华大基因 BGI

劳而思·博伦再生医学研究所

LARS BOLUND INSTITUTE OF REGENERATIVE MEDICINE

SOLIDUM PETIT IN PROFUNDIS · UNIVERSITAS ARHUSIENSIS

华大基因 BGI

FEAM Conference 2018 on Precision Medicine and Personalized Health

Towards Personalized Regenerative Cell Therapy

FEAM, Sep 28, 2018

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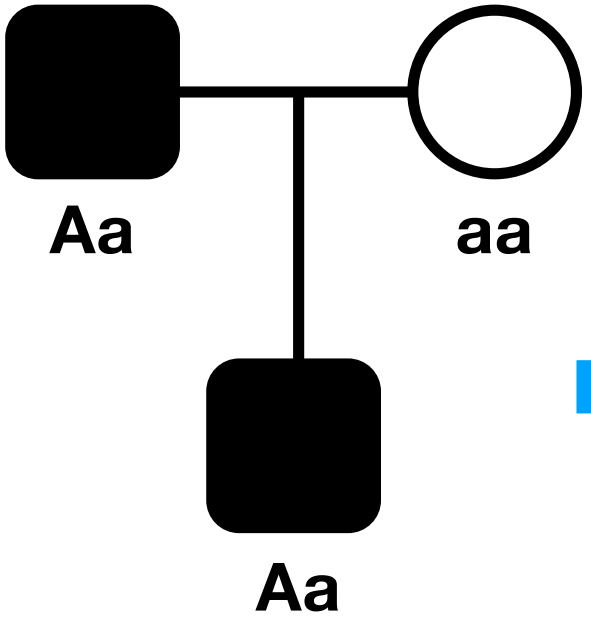
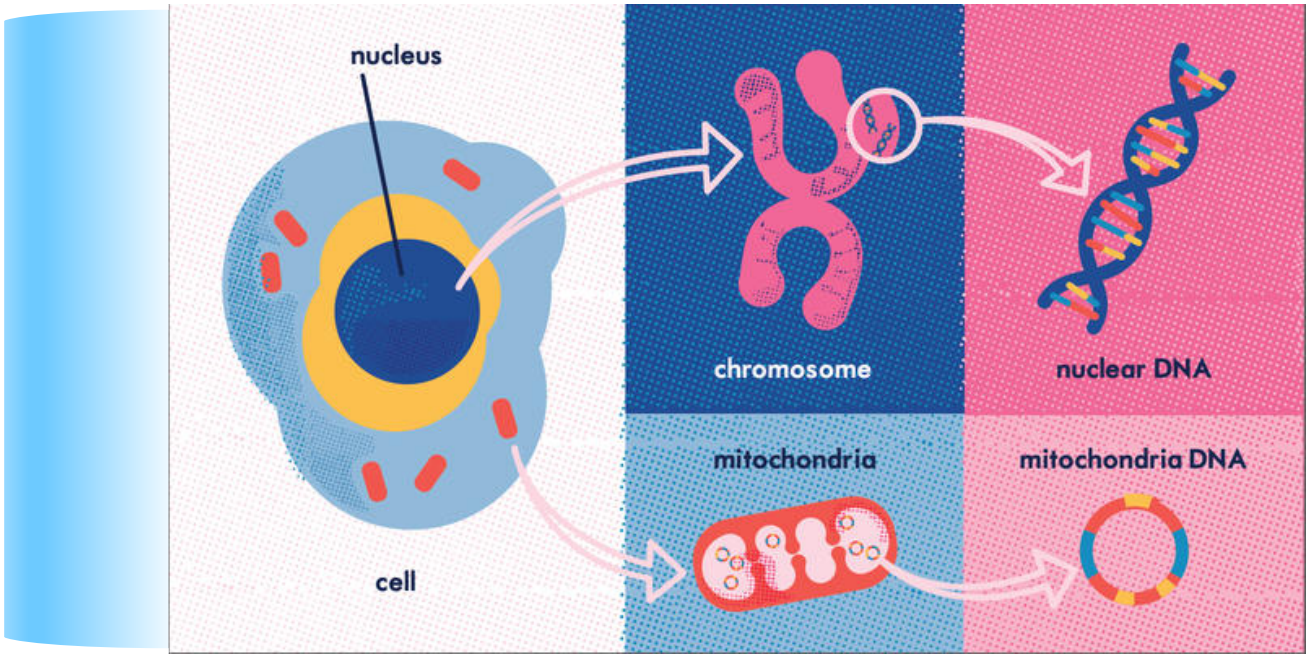
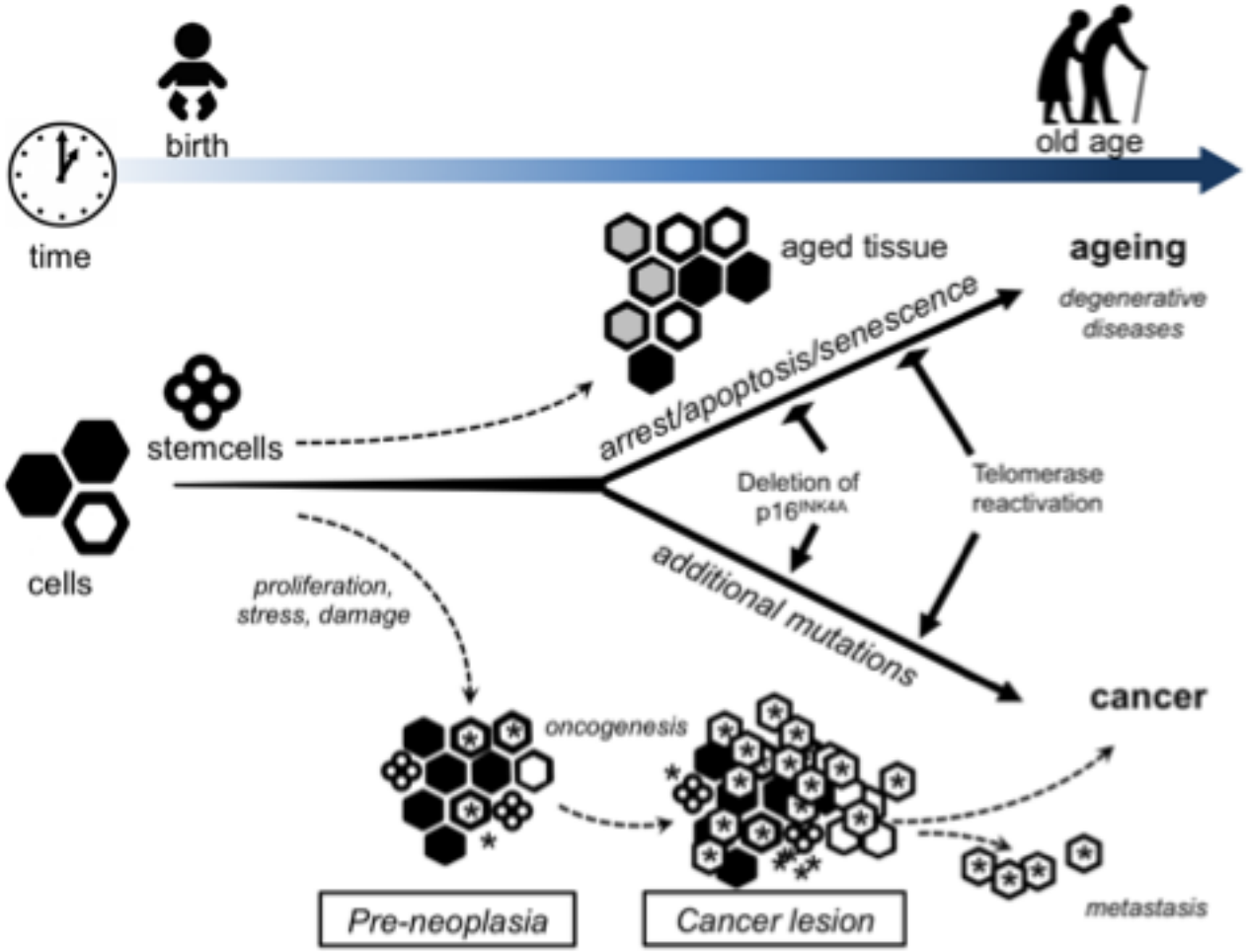
Group page: www.dream.au.dk

Many things already went wrong before development





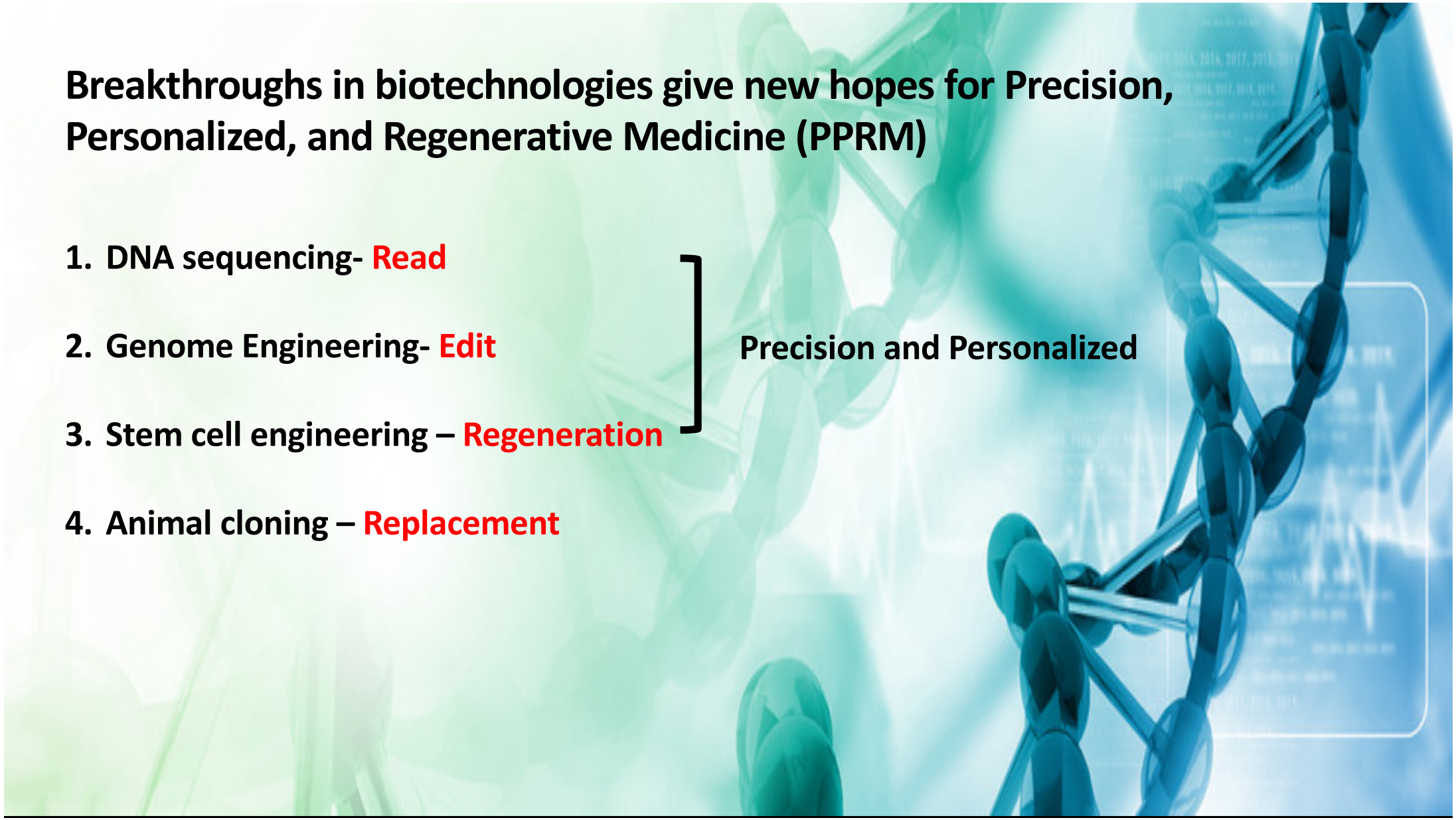
Many things can go wrong during development



