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 , 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 varies in time according to

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

The vector field contains detailed information on regulatory information, e.g.

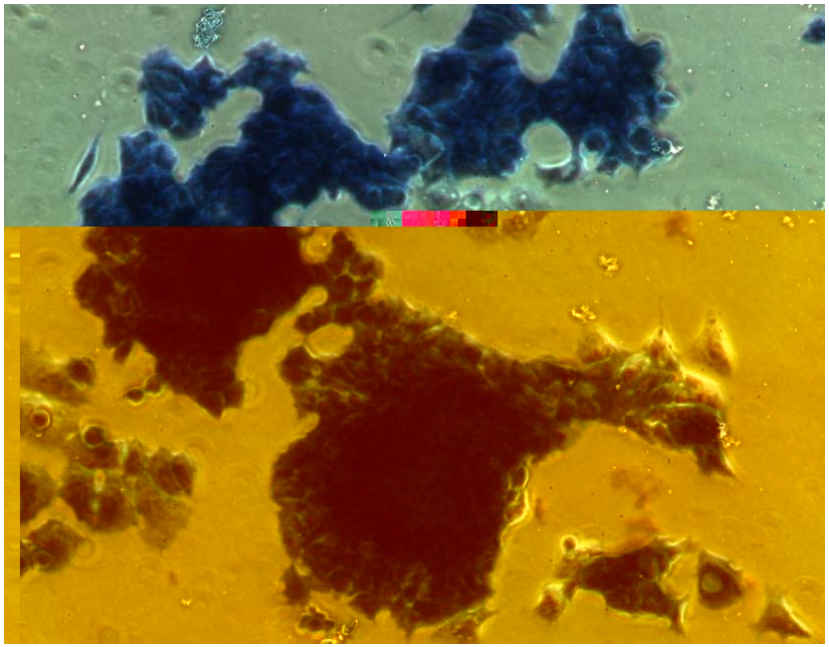
↑ in

However, 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)



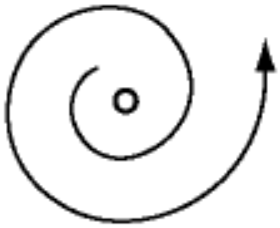
Equilibrium state

A state μ is an equilibrium state if μ

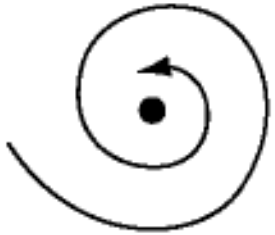
The equilibrium is stable if, once the system comes close to , it will stay close to from then on.

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

unstable



stable



Regulatory network

Suppose then for small

where is the Jacobian matrix:

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

Stability imposes a global constraint on the network: 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 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

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
- Lyapunov stability

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

choose to get an necessary condition

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

- 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 in the vicinity of gene

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

- Define the TFAS weighting factor

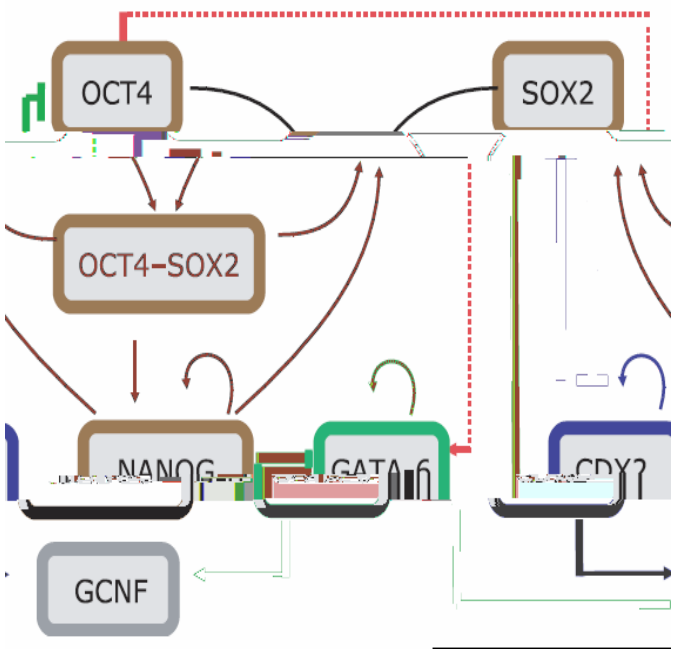
$$c_{ij} = 1 / a_{ij}$$

- 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.



