Genome editing and stem cell engineering for disease modeling

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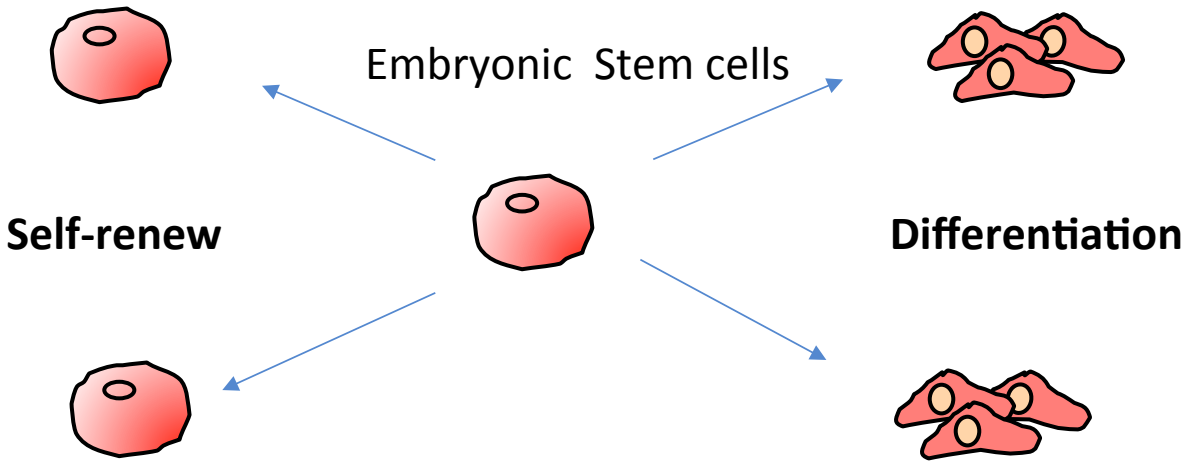
The human Pluripotent Stem Cell core (HPSCC) is committed to accelerating collaborative studies in stem cell biology

The HPSCC mission is to serve as the central platform of technical support for investigators seeking to work with human embryonic and pluripotent stem cells

Pluripotent stem cells



PROS

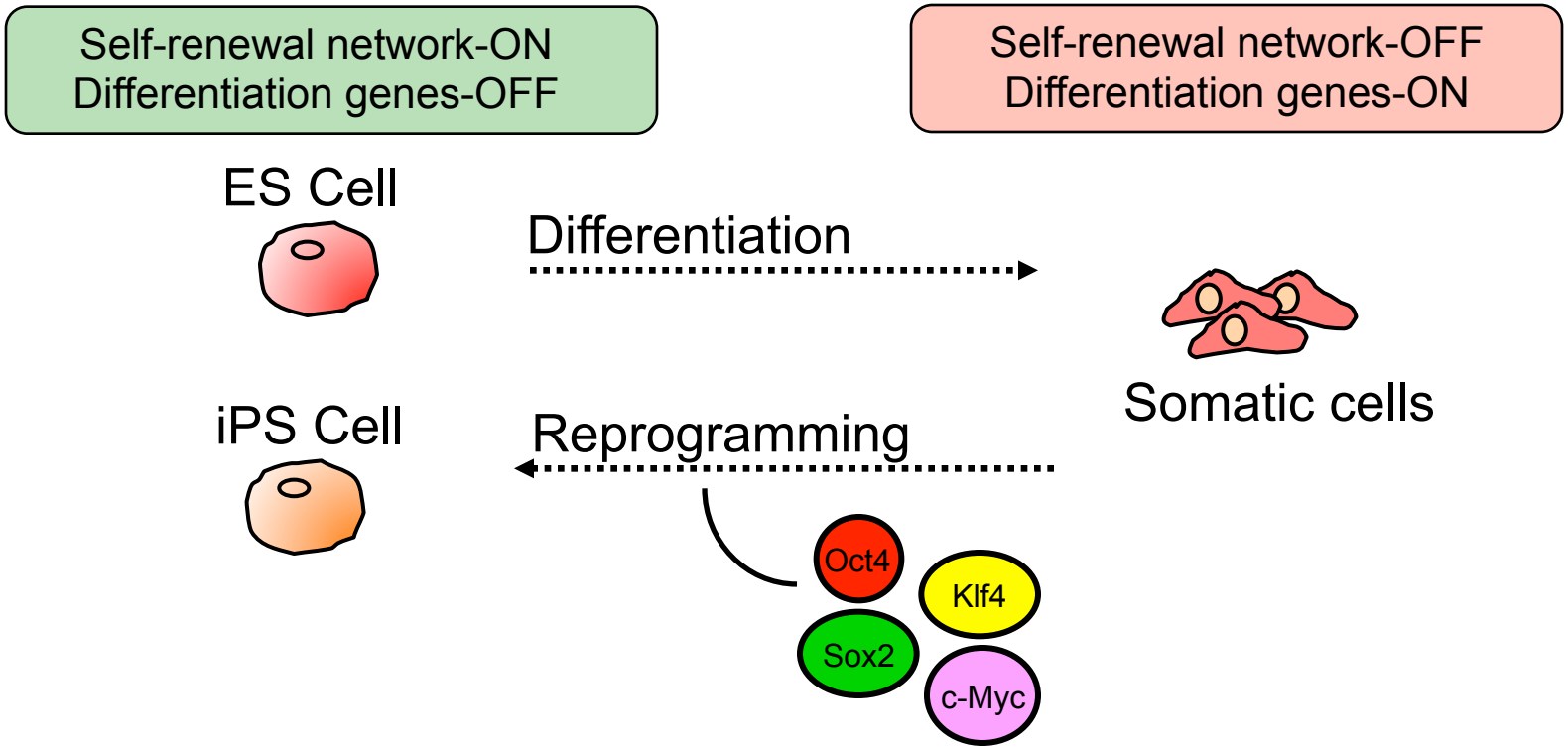
Limitless potential in regenerative medicine and disease

CONS

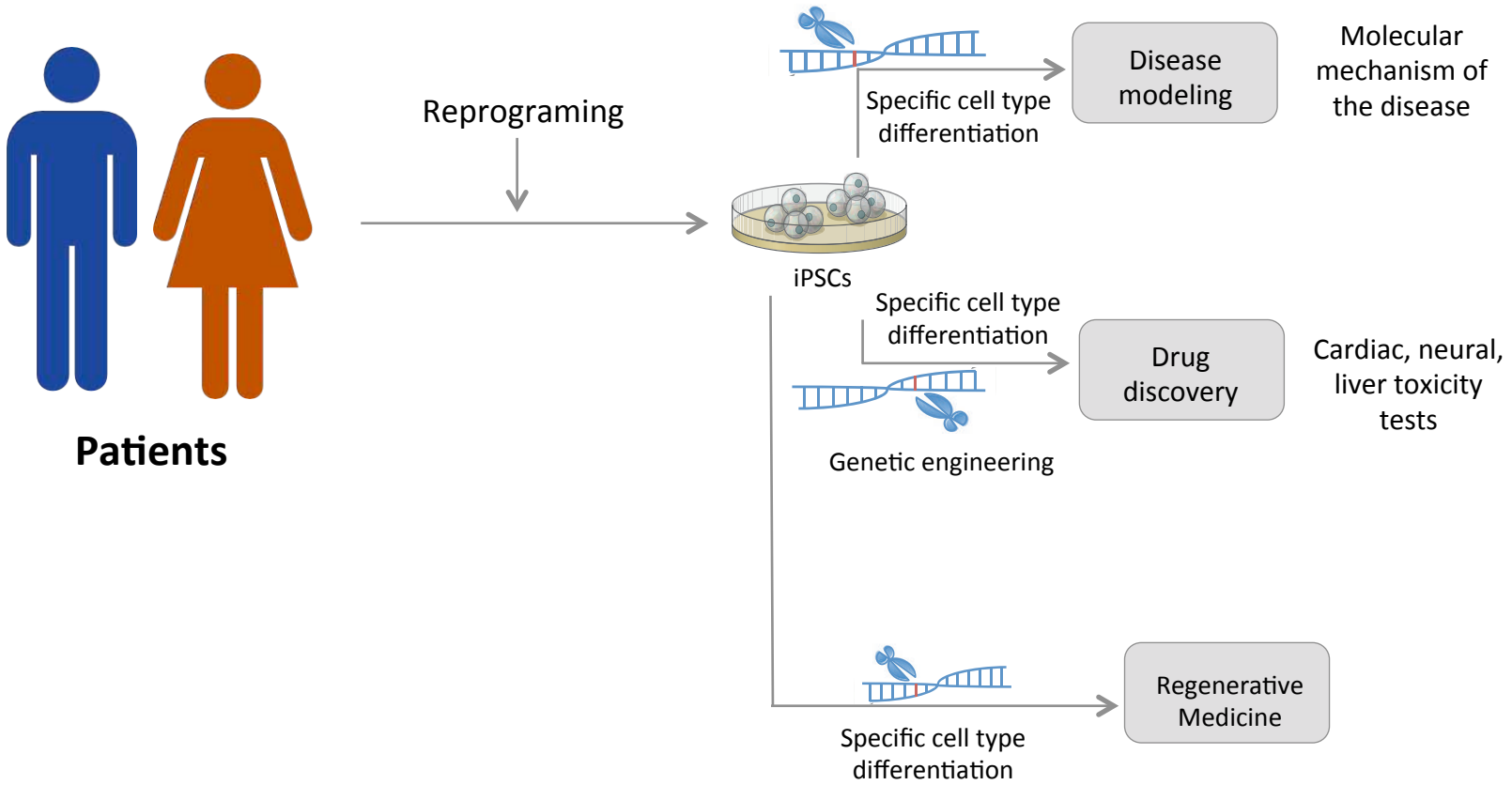
Rejection after transplantation

Usage of human embryos to create them

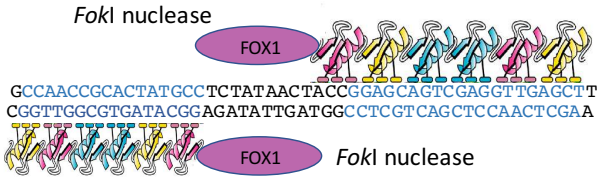
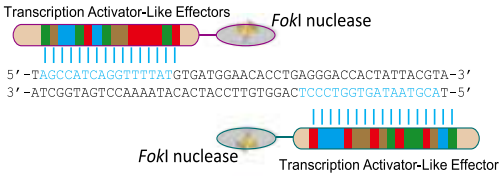
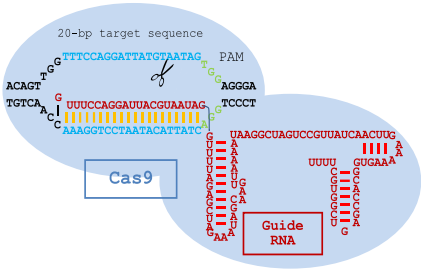
Inducing a pluripotent state (iPS)



