

Gene expression in artificial genomes after perturbations: gene insertion and knock-out

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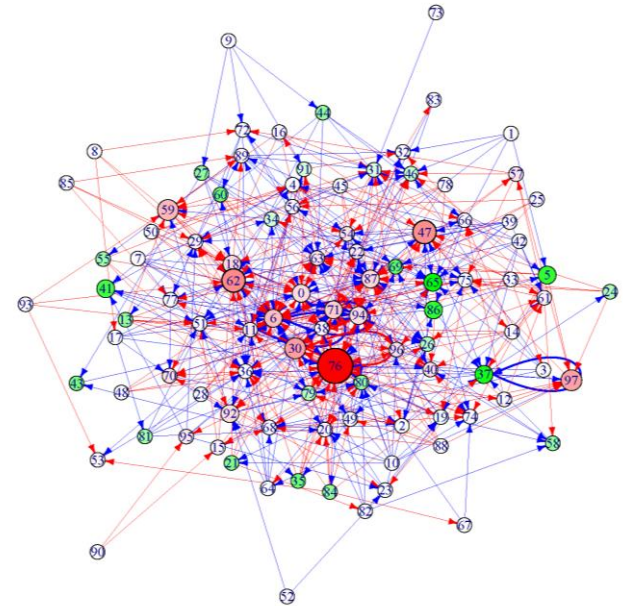
Dr. Hans Binder

Prof. Volkmar Liebscher



Outline

- Objective
- Random genome model
 - Random genome model
 - Thermodynamical model of genomic regulation
- Insertions and knock-out in artificial genomes
- Experimental data
- Summary



Introduction



Random genome model



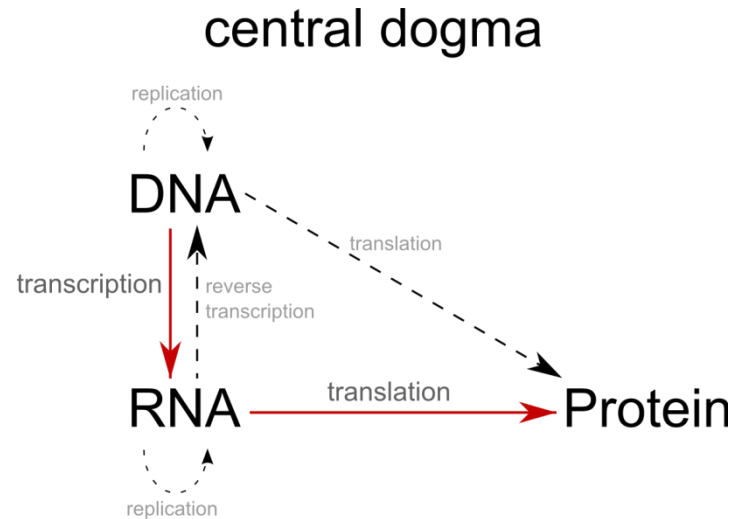
Insertion/KO



Experiments



Objective of the work



- Artificial genome modeled by “Random genome model” (T. Reil)

Objective: analyzing effects of perturbations of artificial genome on its expression

Introduction



Random genome model



Insertion/KO



Experiments



Random genome model (RGM)

2013121103210101022302012 ... 1011213121101201010221223012303120301010020100302 ... 012101
0 L_{genome}

- Random genome of particular length is generated as a chain of numbers (0-3)

Introduction



Random genome model



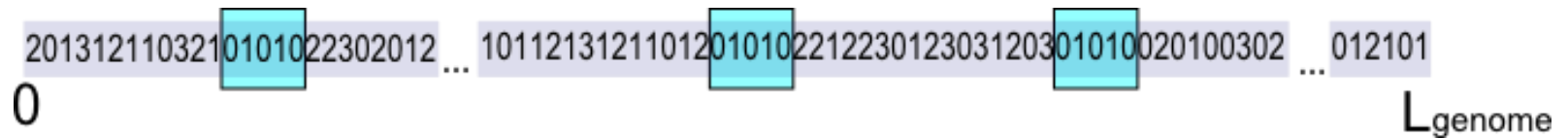
Insertion/KO



Experiments



Random genome model (RGM)



- Random genome of particular length is generated as a chain of numbers (0-3)
- Sequence is scanned for a string which encodes the promoter of a gene