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

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

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

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

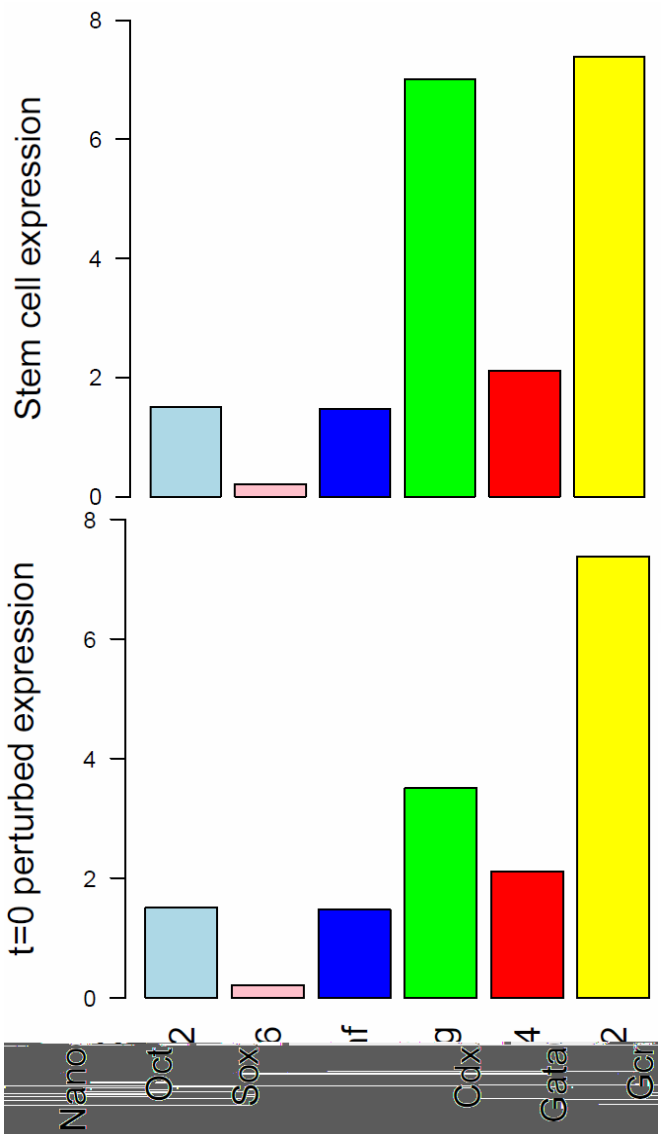
$$\frac{d[GC]}{dt} = \frac{i_0 + i_1[C] + i_2[G]}{1 + j_0[C] + j_1[G]} - \gamma_5[GC]$$

