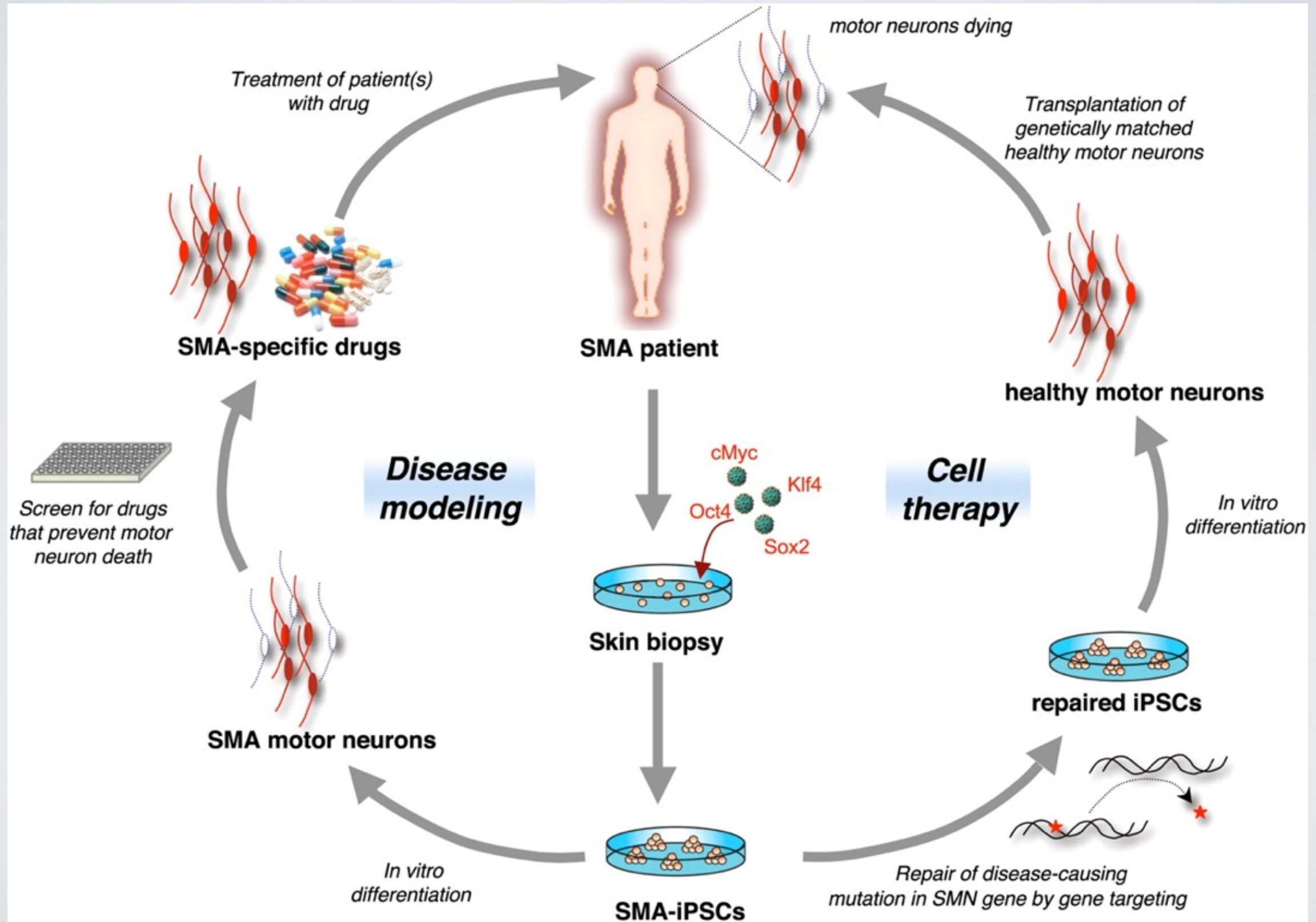


Adapting Induced Pluripotent Stem Cells For Clinical Use

