

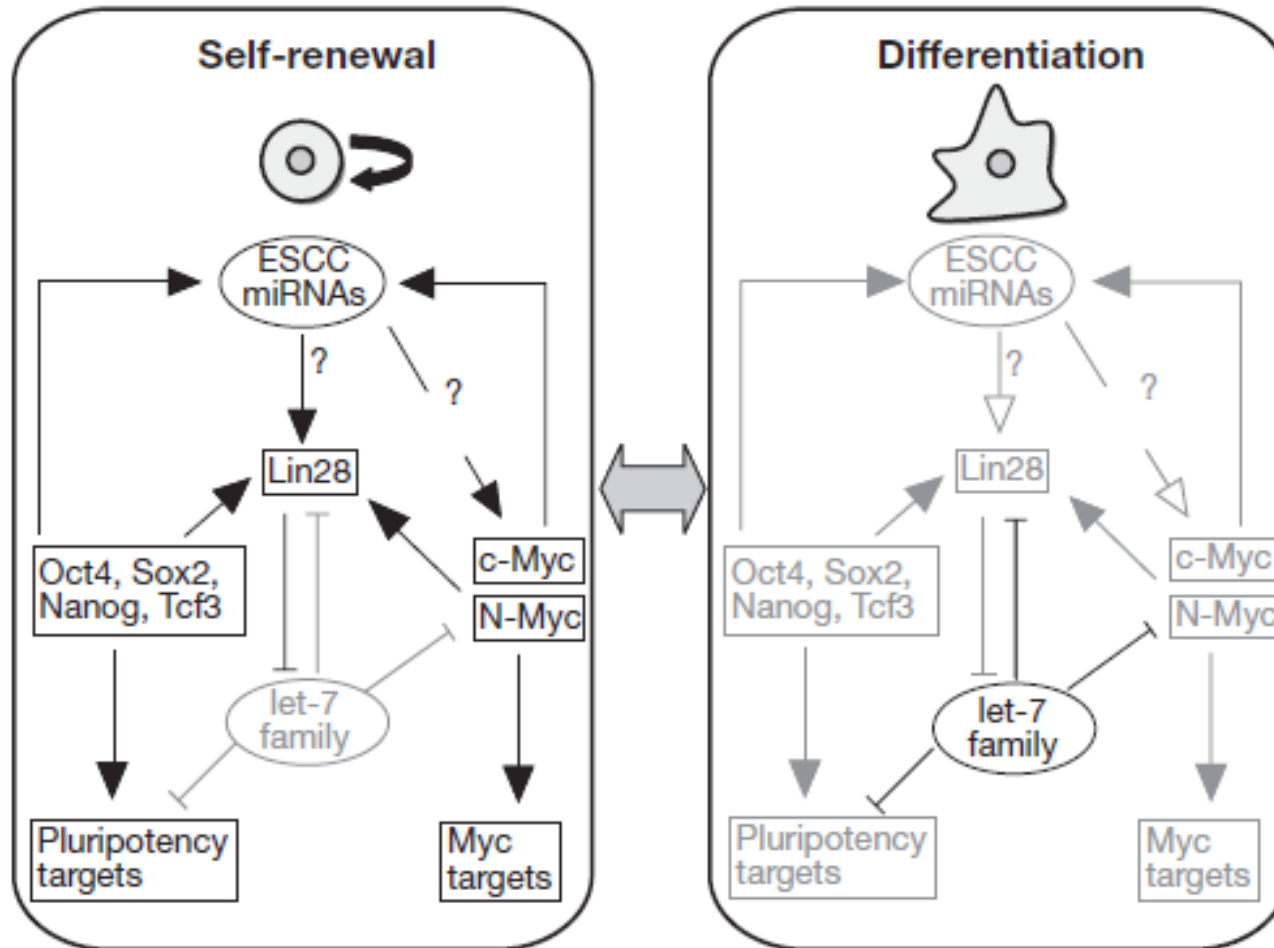
# concept

- **discrimination between two cell types\* based on miRNA “fingerprints”.**
- **regulated reporter system introduced by assembled viral vector construct.**
- **viral selection for miRNA binding patterns with best arrangements.**

