UNIVERSITÀ
DI TRENTO
Department of
Cellular, Computational and Integrative Biology - CIBIO

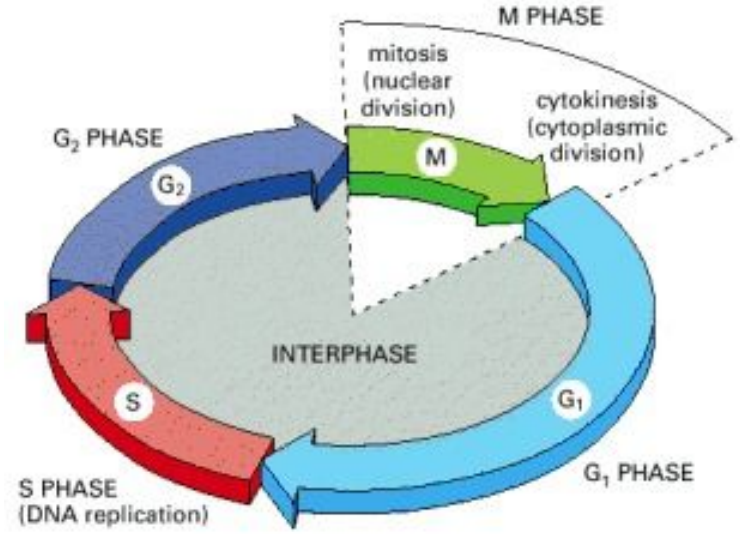
MASTER'S DEGREE IN QUANTITATIVE AND COMPUTATIONAL BIOLOGY

Exploring the integration of genomic data into stochastic modeling of Cell Cycle

Supervisor: Prof. Alessandro Romanel (PhD)

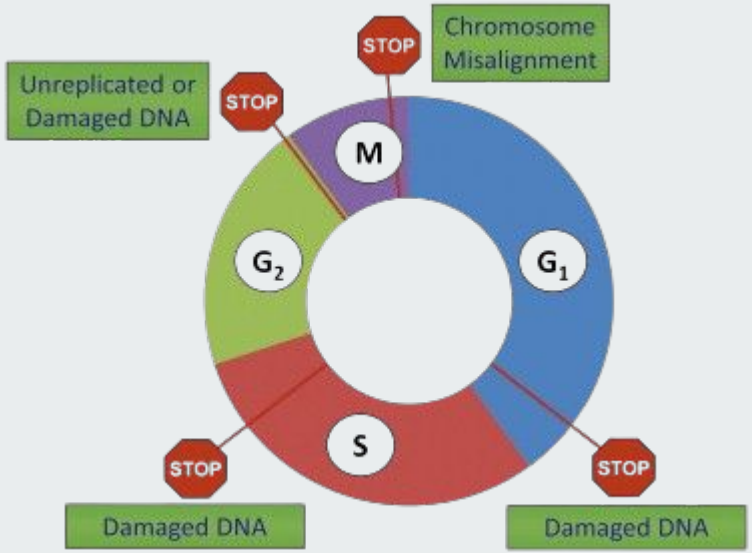
Graduant: Andrea Tonina

Cell cycle



Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. An Overview of the Cell Cycle.

Cell cycle regulations

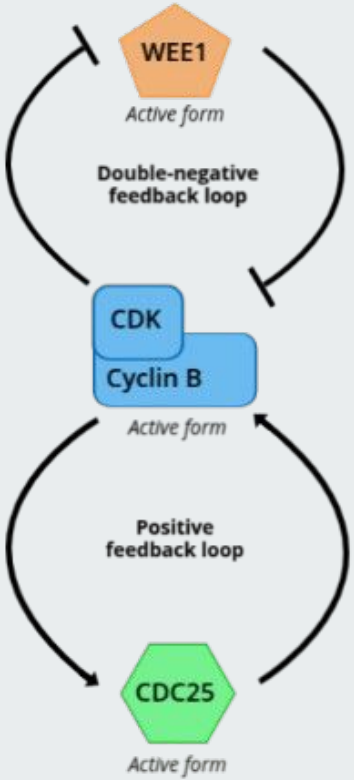


- Cyclin-CDK complexes
- Transcriptional control
- Protein-protein interactions and subcellular localization
- Cell cycle checkpoints

Vincent W. Yang, Chapter 15 - The Cell Cycle, Editor(s): Leonard R. Johnson, Fayez K. Ghishan, Jonathan D. Kaunitz, Juanita L. Merchant, Hamid M. Said, Jackie D. Wood, Physiology of the Gastrointestinal Tract (Fifth Edition), Academic Press, 2012, Pages 451-471.

Cell cycle transitions

- Biological switches
- G2 ⇨ M
- Stochastic or Deterministic approaches

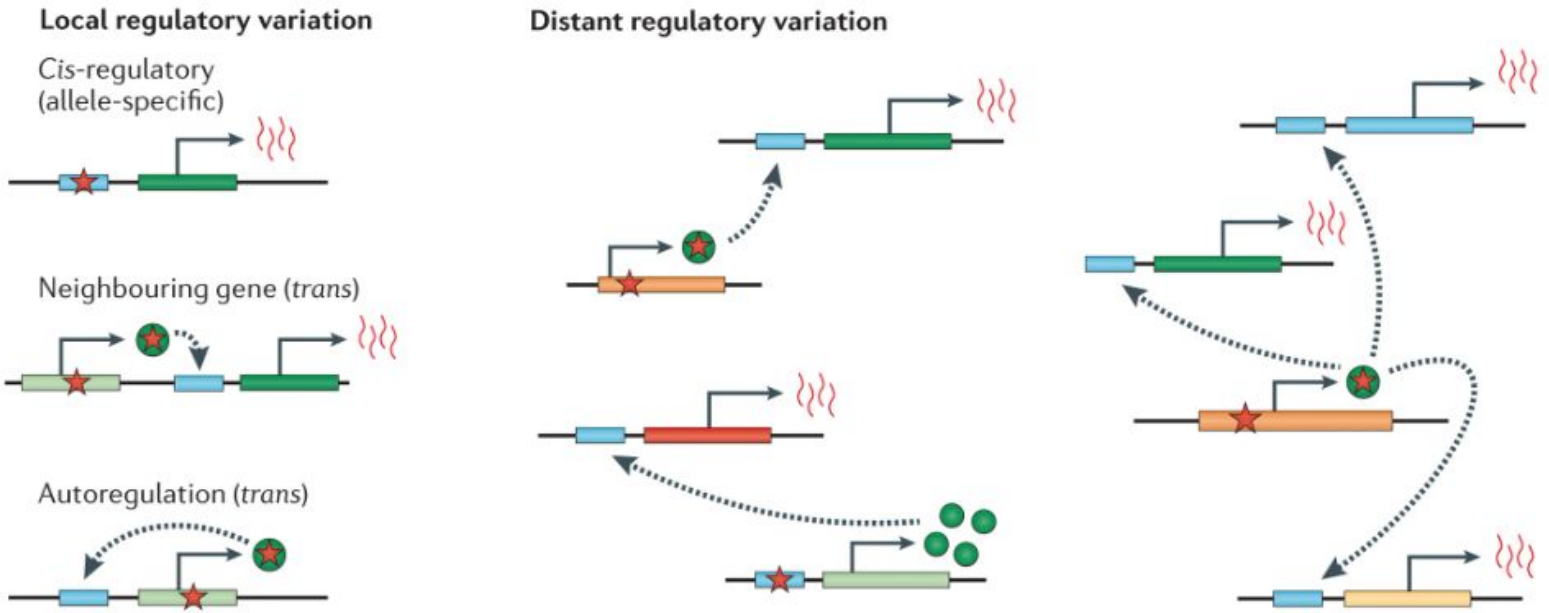


Adapted from Zámborszky, J. (2013). Cell Cycle Transitions, G2/M. In: Dubitzky, W., Wolkenhauer, O., Cho, KH., Yokota, H. (eds) Encyclopedia of Systems Biology. Springer, New York, NY. https://doi.org/10.1007/978-1-4419-9863-7_38