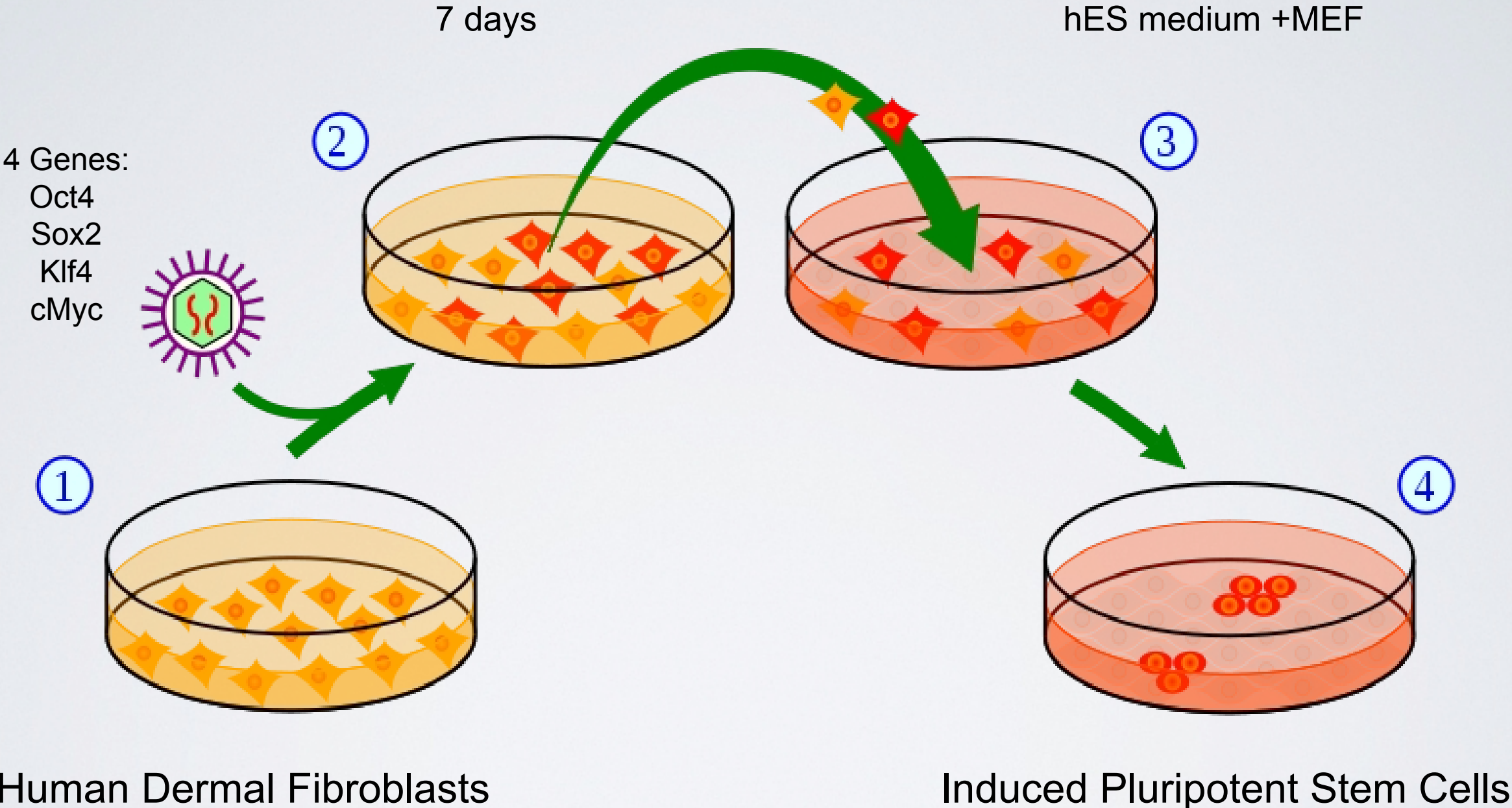


Dana Wyman
Professor Brutlag
Genomics and Medicine

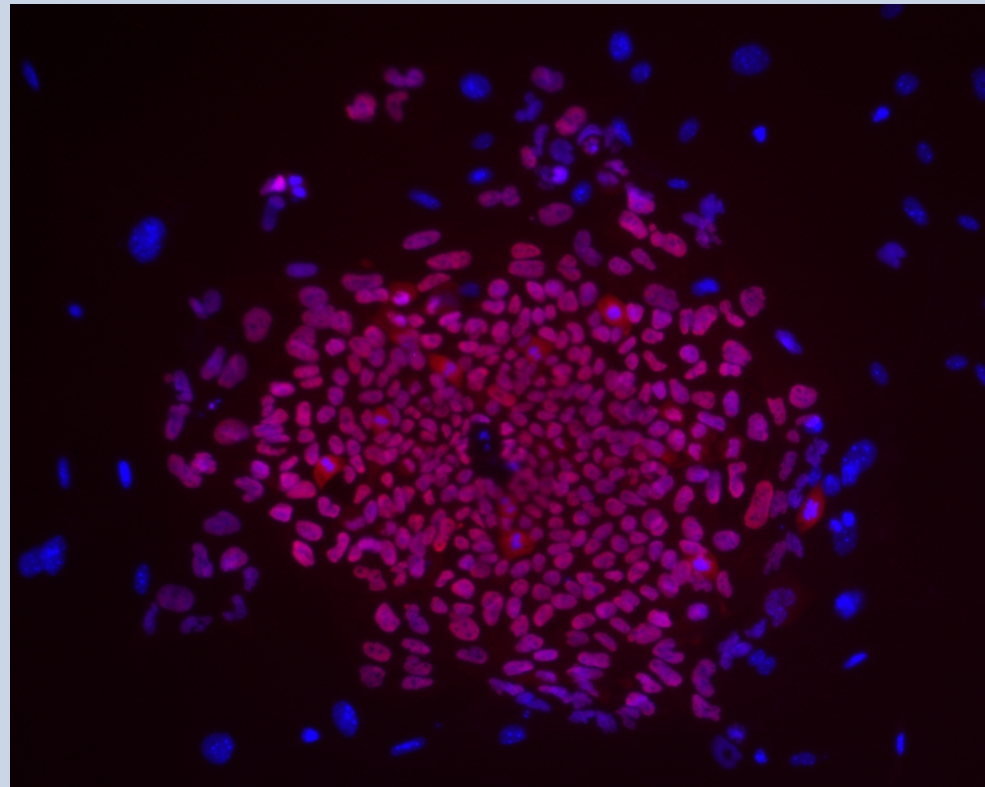
