iPS Cells – a new therapy for Epidermolysis Bullosa

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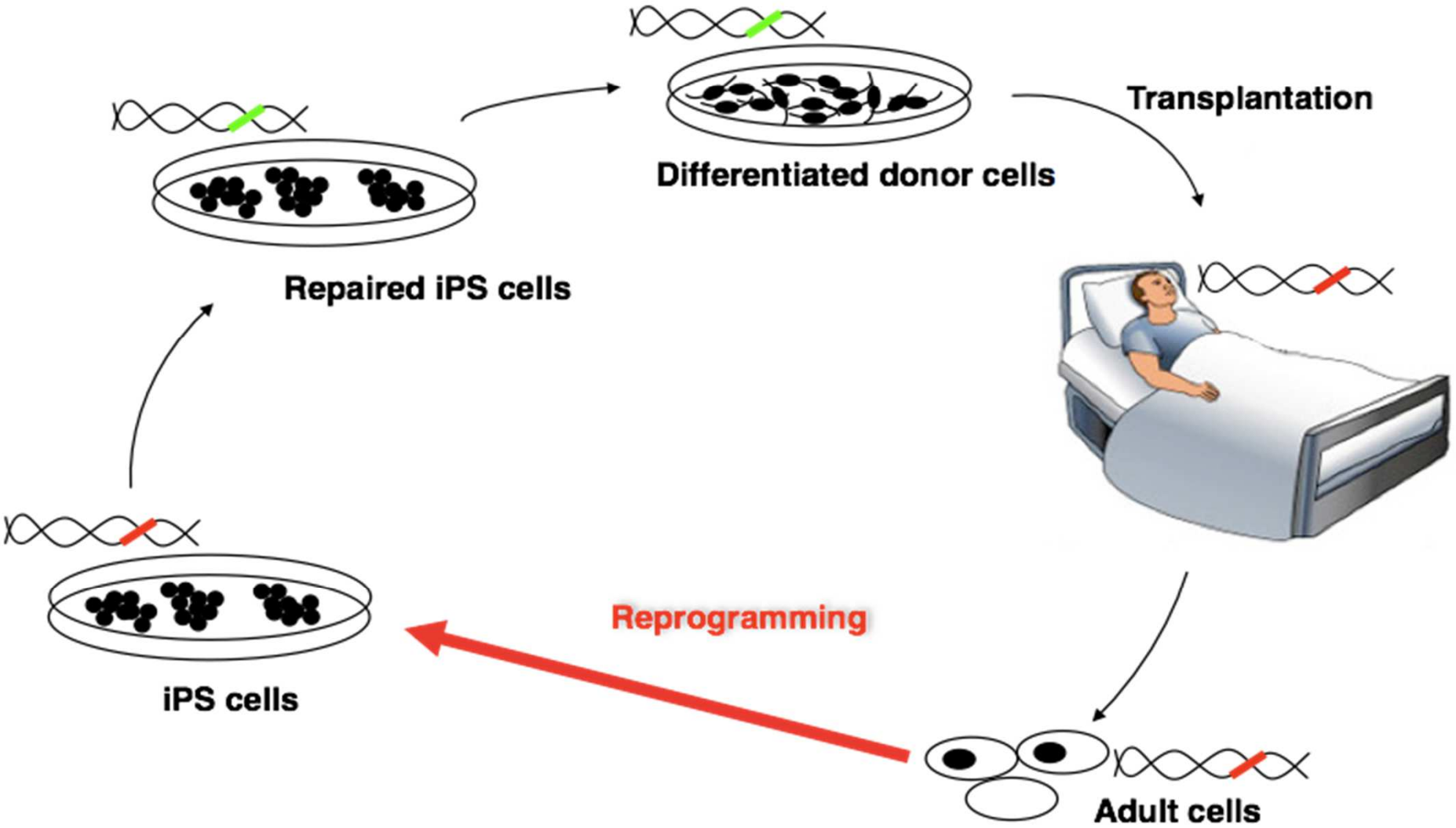
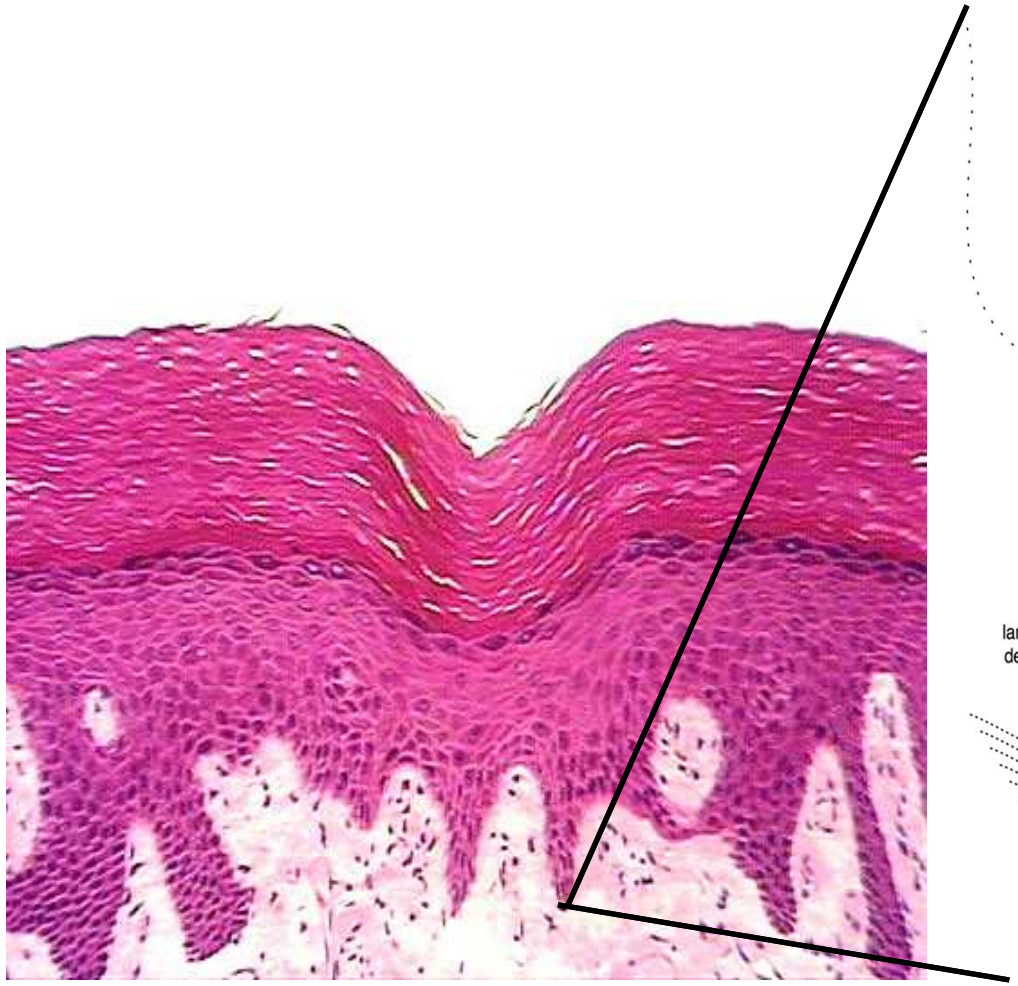
Stanford University

