

# Direct reprogramming

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Direct reprogramming  
= transdiferenciace

# The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles

by J. B. GURDON<sup>1</sup>

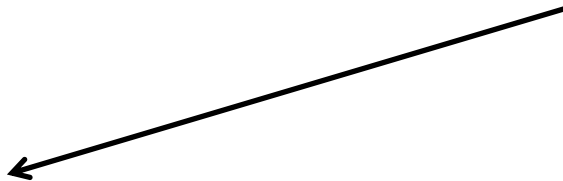
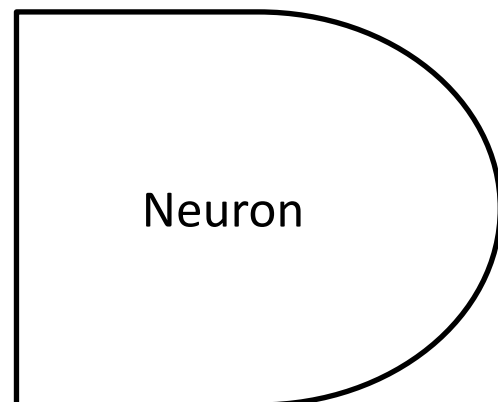
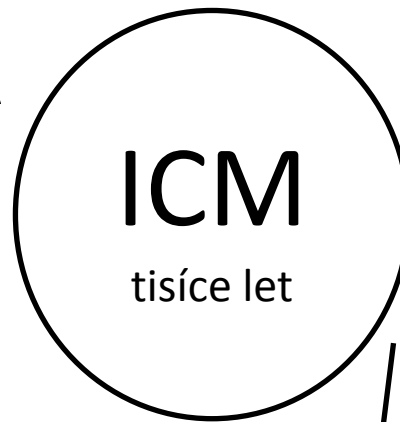
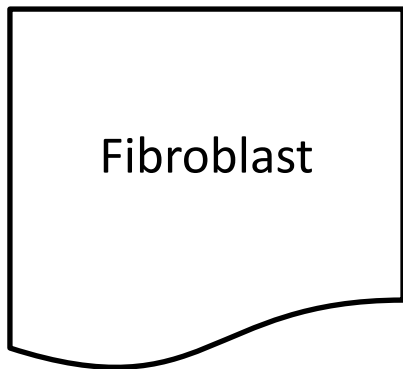
*From the Embryology Laboratory, Department of Zoology, Oxford*

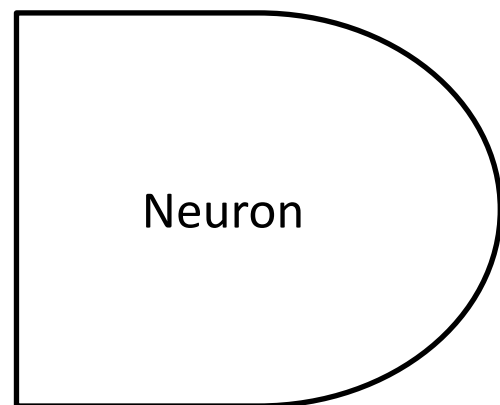
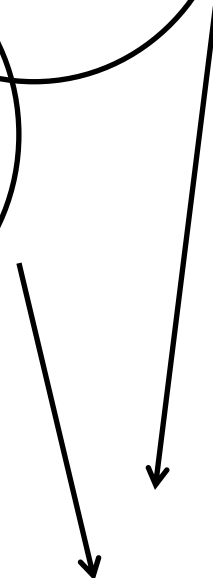
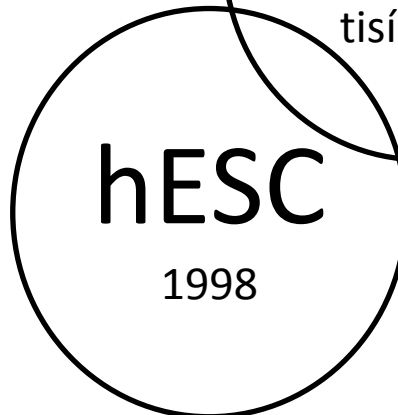
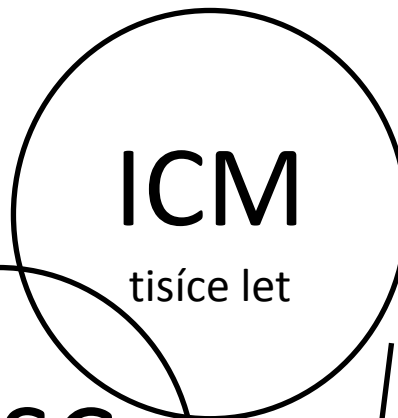
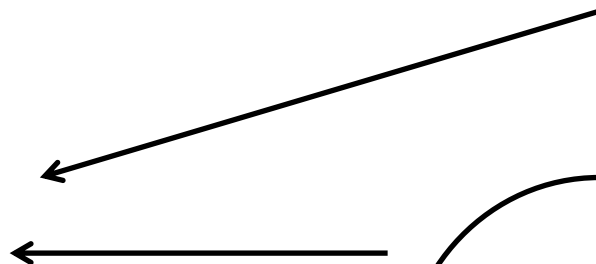
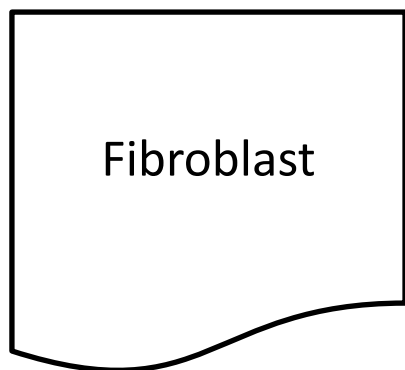
WITH ONE PLATE