Introduction



Random genome model



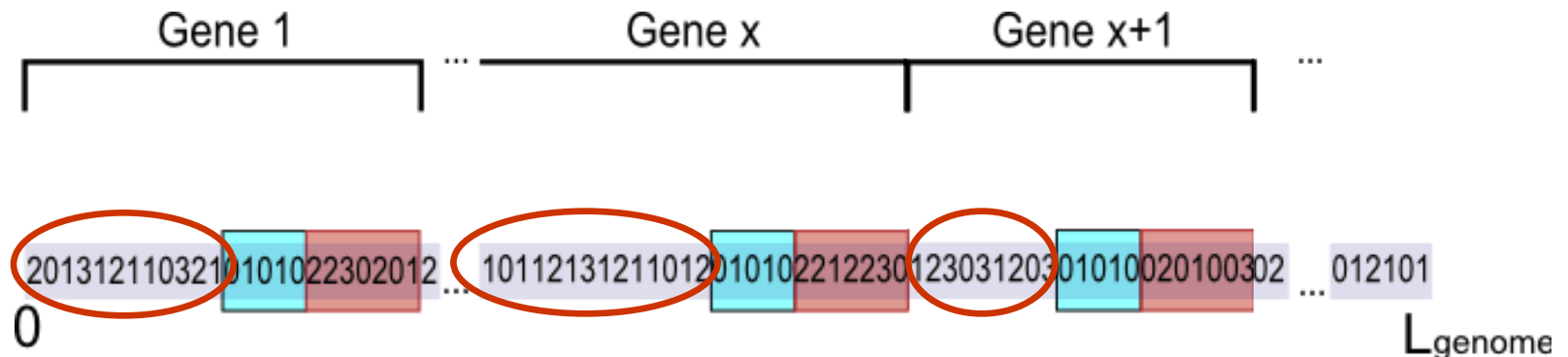
Insertion/KO



Experiments



Random genome model (RGM)



- ⇒ Promoter determines the start of the coding sequence
- ▣ Region between coding region and next promoter determines regulatory region

Introduction



Random genome model



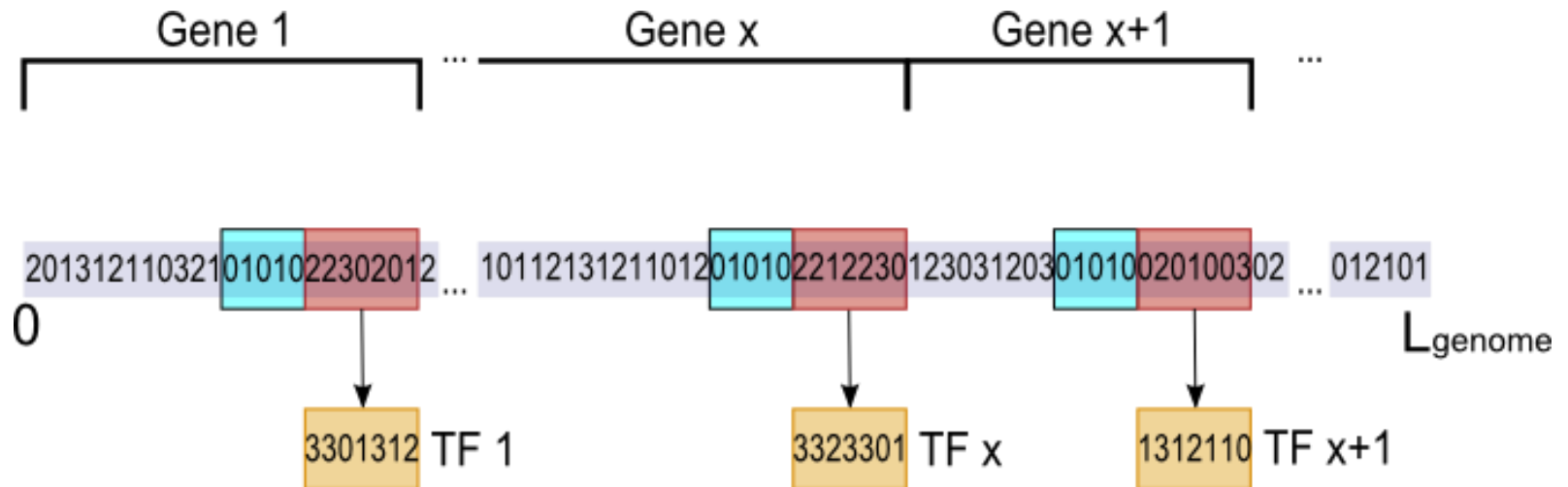
Insertion/KO



Experiments



Random genome model (RGM)



- Transcription factors (TF) are obtained by simple modification of coding sequence

Introduction



Random genome model



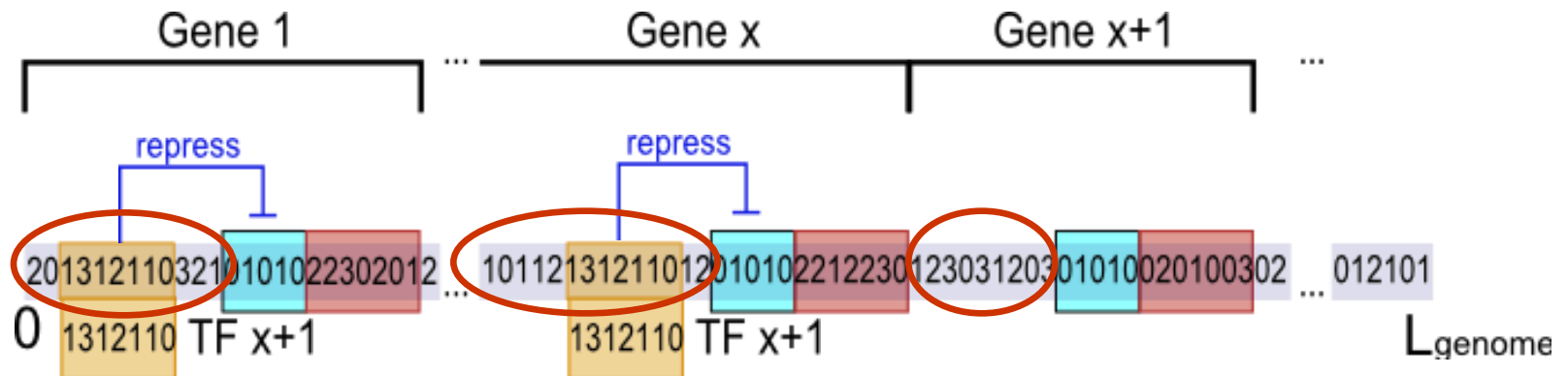
Insertion/KO



Experiments



Random genome model (RGM)



