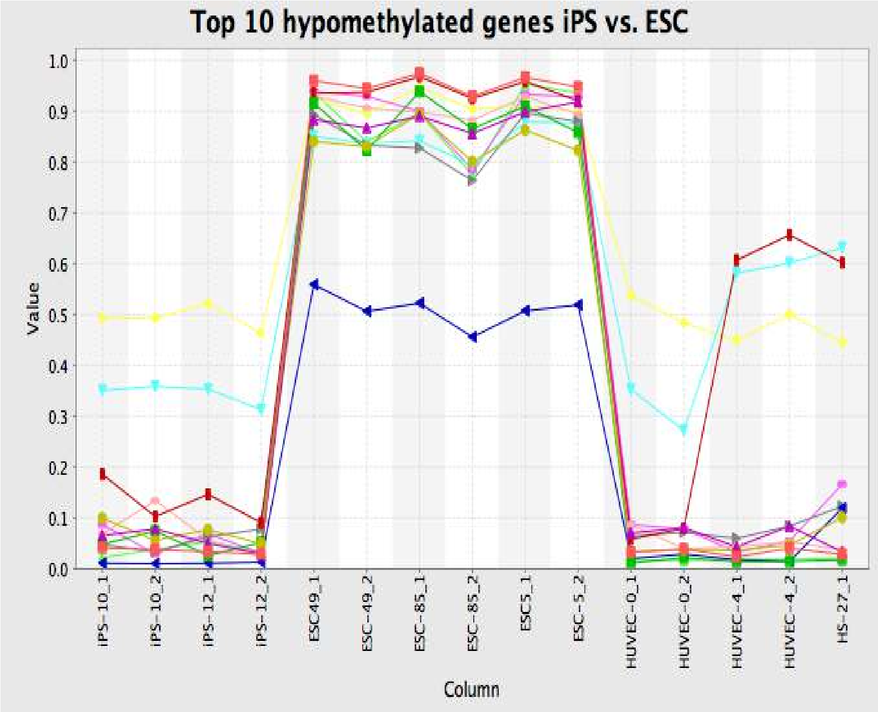


Lagarkova et al, 2010

Differential promoter methylation iPSCs vs. ESCs

Test	0.05	0.01
P-value	102	83
FDR	80/13	54/8
Bonferoni	42	21/2
Q-test	-	-

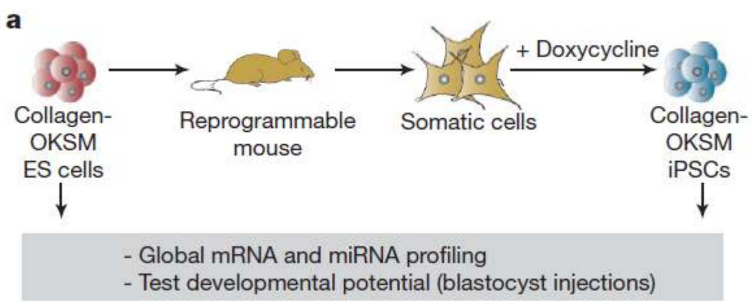


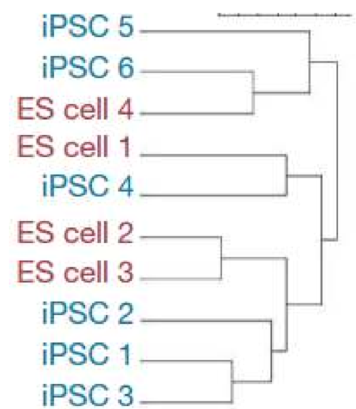


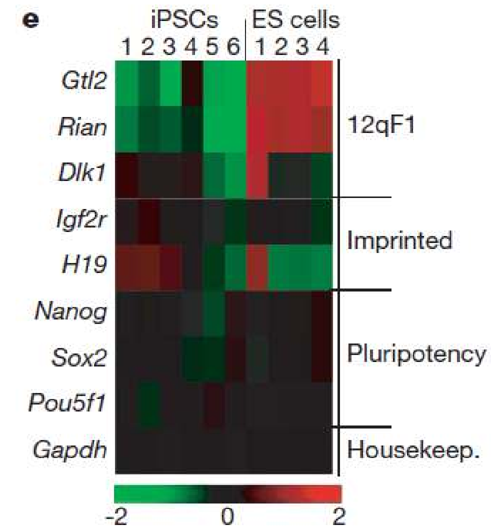
No cancer-related genes were identified

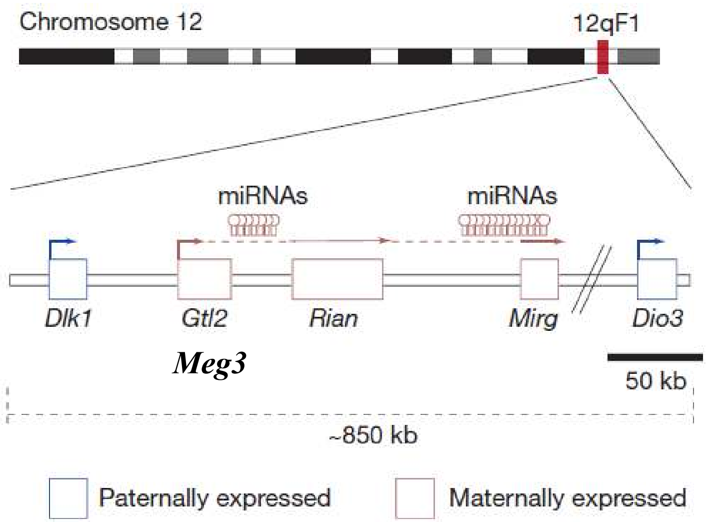
Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells?

Stadtfeld et al 2010

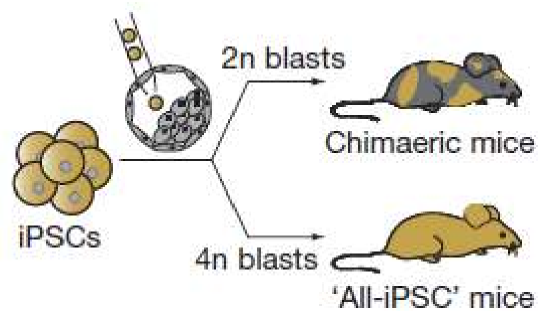


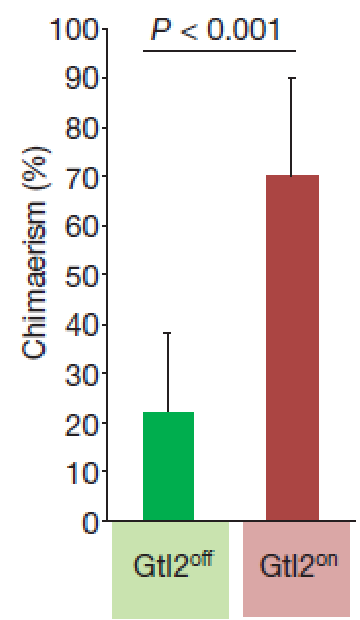






Meg3

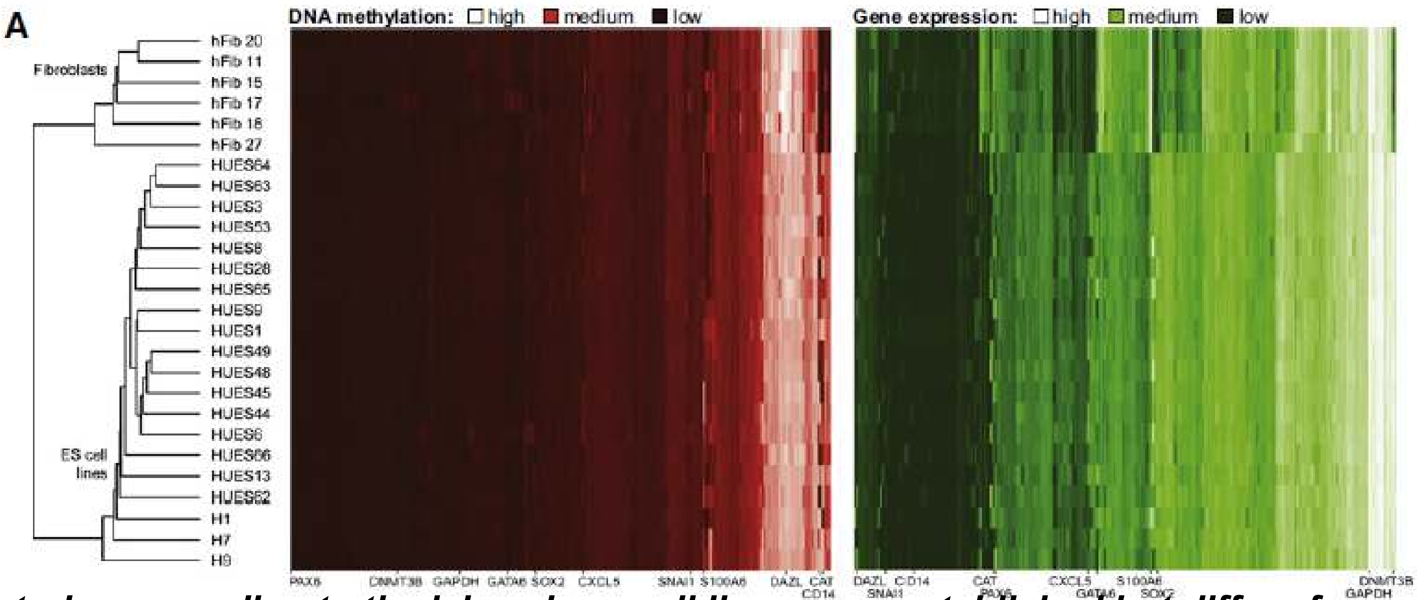




Are iPSCs equal to ESCs or within ESCs diversity?

ESCs diversity

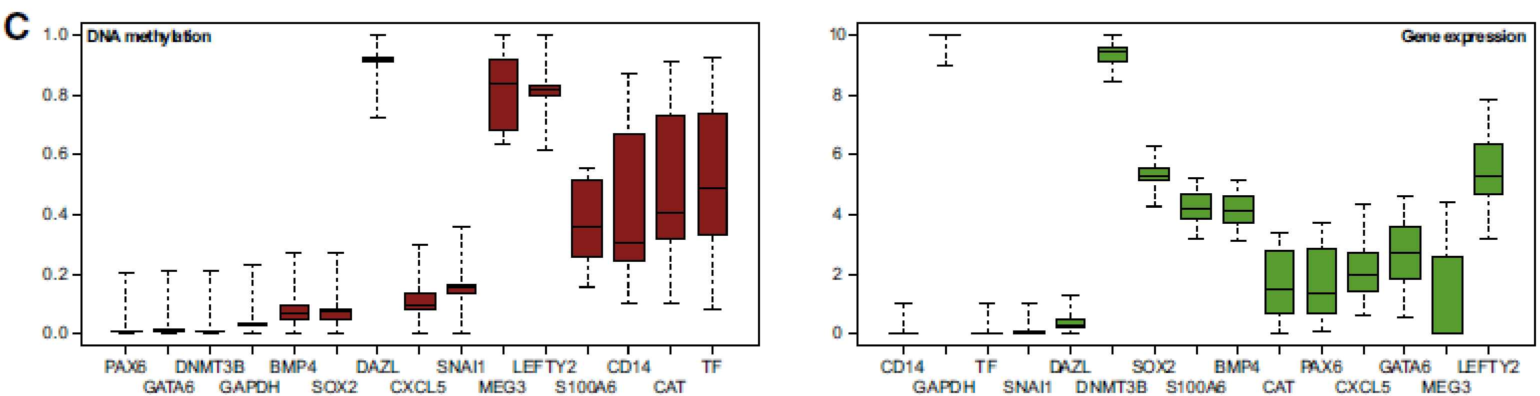
4 mln CpG **15500 genes** *Bock et al., 2011*



! Clustering according to the labs where cell lines were established but differs from somatic !