Introduction



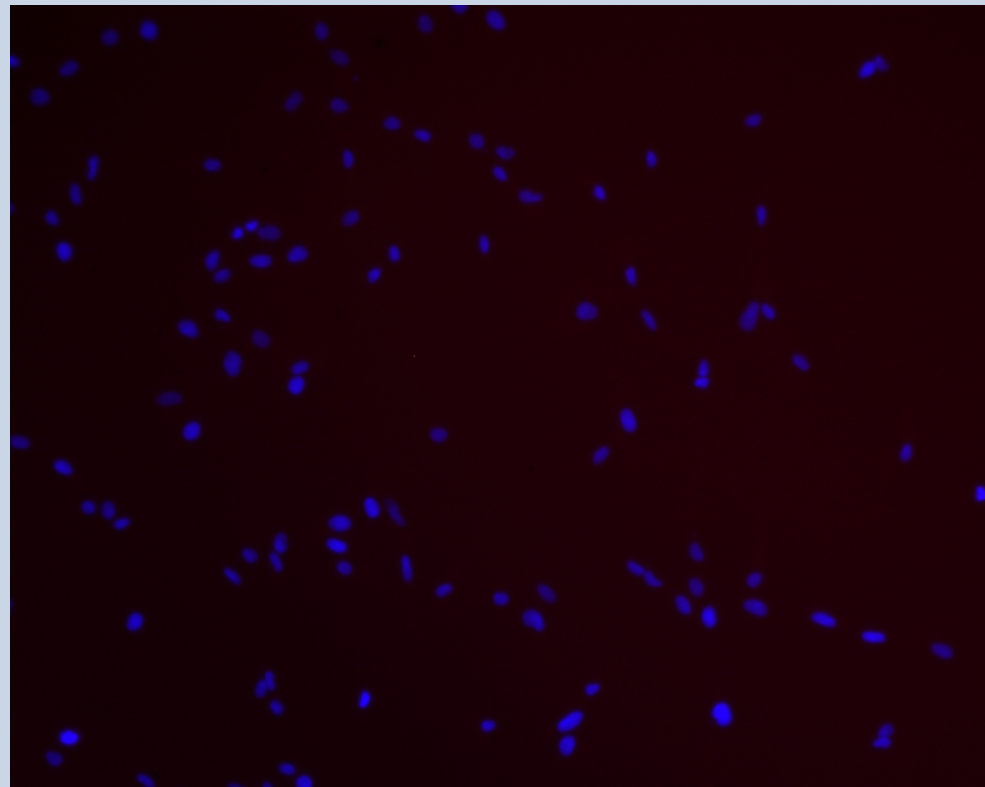
Yamanaka Method



Immunofluorescence Test