- Transcription factors (TF) are obtained by simple modification of coding sequence
- TFs are bound to the regulatory region of a gene in a sequence-specific fashion → regulate gene expression of downstream gene
- TFs can have an enhancing or repressing effect

Introduction



Random genome model



Insertion/KO



Experiments



Thermodynamical model of genomic regulation

- **Regulation factor F_i** of gene i reflects the interactions of the k regulators with RNAP
- **Promoter occupancy Θ_i** of gene i : probability that RNAP binds to promoter of gene i ($0 \leq \Theta_i \leq 1$)
- **Expression rate E_i** is directly proportional to Θ_i
 - $E_i < 1$ repressed gene,
 - $E_i = 1$ unregulated gene,
 - $E_i > 1$ activated gene

Introduction



Random genome model



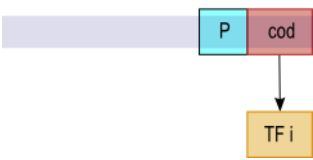
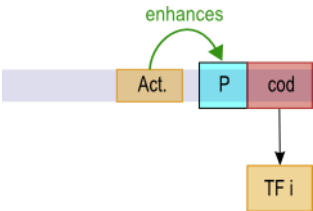
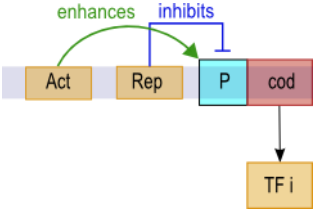
Insertion/KO



Experiments



Regulation

Case	In-degree	Expression E_i
<p>Gene i</p> 	0	$E_i \sim \Theta_i = \frac{X_0}{1+X_0}$ $E_i = 1$
	1	$E_i \sim \Theta_i = \frac{F_i X_0}{1+F_i X_0}$ $E_i > 1$
	2	$E_i \sim \Theta_i = \frac{F_i X_0}{1+F_i X_0}$

Introduction



Random genome model



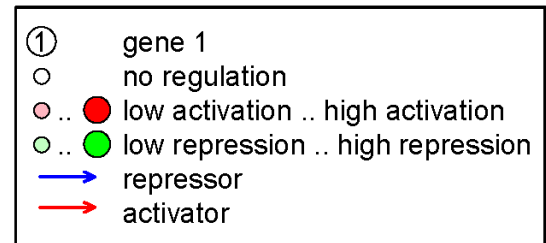
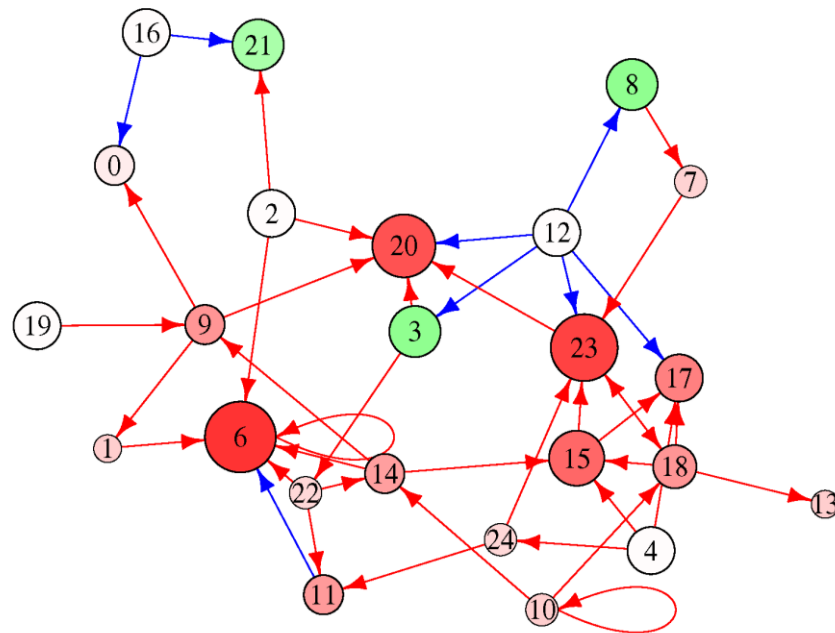
Insertion/KO



Experiments



Network of a random genome



Introduction



Random genome model



Insertion/KO



Experiments



Insertion and knock-out of genes

- ❑ Modifications used to discover the role of the inserted or knocked-out gene in considered organism
- ❑ RGM allows straightforward modeling of insertion/knock-out
- ❑ Effect of modifications on expression rate was analyzed