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

2 stable equilibrium states:
stem cell & endoderm

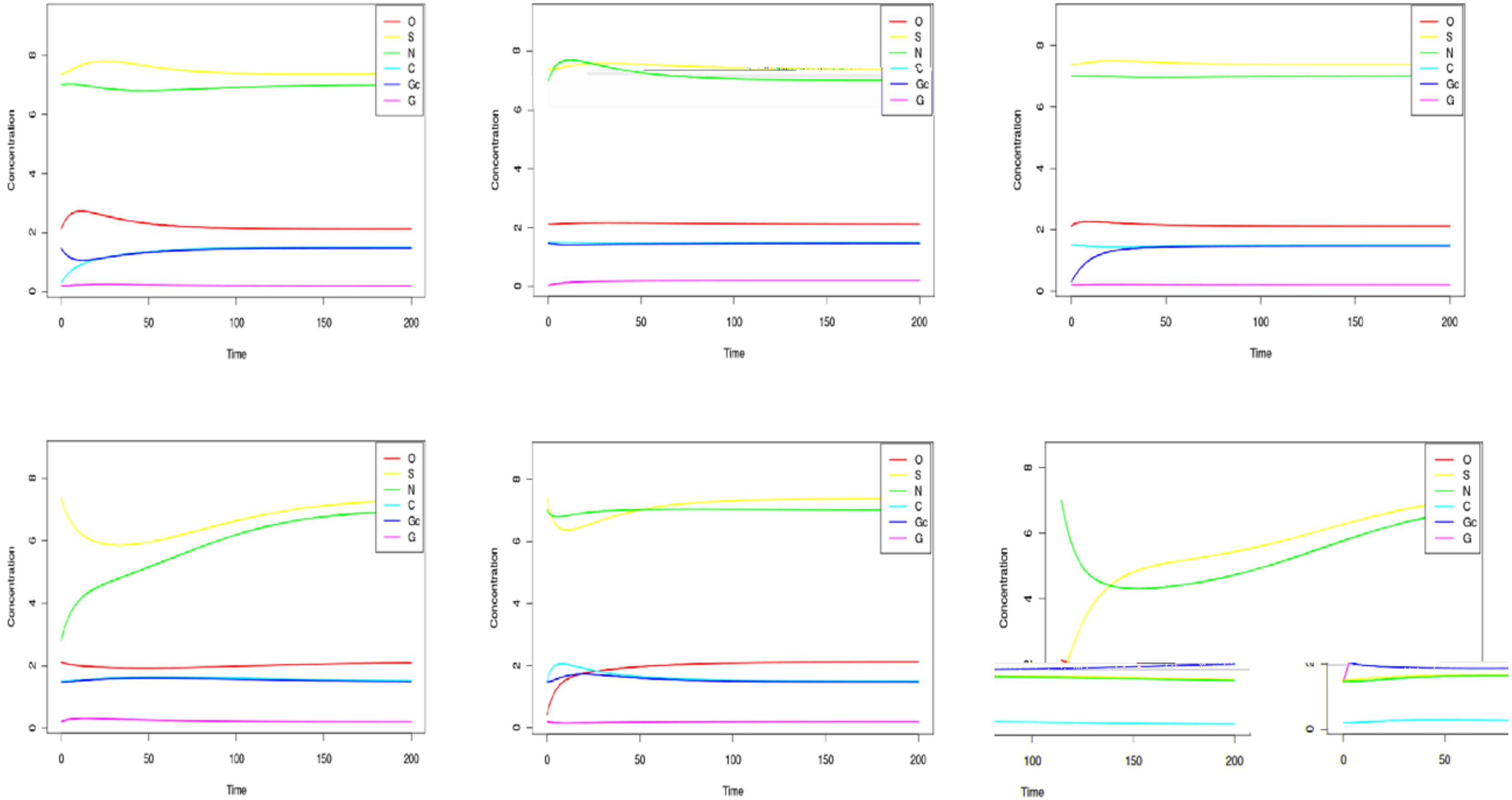
Use symbolic solver to get the two networks

