Transcriptional Regulation of Junctophilin-2 in Cardiomyocytes

心肌细胞中Junctophilin-2转录调控的探究

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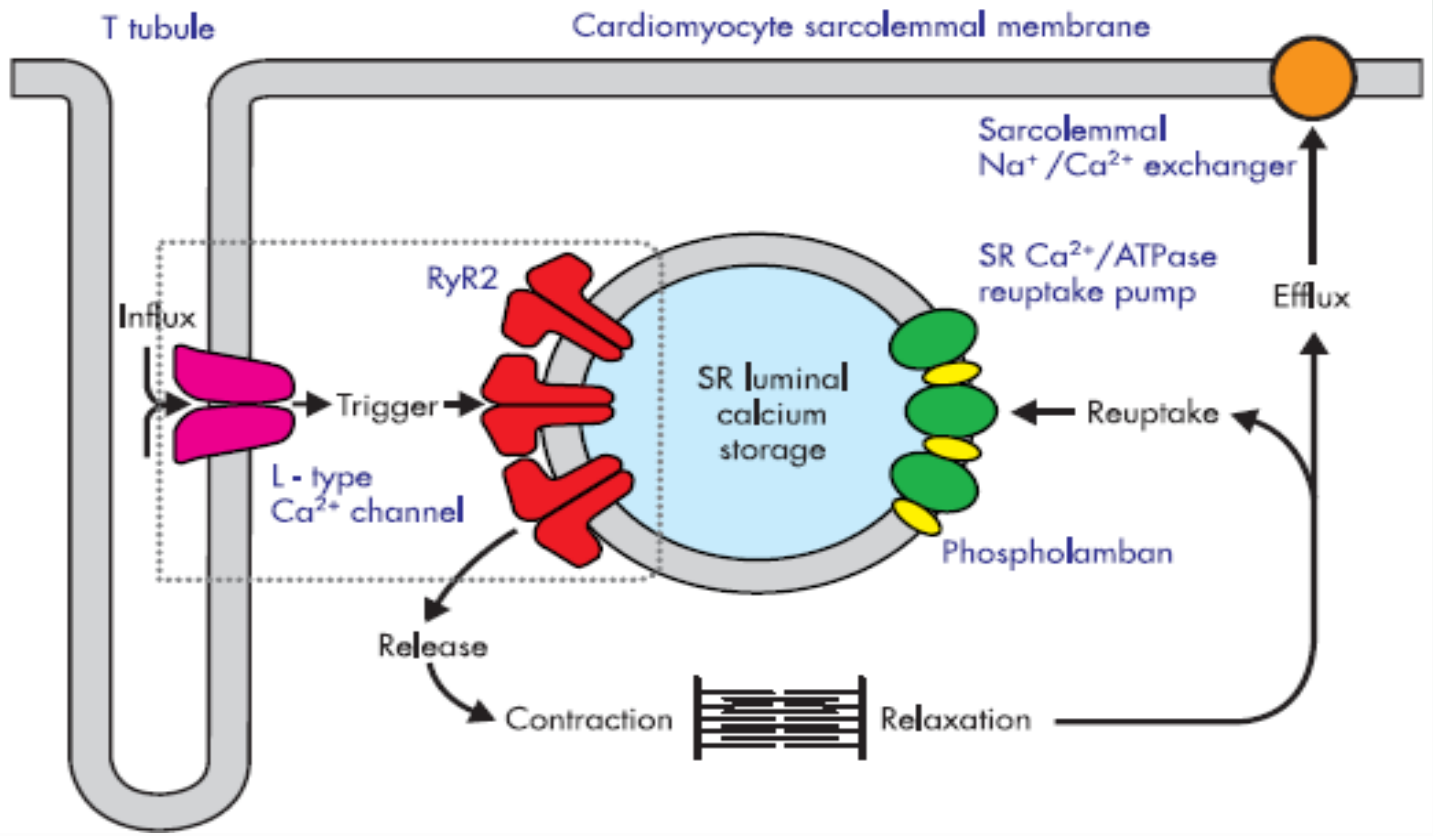
杨碧莹
翟艳芳