The incidence of degenerative diseases increases with aging

The incidence of cancers increases with aging

Jan R. Aunan,William C Cho,Kjetil S, et al. Molecular Hallmarks[J]. A&D, 10.14336/AD.2017.0103



Breakthroughs in biotechnologies give new hopes for Precision, Personalized, and Regenerative Medicine (PPRM)

1. DNA sequencing- **Read**
2. Genome Engineering- **Edit**
3. Stem cell engineering – **Regeneration**
4. Animal cloning – **Replacement**

Precision and Personalized

1. DNA Sequencing - Read

Enable

- The identification of what already went wrong – predisposing mutations, chromosomal abnormalities (Diagnosis)
- The prediction of what will go wrong (Prognosis)
- The selection of best treatment (Precision)
- The development of personalized treatment (personalization)

Comparison of NGS systems

Company	System	read length	Throughput(Gb)	Run on time
MGI tech	BGISEQ-500	2*100	520	10 day
	MGISEQ-200	2*100	60	2 day
	MGISEQ-2000*2	2*150	1050	3 day
illumina	MiSeq	2*150	5.1	1 day
	NextSeq 550	2*150	120	1.2 day
	HiSeq 2000	2*100	200	8 day
	HiSeq 3000	2*150	750	3.5 day
	HiSeq 4000	2*150	1500	3.5 day
	Novaseq 600-S1*2	2*150	1000	1 day
	Novaseq 600-S2*2	2*150	2500	1.5 day
	Novaseq 600-S4*2	2*150	6000	1.9 day
Thermo Fisher(Life)	Ion Proton	200	32	2-4 h
Pacific Biosystems	Sequel	1000	7.5	1 day
Oxford Nanopore tech	Promethlon	real-time	4800	2 day

Our Vision That omics technologies enable all people to live full and healthy lives, free from the burden of disease and hunger

Our Mission To advance the field of genomics with new technologies and by working collaboratively with international partners

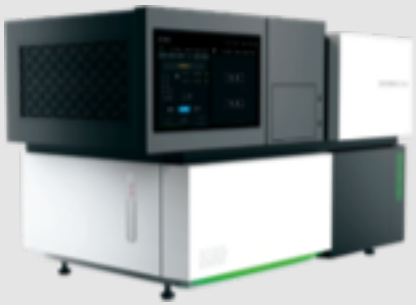
Our Strategy To use our scientific expertise, network, and sequencing assets to form genomics partnerships with key stakeholders in the field of precision medicine and agri-genomics

BGI is the world's largest genomics center, experienced in genomic research, services and sequencer manufacturing. BGI is uniquely positioned to support academia and pharma, agriculture and healthcare industries with highly reliable service for basic research, pharmaceutical drug development, molecular breeding and clinical diagnosis.

Platform

MGI NGS platform Guide

1



BGISEQ-500

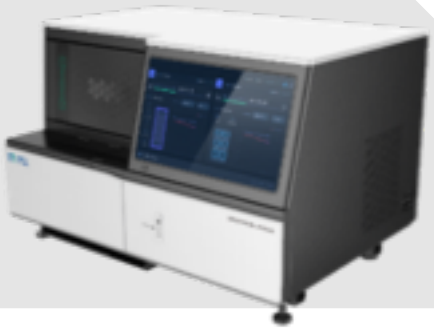
Maximum output: 520 Gb
Effective reads: 1300 Million
Maximum Read Length*: PE100

2

MGISEQ-200

Maximum output: 60Gb
Effective reads:300 Million
Maximum Read Length:PE100

3



MGISEQ-2000

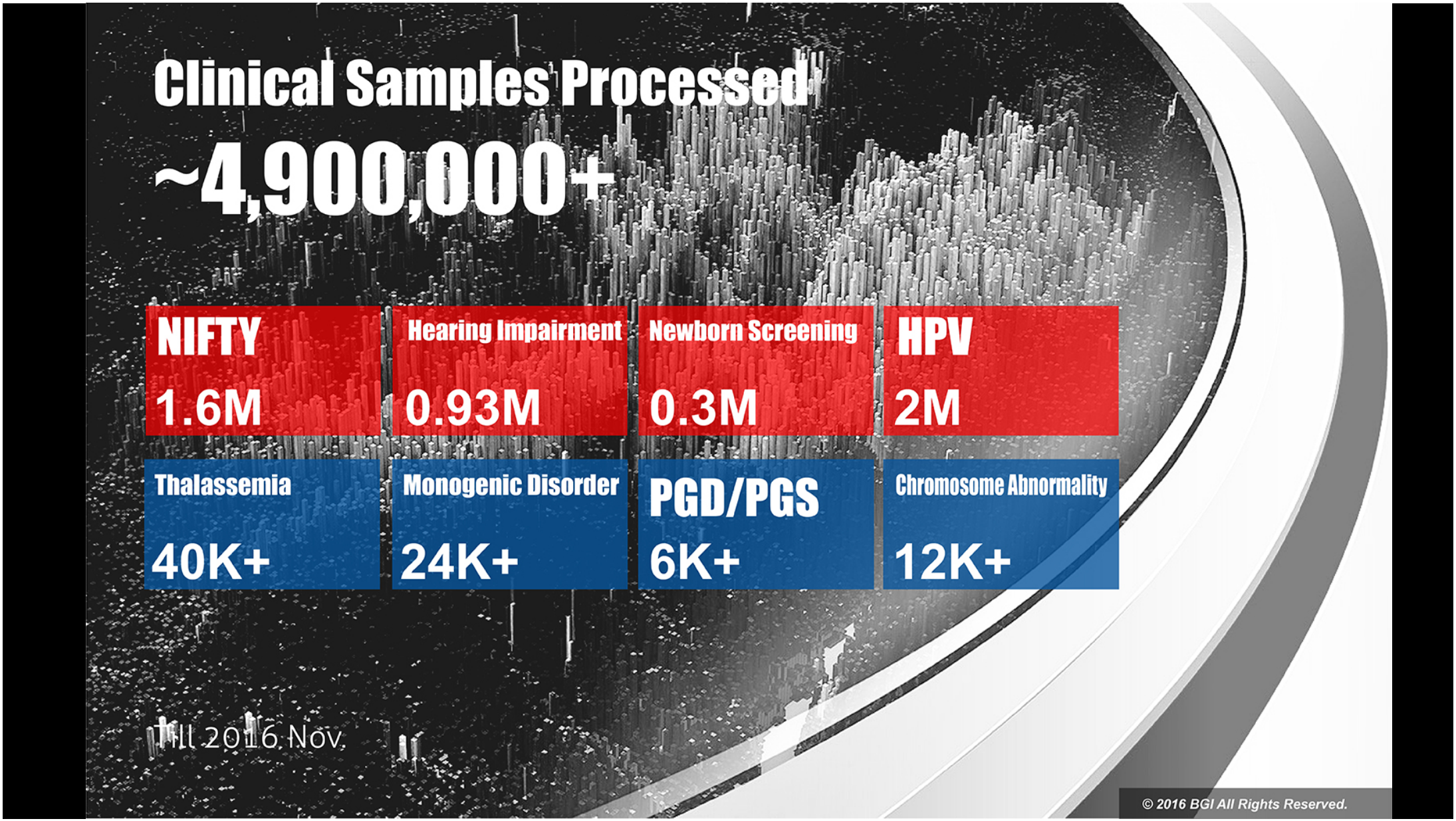
Maximum Output: 600Gb
Effective reads:1500 Million
Maximum Read Length *:PE150

科研 Research

- 全基因组 Whole Genome
- 转录组 Transcriptome
- 表观基因组 Epigenome
- 外显子 Exome
- 分子育种 Molecular Breeding

临床 Clinical

- 生育健康 Reproductive Health
- 肿瘤检测 Oncology Testing
- 病原微生物 Pathogenic Microorganism


Clinical Samples Processed
~4,900,000+
NIFTY
1.6M
Hearing Impairment
0.93M
Newborn Screening
0.3M
HPV
2M
Thalassemia
40K+
Monogenic Disorder
24K+
PGD/PGS
6K+
Chromosome Abnormality
12K+
Till 2016 Nov.
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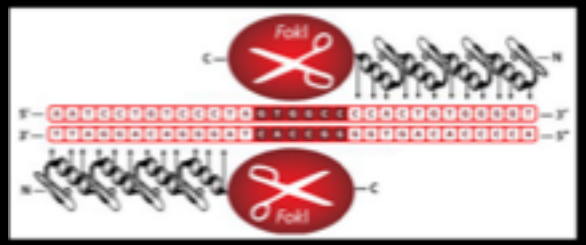
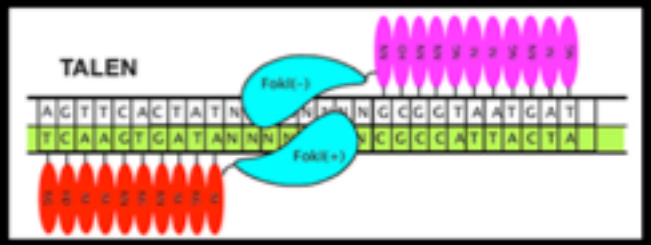
2. Genome Engineering - Edit