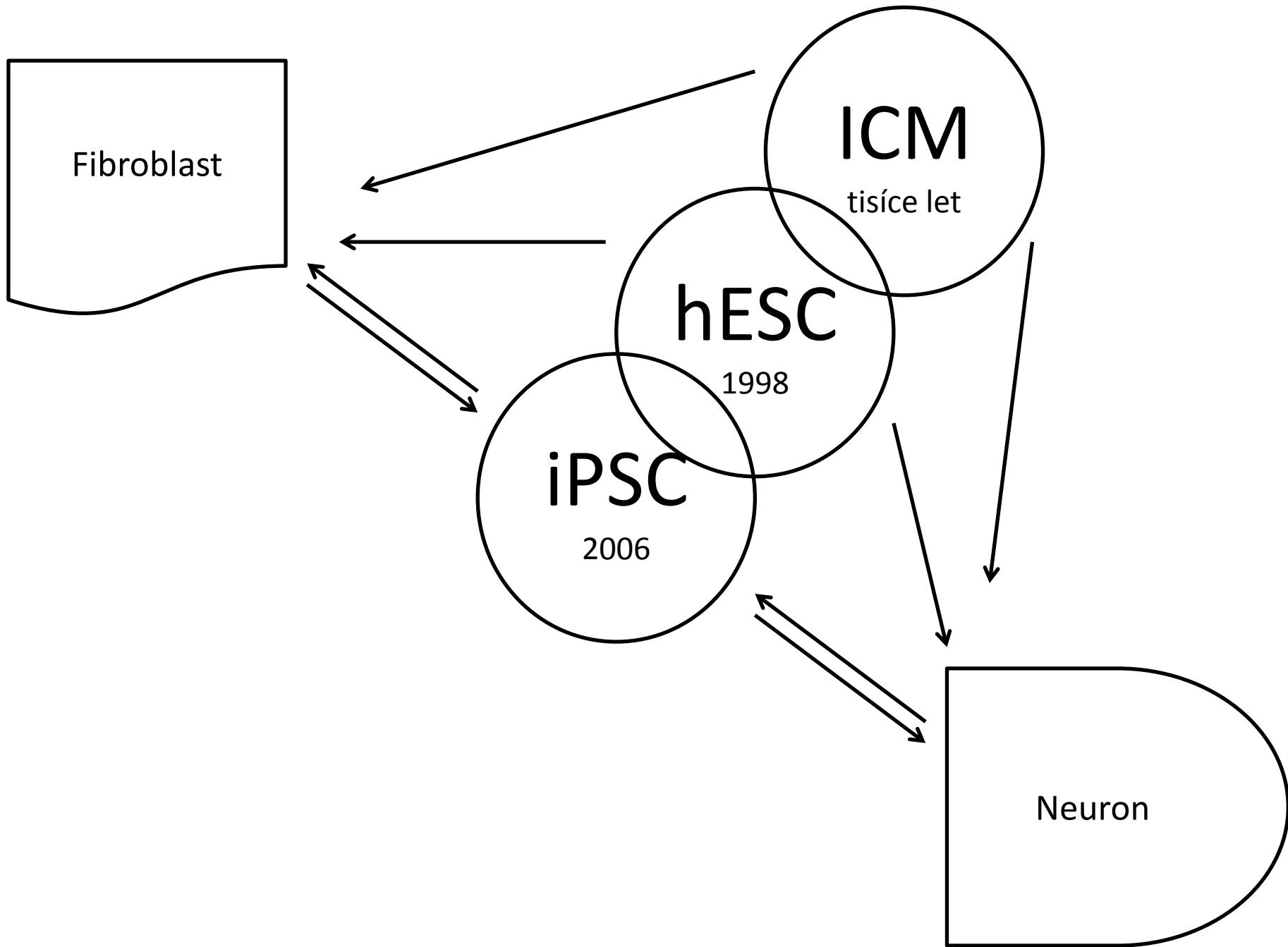
**[J. Embryol. exp. Morph., Vol. 10, Part 4, pp. 622-40 December 1962]**