Some gene (CAT, CD14 etc) ExpMet patterning is variable between lines and maintained through passaging

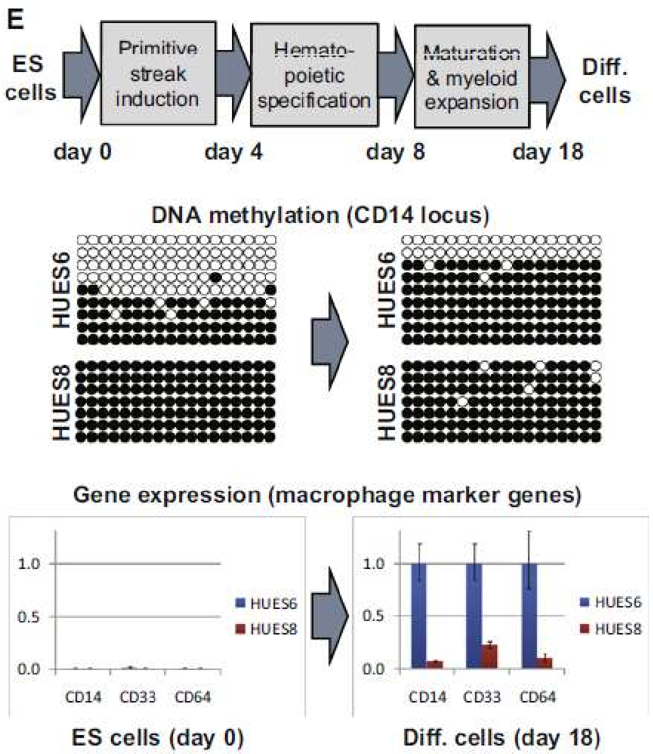
ESCs reference corridore

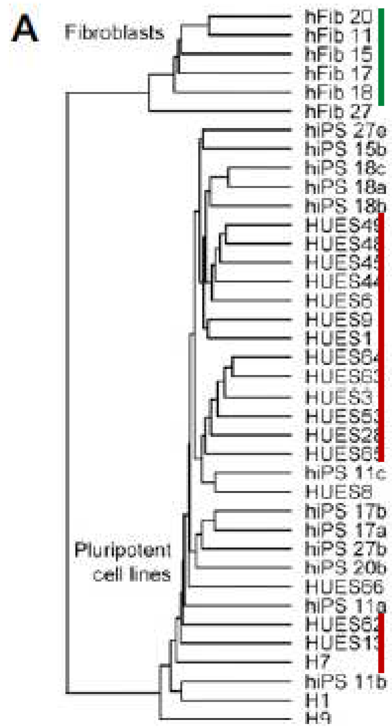


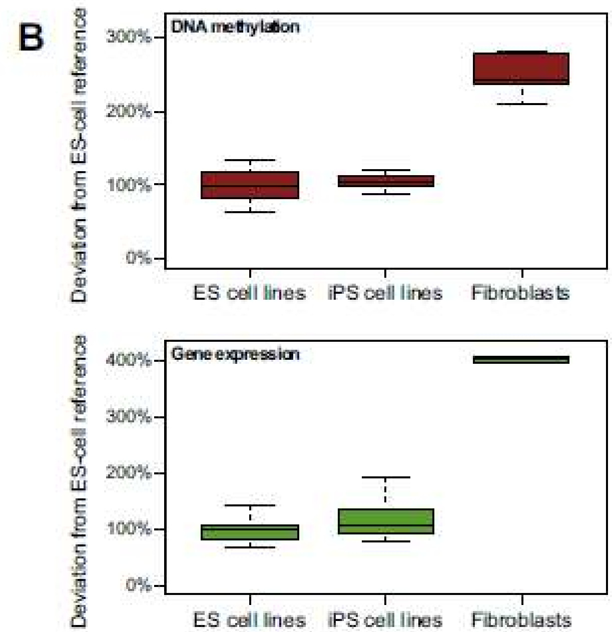
If gene's ExpMet falls out cell line could not be considered ES

Fall outs consequences

iPSCs vs ESCs diversity



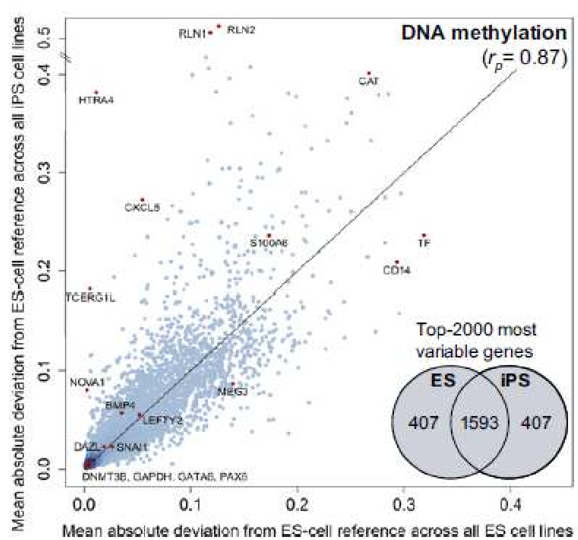


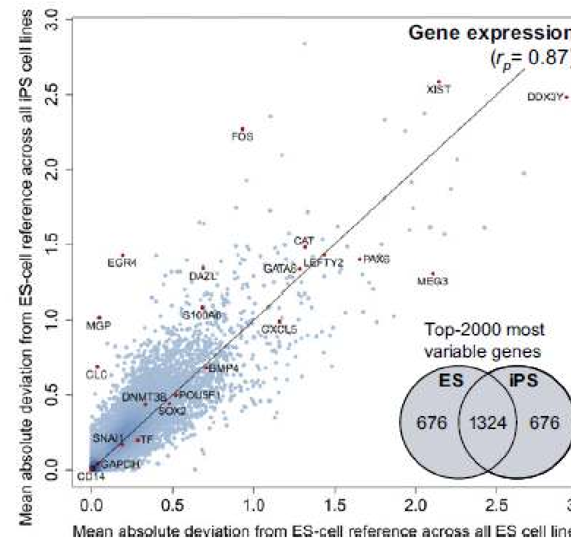


Average deviation among iPS is slightly higher than ES

D

	iPS Cell Classifier	Accuracy	Sensitivity	Specificity
Published iPS cell signatures	Chin2009 gene expression signature	63%	0%	100%
	Doi2009 DNA methylation signature	63%	0%	100%
	Stadtfeld2010 single-gene signature (MEG3)	72%	100%	55%





There is no any particular ExpMet signature that distinguishes iPS from ES cell lines but most of iPSCs might be

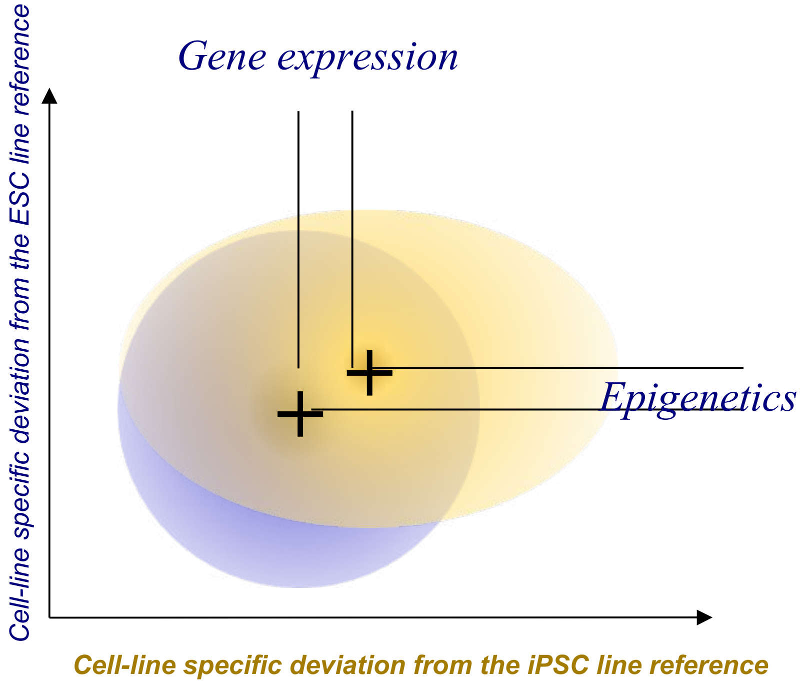
Bock et al., 2011

There are OR there are not notable differences between iPSC and ESC lines?

S. Yamanaka 2012 *Cell Stem Cell*

Table 1. Number of ESC and iPSC Clones Analyzed in Published Studies

Conclusion about the Relationship between ESCs and iPSCs	First Author	Year	Clone Numbers ESC	Clone Numbers iPSC
It is difficult to distinguish between them	A.M. Newman	2010	23	68
	M.G. Guenther	2010	36	54
	C. Bock	2011	20	12
There are notable differences	M. Chin	2009	3	5
	C.M. Marchetto	2009	2	2
	J. Deng	2009	3	4
	Z. Ghosh	2010	6	4
	A. Doi	2011	3	9
	Y. Ohi	2011	3	9
	K. Kim	2011	6	12
	R. Lister	2011	2	5
It is difficult to distinguish.	**Nazor et al.**	**2012.**	**40**	**80.**

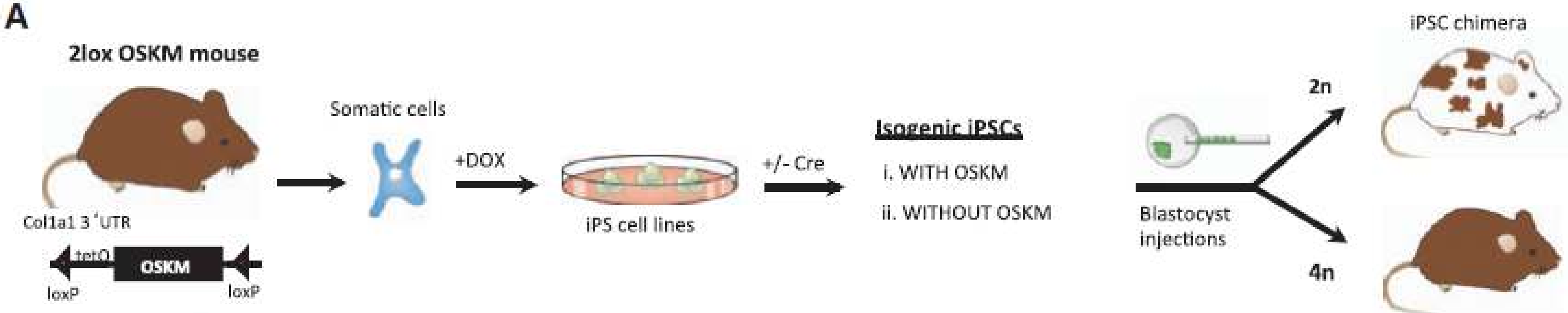


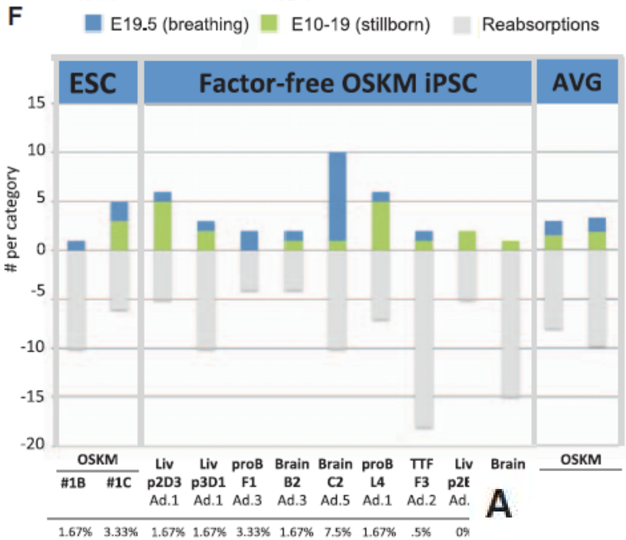
Yamanaka's message: More cell lines we took in comparison less differences we observed