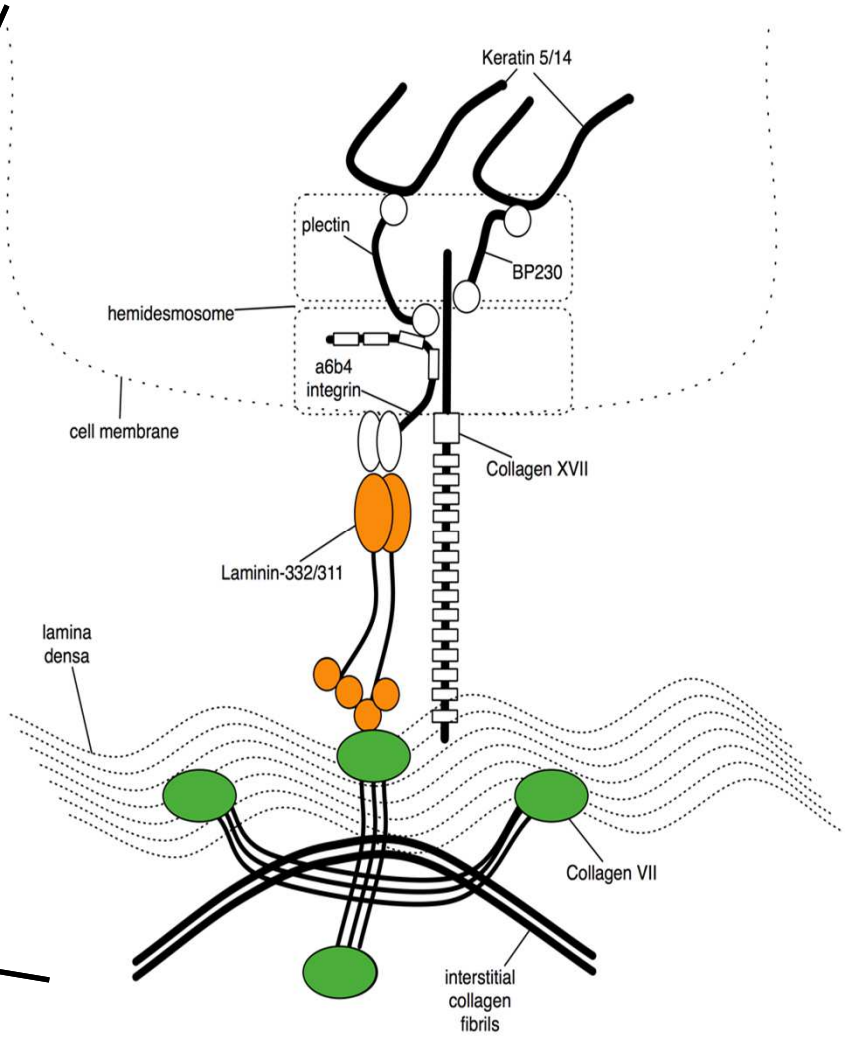
eb clinet meeting, Debra
Salzburg, Austria September 24, 2017

Gene repair in iPS cells



Basal keratinocytes attach to dermis via 12 protein linker complex





Adapted from Fitzpatricks, Marinkovich P, Chapter 62

Dystrophic Epidermolysis Bullosa

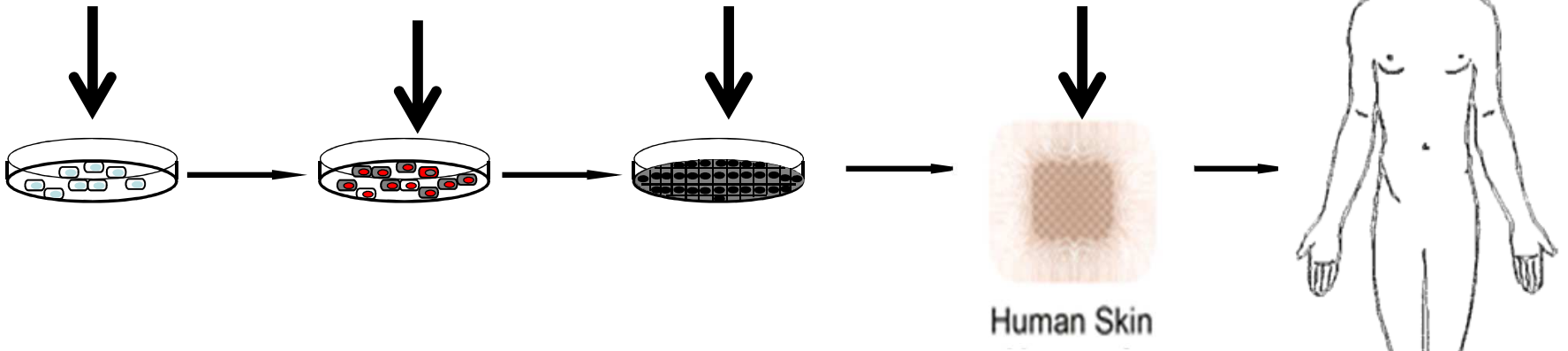
Retroviral gene transfer of COL7A1 cDNA into somatic keratinocytes

RDEB Keratinocytes

Viral transfer.
LZRSE-Col7A1

Epidermal
Sheet
Production

Grafting



6-12 days 3-7 days 2 days 12-30 days

Phase I/IIA Trial IND #13708

(Phase III approved May 2017)

