Gene editing in embryos and germ line

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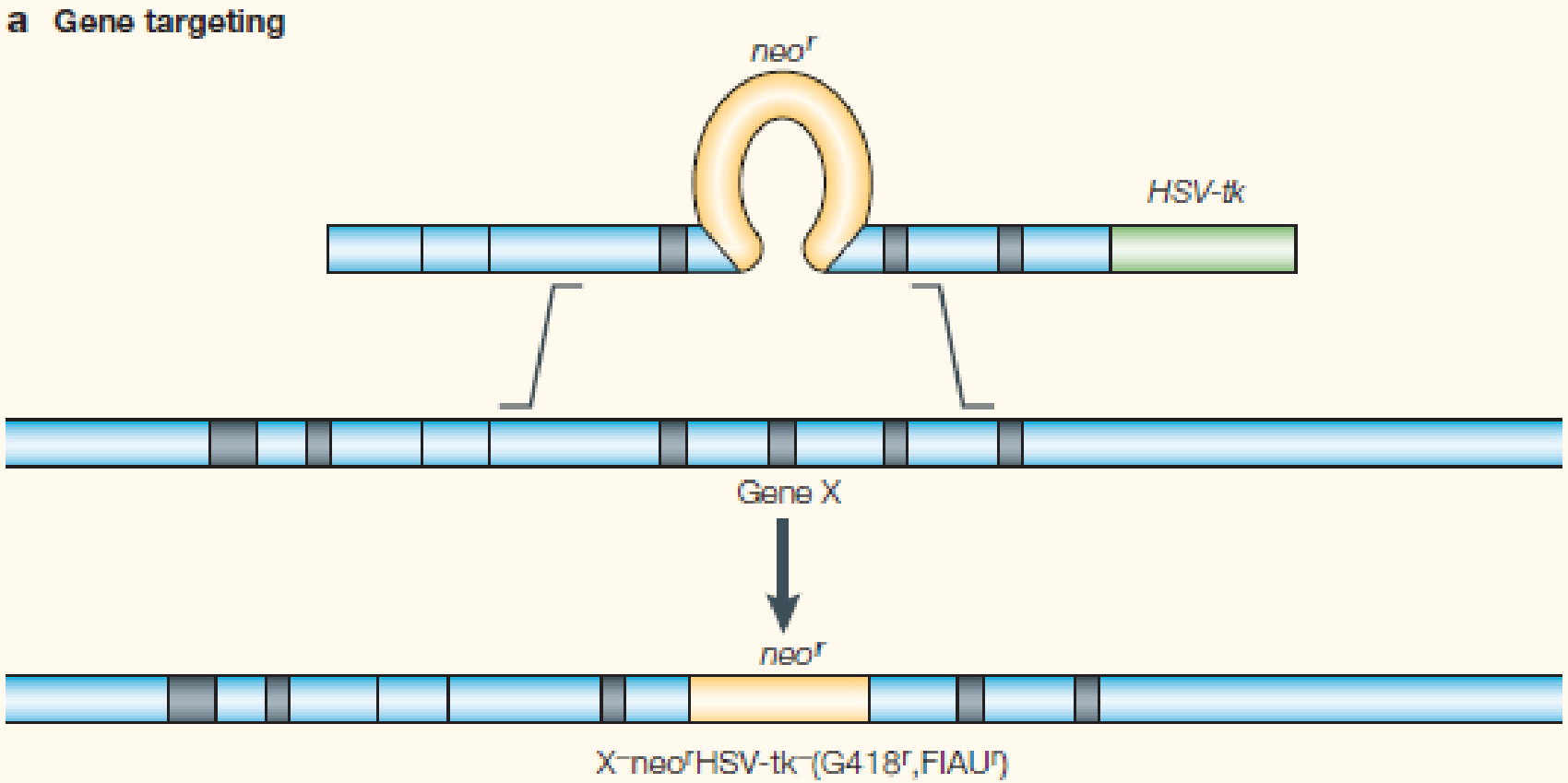
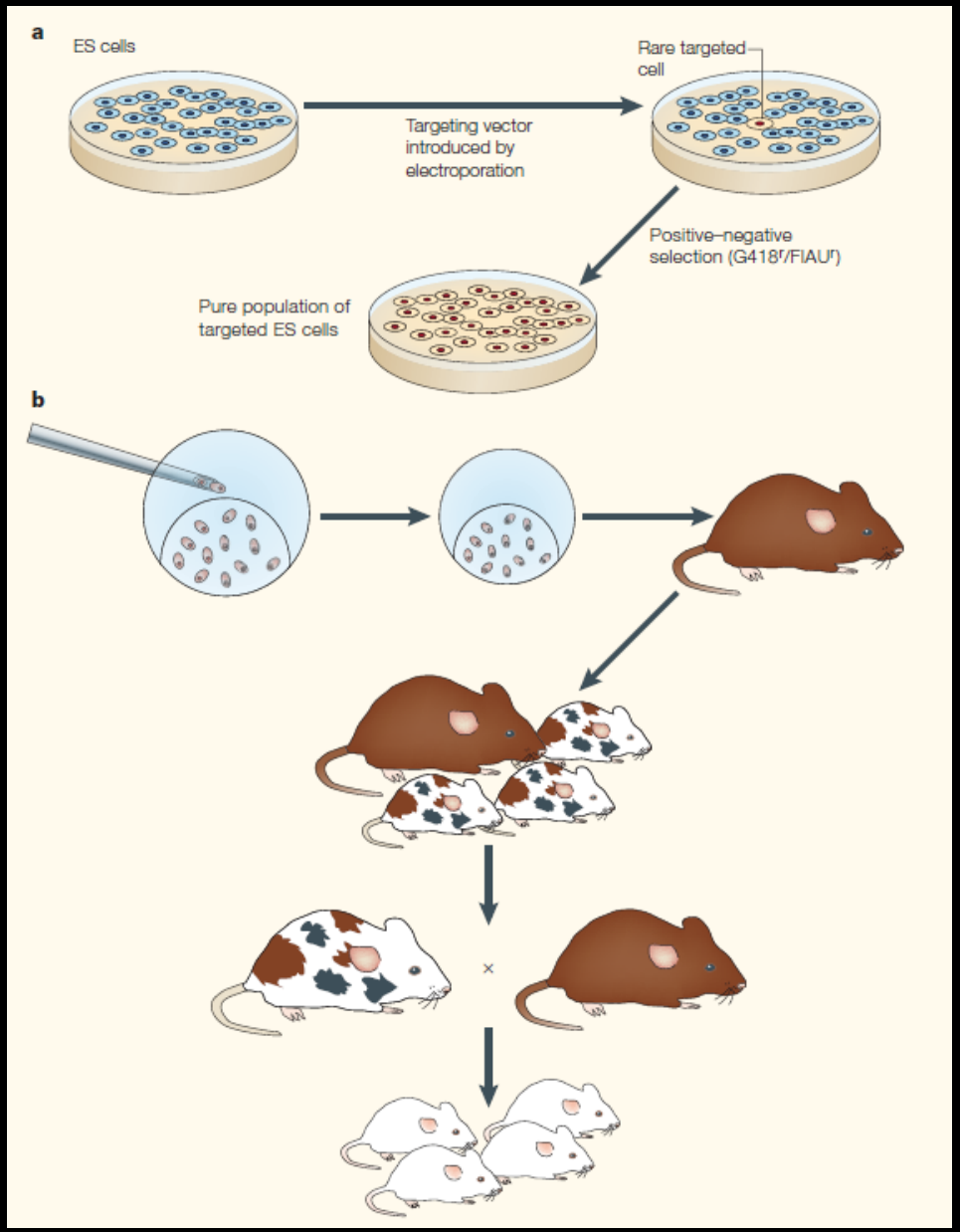
Gene Targeting by homologous recombination