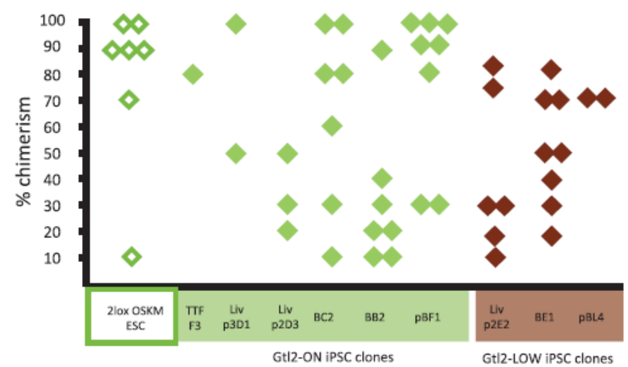
Reprogramming Factor Stoichiometry Influences the Epigenetic State and Biological Properties of Induced Pluripotent Stem Cells

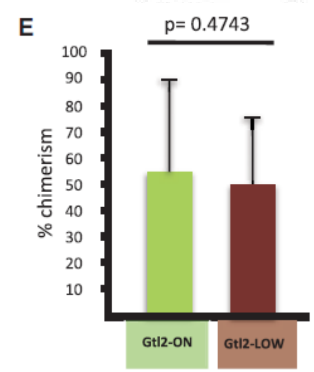
2012

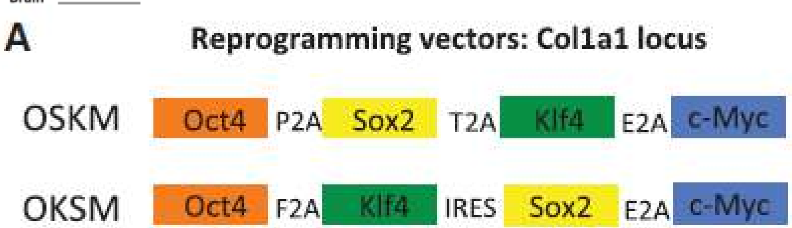
Bryce W. Carey,[1,2] Styliani Markoulaki,[1] Jacob H. Hanna,[3] Dina A. Faddah,[1,2] Yosef Buganim,[1] Jongpil Kim,[1] Kibibi Ganz,[1]





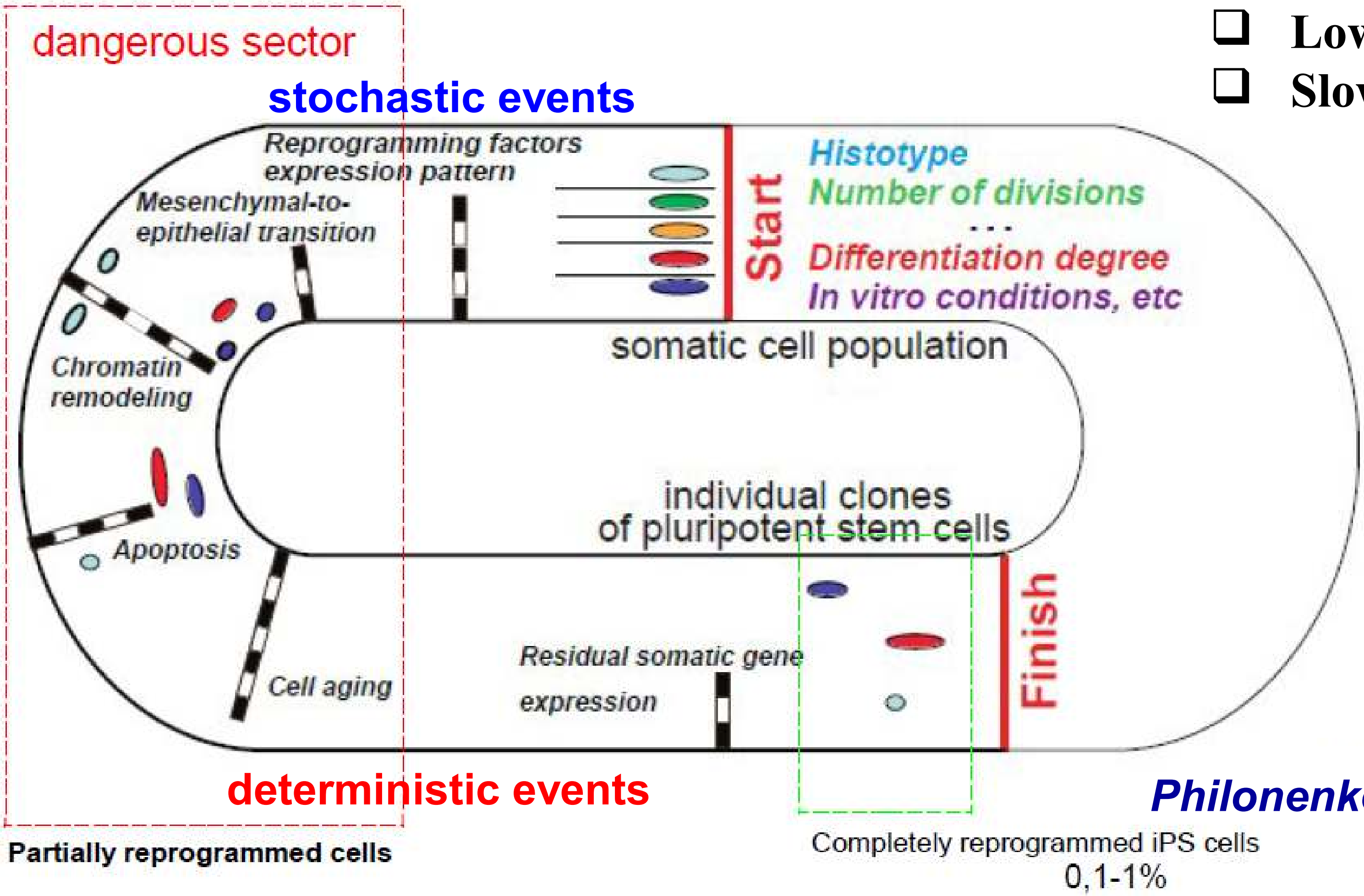






OLYMPIC REPROGRAMMING ARENA

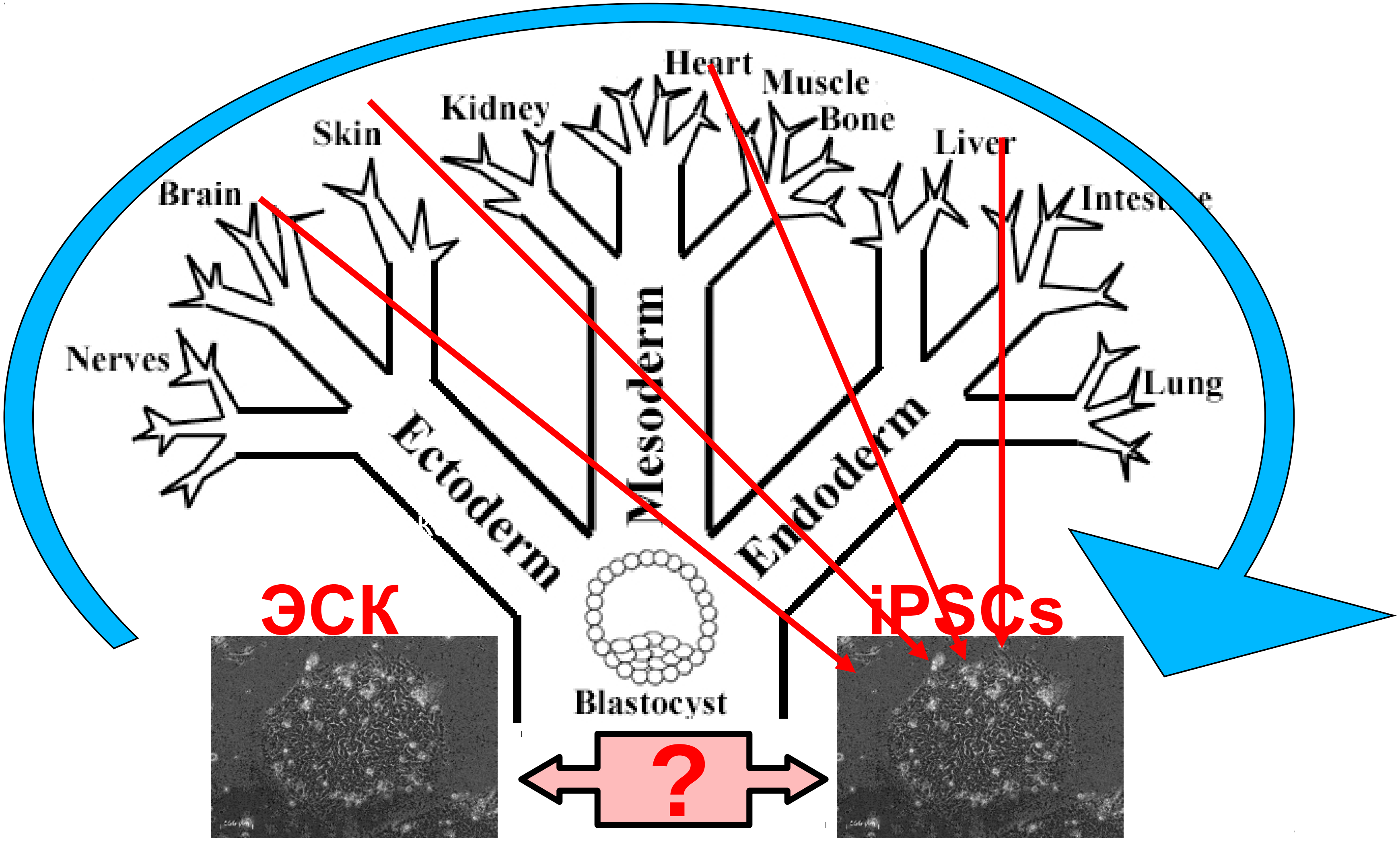
- Low Efficiency
- Slow Kinetics



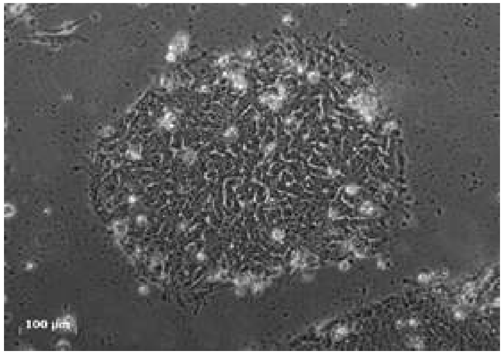
Philonenko et al, 2011

Are iPSCs equal to ESCs or within ESCs diversity?

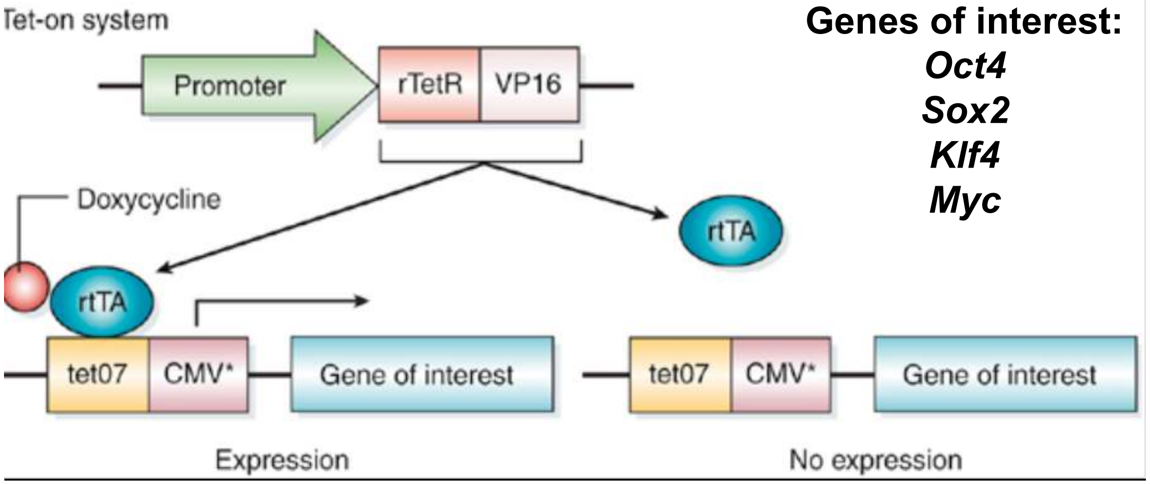
What are the marks of the reprogramming process? What are the differences between human ES and iPS cells?



