

Genetic Screens in Human Cells Using the CRISPR-Cas9 System

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3 JANUARY 2014 VOL 343 SCIENCE www.sciencemag.org

Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

Ophir Shalem,^{1,2*} Neville E. Sanjana,^{1,2*} Ella Hartenian,¹ Xi Shi,^{1,3} David A. Scott,^{1,2} Tarjei S. Mikkelsen,¹ Dirk Heckl,⁴ Benjamin L. Ebert,⁴ David E. Root,¹ John G. Doench,¹ Feng Zhang^{1,2}†

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RNA-programmed genome editing in human cells

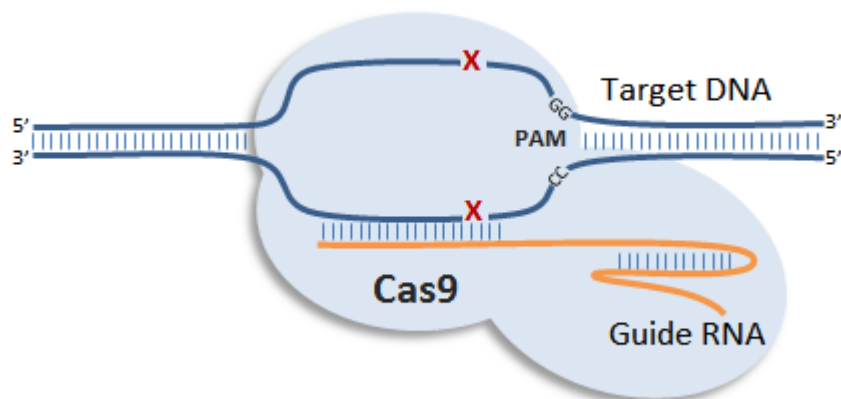
Martin Jinek, Alexandra East, Aaron Cheng, Steven Lin, Enbo Ma, Jennifer Doudna ✉

Howard Hughes Medical Institute, University of California, Berkeley, United States; University of California, Berkeley, United States; Lawrence Berkeley National Laboratory, United States

DOI: <http://dx.doi.org/10.7554/eLife.00471>

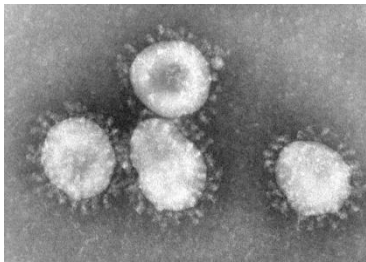
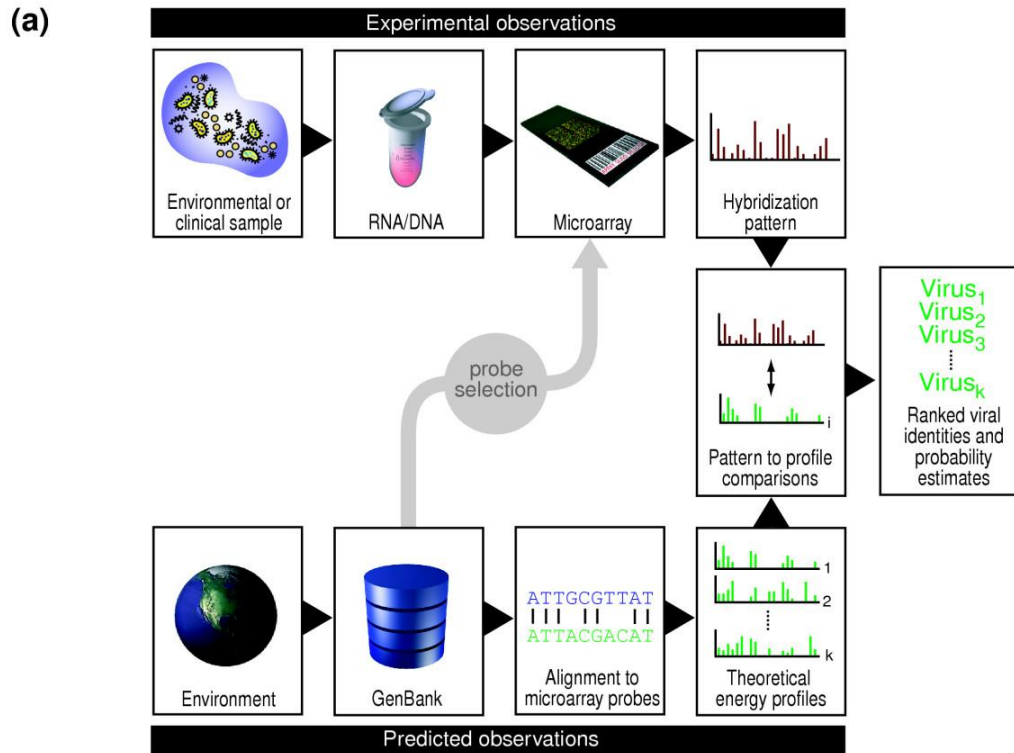
Published January 29, 2013

Cite as eLife 2013;2:e00471

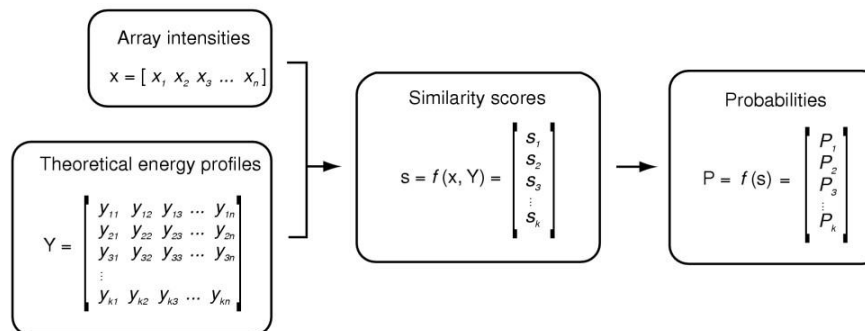


METAGENOMIKA

Primer uporabe DNA mikromrež za diagnostiko virusnih infekcij



(b)



'E-Predict'

Interaktomika

Povej mi, s kom se družiš in povem ti, kdo si.

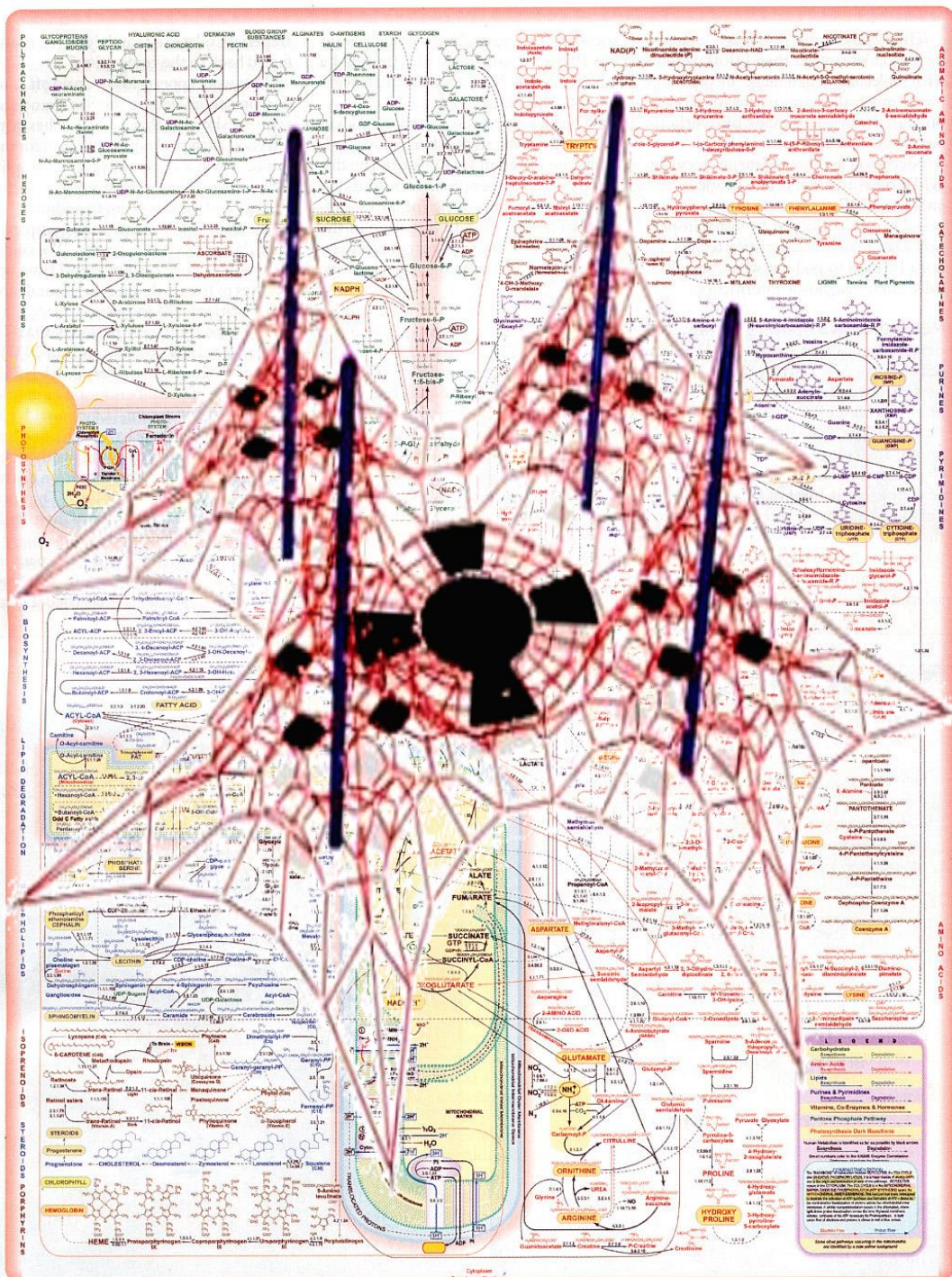
Kaj je življenje?

Kako lahko opišemo delovanje celice?

NETWORK BIOLOGY:
UNDERSTANDING THE CELL'S
FUNCTIONAL ORGANIZATION

Albert-László Barabási & Zoltán N. Oltvai[#]*

Nature Reviews Genetics (2004) 5:101



POLYSACCHARIDES
 HEXONES
 PENTONES
 PHOSPHINES
 DIOSPHINES
 LIPID DEGRADATION
 PROTEOLYSIS
 IONOPHORES
 STEROIDS
 PHENOL
 HEMOGLOBIN

NUCLEOTIDES
 NUCLEIC ACIDS
 AMINO ACIDS
 NEUROTRANSMITTERS
 HORMONES
 VITAMINS
 COENZYMES
 FLAVONOIDS
 ALKALOIDS
 TERPENOIDS
 PHENOLIC COMPOUNDS
 GLYCOSIDES
 SAPONINS
 QUINONES
 ANTIMETABOLITES
 ANTIBIOTICS
 TOXINS
 DRUGS
 METALLOIDS
 METALS
 TRACE ELEMENTS
 WATER
 O₂

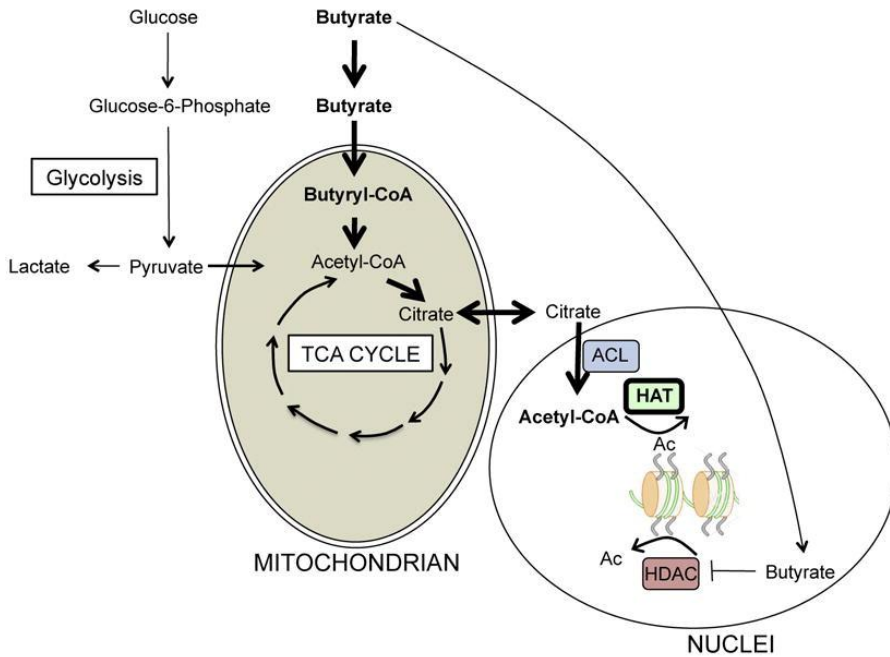
KEY

Carbohydrates	Proteins
Lipids	Nucleic Acids
Purines & Pyrimidines	Metals, Co-Enzymes & Hormones
Purine Phosphate Pathway	Phenolics Dark Reactions

Note: Metabolites involved in the synthesis of DNA and RNA are indicated by a red arrow. Metabolites involved in the synthesis of RNA are indicated by a blue arrow. Metabolites involved in the synthesis of DNA are indicated by a green arrow.