Genetic variability

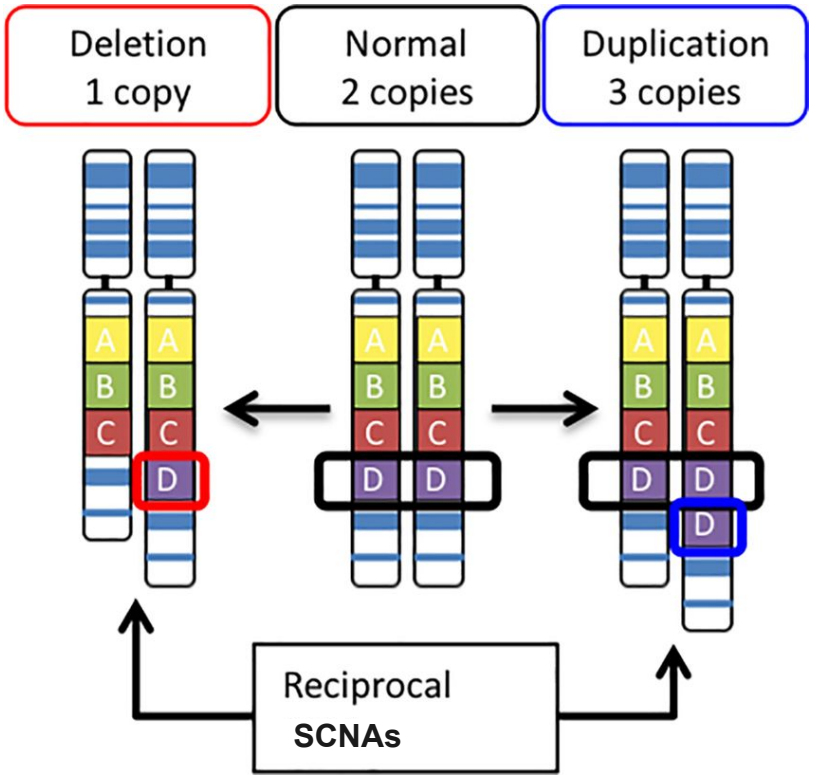
- Difference in DNA sequences within our genomes among individuals
- Mutations are the ultimate source of new genetic variants in DNA
- Spectrum mutation is quite broad and diverse
- Distinction between : germline or somatic

eQTLs



Albert, F., Kruglyak, L. The role of regulatory variation in complex traits and disease. *Nat Rev Genet* 16, 197–212 (2015). https://doi.org/10.1038/nrg3891

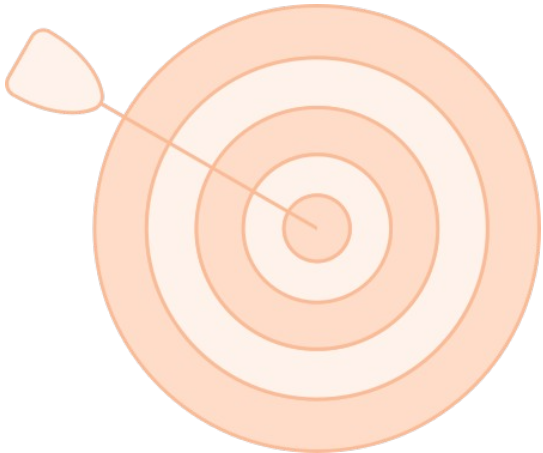
SCNA



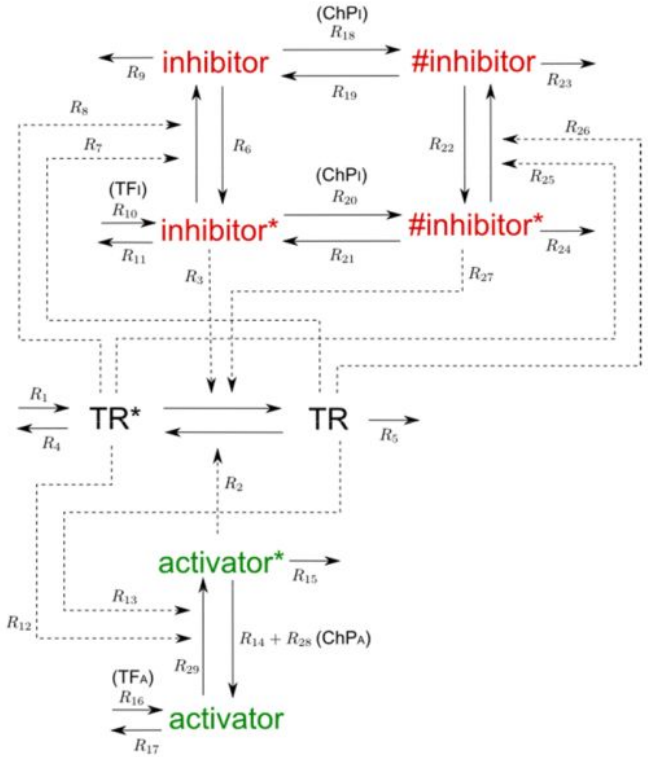
Sønderby IE, Ching CRK, Thomopoulos SI, et al. Effects of copy number variations on brain structure and risk for psychiatric illness: Large-scale studies from the ENIGMA working groups on CNVs. *Hum Brain Mapp*. 2022; 43: 300–328

Aims

- Characterization of a generic stochastic model of cell cycle transition
- Human-specific cell cycle transition model G2/M
- Explore the impact of germline and somatic variants
    - eQTLs
    - Somatic copy number alterations in breast cancer
- Explore how this variants can be utilized to stratify patient in a tumoral context



General and universal cell cycle transition model



Utilized for implementation of the model the stochastic programming language Blenx

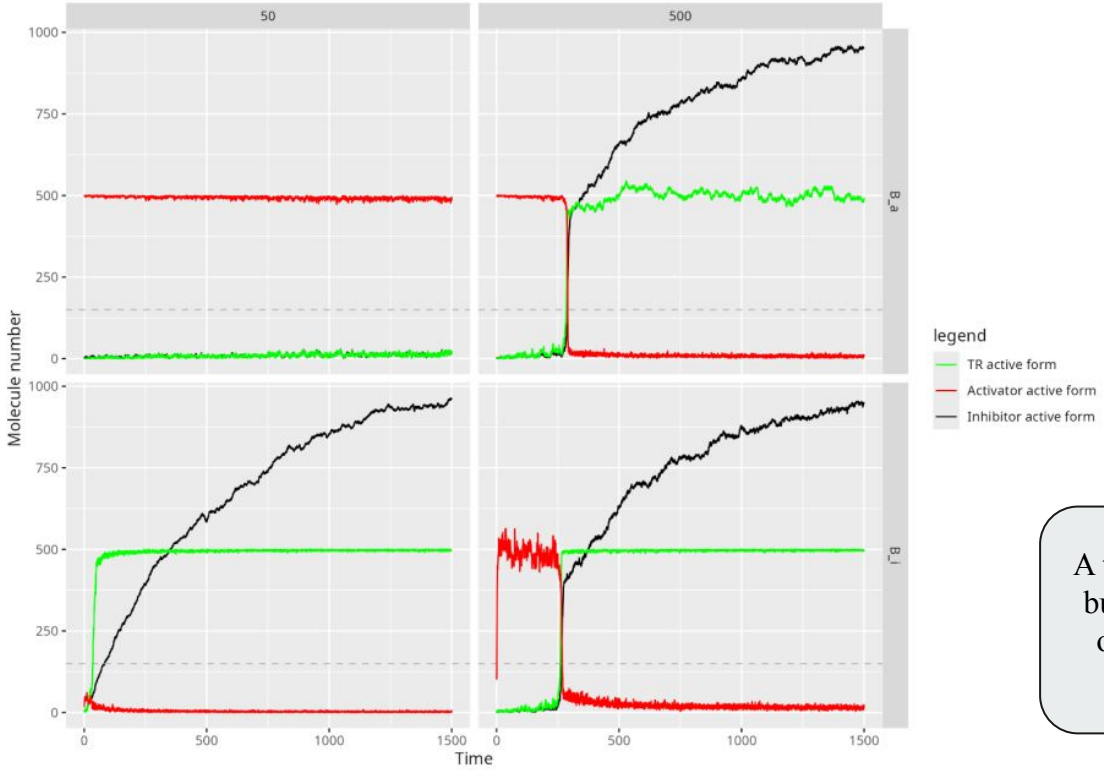
Dematté, L., Priami, C., Romanel, A. (2008). The BlenX Language: A Tutorial. In: Bernardo, M., Degano, P., Zavattaro, G. (eds) Formal Methods for Computational Systems Biology. SFM 2008. Lecture Notes in Computer Science, vol 5016. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-68894-5_9

Romanel A, Jensen LJ, Cardelli L, Csikász-Nagy A (2012) Transcriptional Regulation Is a Major Controller of Cell Cycle Transition Dynamics. PLoS ONE 7(1): e29716. https://doi.org/10.1371/journal.pone.0029716

