An approach to infer the gene regulatory network of a stable cell type

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Cellular state

Let x_i = expression level of gene *i*,
then the cellular state is the vector

$$X = (x_1, x_2, x_3, \ldots, x_n)$$

How are the levels of expression maintained ?
What are the gene regulatory mechanisms?

Our task is to formulate these questions mathematically and find a way to solve them

Dynamical system

Assume *X* varies in time according to

$$\frac{dX(t)}{dt} = A(X(t))$$

The vector field $A: R^n \rightarrow R^n$ contains detailed information on regulatory information, e.g.

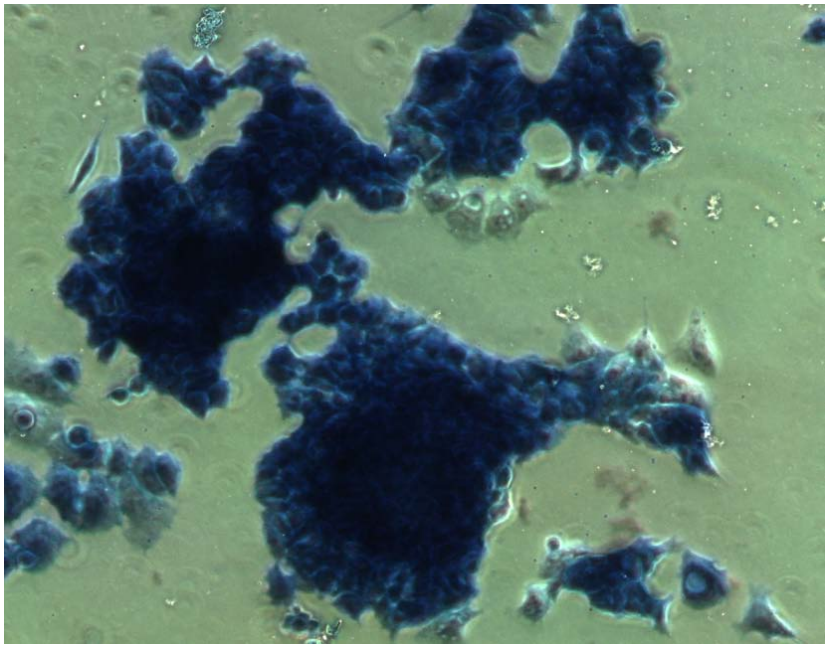
X_j *positively regulates* $X_i \quad \leftrightarrow \quad A_i(x_1 \ldots x_n) \uparrow$ in x_j

However, *A* is too complex to reconstruct from experiments based on current technology.

Stable cell type

A stable cell type can maintain a characteristic pattern of gene expression through a gene regulatory network.

Example:
Mouse embryonic stem cells (on 0.1% gelatin, with LIF)



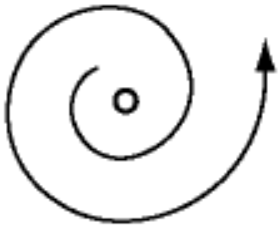
Equilibrium state

A state $\boldsymbol{\mu}$ is an equilibrium state if $\boldsymbol{A(\mu)=0}$

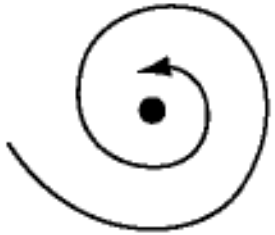
The equilibrium is stable if, once the system comes close to μ, it will stay close to μ from then on.

We identify stable cell types with stable equilibrium states of the dynamical system

unstable



stable



Regulatory network

Suppose $X(0)= \mu+\delta$, then for small t

$X(t)-X(0)\approx t\, A(\mu+\delta) \approx t\, [A(\mu) + T\, \delta\,] = t\, T\, \delta$

where T is the Jacobian matrix: $T_{ij}=(\partial A_i/x_j)(\mu)$

We propose to regard T as the regulatory network that maintains the equilibrium μ

Stability imposes a global constraint on the network: T must be negative definite to ensure stability

An approach to network reconstruction

- Use RNA-interference to knockdown each regulator in the stable cell type
- Measure gene expression after the perturbation
- Infer network based on a regression model
- Incorporate sparsity & stability into the regression
- Incorporate regulator binding data when available