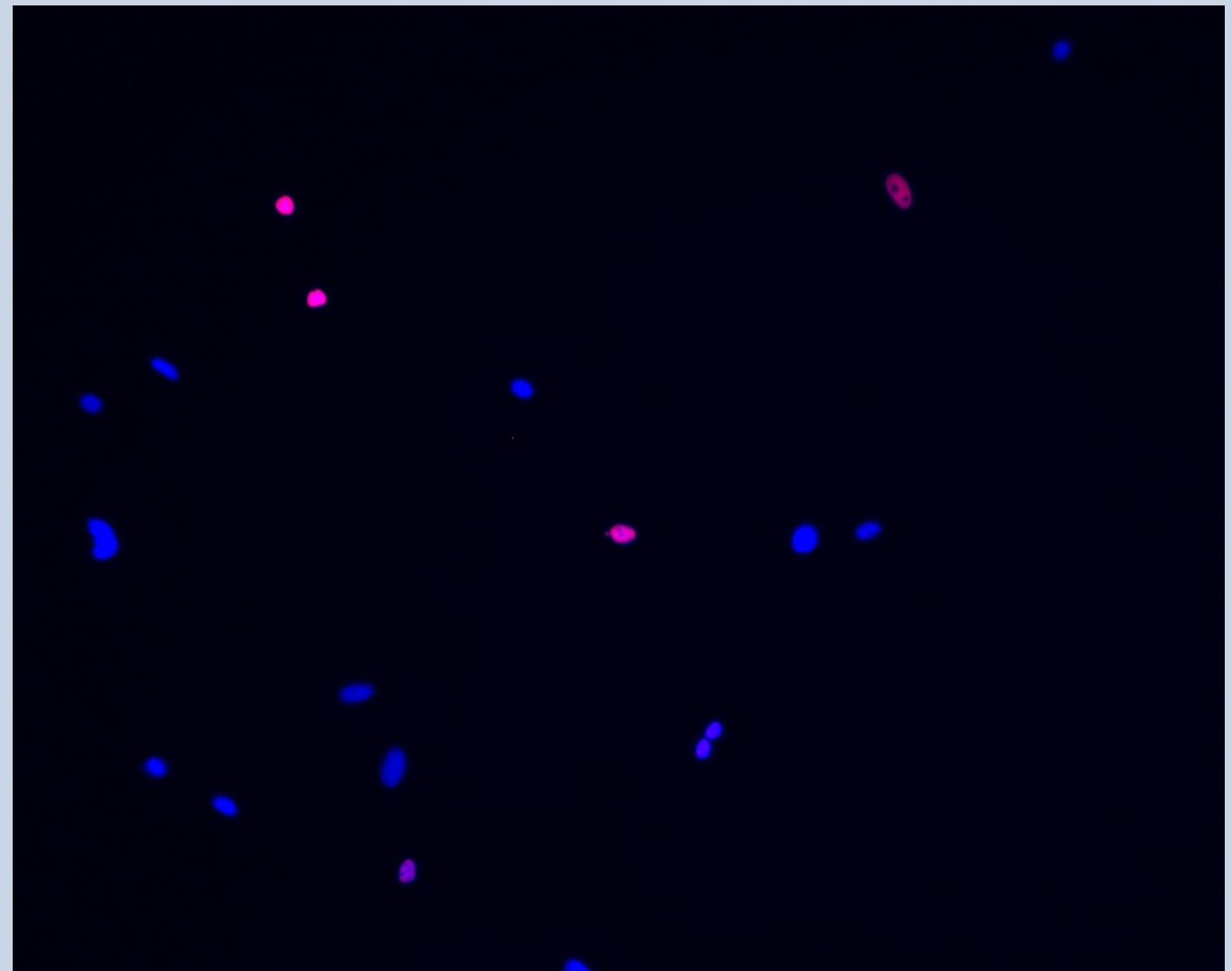


ES Cells (+ control)



Untransduced Fibroblasts (- control)