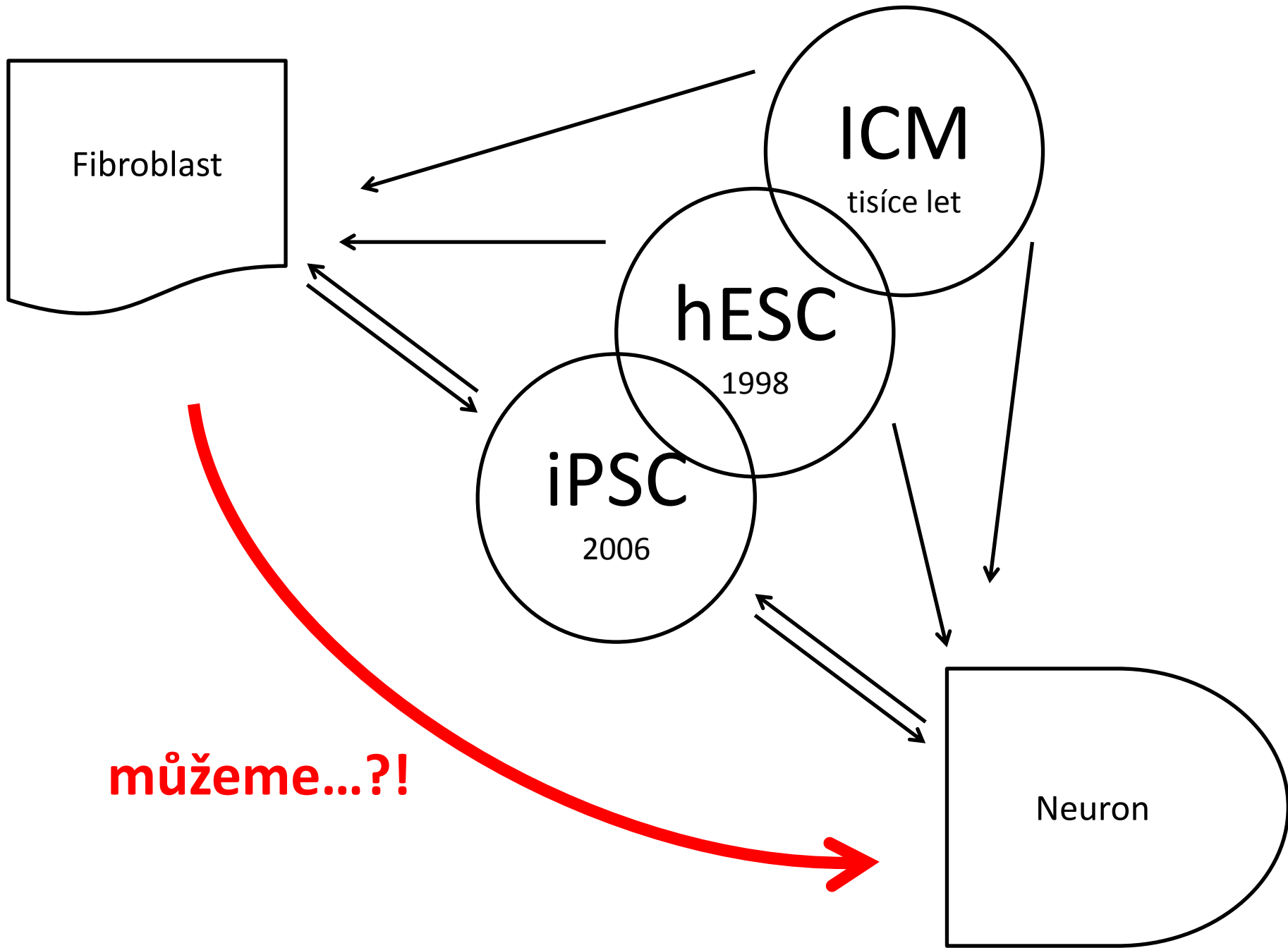


Nobelova cena 2012 (S. Yamanaka)









Fibroblast

ICM

tisíce let

hESC

1998

iPSC

2006

Neuron

**můžeme...?!**

náhrada/oprava buněk, tkání a orgánů

model nemoci na buněčné úrovni



# Medicína



*IN VITRO*

iPSC



Diferencovaná buňka



Implantace

*IN VITRO*

Diferencovaná buňka



Transdiferencovaná  
buňka *in vitro*



Implantace

*IN SITU*

Transdiferenciace *in vivo*

# Jak na to?

- transkripční faktory
- epigenetické regulátory
- miRNA
- malé chemické molekuly

# Jak na to?

## transkripční faktory

- hodiny po transfekci
- stabilní i po odstranění stimulu

# Jak na to?

## epigenetické regulátory

- Dnmt1 (Dhawan et al., 2011)
- Baf60c (Takeuchi and Bruneau, 2009)
- polycomb repressor complex 2  
(PRC2) (Patel et al., 2012)