precision positioning, cleavage, and modification

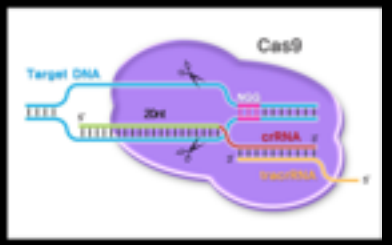
ZFN



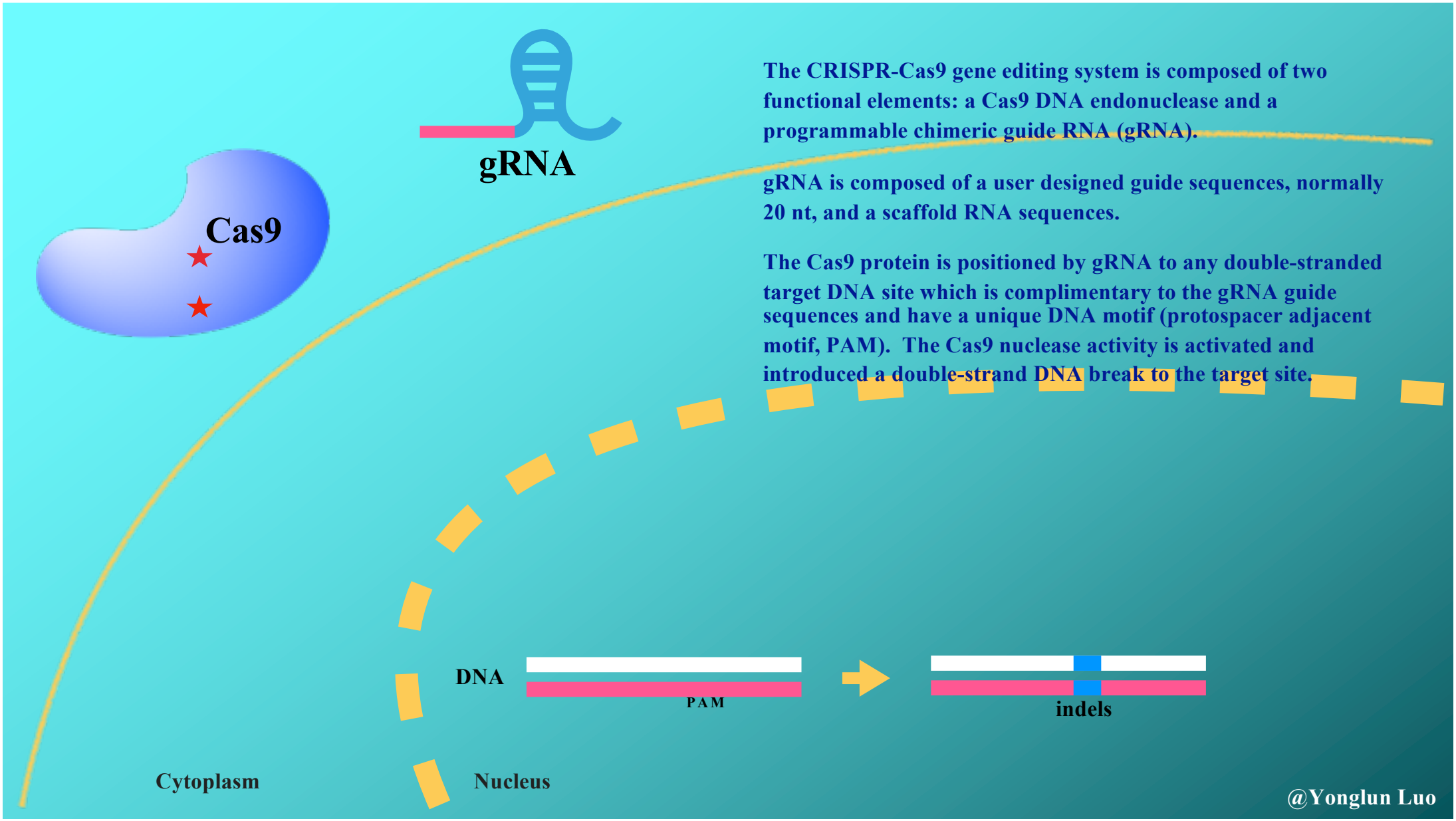
TALEN



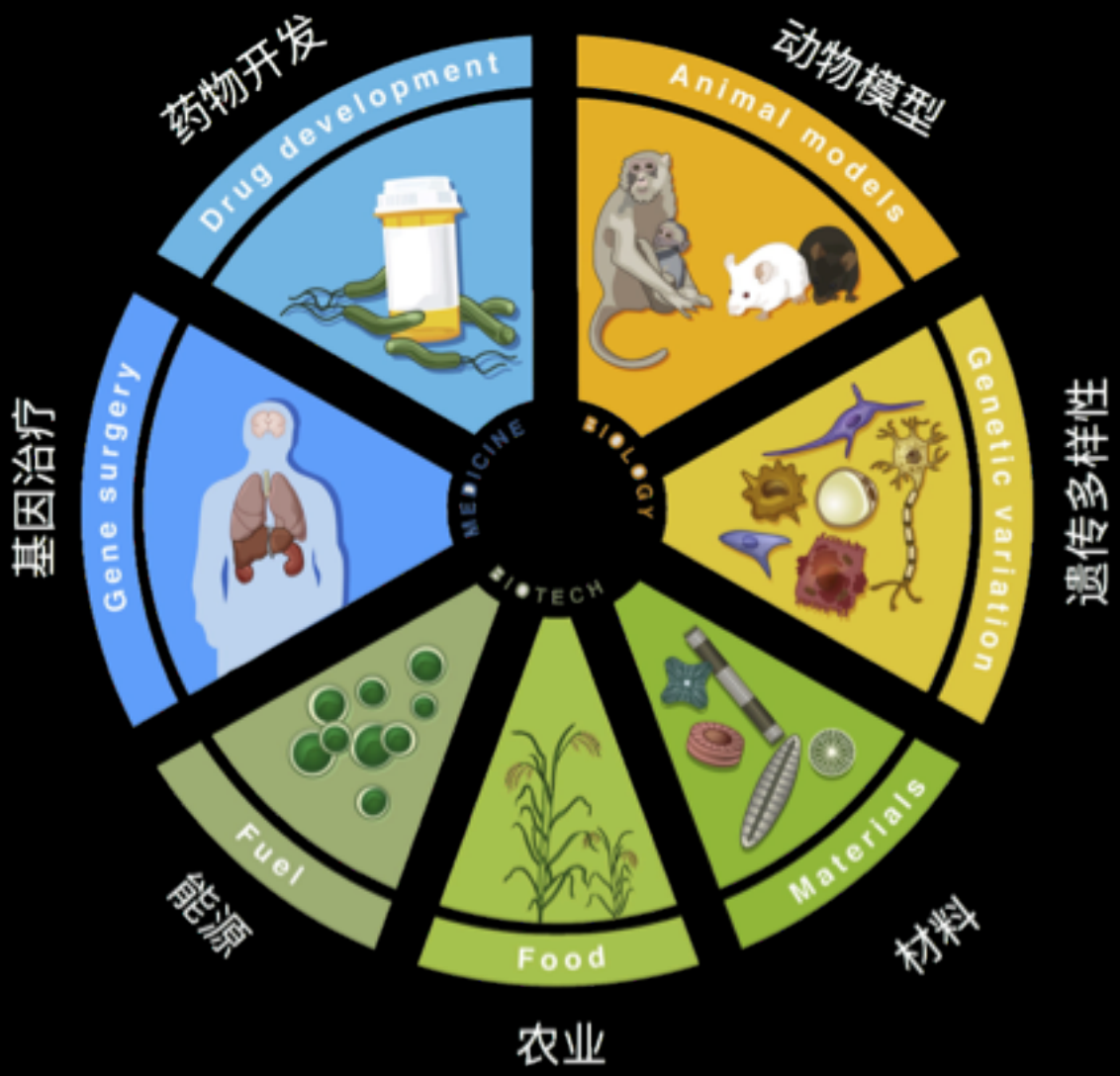
CRISPR-Cas9



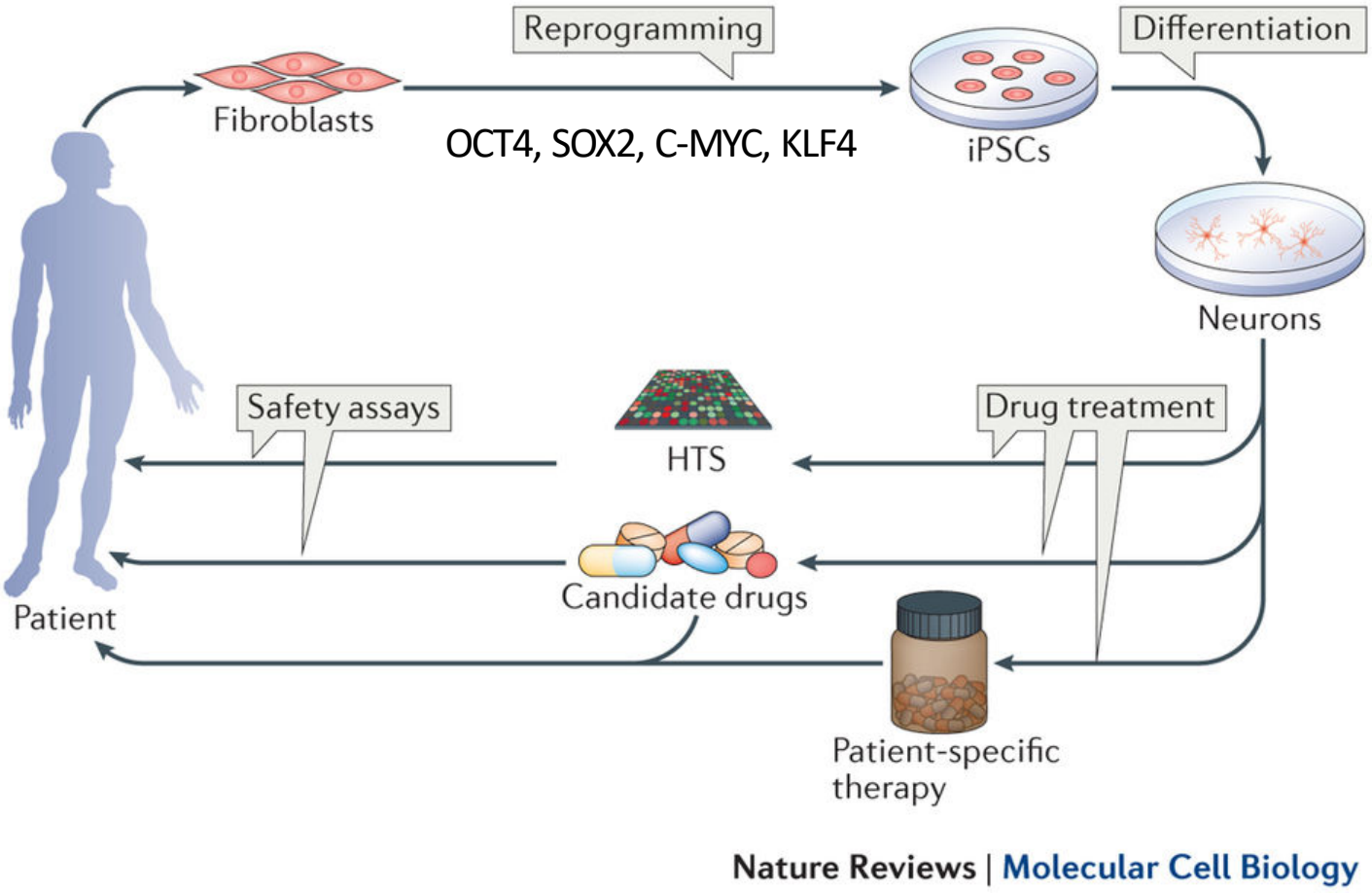
@Yonglun Luo

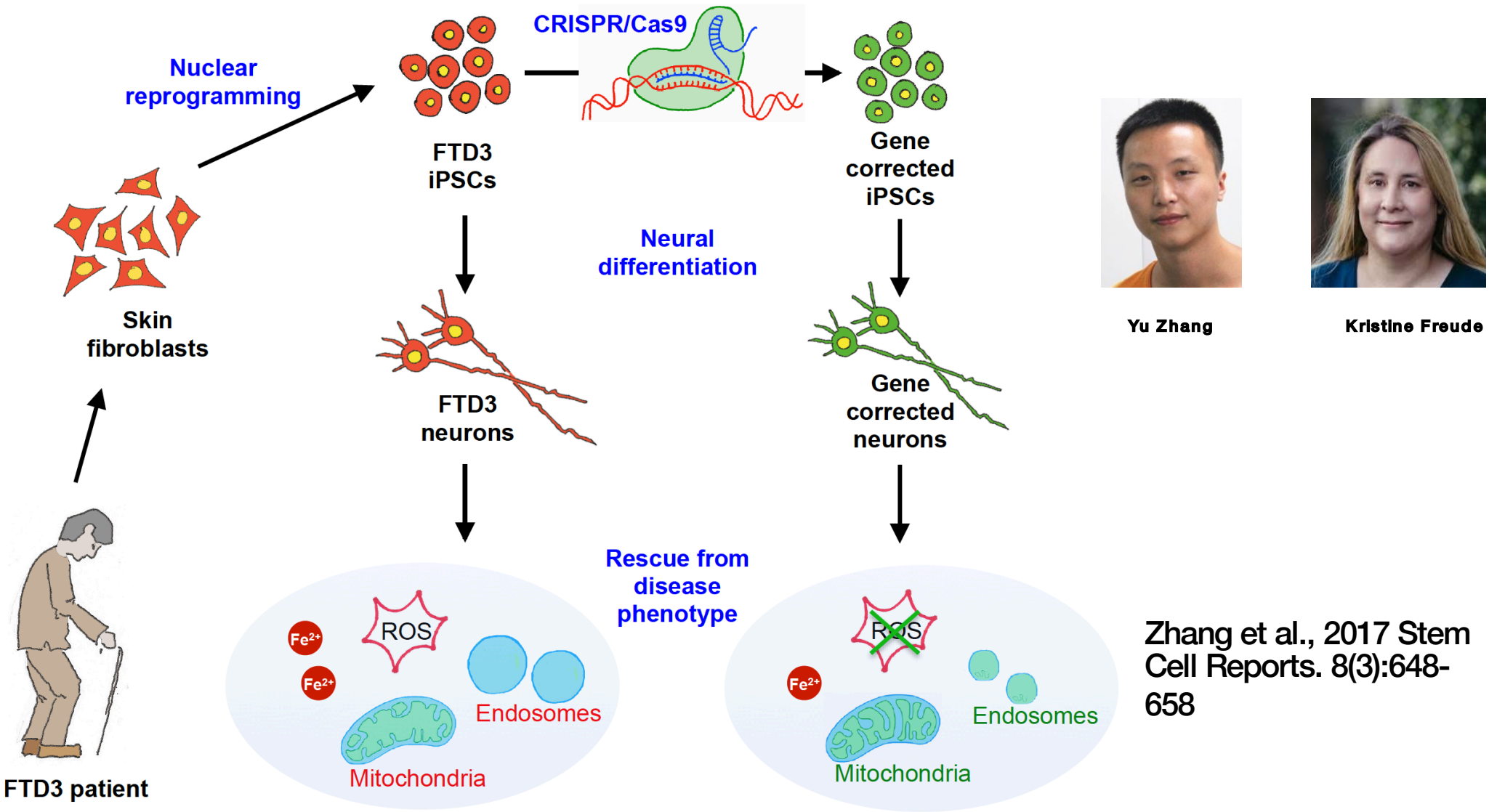

gRNA
Cas9
The CRISPR-Cas9 gene editing system is composed of two functional elements: a Cas9 DNA endonuclease and a programmable chimeric guide RNA (gRNA).
gRNA is composed of a user designed guide sequences, normally 20 nt, and a scaffold RNA sequences.
The Cas9 protein is positioned by gRNA to any double-stranded target DNA site which is complimentary to the gRNA guide sequences and have a unique DNA motif (protospacer adjacent motif, PAM). The Cas9 nuclease activity is activated and introduced a double-strand DNA break to the target site.
DNA
PAM
indels
Cytoplasm
Nucleus
@Yonglun Luo