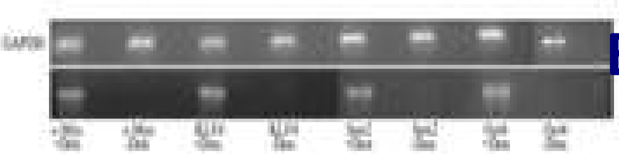
Transgenic human ESCs for pluripotency induction



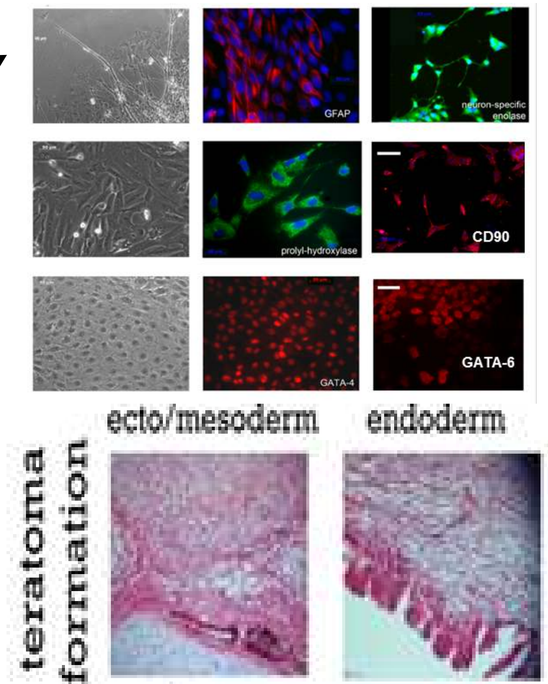
+



Genes of interest:
Oct4
Sox2
Klf4
Myc

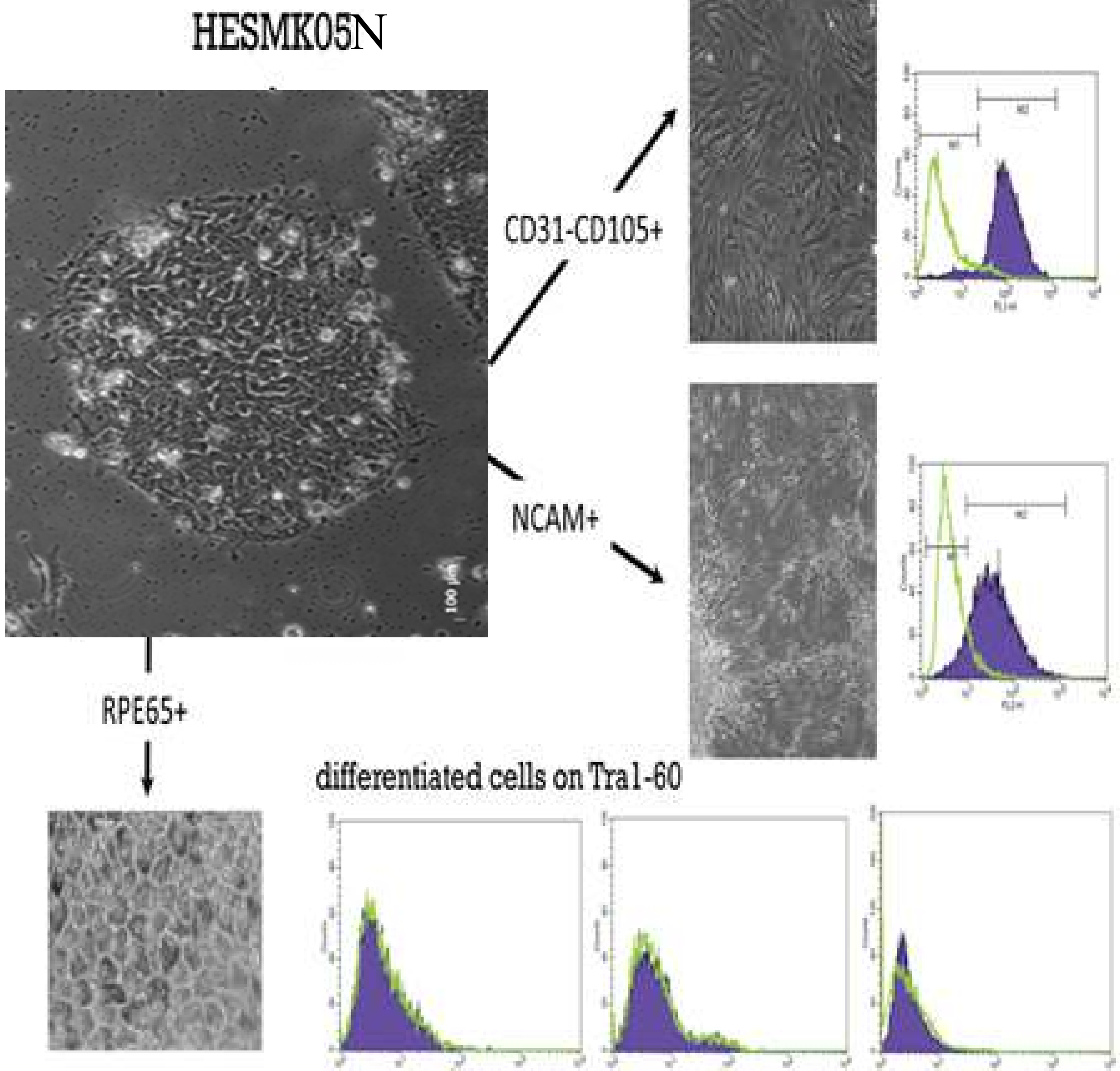


ESM05N



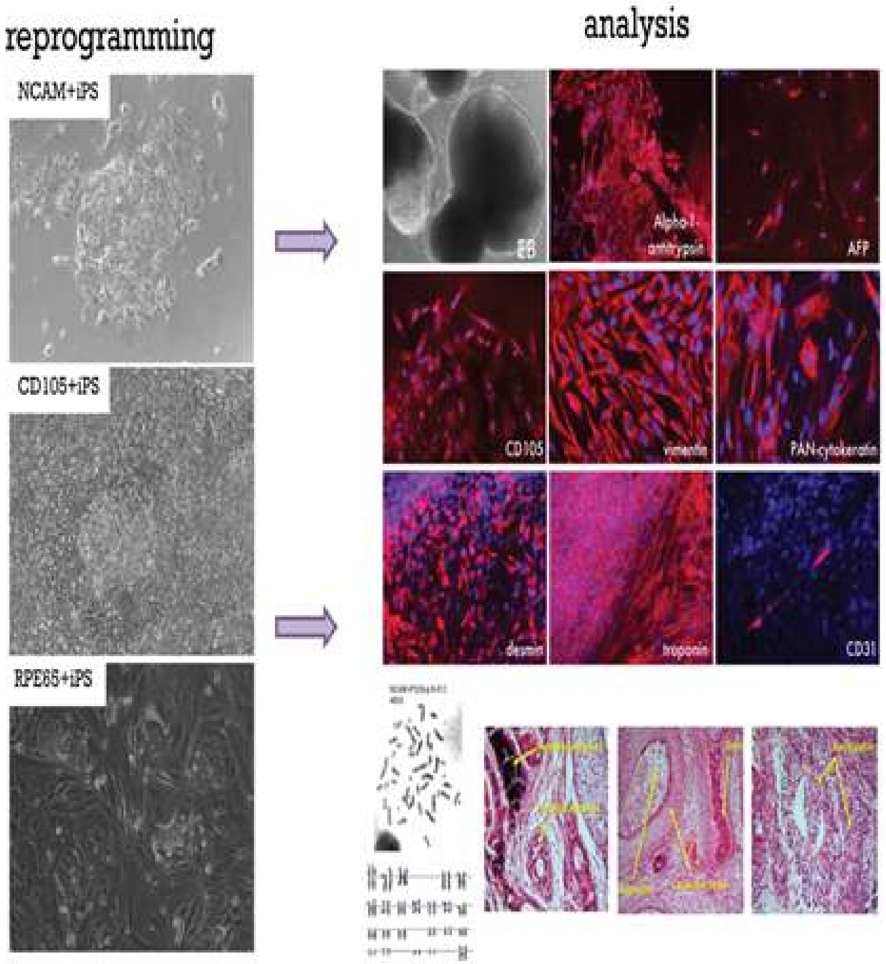
No transgene leakage in pluripotent and differentiated cells but inducible

Transgenic human ESCs differentiation and selection



+ *DOX*

Transgenic human ESCs differentiation, selection and pluripotency induction

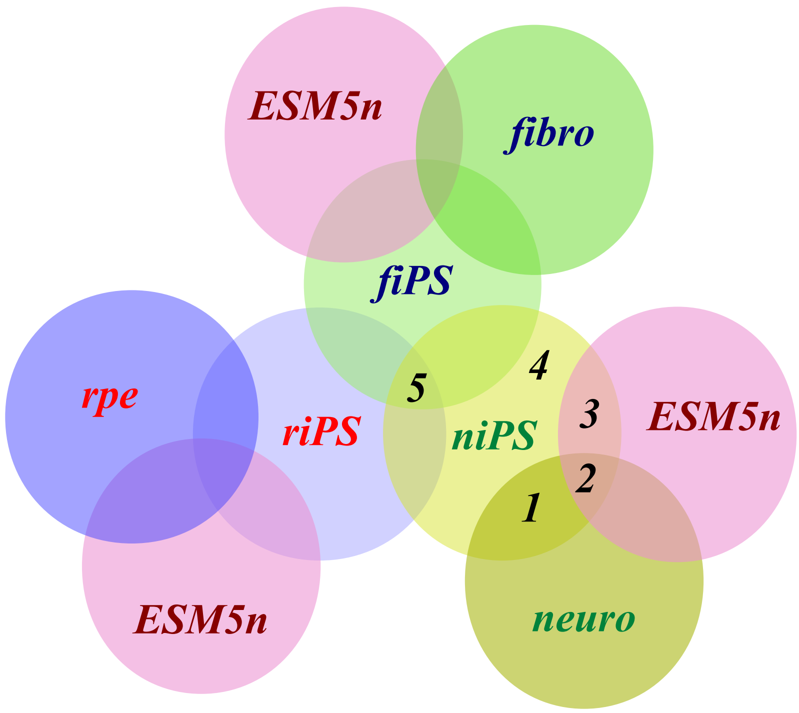


iPS7
iPS47
iPS27
iPS22
iPS29
iPS14

Illumina 450K Infinium DNA Methylation BeadChip

Illumina HT12v3,4 Transcription Bead Chip

Search for reprogramming traces in human iPSCs



1. *Somatic memory*
2. *Intact in all cells*
3. *Pluripotency specific*
4. *iPSC line specific*
5. *Reprogramming tracing, minimal distance between iPSC and ESC*

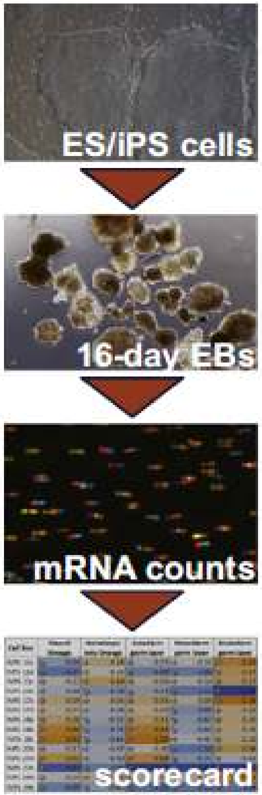
MEG3 over methylation and under expression (Stadtfeld et al., 2010, Chin et al 2009, Doi et al 2009)