Peking University　Group 07

Outline

- Background
- Purpose
- Design & Results
- Acknowledgement

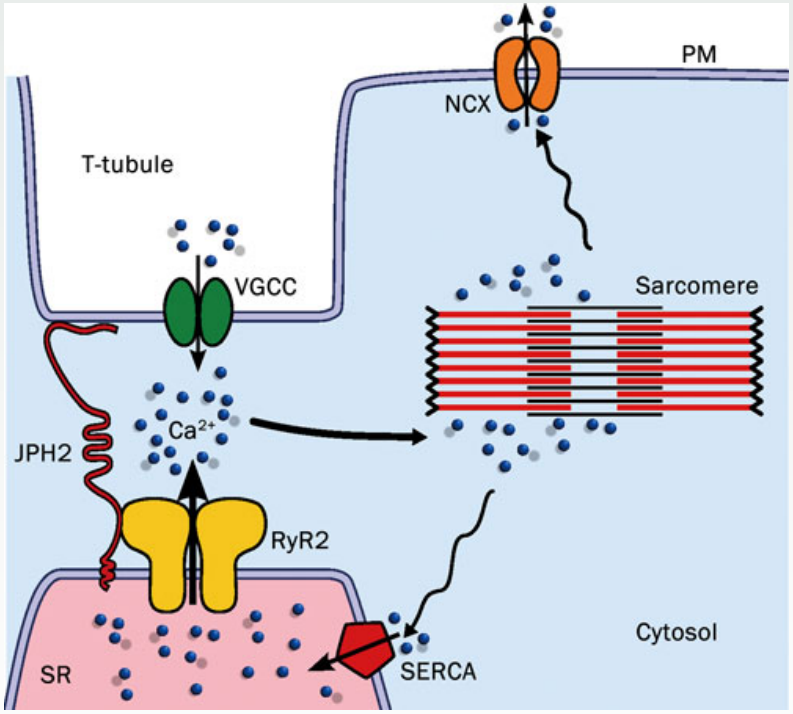
Introduction



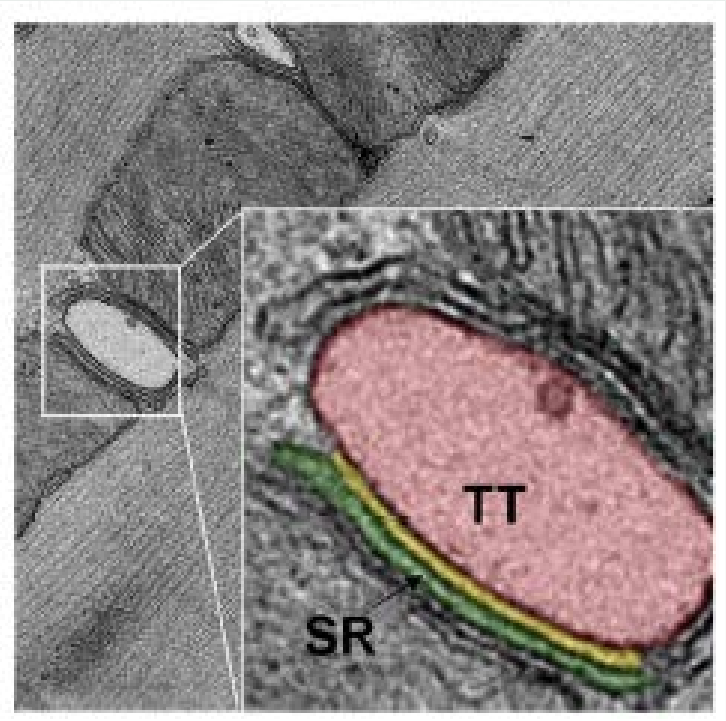
——Heart, 2003, 89(4): 371-376.

Junctophilin-2 is protein of TT-SR coupling structure

JP2 是T管-肌质网欧联结构中重要的连接蛋白



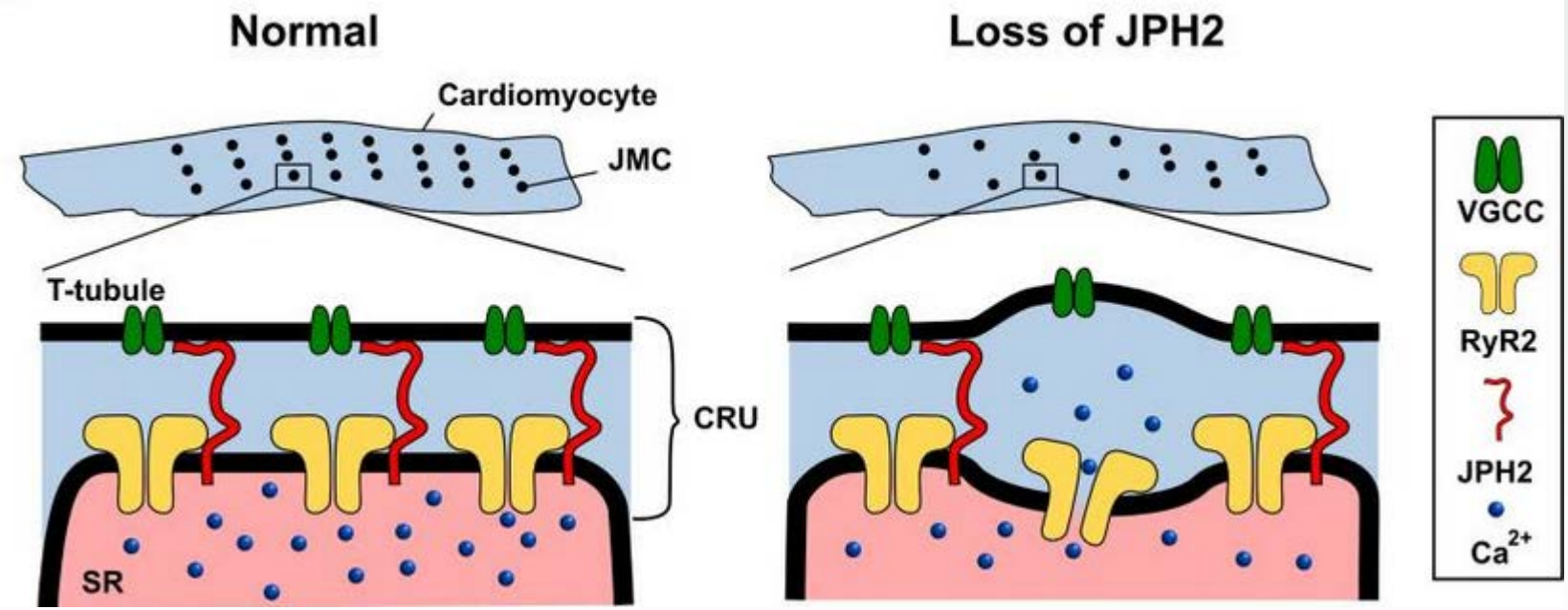
——Acta pharmacologica Sinica, 2010, 31(9): 1019-1021.



——Circulation research, 2012, 111(7): 837-841.

JP-2 is important in the process of E-C coupling

JP2 对兴奋收缩偶联机制的正常功能有很重要的作用



Circulation. Mar 8, 2011; 123(9): 979–988.

Summary

Abnormal E-C coupling is the main reason to lead to heart failure. Junctophilin-2 (JP2) is very important protein to link transverse tubules (TTs) and SR structural coupling. The decrease expression of JP2 can cause the defective E-C coupling. According to recent reports, during the process of heart failure, JP2 down regulation lead to TT-SR structural coupling disrupted so that affect the activity of intracellular Ca^{2+}.

Question:

What’s the mechanism of loss of Junctophilin-2?