PI: Peter Marinkovich, MD

4 Patients 6 wounds / patient 18 mo follow-up

Safety Efficacy Pain

The Need for Improved Cell-Based Therapy

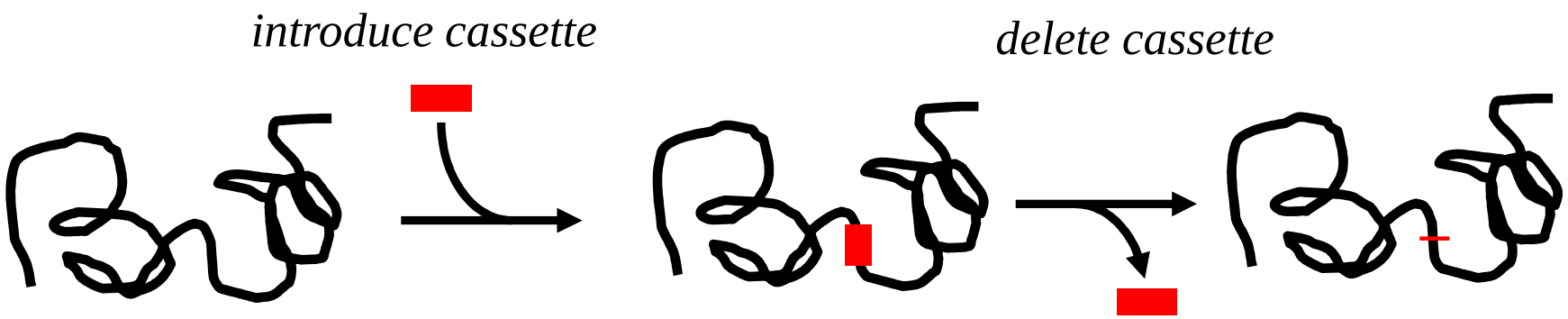
- Low keratinocyte stem cell number in patients
- Limited keratinocyte growth capacity
- Gene Transfer ineffective for dominant negative diseases
- Potential oncogene activation by randomly integrated retroviruses (squamous cell carcinoma!)
- Constitutive COL7A1 overexpression: Potential immunological side effects

everything addressed by iPS cell approach!

DEBCT v1 - iPS cell reprogramming: excisable lentiviral cassette

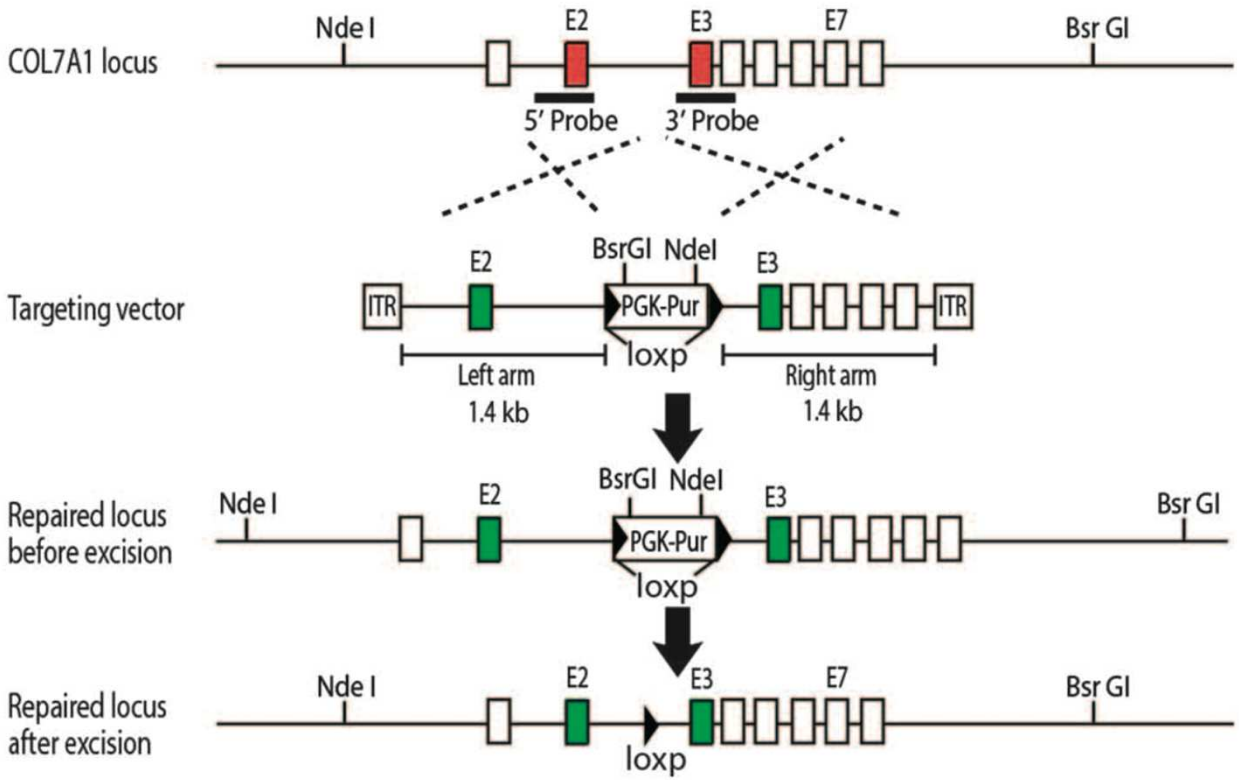
Floxed 4F constructs: EF1a-Oct4 $_{2A}$ Klf4 $_{IRES}$ Myc $_{2A}$ Sox2

(Sommer et al. 2009)