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\* i. e. cancer cell / non-cancer cell or stem cell / differentiated cell, explained as followed

# stem cell differentiation



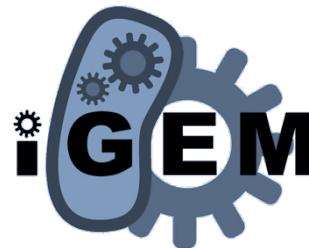
**Opposing microRNA families regulate self-renewal in mouse embryonic stem cells**

Collin Melton<sup>1,2</sup>, Robert L. Judson<sup>1,2</sup> & Robert Blelloch<sup>1,2</sup>

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# aspects

- selection according to the cells' differentiation state
  - successful approaches already reported<sup>1</sup>
    - ✓ feasibility & broad interests
  - still open questions:
    - concerning number and complementarities of target sites<sup>2</sup>
    - crucial role of linker sequences, surrounding regions ...
- modular structure allows **engineering** of target site compositions




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[1] Brown BD, Gentner B, Cantore A, Colleoni S, Amendola M, Zingale A, Baccharini A, Lazzari G, Galli C, Naldini L (2007). Endogenous microRNA can be broadly exploited to regulate transgene expression according to tissue, lineage and differentiation state. *Nat Biotechnol.* **25**, 1457-1467.

[2] Brown BD, Naldini L (2009). Exploiting and antagonizing microRNA regulation for therapeutic and experimental applications. *Nat Rev Genet.* **10**, 578-585.

# connections

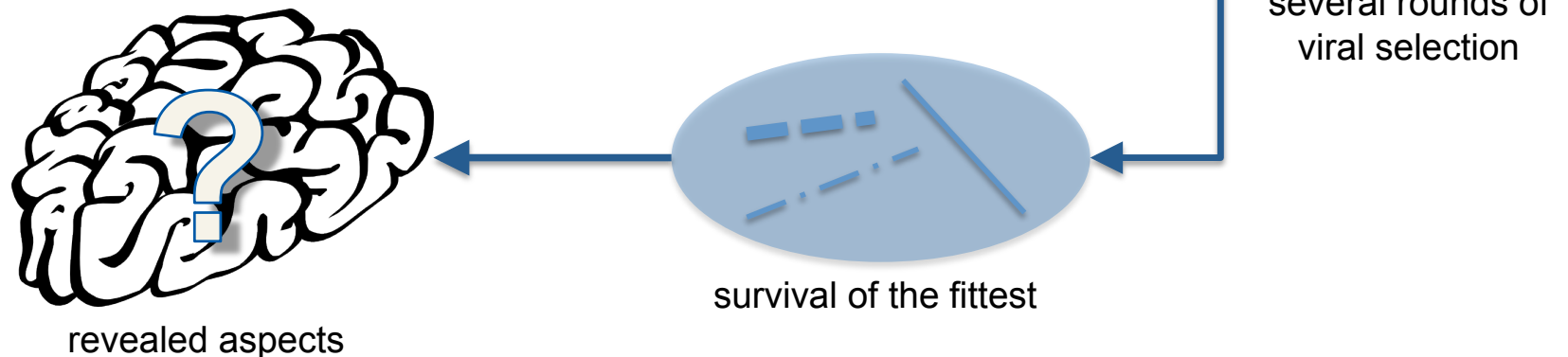
- synthetic biology:
  - **theory**: scan of databases for suitable libraries
  - **modeling** of miRNA-mRNA interaction
    - predictions for thresholds and saturation
  - **construction** of diverse target combinations; shuffling
  - experimental **validation**
- viral selection for best miRNA targets
  - analysis for identification of predominant patterns: bioinformatics

# selection process principle

- population of viral vectors
  - each with unique combination of miRNA target sequences
  - best patterns allow viral replication
    - survival in progeny

after generations and multiple selection rounds<sup>3</sup>:

- “fittest” sequences can be analyzed
  - identification of characteristic properties



[3] Tschulena U, Peterson KR, Gonzalez B, Fedosyuk H, Barbas CF, 3rd (2009). Positive selection of DNA-protein interactions in mammalian cells through phenotypic coupling with retrovirus production. *Nat Struct Mol Biol.* **16**, 1195-1199.

# mechanism of viral selection