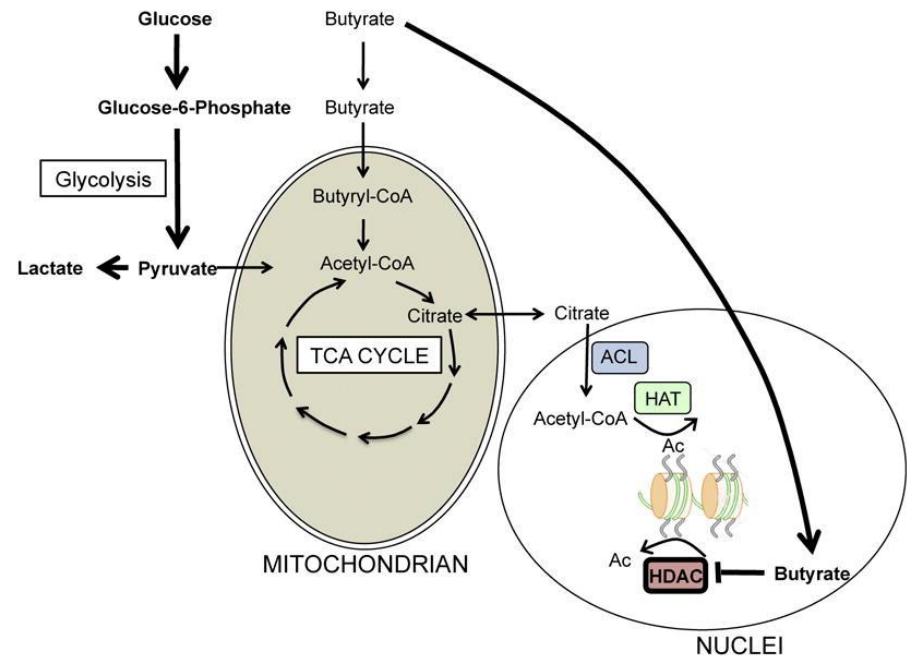
Warburgov efekt

Normal Colonocyte
(-) Warburg effect

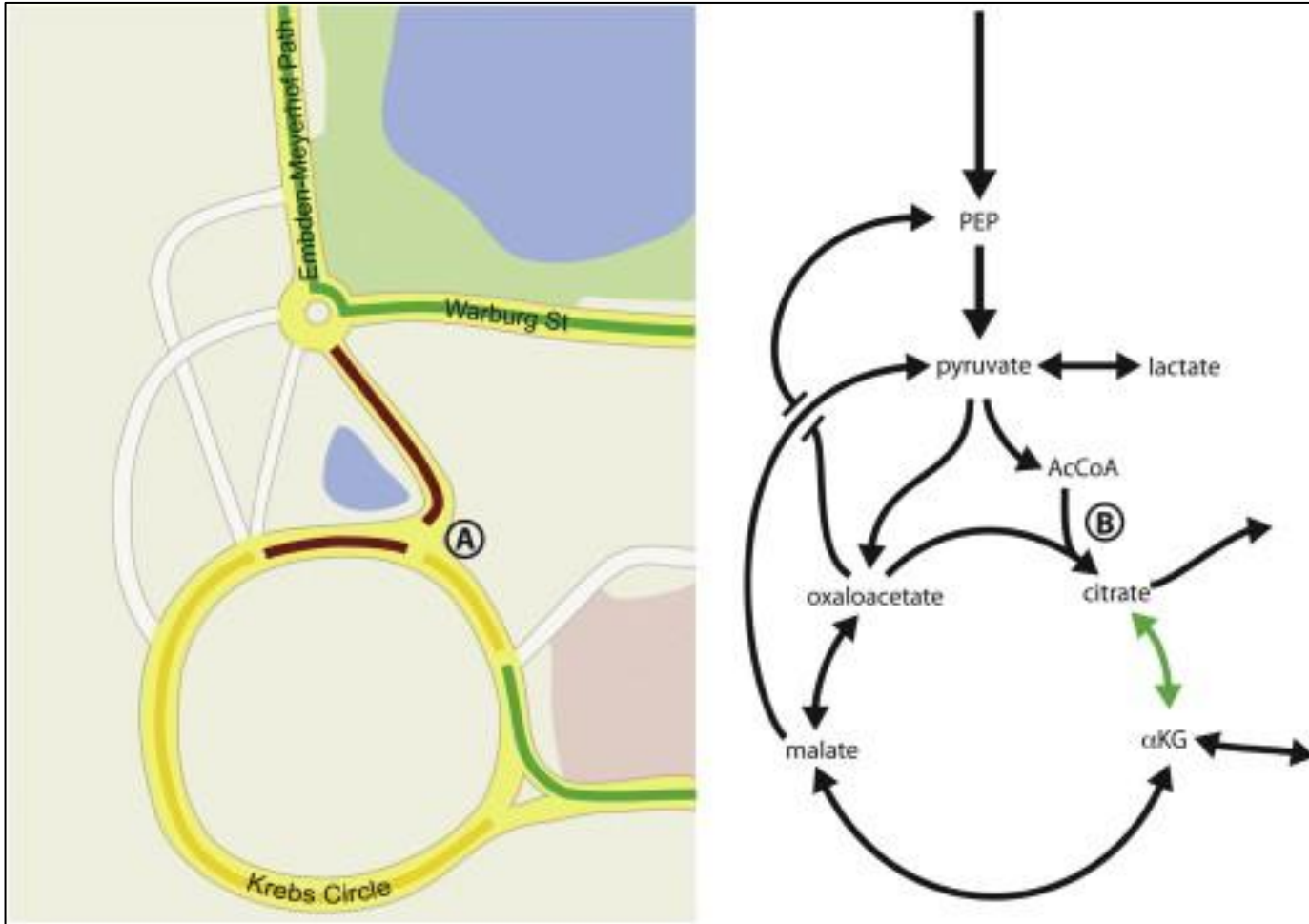


- Butyrate Oxidation/Energetics
- HAT Activity
- Proliferation

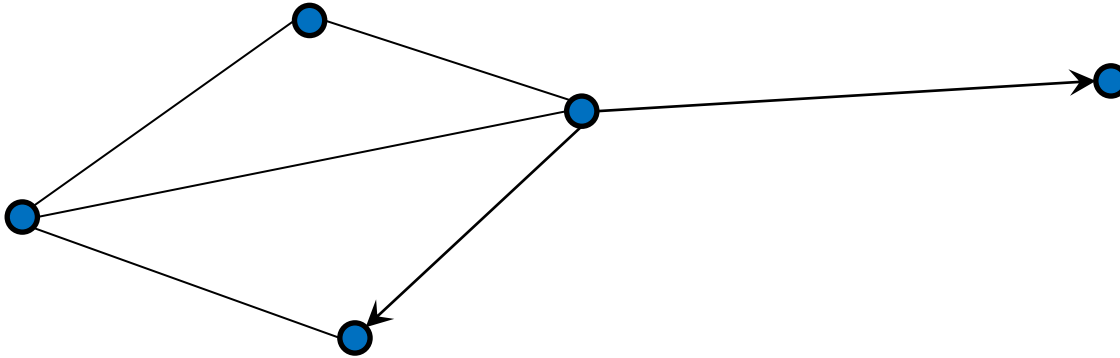
Cancerous Colonocyte
(+) Warburg effect



- Butyrate Accumulation
- HDAC inhibition
- Apoptosis



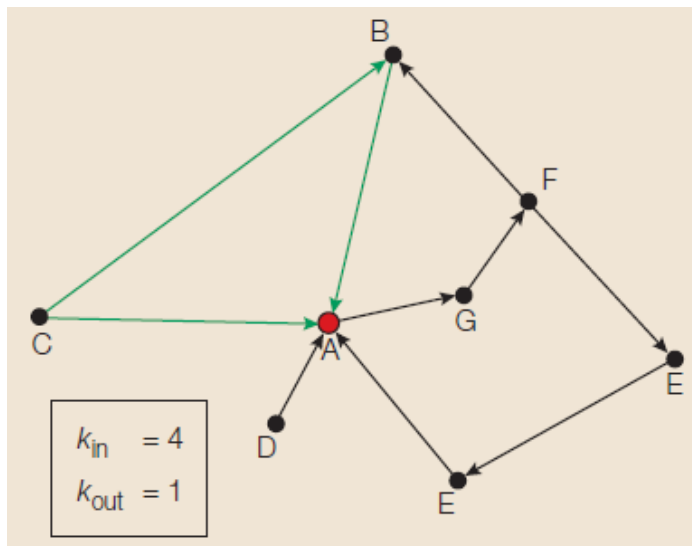
Mreže kot grafi



Točke (vozlišča, vozli; “**nodes, vertices**”): protein, geni, metaboliti...

Povezave (robovi, veje; “**edges**”): fizične interakcije, genetske i., encimske reakcije...

Lastnosti grafov, relevantne za biološke mreže

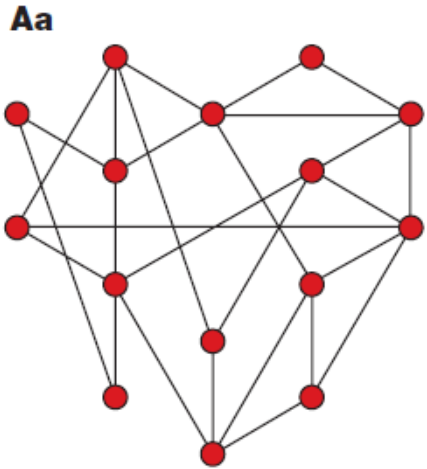


Stopnja vozlišča (“degree, connectivity”)

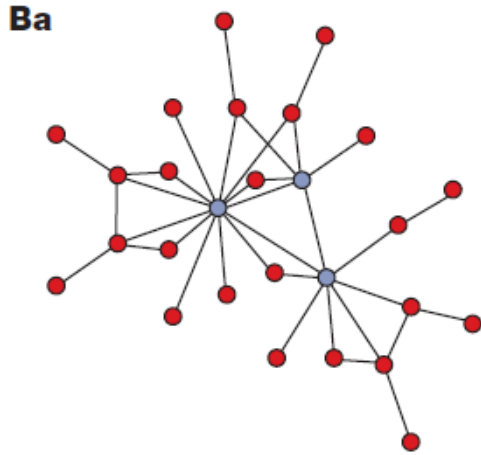
Najkrajša pot (“shortest path”)

Koeficient povezanosti (“clustering coefficient”)