A broad application spectrum of genome editing technology



3. Stem Cell Engineering – Regeneration



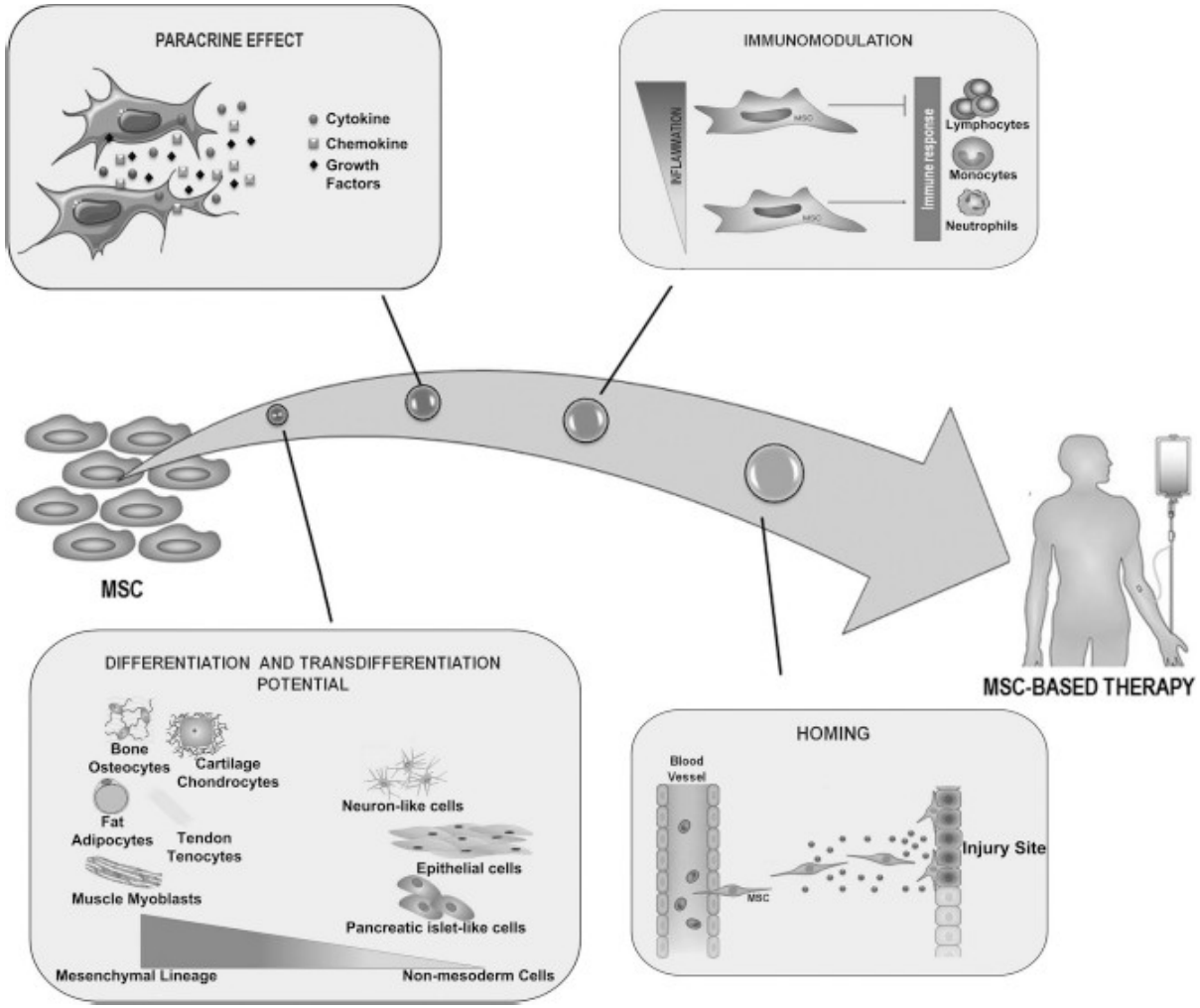


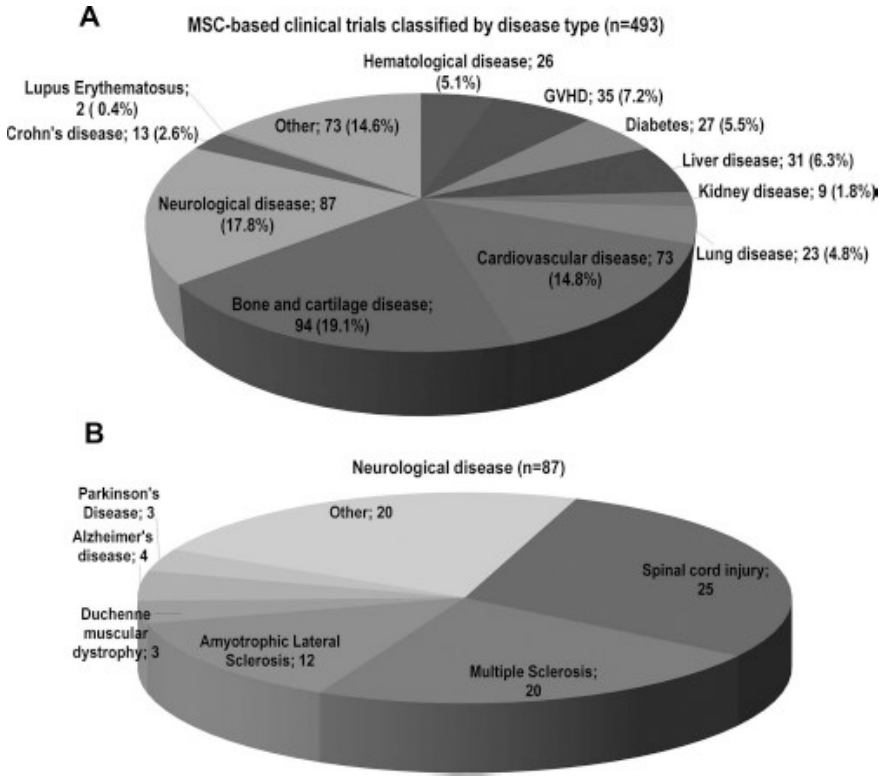
Yu Zhang

Kristine Freude

Zhang et al., 2017 Stem Cell Reports. 8(3):648-658

Mesenchymal Stem Cells （MSC） hold great potential in RM





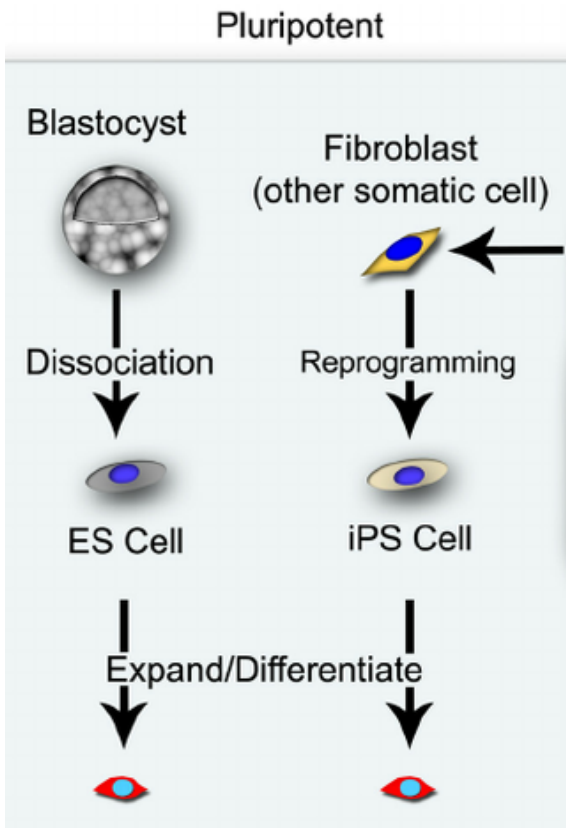
Cell Transplant. 2016;25(5):829-48.

Broad resources for deriving MSCs

Adult tissues
- Bone marrow
- Deciduous teeth
- Fat
- Hair follicles
- Peripheral blood
- Periodontal ligament
- Trabecular bone
- Scalp subcutaneous tissue
- Skeletal muscle

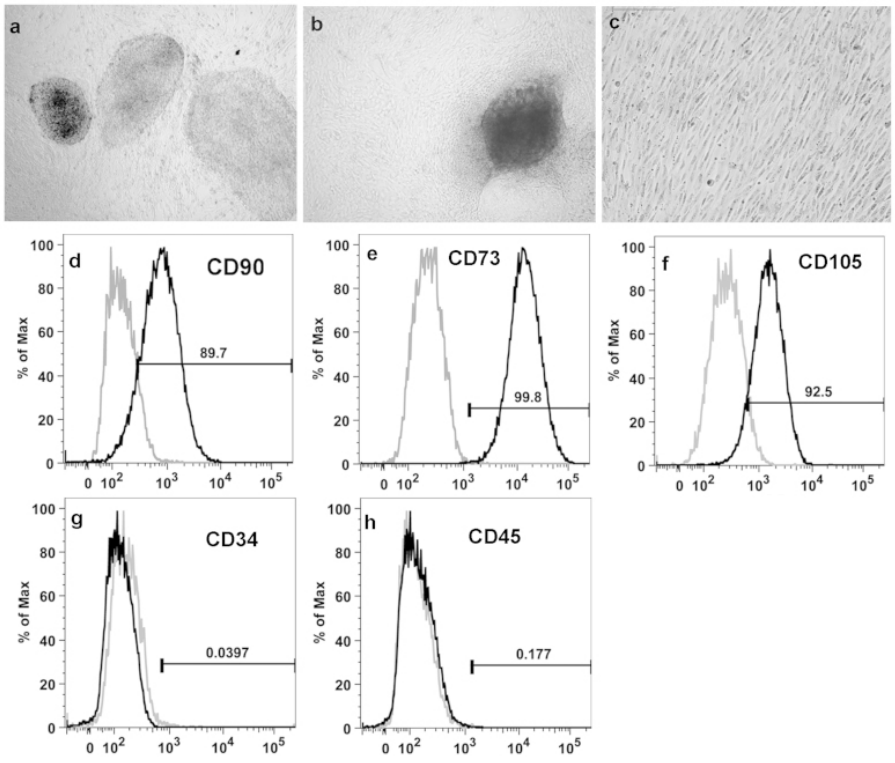
Fetal tissues
- Bone marrow
- Liver
- Lung
- Placenta
- Spleen
- Umbilical cord

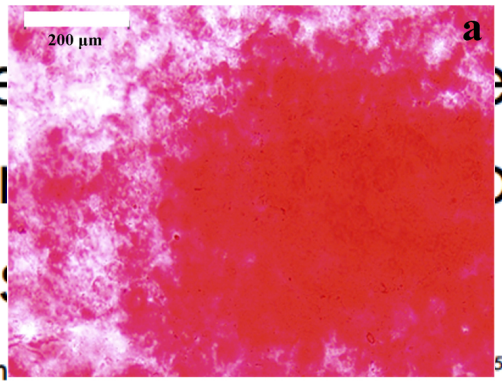
MSC

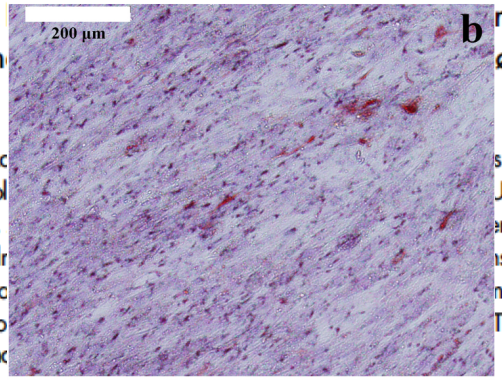


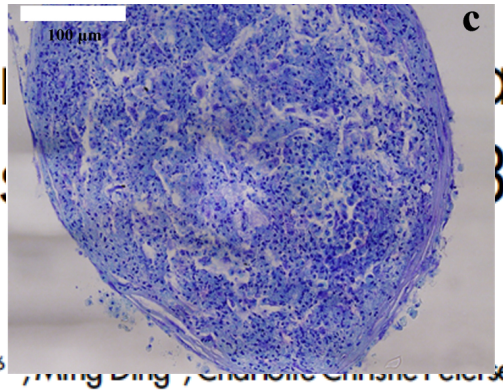
Pluripotent Stem cell-derived MSCs

SCIENTIFIC REPORTS









mple e al

Cs ar os 3D

folds

*, Yonglun 5,6 ,Ming Ding[7], Charlotte Christine Petersen[3,8],

, Frederik nghua Lv[2], Qing Ma[5,6], Dang Q. S. Le[1],

Besenbach ørgen Kjems[4], William T. Pu[5,6] & Cody Bünger[1]

c Research La s University Hospital, Aarhus, DK, [2]Department of Burn

Gastroenterol Jniversity, Nanchang, CN, [3]Department of Biomedicine,

ersity, 8000, enter, Aarhus University, Aarhus, DK, [5]Department of

Boston Childr stitute, Harvard University, Cambridge, MA, USA,

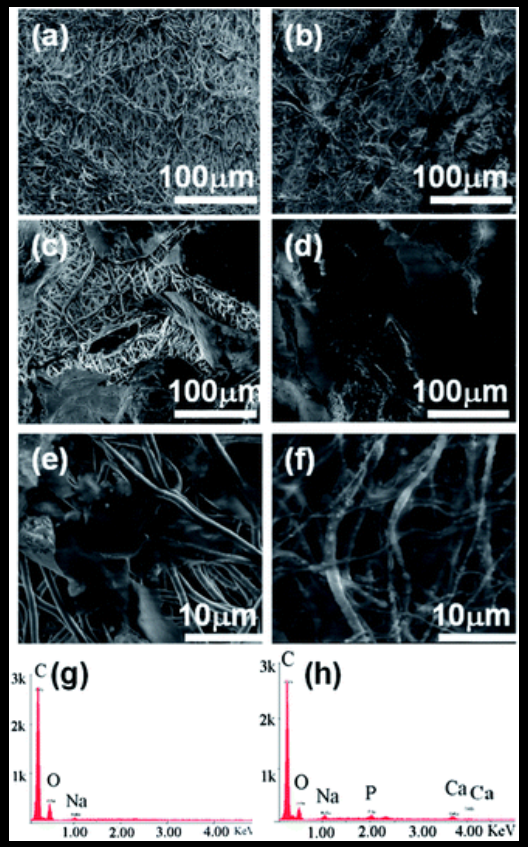
c Research La natology, Odense University Hospital, Institute of Clinical

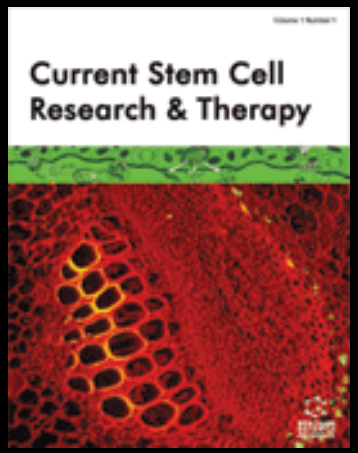
iversity of So The FACS Core Facility, Faculty of Health, Aarhus