Perturbation of stem cell state



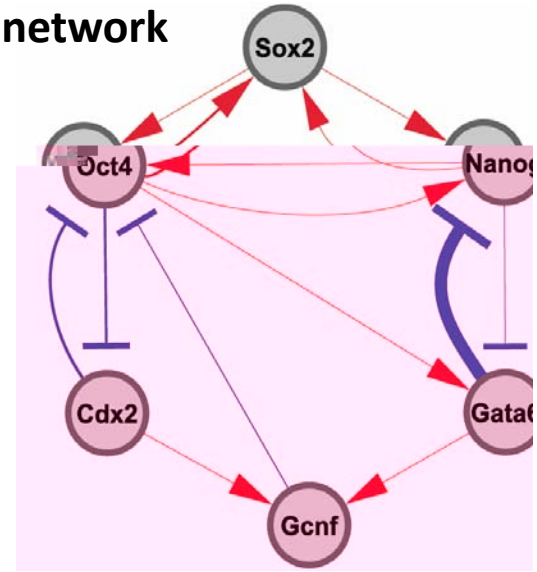
- Knockdown one of the six TFs in each experiment
- The TF expression is reduced by 50% (Nanog) at time =0
- Simulate evolution of expression after perturbation

Time evolution after perturbation

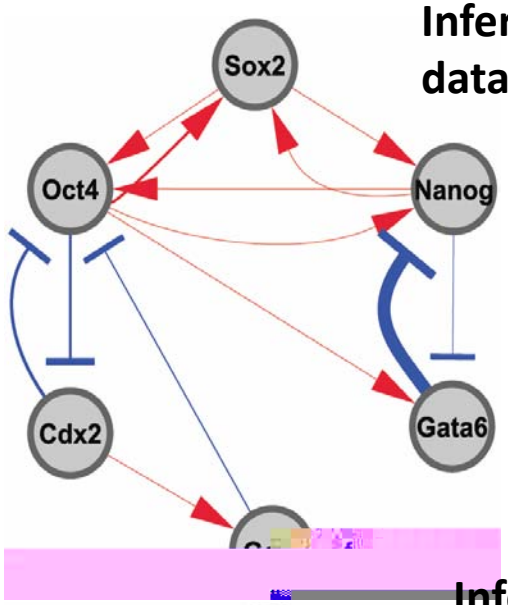


Network reconstruction results

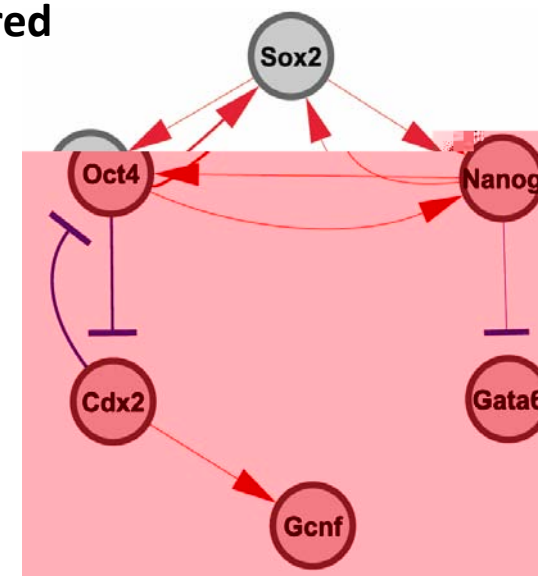
True network



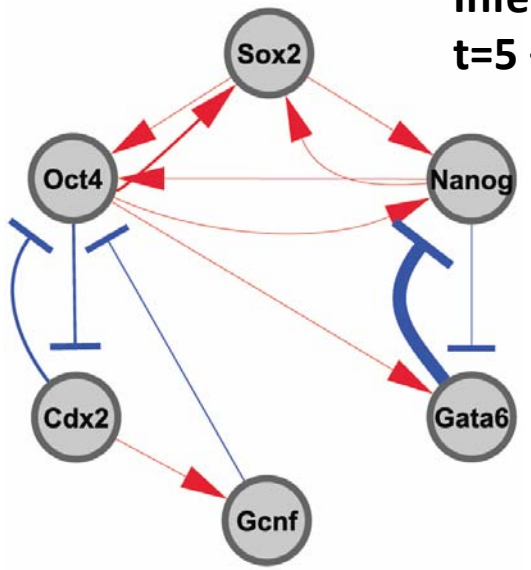