Regression model

- Response: gene expression changes on I genes

$$Y = \{Y_1, Y_2, ..., Y_I\}$$

where $Y_i = X_i(t) - X_i(0)$

- Predictor: perturbation on *J* regulators

$$Z = \{Z_1, Z_2, ..., Z_J\}$$

e.g. $Z = ((0.5)\mu_1, 0, 0, ...0)'$

- Model: $E(Y_i) = \sum_{j=1}^{J} T_{ij} Z_j$, for $i = 1, ...I$

- Goal: identify non-zero elements in T_{ij}

Sparsity

- The true network is likely to be sparse
- Lasso-type regularization with L_1 penalty
- Penalized loss function

$$L(T, \lambda_1) = \sum_{per} \sum_{i=1}^{I} \left\| Y_i - \sum_{j=1}^{J} T_{ij} Z_j \right\|_2^2 + \lambda_1 \|T\|_1$$

here the outer sum is over all perturbation experiments

Stability

- Stability imposes useful constraints on *T*
- Lyapunov stability

$$\left\| X(t) - \mu \right\|^2 = \left\| (I + T)(X(0) - \mu) \right\|^2 \le \left\| X(0) - \mu \right\|^2$$

choose *X(0)-μ* to get an necessary condition

$$T_{jj} \le 0; \qquad \left\| {}^{(-j)}T_j \right\|_2^2 \le 1, \text{for } j = 1, \ldots, J$$

- This leads to the optimization of

$$L(T, \lambda_1, \lambda_2) = \sum_{per} \sum_{i=1}^{I} \left\| Y_i - \sum_{j=1}^{J} T_{ij} Z_j \right\|_2^2 + \lambda_1 \sum_{j=1}^{J} \left\| T_j \right\|_1 + \lambda_2 \sum_{j=1}^{J} \left\| {}^{(-j)}T_j \right\|_2^2$$

- Alternative formulations are possible

Incorporate TF binding location data

- TF association strength (TFAS) integrates the ChIP-seq peak intensities of TF *j* in the vicinity of gene *i*

$$a_{ij} = \sum_k g_k e^{-d_k / d_0}$$

- Define the TFAS weighting factor

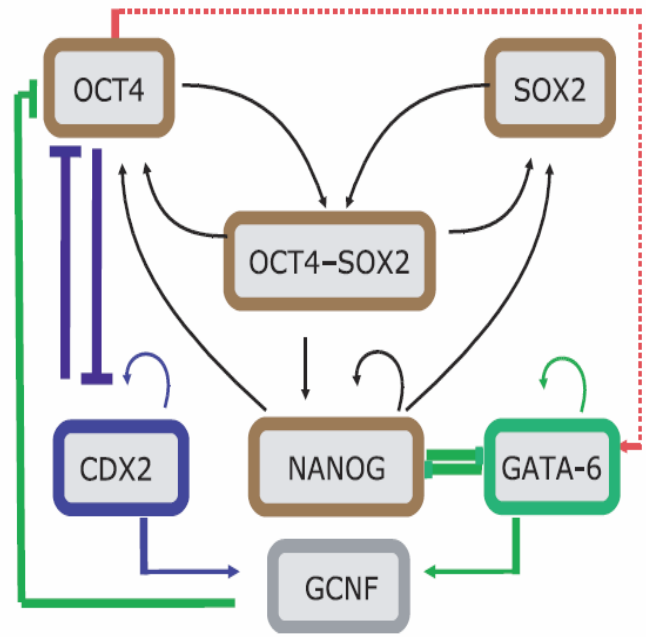
$$c_{ij} = 1 / a_{ij}$$

- Penalized loss function

$$L(T, \lambda_1, \lambda_2, c) = \sum_{per} \sum_{i=1}^{I} \left\| Y_i - \sum_{j=1}^{J} T_{ij} Z_j \right\|_2^2 + \lambda_1 \sum_{j=1}^{J} \left\| c_j \cdot T_j \right\|_1 + \lambda_2 \sum_{j=1}^{J} \left\| c_j \cdot^{(-j)} T_j \right\|_2^2$$

Simulated data:

Manually constructed by Chickarmane et al., (2008) PloS One.