arhus, Denm

Achievements in MSC RM by the DREAM team

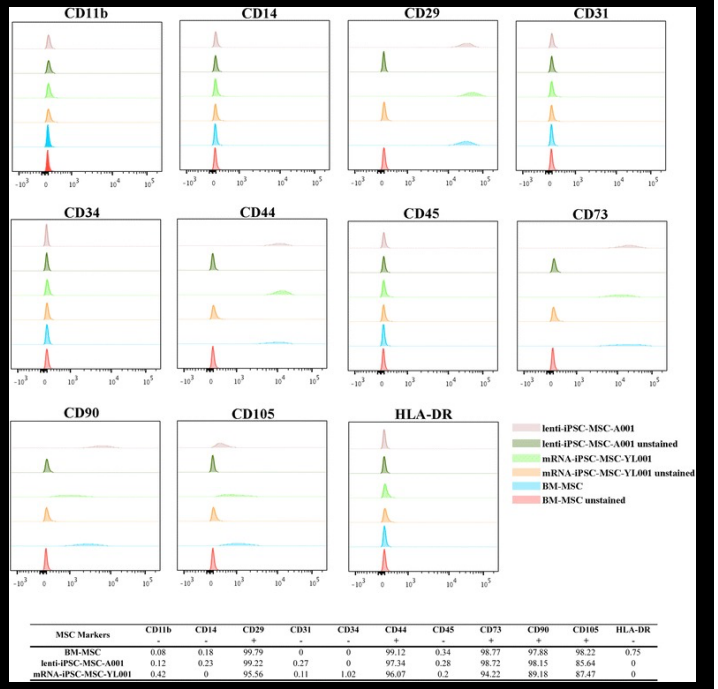
Kang et al. 2015





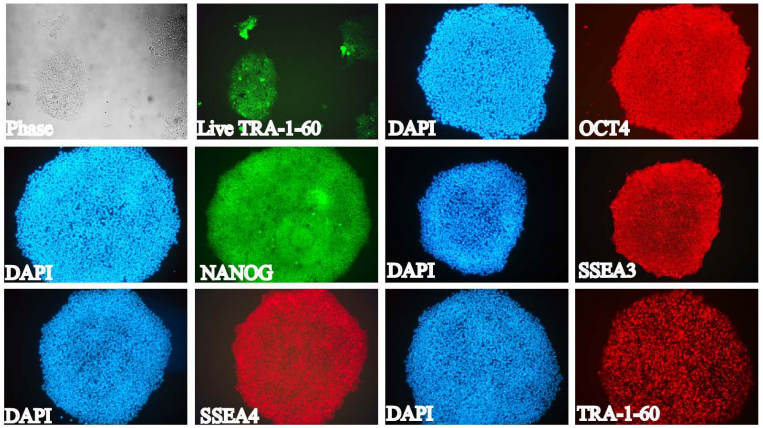
Lin et al. 2015

Kang et al. 2016



MSC Markers	CD11b	CD14	CD29	CD31	CD34	CD44	CD45	CD73	CD90	CD105	HLA-DR
	-	-	+	-	-	+	-	+	+	+	-
BM-MSC	0.08	0.18	99.79	0	0	99.12	0.34	98.77	97.88	98.22	0.75
lenti-iPSC-MSC-A001	0.12	0.23	99.22	0.27	0	97.34	0.28	98.72	98.15	85.64	0
mRNA-iPSC-MSC-YL001	0.42	0	95.56	0.11	1.02	96.07	0.2	94.22	89.18	87.47	0

A new method for safer iPS-MSC derivation developed by us



A

MSC markers (%)	CD11b	CD14	CD29	CD31	CD34	CD44	CD45	CD73	CD90	CD105	HLA-DR	CD166
C2N2iPSC-MSC-P5	0.796	1.12	99.9	1.30	0.224	100	1.10	100	99.5	95.0	0.16	NA
C2N2iPSCR2-MSC-P5	4	0.081	99.7	0.136	0.258	99.9	0.159	99.9	97.5	96.5	7.36	93.2
H9ES-MSC-P5	4.76	1.79	99.9	1.35	0.628	99.9	1.57	99.9	97.1	90.9	2.02	96.0
BM-MSC-P6	1.48	1.55	100	1.51	0	100	1.47	100	99.3	99.9	1.89	100

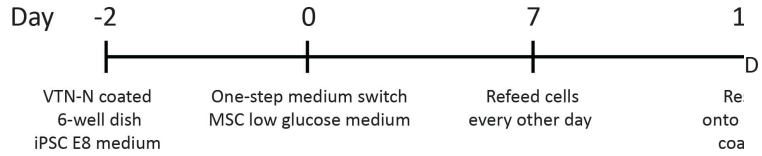
B

MSC markers (%)	CD11b	CD14	CD29	CD31	CD34	CD44	CD45	CD73	CD90	CD105	HLA-DR	CD166
C2N2iPSC-MSC-P10	0.116	0.780	99.7	1.66	0	99.8	1.09	99.9	99.4	98.5	0.177	NA
C2N2iPSCR2-MSC-P10	1.70	0.792	99.9	0.520	1.35	99.7	0.246	96.8	97.2	87.8	0.985	98.1
H9ES-MSC-P10	1.08	2.45	99.6	1.47	1.31	96.9	1.37	95.4	98.6	85.2	1.20	90.8
BM-MSC-P12	1.58	1.43	99.8	1.26	0.742	99.2	0.995	99.8	98.8	98.7	0.847	96.3

C

MSC markers (%)	CD11b	CD14	CD29	CD31	CD34	CD44	CD45	CD73	CD90	CD105	HLA-DR	CD166
C2N2iPSC-MSC-P20	12.4	10.9	99.9	10.5	17.0	99.8	11.9	100	97.0	94.9	7.66	99.4

A



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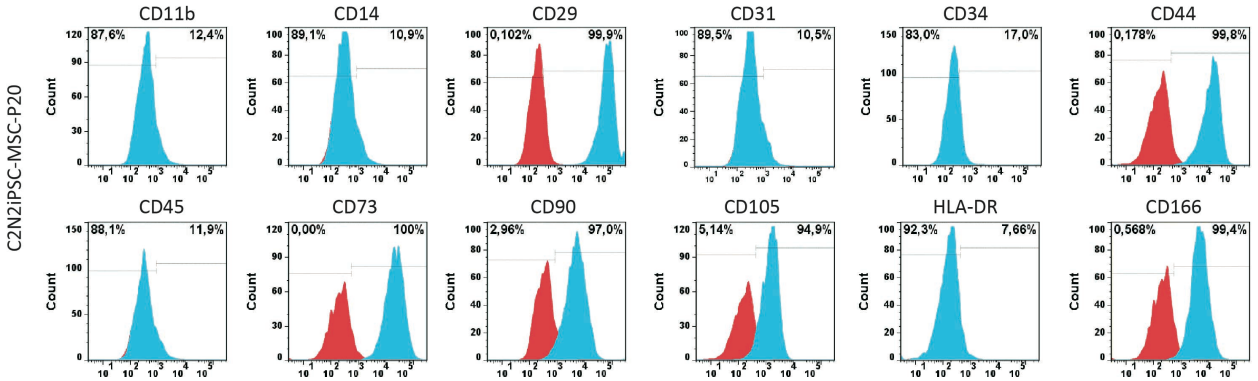
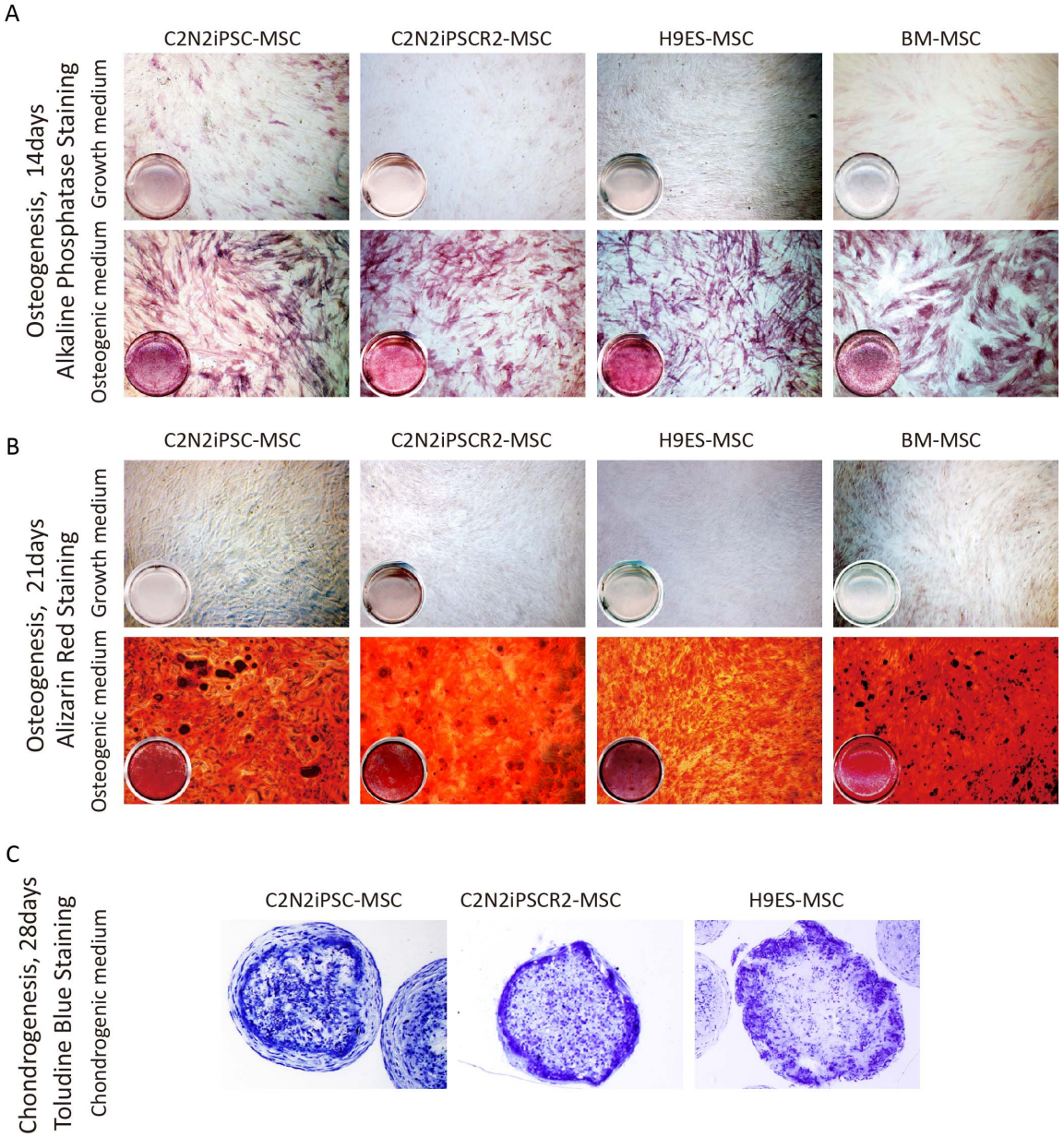
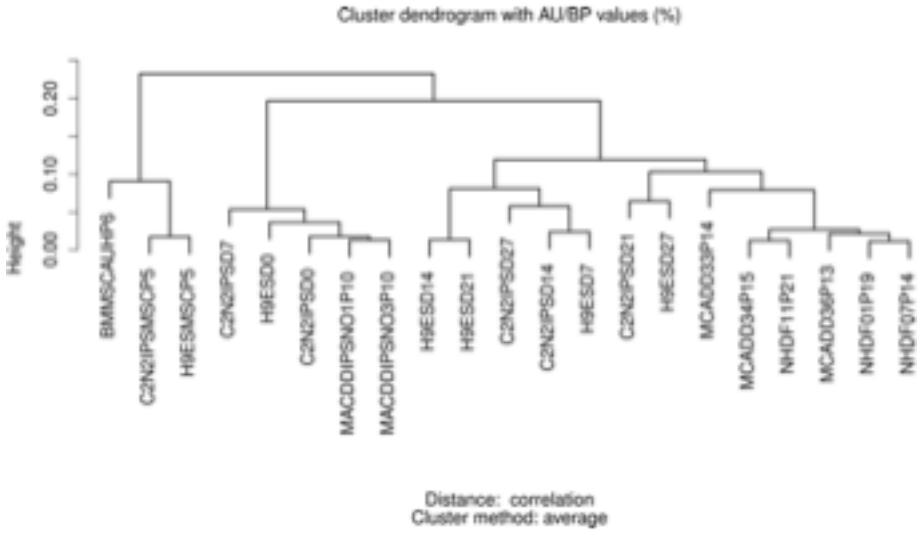
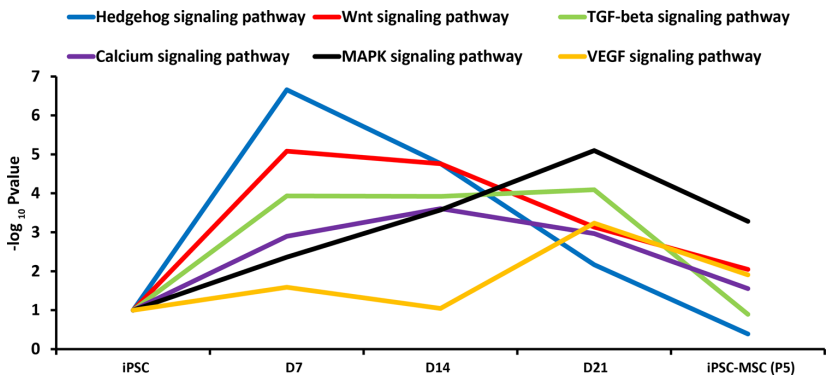