Post transcription

Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

Mir-24 Regulates Junctophilin-2 Expression in Cardiomyocytes

Ming Xu, Hao-Di Wu, Rong-Chang Li, Hai-Bo Zhang, Meng Wang, Jin Tao, Xin-Heng Feng, Yun-Bo Guo, Su-Fang Li, Shao-Ting Lai, Peng Zhou, Lin-Lin Li, Hua-Qian Yang, Guan-Zheng Luo, Yan Bai, Jianzhong J. Xi, Wei Gao, Qi-De Han, You-Yi Zhang, Xiu-Jie Wang, Xu Meng and Shi-Qiang Wang

Proteolysis

J Physiol 591.3 (2013) pp 719–729

RAPID REPORT

Ca^{2+}-dependent proteolysis of junctophilin-1 and junctophilin-2 in skeletal and cardiac muscle

R. M. Murphy[1], T. L. Dutka[1], D. Horvath[2], J. R. Bell[3], L. M. Delbridge[3] and G. D. Lamb[1]

Departments of [1]Zoology and [2]Human Biosciences, La Trobe University, Melbourne, Victoria 3086, Australia
[3]Department of Physiology, University of Melbourne, Victoria 3010, Australia

Trafficking

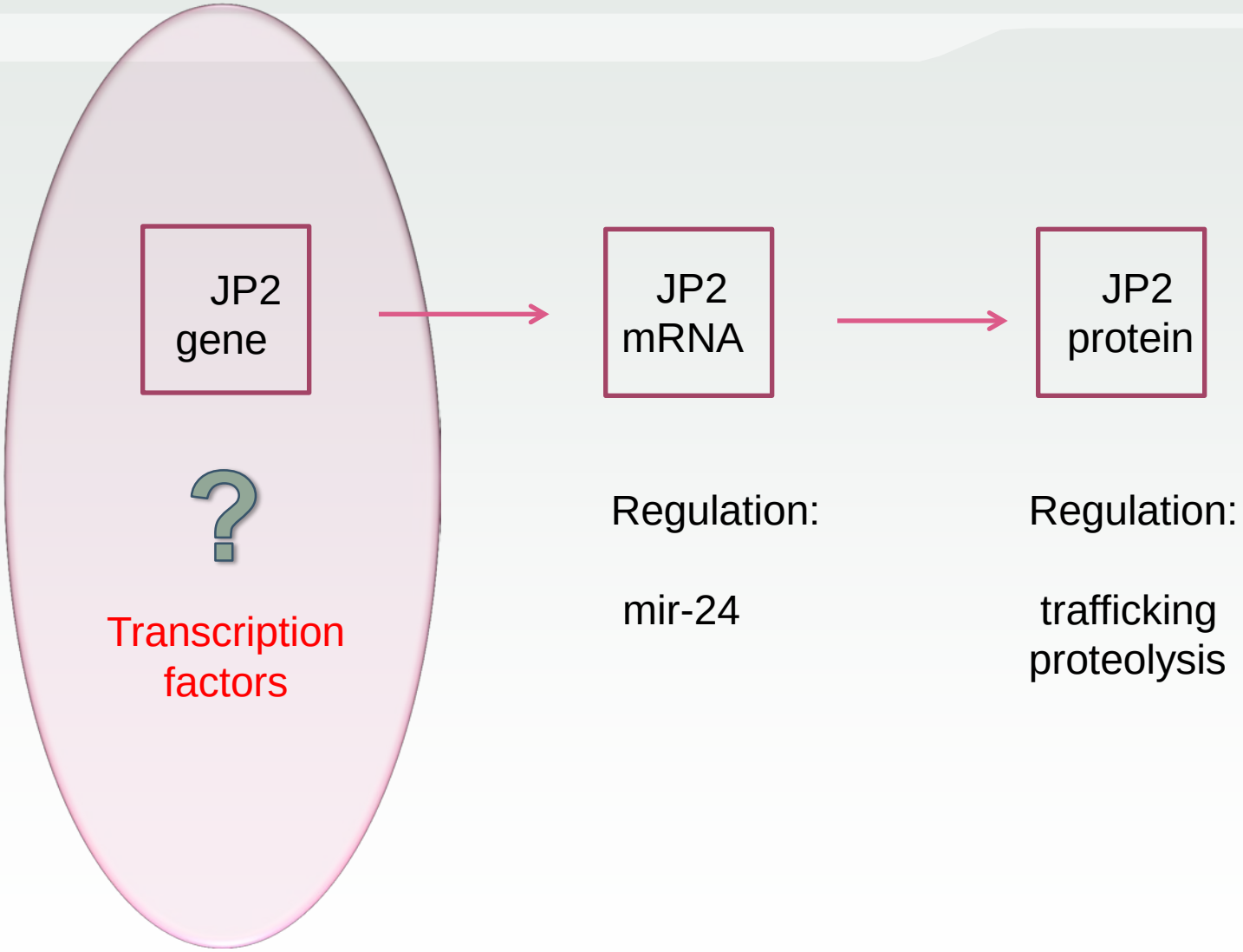
Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