2 stable equilibrium states:
stem cell & endoderm

Use symbolic solver to get the two networks

$$\frac{d[O]}{dt} = \frac{a_0 + a_1[A] + a_2[O][S] + a_3[O][S][N]}{1 + b_0[A] + b_1[O] + b_2[O][S] + b_3[O][S][N] + b_4[C][O] + b_5[GC]} - \gamma_1[O] \quad (2)$$

$$\frac{d[S]}{dt} = \frac{c_0 + c_1[O][S] + c_2[O][S][N]}{1 + d_0[O] + d_1[O][S] + d_2[O][S][N]} - \gamma_2[S]$$

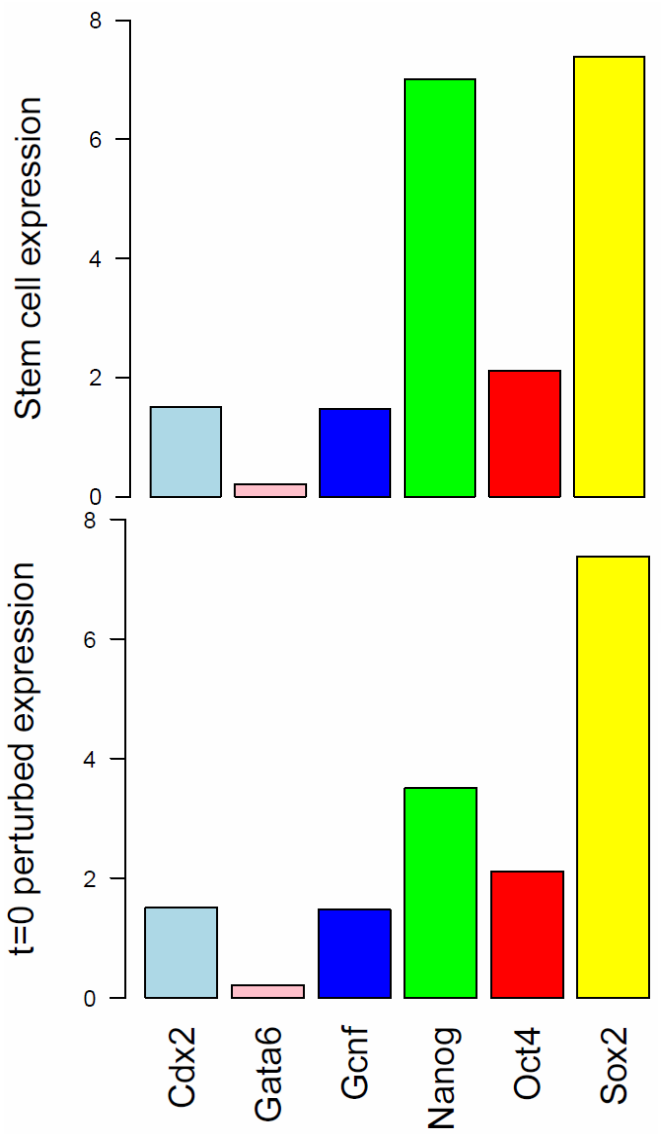
$$\frac{d[N]}{dt} = \frac{e_0 + e_1[O][S] + e_2[O][S][N]}{1 + f_0[O] + f_1[O][S] + f_2[O][S][N] + f_3[O][G]} - \gamma_3[N]$$

$$\frac{d[C]}{dt} = \frac{g_0 + g_1[C]}{1 + h_0[C] + h_1[C][O]} - \gamma_4[C]$$

$$\frac{d[GC]}{dt} = \frac{i_0 + i_1[C] + i_2[G]}{1 + j_0[C] + j_1[G]} - \gamma_5[GC]$$