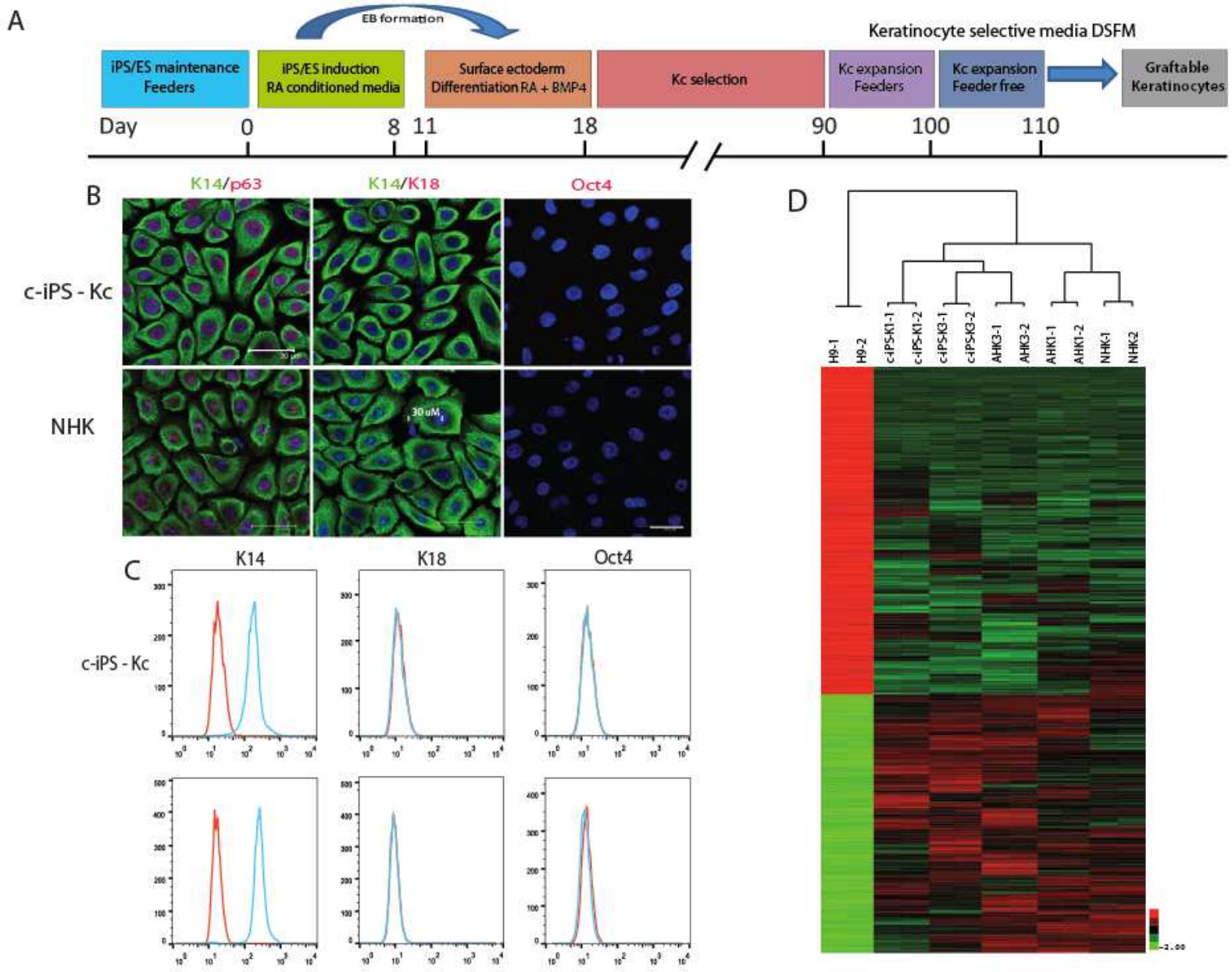
- Reprogramming requires presence of mouse feeders
- Expansion of iPS cells while maintaining normal KT requires mouse feeders

DEBCT v1 – gene targeting: AAV-DJ mediated



Method	Line	Picked clones	Targeted	Corrected allele	Efficiency (%)	Correction (%)
Conventional	K3-4	168	19	5	11.3	26
Conventional	F1-2	110	4	3	3.6	75
AAV	K3-1	30	17	1	56.7	5.9
AAV	F1-1	6	1	1	16.7	100

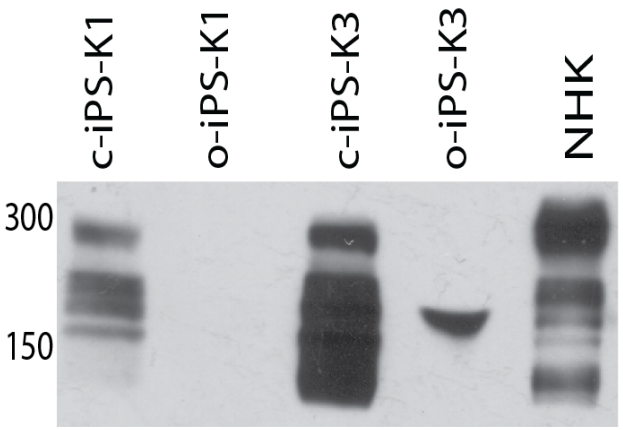
DEBCT v1 – differentiation



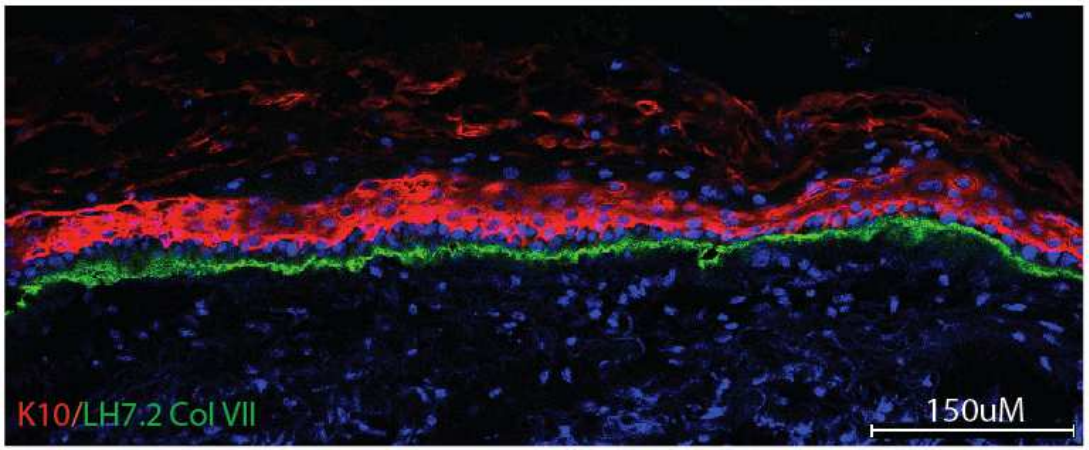
Sebastiano, Zhen et al., 2014 *Sci Transl. Med.*

DEBCT efficacy:
COLLAGEN VII production and stratification of corrected keratinocytes *in vitro*