Introduction



Random genome model



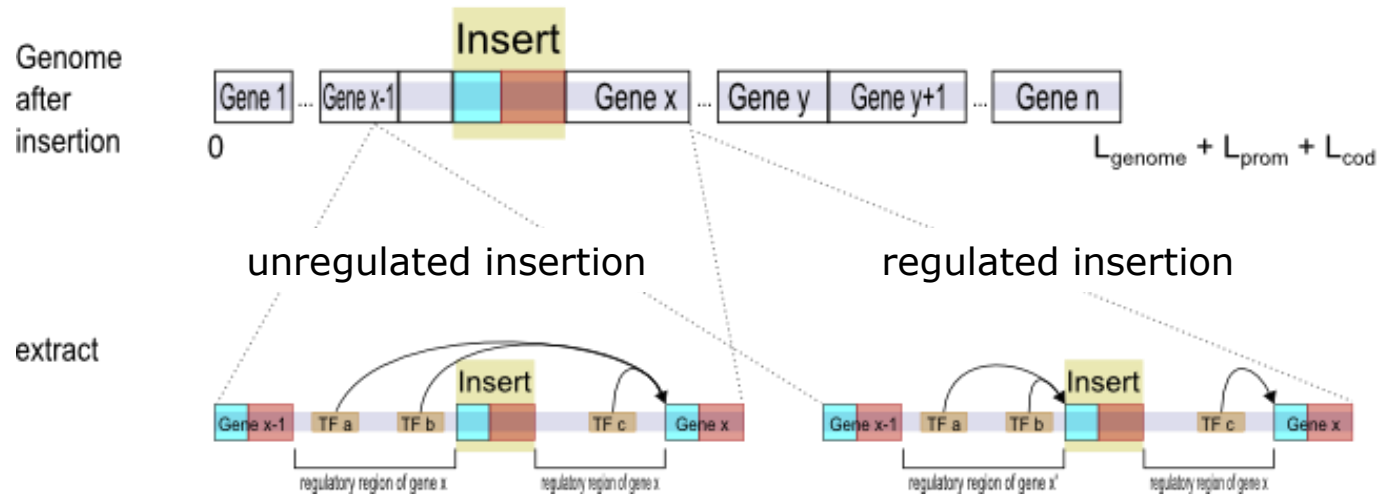
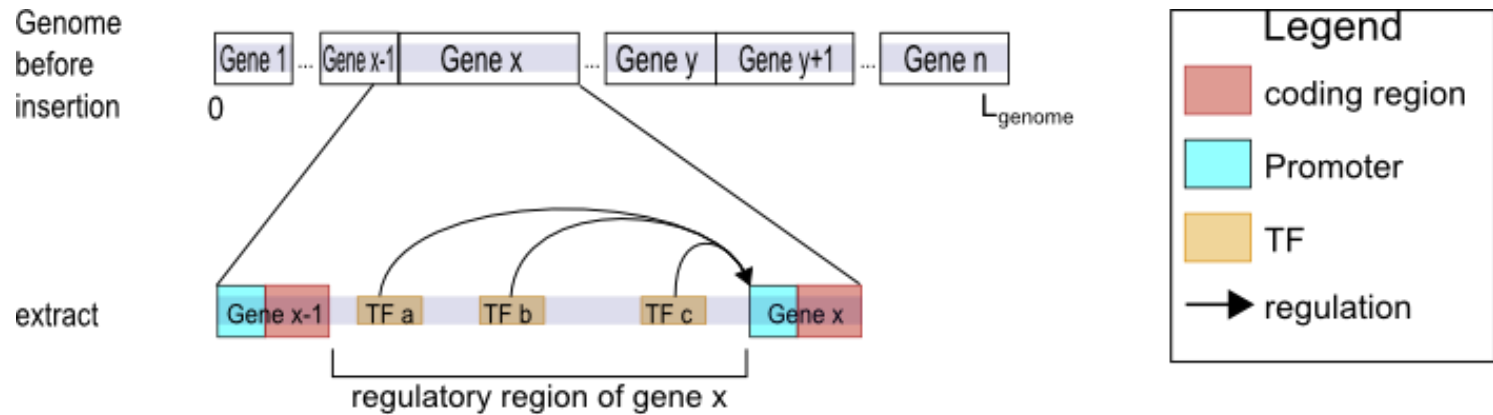
Insertion/KO



