Methods for generating induced pluripotent stem cells (iPSC)

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INTRODUCTION

Reprogramming of somatic cells to obtain induced pluripotent stem cells (iPSC) was achieved for the first time by Shinya Yamanaka and Kazutoshi Takahashi (2006). Since then, there have been developed many different methods to reprogram somatic cells into iPSCs. All these techniques require incorporation of the reprogramming factors (with different delivery methods and factor combinations) into a somatic cell type.

The reprogramming process involves choosing a suitable reprogramming factors "cocktail", cell type and method. The main problem of the methods used for generation iPSC is their low efficiency, but also other ones (screening problems, tumor, etc). Nowadays iPSCs research lines are routed to their use for medicine, not yet known if they will fit this goal.

This poster shows an overview of all these aspects and explains currently used methods for generating iPSC (including their advantages, disadvantages and average efficiency).

REPROGRAMMING FACTORS

The proteins that trigger the first steps for iPSC generation are transcriptional factors. They can be combined in "cocktails" to success in this process. Here is shown a list with the most used nowadays and their main functions:

- **Sox2** → Controls Oct3/4 expression.
- **Oct3/4** → Essential for keeping pluripotency.
- **Klf4** → Differentiation and cell proliferation.
- **c-Myc** → Proto-oncogene, increases efficiency.
- **NANOG** → Essential for dedifferentiation and X chromosome reactivation.
- **LIN28** → Regulates self renewal.

STARTING CELL TYPES

The process starts from a somatic cell and can not be any cell type. There are cells easier to extract from an animal and to reprogram:

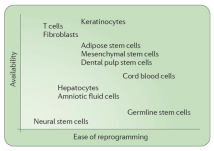


Figure 1: Different cell types sorted by its availability and ease of reprogramming. *SOURCE: "Methods for making induced pluripotent stem cells: reprogramming à la carte"*

REPROGRAMMING STEPS (FIBROBLAST)

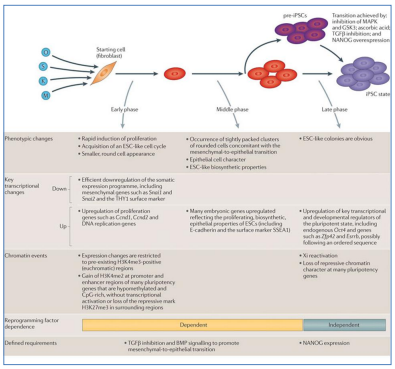


Figure 2: Steps that cells go through during the reprogramming process. There are 3 main steps and each one has specific characteristics such as phenotypic changes, transcriptional modifications, chromatin evens, dependence/independence of reprogramming factors and molecular requirements. *SOURCE: "Progress in understanding reprogramming to the induced pluripotent state".*

EFFICIENCY IMPROVEMENT USING SMALL MOLECULES

Low efficiencies during iPSC generation is the main problem of these methods. Small molecules can be used to improve it:

- **Histone deacetylase (HDAC) inhibitors**: Valproic acid (VPA), butyrate and trichostatin A (TSA).
- **Methyltransferase inhibitors**: BIX-01294 and Parnate.
- **MicroRNA blockers**.

PROBLEMS DERIVED FROM REPROGRAMMING

- Latent retrovirus/lentivirus are a major risk for the medical use of iPSC.
- Tumor generation.
- Starting cell type differentiation level.
- Variability problems and quality.
- Chromosomal instability.
- Low efficiency.
- Mutagenesis owing to integrative methods.

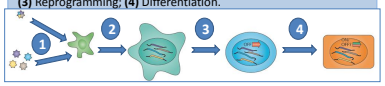
SCREENING TECHNIQUES

- Whole genome sequencing analysis.
- Embryoid body formation.
- Teratoma formation.
- Alkaline phosphatase.
- Methylation analysis (Differentially methylated regions).

REPROGRAMMING FACTORS: DELIVERY METHODS

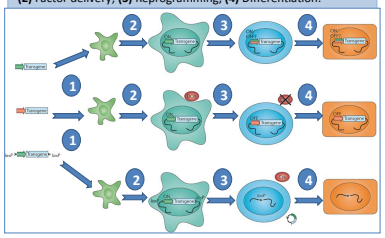
Viral delivery methods

MMLV retrovirus: Using a retrovirus to deliver the reprogramming factors was the first method to be used. STEPS: **(1)** Infection with single/polycistronic retrovirus; **(2)** Factor delivery; **(3)** Reprogramming; **(4)** Differentiation.