$R_1 = \frac{kms}{\alpha}$	$R_{16} = kcs \times TF$
$R_2 = \frac{kma \times \alpha \times activator^* \times TR}{jma + (\alpha \times TR)}$	$R_{17} = kcd_1 \times activator$
$R_3 = \frac{kmi \times \alpha \times inhibitor^* \times TR^*}{jmi + (\alpha \times TR^*)}$	$R_{18} = \frac{kcp_7 \times \alpha \times ChPI \times inhibitor}{jcp_7 + (\alpha \times inhibitor)}$
$R_4 = kmd \times TR^*$	$R_{19} = \frac{kcp_6 \times \alpha \times Pho \times \#inhibitor}{jcp_6 + (\alpha \times \#inhibitor)}$
$R_5 = kmd_1 \times TR$	$R_{20} = \frac{kcp_1 \times \alpha \times ChPI \times inhibitor^*}{jcp_1 + (\alpha \times inhibitor^*)}$
$R_6 = \frac{kwa \times \alpha \times E_1 \times inhibitor}{jwa + (\alpha \times inhibitor)}$	$R_{21} = \frac{kcp_2 \times \alpha \times Pho \times \#inhibitor^*}{jcp_2 + (\alpha \times \#inhibitor^*)}$
$R_7 = \frac{kwi \times \alpha \times TR^* \times inhibitor^*}{jwi + (\alpha \times inhibitor^*)}$	$R_{22} = \frac{kcp_5 \times \alpha \times E_1 \times \#inhibitor}{jcp_5 + (\alpha \times \#inhibitor)}$
$R_8 = \frac{kwi \times \alpha \times perc \times TR \times inhibitor^*}{jwi + (\alpha \times inhibitor^*)}$	$R_{23} = kwd_1 \times \#inhibitor$
$R_9 = kwd_1 \times inhibitor$	$R_{24} = kwd \times \#inhibitor^*$
$R_{10} = kws \times TF$	$R_{25} = \frac{kcp_3 \times \alpha \times TR^* \times \#inhibitor^*}{jcp_3 + (\alpha \times \#inhibitor^*)}$
$R_{11} = kwd \times inhibitor^*$	$R_{26} = \frac{kcp_4 \times \alpha \times perc \times TR \times \#inhibitor^*}{jcp_4 + (\alpha \times \#inhibitor^*)}$
$R_{12} = \frac{kca \times \alpha \times TR^* \times activator}{jca + (\alpha \times activator)}$	$R_{27} = \frac{kmi_1 \times \alpha \times \#inhibitor^* \times TR^*}{jmi_1 + (\alpha \times TR^*)}$
$R_{13} = \frac{kca \times \alpha \times perc \times TR \times activator}{jca + (\alpha \times activator)}$	$R_{28} = \frac{kcp_8 \times \alpha \times ChPA \times activator^*}{jcp_8 + (\alpha \times activator^*)}$
$R_{14} = \frac{kci \times \alpha \times E_2 \times activator^*}{jci + (\alpha \times activator^*)}$	$R_{29} = \frac{kca \times \alpha \times S \times activator}{jca + (\alpha \times activator)}$
$R_{15} = kcd \times activator^*$	

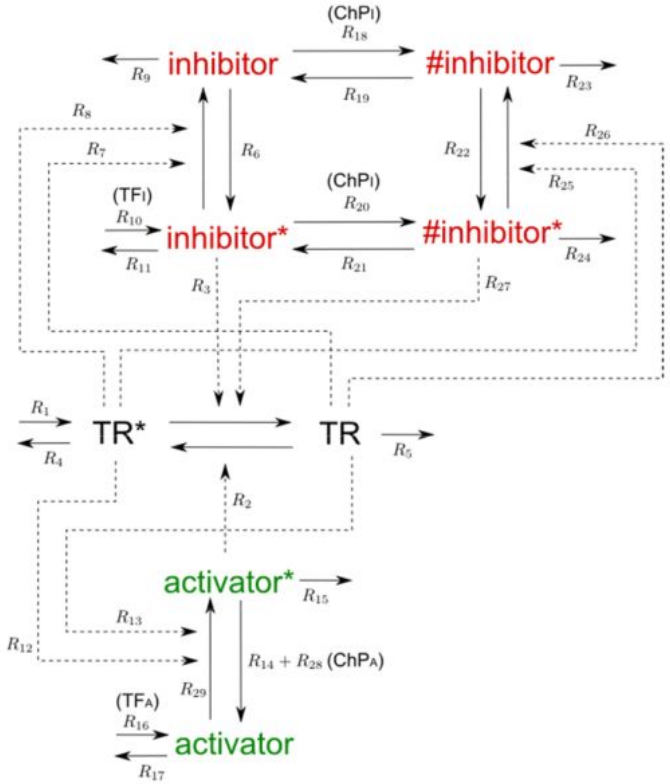
Table 1 . Kinetic laws associated to model reactions. Table adapted from *Romanel et al*[187]**.** Parameters with letter *k* are catalytic constants with dimension 1/min, whereas the ones starting with letter *j* are Michaelis constants as dimensionless.

Simulations of GO and STOP transcriptional regulations



A total of 24 models were build using combination of the three regulatory processes

Sensitivity analysis of the general model



Species :

- *E2* variations impact transition in each models
- TF_A variations more impactful on models than TF_I
- ChP_A variations on PFB on inhibitors or both

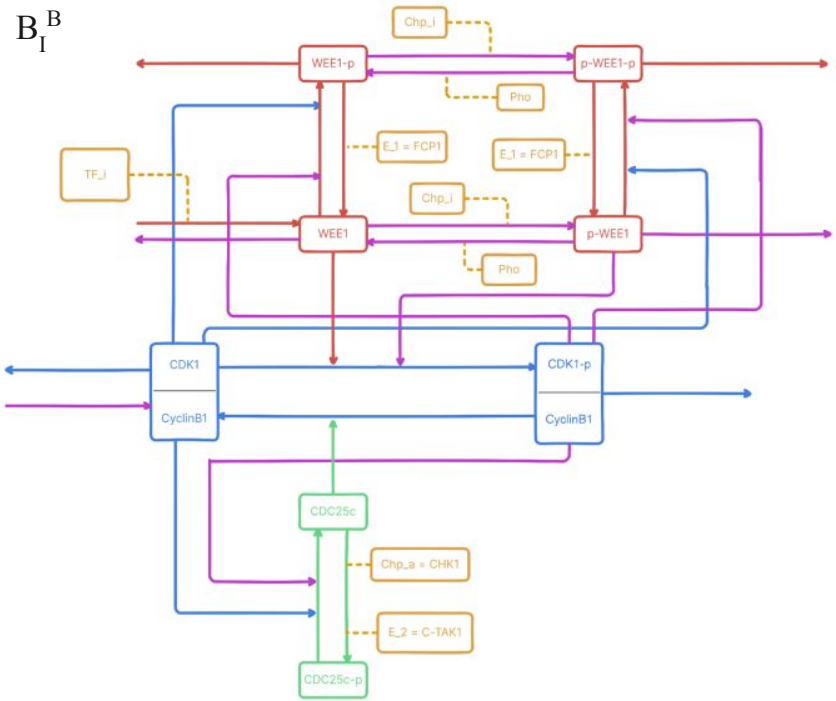
Parameters:

- *kma* variations impact transition on all models
- *jci* variations impact model with transcriptional control on the activator only

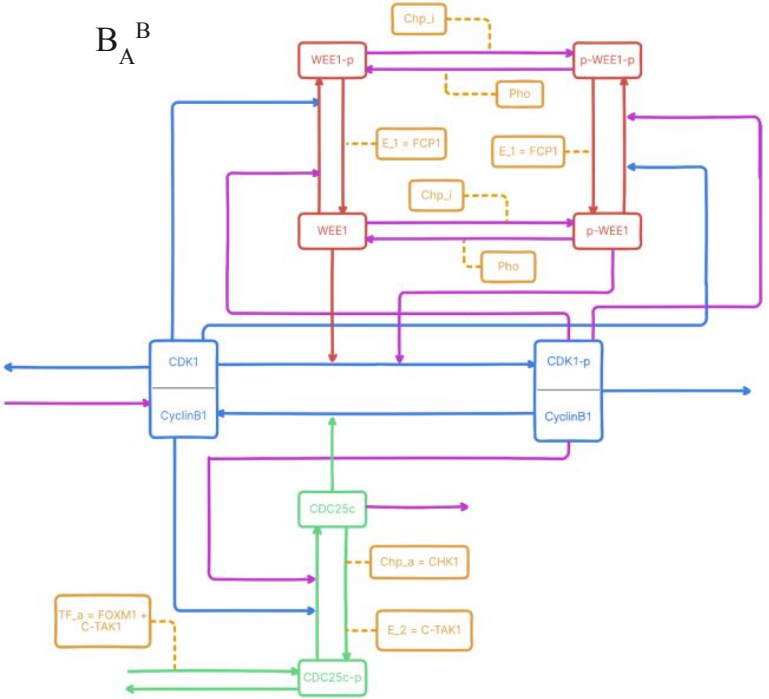
Romanel A, Jensen LJ, Cardelli L, Csikász-Nagy A (2012) Transcriptional Regulation Is a Major Controller of Cell Cycle Transition Dynamics. PLoS ONE 7(1): e29716. https://doi.org/10.1371/journal.pone.0029716

Human specific cell-cycle model G2/M

B_I^B



B_A^B



eQTLs

Adult Genotype-Tissue Expression (GTEx) project

- median gene-levels expression in TPM for each tissue
- eQTLs information for each gene of interest involved in the cell cycle G2/M transition

First part

$$\begin{cases} e_{tot} = (1 - MAF)e_r + (MAF)e_a \\ aFC = log_2(\frac{e_a}{e_r}) \end{cases}$$

$$\begin{cases} e_{tot} = (1 - MAF)e_r + (MAF)e_a \\ 2^{aFC} * e_r = e_a \end{cases}$$

$$\begin{cases} e_{tot} = e_r * (1 - MAF + MAF * 2^{aFC}) \\ 2^{aFC} * e_r = e_a \end{cases}$$