Experiments



Insertion



Introduction



Random genome model



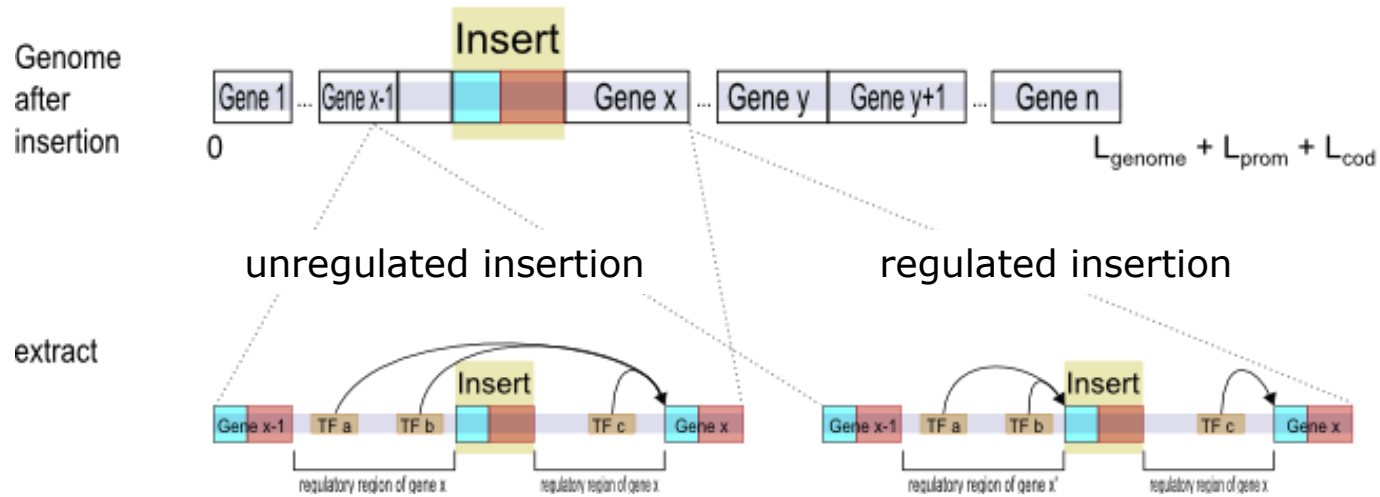
Insertion/KO



Experiments



Insertion



- ▣ In-degree of downstream gene doesn't change
- ▣ Expression $E=500$
- ▣ Expressions of insert and original are summed up \rightarrow overexpression
- ▣ In-degree of downstream gene may change
- ▣ May differ from $E=500$ if regulated

Introduction



Random genome model



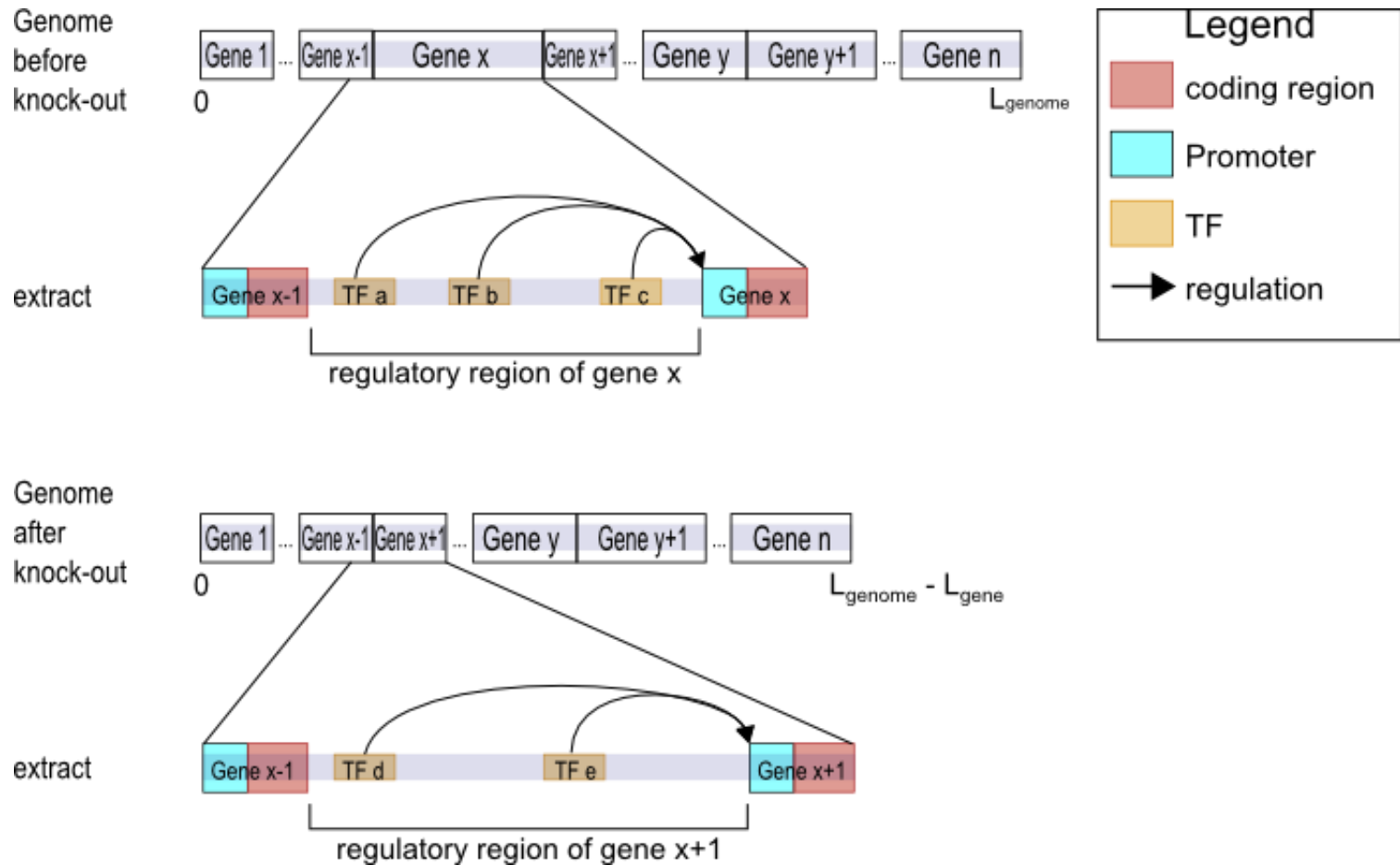
Insertion/KO



Experiments



Knock-out (KO)