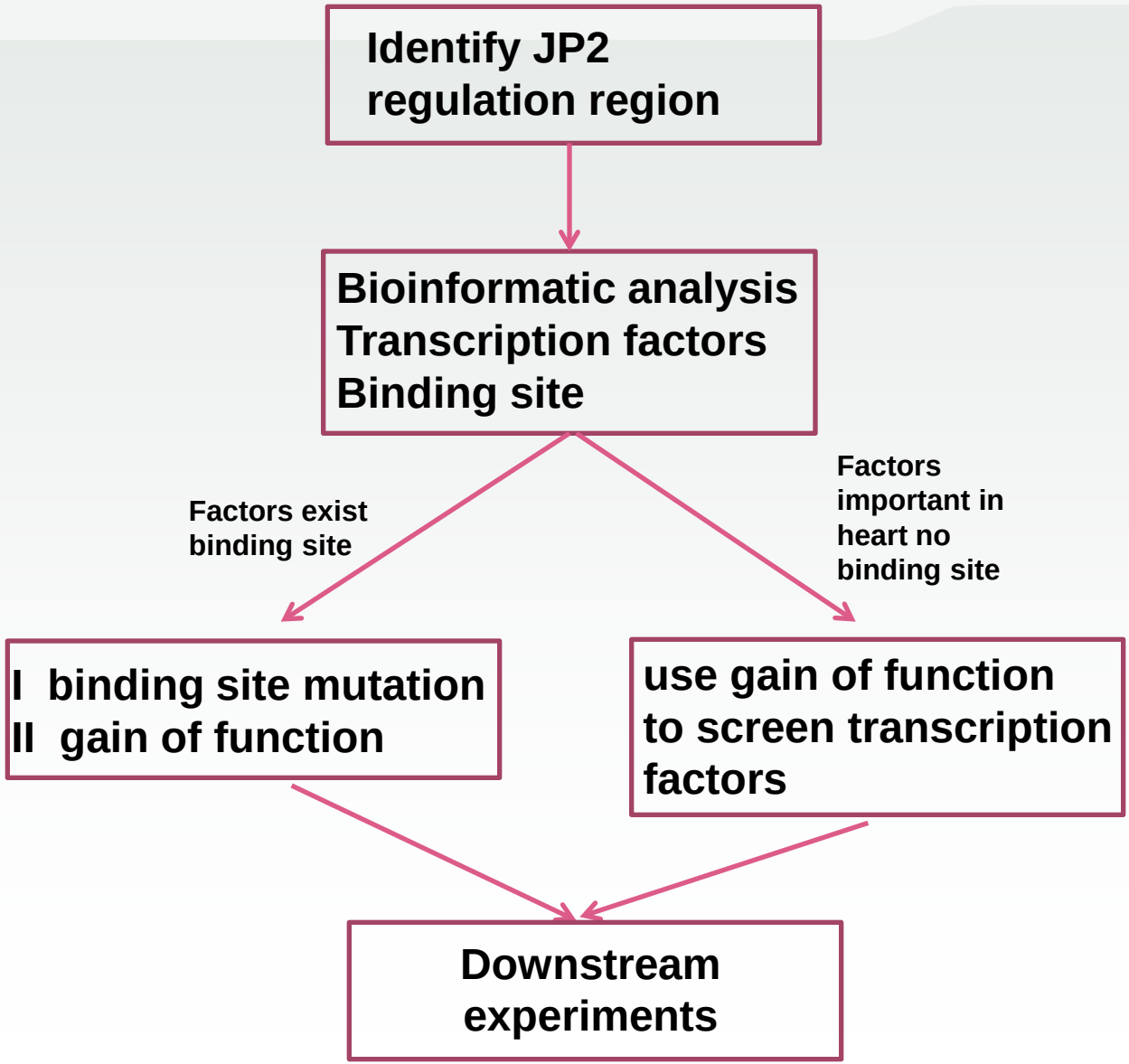
Microtubule-Mediated Defects in Junctophilin-2 Trafficking Contribute to Myocyte T-Tubule Remodeling and Ca^{2+} Handling Dysfunction in Heart Failure

Caimei Zhang, Biyi Chen, Ang Guo, Yanqi Zhu, Jordan D. Miller, Shan Gao, Can Yuan, William Kutschke, Kathy Zimmerman, Robert M. Weiss, Xander H. T. Wehrens, Jiang Hong, Frances L. Johnson, Luis F. Santana, Mark E. Anderson and Long-Sheng Song

What do we focus on?