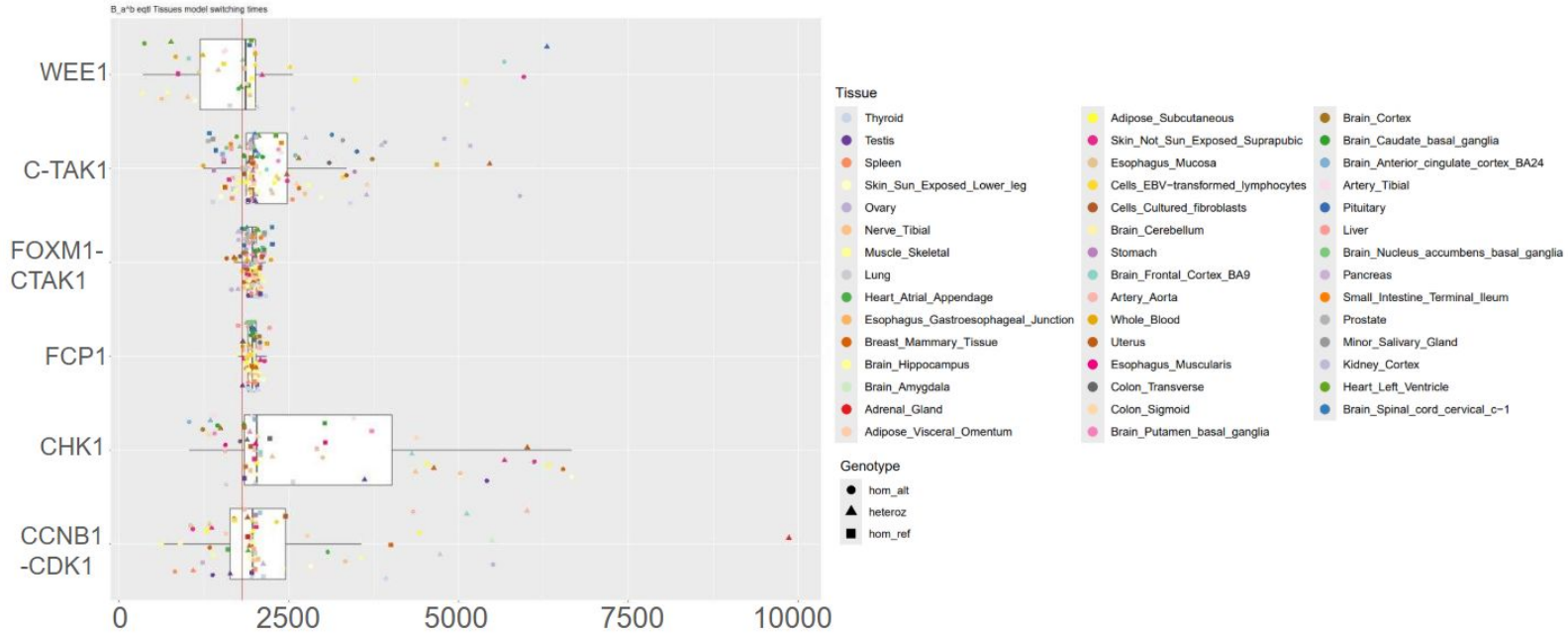
$$\begin{cases} e_r = \frac{e_{tot}}{(1-MAF+MAF*2^{aFC})} \\ 2^{aFC} * e_r = e_a \end{cases}$$

Second part Calculate e_a and e_r

$$\begin{cases} e_r = \frac{e_{tot}}{(1-MAF+MAF*2^{aFC})} \\ e_a = \frac{2^{aFC} * e_{tot}}{(1-MAF+MAF*2^{aFC})} \end{cases}$$