Introduction



Random genome model



Insertion/KO

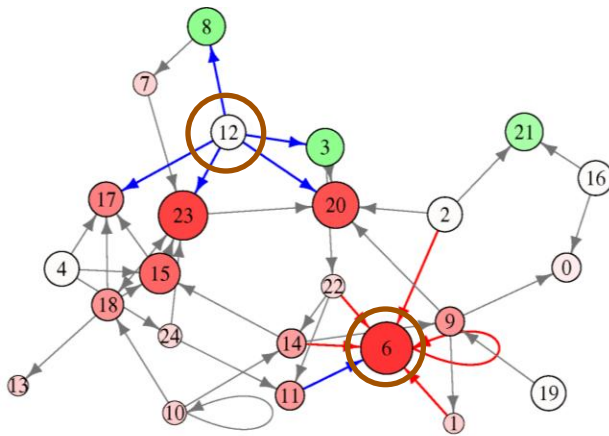


Experiments

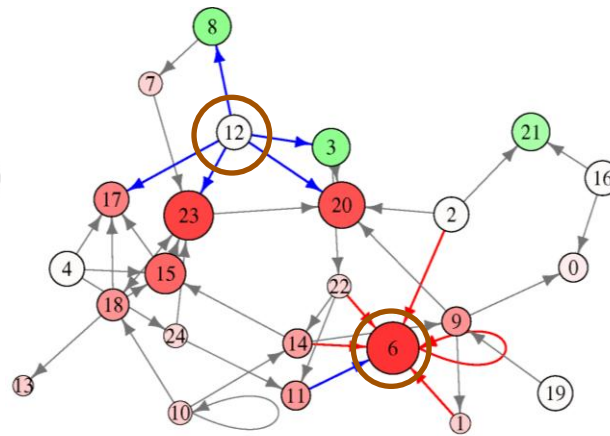


Perturbations of the genome after modifications

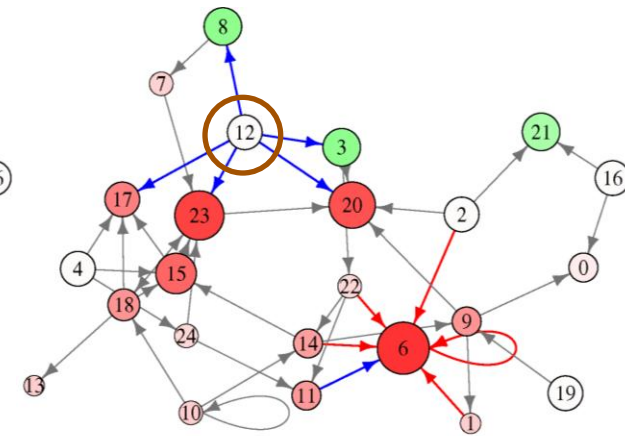
Unregulated insertion



Regulated insertion



Knock-out



Introduction



Random genome model



Insertion/KO

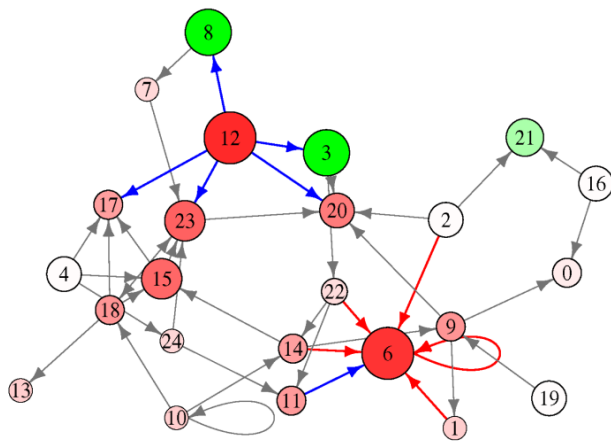


Experiments

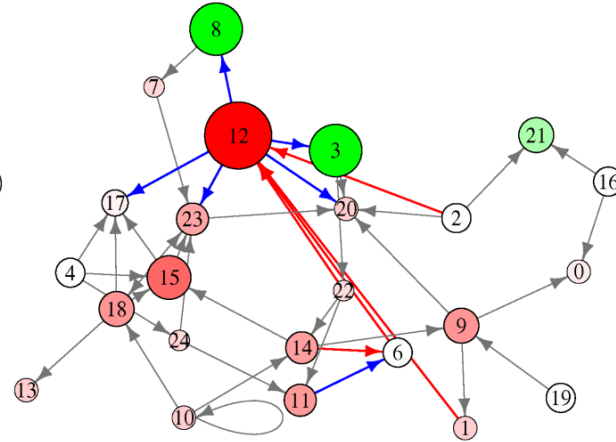


Perturbations of the genome after modifications

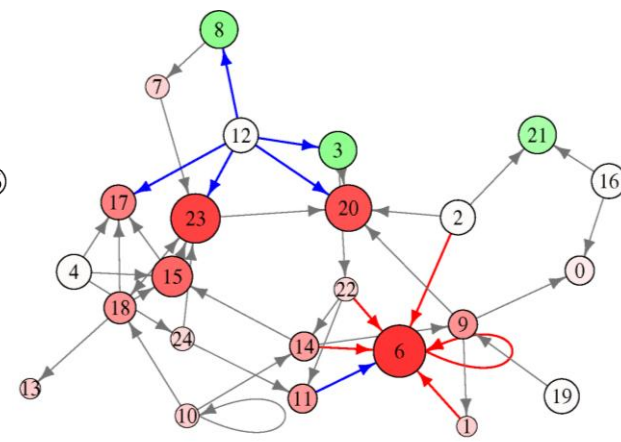
Unregulated insertion



Regulated insertion



Knock-out



Introduction



Random genome model



Insertion/KO

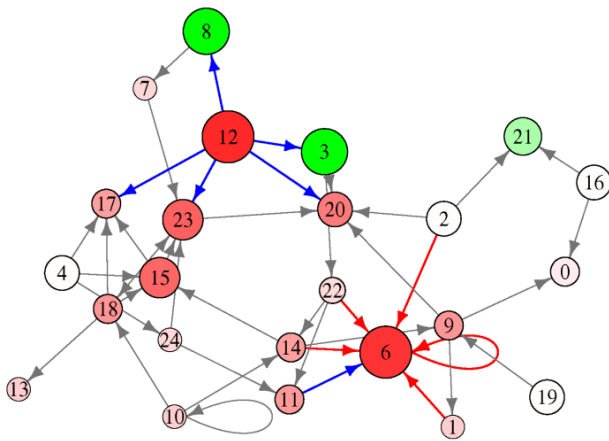


Experiments



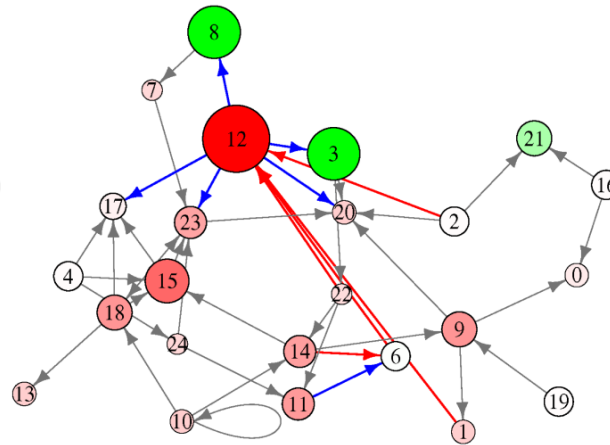
Perturbations of the genome after modifications

Unregulated insertion



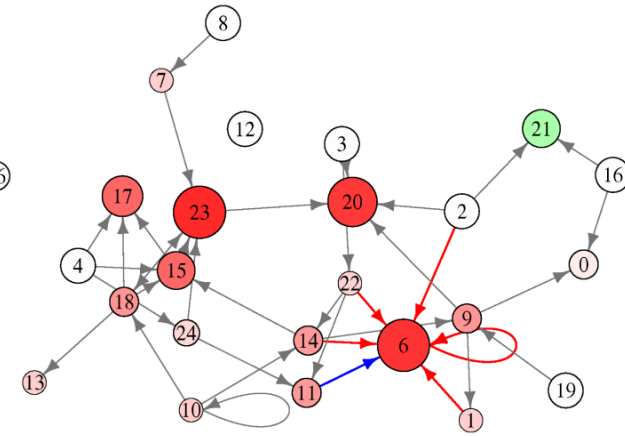
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Regulated insertion



5

Knock-out



5

Hypothesis: Insertion has global effect, KO has local effect on expression of the random genome

Introduction



Random genome model



Insertion/KO



Experiments



Modeling inhomogeneous networks by introducing Hubs

