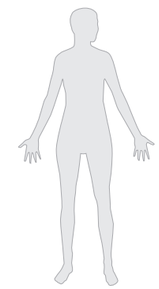
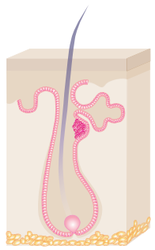
# ADULT . GENETICALLY REPROGRAMMED . *Induced Pluripotent Stem Cells*

*in vitro*



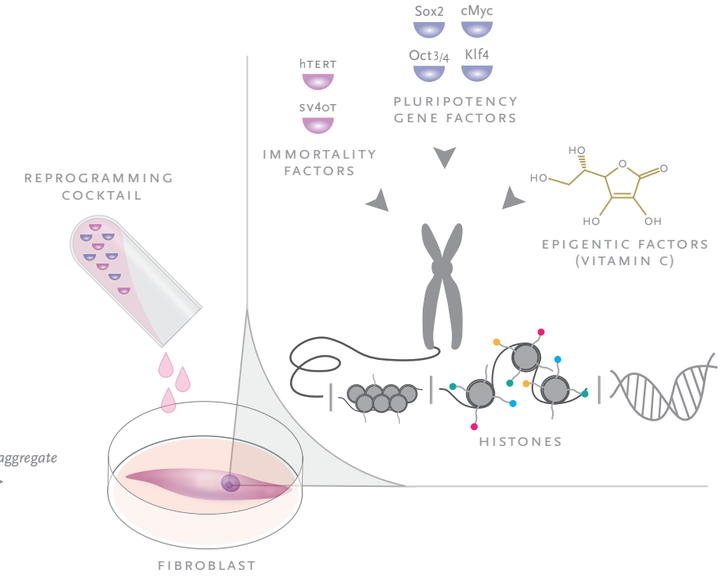
*skin biopsy*



## PATIENT DONOR & TISSUE PREP

After providing informed consent, the patient undergoes surgical biopsy. The tissue environment (*in vivo*) surrounding cells maintains them in a differentiated state. To induce pluripotency, enzymatic digestion & physical manipulation are used to dislodge cells from tissue so that they can respond to molecular signals being introduced in the lab. Fresh tissue samples from young donors exhibit enhanced reprogramming efficiency as compared to frozen tissue or samples from older donors.

*disaggregate*




## CELL CULTURE & DNA REPROGRAMMING

***Inducing Pluripotency:*** In 2007, Yamanaka identified four protein factors that induce differentiated cells to adopt stem cell behaviors. These "Yamanaka factors," Oct3/4, Sox2, Klf4, and cMyc, reprogram the cells by changing chromosomal architecture. The relaxation of the DNA in some regions results in activation of "stemness" genes while the compaction of other regions results in inhibition of differentiation-associated genes. More recently, epigenetic factors, such as Vitamin C, have been shown to stabilize this reprogrammed chromosomal state and the manipulation of MDB3 enhances induction of pluripotency.

***Inducing Immortality:*** "Immortality factors," including telomere-related enzymes and viral components, enhance cellular reprogramming efficiency and stabilize chromosome ends. These factors were essential in the immortalization of HeLa, the first human cell line, established in 1951.

***Delivering the factors:*** Yamanaka used viruses to deliver the genes that code for OCT3/4, SOX2, KLF4, cMYC. To reduce cancer risk associated with viruses and cMyc, clinical researchers directly expose cells to induction factors using chemicals, proteins, and RNA.

*reprogramming*

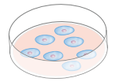

## TESTING FOR PLURIPOTENCY

***Cell Health:*** Cell death, incomplete reprogramming, and cell transformation (becoming cancerous) can occur. Scientists use microscopic analysis and a blue dye to evaluate cell viability and cellular architecture.

***Proteins for Pluripotency:*** As cells move into the "stemmy" state they lose specialized structures and adopt a rounded shape. The presence of proteins resulting from these shape changes is detected using immunofluorescence microscopy.

***Genomic Structure for Pluripotency and Immortality:*** Genomic structural changes that activate "stemness" genes and inhibit differentiation-associated genes are detected indirectly by the presence and location of epigenetic reprogramming factors on DNA. Gene expression changes are visualized using microarray technology.

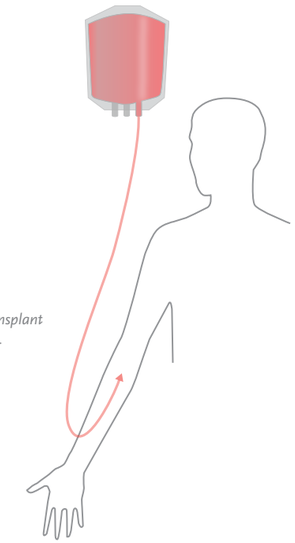
*mitosis*
*expansion*



**iPSCs LINE**

*differentiation*


*transplant*



***Patient-derived Stem Cell Therapy:***
Scientists use iPSCs to understand the basics of cell differentiation. When cells are partially differentiated to a specific cell type (a progenitor) they can be used for cell transplant therapy. In addition, researchers are exploring factors that directly differentiate or transdifferentiate cells in the body (*in vivo*), skipping induced pluripotency, which reduces the risk of cancer.

**iPSCs:** ***A patient's cells are reprogrammed into "healing" cells*** *that upon transplantation back into the body alter the tissue environment to repair degenerative tissue. Yamanaka and Gurdon challenged the static epigenetic landscape proposed by Waddington in 1957, and won the 2012 Nobel Prize for identifying pluripotency reprogramming factors. In 2013, Takebe's team created human liver buds from iPSCs (in vitro) that were capable of restoring liver function in mice (in vivo). Takahashi launched the first clinical trial for iPSC treatment of macular degeneration, and Serrano successfully induced pluripotency in vivo in a mouse model.*