(unpublished results) Zhou Y. et al.





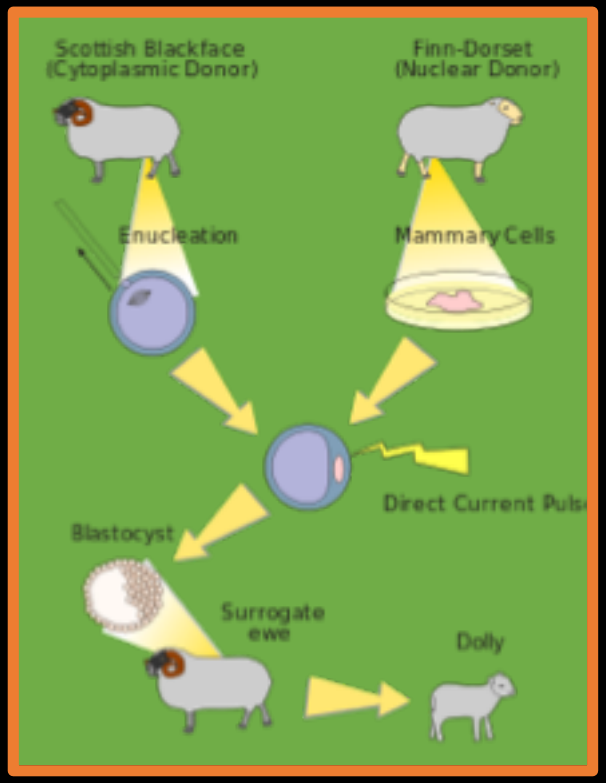


(unpublished results) Zhou Y. et al.

Towards personalized regenerative cell therapy

Current Stem Cell Research & Therapy, 2015, 10, 1-9

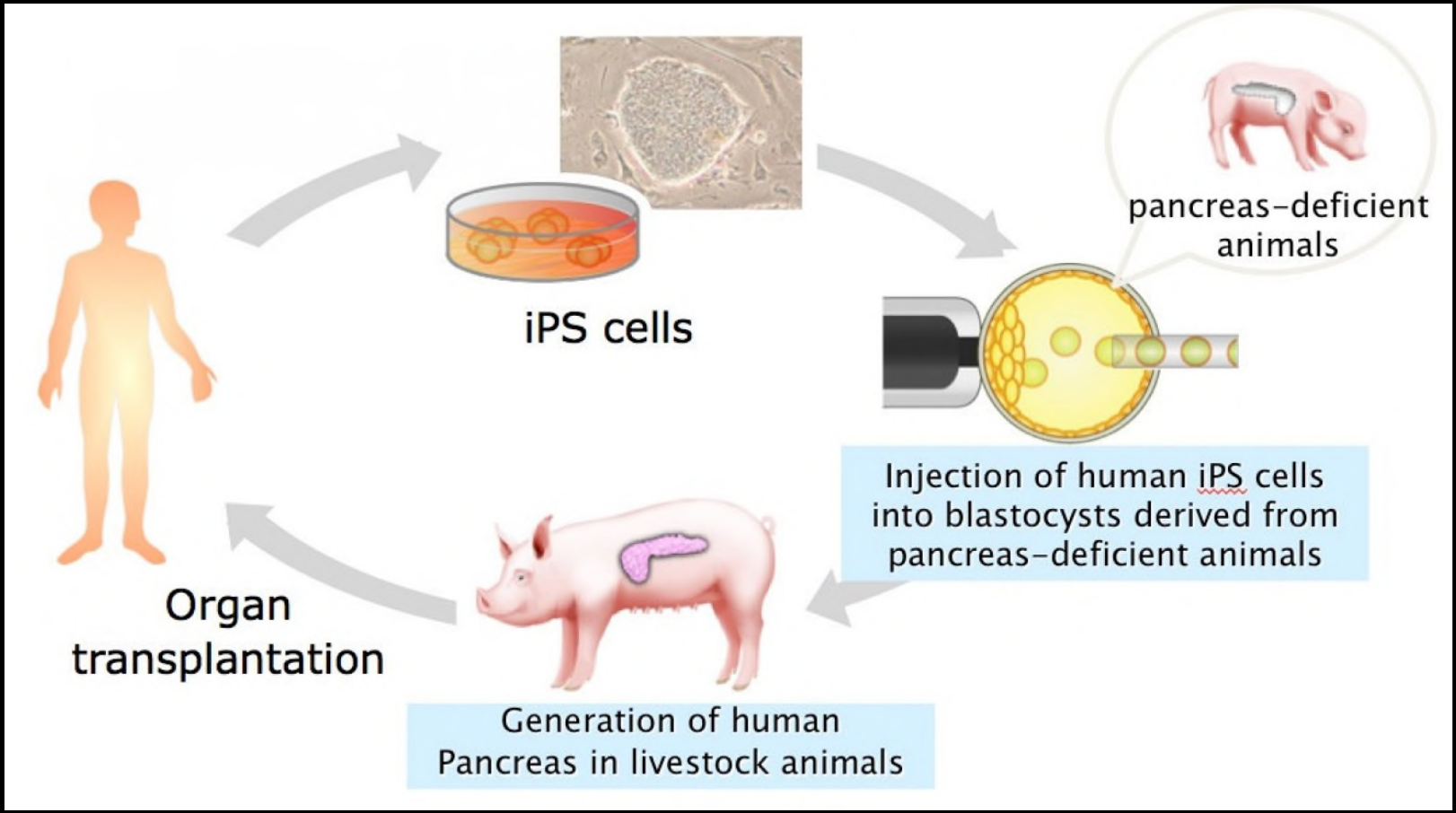
4. Animal biotechnology - Replacement



Somatic Cell Nuclear Transfer
SCNT, 体细胞核移植技术，简称为 “克隆”

第一头克隆样：多莉及克隆羊之父 “Sir Ian Wilmut”

RM based on human-pig chimera technology



An illustration of a potential process for harvesting human organs from pigs using chimera embryos. (Hiro Nakauchi)

Article

Interspecies Chin
with Mammalian

Jun Wu,[1] Aida Platero-Luengo,[1] Mas
Keiichiro Suzuki,[1] Yanina Soledad B
Jingping Luo,[1] Marcela Vilariño,[3] Inr
Sonia Sánchez-Bautista,[4] M. Llanos
Paloma Martinez-Redondo,[1] Alejanc
Concepcion Rodriguez Esteban,[1] W
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[5]Clinica Centro Fundación Pedro Guillén,
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Nara 631-8505, Japan
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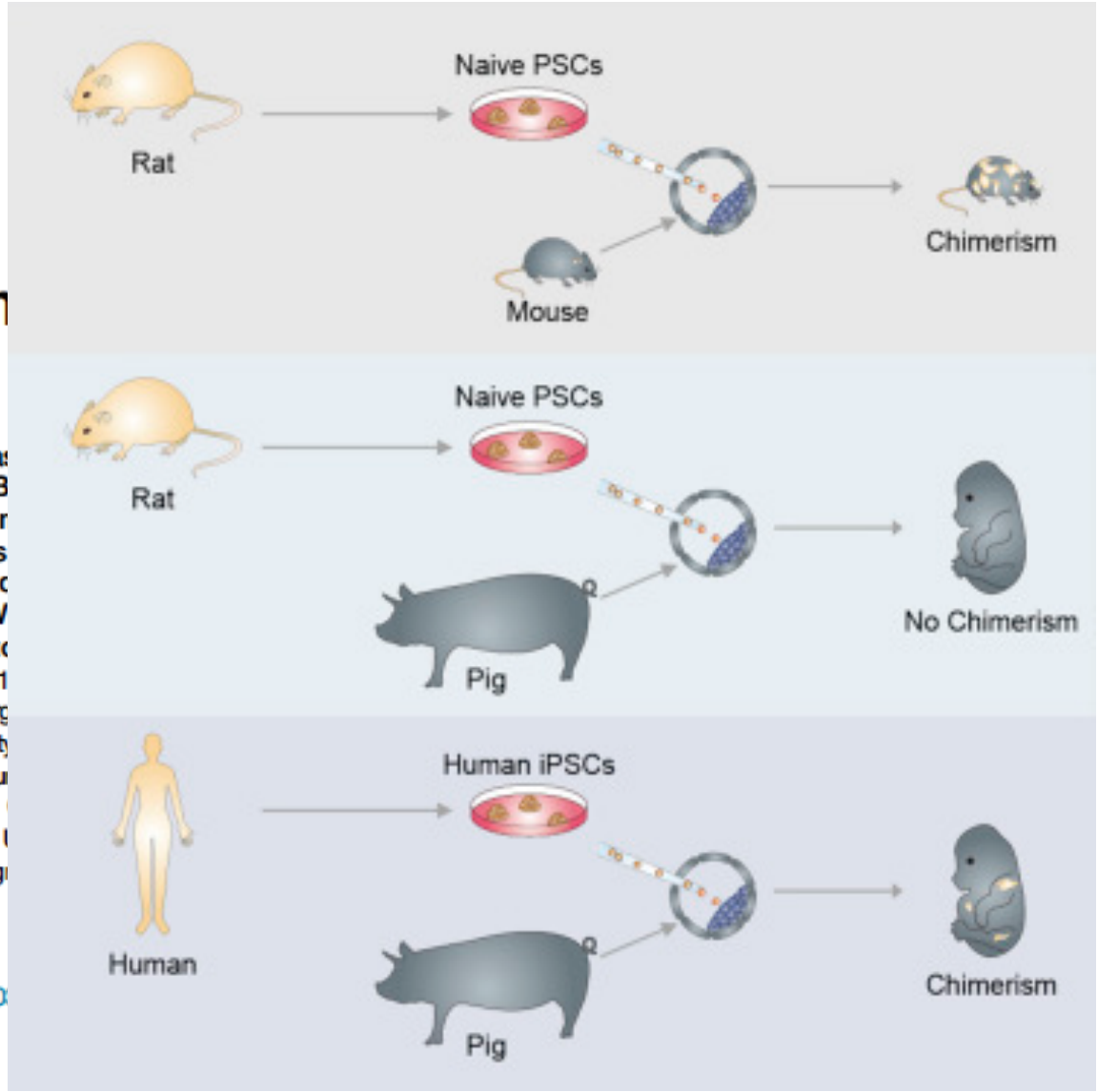
*Correspondence: belmonte@salk.edu
http://dx.doi.org/10.1016/j.cell.2016.12.0