COL7 Western blot

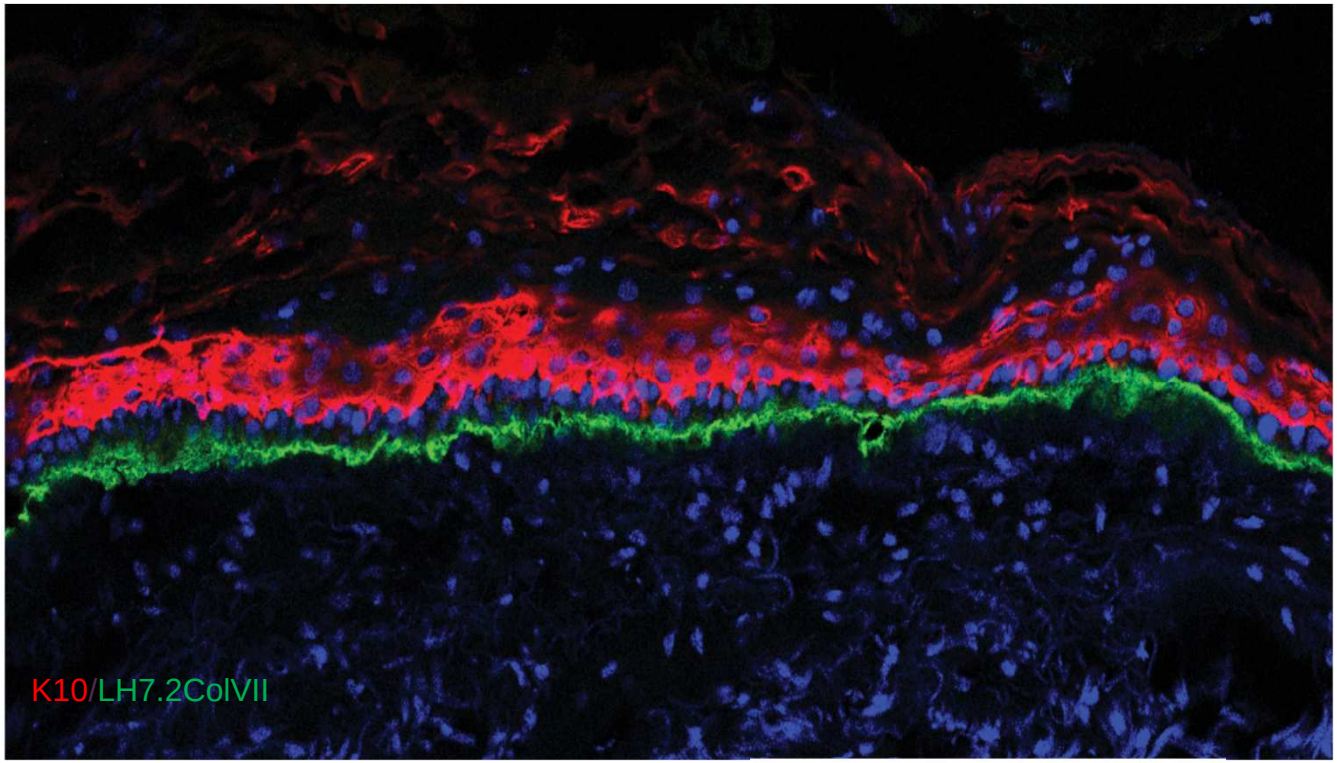


organotypic cultures



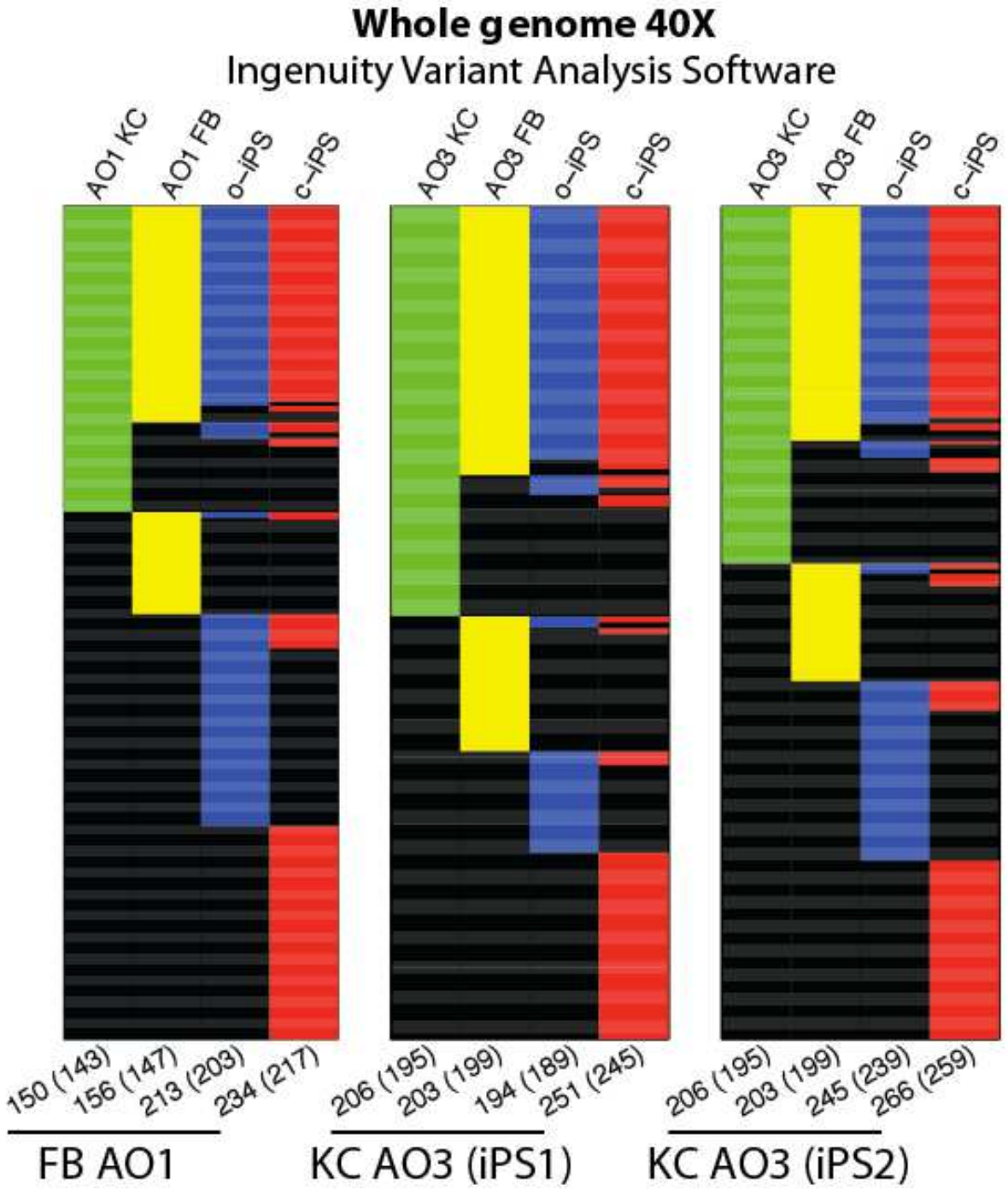
Sebastiano, Zhen et al., *Sci Transl. Med*. 2014