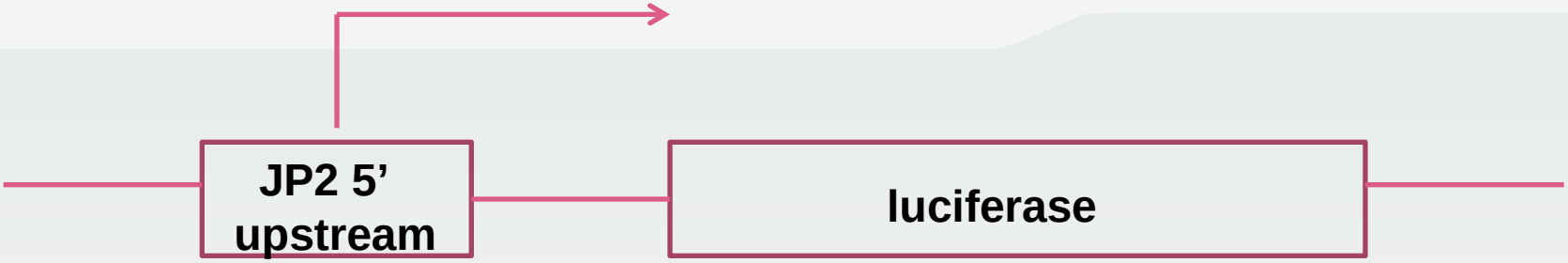


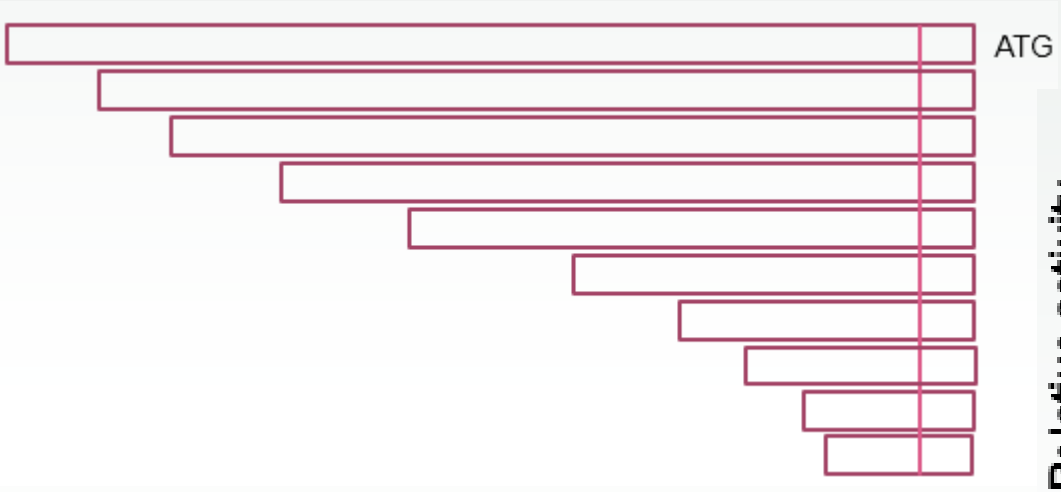
Research strategy

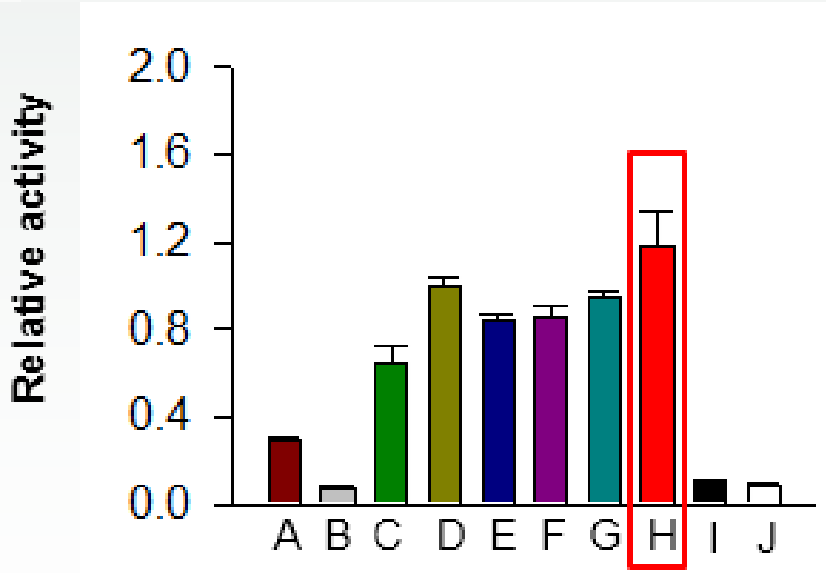


Results

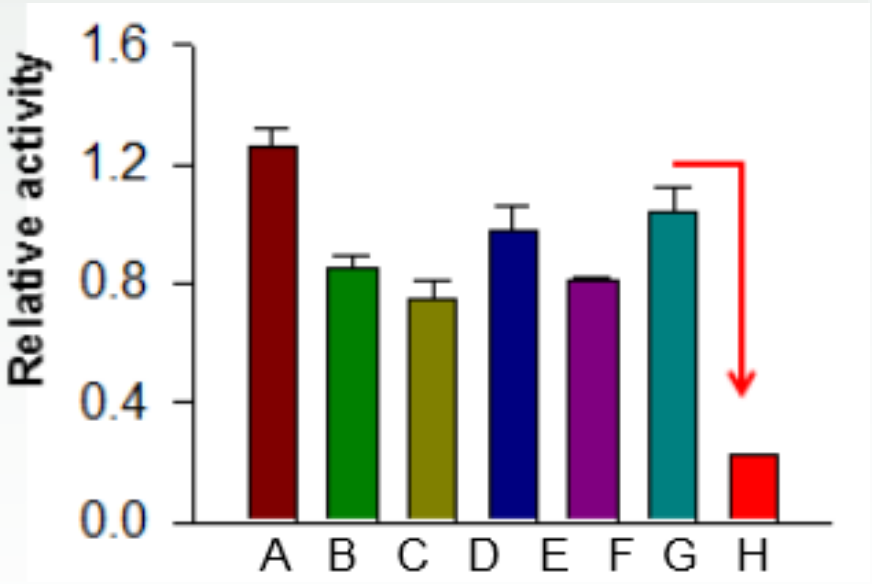
Identify the JP2 regulation region



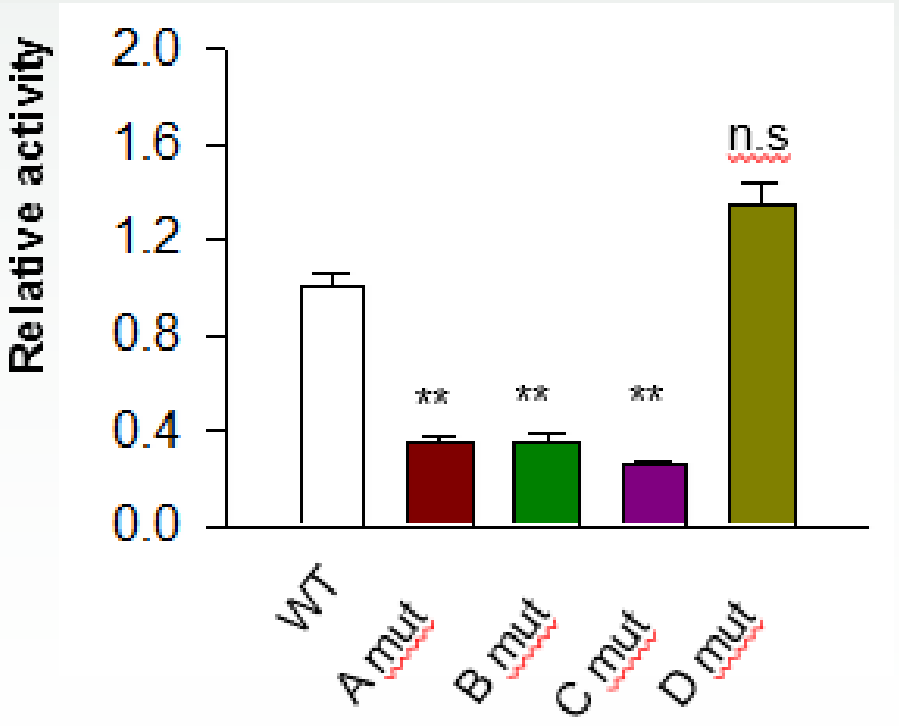




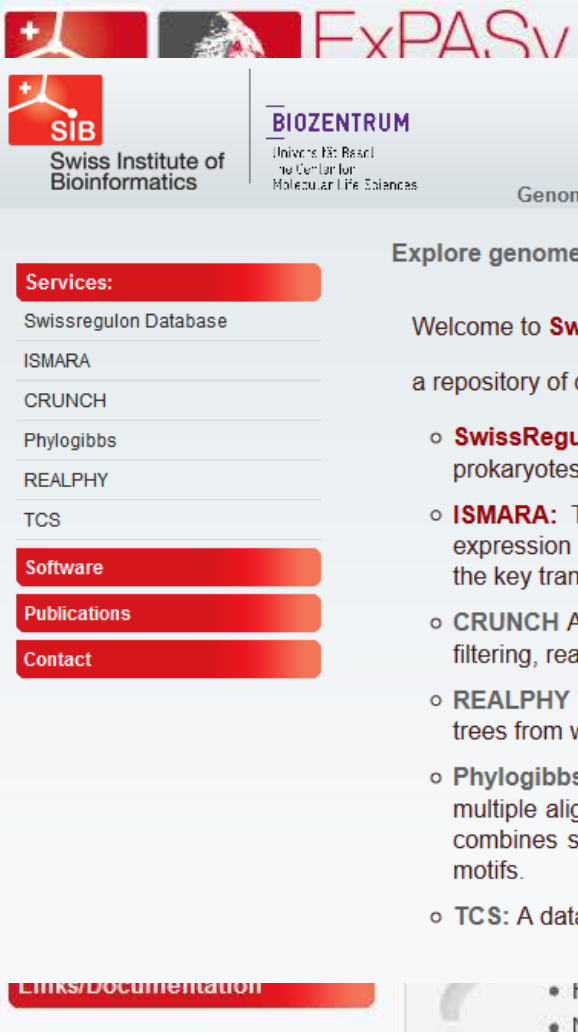
Different length of JP2 5' upstream's promoter activity