Conclusion: there are some marks that determine reprogramming process in human iPSCs but! major input in the differences between iPSCs and ESCs comes out from somatic memory

Maria Shutova presentation

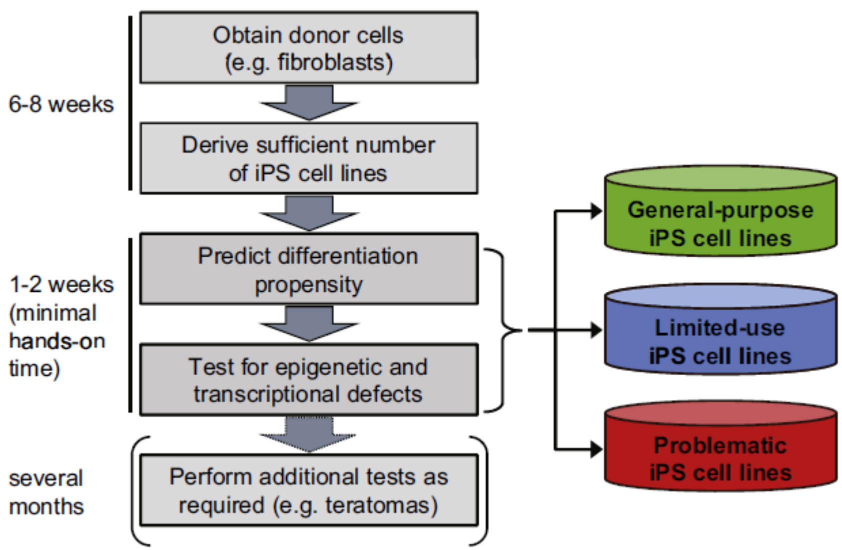
iPSCs scorecard profiling enables their utility

Bock et al., 2011



Cell line	Neural lineage	Hematopo-ietic lineage	Ectoderm germ layer	Mesoderm germ layer	Endoderm germ layer
hiPS 11a	-0.69	0.18	-0.37	-0.23	0.83
hiPS 11b	-1.17	-0.23	-0.96	-1.03	0.47
hiPS 11c	-0.22	0.40	-0.03	-0.16	0.37
hiPS 15b	-0.48	-0.78	-0.63	-1.11	-2.49
hiPS 17a	0.19	0.05	0.33	0.00	1.16
hiPS 17b	-0.07	-0.48	-0.02	-0.83	0.20
hiPS 18a	0.28	-0.52	0.31	-0.67	0.20
hiPS 18b	0.80	-0.72	0.84	-0.62	0.15
hiPS 18c	0.93	-0.65	1.05	-0.41	0.10
hiPS 20b	-0.37	-0.47	-0.30	-1.16	0.56
hiPS 27b	0.52	-0.50	0.68	-0.71	-0.42
hiPS 27e	-1.61	-1.04	-2.12	-1.82	-3.27
hiPS 29d	-0.25	-0.04	0.00	-0.11	0.83
hiPS 29e	-0.99	-0.60	-1.15	-1.14	-1.08

Differentiation propensity: high medium low



iPSCs practical application

- *Strong criteria for iPSC lines selection*
- *Robust and scalable protocols of iPSC maintaining*
- *Robust and reproducible differentiation protocols*
- *Functional similarity of differentiated derivatives to their natural counterparts*
- *Standard and scalable protocols for differentiated cells production*

Acknowlegments

<u>VIGG RAS</u>

<u>Lagarkova Maria</u>
Bogomazova Alexandra
Shutova Maria
Philonenko Elena
Vasina Ekaterina
Chestkov Ilyia

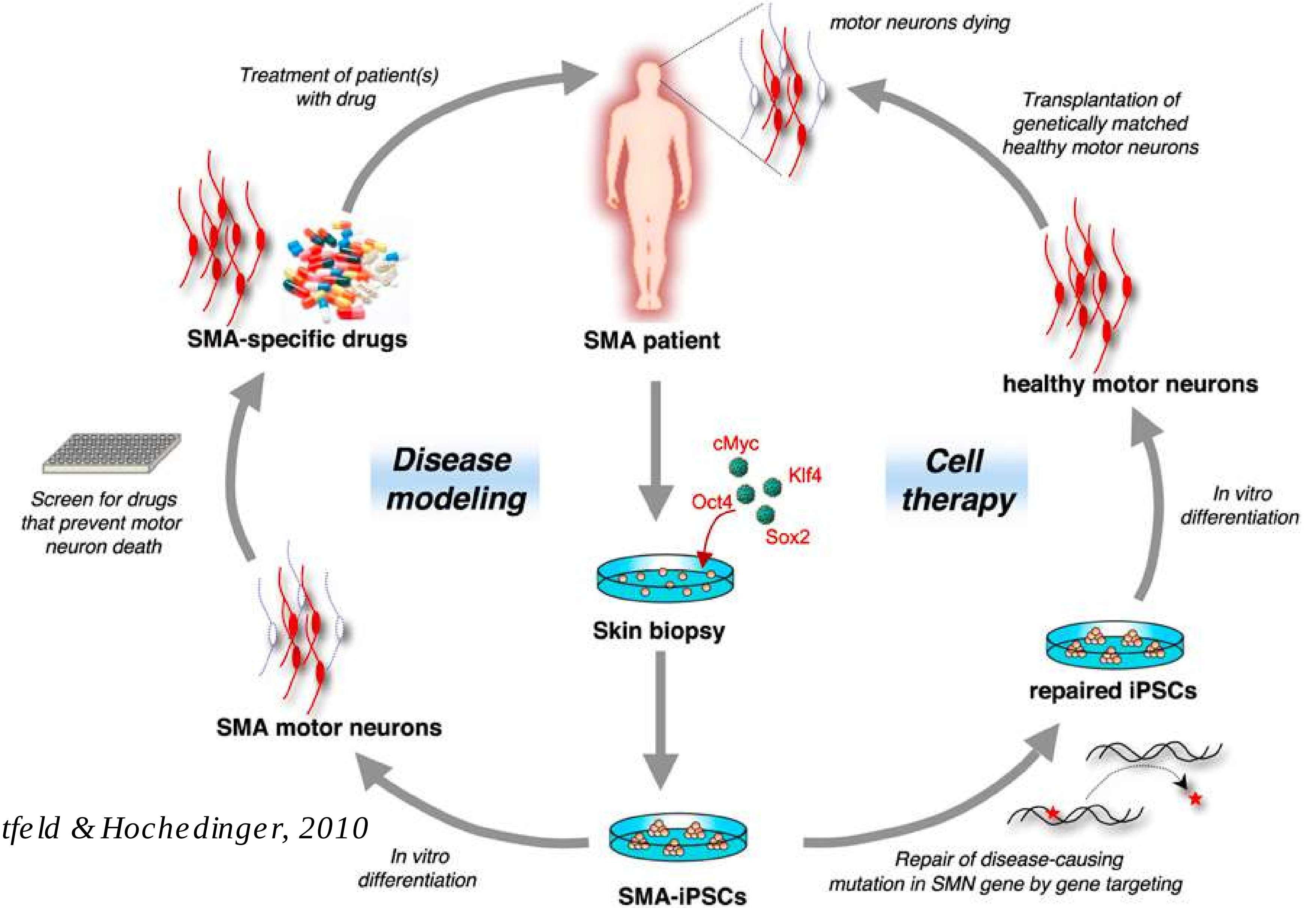
<u>Institute Cytology RAS</u>
Tomilin Alexey

<u>SRI PCM FMBA</u>
Naumov Vladimir
Ischenko Dmitrii

WWW.SKOLSTEMCELLS.ORG

We are looking for motivated students and postdocs

iPSC promises



Stadtfeld & Hochedinger, 2010

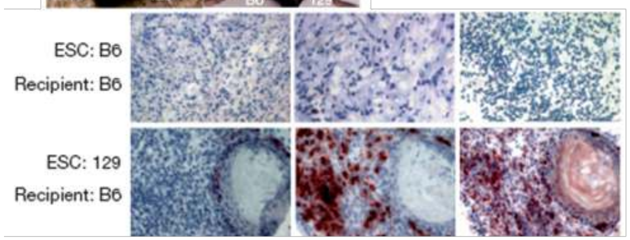
Immunogenicity of iPSC and their differentiated derivatives

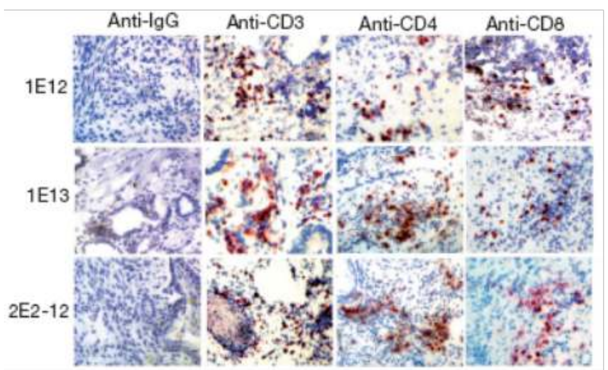
LETTER

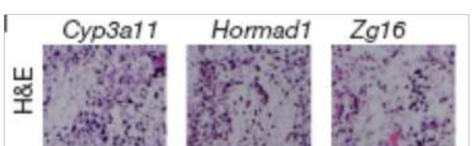
doi:10.1038/nature10135

Immunogenicity of induced pluripotent stem cells

Tongbiao Zhao[1], Zhen-Ning Zhang[1], Zhili Rong[1] & Yang Xu[1]





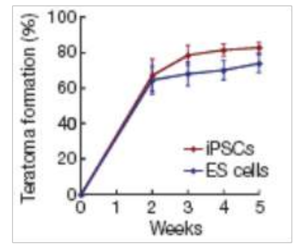


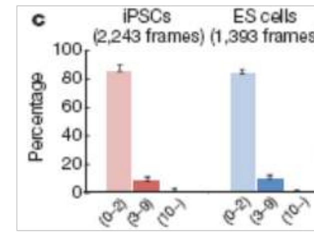
LETTER

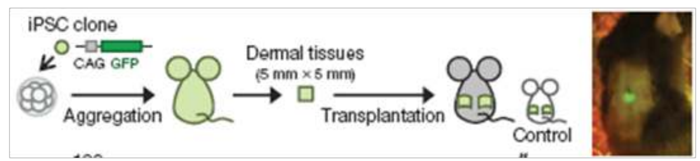
doi:10.1038/nature11807

Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells

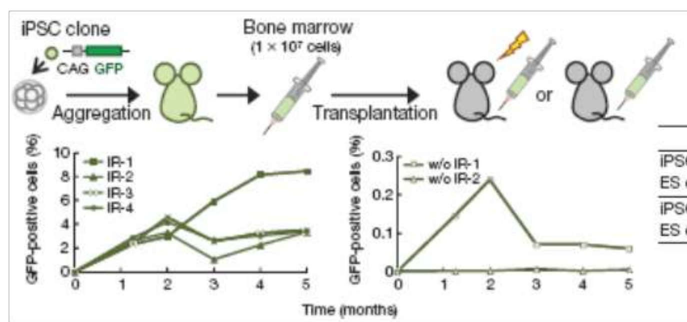
Ryoko Araki[1,2], Masahiro Uda[1], Yuko Hoki[1], Misato Sunayama[1], Miki Nakamura[1], Shunsuke Ando[1], Mayumi Sugiura[1], Hisashi Ideno[1,3], Akemi Shimada[3], Akira Nifuji[1,3] & Masumi Abe[1]







	Graft survival rate	
	C57BL/6	Balb/c
IPSCs (5 lines)	37/38 (98.3 ± 1.7%)	0/20 (0%)
ES cells (5 lines)	37/40 (92.5 ± 5.0%)	0/18 (0%)