DEBCT efficacy:
Skin formation and Collagen VII production *in vivo*



3-week xenografts on NSG mice

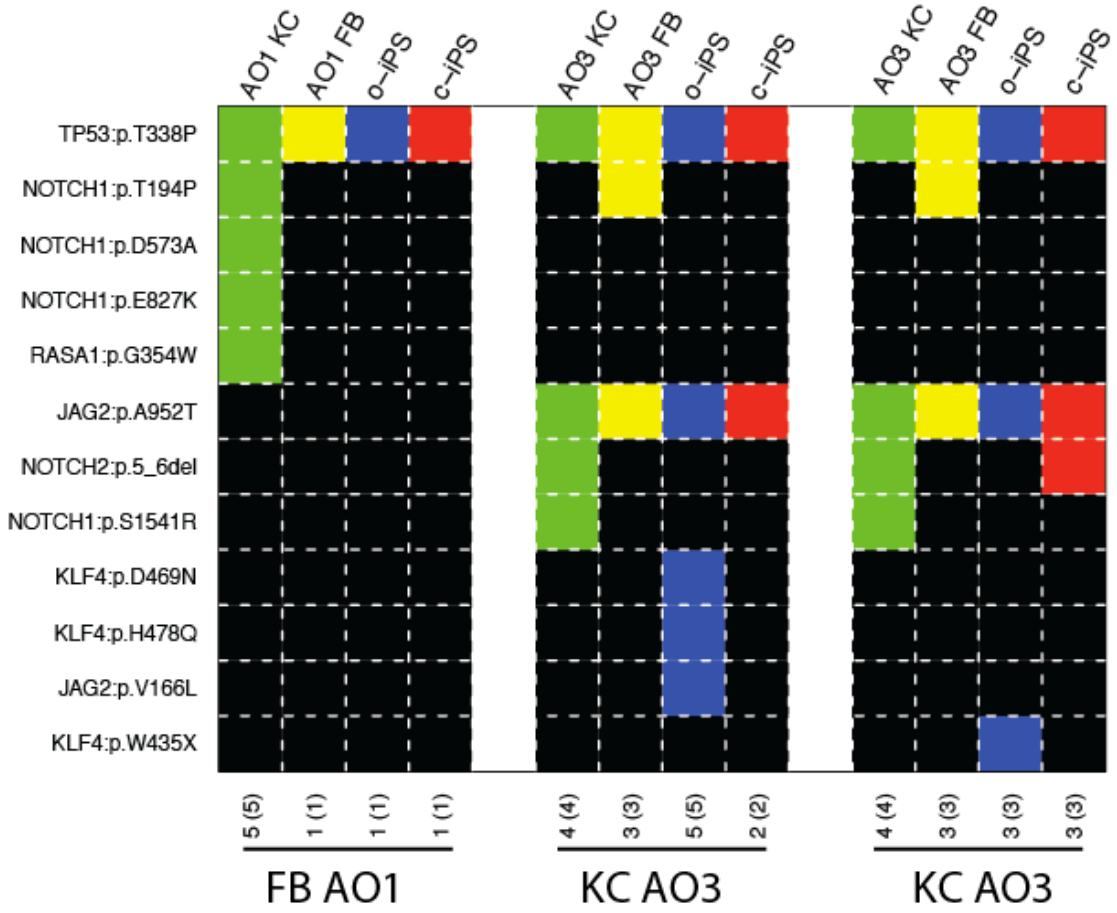
Sebastiano et al., 2014 *Sci Transl. Med.*


Whole genome 40X
Ingenuity Variant Analysis Software
AO1 KC
AO1 FB
o-iPS
c-iPS
AO3 KC
AO3 FB
o-iPS
c-iPS
AO3 KC
AO3 FB
o-iPS
c-iPS
150 (143)
156 (147)
213 (203)
234 (217)
206 (195)
203 (199)
194 (189)
251 (245)
206 (195)
203 (199)
245 (239)
266 (259)
FB AO1
KC AO3 (iPS1)
KC AO3 (iPS2)

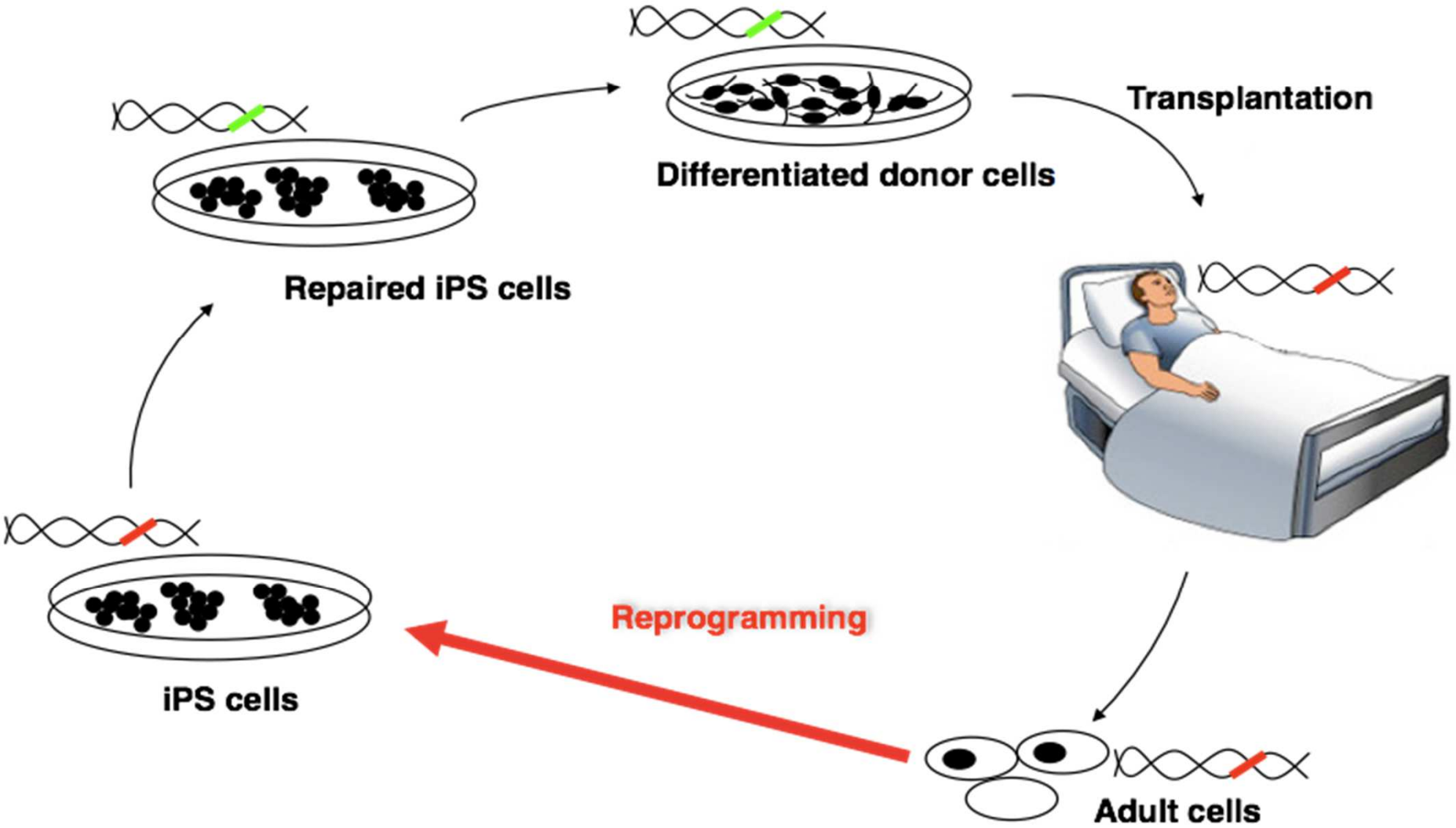
SCC Predisposition 700X
Targeted Resequencing