Inferred from data at t=1



Inferred t=5

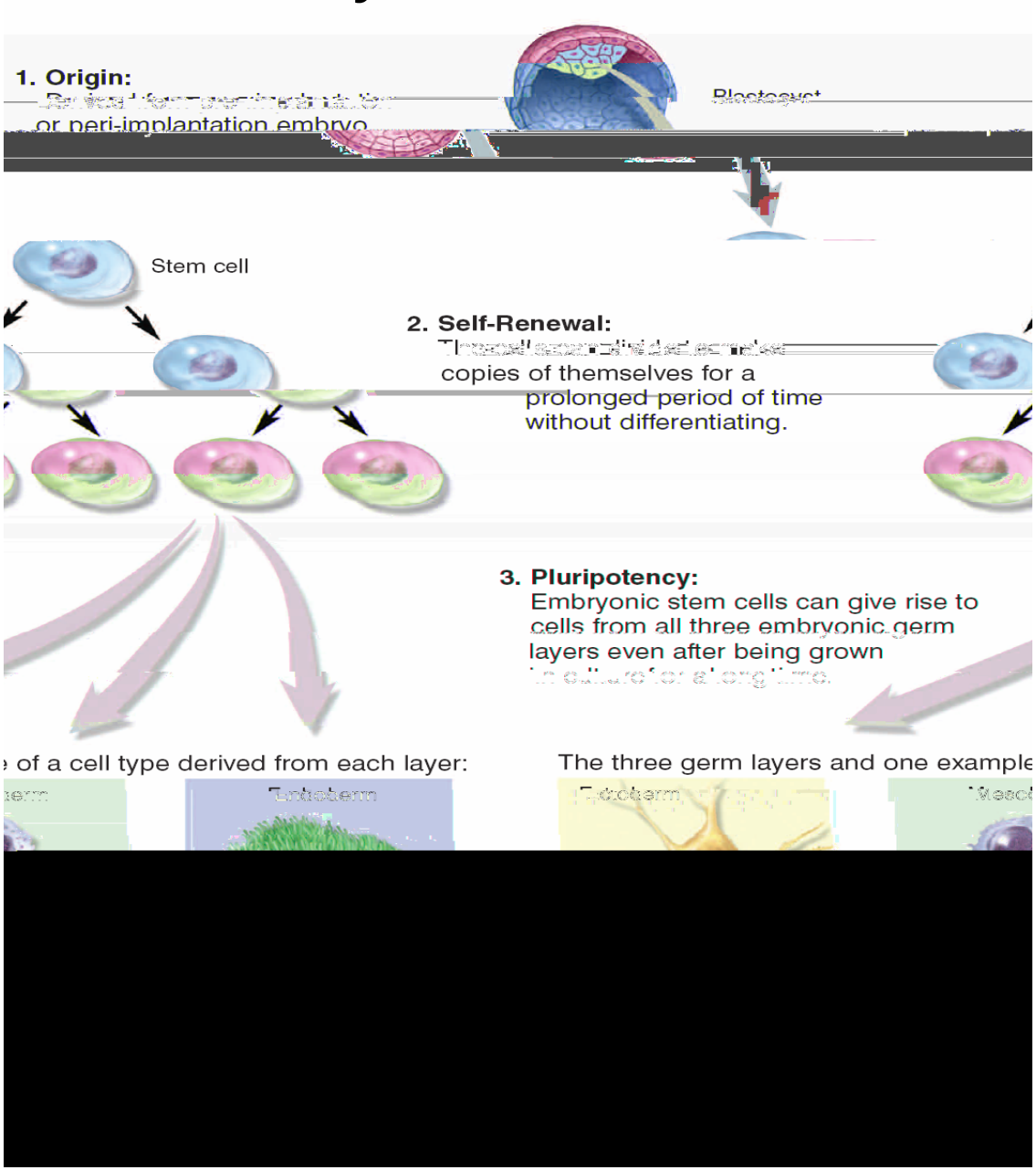


Inferred t=5 + prior



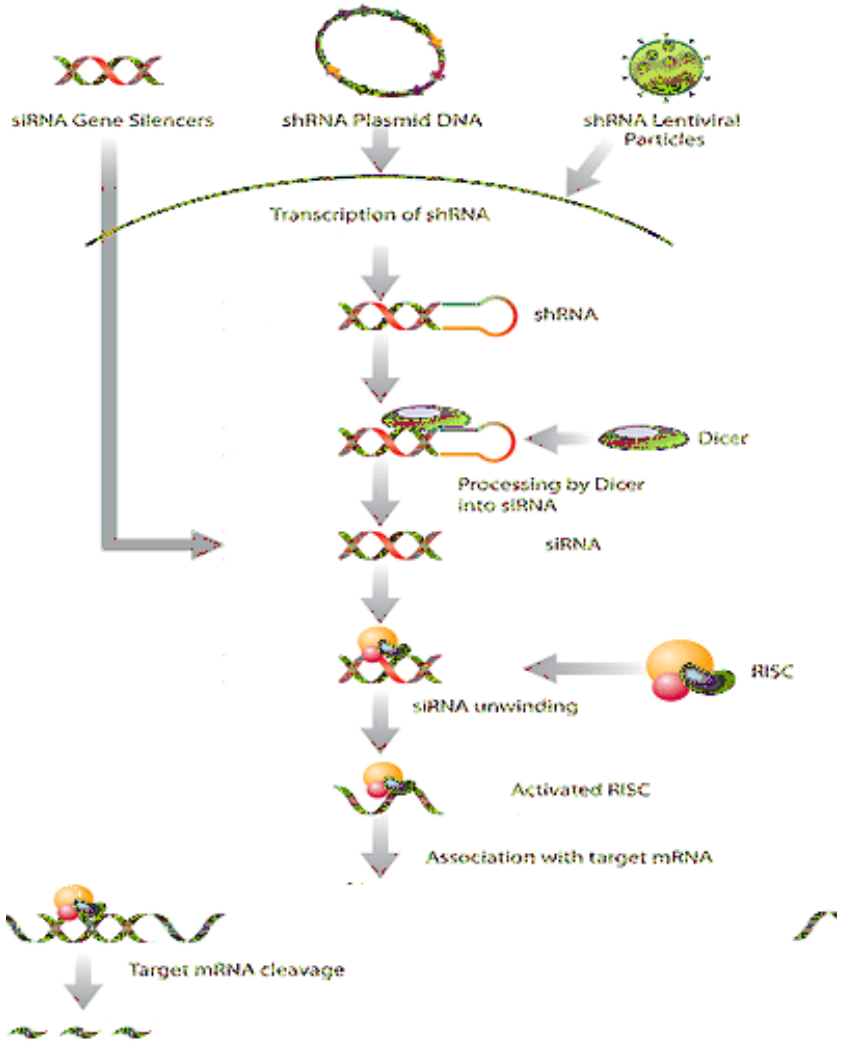
Real data

Embryonic stem cell



August 2006.