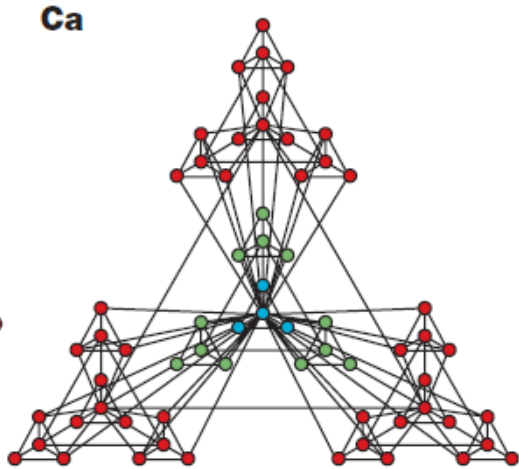
A Random network



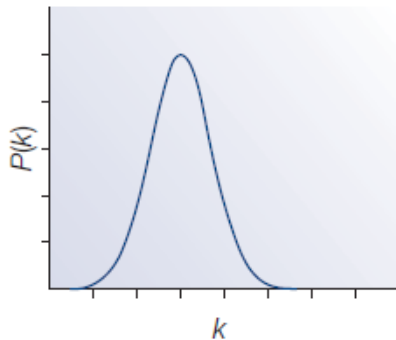
B Scale-free network



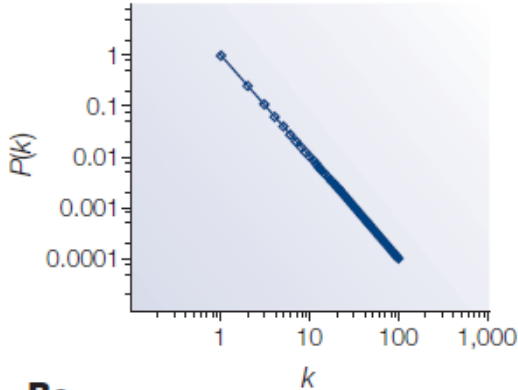
C Hierarchical network



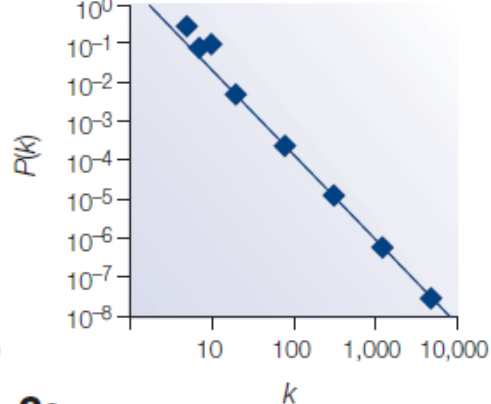
Ab



Bb

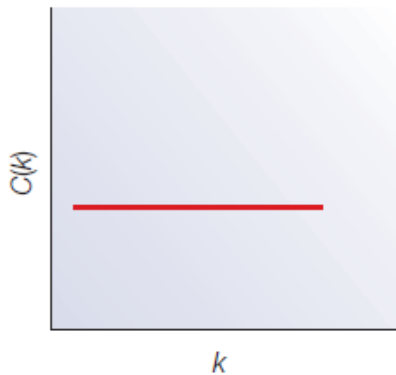


Cb

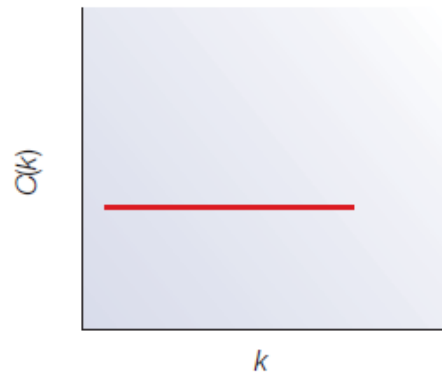


Razporeditev **stopenj**
Degree (k) distribution

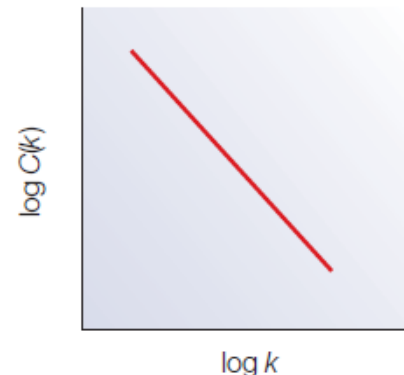
Ac



Bc



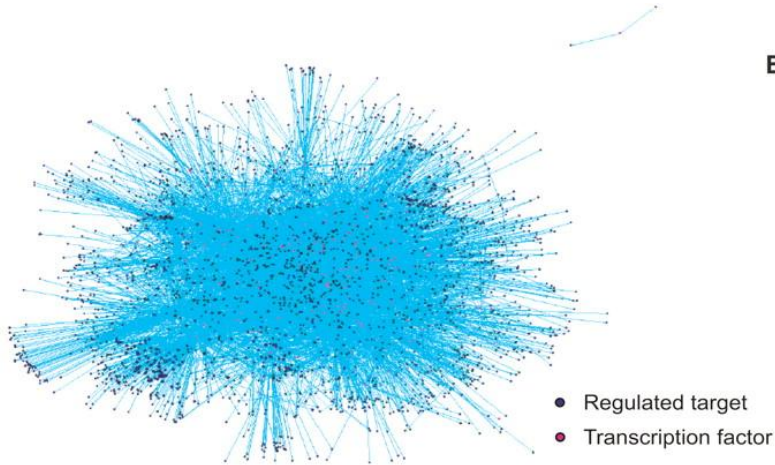
Cc



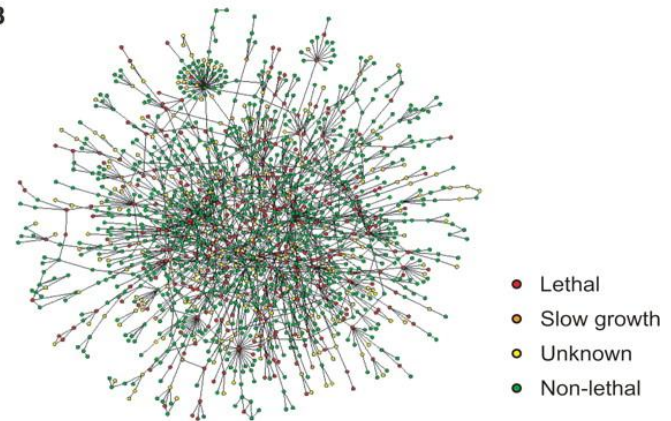
Razporeditev **koeficientov**
povezanosti
Clustering coefficient
 $(C(k))$

Examples of the five major biological networks.

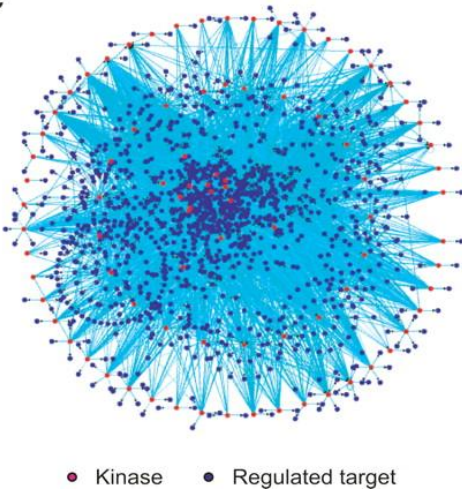
A



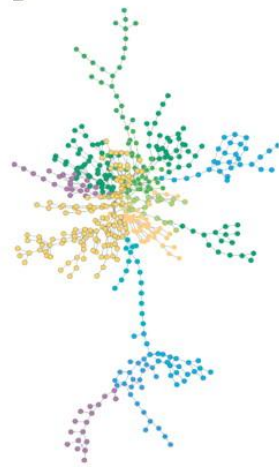
B



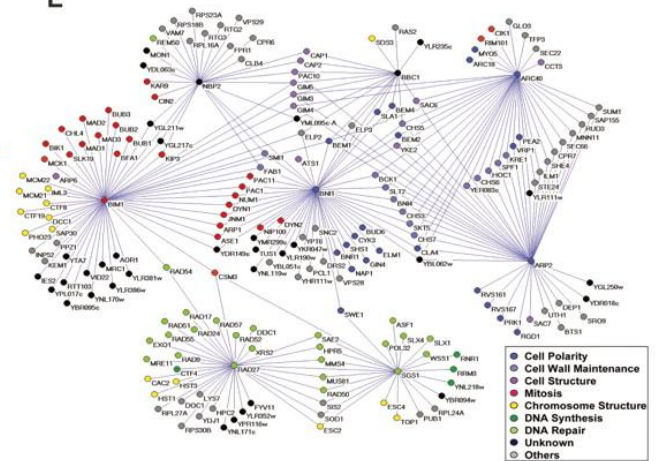
C



D



E



Zhu X et al. *Genes Dev.* 2007;21:1010-1024



Andrej Mrvar

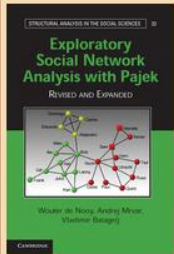
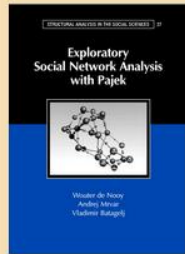


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Curriculum Vitae

Lectures



andrej.mrvar@fdv.uni-lj.si

Vlado Vladimir Batagelj



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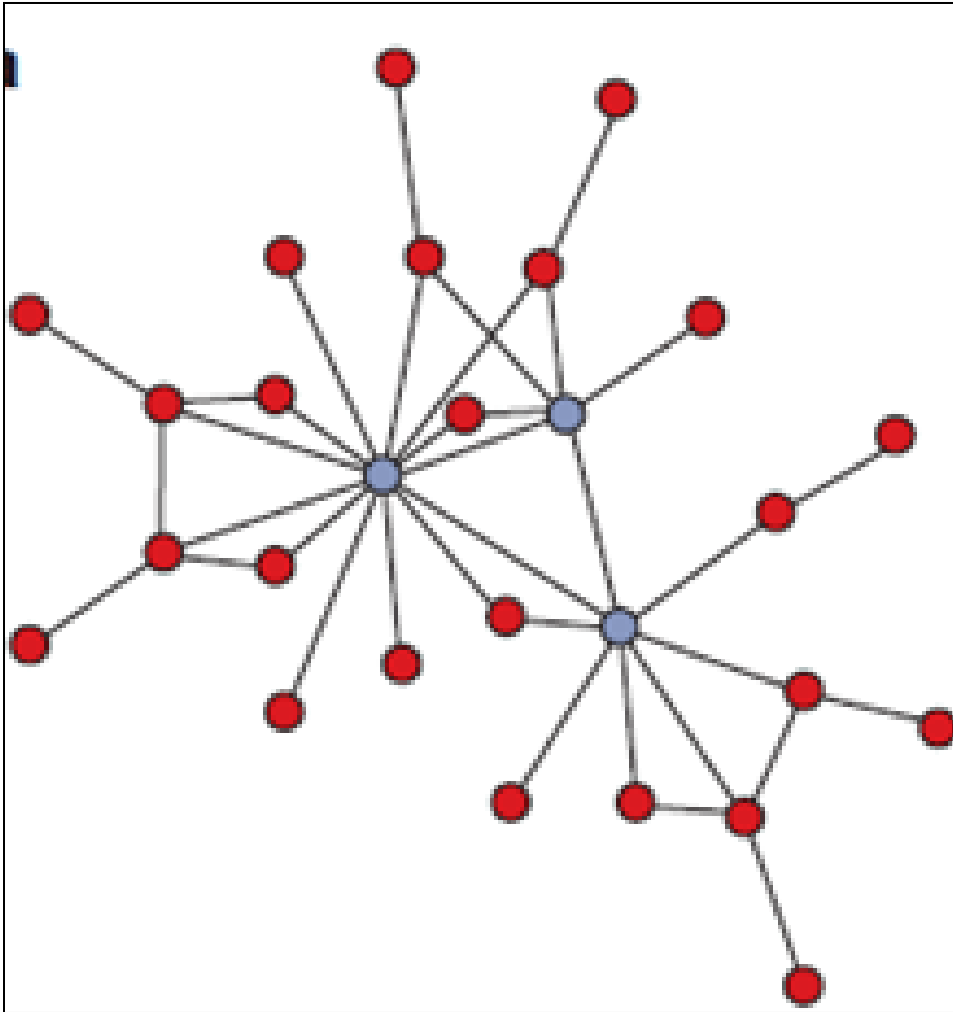
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[SquirrelMail FMF](#)

[Kronika](#); [Server statistics](#) [Opentype](#) / [Unicode](#)



središčne (centralne) točke
(neusmerjene interakcije)

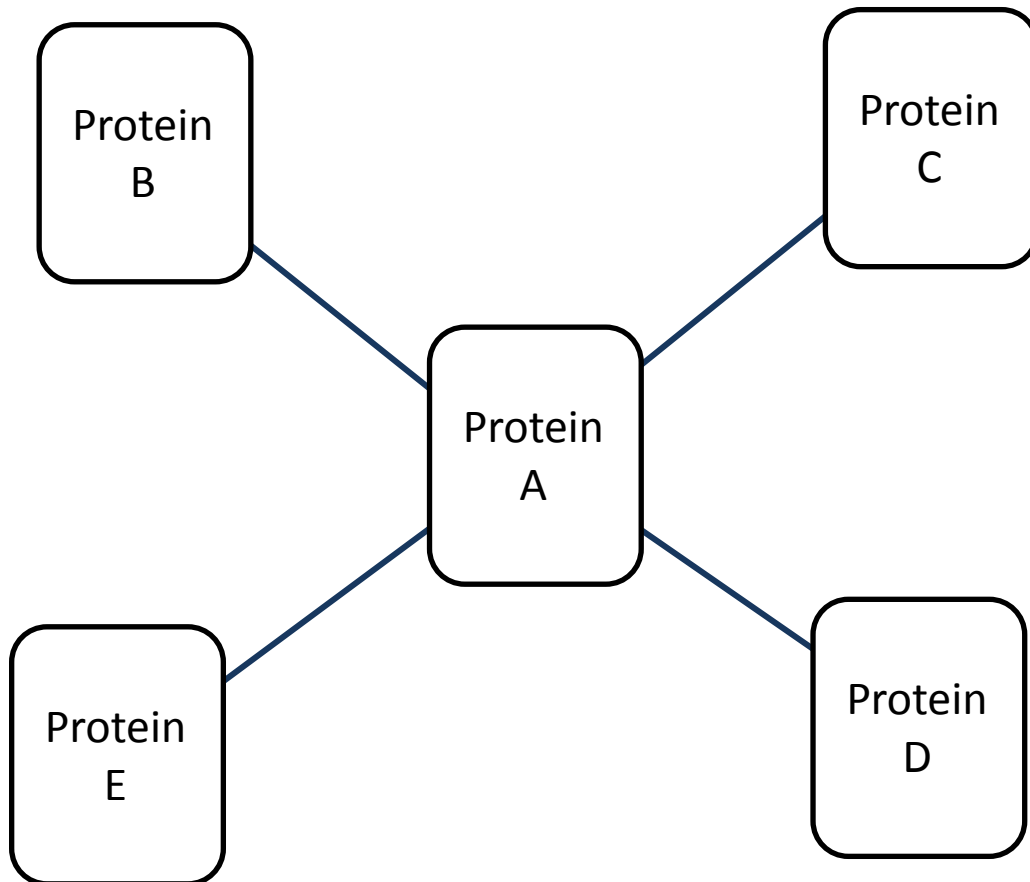


pomembne točke
(usmerjene interakcije)

zvezdišča

'Hubs'