- Connectivity is defined by the length of the coding sequence
- Higher connectivity of a gene is achieved by reducing its coding length



Introduction



Random genome model



Insertion/KO



Experiments



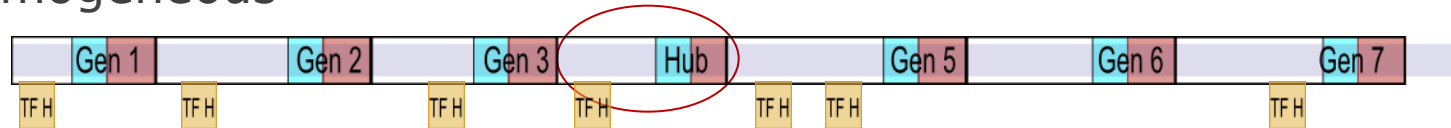
Modeling inhomogeneous networks by introducing Hubs

- Connectivity is defined by the length of the coding sequence
- Higher connectivity of a gene is achieved by reducing its coding length

Homogeneous



Inhomogeneous



- ⇒ increased probability of autoregulation of modified gene
- This gene is called **hub**

Introduction



Random genome model



Insertion/KO



Experiments



Experiments

- Microarray datasets of gene overexpression/ KO were analyzed
- Datasets contained raw intensities of genes of chips:
 - At least 1 “control-chip” (no overexpression/KO of the gene)
 - At least 1 chip containing measurements of the overexpression/KO
- After data preprocessing difference spectra were established

$$\log \text{ ratio} = \ln E_{Treatment} - \ln E_{control}$$

- ⇒ Analysis of the effect of a gene on the expression profile of the cell

Introduction



Random genome model



Insertion/KO



Experiments



Experiments-

Overexpression of PRDM5 (H. sapiens)