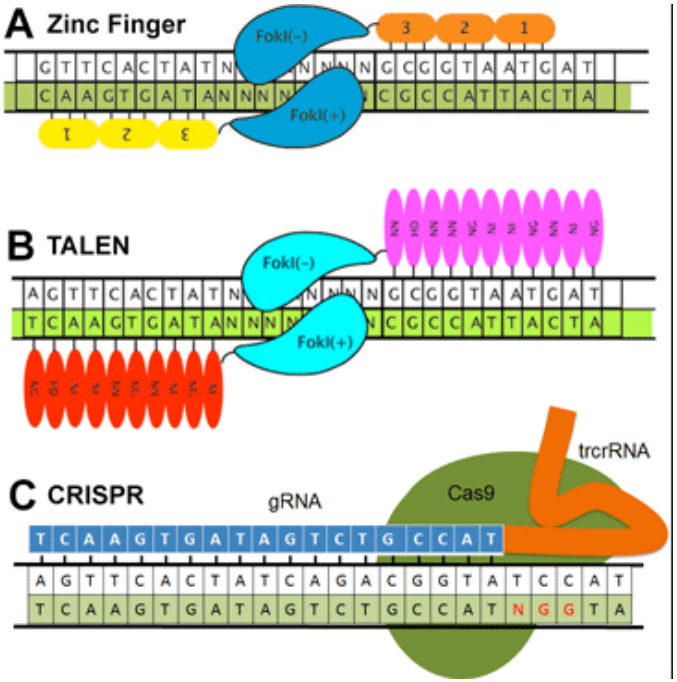
Capecchi, 2005

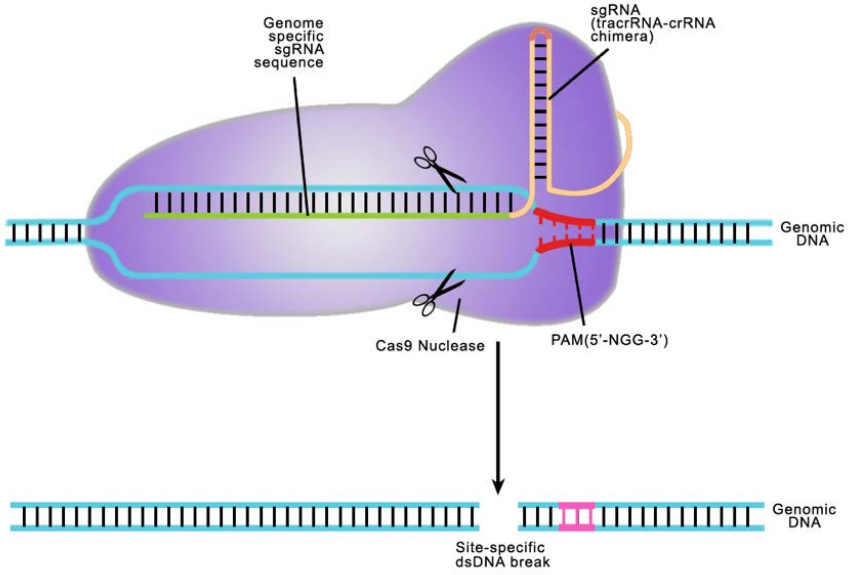


- Gene targeting in mouse ES: very efficient.
- But generation of mice is time consuming
- Applicable *only* to mice: no robust chimera-competent ES cells in other species
- Homologous recombination is inefficient in human ES cells

Capecchi 2005

Genome Engineering:
The Age of Crispr/Cas





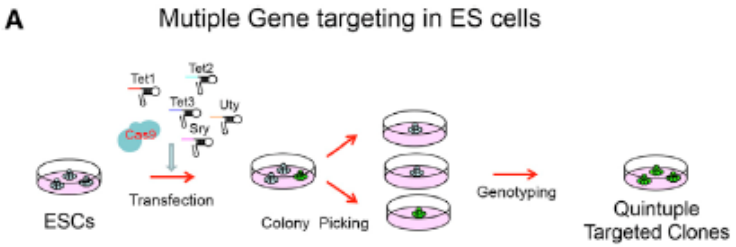
How efficient is this technology for gene editing in animals and human ES / iPS cells?