Od merila neodvisne mreže
'Scale-free network'

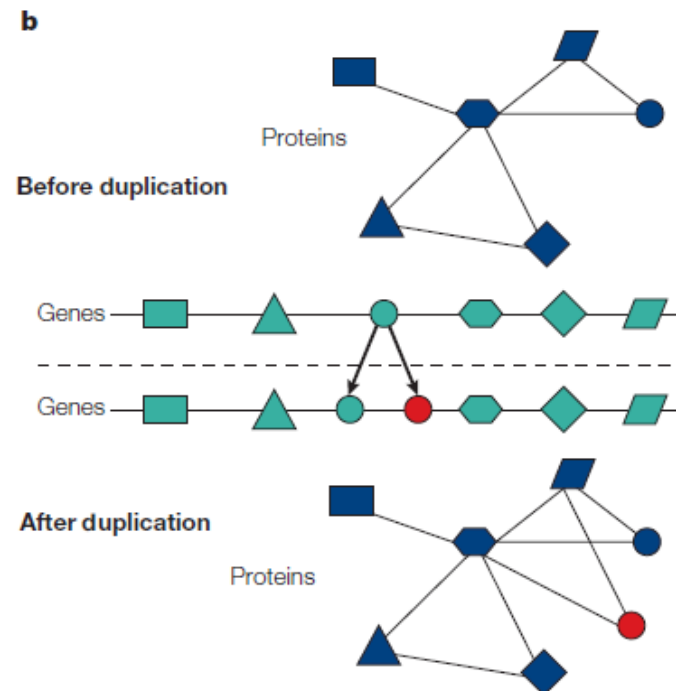
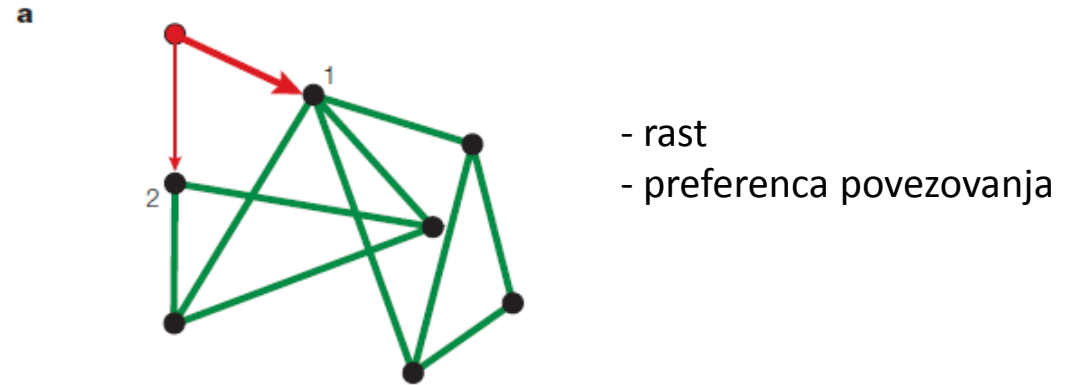


“Nothing in Biology Makes Sense Except in the Light of Evolution”



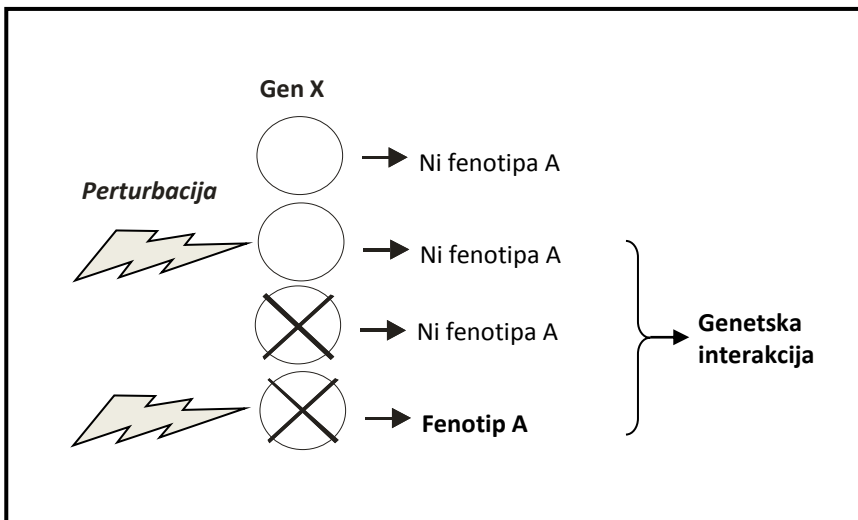
Teodosij Grigorovič
Dobžanski
1900-1975

American Biology Teacher (1973) 35:125

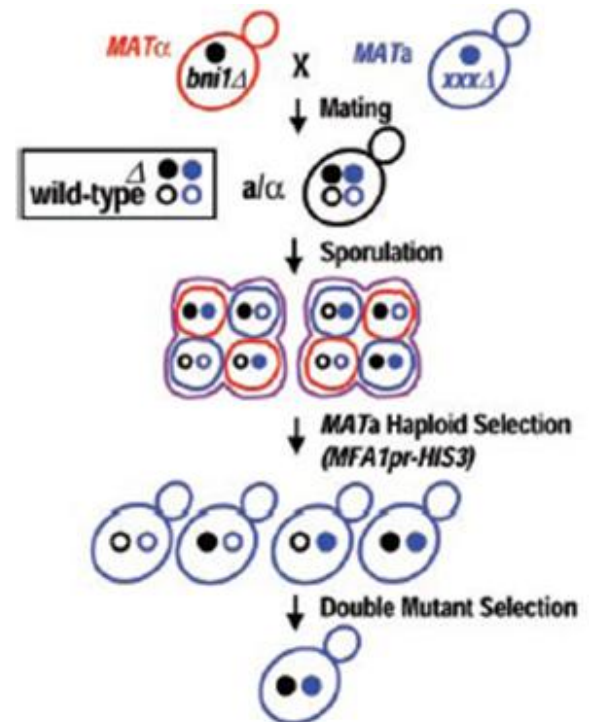


Nature Reviews
Genetics (2004) 5:101

Genetske (in kemogenetske) interakcije



Genetske interakcije med geni



Kvantitativne genetske interakcije

Fitness divjega tipa (F_{AB}) = 1

$$F_{aB} = 0,9$$

$$F_{Ab} = 0,7$$

$$F_{ab} = 0,63$$

$$F_{ab} \ll 0,63$$

Negativna g. i.
(npr. gena sta v para-lelnih
genetskih poteh)

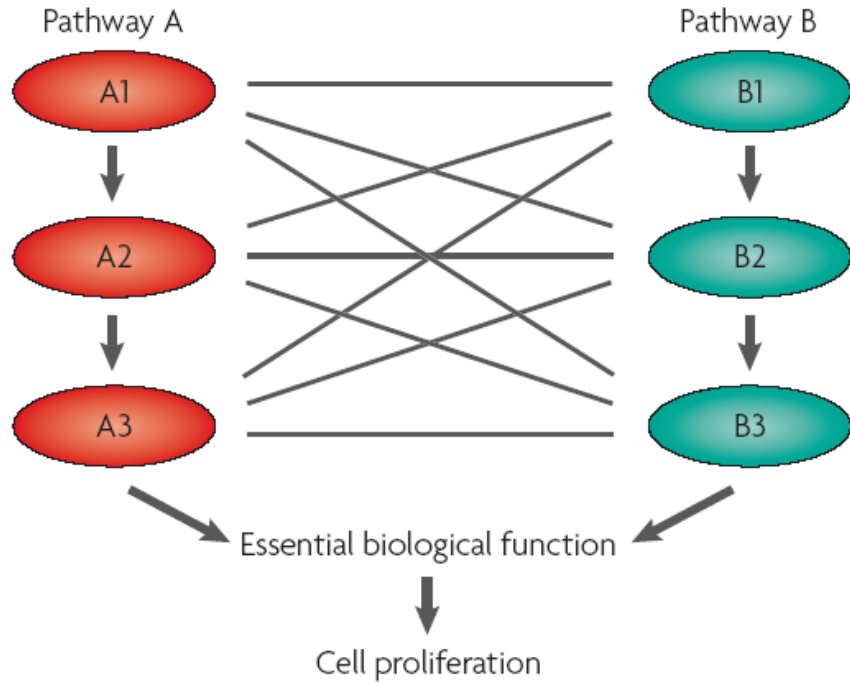
Ni genetske interakcije (gena
sta neodvisna)

$$F_{ab} \gg 0,63$$

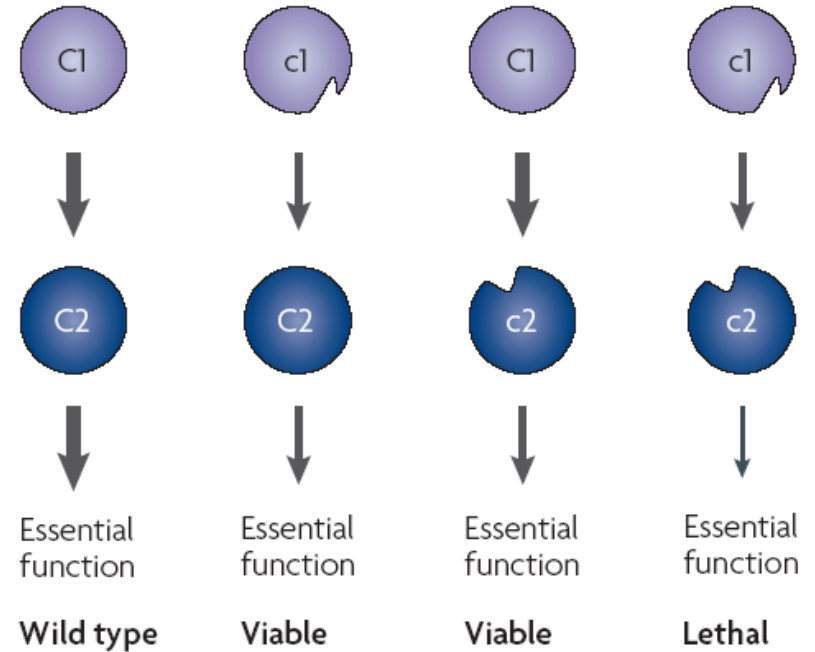
Pozitivna g. i.
(npr. gena sta v isti genetski
poti)