iPSC as patient-specific pre-clinical disease models

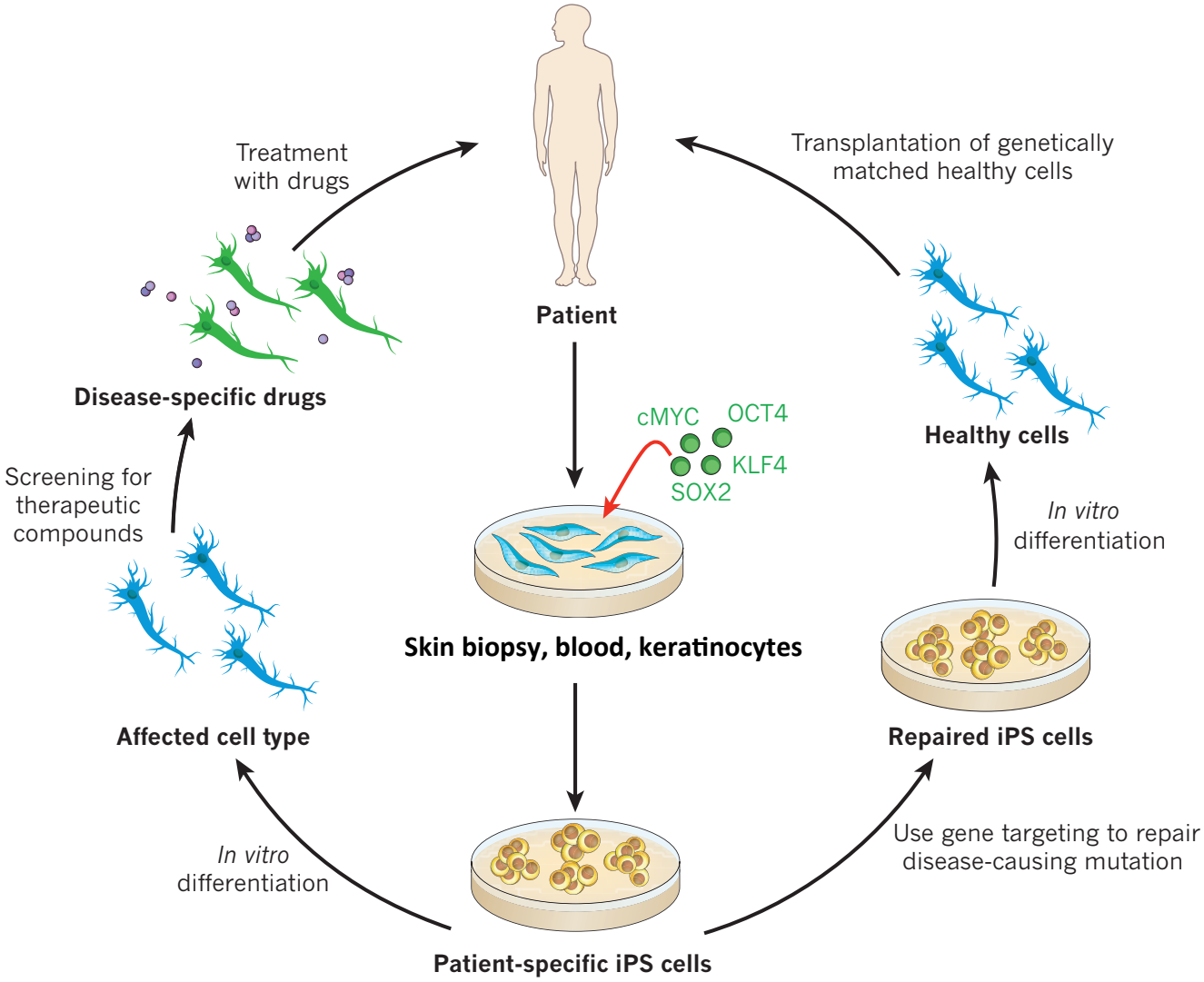


Tools for genome editing

	Zinc Finger nucleases (ZFN)	TAL effector nucleases (TALENs)	CRISPR/Cas
			
Advantage:	Long experience in using Clinical trial	Easy to design High specificity	Simplicity High efficiency
Disadvantage:	Difficult to designed and Construct Target site restrictions	Labor intense to construct "T" requirement Sensitive to 5'methylcytosine	PAM requirement Off-target concerns

Mashimo T. 2014, 56, 46-52

Medical applications of iPS cells



Adapted from: Robinton & Daley, Nature 2012

Human pluripotent stem cell services

Cell derivation and characterization:

- IPSC generation and characterization
- hES/hiPS directed differentiation into specialized cell types
- hES/hiPS maintenance, expansion and banking

Genome engineering:

- Genome editing of mammalian cells (hESC, iPSC, CSC)
- Engineering effector molecules for gene regulation

Training in stem cell culture techniques

Source materials

Fibroblast

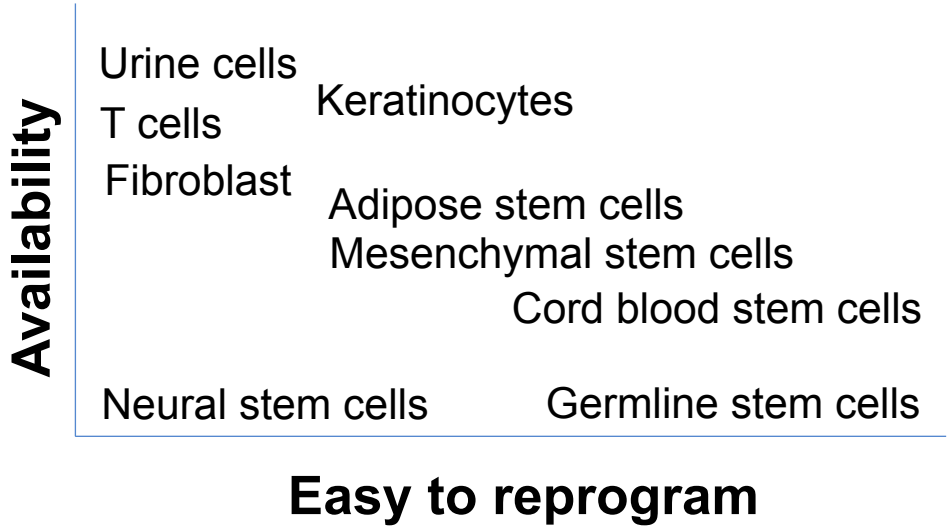
Neural cells

Hair follicle

Keratinocytes

Blood

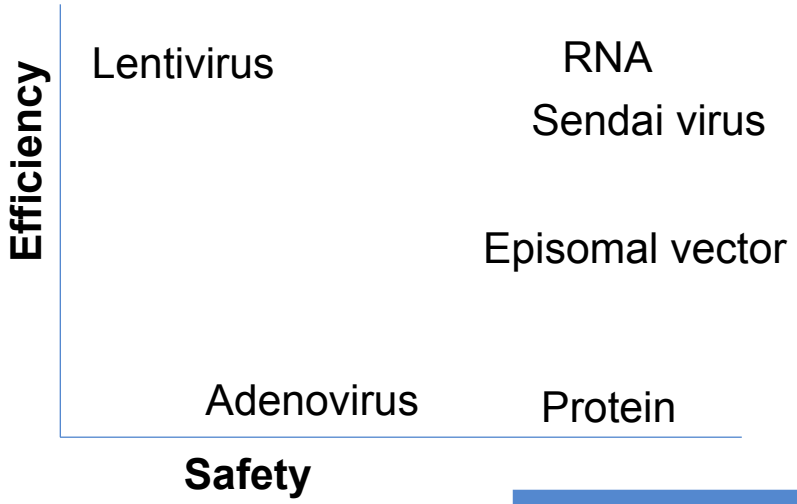
Urine



Blood: easy to obtain and easy to reprogram
Fibroblast: easy to obtain, frozen stock and expandable

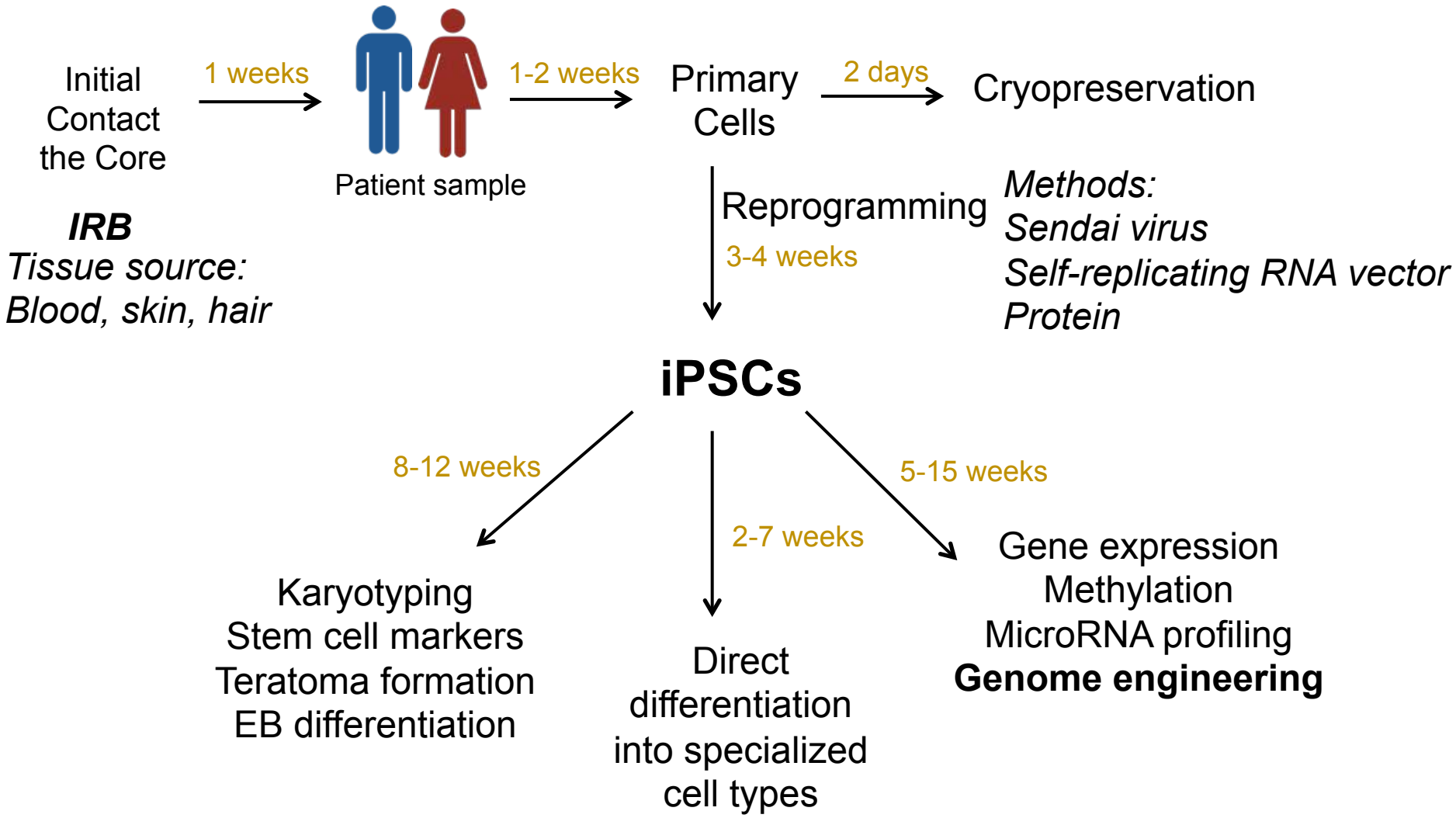
Urine: easiest to obtain, easy to reprogram, potential contamination