Gene knockdown by RNA interference



Summary of experiments in our lab

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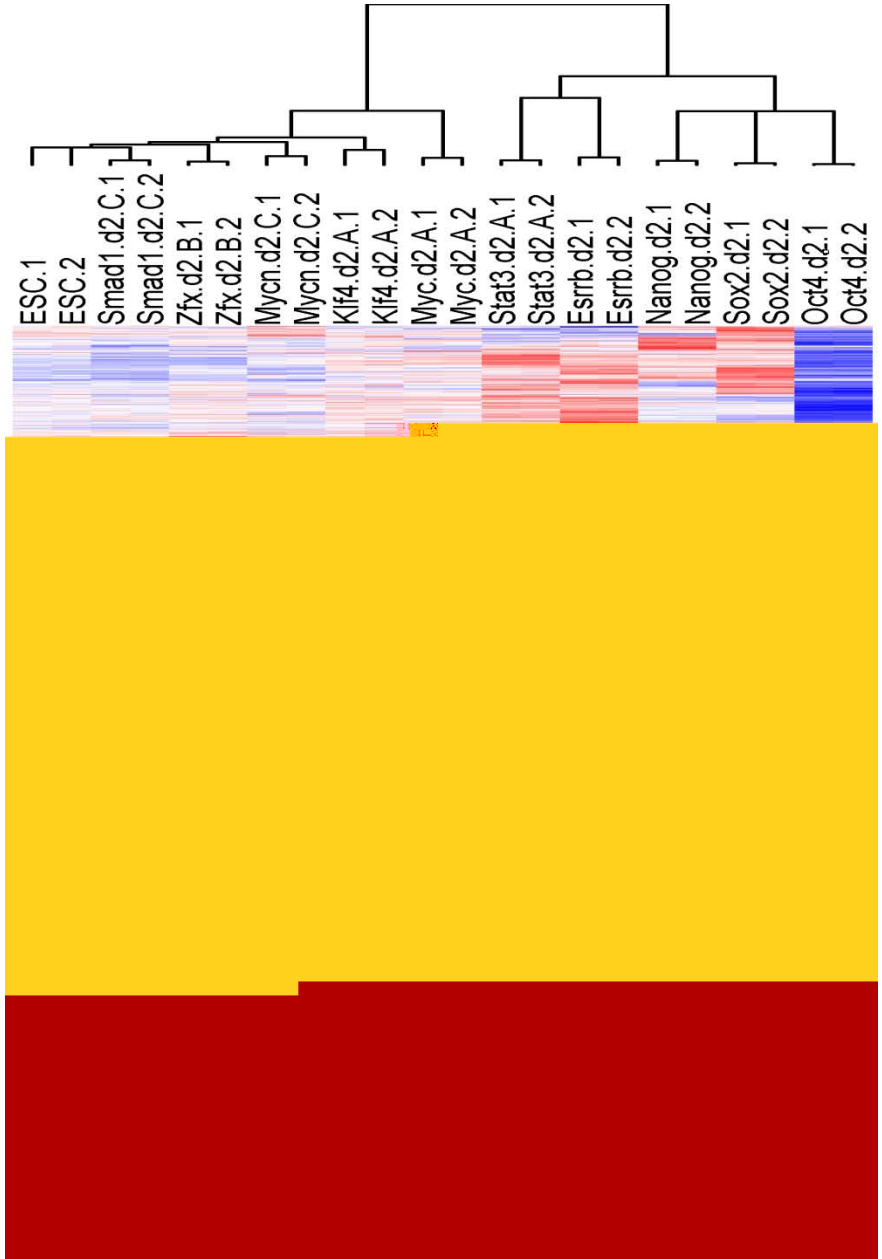
Sample clustering (after batch effect correction)



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)