Box 2 | Mechanisms of synthetic-lethal interactions

a Between-pathway genetic interactions



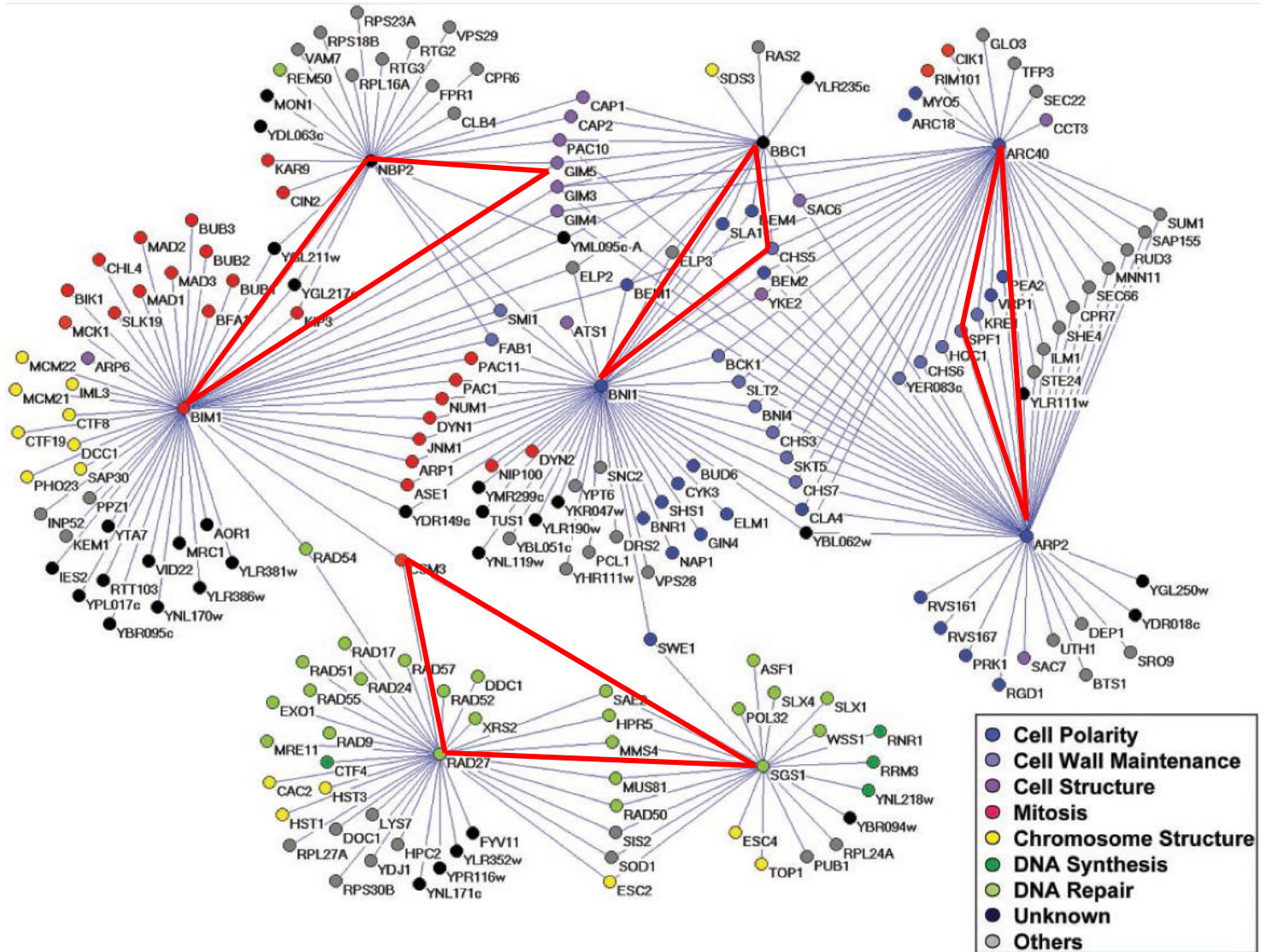
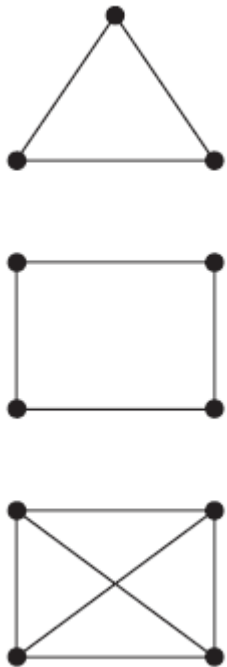
b Within-pathway genetic interactions

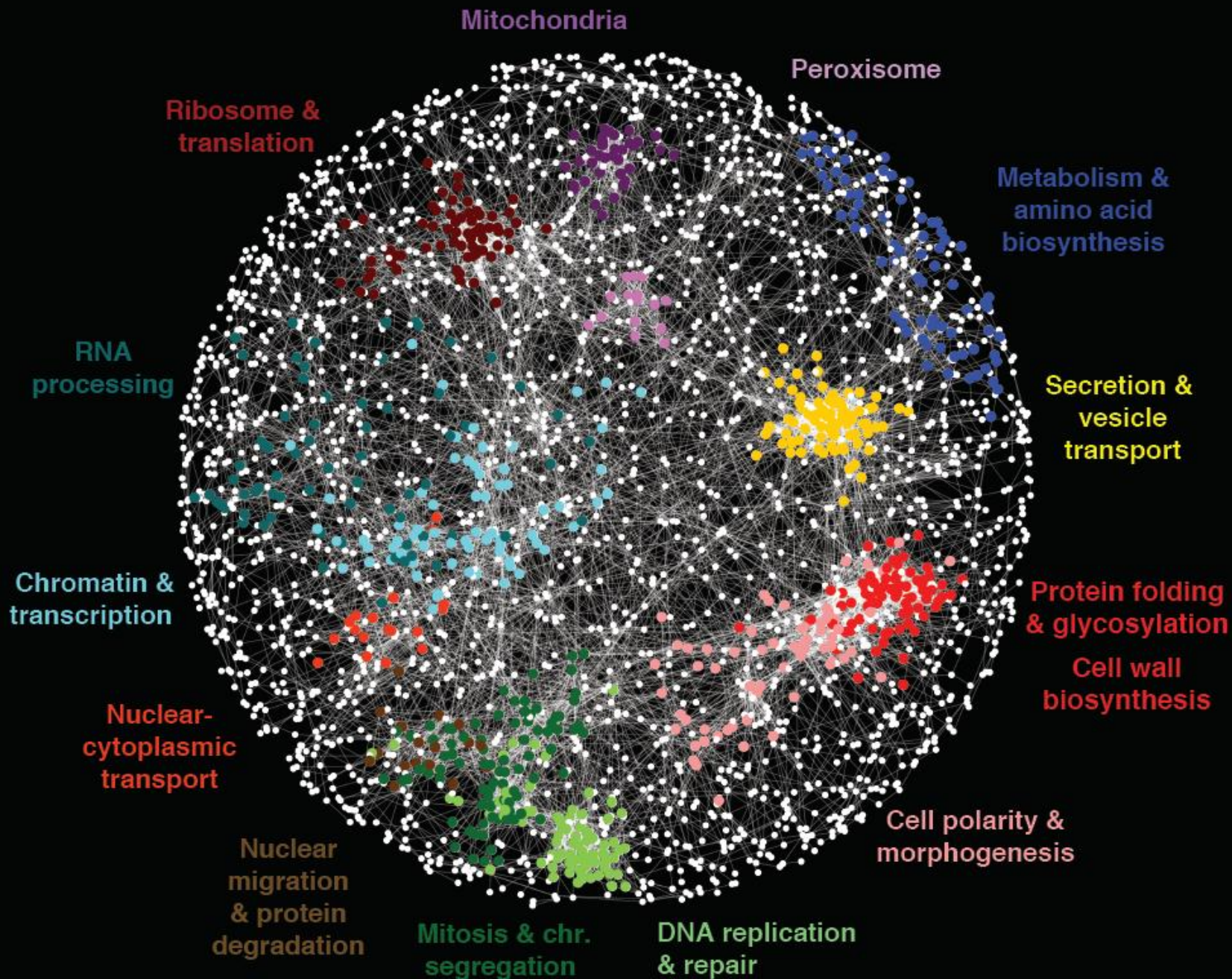


Nat Rev Genet (2007) 8:437

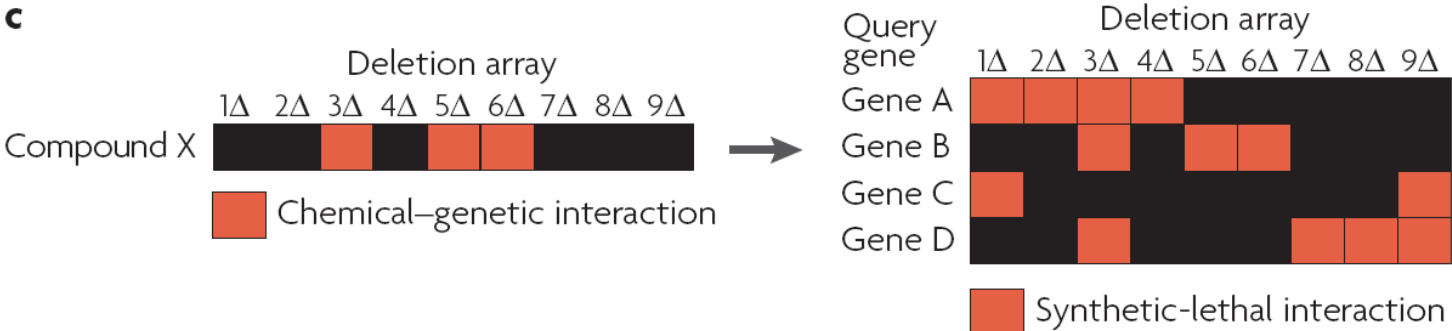
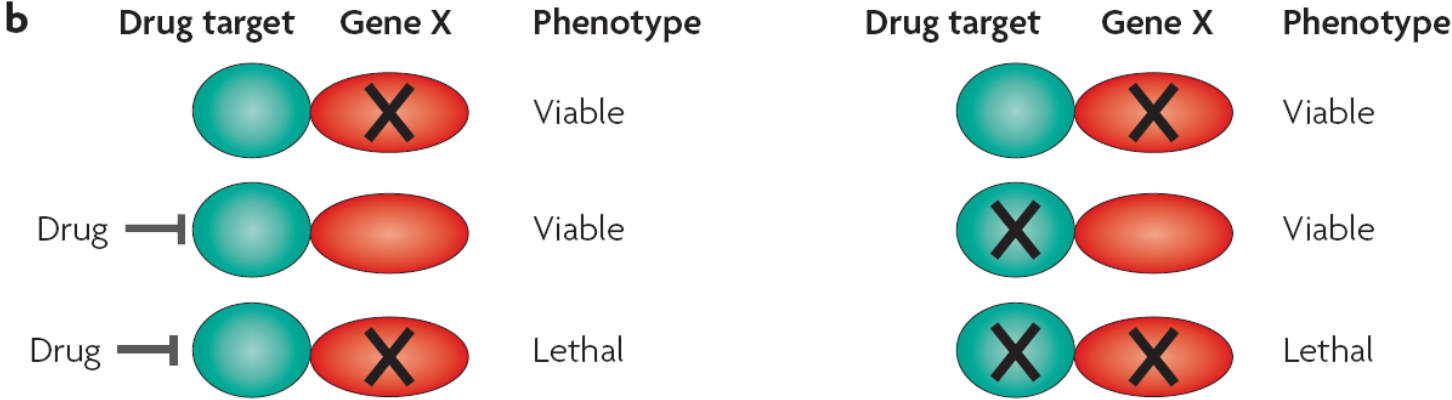
Kakšno razporeditev **koeficientov povezanosti** napovedujeta zgornja modela?

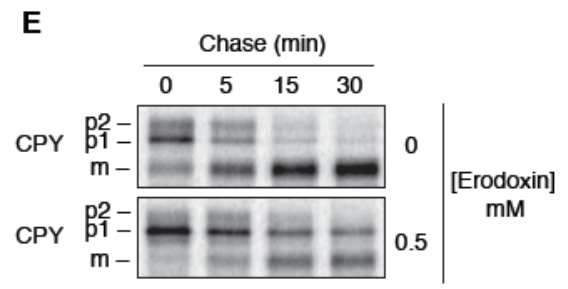
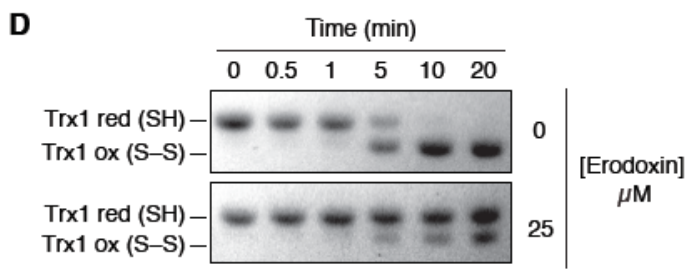
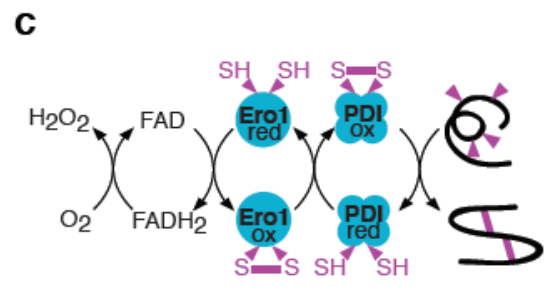
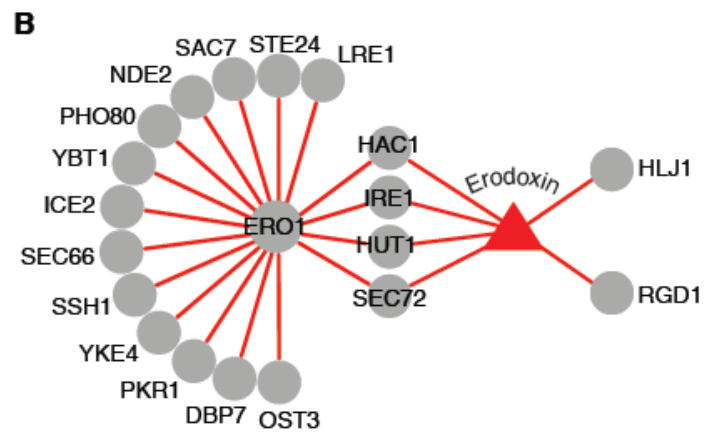
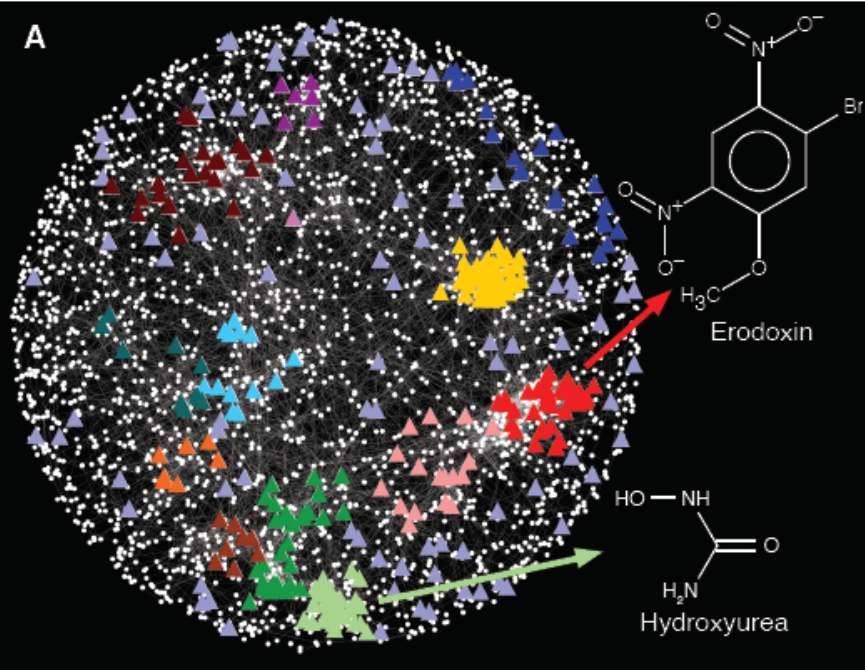
Moduli v mrežah