Comparison of IPSC derivation technology

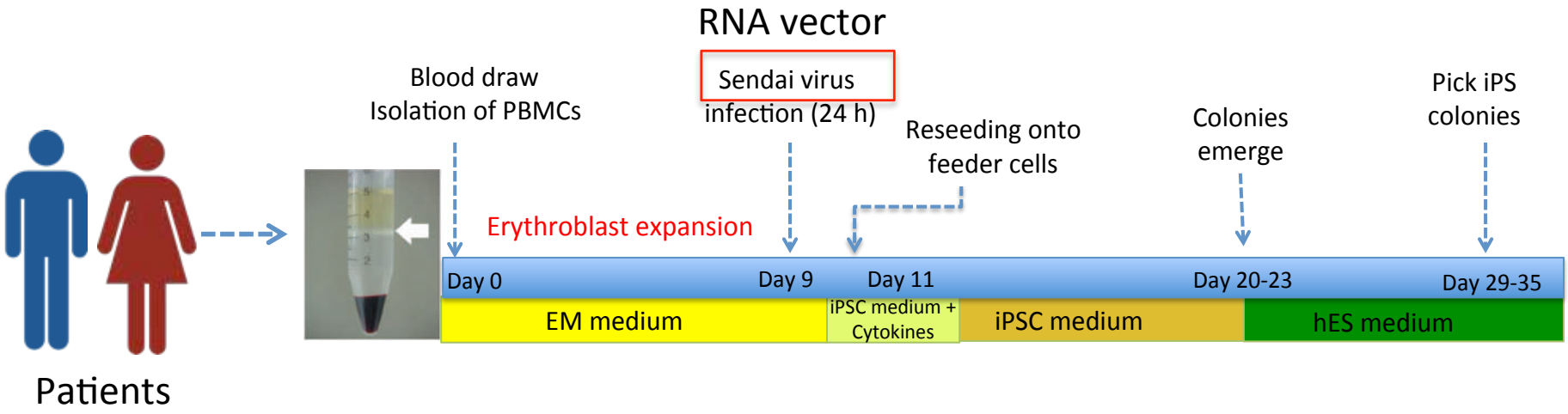


Method	Integration	Efficiency	Cost
Retrovirus	Yes	0.001 – 0.01%	++
Lentivirus	Yes	0.001 – 0.01%	++
Adenoviral	Possible	0.0001 – 0.001%	++++
Sendai virus	No	0.01 – 1%	++++
Episomal	Possible	0.0001 – 0.01%	+
mRNA	No	>1%	++++
Protein	No	0.00001%	++++

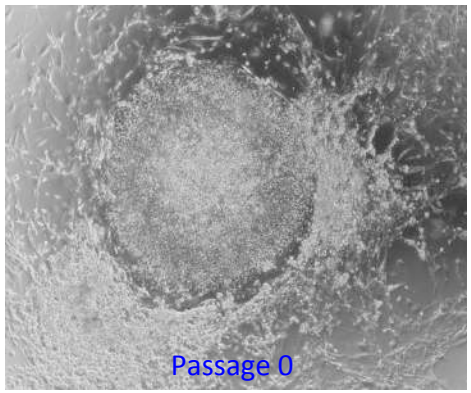
Timeline for iPSC derivation

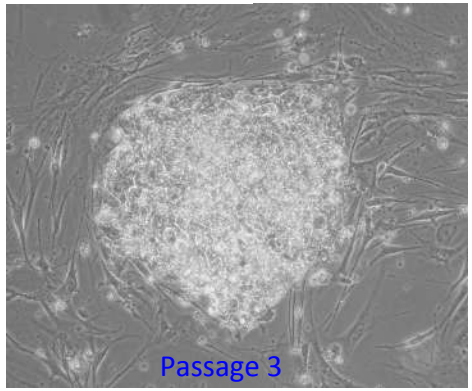


Reprogramming blood to iPS cells using a integration-free method

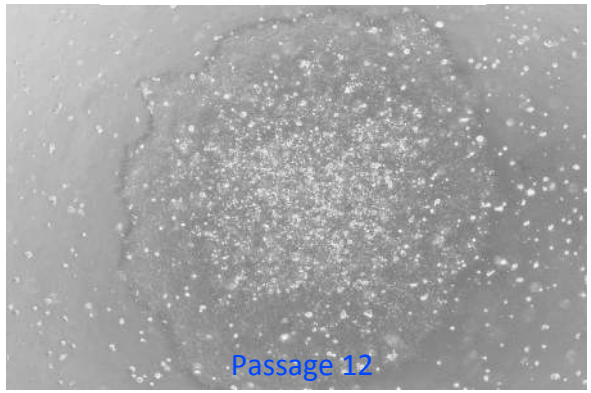


iPSC colonies in Feeders



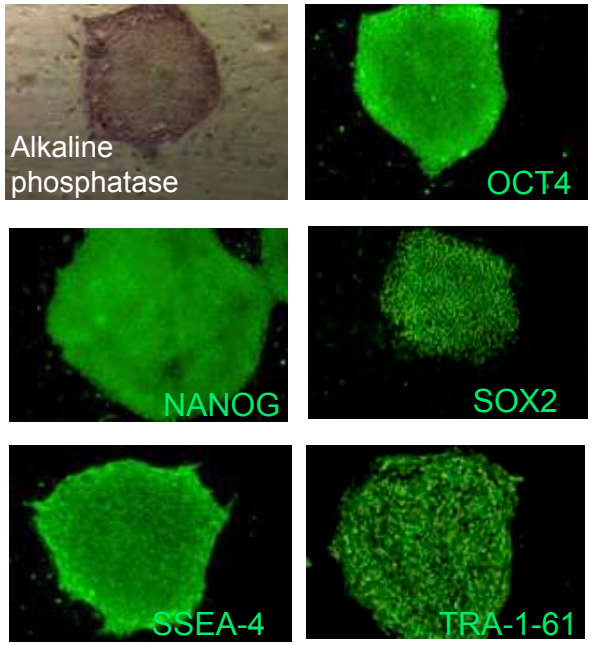


iPSCs feeder-free

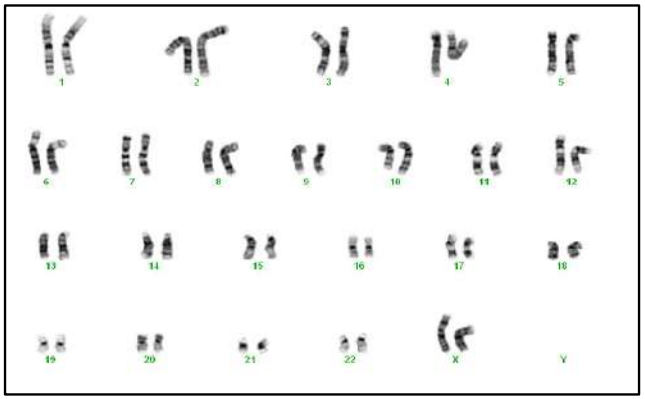


Quality assurance: characterization of iPSCs

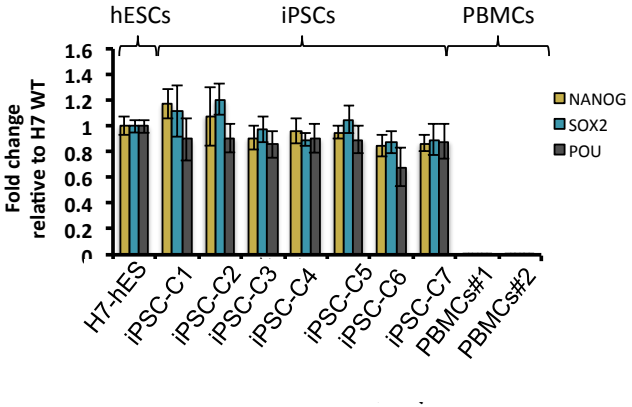
Immunofluorescence of pluripotency markers



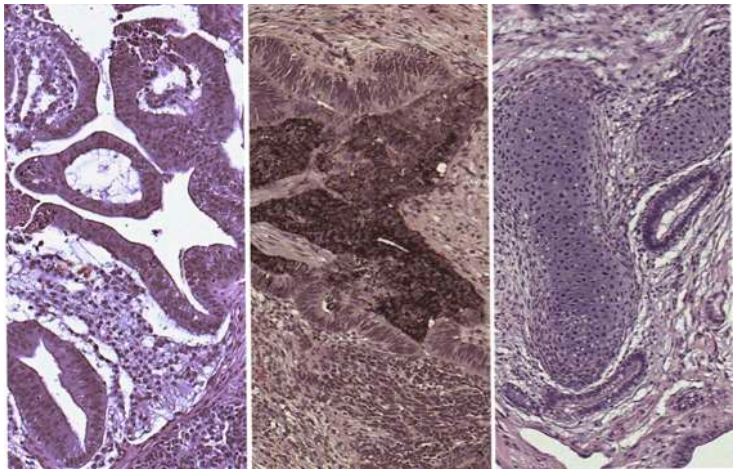
Karyotype



qRT-PCR of pluripotency markers



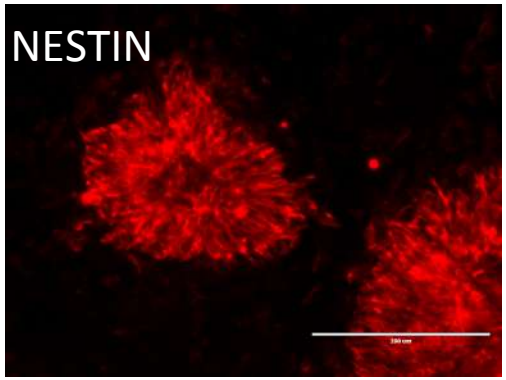
Teratoma formation

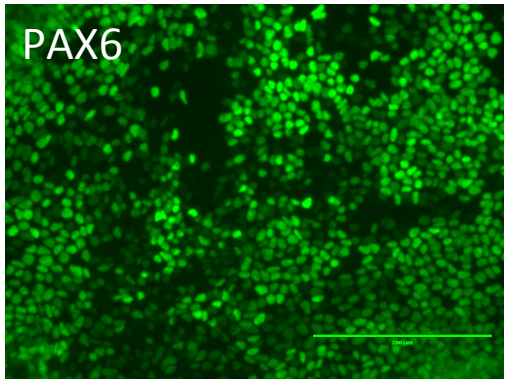


Adriana Beltran, Bryan Richardson

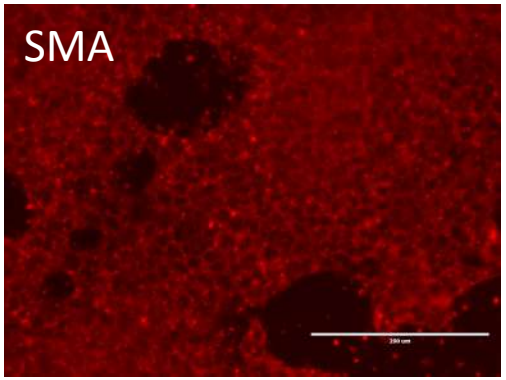
Differentiation of hES and IPS into three germ layers