- Expression profiles of overexpression of tumorsuppressor PRDM5 were analyzed after 8, 24 and 48 h
- Dataset contained 12 chips

Introduction



Random genome model



Insertion/KO

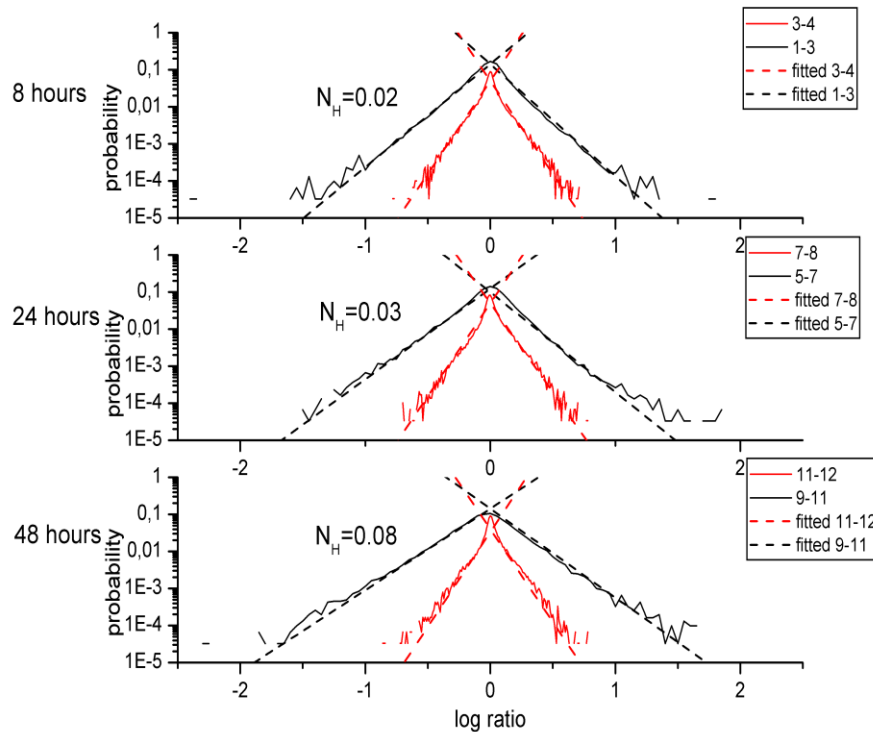


Experiments



Experiments-

Overexpression of PRDM5 (H. sapiens)



- Black curve: distribution of differential expression
- Red curve: noise distribution (difference of expression rates of two control chips)
- PRDM5 rank 1 of differentially expressed genes
- ⇒ PRDM5 overexpressed
- ⇒ Connectivity of PRDM5 could be calculated
- With increasing time the connectivity increases

⇒ PRDM5 highly connected gene, which affected diff. exp. of other genes

Introduction



Random genome model



Insertion/KO



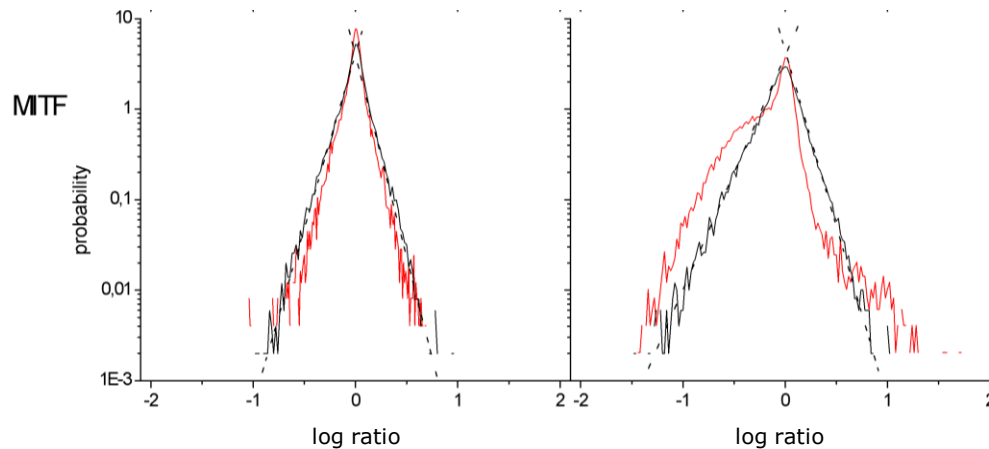
Experiments



Experiments-

KO of genes

- ❑ Considered KO experiments didn't result in differential expression of the genome
- ❑ Initially the knocked-out gene itself wasn't highly expressed
- ❑ Often: difference spectra of differential expression has the same slope as noise spectra



Introduction



Random genome model



Insertion/KO



Experiments



Summary

- ❑ Gene insertion/KO in artificial genome was modeled → Insertion has global effects, KO local effects on expression
 - ❑ Inhomogeneous networks modeled with the help of hubs → bigger effect on expression profile
 - ❑ In KO experiments chosen the considered genes had local effects on expression as the gene wasn't highly connected
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Thank you for your
attention!