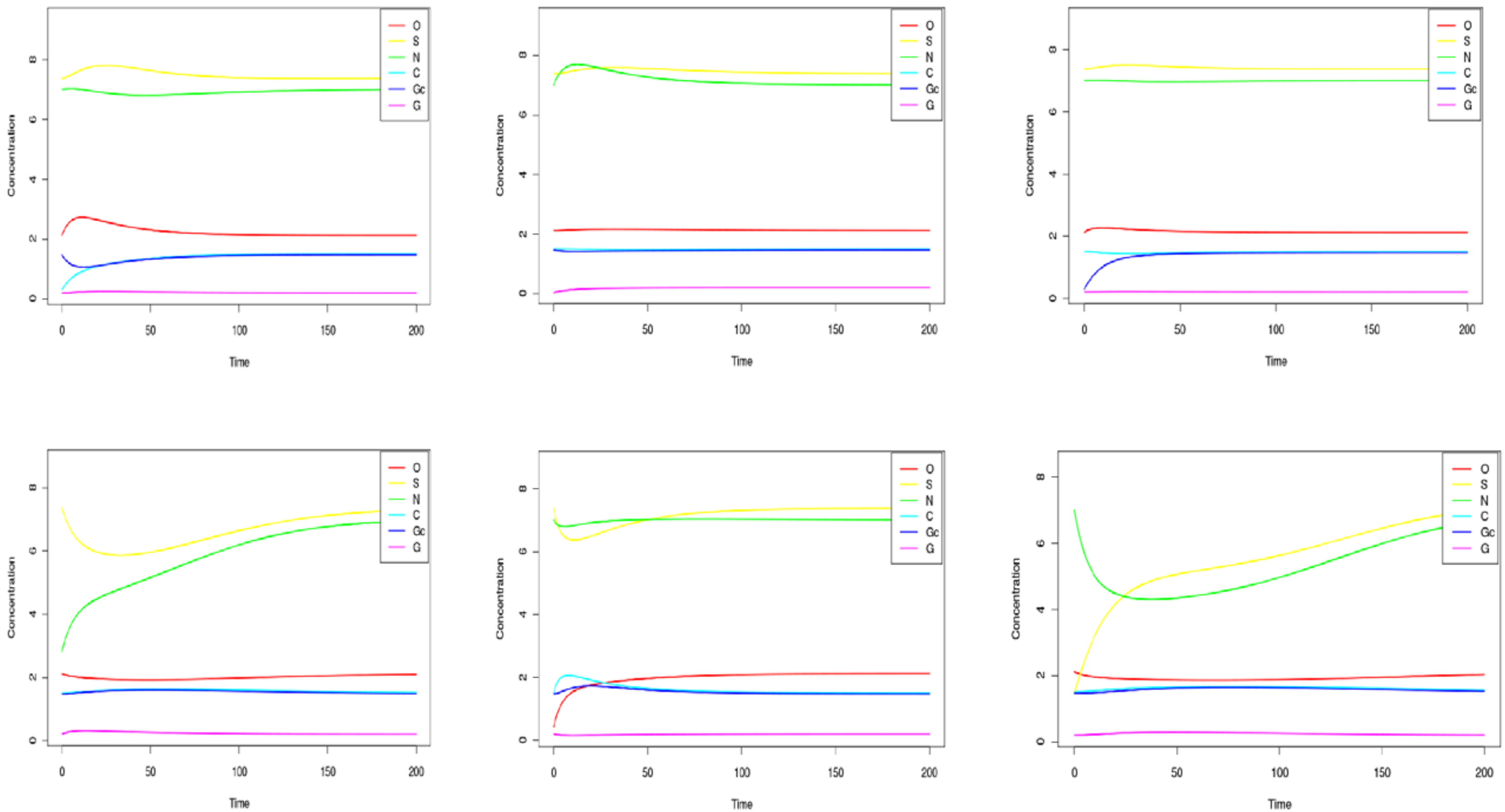
$$\frac{d[G]}{dt} = \frac{p_0 + p_1[O] + p_2[G]}{1 + q_0[O] + q_1[G] + q_2[N]} - \gamma_g[G],$$

Perturbation of stem cell state



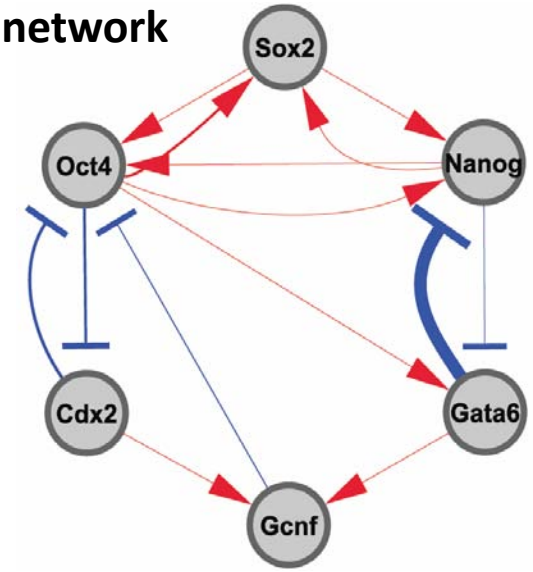
- Knockdown one of the six TFs in each experiment
- The TF expression is reduced by 50% (Nanog) at time *t*=0
- Simulate evolution of expression after perturbation