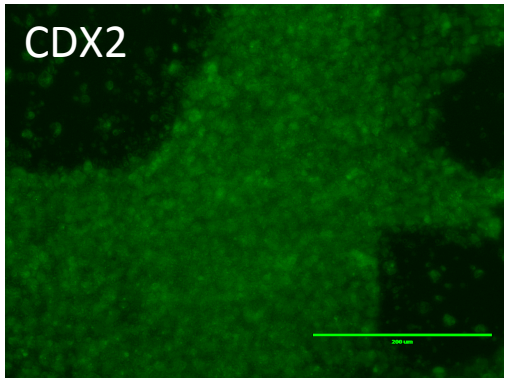
Ectoderm



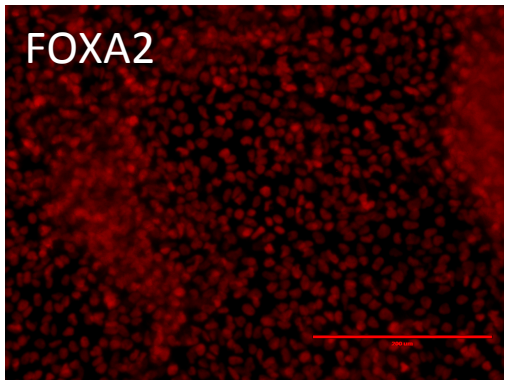


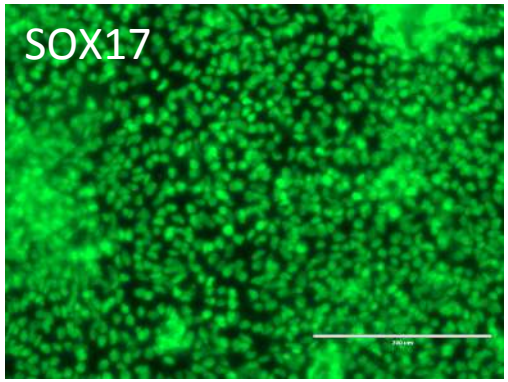
Mesoderm



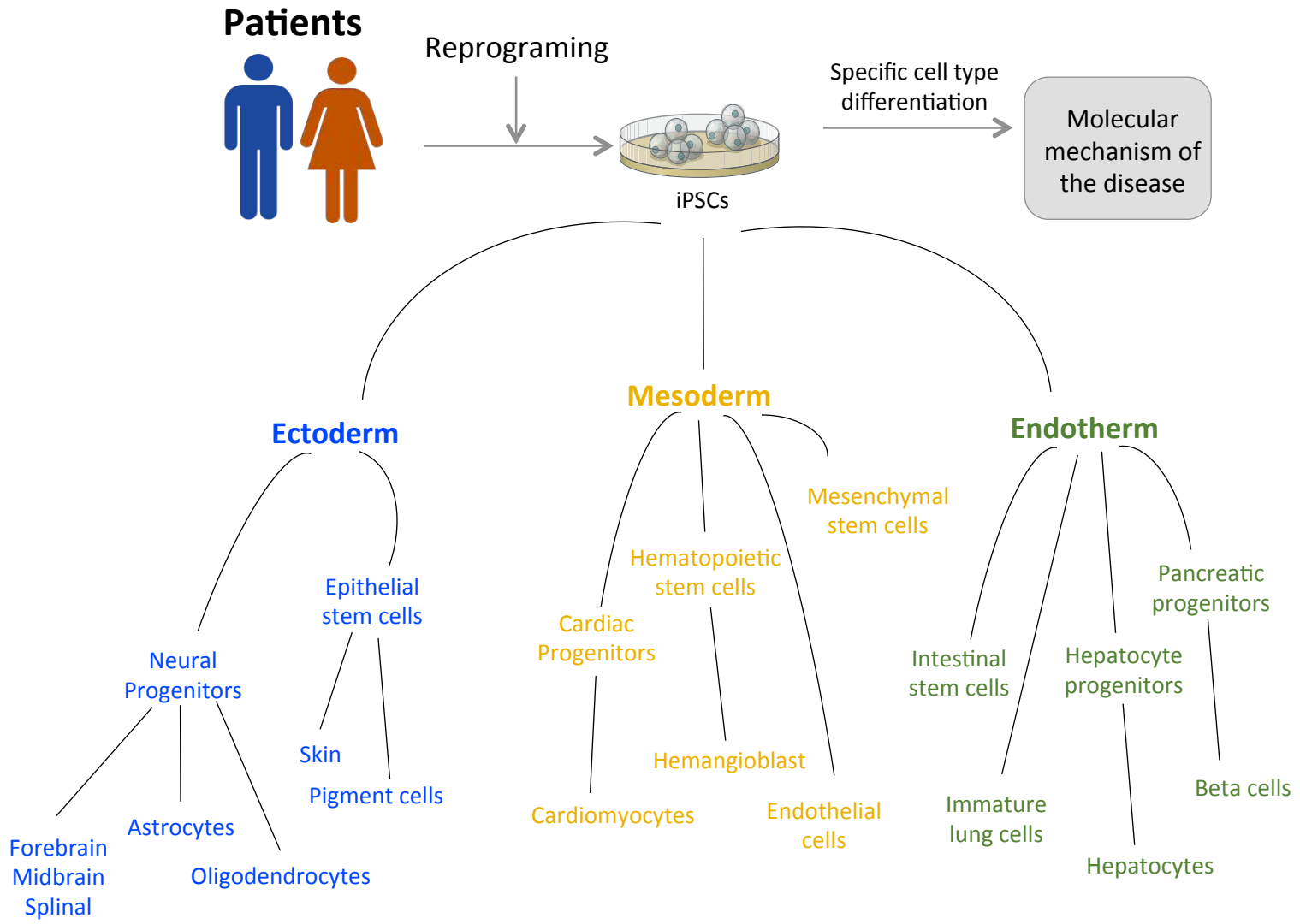


Endoderm

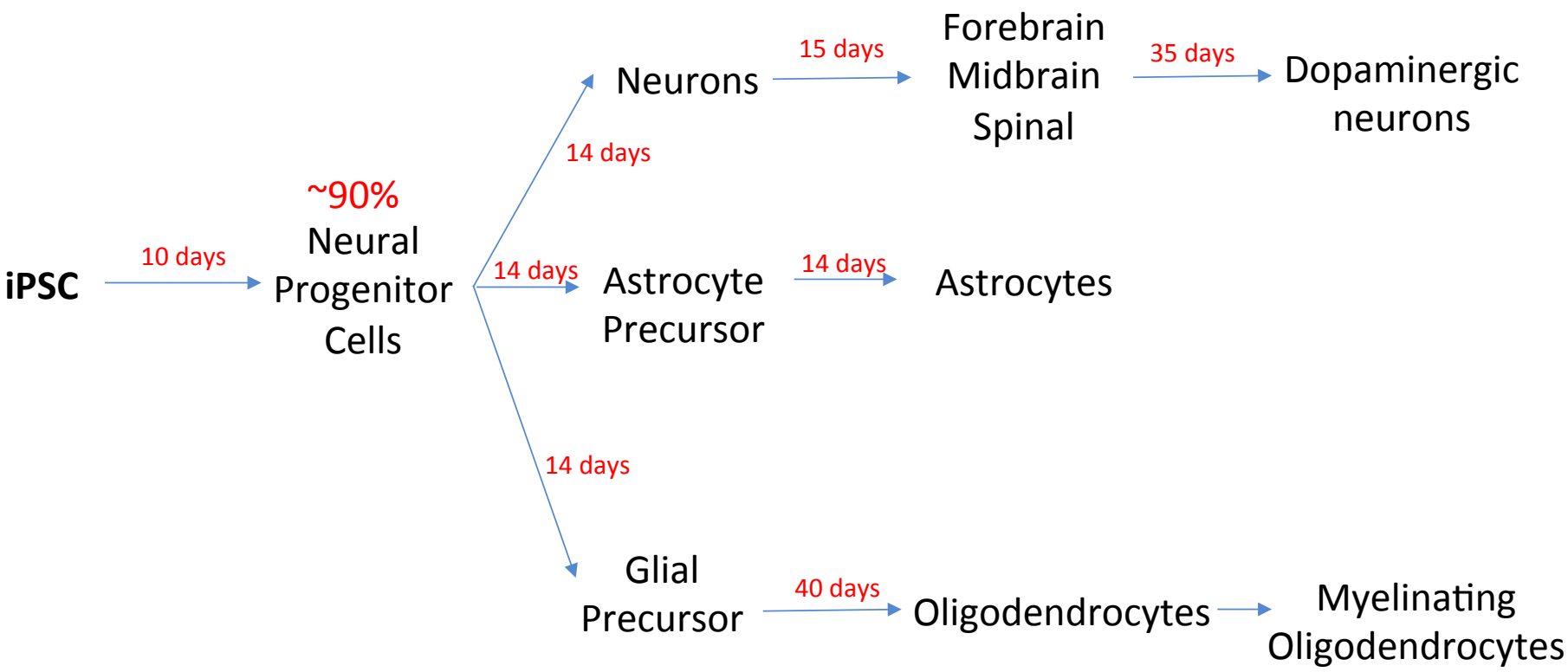




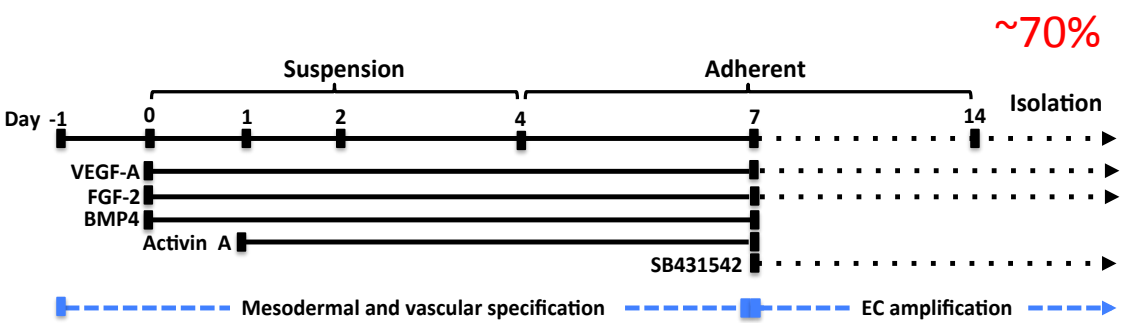
Specific cell type differentiation

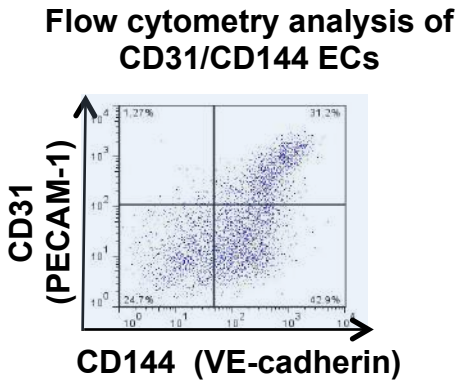


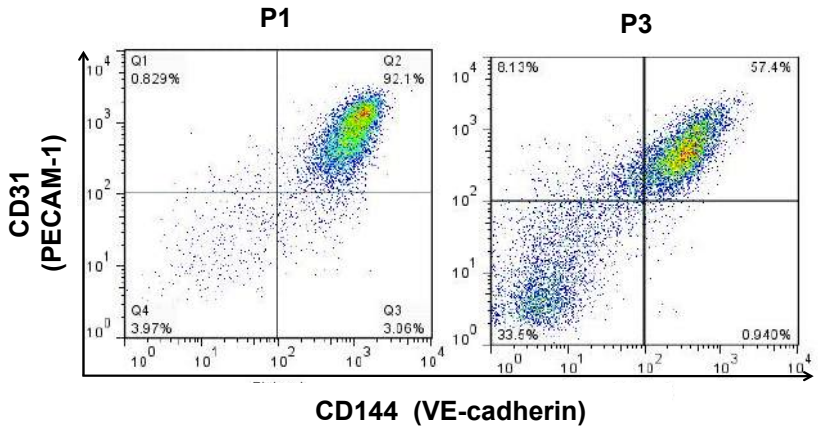
Neural differentiation timeline

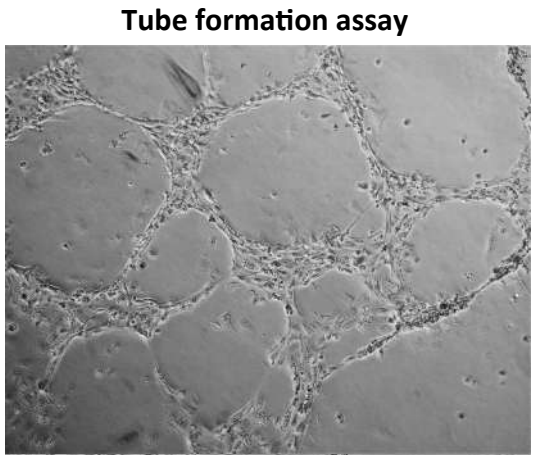


Derivation of functional endothelial cells from iPSCs









Maintenance, expansion and banking of hES and hiPS cells

Embryonic stem cell lines distributed by the core:

H1, H7 and H9

iPSC control lines

IMPORTANT:

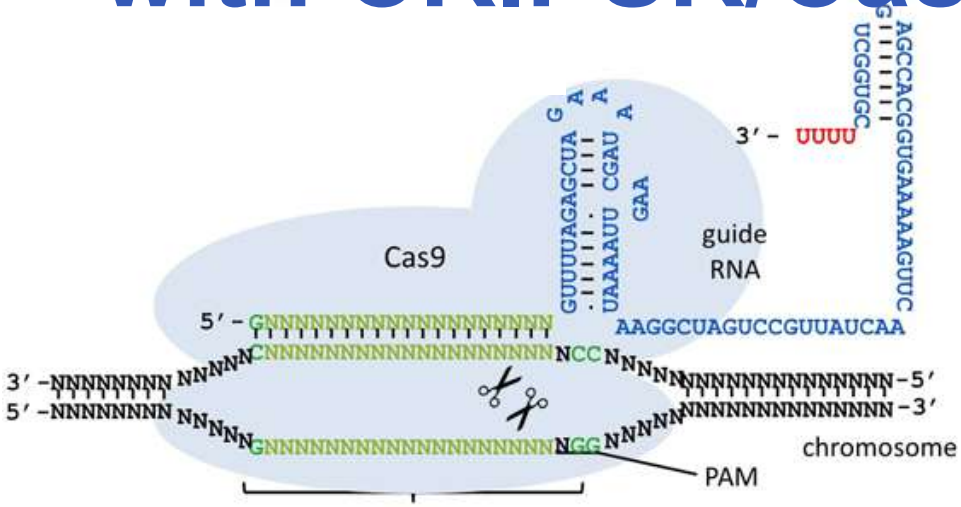