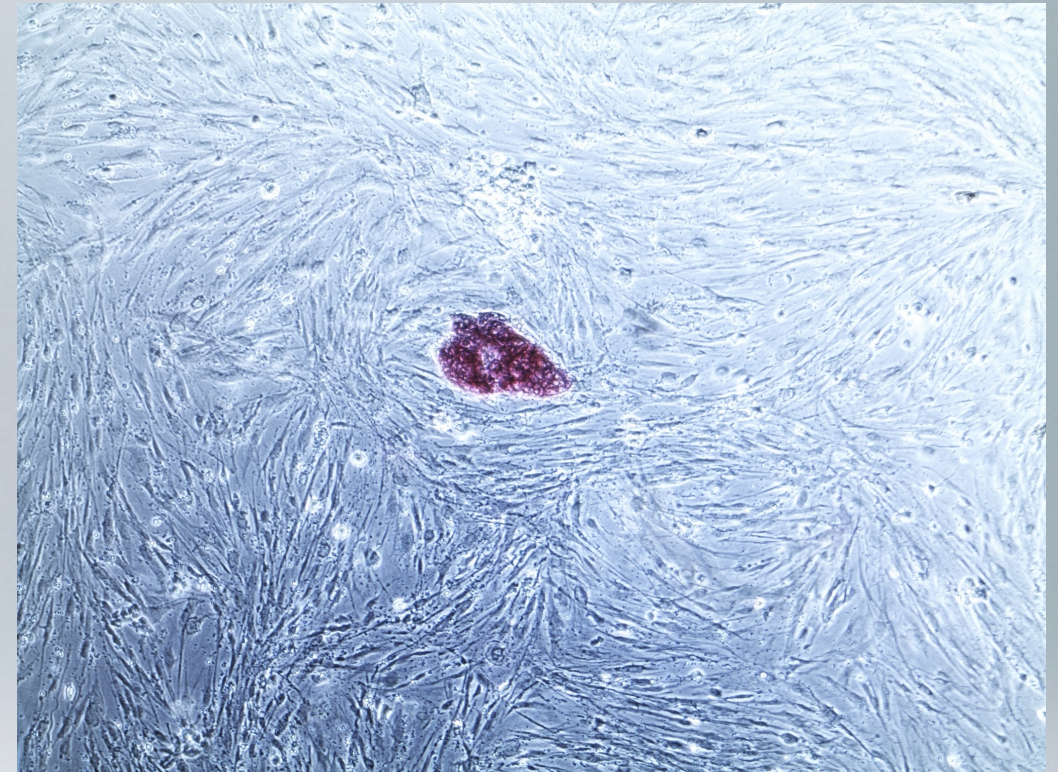
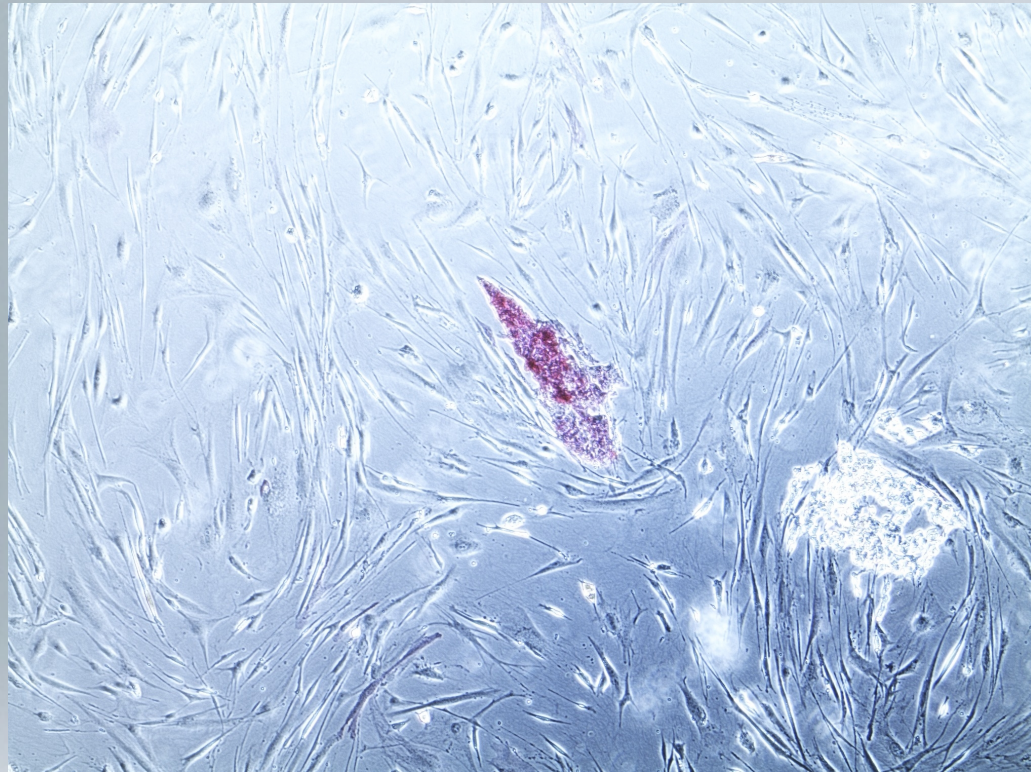
Oct 4-transduced Fibroblasts



Blue (DAPI): nuclear membrane of all cells

Red : Cells containing the Oct 4 protein (expressing the Oct 4 gene)

Alkaline Phosphatase (AP) Stain



These AP positive iPSC colonies are expressing surface antigens characteristic of ES cells, as indicated by the red stain.