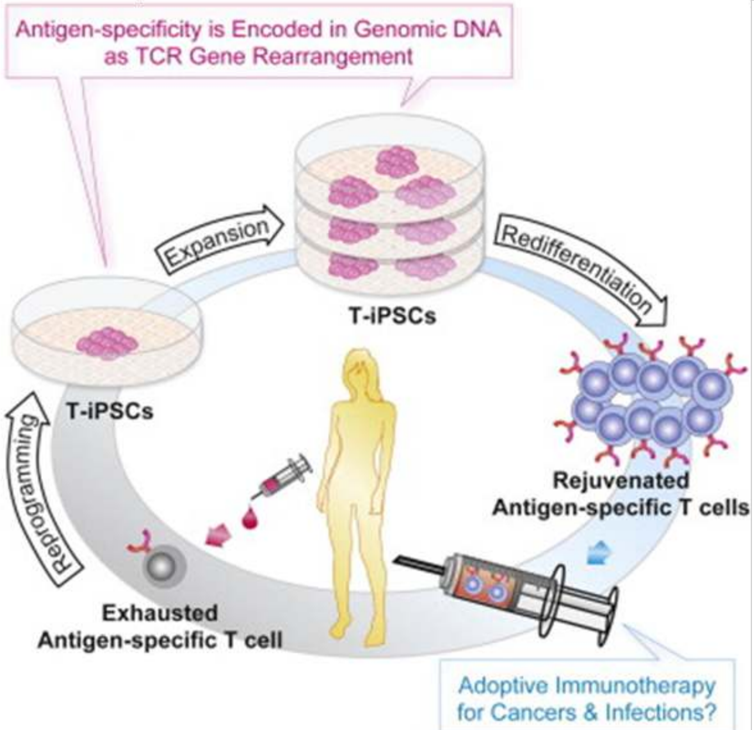


	Recipient	Graft survival rate
iPSCs (1 line)	with IR	4/4 (100%)
ES cells (1 line)	with IR	4/4 (100%)
iPSCs (5 lines)	w/o IR	20/21 (95.2%)
ES cells (3 lines)	w/o IR	11/12 (91.7%)

Не обнаружено экспрессии *Hormad1* и *Zg16*

Generation of Rejuvenated Antigen-Specific T Cells by Reprogramming to Pluripotency and Redifferentiation

Cell Stem Cell 12, 114–126, January 3, 2013 ©2013 Elsevier Inc.



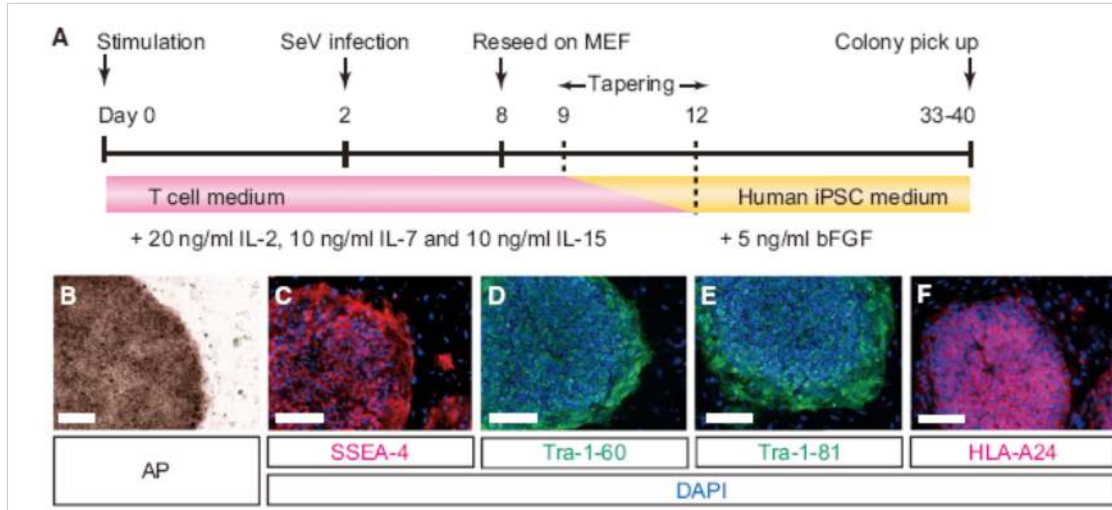
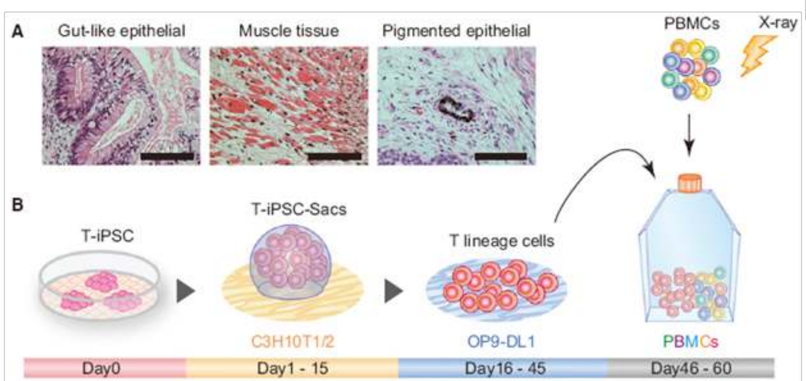
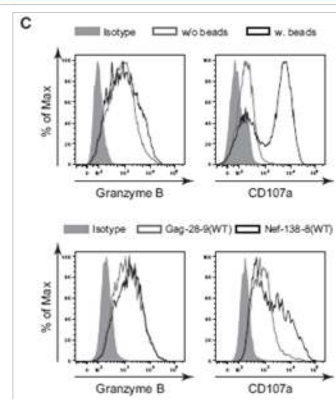
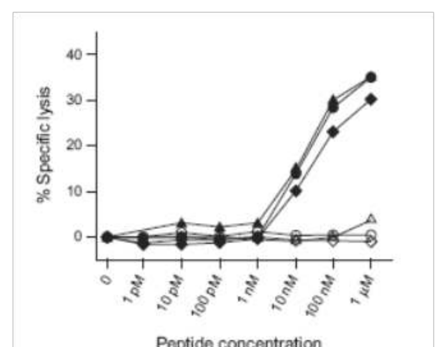


Table 1. Generation of Human T-iPSCs from Various Patient-Derived T Cell Specimens

Antigen	T Cell Source	Initial Cell Number	No. of ESC-like Colonies	No. of Colonies Picked up for Establishing T-iPSC Clones	Date (MM/YYYY)
HIV-1 Nef	monoclonal T cell clone	4×10^5	7	7	05/2011
CMV pp65	polyclonal tetramer-sorted cells	~5,000	15	15	07/2011
GAD	monoclonal T cell clone	1×10^6	>100	not picked up	08/2012
		5×10^5	>100	19	08/2012
α-GalCer	FACS-sorted Vα24⁺ cells	1×10^6	>100	not picked up	08/2012
		5×10^5	>100	7	08/2012

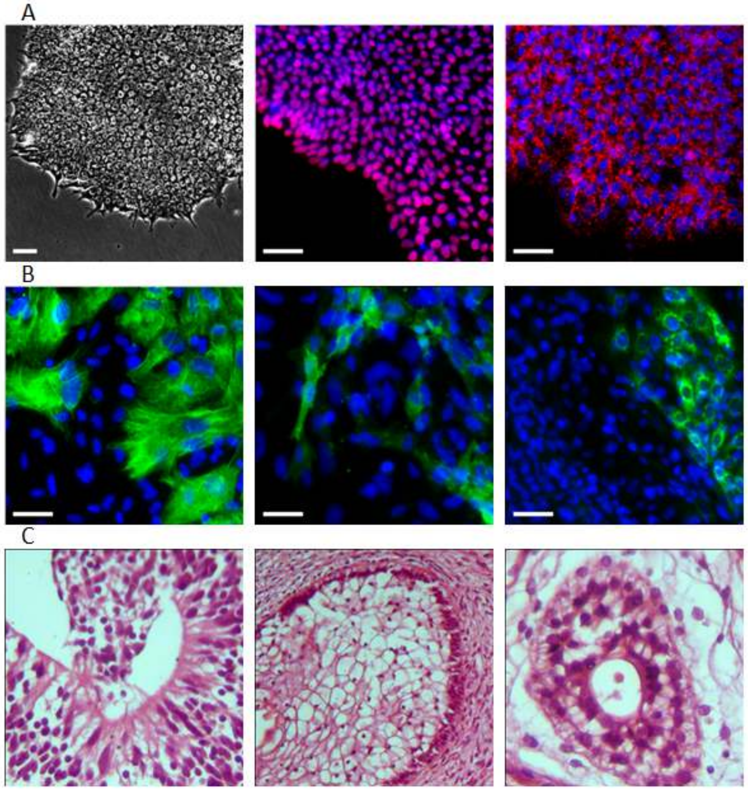




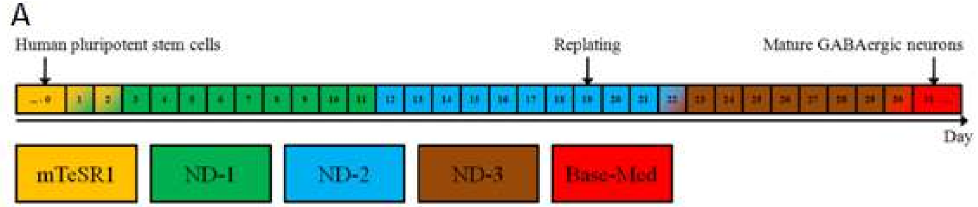


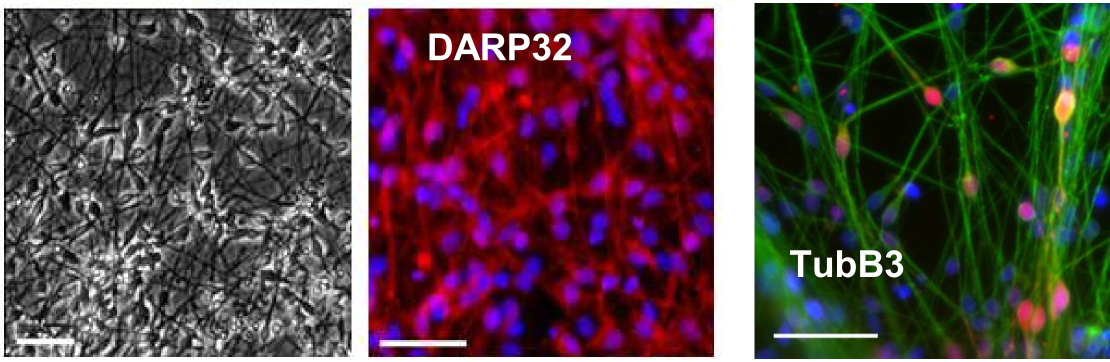
Huntington disease skin fibroblasts reprogramming

iPSC lines: *3 fibro iPSCs from three female patients with 41-47 CAG-repeats in the mutant allele of HTT gene.*



GABAergic medium spiny neurons differentiation (60-80%)