hES lines acquired from the core must be checked for pluripotency every month and be karyotyped every 20 passages.

Human pluripotent stem cell services

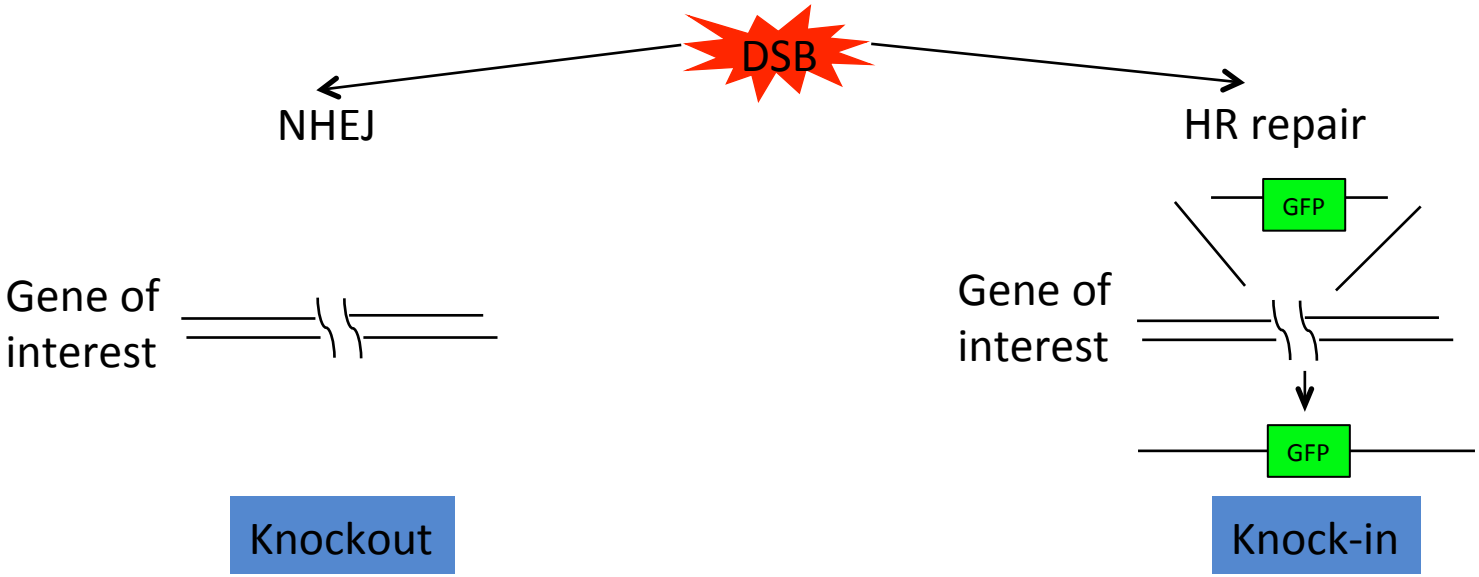
Genome engineering:

- Genome editing of mammalian cells (hESC, iPSC, CS)
- Engineering effector molecules for gene regulation

Fast and easy genome engineering with CRIPSR/Cas9



23 bp genomic target sequence



Genome editing possibilities

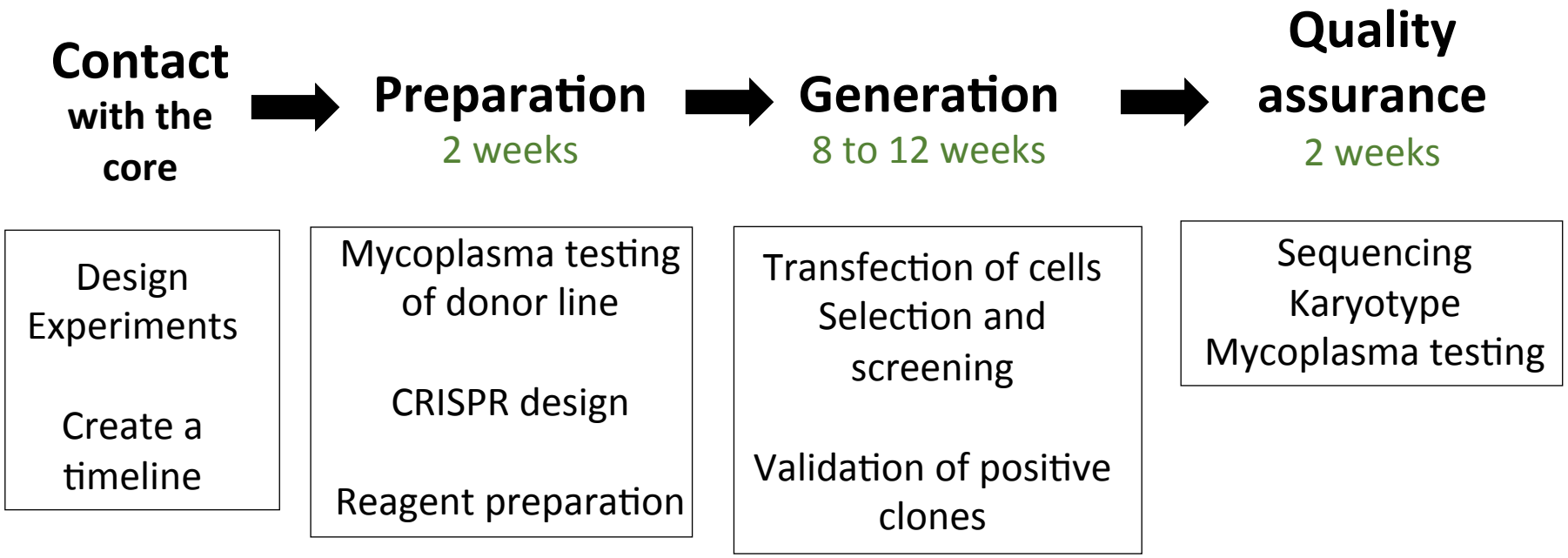
Genome editing using CRISPR/Cas9 based technologies are applied to any mammalian cell type (hES, hIPS and cancer cell lines).

•CRISPR-mediated gene knock-out.

•CRISPR-mediated gene knock-in.

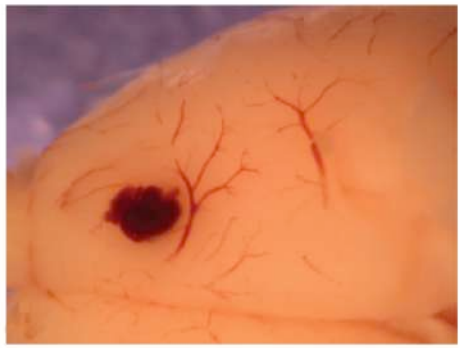
•CRISPR-mediated point mutation introduction and/or repair to create isogenic cell lines.