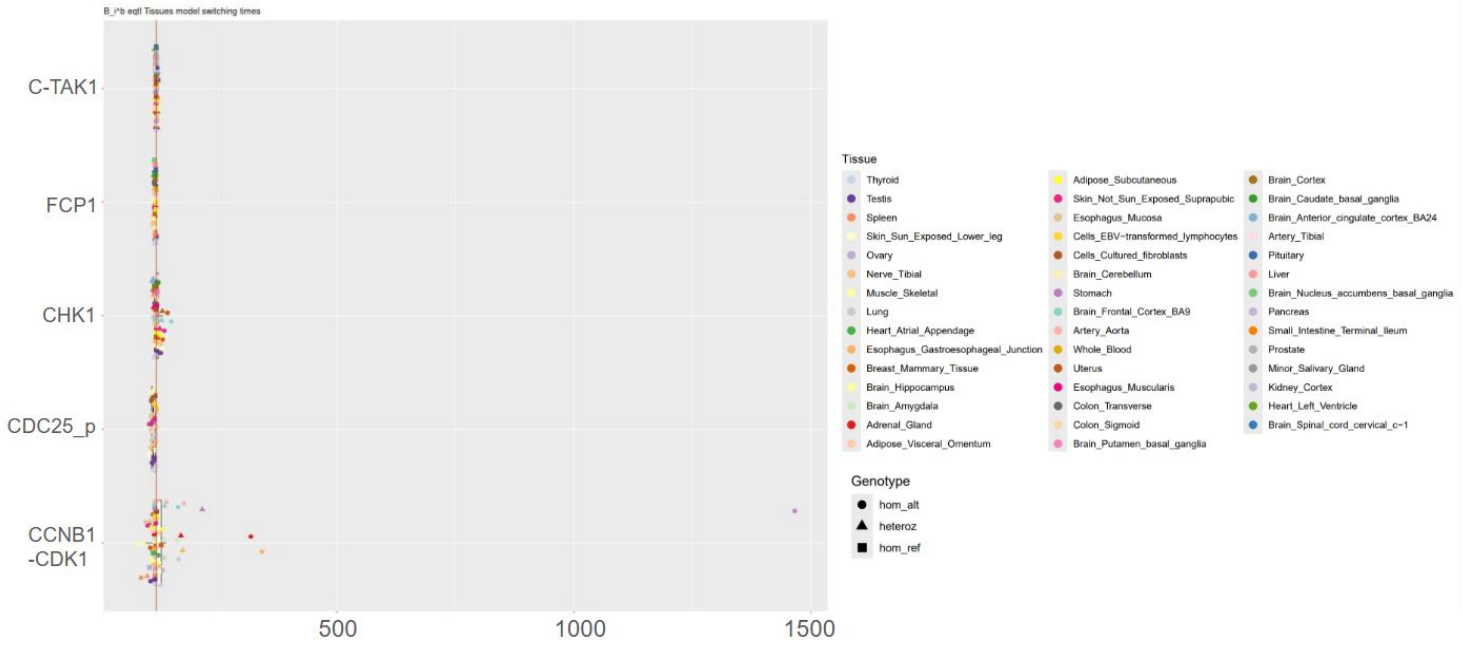
eQTLs

B_A^B

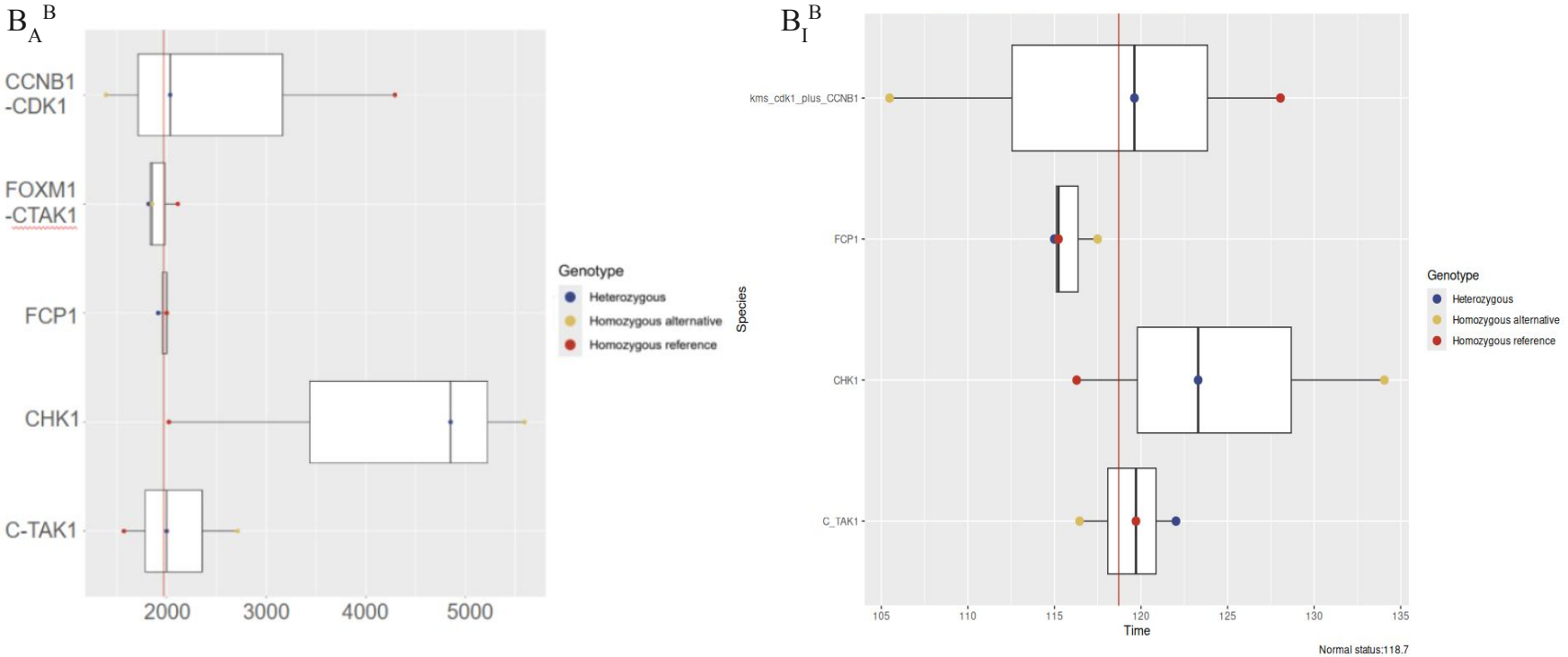

B_a^b eqtl Tissues model switching times
WEE1
C-TAK1
FOXM1-
CTAK1
FCP1
CHK1
CCNB1
-CDK1
0
2500
5000
7500
10000
Tissue
Thyroid
Testis
Spleen
Skin_Sun_Exposed_Lower_leg
Ovary
Nerve_Tibial
Muscle_Skeletal
Lung
Heart_Atrial_Appendage
Esophagus_Gastroesophageal_Junction
Breast_Mammary_Tissue
Brain_Hippocampus
Brain_Amygdala
Adrenal_Gland
Adipose_Visceral_Omentum
Adipose_Subcutaneous
Skin_Not_Sun_Exposed_Suprapubic
Esophagus_Mucosa
Cells_EBV−transformed_lymphocytes
Cells_Cultured_fibroblasts
Brain_Cerebellum
Stomach
Brain_Frontal_Cortex_BA9
Artery_Aorta
Whole_Blood
Uterus
Esophagus_Muscularis
Colon_Transverse
Colon_Sigmoid
Brain_Putamen_basal_ganglia
Brain_Cortex
Brain_Caudate_basal_ganglia
Brain_Anterior_cingulate_cortex_BA24
Artery_Tibial
Pituitary
Liver
Brain_Nucleus_accumbens_basal_ganglia
Pancreas
Small_Intestine_Terminal_Ileum
Prostate
Minor_Salivary_Gland
Kidney_Cortex
Heart_Left_Ventricle
Brain_Spinal_cord_cervical_c−1
Genotype
hom_alt
heteroz
hom_ref

eQTLs

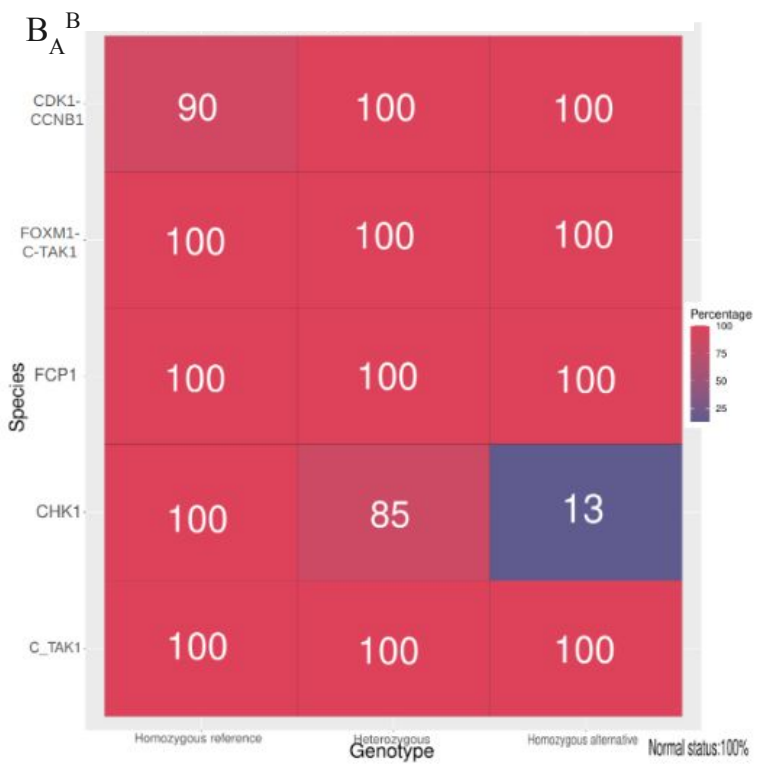
B_I^B

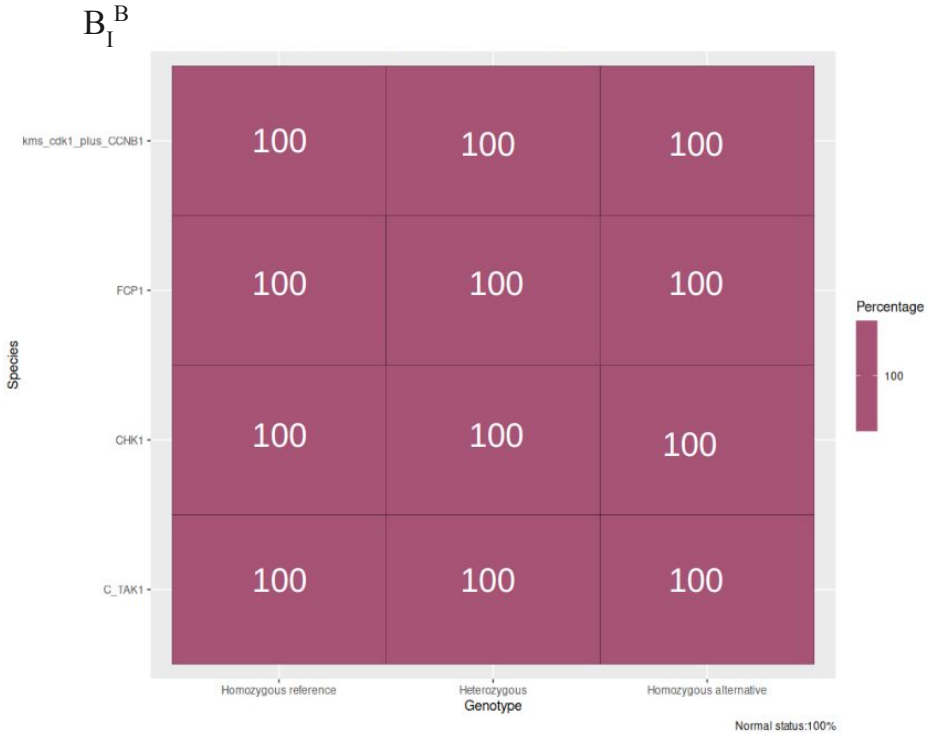

B_i^b eqtl Tissues model switching times
C-TAK1
FCP1
CHK1
CDC25_p
CCNB1
-CDK1
500
1000
1500
Tissue
Thyroid
Testis
Spleen
Skin_Sun_Exposed_Lower_leg
Ovary
Nerve_Tibial
Muscle_Skeletal
Lung
Heart_Atrial_Appendage
Esophagus_Gastroesophageal_Junction
Breast_Mammary_Tissue
Brain_Hippocampus
Brain_Amygdala
Adrenal_Gland
Adipose_Visceral_Omentum
Adipose_Subcutaneous
Skin_Not_Sun_Exposed_Suprapubic
Esophagus_Mucosa
Cells_EBV-transformed_lymphocytes
Cells_Cultured_fibroblasts
Brain_Cerebellum
Stomach
Brain_Frontal_Cortex_BA9
Artery_Aorta
Whole_Blood
Uterus
Esophagus_Muscularis
Colon_Transverse
Colon_Sigmoid
Brain_Putamen_basal_ganglia
Brain_Cortex
Brain_Caudate_basal_ganglia
Brain_Anterior_cingulate_cortex_BA24
Artery_Tibial
Pituitary
Liver
Brain_Nucleus_accumbens_basal_ganglia
Pancreas
Small_Intestine_Terminal_Ileum
Prostate
Minor_Salivary_Gland
Kidney_Cortex
Heart_Left_Ventricle
Brain_Spinal_cord_cervical_c-1
Genotype
hom_alt
heteroz
hom_ref

eQTLs


B_A^B
CCNB1
-CDK1
FOXM1
-CTAK1
FCP1
CHK1
C-TAK1
2000
3000
4000
5000
Genotype
Heterozygous
Homozygous alternative
Homozygous reference
Species
B_I^B
kms_cdk1_plus_CCNB1
FCP1
CHK1
C_TAK1
105
110
115
120
125
130
135
Time
Normal status:118.7
Genotype
Heterozygous
Homozygous alternative
Homozygous reference

eQTLs

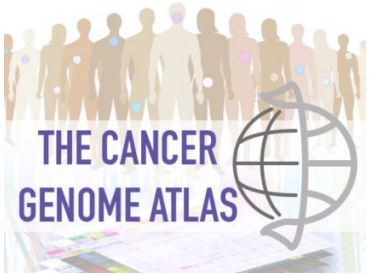

CDK1-CCNB1
FOXM1-C-TAK1
FCP1
CHK1
C_TAK1
Species
90
100
100
100
100
100
100
100
100
100
85
13
100
100
100
Homozygous reference
Heterozygous
Homozygous alternative
Genotype
Percentage
100
75
50
25
Normal status:100%