Kemogenomika



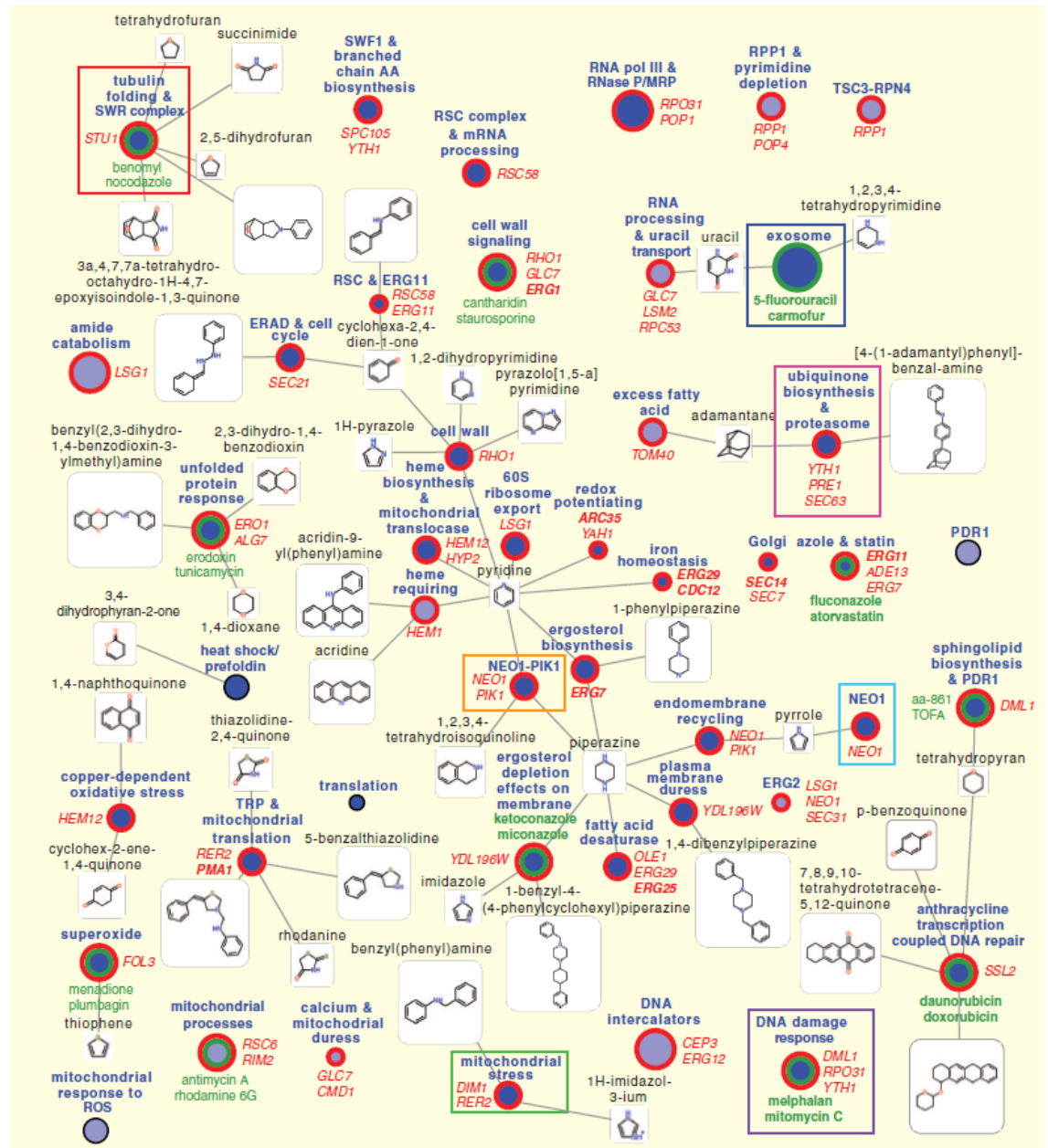


Mapping the Cellular Response to Small Molecules Using Chemogenomic Fitness Signatures

Anna Y. Lee,^{1,2*} Robert P. St-Onge,^{3*} Michael J. Proctor,³ Iain M. Wallace,¹ Aaron H. Nile,⁴ Paul A. Spagnuolo,⁵ Yulia Jitkova,⁶ Marcela Gronda,⁶ Yan Wu,⁶ Moshe K. Kim,^{7,8} Kahlin Cheung-Ong,^{1,2} Nikko P. Torres,^{1,7} Eric D. Spear,⁹ Mitchell K. L. Han,¹⁰ Ulrich Schlecht,³ Sundari Suresh,³ Geoffrey Duby,¹¹ Lawrence E. Heister,¹ Anuradha Surendra,¹ Eula Fung,³ Malene L. Urbanus,² Marinella Gebbia,¹ Elena Lissina,^{1,2} Molly Miranda,³ Jennifer H. Chiang,¹² Ana Maria Aparicio,³ Mahel Zeghouf,¹³ Ronald W. Davis,³ Jacqueline Cherfils,¹³ Marc Boutry,¹¹ Chris A. Kaiser,⁹ Carolyn L. Cummins,¹⁰ William S. Trimble,^{7,8} Grant W. Brown,^{1,7} Aaron D. Schimmer,⁶ Vytas A. Bankaitis,⁴ Corey Nislow,^{1,2,12} Gary D. Bader,^{1,2} Guri Giaever^{1,2,10,12†}

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3250 kemikalij × 6000 genov



Monogenske : poligenske lastnosti

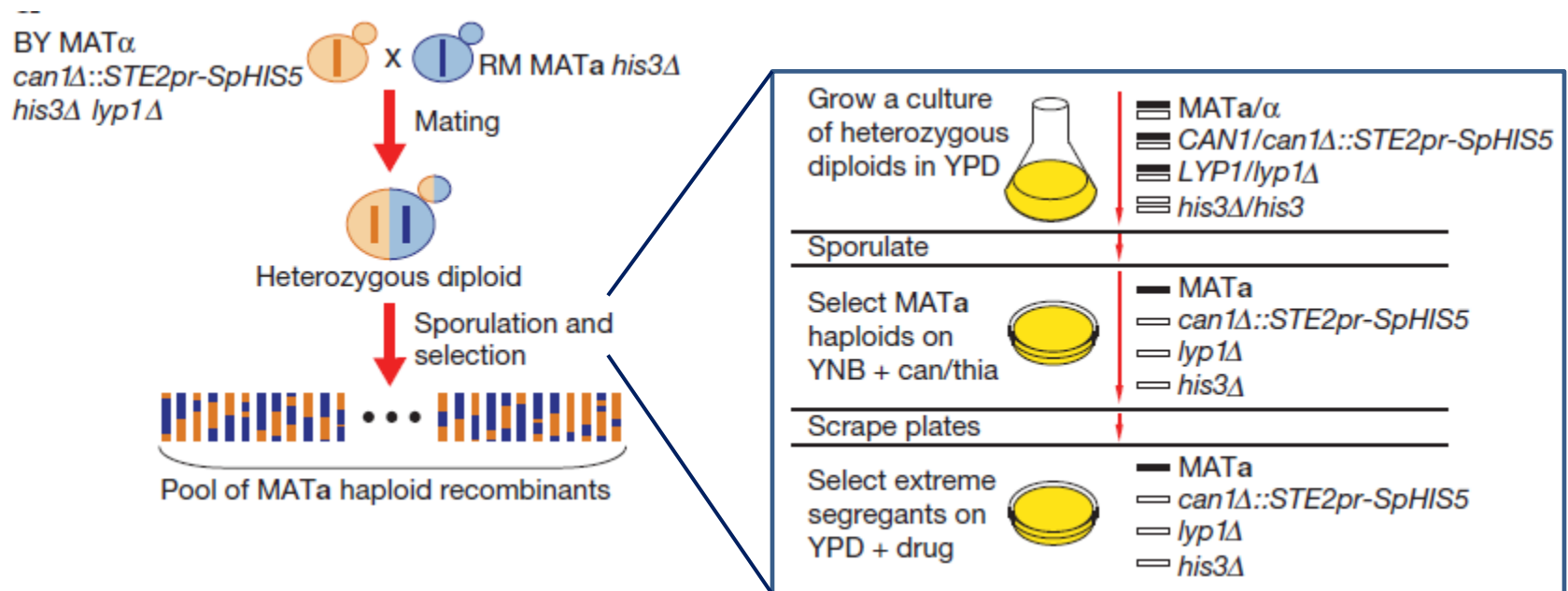
Poligenske lastnosti – kvantitativni lokusi lastnosti (QTL)

Vol 464 | 15 April 2010 | doi:10.1038/nature08923

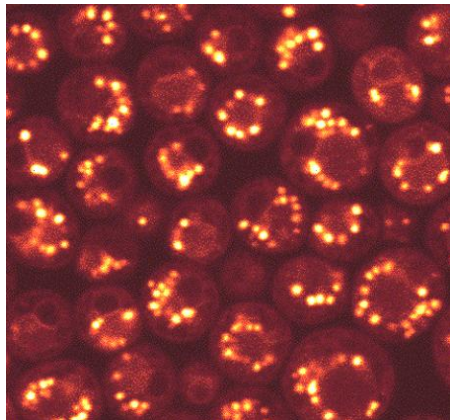
nature

Dissection of genetically complex traits with extremely large pools of yeast segregants

Ian M. Ehrenreich^{1,2,3}, Noorossadat Torabi^{1,4}, Yue Jia^{1,3}, Jonathan Kent¹, Stephen Martis¹, Joshua A. Shapiro^{1,2,3}, David Gresham^{1†}, Amy A. Caudy¹ & Leonid Kruglyak^{1,2,3}

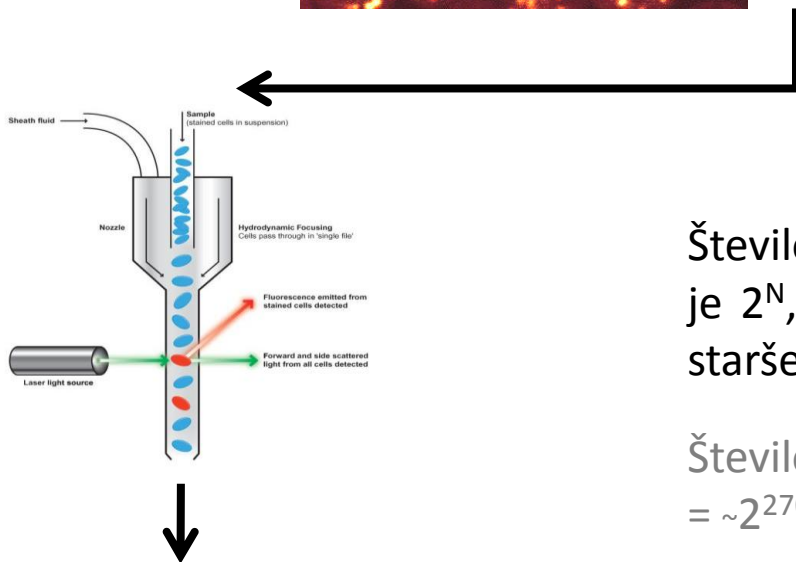
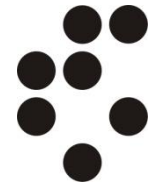
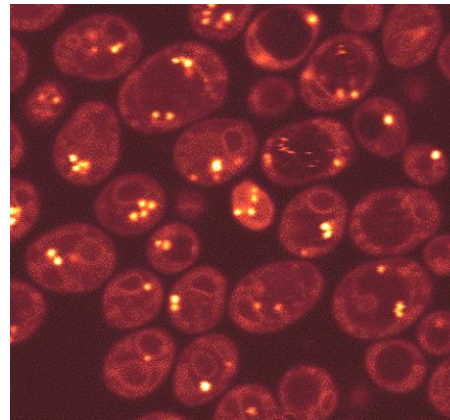


'Debeli'
starš



X

'Suhi'
starš



Število možnih kombinacij različnih genomov je 2^N , če je N = število genetskih razlik med starševskima sevoma.