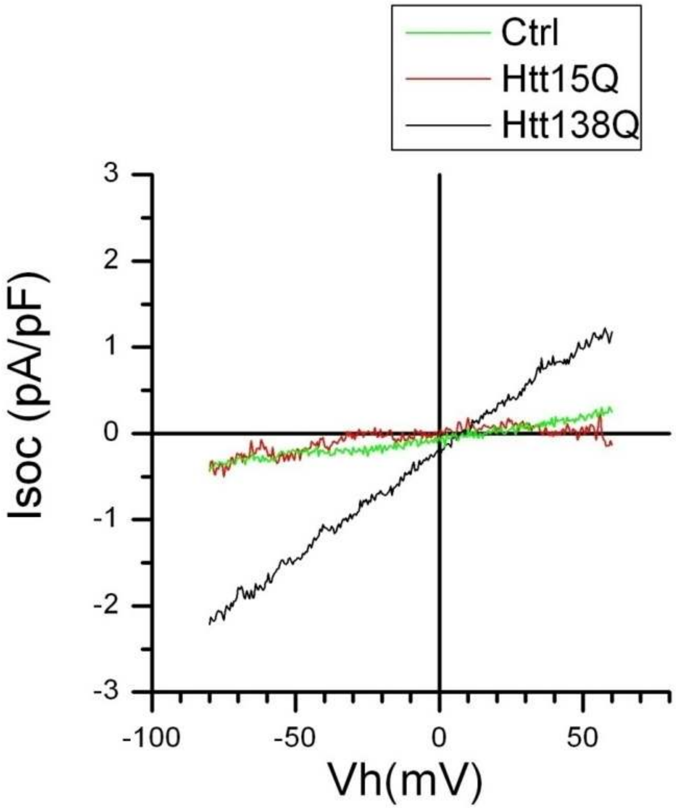


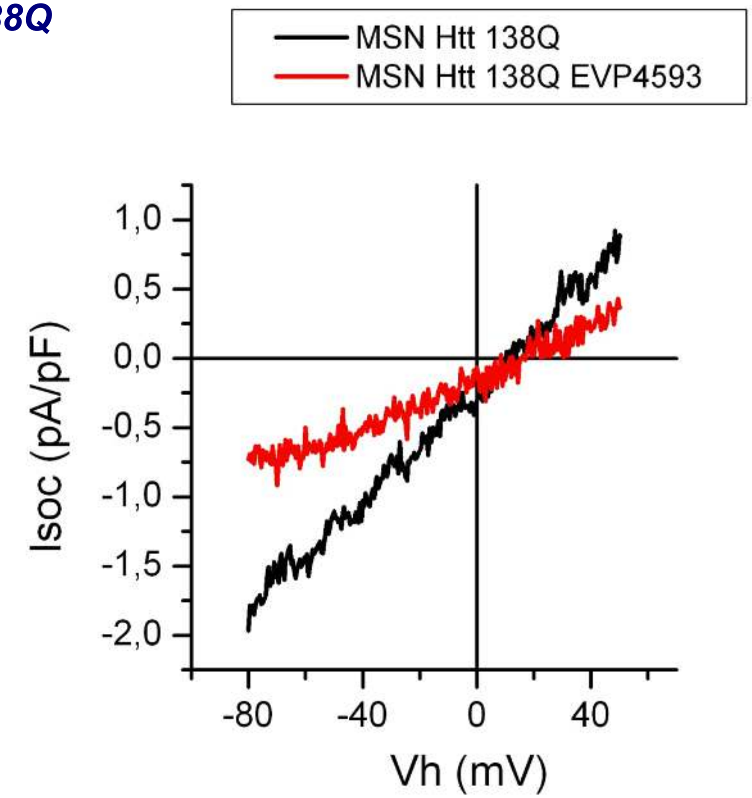


Phase contrast display mature GABAergic medium spiny neurons. Immunofluorescence image shows expression of DARP32 (red) in mature neurons. DAPI (blue) was used for counter-staining the cells. Scale bar, 50 μm. (D): Immunofluorescence image shows co-expression of DARP32 (red) and TUBB3 (green) in mature neurons. DAPI (blue) was used for counter-staining the cells. Scale bar, 50 μm.

Store operated calcium entry is increased in the model system of HD: SK-N-SH neuroblastoma cell line transfected with Htt138Q

Депо-управляемый вход кальция увеличен в модельной системе нейробластомы SK-N-SH Htt138Q

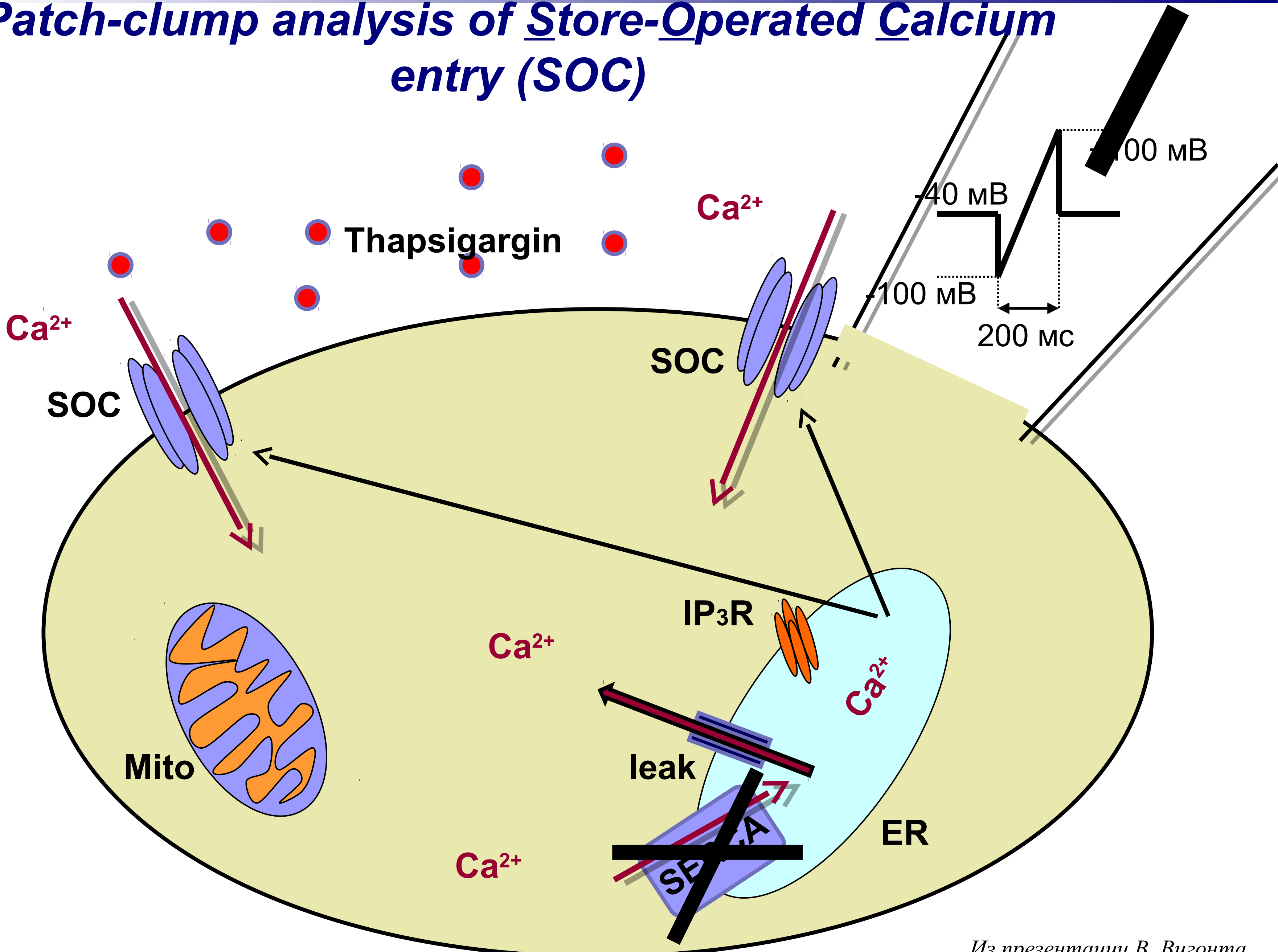




EVP4593 blocks glutamate-induced neuron apoptosis

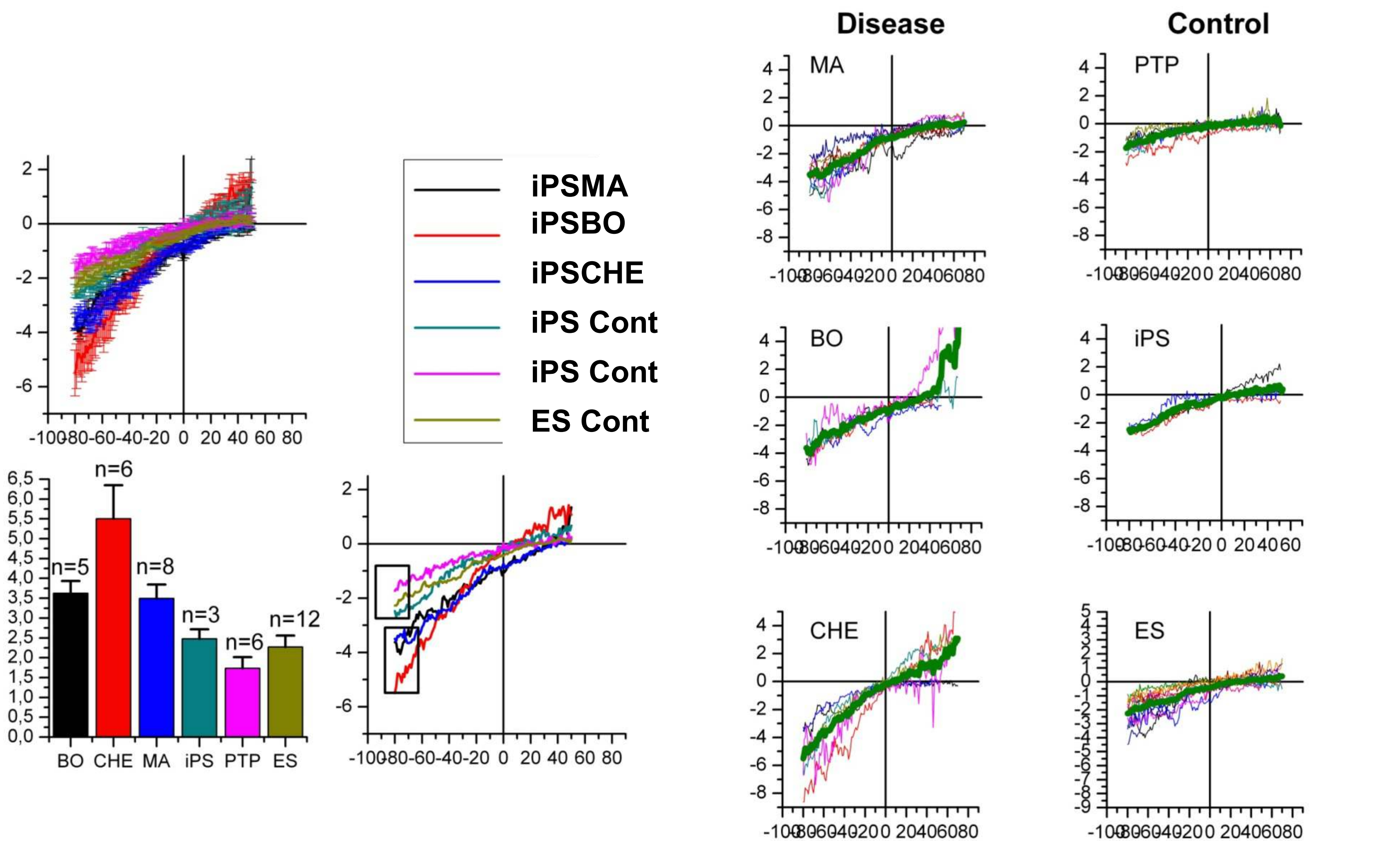
Wu et all, *Chem Biol*; 2011

Patch-clump analysis of Store-Operated Calcium entry (SOC)