Time evolution after perturbation

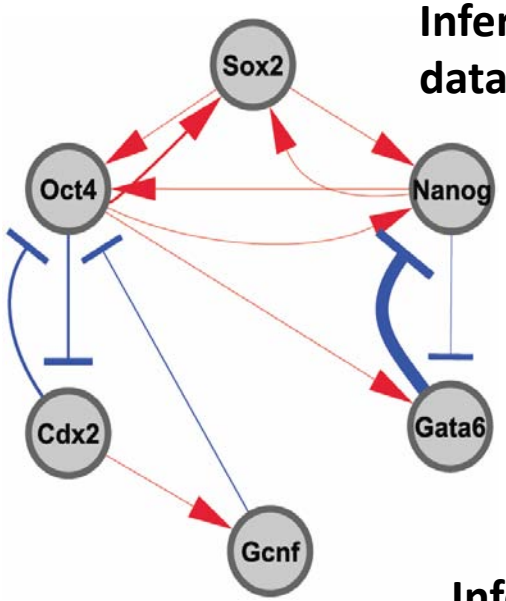


Network reconstruction results

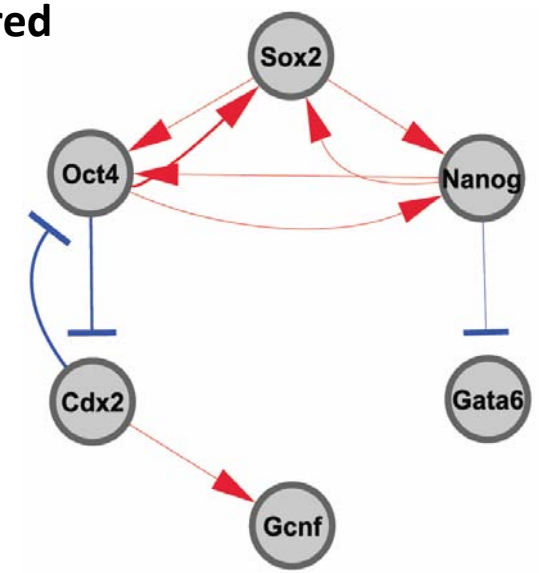
True network



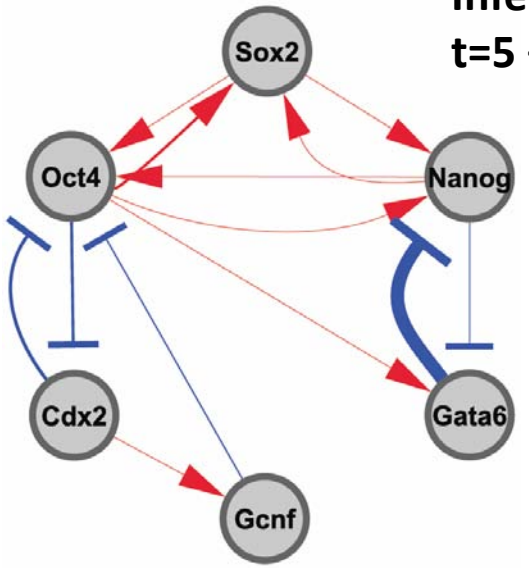
Inferred from data at t=1



Inferred t=5



Inferred t=5 + prior



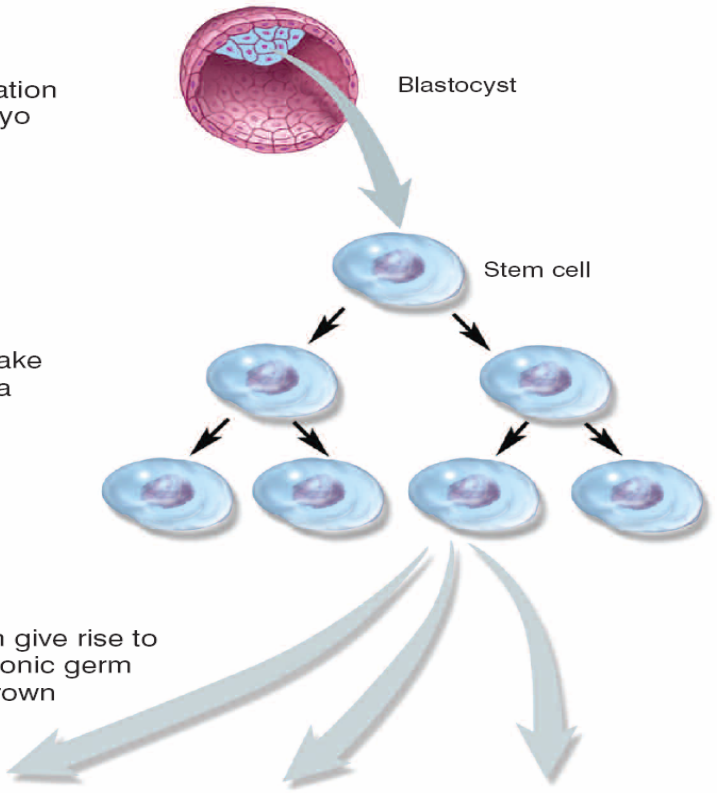
Real data

Embryonic stem cell

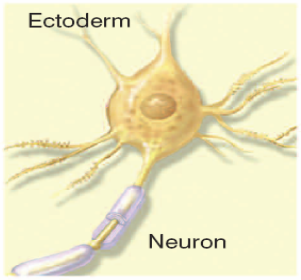
1. **Origin:**
   Derived from pre-implantation or peri-implantation embryo

2. **Self-Renewal:**
   The cells can divide to make copies of themselves for a prolonged period of time without differentiating.