Število atomov v vesolju ocenjujemo na $\sim 10^{82}$
 $= \sim 2^{270}$.

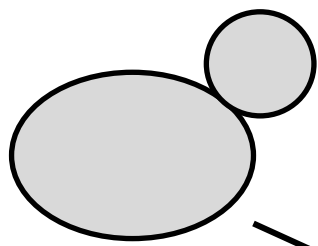


NGS – sekvenciranje
nove generacije

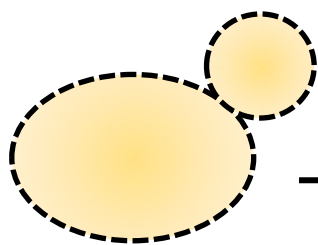
Identifikacija relevantnih/kavzalnih QTL/SNV-jev



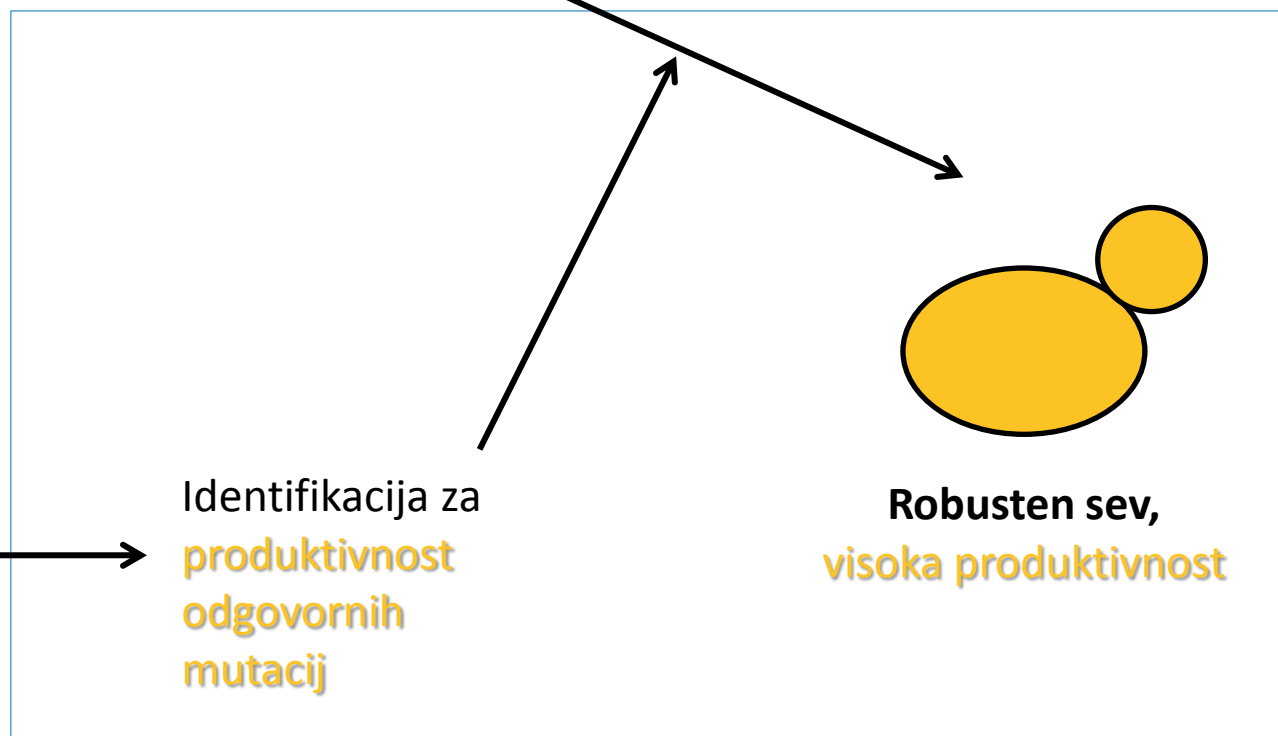
Robusten sev,
nizka produktivnost



Naključne
mutacije

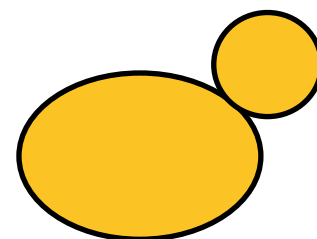


Ne-robusten sev,
visoka produktivnost



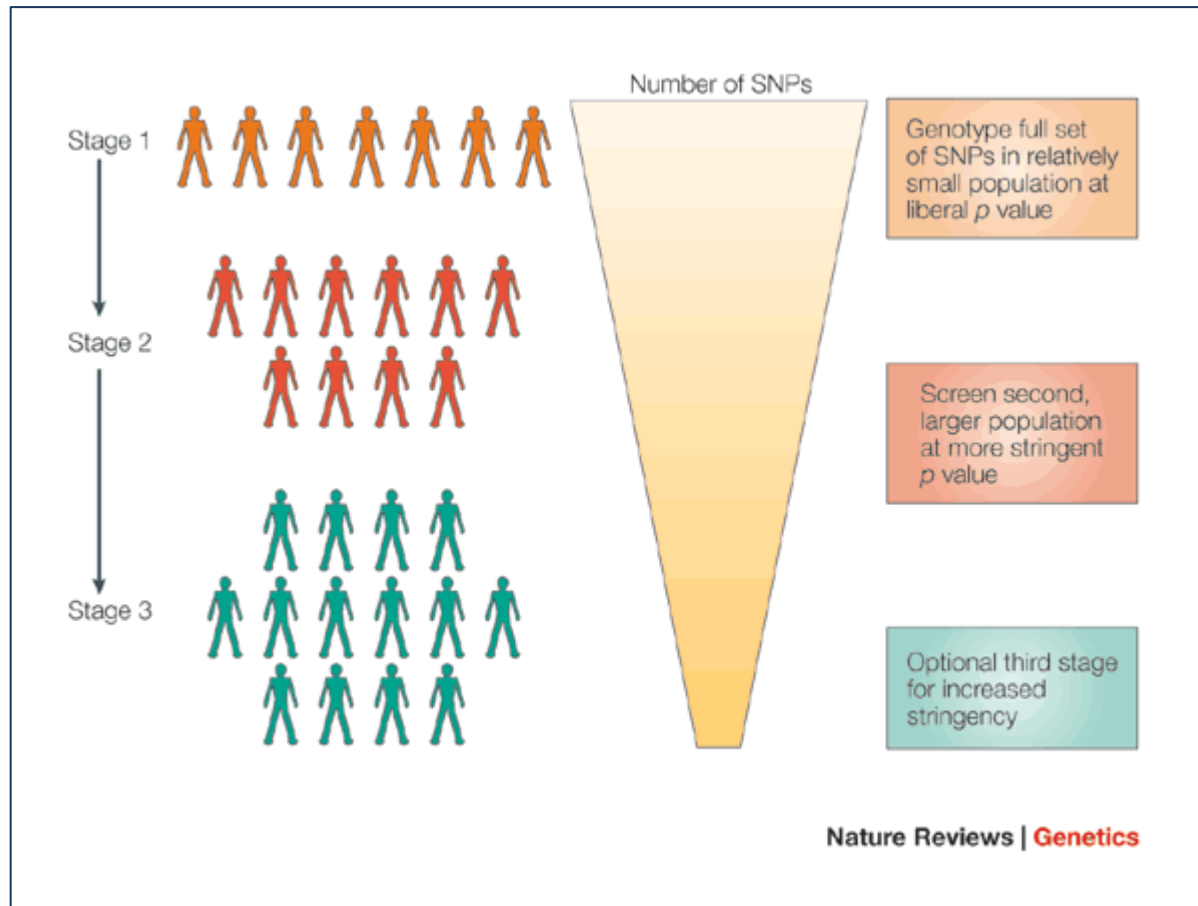
Identifikacija za
produktivnost
odgovornih
mutacij

Robusten sev,
visoka produktivnost



Nova generacija industrijskih mikroorganizmov

Genome-wide association studies (GWAS)



LETTER

14th February, 2013

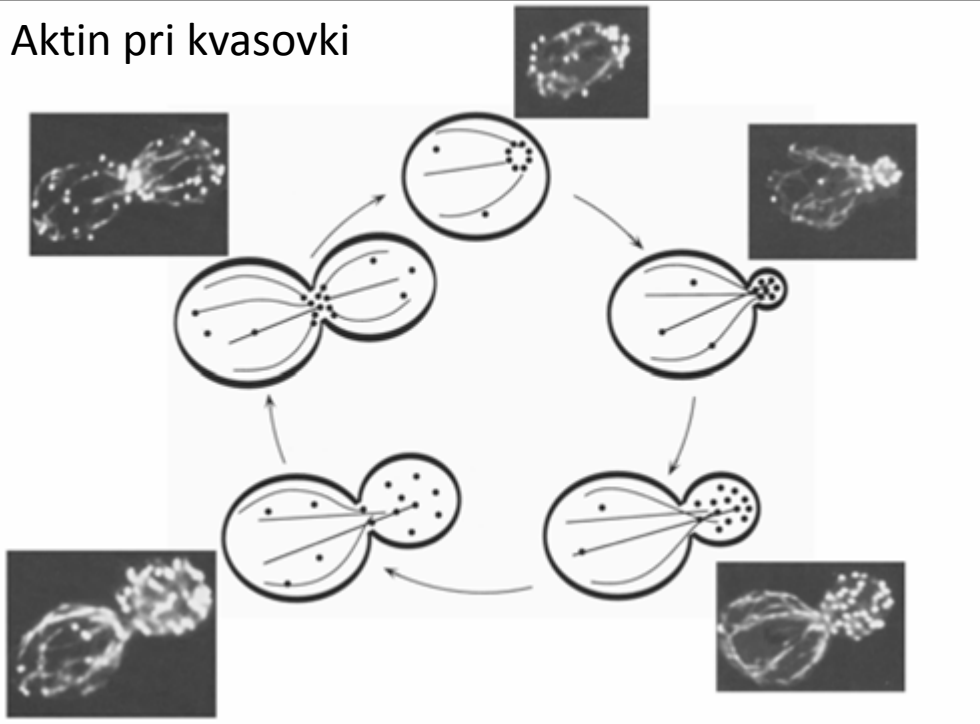
doi:10.1038/nature11867

Finding the sources of missing heritability in a yeast cross

Joshua S. Bloom^{1,2}, Ian M. Ehrenreich^{1,3}, Wesley T. Loo^{1,2}, Thùy-Lan Võ Lite^{1,2} & Leonid Kruglyak^{1,4,5}

Koliko funkcij (lahko) ima posamezen igralec (gen/protein)?