Problems with the Yamanaka Method

- Carcinogenic reprogramming factors
- Viral integration into the genome
- Interferes with endogenous gene expression
- Can lead to tumors
- Large number of viral integrations per cell

Approaches to iPSC Generation

Integrating Vectors

- Retro/Lentiviruses

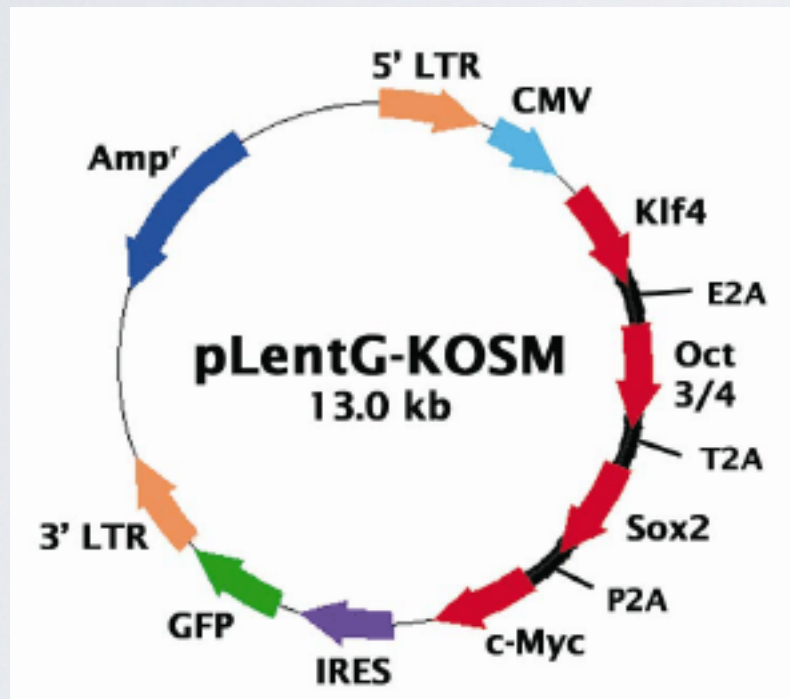
Excisable Vectors

- Lox-CRE mediated excisions
- Transposons

Non-Integrating Vectors

- Direct Delivery of Proteins
- MicroRNA

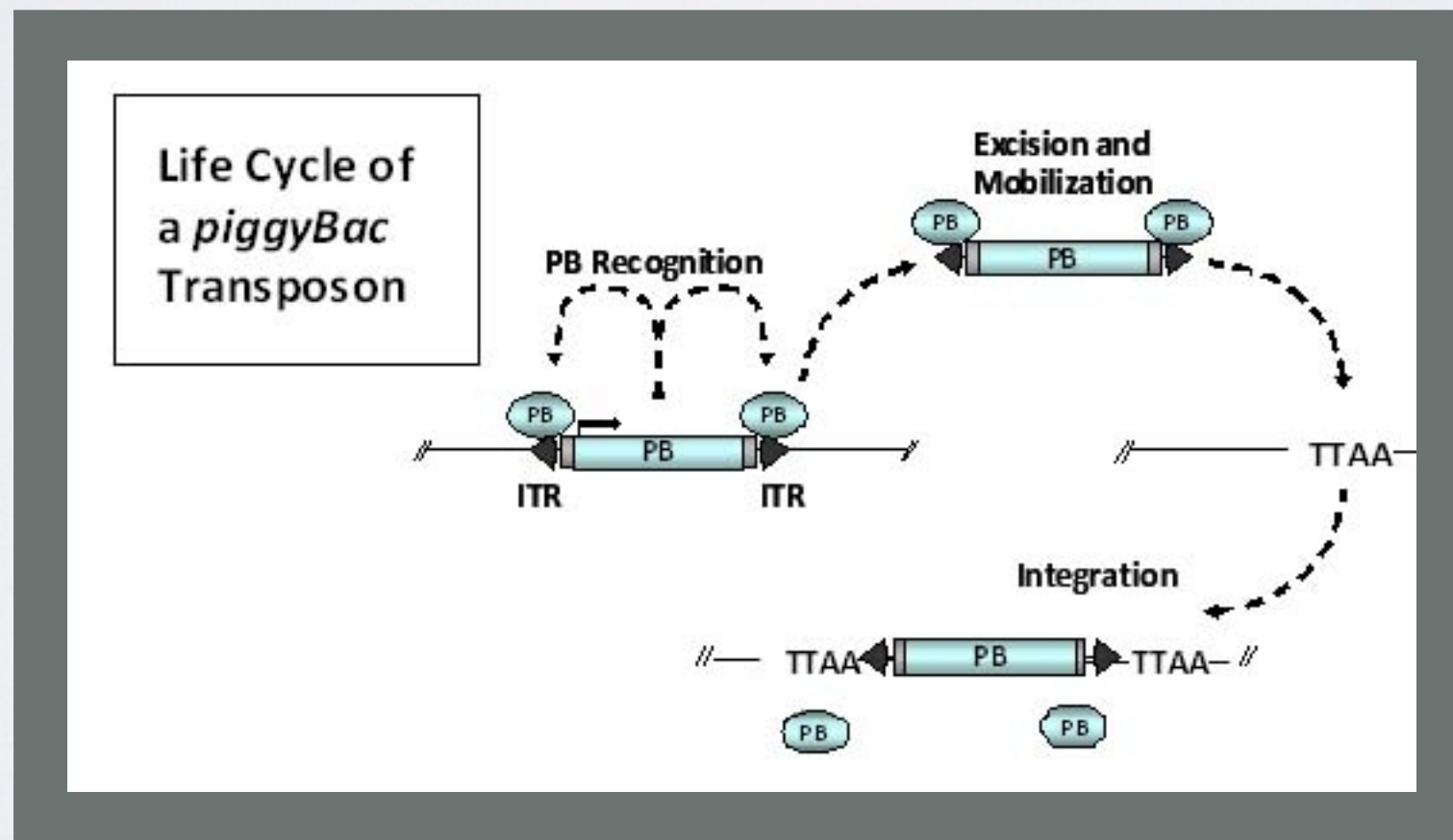
Safer Viral Integration



- Using fewer reprogramming factors
- Specifically avoiding Klf4 and c-Myc
- Using a single expression “cassette” rather than multiple vectors