Genome editing timeline

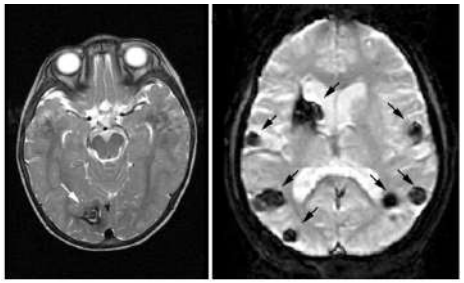


CRIPSR mediated gene KO
Editing the CCM3 locus

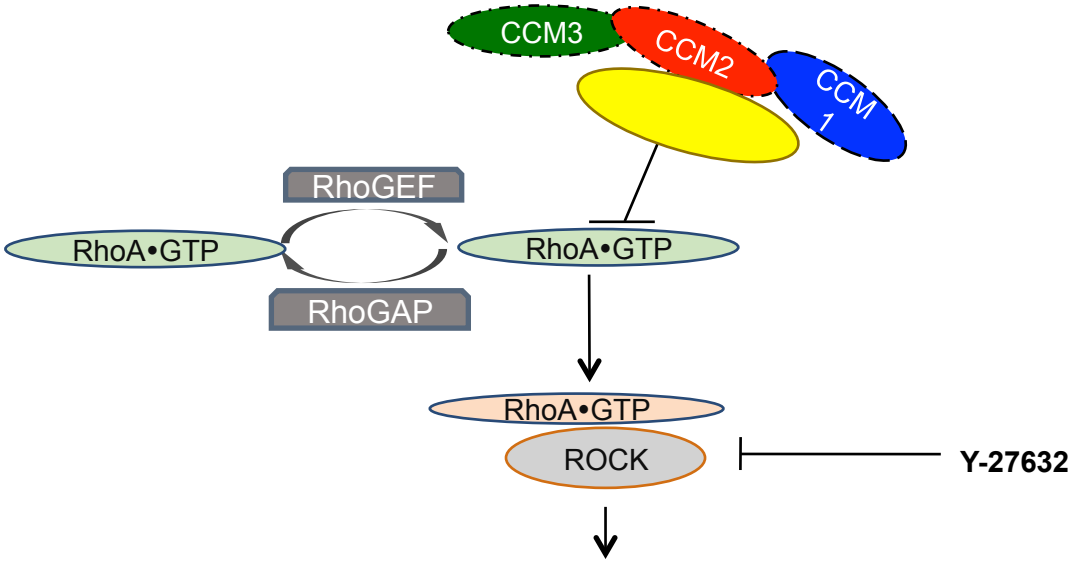
Cerebral Cavernous Malformation (CCM)



Plummer NW, et al. Am J Pathol, 2004.

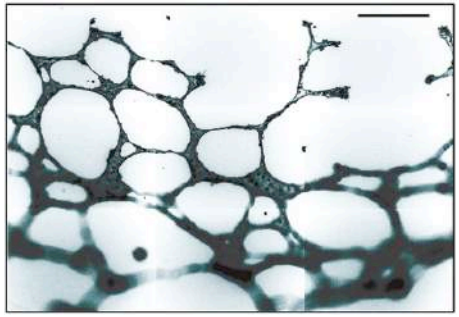


Revencu, et al., J Med Genet, 2006

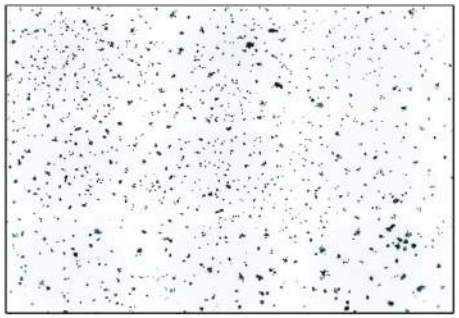


Actin stress fiber formation and loss of endothelial function

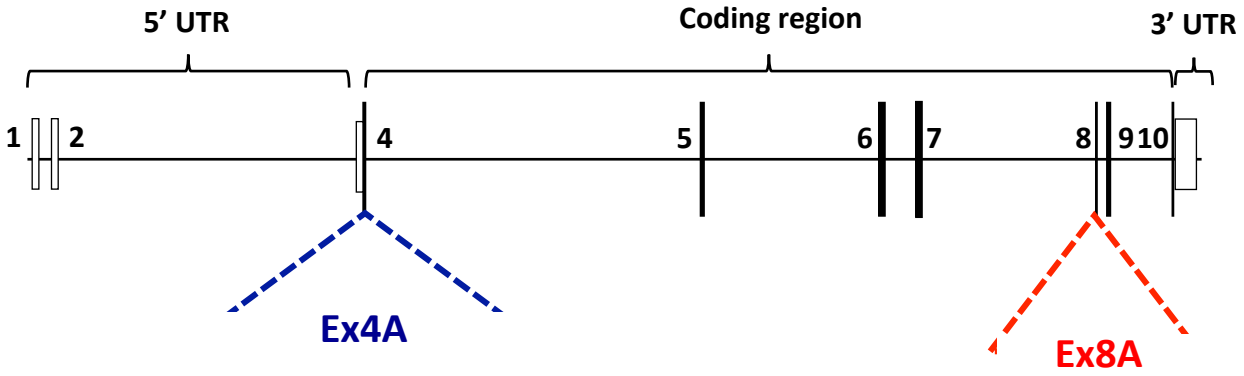
Control

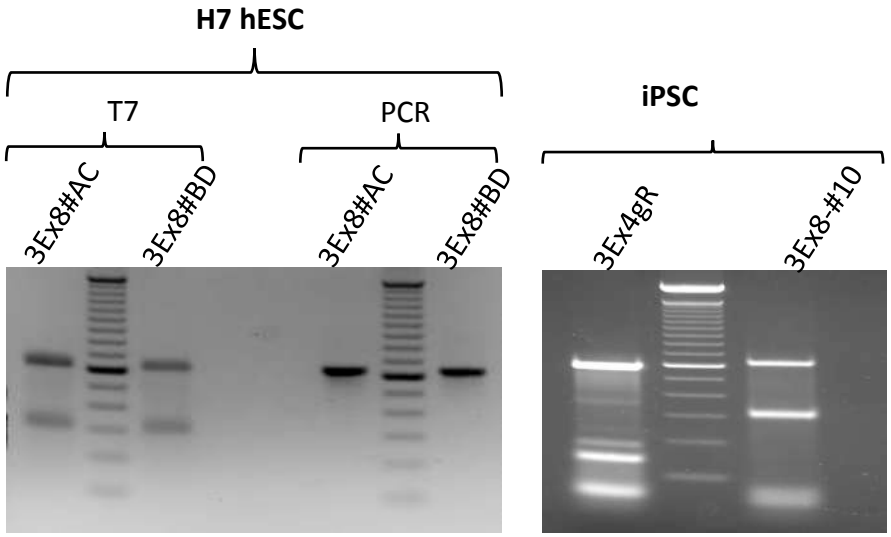


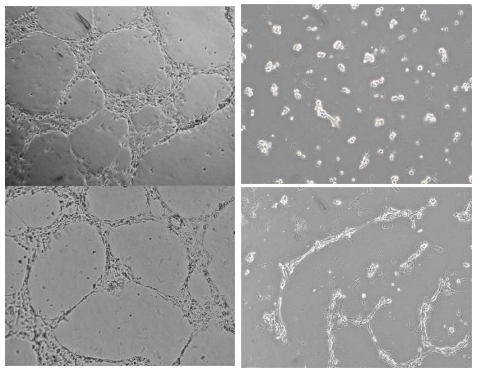
CCM3 loss



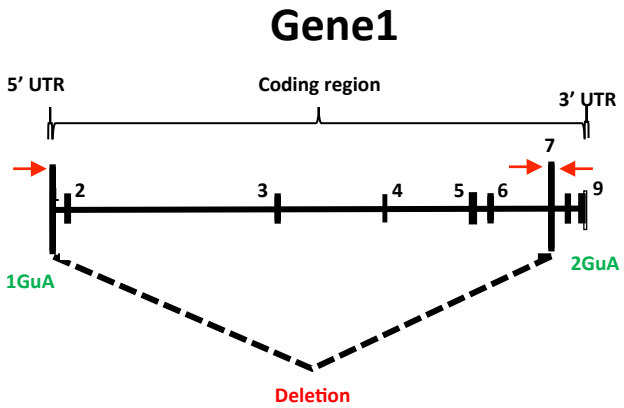
CRIPSR mediated gene KO

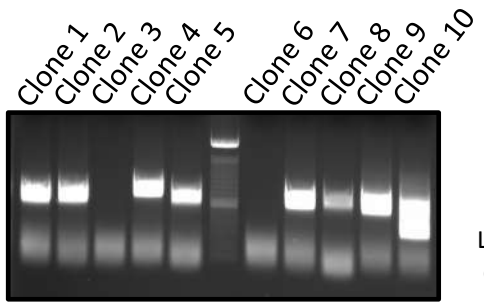




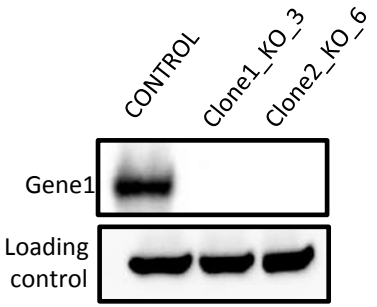


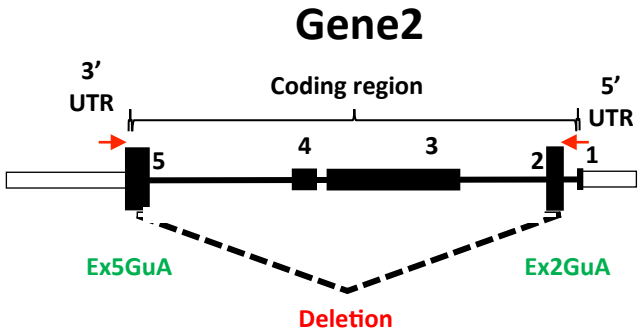
CRIPSR mediated gene KO
High efficient double allele KOs