Multiple gene knockouts:

One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering

Haoyi Wang,[1,6] Hui Yang,[1,6] Chikdu S. Shivalila,[1,2,6] Meelad M. Dawlaty,[1] Albert W. Cheng,[1,3] Feng Zhang,[4,5] and Rudolf Jaenisch[1,3,*]

Cell *153*, 910–918, May 9, 2013



ES cells:

- Single transfection: targeting up to 5 genes *(8 mutant alleles, ~ 50%)*

One step generation of mice with:

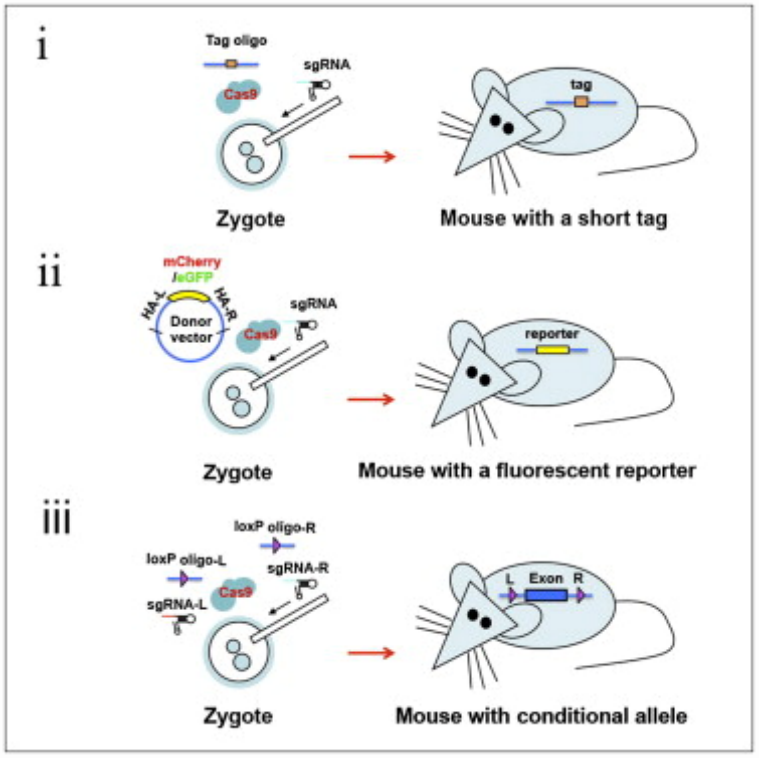
- Homozygous mutations in several genes *(80% efficiency)*
- Predetermined point mutations in several genes *(60% efficiency)*

Reporters, Tags, and Conditional mutants

One-Step Generation of Mice Carrying Reporter and Conditional Alleles by CRISPR/Cas-Mediated Genome Engineering

Hui Yang,[1,4] Haoyi Wang,[1,4] Chikdu S. Shivalila,[1,2,4] Albert W. Cheng,[1,3] Linyu Shi,[1] and Rudolf Jaenisch[1,3,*]

Cell *154*, 1370–1379, September 12, 2013



One step generation of mice with reporters inserted into genes:

- GFP into Oct4, Sox2, Nanog (10 - 30% efficiency)

One step generation of mice conditional mutant mice:

- MECP2 (16% efficiency)

Mice with defined deletions (30% efficiency)

Off-target mutations

(Unintended genetic alterations)