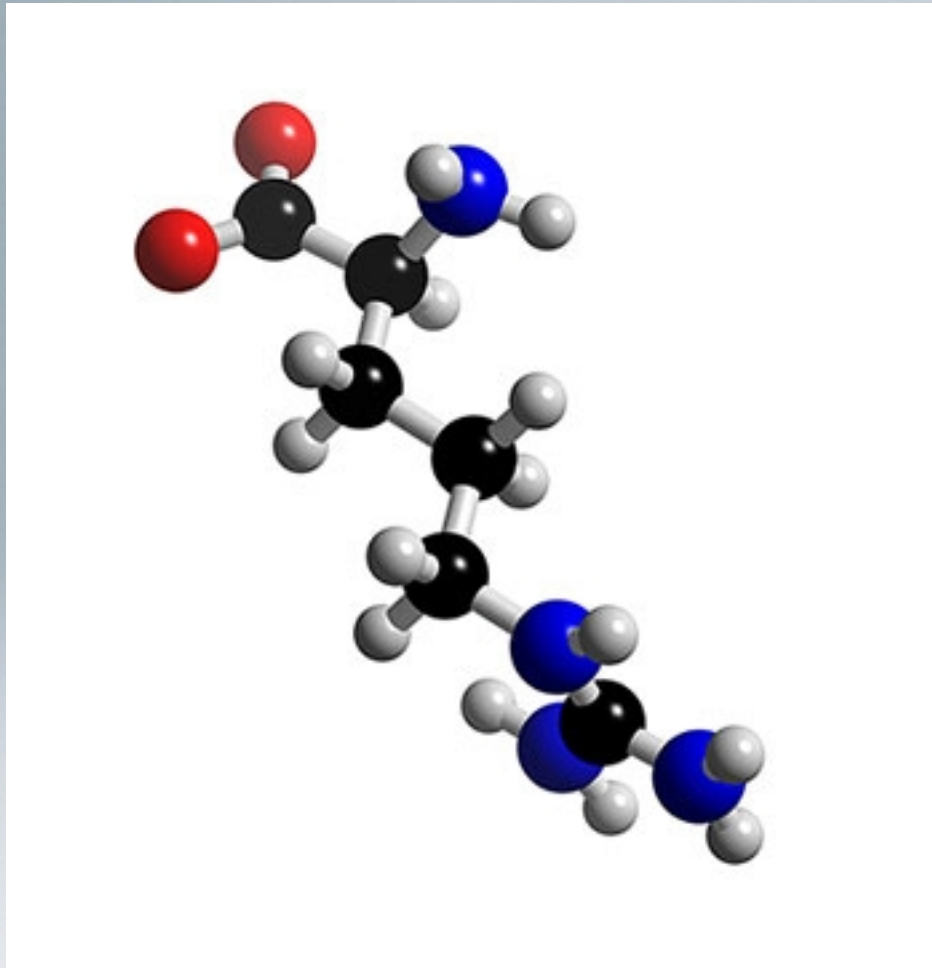
Excisable Vectors

- piggyBac transposons
- Capable of carrying 10 kilobases of DNA
- Can be excised seamlessly using piggyBac transposase



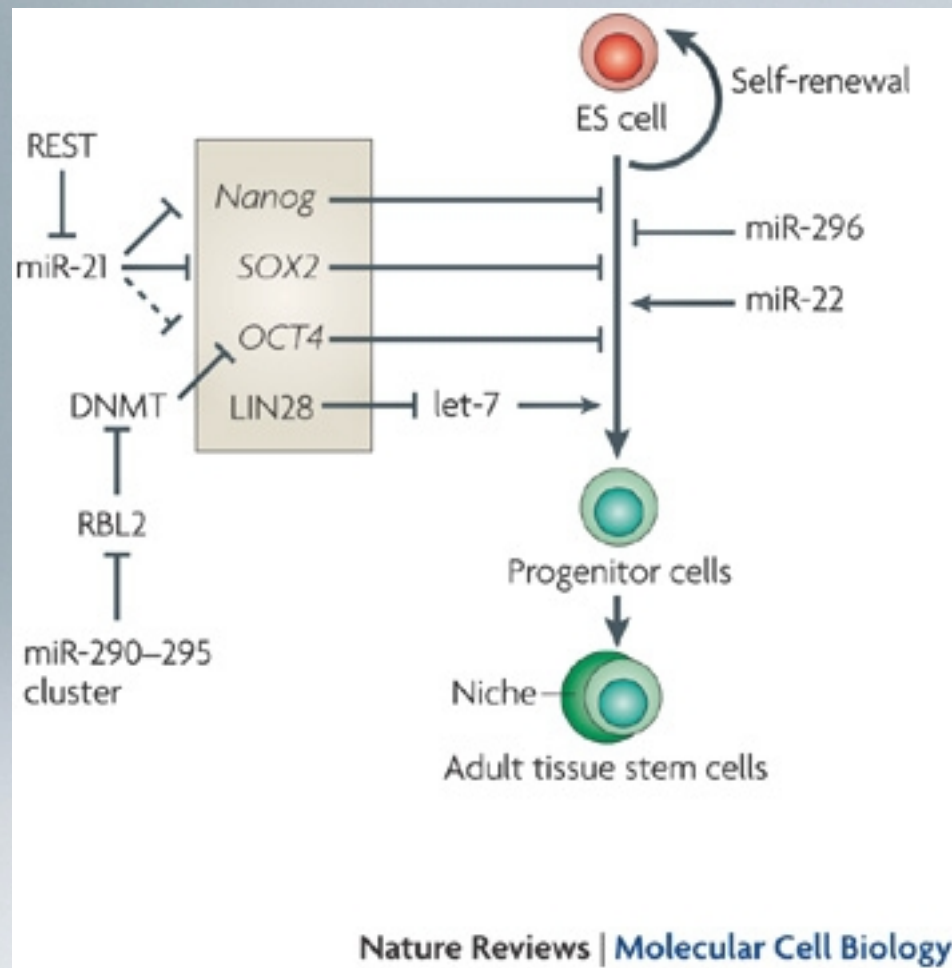
Protein Delivery

- Purified reprogramming proteins are attached to a cell-penetrating peptide



- No integration into the genome
- No nucleic acids involved
- More time consuming and less efficient than viral integration
- Multiple rounds of protein treatment may be required for full reprogramming to occur

MicroRNA



- A subset of microRNA exclusively found in ESCs is thought to regulate expression of developmentally important genes
- Used with other methods to increase their efficiency
- Can replace the reprogramming factor c-Myc

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- picture on first slide: <http://btech-info.blogspot.com>