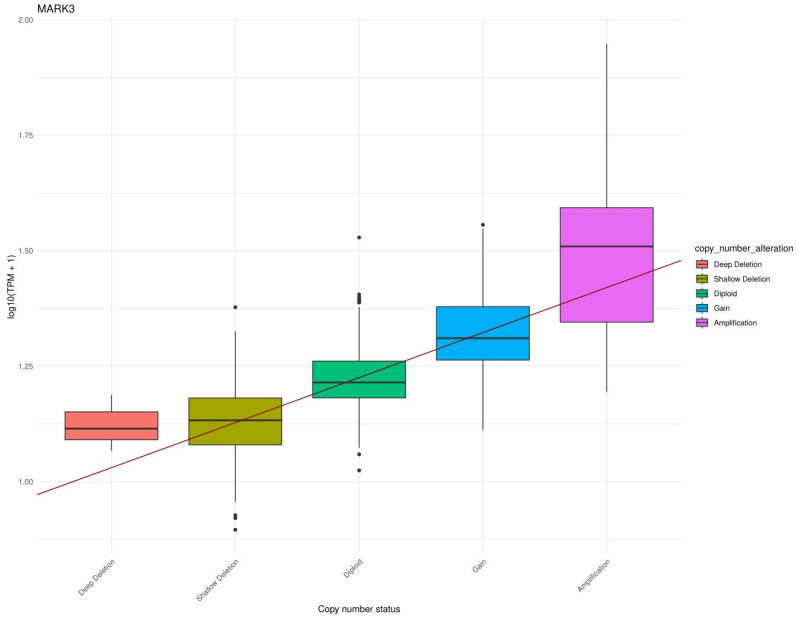

kms_cdk1_plus_CCNB1
FCP1
CHK1
C_TAK1
Species
100
100
100
100
100
100
100
100
100
100
100
100
Homozygous reference
Heterozygous
Homozygous alternative
Genotype
Percentage
100
Normal status:100%

SCNAs

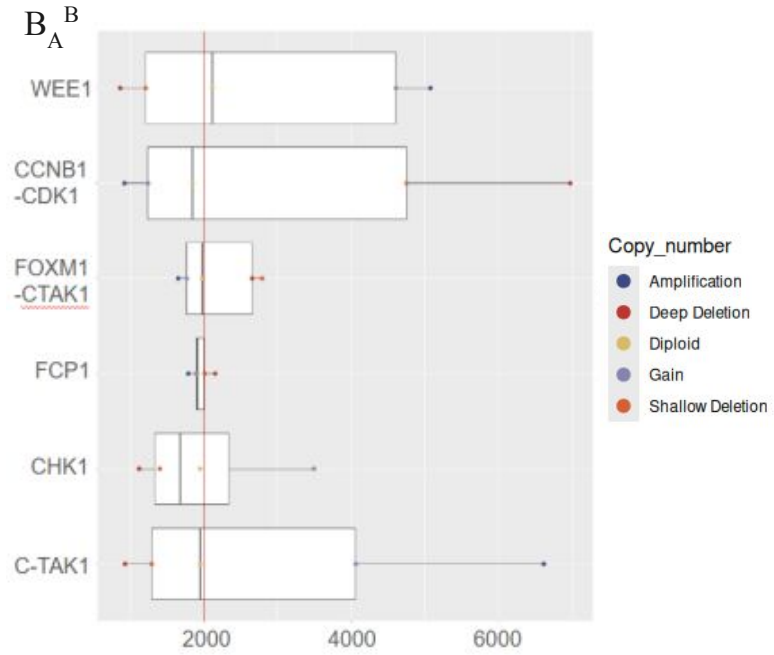
Invasive Breast Carcinoma (TCGA, PanCancer Atlas)

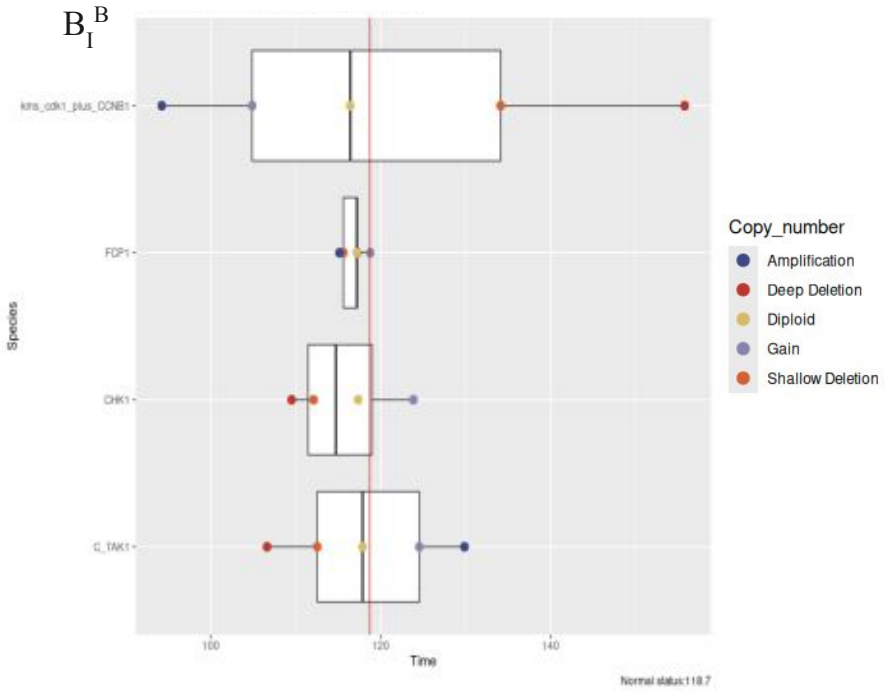
- batch-corrected RNA-seq data
- somatic copy number alterations data