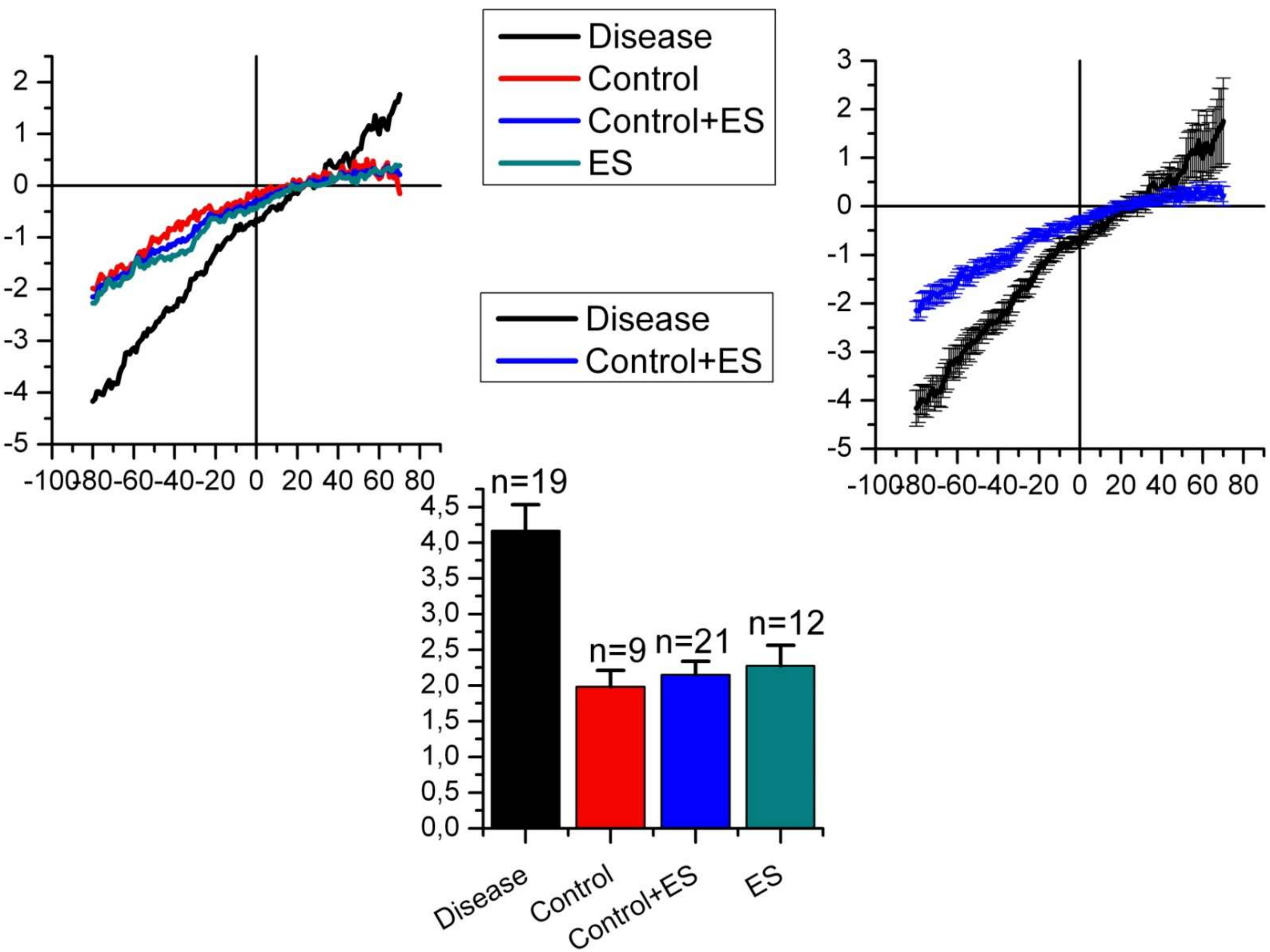


Из презентации В. Вигонта

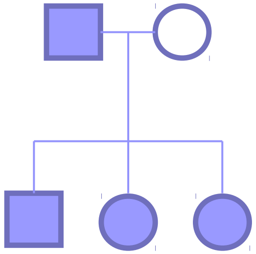
SOC entry patch-clump analysis of control and HD iPSC derived GABAergic neurons



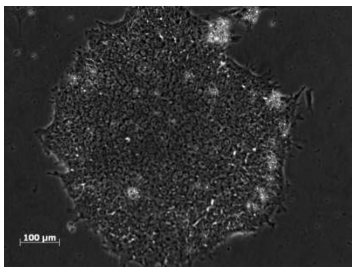
In Htt41-47 iPSC derived GABA-ergic neurons SOC entry is increased at least 2 folds in comparison with normal counterparts

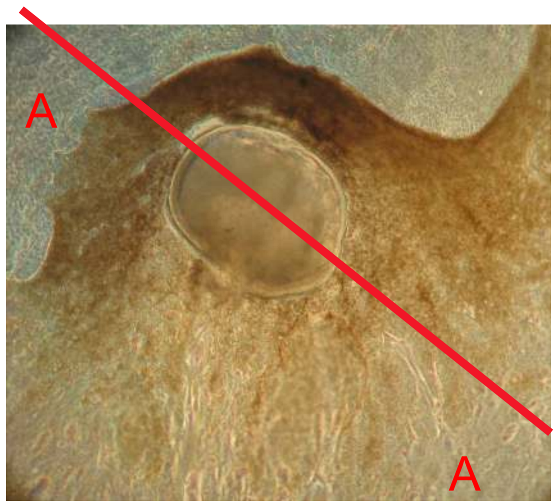


Заболевание Штатгардта- наследственная макулодистрофия сетчатки

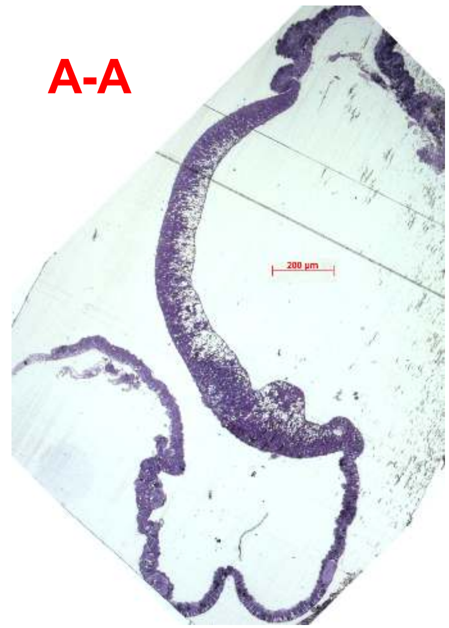


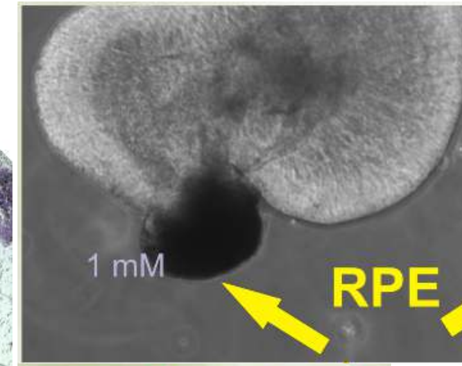
iPSCs

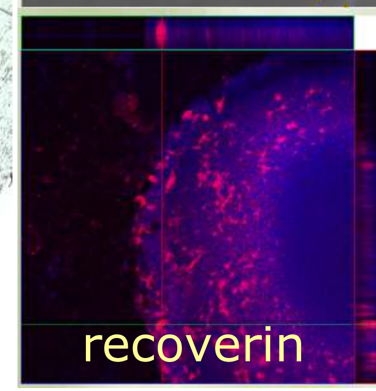




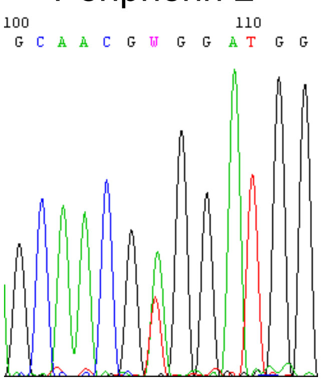
A-A

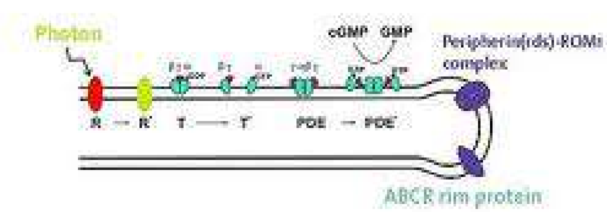


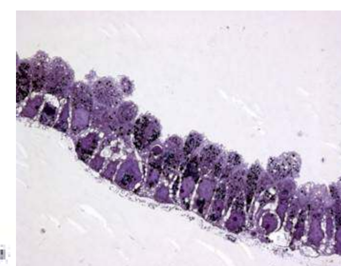


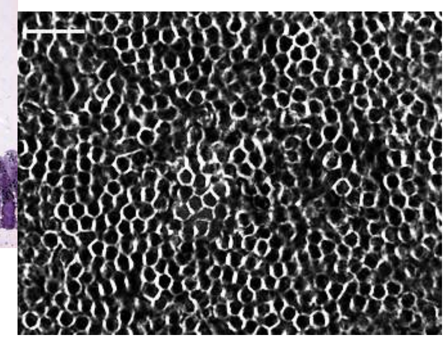


Peripherin 2





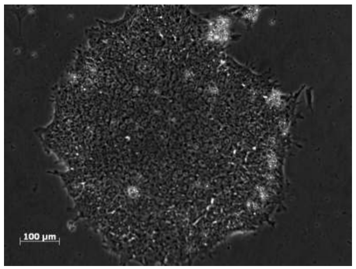




RPE (retinal pigment epithelium)

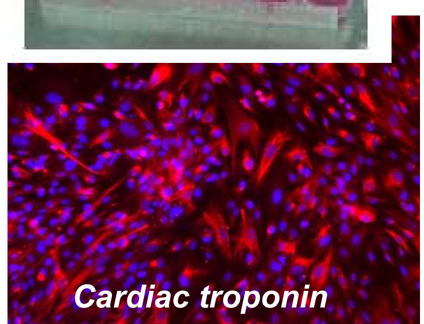
Производные плюрипотентных стволовых клеток в доклинических исследованиях

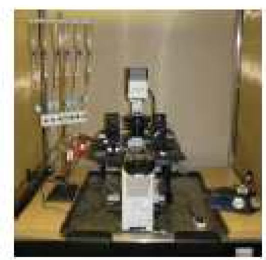
iPSCs

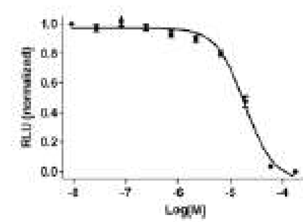


Приобрести

Разморозить





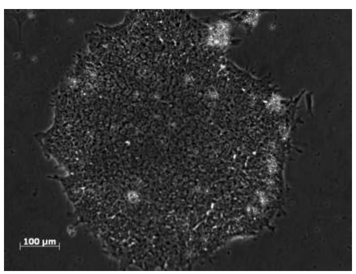


Провести тестирование

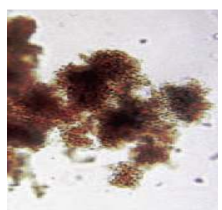
Использование iPS клеток для получения компонентов крови

-для удовлетворения потребности в донорах в РФ необходимо иметь 28 доноров на 1000 чел. населения, в настоящее время около 13 доноров.
-риск инфицирования доноров
-Giarratana et al., 2011, Proof of principle for transfusion of in vitro generated red blood cells, *Blood,* Sep.1

iPSCs

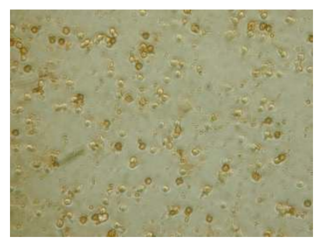


BFU-E

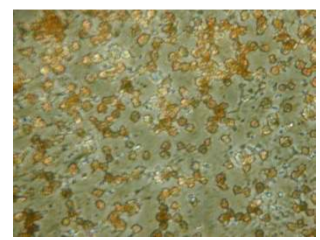


метилцеллюлоза

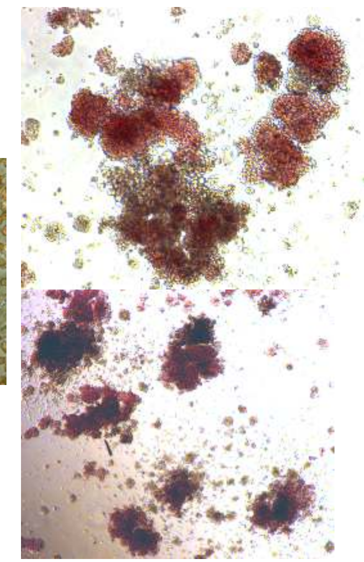
5 дней



10 дней



21 день



Только потребность в эритроцит содержащих компонентах крови сегодня в России превышает 2 млн. л в год. Потребность удовлетворяется на 47-50 % из-за нехватки донорских кадров и стоит 5 млрд.рублей в год