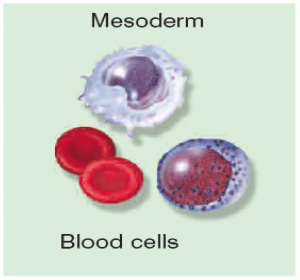
3. **Pluripotency:**
   Embryonic stem cells can give rise to cells from all three embryonic germ layers even after being grown in culture for a long time.



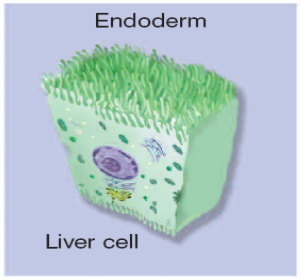
The three germ layers and one example of a cell type derived from each layer:



Ectoderm gives rise to: brain, spinal cord, nerve cells, hair, skin, teeth, sensory cells of eyes, ears nose, and mouth, and pigment cells.



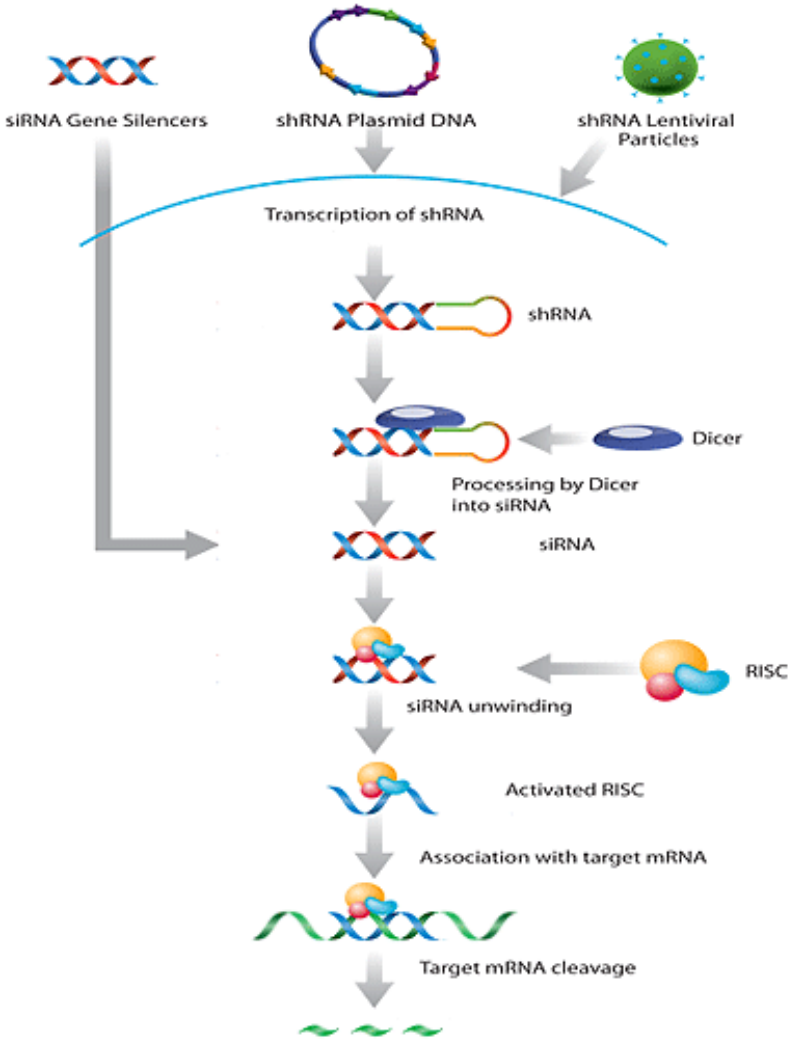
Mesoderm gives rise to: muscles, blood, blood vessels, connective tissues, and the heart.



Endoderm gives rise to: the gut (pancreas, stomach, liver, etc.), lungs, bladder, and germ cells (eggs or sperm)

Regenerative Medicine. August 2006.