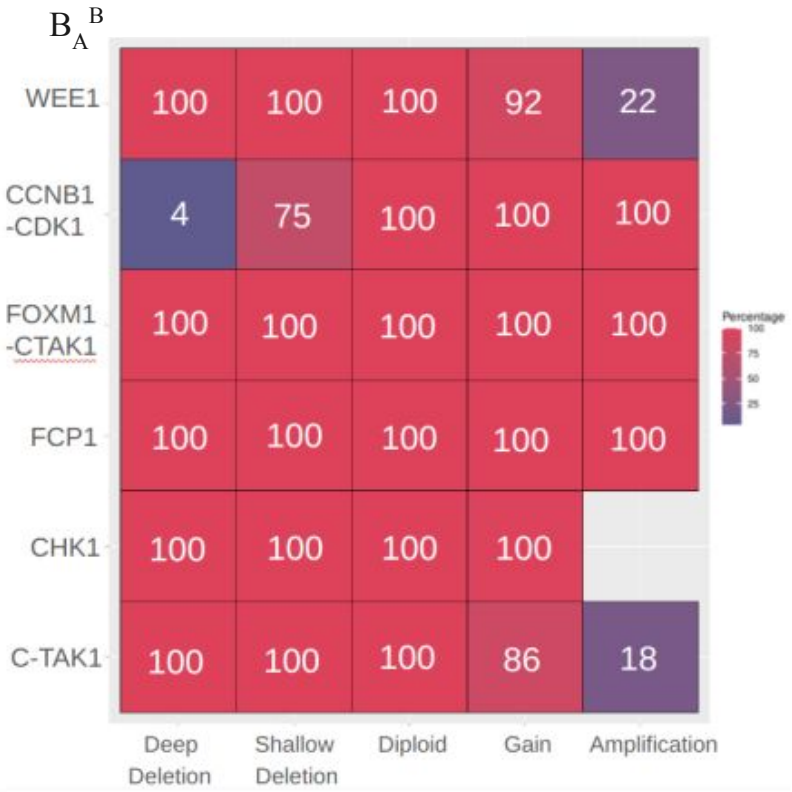


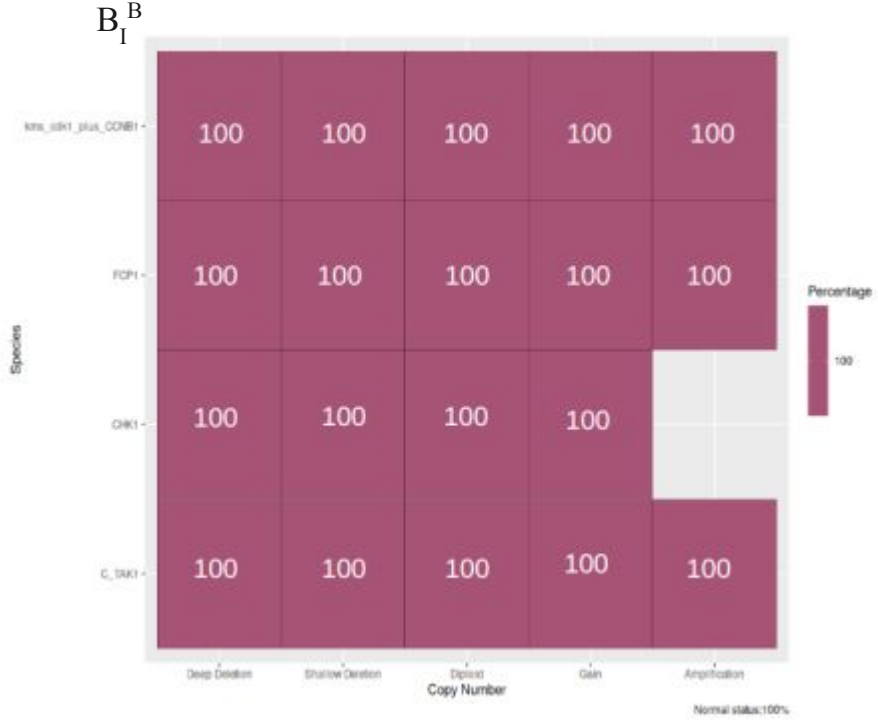
SCNAs


B
A
B
WEE1
CCNB1
-CDK1
FOXM1
-CTAK1
FCP1
CHK1
C-TAK1
2000
4000
6000
Copy_number
Amplification
Deep Deletion
Diploid
Gain
Shallow Deletion

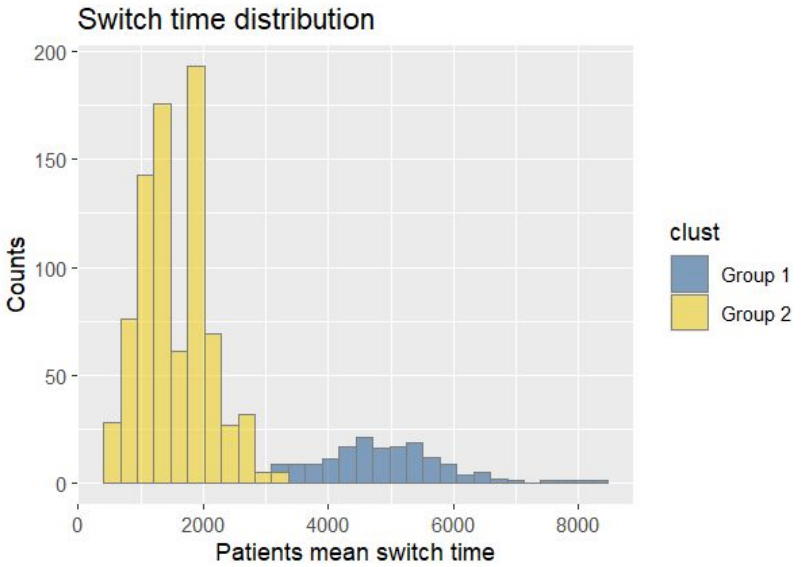

B
I
B
kins_cdk1_plus_CCNB1
FCP1
CHK1
C_TAK1
Species
100
120
140
Time
Normal status:118.7
Copy_number
Amplification
Deep Deletion
Diploid
Gain
Shallow Deletion

SCNA

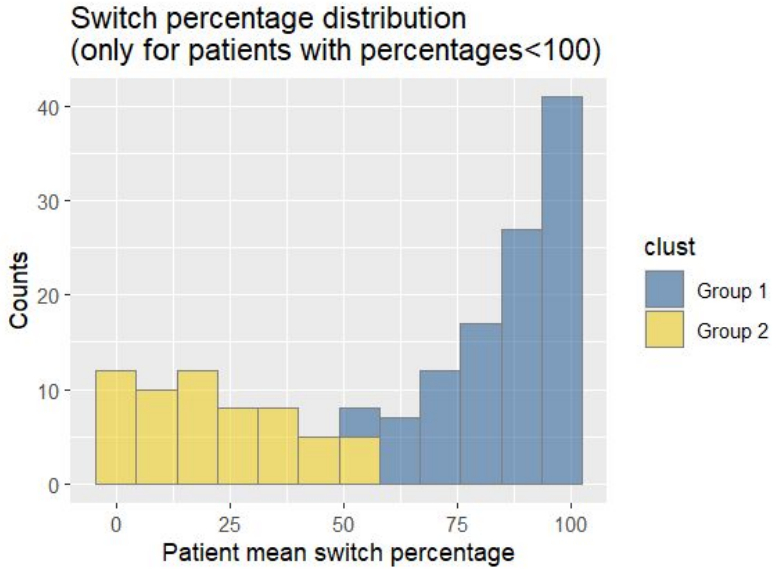

WEE1
CCNB1 -CDK1
FOXM1 -CTAK1
FCP1
CHK1
C-TAK1
100 100 100 92 22
4 75 100 100 100
100 100 100 100 100
100 100 100 100 100
100 100 100 100
100 100 100 86 18
Deep Deletion
Shallow Deletion
Diploid
Gain
Amplification
Percentage
100
75
50
25


Species
100 100 100 100 100
100 100 100 100 100
100 100 100 100
100 100 100 100 100
Deep Deletion
Shallow Deletion
Diploid
Gain
Amplification
Copy Number
Percentage
100