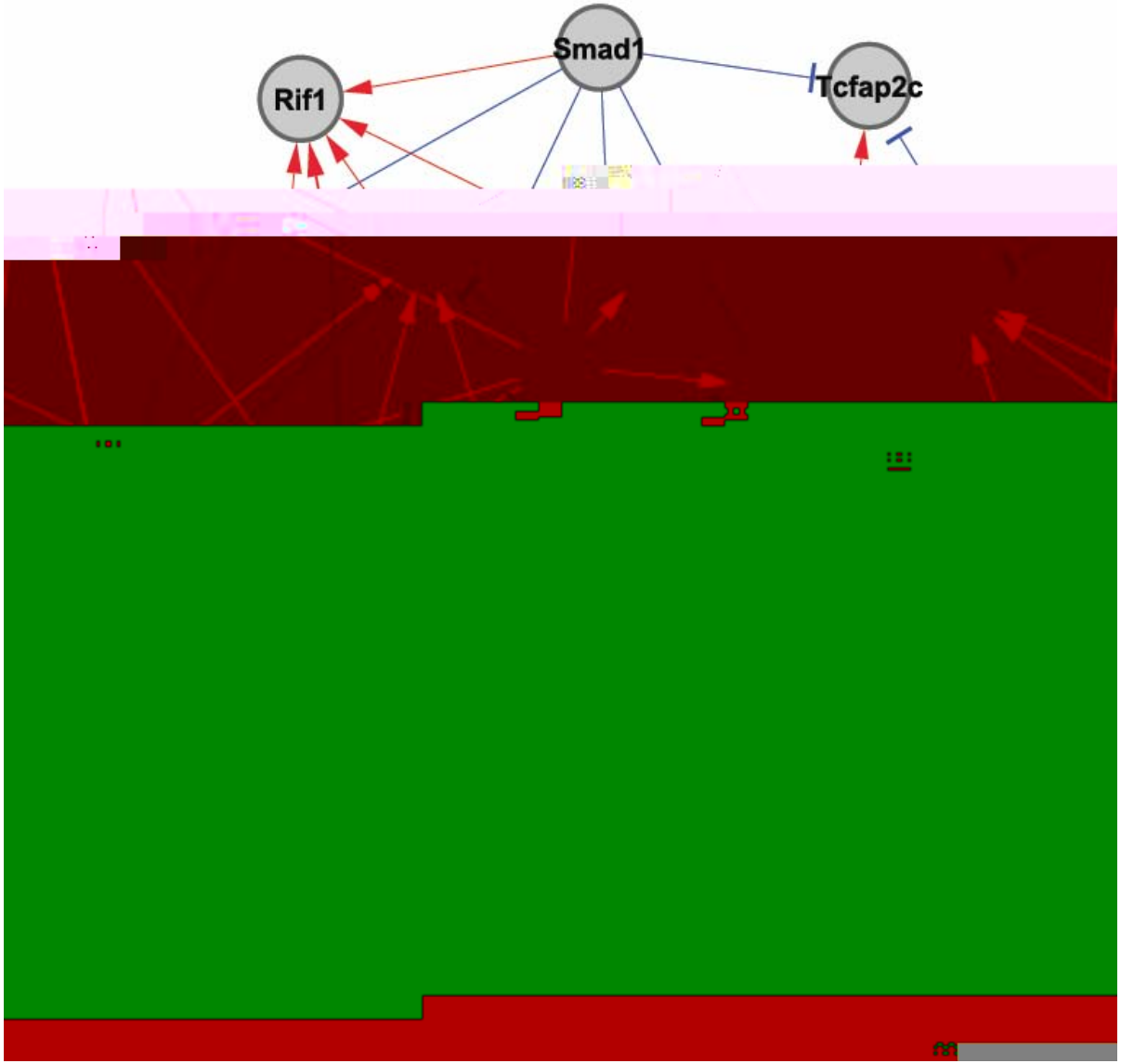
- Cross-validated for choice of λ

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

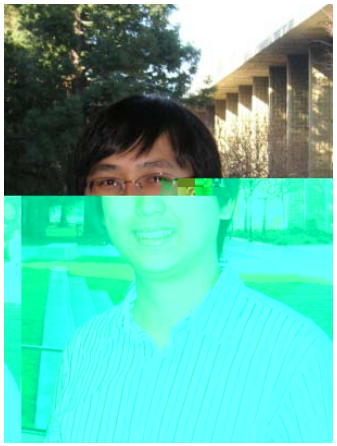
A subnetwork for important TFs



Acknowledgement

Gene perturbation:
Xi Chen

Methods & data analysis:
Zhengqing Ouyang



Methods:
Bokyung Choi