Evidence obtained in cultured cancer cells argued for a very high rate of off-target cleavage

(mutations in other than the intended genomic sites: Fu et al, 2013; Hsu et al, 2013)

Off-target (OT) analysis in gene-edited mice

- We tested off-targets (1 – 3 mismatches) of 7 sgRNAs targeting 6 genes in 40 mutant mice
- *Three off-target cleavages at sites that differed at the 5' position of the guide RNA*

High specificity of CRISPR/Cas mediated cleavage when using well-designed guide RNAs

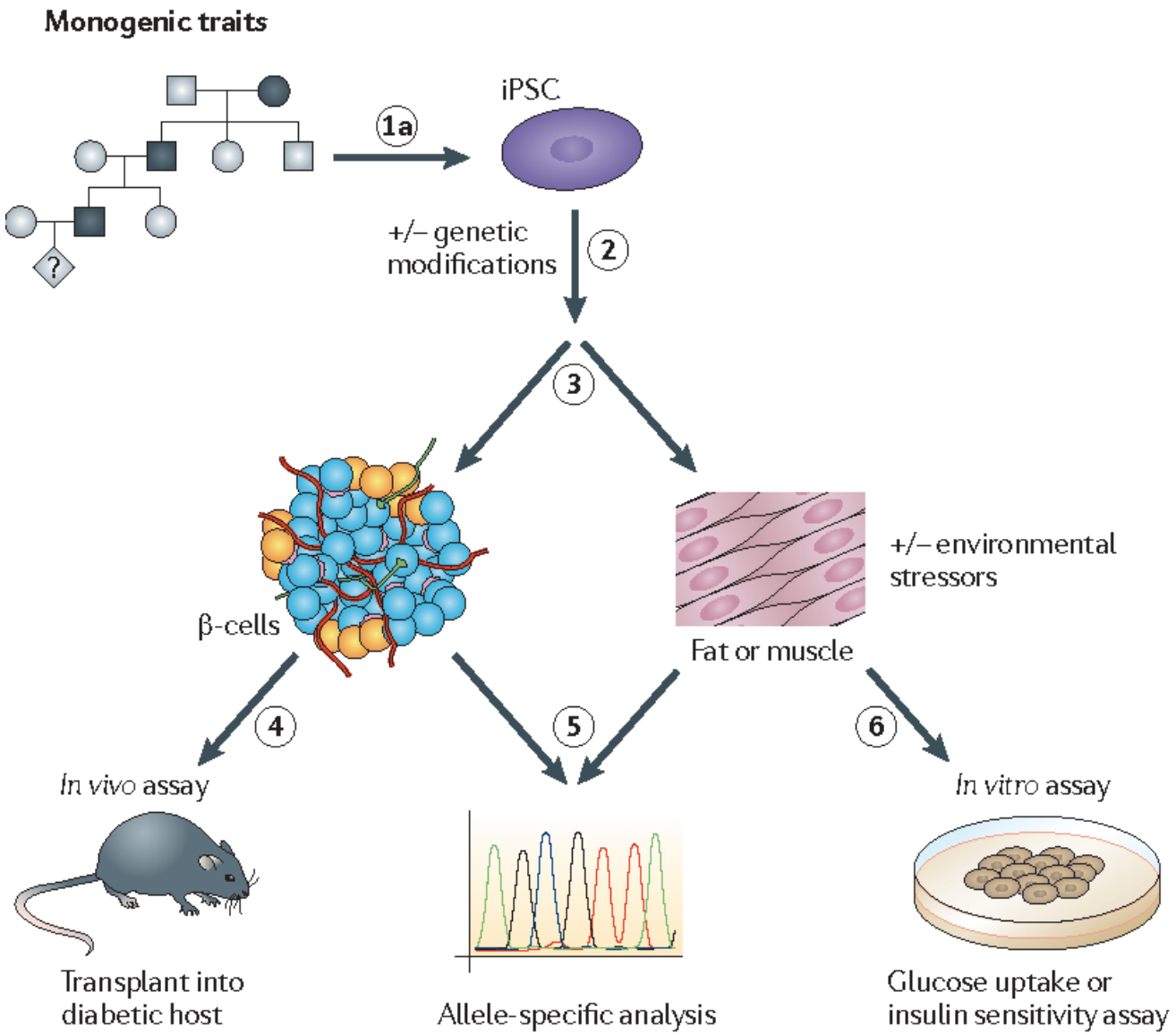
Timing of gene insertion / editing

In more than 50% of manipulated embryos integration of donor DNA DNA occurs later than at the 1-cell stage resulting in

mosaic embryos

Only part of the embryo's cells carry the insert

Mosaicism is more serious for *insertion* of DNA (for gene correction) than *inactivating* a gene (CCR5)