Patient stratification

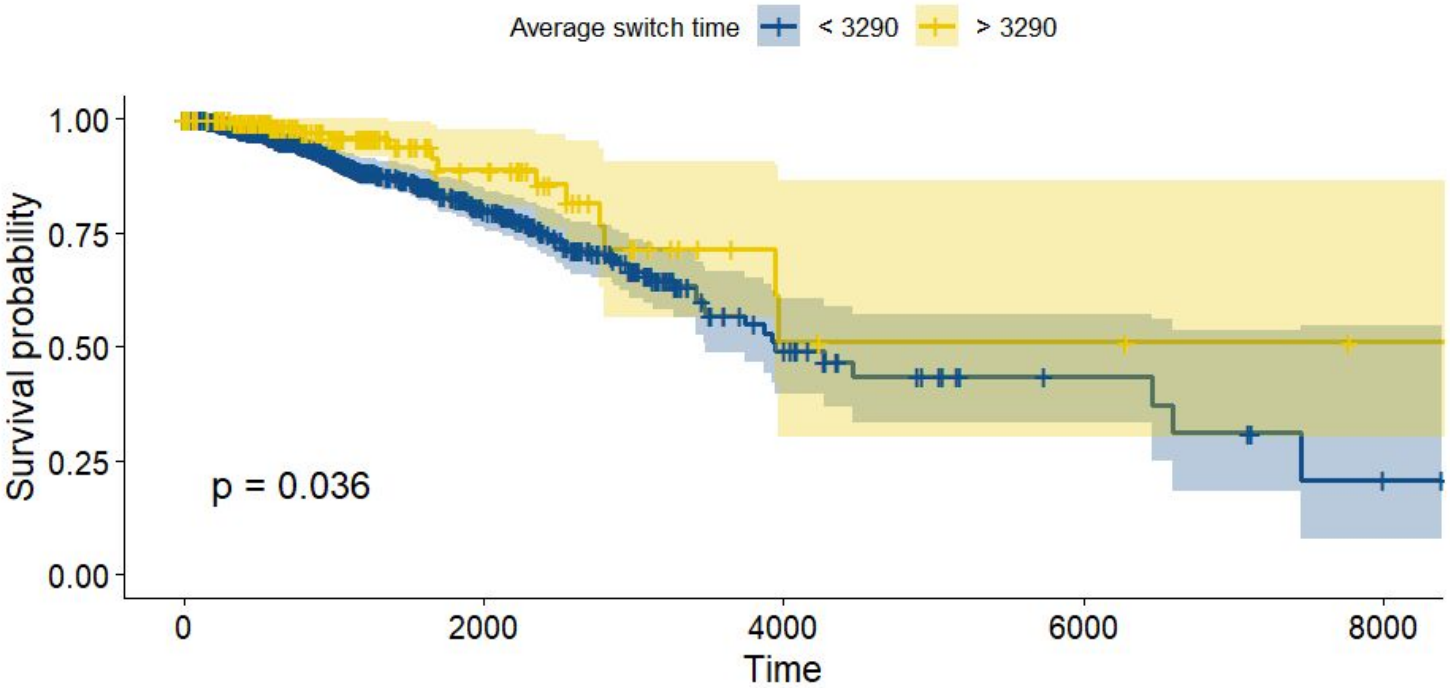


threshold = 3290

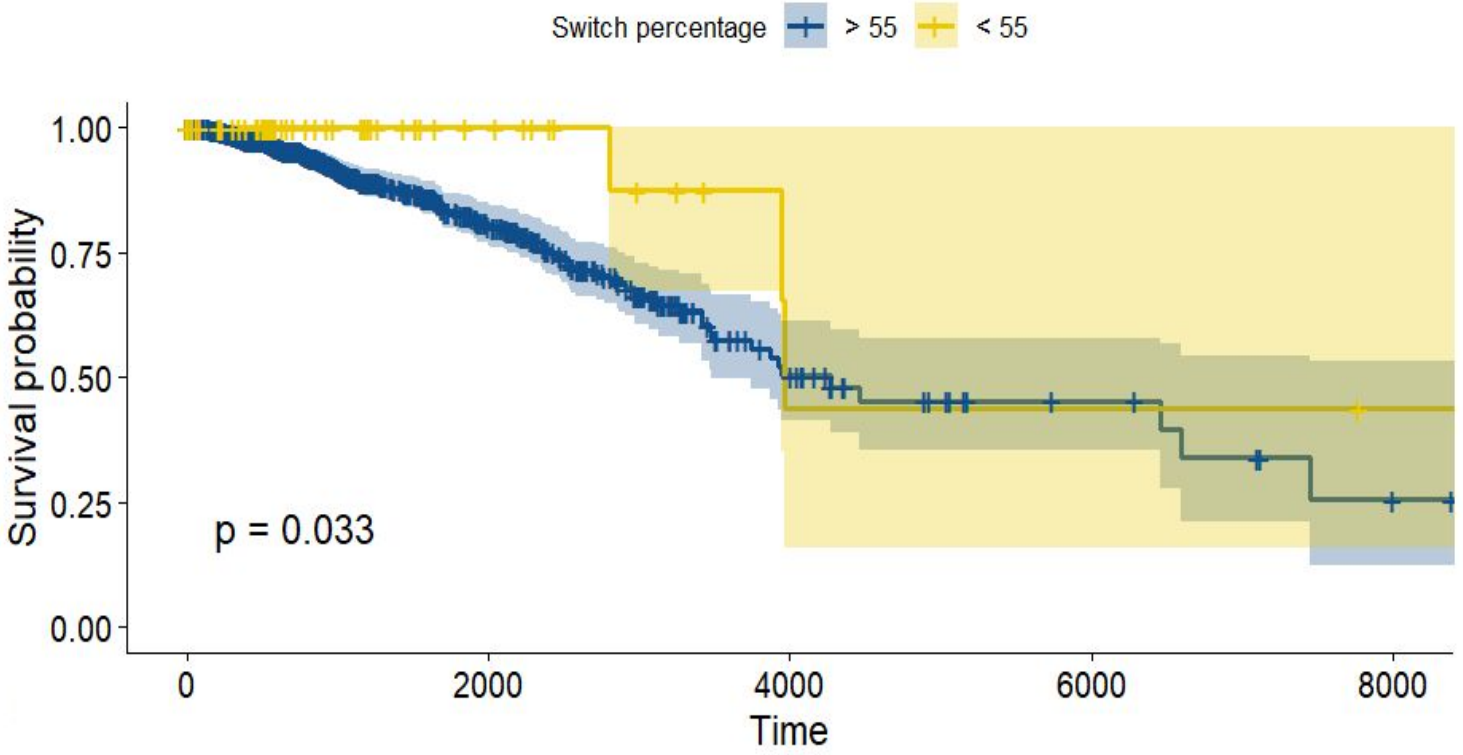


threshold = 55%

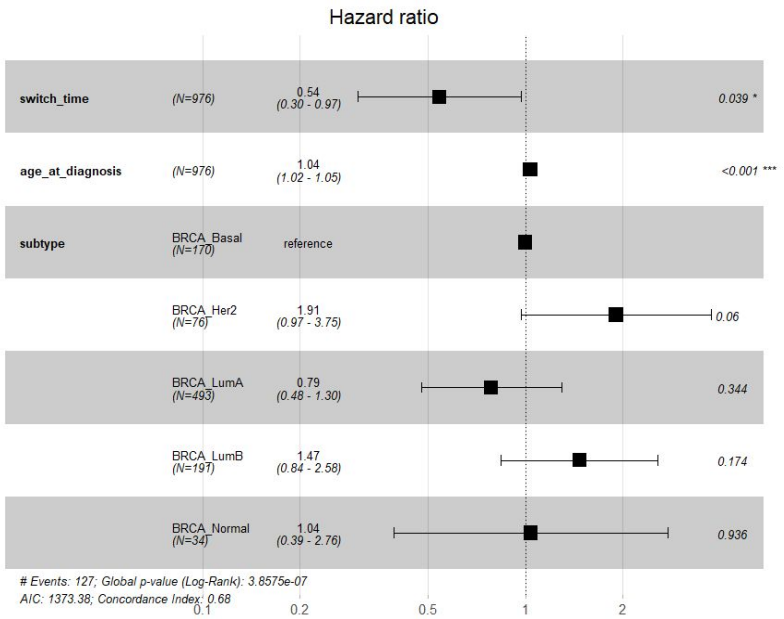
Survival Analysis (OS)

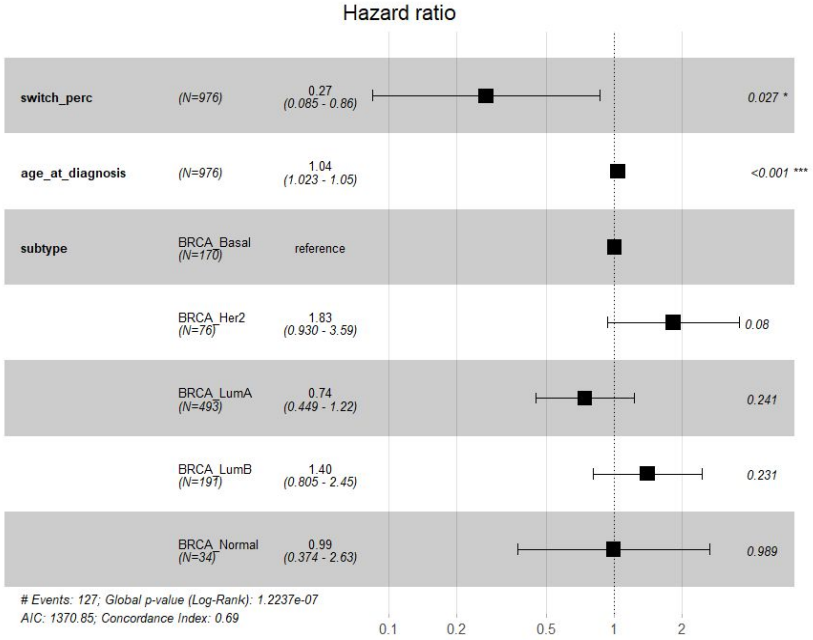


Survival Analysis (OS)



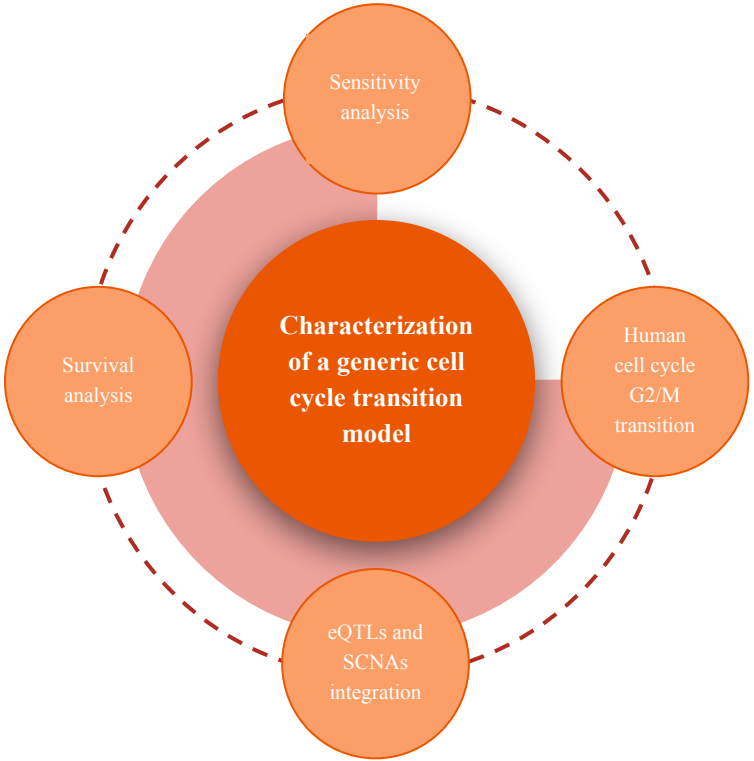
Survival Analysis





Conclusions

- Insight applying stochastic model to represent cell cycle transition
- Integrating mutation information when developing model
- Mathematical model used describe impact mutation at patient level



Future Perspectives

- Increase the number of patients considered
  - subtype-specific survival analysis
- G1/S and M/G1 checkpoints
- Integrating eQTL and SCNA together (also other variants)
- Extending this approach to other tumor types
- Refinement and expansion of general cell cycle transition models

Thank you