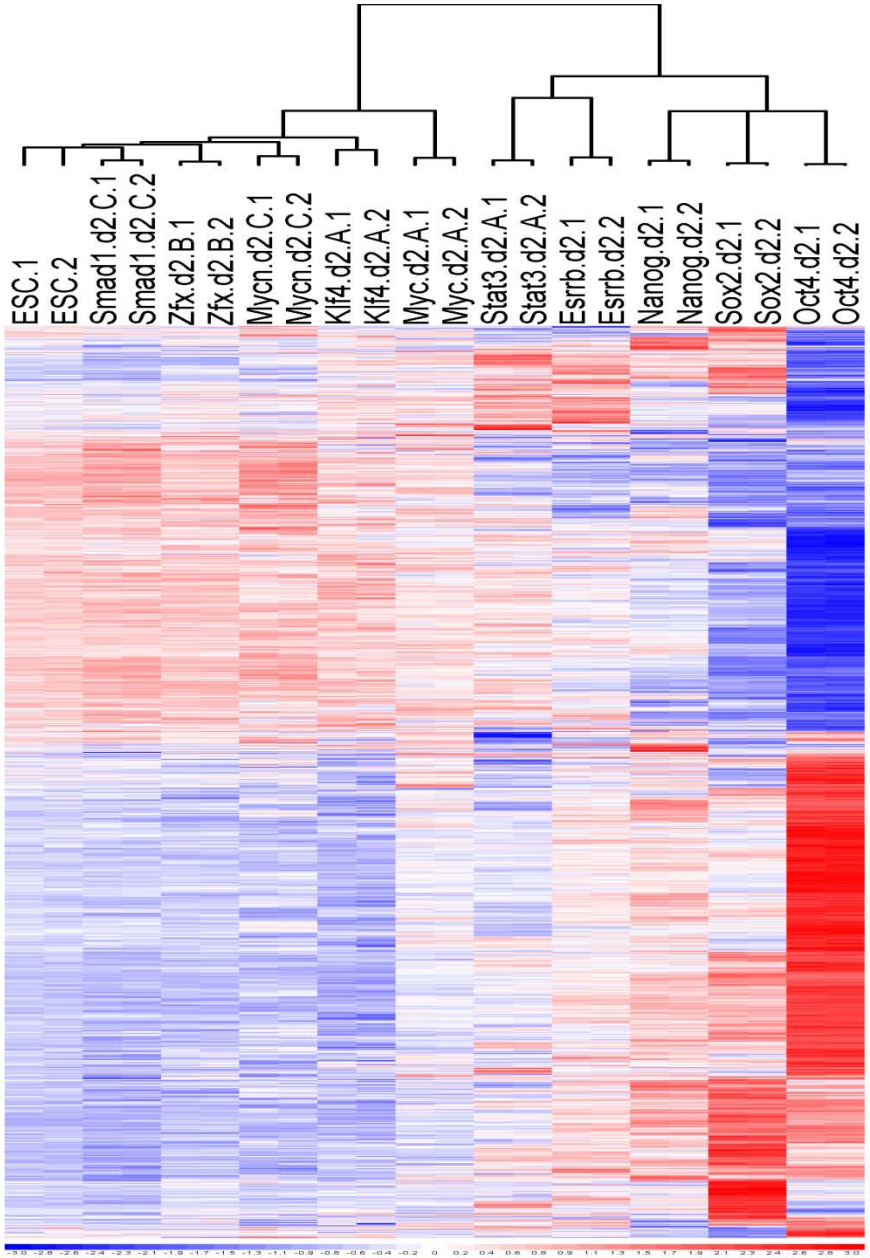
Gene knockdown by RNA interference



Summary of experiments in our lab

- Identification of key TFs
  - Literature
  - Expression
  - ChIP data availability
- RNAi performed on many TFs but is still on going
  - Oct4, Nanog, Sox2, Esrrb, Stat3, Klf4, Myc, Mycn, Zfx, Smad1
- Measure gene expression by microarray at day 2

Sample clustering (after batch effect correction)



some details

- Quantile normalization
- Batch effect modeling
- No gene filtering
  - All 18138 genes entering into the model fitting
  - Perhaps the first attempt on gene regulatory network inference at the whole genome level in a mammalian cell type

- Network reconstruction with ChIP information
  (ChIP-seq data from Chen et al 2008)