BWA Pipeline Analysis

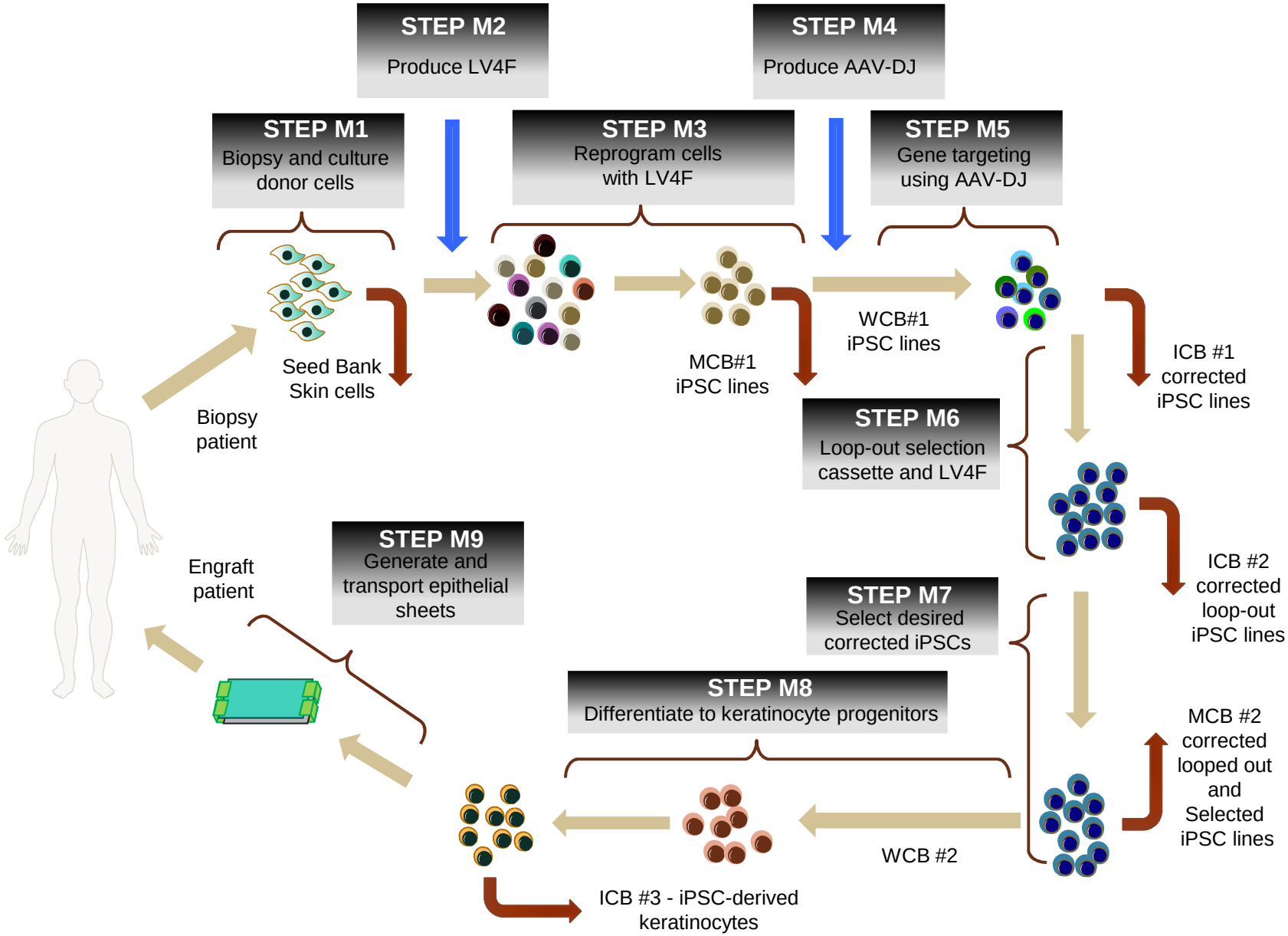
Notch1	*H-ras*
Notch2	*K-ras*
Notch3	*RasA1*
Notch4	*CDKN2A*
Jagged1	*TP53*
Jagged2	*KLF4*
CHUK	