# Jak na to?

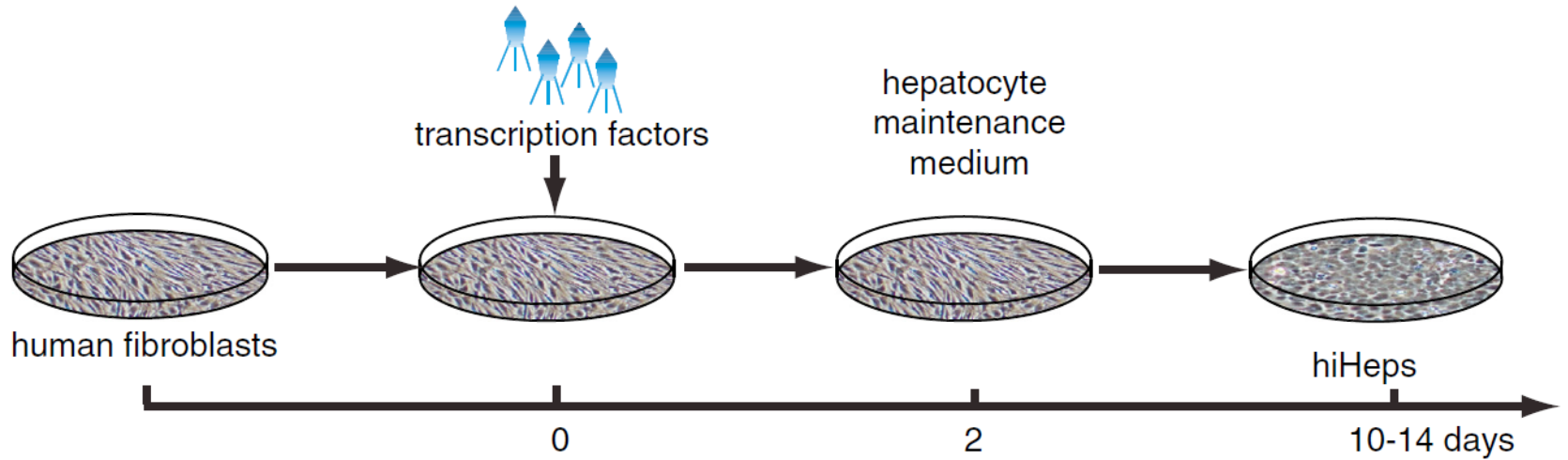
## miRNA

- miR-9/9 and miR-124 (Yoo et al., 2011)
- není tak efektivní jako TF

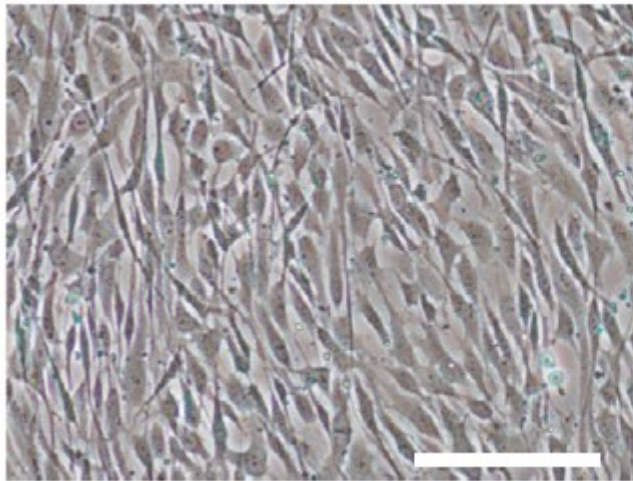
# Jak na to?

malé chemické molekuly

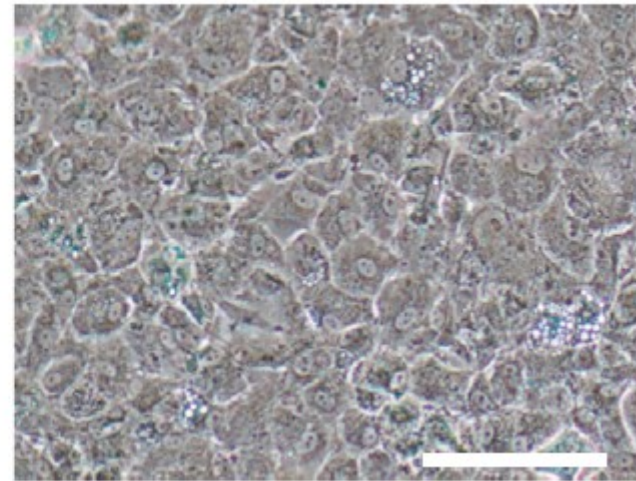
- především u iPSC
- předmět výzkumu



HFF1



hiHep



HFF = human fetal limb fibroblast

Huang P., Zhang L., Gao Y. et al. (2014). Cell Stem Cell 14, 370–384

- Exosome Research
- CRISPR/Cas9
- Genome Engineering
- Lentiviral Technology
- AAV Technology
- Episomal Vectors
- Immunology Research
- MicroRNA Research
- LncRNA Research
- Stem Cell Research
  - Growth Factors and Media
  - iPSC Reprogramming Systems
  - Antibody and AP Staining Kits
  - iPS Cell Lines
  - Pluripotency Reporters
  - > Direct Reprogramming Factors
  - Differentiation Reporters

TD Factor combinations



## Direct Reprogramming Factors

Expression vectors to directly convert one cell type to another

### Reprogram cellular identities

- Collection of transcription factors and microRNAs
- Explore novel conversion cocktails
- Make patient-specific cells and tissues

Overview Methods Sample data **Factor Combinations** Protocols Ordering

### TD Factors and Known Combinations

CELL TYPE	TD FACTOR	CATALOG#	REFERENCE
	Oct4	TD1002PA-1	Takahashi, K., K. Tanabe, et al. (2007). "Induction of pluripotent stem cells from adult human fibroblasts by defined factors." <i>Cell</i> 131(5): 861-72.
	Sox2	TD1003PA-1	
	c-Myc	TD1007PA-1	
	Klf4	TD1006PA-1	
	Lin28	TD1004PA-1	Yu, J., M. A. Vodyanik, et al. (2007). "Induced pluripotent stem cell lines derived from human somatic