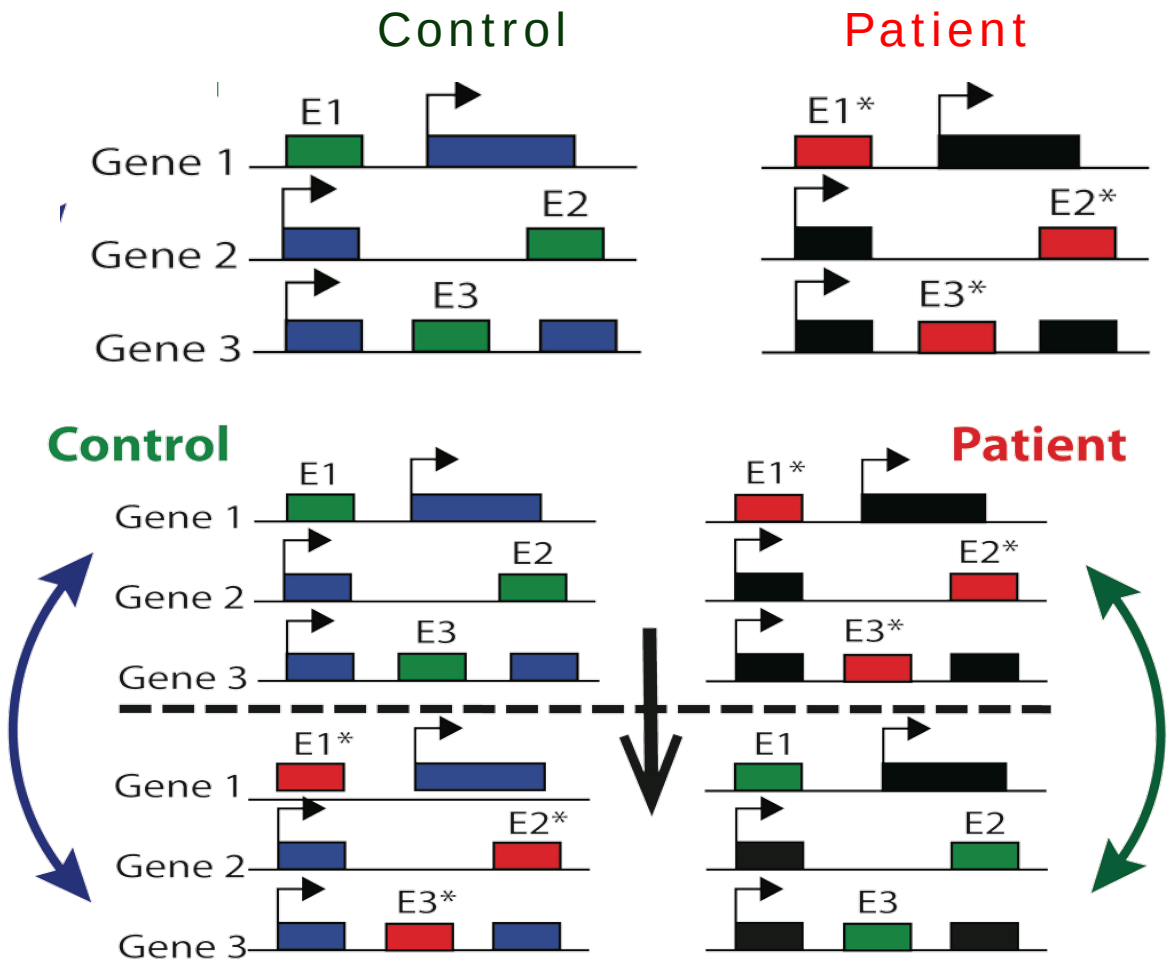


Zhu & Daley, 2011

GWA studies to define genomic loci involved in diseases

Sporadic ‘idiopathic’ forms
of Parkinson disease
(>90%)

Many regulatory elements (enhancers) affect gene expression and contribute to disease



These "isogenic" cell lines differ exclusively at the disease causing mutation

Applications of Genome Engineering

Rodents, pigs, monkeys

Human ES / iPS cells

Disease Modeling

Resource

Cell *156*, 1–8, February 13, 2014 ©2014 E

Cell

Human embryos, germ line?

Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

Yuyu Niu,[1,5,7] Bin Shen,[2,7] Yiqiang Cui,[3,7] Yongchang Chen,[1,5,7] Jianying Wang,[2] Lei Wang,[3] Yu Kang,[1,5] Xiaoyang Zhao,[4] Wei Si,[1,5] Wei Li,[4] Andy Peng Xiang,[6] Jiankui Zhou,[2] Xuejiang Guo,[3] Ye Bi,[3] Chenyang Si,[1,5] Bian Hu,[2] Guoying Dong,[3] Hong Wang,[1,5] Zuomin Zhou,[3] Tianqing Li,[1,5] Tao Tan,[1,5] Xiuqiong Pu,[1,5] Fang Wang,[1,5] Shaohui Ji,[1,5] Qi Zhou,[4] Xingxu Huang,[2,*] Weizhi Ji,[1,5,*] and Jiahao Sha[3,*]

vivo genome liting

Combined with cell therapy

Some applications for gene editing in the human embryo/germ line

I. Inactivation of susceptibility genes to achieve disease resistance

II. Correction of disease causing mutations

I. Enhancement

I. Disease resistance by inactivation of a susceptibility gene

Two examples:

- Inactivation of HIV receptor to achieve resistance of blood cells to AIDS
- Inactivation of PCSK9 to lower risk of heart disease