Binding site mutation



Here, we have got the core region, but what is the TF binding the sequence?


ExPASy
SIB
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BIOZENTRUM
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Documentation
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Search mammal promoters and motifs:
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REALPHY
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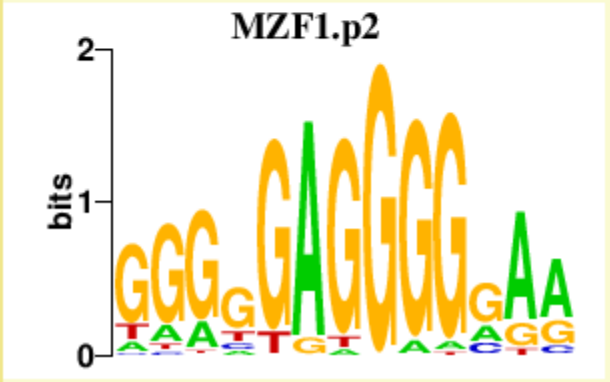
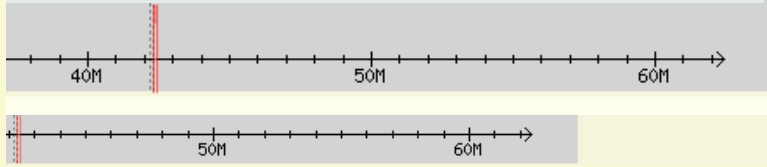
Publications
Contact
Welcome to SwissRegulon portal ,
a repository of datbases and bioinformatic tools related to transcription regulatory processes and includes:
SwissRegulon: A database of genome-wide annotations of regulatory sites. We currently have annotations for 17 prokaryotes and 3 eukaroytes in our collection.
ISMARA: The Integrated System for Motif Activity Response Analysis is a free online tool that models genome-wide expression data in terms of our genome-wide annotations of regulatory sites. For an given input expression data-set it infers the key transcription regulators, their sample-dependent activities, and their genome-wide targets.
CRUNCH A completely automated pipe-line for ChIP-seq data analysis, starting from raw sequencing reads, through quality filtering, read mapping, fragment size estimation, peak calling, peak annotation and comprehensive regulatory motif analysis.
REALPHY The Reference sequence Alignment based Phylogeny builder is a free online pipeline that can infer phylogenetic trees from whole genome sequence data.
Phylogibbs: An algorithm for inferring regulatory motifs and regulatory sites from collections of DNA sequences, including multiple alignments of orthologous sequences from related organisms. Phylogibbs uses a rigorous Bayesian approach that combines search for overrepresented sequence-motifs with sequence conservation analysis of the putative sites for these motifs.
TCS: A data-base of predicted two-component signalling interactions across bacterial genomes.
Links/Documentation
Features and updates
New to ExPASy
Release
547 357

MZF1.p2-135058 Details

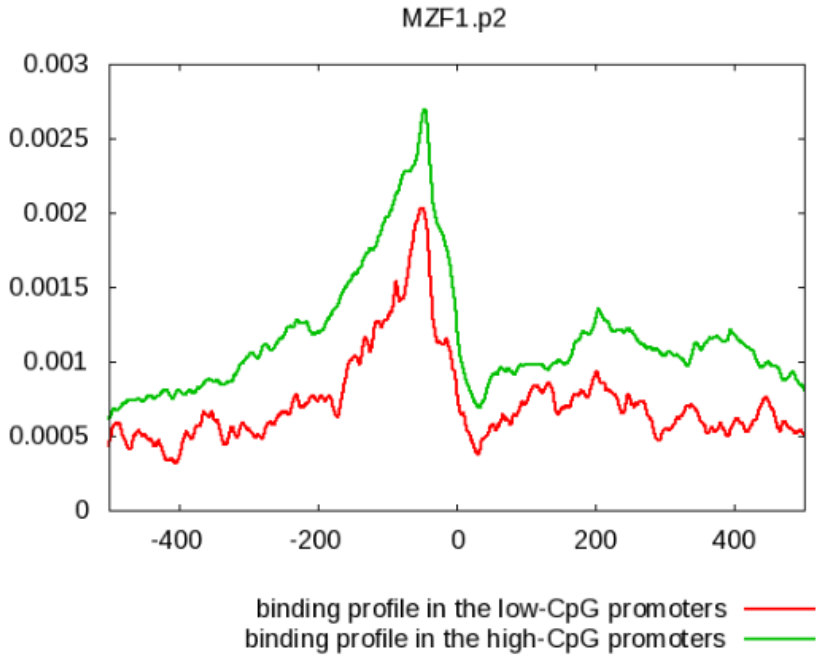
Name:	**MZF1.p2-135058**
Type:	TFBS
Description:	
Source:	Motevo
Position:	chr20:42249585..42249597 (+ strand)
Length:	13
Score:	0.898 (Posterior probability)
Alignments:	hg18: GGGAGAGGGAGAC canFam2: TGGGGAGGAGGAC
Motif:	**MZF1.p2**

Motif logo:





Distance distribution:



Sumr

OfMotifDescription: None

MotifMembers: MZF1: myeloid zinc finger 1

Promoters: hg18_v1_chr20_-_42249146_42249269

hg18_v1_chr20_-_42249631_42249631

Sequence: GGGAGAGGGAGAC

oad_id: MZF1.p2-135058

rimary_id: 772202

gbrowse_dbid: hg18:database

Promoters

Bioinformatic analysis

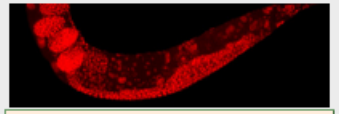
Version:
5.0_ALPHA

Visit the stable JASPAR server: jaspar.genereg.net

The high-quality transcription factor binding profile database

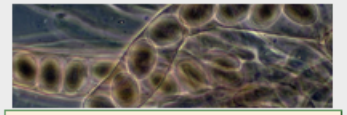
Browse the JASPAR CORE database directly:

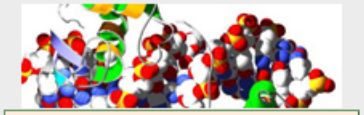

JASPAR CORE Vertebrata


JASPAR CORE Nematoda


JASPAR CORE Insecta


JASPAR CORE Plantae


JASPAR CORE Fungi


JASPAR CORE by Structural Class

DOCUMENTATION | DOWNLOAD | TOOLS | CHANGELOG | CONTACT

Browse/search a JASPAR database

BRCA1 Quick Search

Select a JASPAR database ?

JASPAR CORE
JASPAR Collections
JASPAR CNE
JASPAR FAM
JASPAR PBM
JASPAR PBM_HLH
JASPAR PBM_HOMEO

The JASPAR CORE database contains a curated, non-redundant set of profiles, derived from published collections of experimentally defined transcription factor binding sites for eukaryotes. The prime difference to similar resources (TRANSFAC, etc) consist of the open data acess, non-redundancy and quality.
When should it be used? When seeking models for specific factors or structural classes, or if experimental evidence is paramount

Browse sorted by ?

ID
Species
Structural class
Taxonomic group

Browse

Search by ?

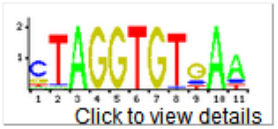
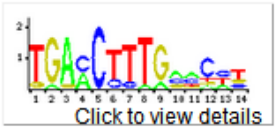
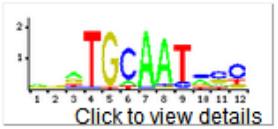
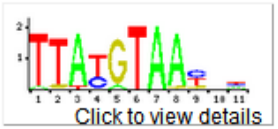
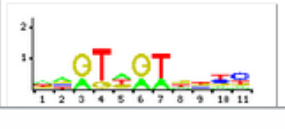
Name BRCA1 AND
Type AND
Species Search

Align to a custom matrix or IUPAC string ?

Fill in an example matrix | Align

SEARCH Name AND Species AND Class SEARCH ?

JASPAR matrix models:

TOGGLE	ID	name	species	class	family	Sequence logo
☑	MA0004.1	Arnt	Mus musculus	Zipper-Type	Helix-Loop-Helix	 Click to view details
☑	MA0006.1	Arnt::Ahr	Mus musculus	Zipper-Type	Helix-Loop-Helix	 Click to view details
☑	MA0009.1	T	Mus musculus	Beta-Hairpin-Ribbon	T	 Click to view details
☑	MA0017.1	NR2F1	Homo sapiens	Zinc-coordinating	Hormone-nuclear Receptor	 Click to view details
☑	MA0019.1	Ddit3::Cebpa	Rattus norvegicus	Zipper-Type	Leucine Zipper	 Click to view details
☑	MA0025.1	NFIL3	Homo sapiens	Zipper-Type	Leucine Zipper	 Click to view details
☑	MA0027.1	En1	Mus musculus	Helix-Turn-Helix	Homeo	

ANALYZE selected matrix models:

CLUSTER ? selected models using STAMP

Create RANDOM matrix models based on selected models

Number of matrices: 200 Format: Raw

RANDOMIZE ?

Create models with PERMUTED columns from selected:

Type: Within each matrix Format: Raw

PERMUTE ?

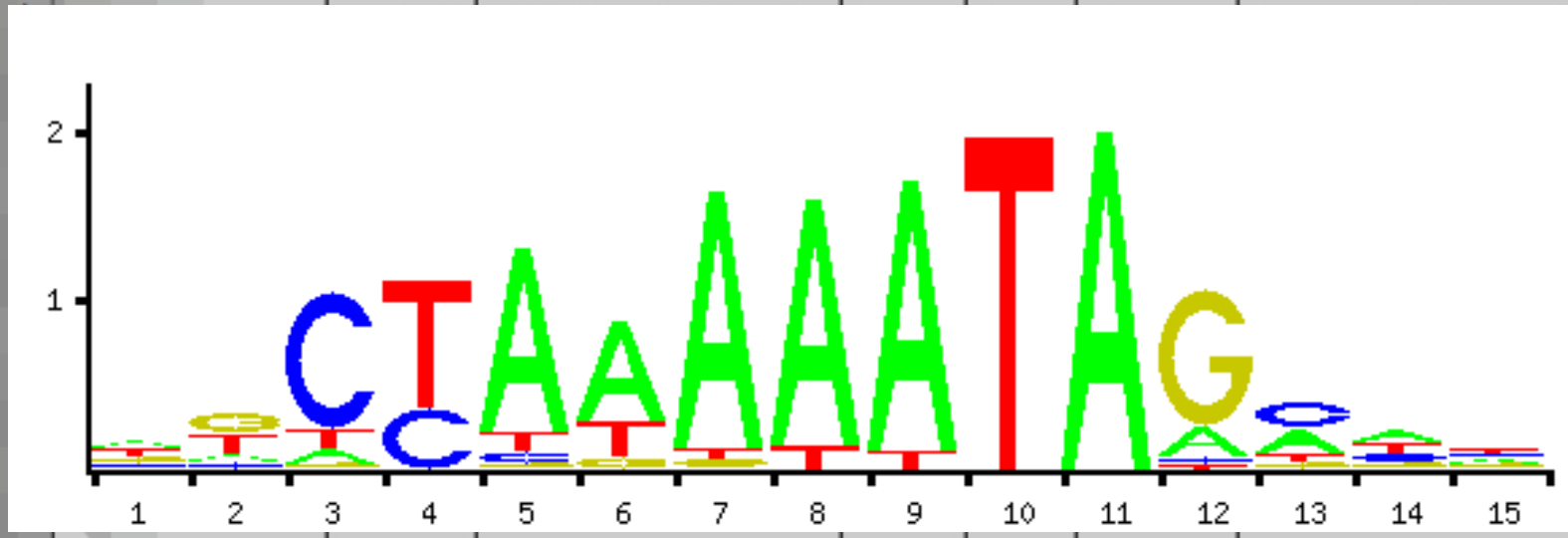
SCAN this (fasta-formatted) sequence with selected matrix models

Relative profile score threshold 80 %

SCAN ?