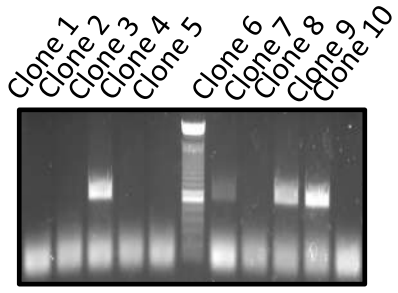




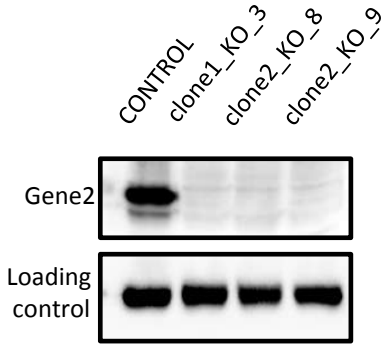
20% efficiency







40% efficiency

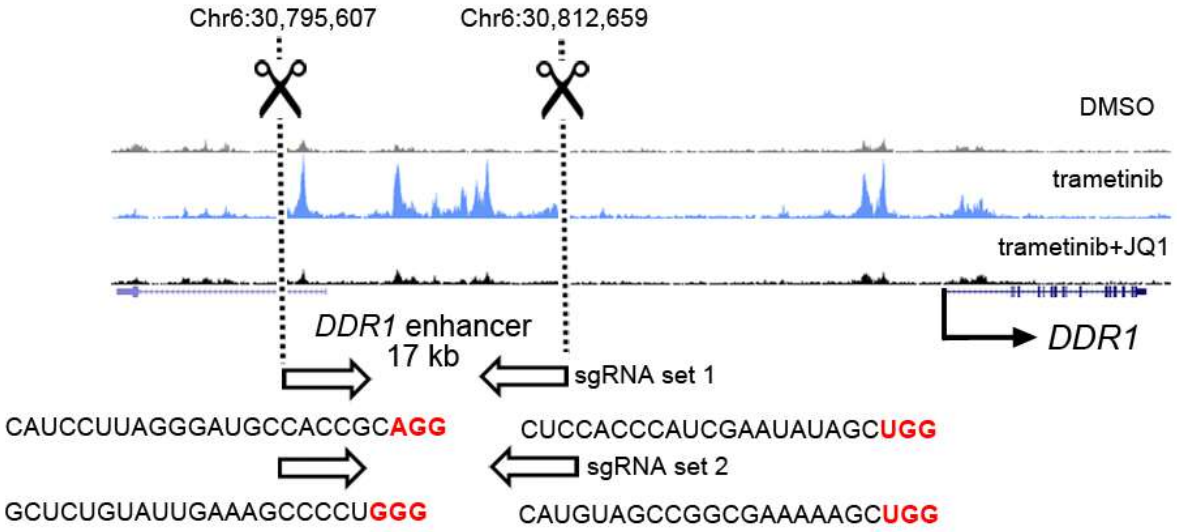


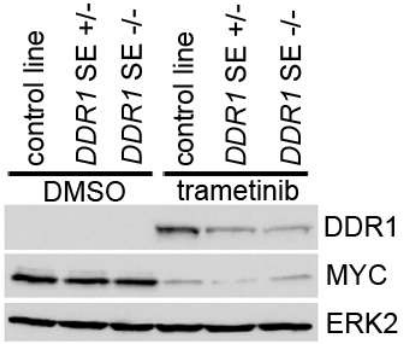
CRIPSR mediated gene KO deletion of large genomic regions

Enhancer Remodeling During Adaptive Bypass to MEK Inhibition Is Attenuated by Pharmacological Targeting of the P-TEFb Complex

Jon S. Zawistowski, Samantha M. Bevill, Daniel R. Goulet, Timothy J. Stuhlmiller, Adriana S. Beltran, Jose F. Olivares-Quintero, Darshan Singh, Noah Sciaky, Joel S. Parker, Naim U. Rashid, Xin Chen, James S. Duncan, Martin C. Whittle, Steven P. Angus, Sara Hanna Velarde, Brian T. Golitz, Xiaping He, Charlene Santos, David B. Darr, Kristalyn Gallagher, Lee M. Graves, Charles M. Perou, Lisa A. Carey, H. Shelton Earp, and Gary L. Johnson

DOI: 10.1158/2159-8290.CD-16-0653 Published January 2017

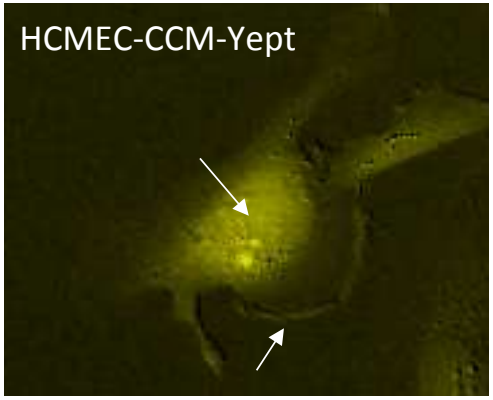




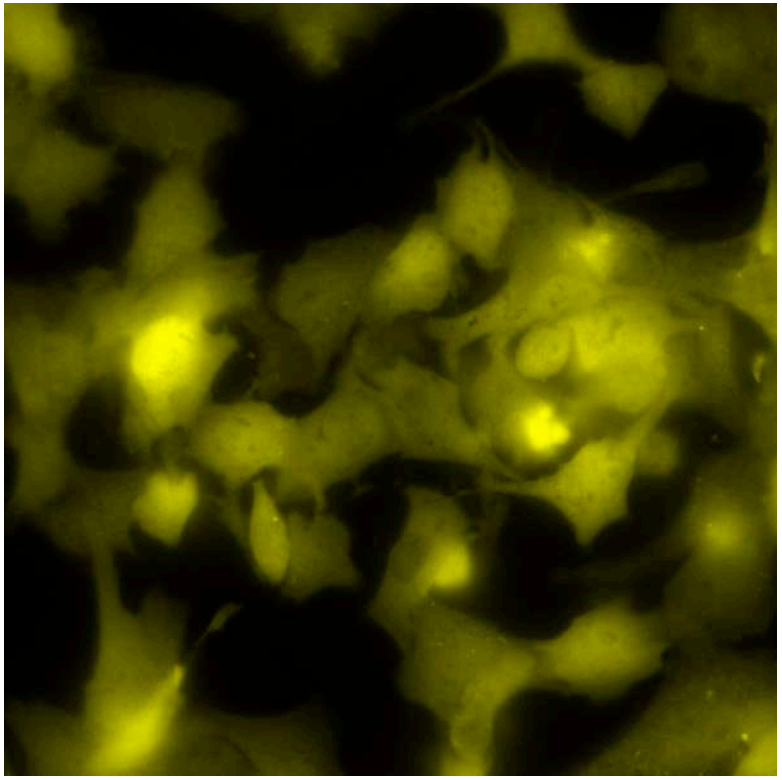
Zawistowski et al. Cancer Discovery 2017

CRIPSR mediated gene KI Fluorescent reporter

CCM-YPET fusion protein cellular distribution mimic CCM protein



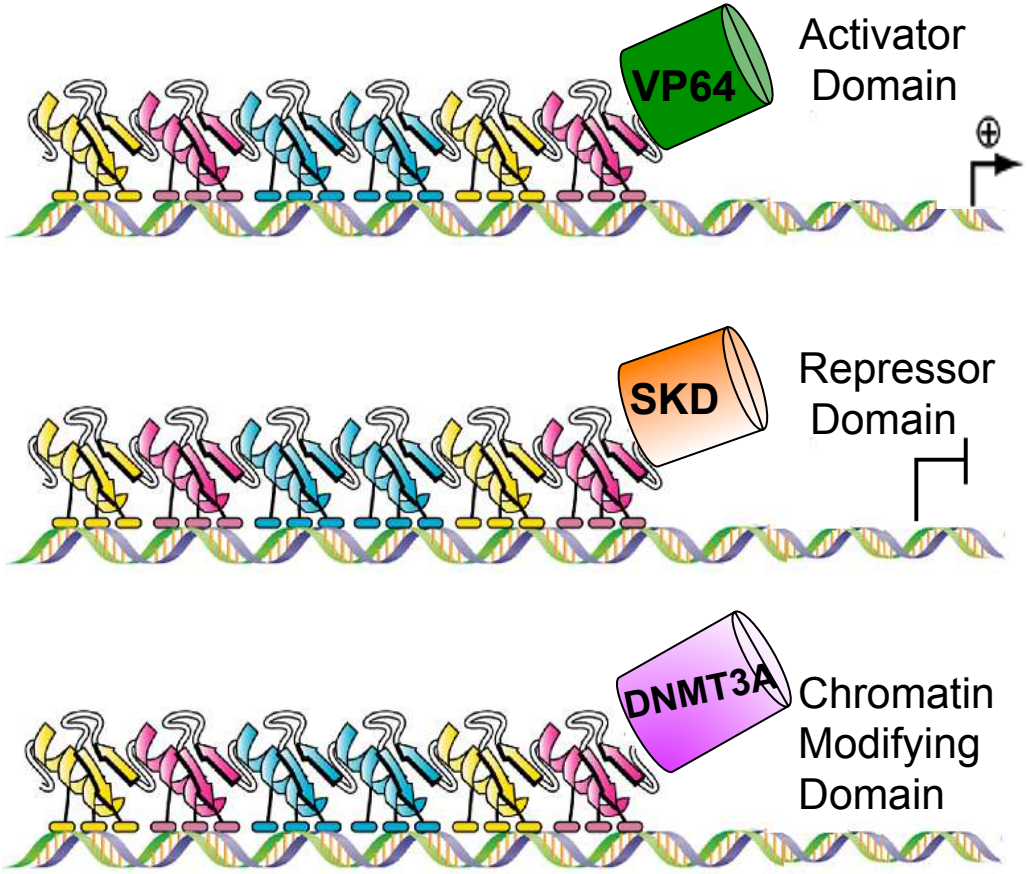
CCM-YPET reporter real-time imaging



Felix Olivares and Adriana Beltran

Engineering effector molecules for gene regulation

Artificial Transcription Factors (ATFs)
Synthetic transcription factors



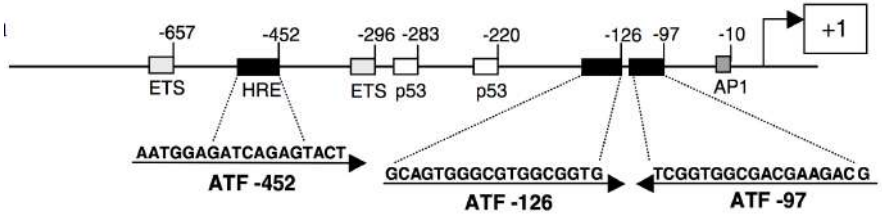
A tumor suppressor (Maspin)

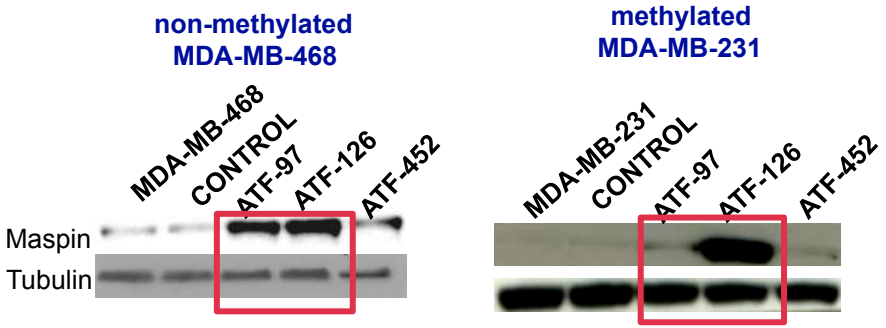
An oncogene (Sox2)