Gene repair in iPS cells

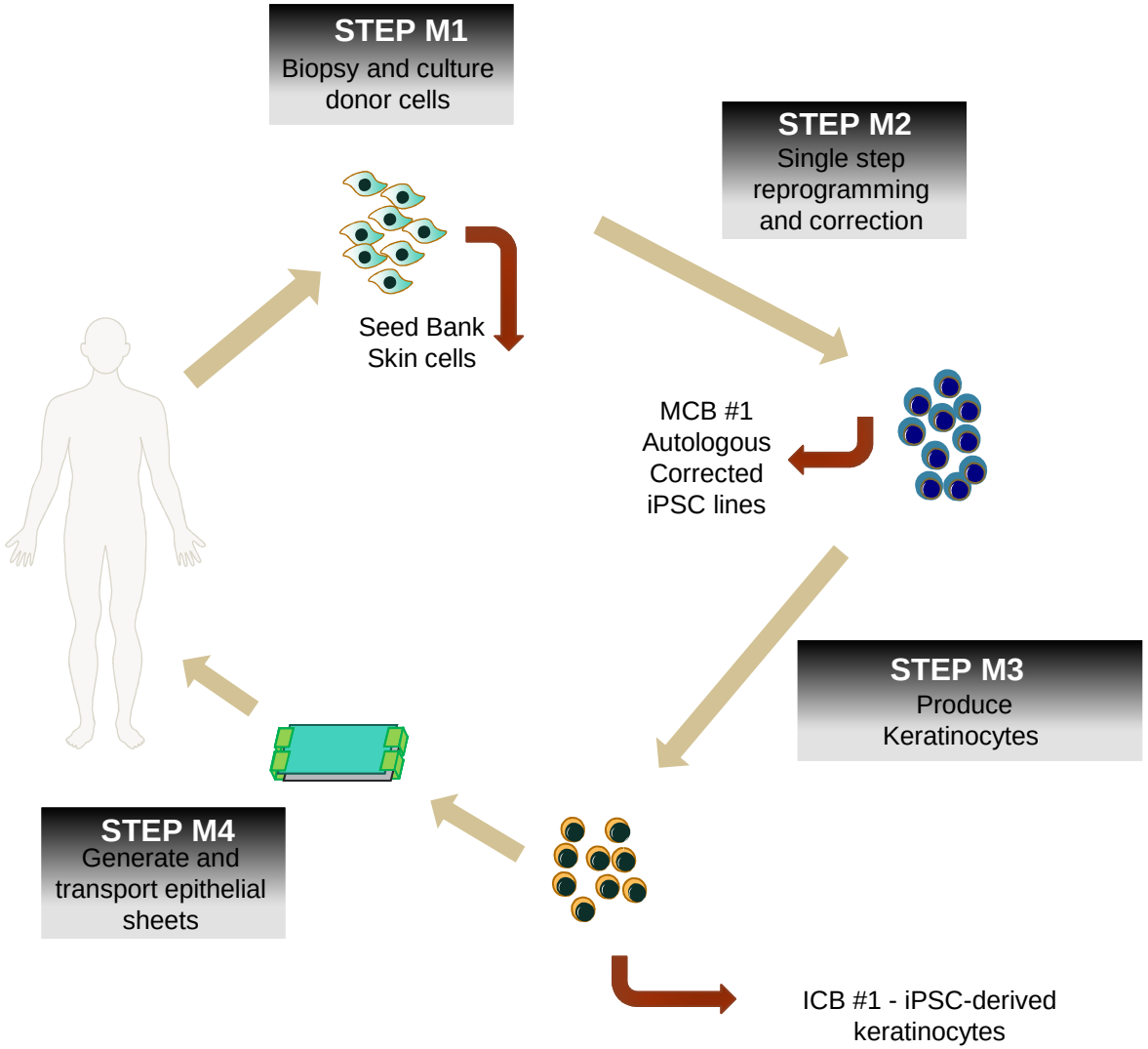


DEBCT v1 – manufacturing process

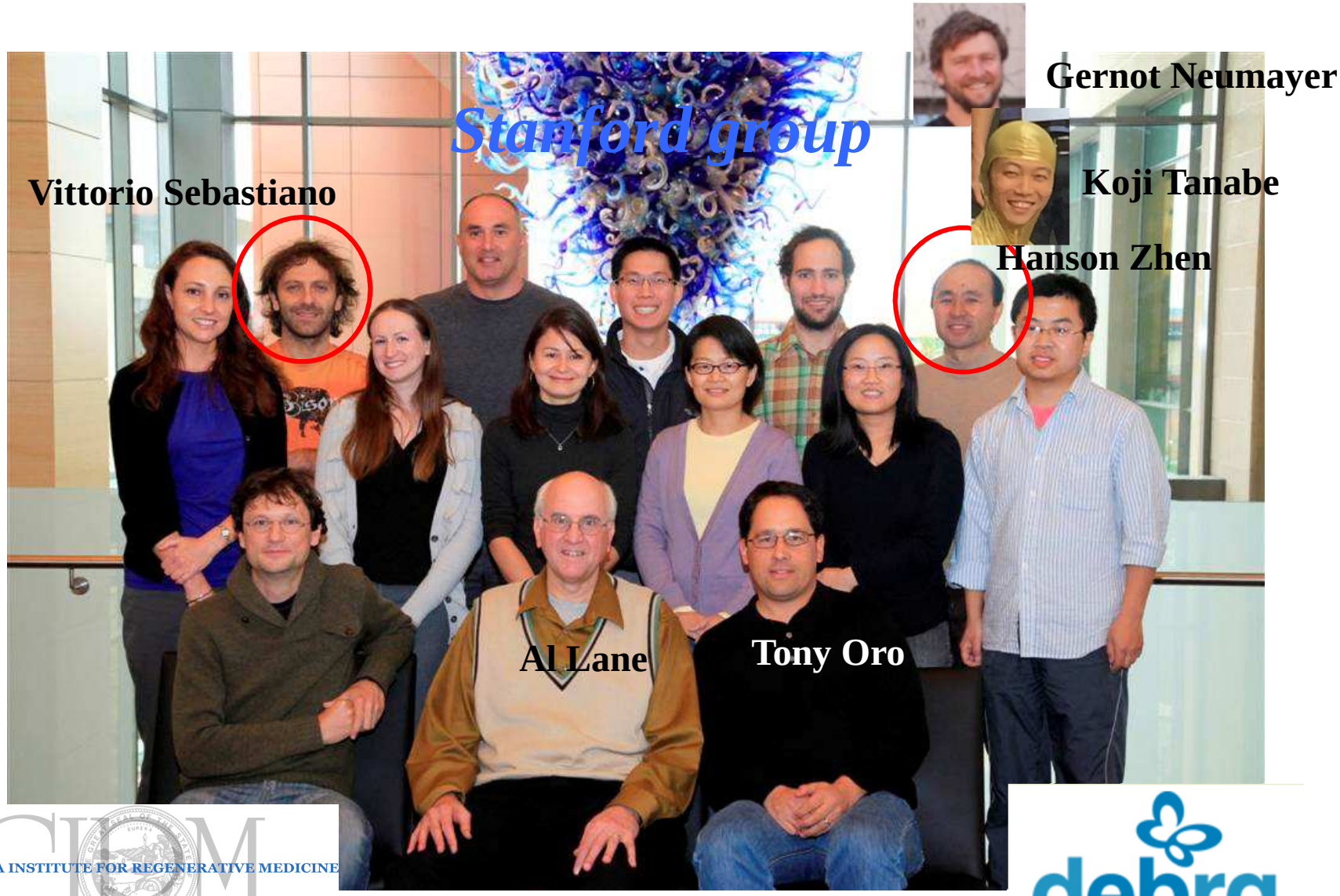


WCB-In process cell bank **MCB**-Master cell bank **ICB**-In process cell bank

Proposal for DEBCT **version 2**



The lab



CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

debra International

EB partnership:

Dennis Roop, UC Denver

Angela Christiano, Columbia University