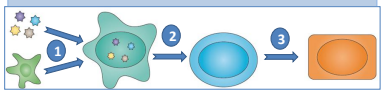
Advantages	Disadvantages	Average efficiency
•Very efficient infection (~90%) •Stable.	•Multiple viral integrations. •Transgenes remain in the genome. •Partially reprogrammed iPSC.	0,01%

Lentivirus: Different modified lentivirus can be used to deliver the reprogramming factors in a more controlled manner, specially with DOX-inducible promoter and Cre recombinase. STEPS: **(1)** Infection with constitutive/DOX-inducible/Excisable lentiviruses (respectively); **(2)** Factor delivery; **(3)** Reprogramming; **(4)** Differentiation.



•Very stable.	•Genome integration. •Residual expression of the transgenes. •Low efficiency.	-
•Very stable.	•Genome integration.	0,005%
•Transgene-free.	•Requires specific recombinases and safe insertion sites. •Little scar on the genome.	0,03%

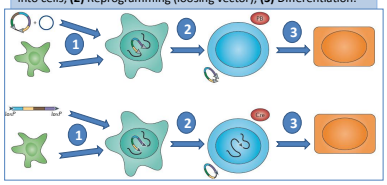
Sendai virus: This ssRNA (-) virus does not integrate in the genome and has shown to be a very good method for iPSC production. **(1)** Infection with Sendai virus; **(2)** Reprogramming; **(3)** Differentiation.



•Integration-free. •Easy screening (HN protein).	•Special facilities required for Sendai virus.	>0,1%

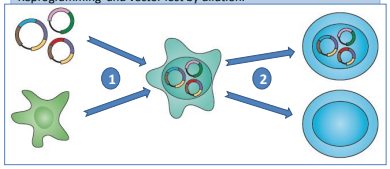
DNA-based methods

Piggybac transposon/Lineal DNA transfection: Using excisable vectors, reprogramming factors can be easily removed after integration. STEPS: **(1)** Liposome/electroporation to insert the vector into cells; **(2)** Reprogramming (loosing vector); **(3)** Differentiation.



Advantages	Disadvantages	Average efficiency
•Transgene-free and vector-free. •Reproducible.	•Genomic integration. •Negative selection advised.	0,001%
•Transgene-free and vector-free.	•Genomic integration. •Negative selection advised. •Low efficiency.	-

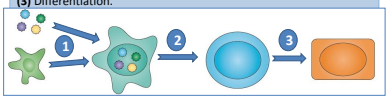
Transcient episomal delivery: Reprogramming factors transfected in plasmid vectors (single/polycistronic).STEPS: **(1)** Liposome/electroporation to introduce the plasmid into cells; **(2)** Reprogramming and vector lost by dilution.



•Transgene-free and vector-free. •No genomic integration. •Vectors are lost by dilution.	•Slow and inefficient. •Need to check numerous lines to find integration-free ones. •Labor-intensive. •Only for SV40T+ cell types.	Single $1{\cdot}10^{-6}$ Polycist. 0,01%

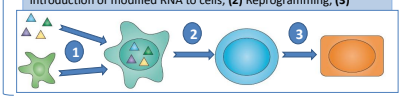
Non DNA-based methods

Protein delivery: Reprogramming can be achieved by introducing the factors (as proteins) directly to the cells. STEPS: **(1)** Introduction of modified reprogramming factors directly to cells; **(2)** Reprogramming; **(3)** Differentiation.



Advantages	Disadvantages	Average efficiency
•Transgene-free and vector-free. •No genomic integration. •No need to screen numerous colonies.	•Slow and inefficient.	>0,001%

RNA delivery: Delivering modified RNA can induce a very efficient method causing translation of reprogramming factors. STEPS: **(1)** Introduction of modified RNA to cells; **(2)** Reprogramming; **(3)**



•Transgene-free and vector-free. •No genomic integration. •No need to screen numerous colonies. •Highest efficiency method.	•Multiple transfections required.	2%

CONCLUSIONS

- Actually iPSCs generation methods are still **not optimized** and efficiency must be improved.
- **RNA delivery** is by far the most efficient and safe method nowadays.
- Using **feeder layers** is a problem for FDA/EMA approval of iPSC for regenerative medicine techniques.
- **Dedifferentiation mechanisms** remain broadly unknown.
- Two or more screening techniques are required for each iPSCs generation process. Since its use for medicine is the main goal, better screening methods and biomarkers are required to be as close to 100% safety as possible, in order to avoid possible teratoma formation in the patient. At this topic, **transdifferentiation** is safer and probably will replace iPSC for regenerative medicine.

APLICATIONS

- **Basic research**: Differentiation, stem cell study and biological model.
- **Disease modeling**: Study of disease physiopathology and personalized medicine.
- **Drug testing and personalized medicine**: Test drug toxicity, doses, cell types affected as well as cellular response to a drug, resistance studies, alternative signaling pathways activation, correction of genetic mutations, etc.
- **Cellular therapy**: Tissue regeneration, artificial organ production (or parts of an organ, such as artificial heart valves) and biological pacemakers.

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