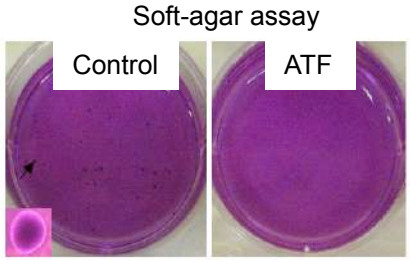
An oncogene (Sox2)

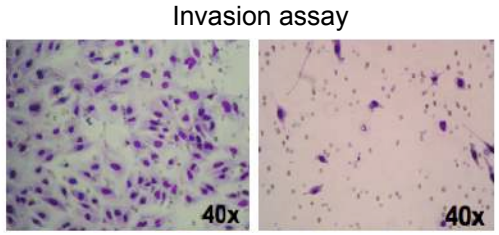
Re-activation of a dormant tumor suppressor gene *maspin* by designed transcription factors

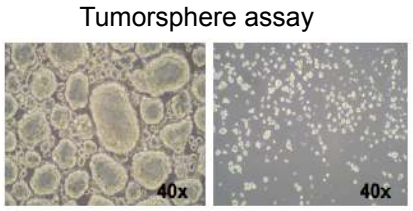
A Beltran[1], S Parikh[1], Y Liu[1], BD Cuevas[1], GL Johnson[1], BW Futscher[2] and P Blancafort[1]

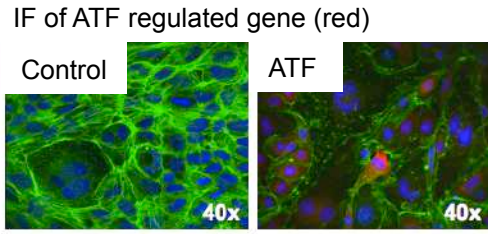


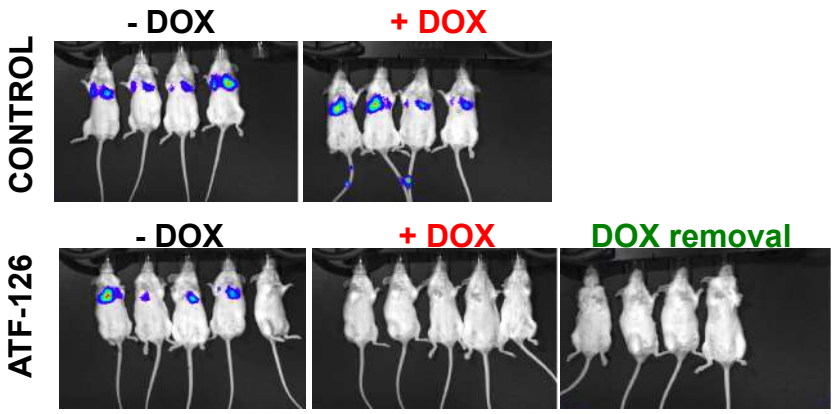








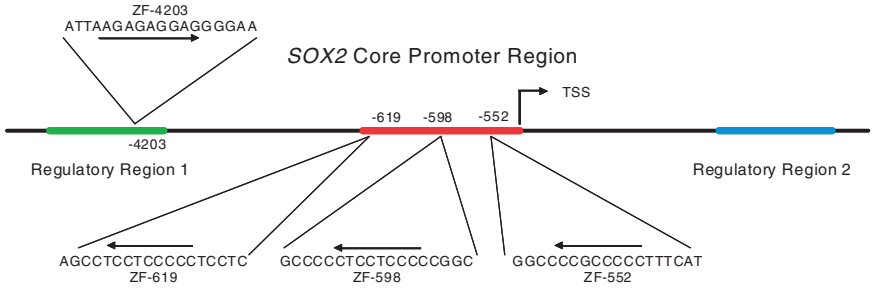


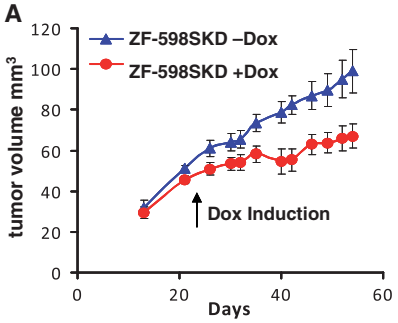


Beltran, A, et al, Oncogene 2006

Targeted silencing of the oncogenic transcription factor *SOX2* in breast cancer

Sabine Stolzenburg[1,2], Marianne G. Rots[1], Adriana S. Beltran[2], Ashley G. Rivenbark[2,3], Xinni Yuan[2], Haili Qian[4], Brian D. Strahl[3,5] and Pilar Blancafort[2,5,*]

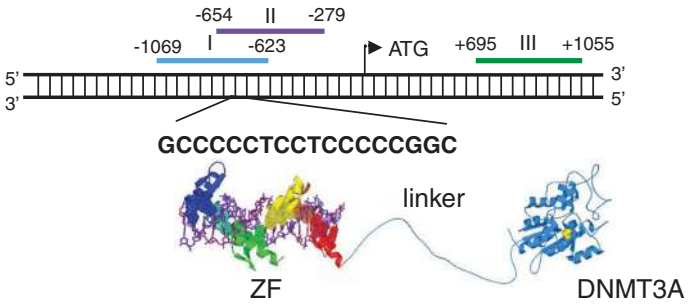


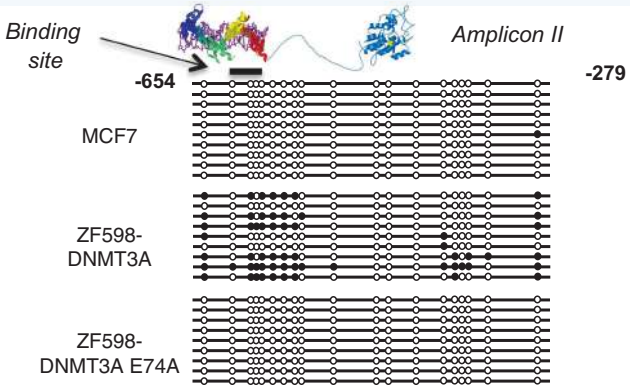


ORIGINAL ARTICLE

Stable oncogenic silencing *in vivo* by programmable and targeted *de novo* DNA methylation in breast cancer

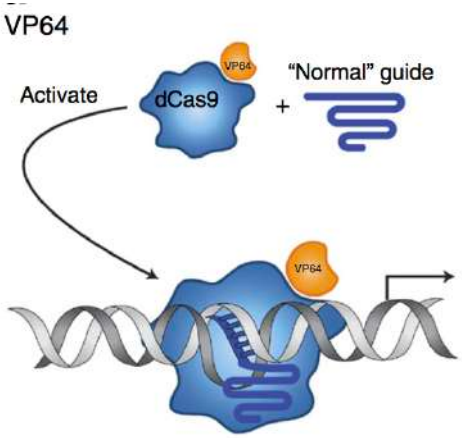
S Stolzenburg[1,2], AS Beltran[2], T Swift-Scanlan[3], AG Rivenbark[4], R Rashwan[1] and P Blancafort[1,2]





Comparison of Cas9 activators in multiple species

Alejandro Chavez[1–3,11], Marcelle Tuttle[1,11], Benjamin W Pruitt[1], Ben Ewen-Campen[3], Raj Chari[3], Dmitry Ter-Ovanesyan[1,3], Sabina J Haque[4,5], Ryan J Cecchi[1], Emma J K Kowal[1], Joanna Buchthal[1], Benjamin E Housden[3], Norbert Perrimon[3,6], James J Collins[1,7–10] & George Church[1,3]



The FEBS Journal

FEBS PRESS science publishing by scientists

REVIEW ARTICLE

Next stop for the CRISPR revolution: RNA-guided epigenetic regulators

Suhani Vora[1,2,3], Marcelle Tuttle[1], Jenny Cheng[1] and George Church[1,2]

1 Wyss Institute for Biologically Inspired Design, Center for Life Sciences Boston, Boston, MA, USA
2 Department of Genetics, Harvard Medical School, Boston, MA, USA
3 Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

Highly specific epigenome editing by CRISPR-Cas9 repressors for silencing of distal regulatory elements

Pratiksha I Thakore[1,2], Anthony M D'Ippolito[2,3], Lingyun Song[2,4], Alexias Safi[2,4], Nishkala K Shivakumar[1], Ami M Kabadi[1,2], Timothy E Reddy[2,5], Gregory E Crawford[2,4] & Charles A Gersbach[1,2,6]

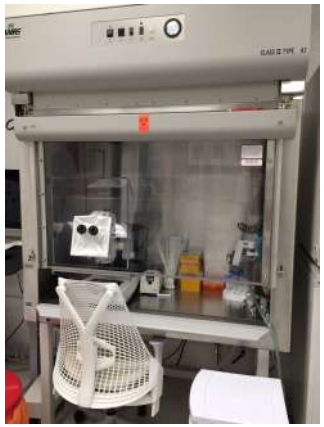
Training in stem cell culture techniques

The Stem Cell Core Facility offers a one-to-one training course in basic human stem cell culture techniques.

- Introduction to stem cell biology
- Morphology of hES and hiPSCs
- Media preparation
- Feeder preparation
- Feeder-free ECM coating
- Passaging hES and hiPSCs by enzyme and manual picking
- Freezing/thawing hiPSCs

Available technology

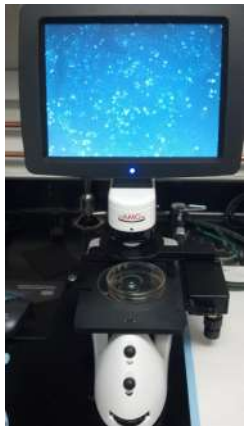
Two Leica stereomicroscopes housed in two biosafety cabinets



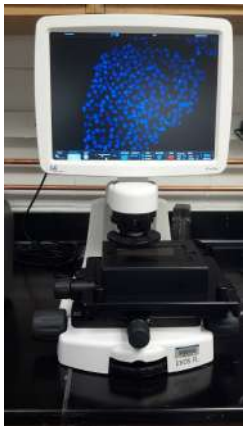
Copper-lined CO2 incubators

QuantStudio™ 7 Flex Real-Time PCR System

EVOS xl Core



EVOS FL cell imaging system



Veriti® 96-Well Fast Thermal Cycler

Countess® II FL Automated Cell Counter

Neon Electroporator

Qubit 3.0 Fluorometer

Going forward

Collaborative research

Expand technological footprint

Build long term relationships

The HPSC core is tightly coupled to the success of its users and collaborators

Take home message

The Human stem cell core can support your stem cell studies by:

1. Reprogramming somatic cells into iPS cells.
2. Differentiate hES and iPS cells into specialized cell types.
3. Develop protocols for directed differentiation.
4. Design and engineer gene editing tools for:
    - Making isogenic iPS cells.
    - Gene knockouts.
    - Deletion of long enhancers and/or short regulatory elements.
    - Making fluorescent reporter lines.
5. Design and engineer synthetic transcription factors for gene regulation

Thank you