# IN VITRO

Kardiomyocyt	Tbx5, Mef2c, Gata-4, Mesp1	Ieda, M., J. D. Fu, et al. (2010). "Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors." Cell 142(3): 375-86.
	Oct4, Sox2, Klf4, C-Myc	Efe, J. A., S. Hilcove, et al. (2011). "Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy." Nat Cell Biol 13(3): 215-22.
Pankreas	Ngn3, Pdx1, MafA, VP16	Zhou, Q., J. Brown, et al. (2008). "In vivo reprogramming of adult pancreatic exocrine cells to beta-cells." Nature 455(7213): 627-32.
Krevni progenitory	Oct4, Gata1, Gata2, Gata3, Gata-4	Szabo, E., S. Rampalli, et al. "Direct conversion of <b>human</b> fibroblasts to multilineage blood progenitors." Nature 468(7323): 521-6
Osteoblasty	Mir-2861	Ivey, K. N. and D. Srivastava (2011). MicroRNAs as regulators of differentiation and cell fate decisions." Cell Stem Cell 7(1): 36-41.

# IN VITRO

Brn2, Ascl1, Mytl1, Zic1

Vierbuchen, T., et al. (2010). "Direct conversion of fibroblasts to functional neurons by defined factors." *Nature* 463(7284): 1035-41.

Brn2, Ascl1, Mytl1,  
NeuroD1

Pang, Z. P., et al. (2011). "Induction of **human** neuronal cells by defined transcription factors." *Nature* 476(7359): 220-3.

Mir-9, Mir-124, Ascl1,  
Mytl1

Yoo, A. S., et al. (2011). "MicroRNA-mediated conversion of **human** fibroblasts to neurons." *Nature* 476(7359): 228-31.

Ascl1, Brn2, Mytl1, Lmx1a,  
FoxA2

Caiazzo, M. et al. (2011). "Direct generation of functional dopaminergic neurons from mouse and human fibroblasts." *Nature* 476(7359): 224-7.

Mytl1, Brn2, Mir-124

Ambasudhan, R., M. Talantova, et al. (2011). "Direct Reprogramming of Adult **Human** Fibroblasts to Functional Neurons under Defined Conditions." *Cell Stem Cell* 9(2): 113-8.

Oct4, Sox2, Klf4, C-Myc

Kim, J., J. A. Efe, et al. (2011). "Direct reprogramming of mouse fibroblasts to neural progenitors." *Proc Natl Acad Sci.* 108(19): 7838-43.

# IN VITRO

**Dopaminergní  
neurony**  
Ascl1, Brn2, Myt1l, Foxa2,  
Lmx1a

Pfisterer, U., A. Kirkeby, et al. (2011). "Direct conversion of **human** fibroblasts to dopaminergic neurons." Proc Natl Acad Sci.108(25):10343-8.

**Motorické  
neurony**  
Lhx3, Ascl1, Brn2, Myt1l,  
Ngn2, Hb9, Isl1, NeuroD1

Son, E. Y., J. K. Ichida, et al. (2011). "Conversion of Mouse and Human Fibroblasts into Functional Spinal Motor Neurons." Cell Stem Cell.

**Hepatocyty**  
Gata-4, HNF1-alpha, Foxa3  
HNF4-alpha, Foxa1, Foxa2,  
Foxa3

Huang, P., Z. He, et al. (2011). "Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors." Nature 475(7356): 386-9.

Sekiya, S. and A. Suzuki (2011). "Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors." Nature 475(7356): 390-3.

Foxa3, HNF1A, HNF4A

Huang P., Zhang L., Gao Y. et al.(2014). Direct Reprogramming of **Human** Fibroblasts to Functional and Expandable Hepatocytes. Cell Stem Cell 14, 370–384

# IN SITU

2008 D. Melton: exokrinní buňky slinivky břišní > beta buňky (Ngn3, Pdx1 a Mafa)

2012 D. Srivastava: fibroblasty z jizvy srdce po infaktu > bijící kardiomyocyty (Gata4, Mef2c and Tbx5)

2014 Guo: gliové buňky > neurony (NeuroD1)

2015 Weider: satelitní gliové buňky > oligodendrocyte-like buňky (Sox10)

# Překážky ve využití

- udržení/expanze buněk
- nízká efektivita
- bezpečnost použití

YEAR  
2 BOOK