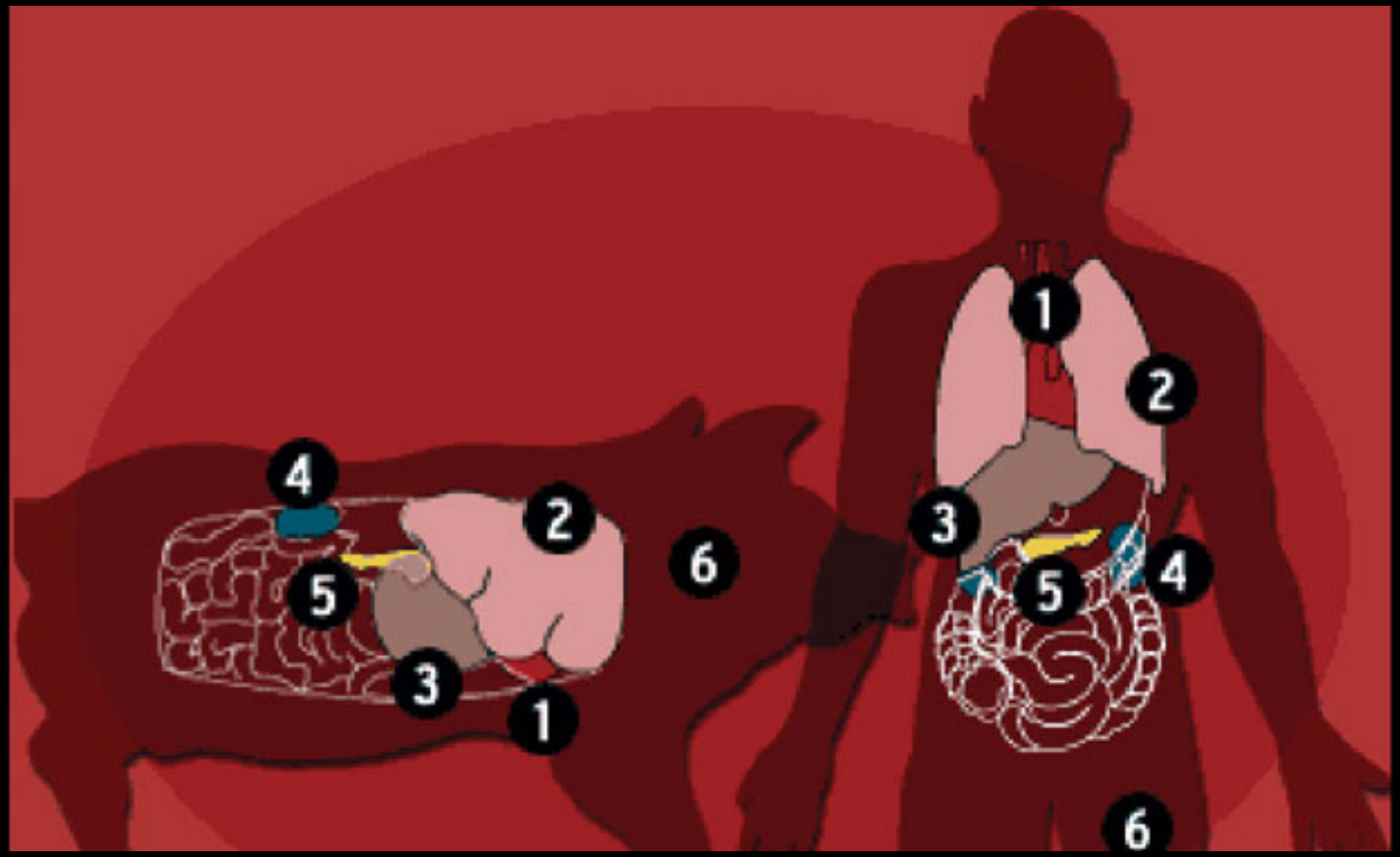


Cell

pig-to-human xenotransplantation

George Church

Luhan Yang



Purging PERVs in pigs by CRISPR

GENOME EDITING

Genome-wide inactivation of porcine endogenous retroviruses (PERVs)

Luhan Yang,[1,2,3]*† Marc Güell,[1,2,3]† Dong Niu,[1,4]† Haydy George,[1]† Emal Lesha,[1] Dennis Grishin,[1] John Aach,[1] Ellen Shrock,[1] Weihong Xu,[6] Jürgen Poci,[1] Rebeca Cortazio,[1] Robert A. Wilkinson,[5] Jay A. Fishman,[5] George Church[1,2,3]*

The shortage of organs for transplantation is a major barrier to the treatment of organ failure. Although porcine organs are considered promising, their use has been checked by concerns about the transmission of porcine endogenous retroviruses (PERVs) to humans. Here we describe the eradication of all PERVs in a porcine kidney epithelial cell line (PK15). We first determined the PK15 PERV copy number to be 62. Using CRISPR-Cas9, we disrupted all copies of the PERV *pol* gene and demonstrated a >1000-fold reduction in PERV transmission to human cells, using our engineered cells. Our study shows that CRISPR-Cas9 multiplexability can be as high as 62 and demonstrates the possibility that PERVs can be inactivated for clinical application of porcine-to-human xenotransplantation.

GENOME ENGINEERING

Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9

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Xenotransplantation is a promising strategy to alleviate the shortage of organs for human transplantation. In addition to the concerns about pig-to-human immunological compatibility, the risk of cross-species transmission of porcine endogenous retroviruses (PERVs) has impeded the clinical application of this approach. We previously demonstrated the feasibility of inactivating PERV activity in an immortalized pig cell line. We now confirm that PERVs infect human cells, and we observe the horizontal transfer of PERVs among human cells. Using CRISPR-Cas9, we inactivated all of the PERVs in a porcine primary cell line and generated PERV-inactivated pigs via somatic cell nuclear transfer. Our study highlights the value of PERV inactivation to prevent cross-species viral transmission and demonstrates the successful production of PERV-inactivated animals to address the safety concern in clinical xenotransplantation.

Niu et al., Science 357, 1303–1307 (2017)

Lin Lin

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Cells

Animals

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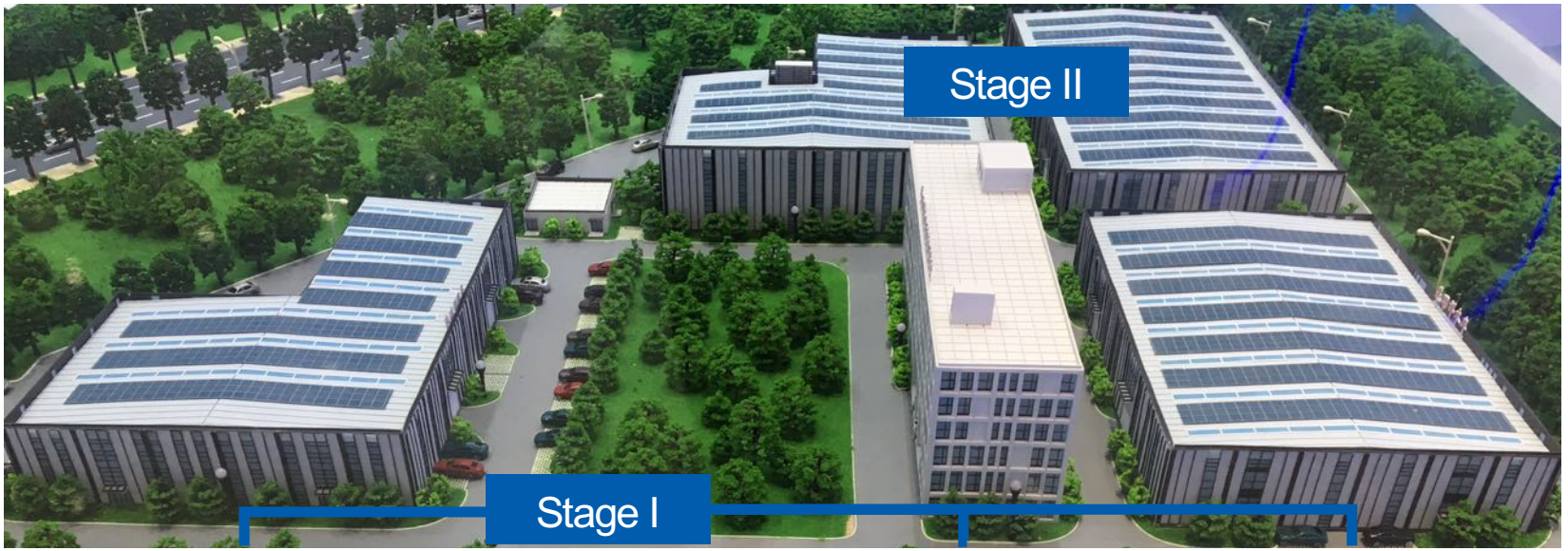
LARS BOLUND INSTITUTE
OF
REGENERATIVE MEDICINE

BGI-Qingdao Overview

- **BGI-Qingdao Park：** We have constructed 3 buildings in BGI-Qingdao park at phase I with totally of 20,000㎡ lab and office area.



#1 building

Super-computing facility

#2 building

Research Platform;

Office

#3 building

Biobank; Animal experiment lab;

Sequencing Platform

Regenerative Medicine with Salamanders

- **Harvard Medical School**

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Dong Niu

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THANKS/谢谢/TAK

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