9 putative sites were predicted with these settings (80%) in sequence named **jph2**

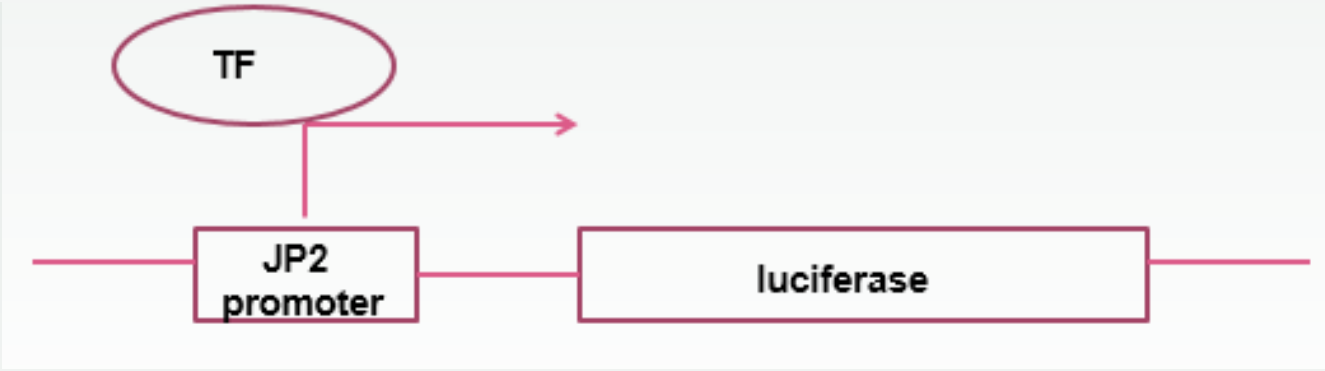
Model ID	Model name	Score	Relative score	Start	End	Strand	predicted site sequence
MA0		4.619	0.821038779492873	2	8	-1	ATAACCC
MA0		10.503	0.869249329307178	2	16	-1	CTCCCAAAATAACCC
MA0							CC
MA0							
MA0							
MA0							
MA0							
MA0							
MA00							



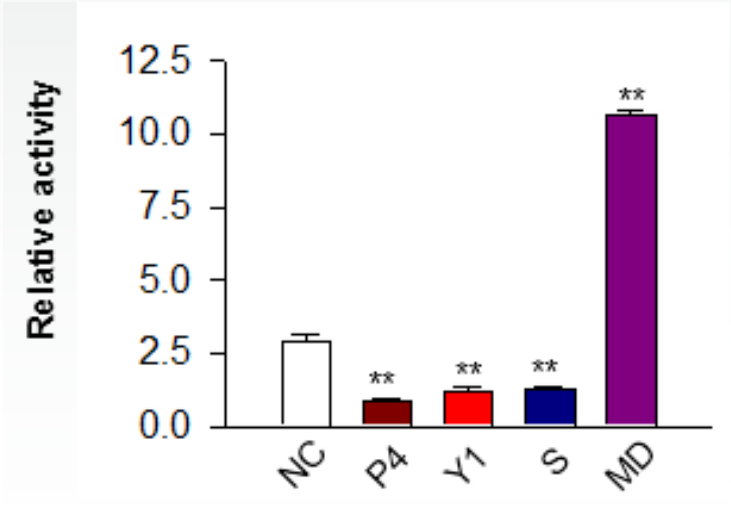
Comment: This type of analysis has a high sensitivity but abysmal selectivity. In other words: while true functional will be detected in most cases, most predictions will correspond to sites bound in vitro but with no function in vivo. A number of additional contraints of the analysis can improve the prediction; phylogenetic footprinting is the most common. We recommend using the ConSite service, which uses the JASPAR datasets.

The review Nat Rev Genet. 2004 Apr;5(4):276-87 gives a comprehensive overview of transcription binding site prediction

Screening the important transcription factors in heart

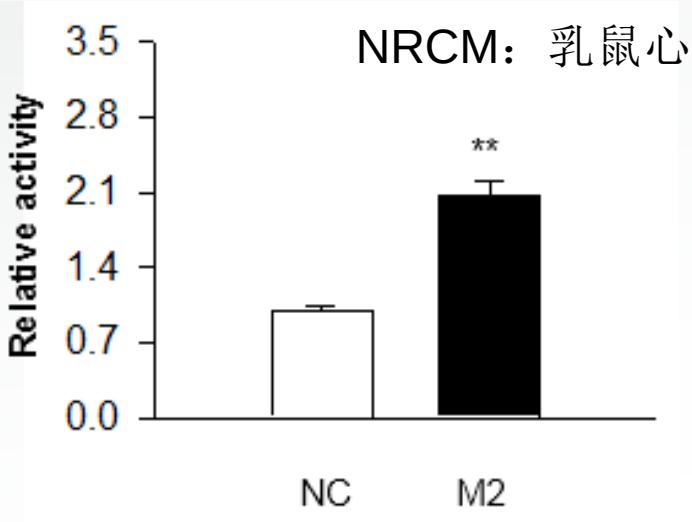


Different TFs effect on JP2 promoter



Gain of function (overexpress M2 in NRCM)

NRCM：乳鼠心肌细胞



Future plan

- Knock down M2 and test JP2 expression level
- Other methods to find JP2 transcription factors

Acknowledgement

- 感谢罗老师一学期以来的辛苦教学及罗老师对我课题的指导。
- 感谢师兄一直以来的照顾，让我慢慢适应研究生的生活。
- 感谢我们组所有人，一学期以来在各个方面互帮互助。

Half a day on the web, saves you half a month in the lab !

Thank you !

Happy New Year!