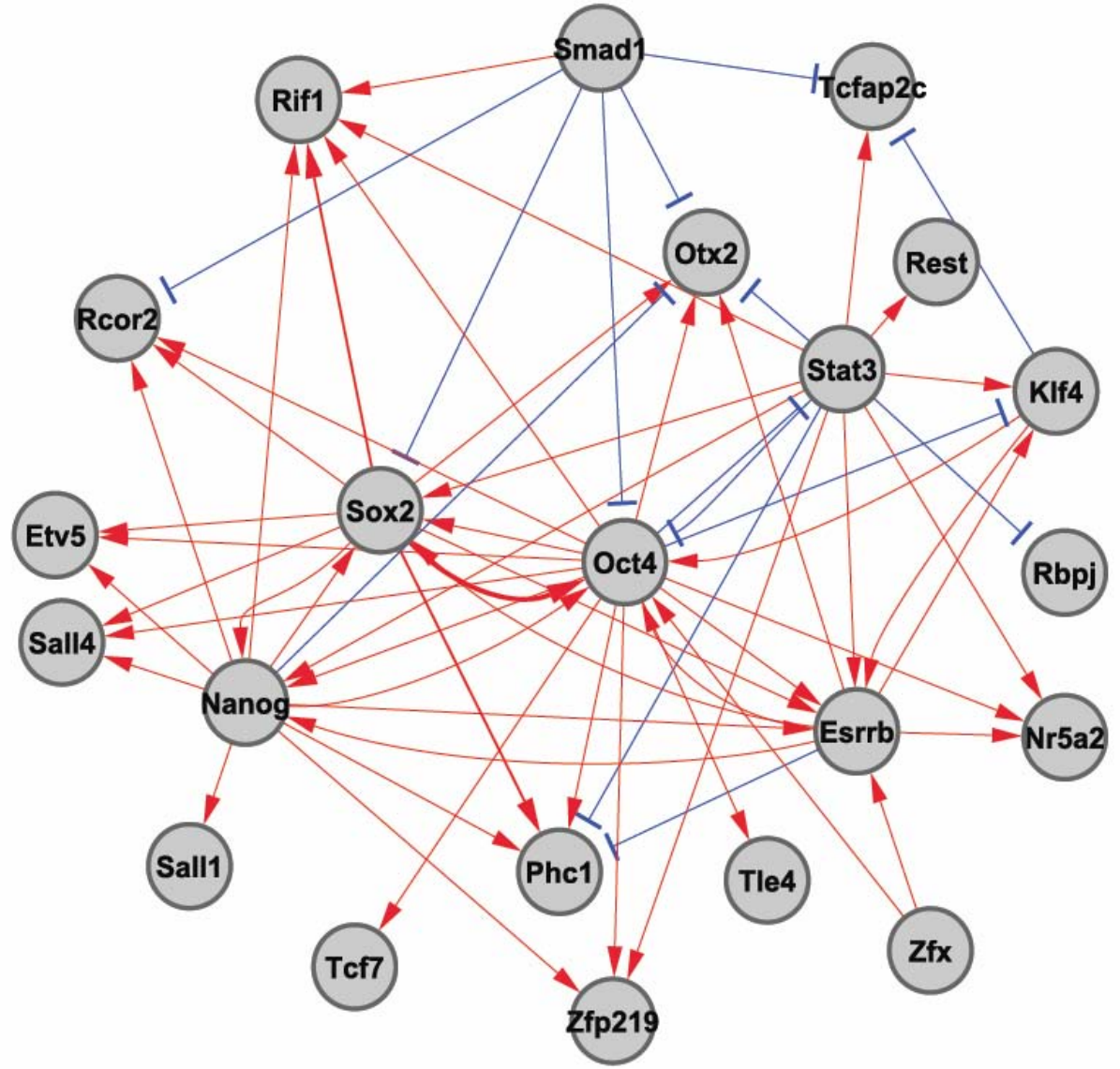
- Cross-validated for choice of λ

TF targets identified

- 3764 targets regulated by 10 TFs

Oct4	Nanog	Sox2	Esrrb	Stat3	Klf4	Myc	Mycn	Zfx	Smad1
2362	588	461	1169	895	277	0	0	72	163

A subnetwork for important TFs



Acknowledgement

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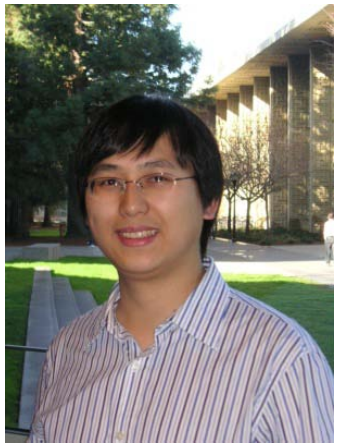

Methods:
Bokyung Choi