-> This is a rather straightforward and efficient

A consideration:

- Blood cells and liver can be manipulated in postnatal individuals by somatic cell gene editing
- *Thus, germ line editing may not be required*

II. Correction of disease causing mutation: *Issues*

Genotype of embryos:

- For recessive diseases 75% of the embryos will be normal
- For dominant diseases 50% of the embryos will be normal
- If one parent is homozygous mutant: 0% of embryos will be normal

→ *How to distinguish mutant from wt embryos?*

Potential complications of gene correction

- *No possibility* to distinguish mutant and normal embryos

→ *Any manipulation will alter genes in 50% or 75% normal embryos (unless one parent is homozygous)*

- In any embryo with one corrected allele, the other allele will likely be mutated by NHEJ

→ *transmission of one corrected and one newly mutated allele to next generation*

. Majority of manipulated embryos will be mosaics

→ *This precludes identification of correctly manipulated embryos by PGD*

III. Enhancement

Example:

- Insertion of genes into expression locus (AAVS1)

Growth hormone: increased height

- Will work: predictable transgene expression
- *Enhancement poses not as much of a scientific than an ethical issue*

Gene editing of human embryos

1. Clinical / therapeutic:
   - *While possible, may have limited if any therapeutic application*
2. Basic research:
   - *Will likely give useful information*