Aktin pri kvasovki



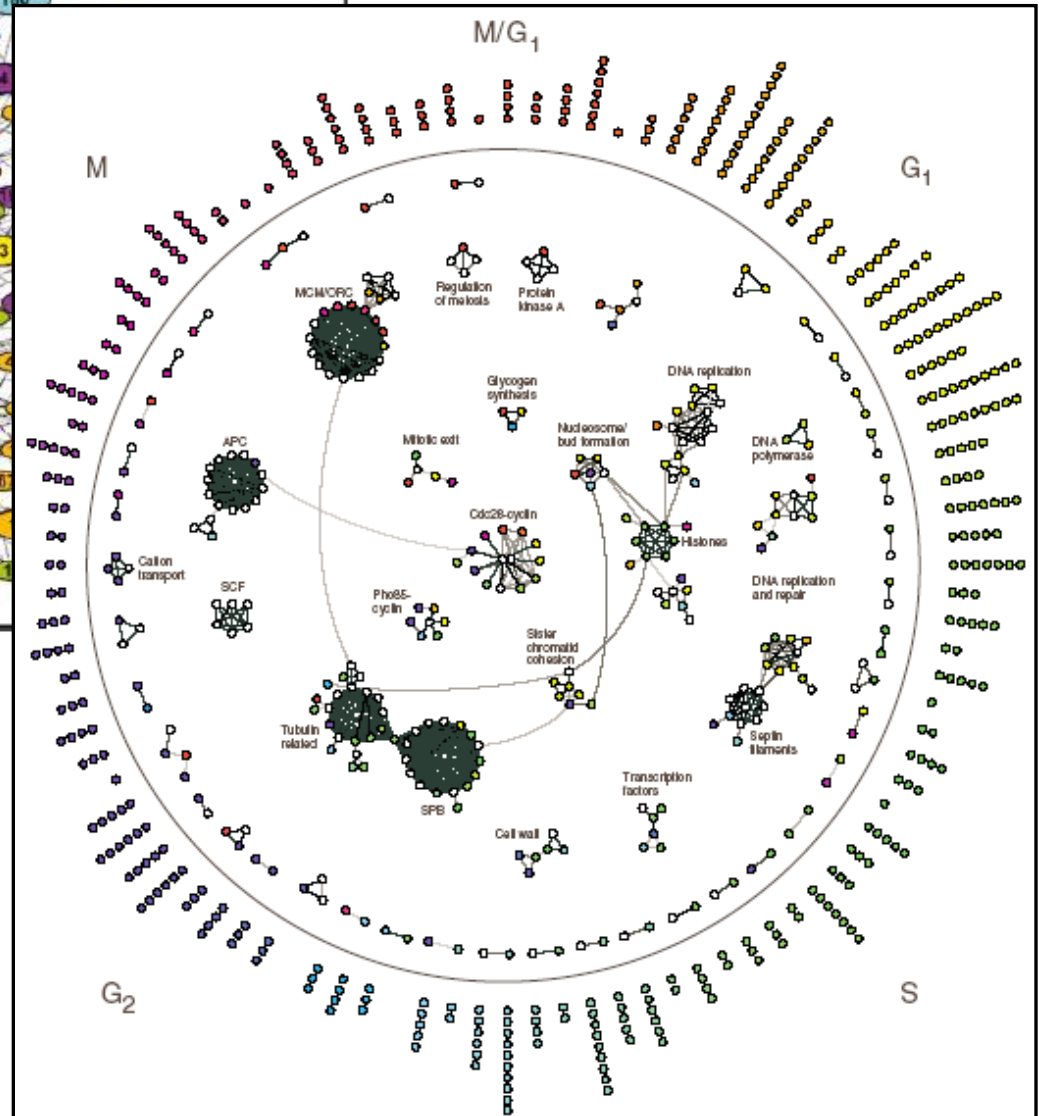
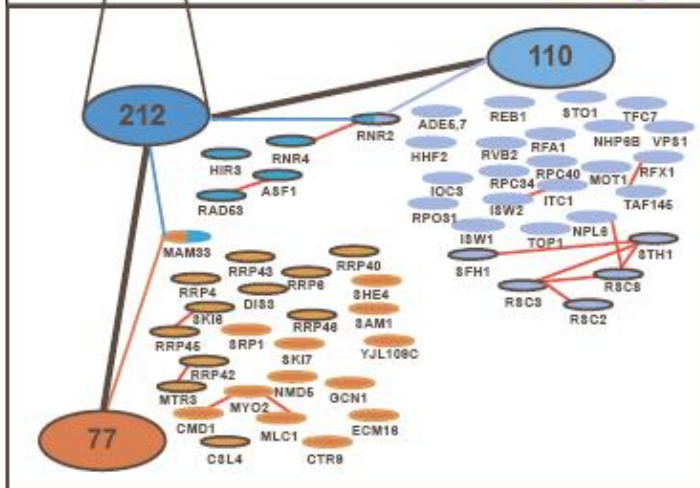
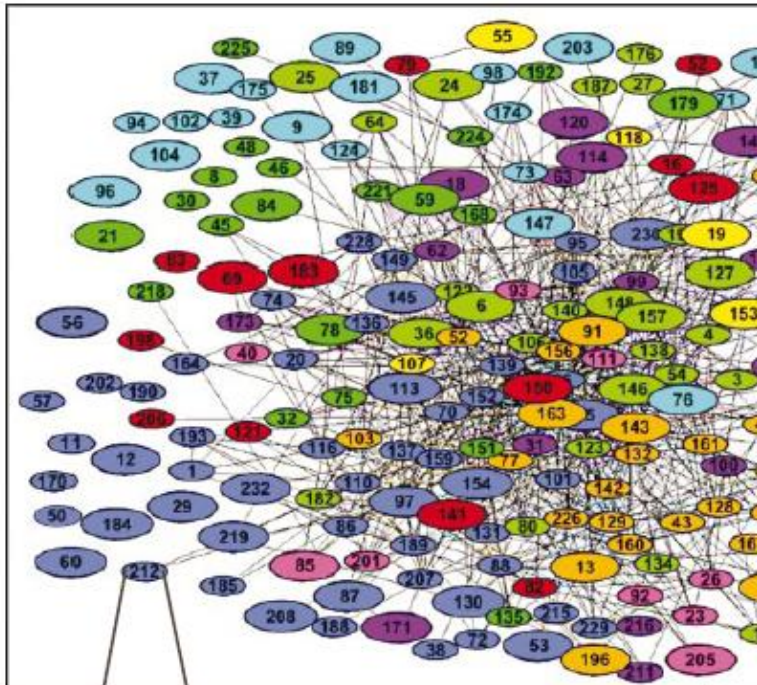
Evidence for monomeric actin function in INO80 chromatin remodeling

Prabodh Kapoor, Mingming Chen, Duane David Winkler, Karolin Luger & Xuetong Shen

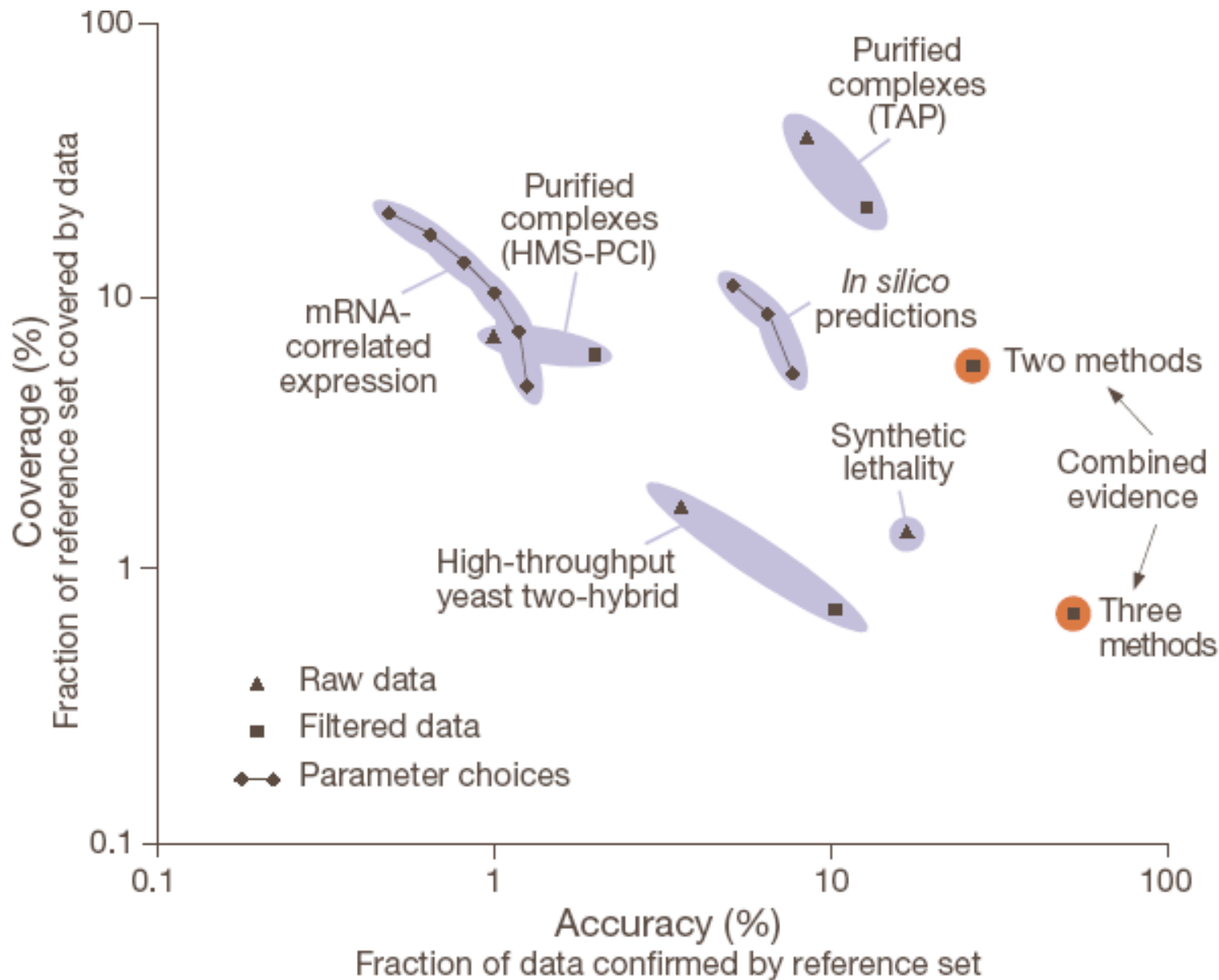
Nature Structural & Molecular Biology **20**, 426–432 (2013) | doi:10.1038/nsmb.2529

Received 27 November 2012 | Accepted 06 February 2013 | Published online 24 March 2013

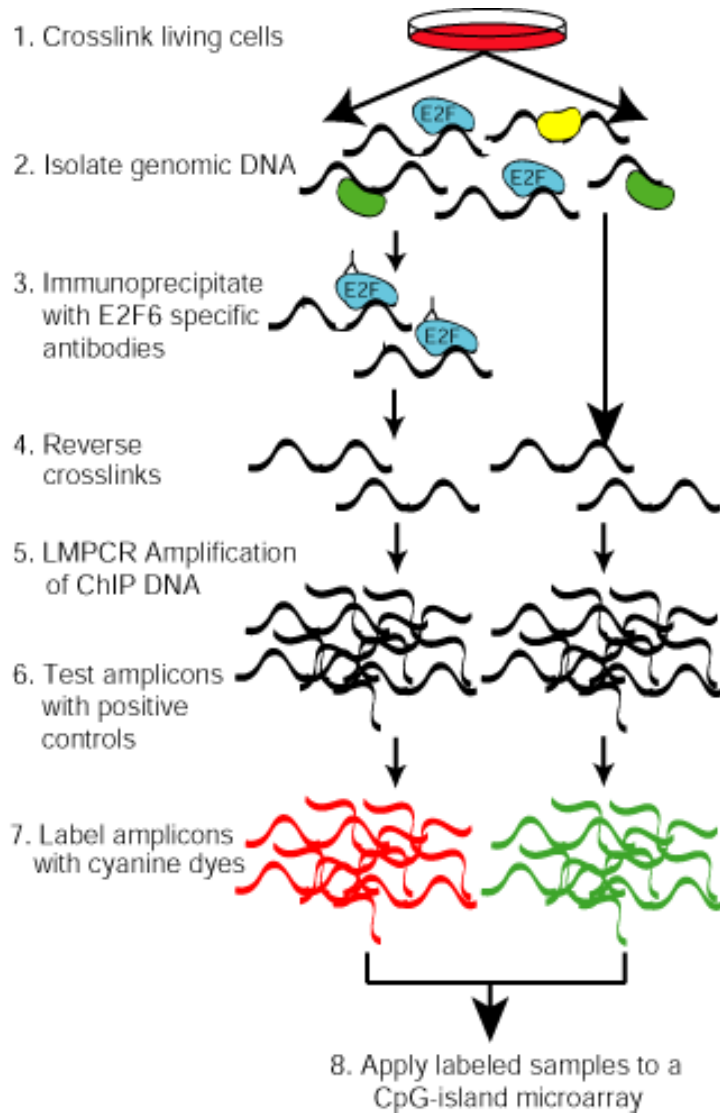
Primeri uporabe interaktomike



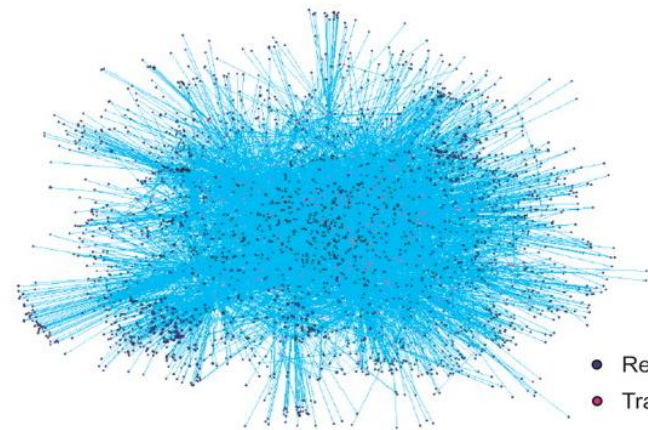
Kombiniranje različnih tipov podatkov



Kromatinska imunoprecipitacija na "čipu" (ChIP chip)

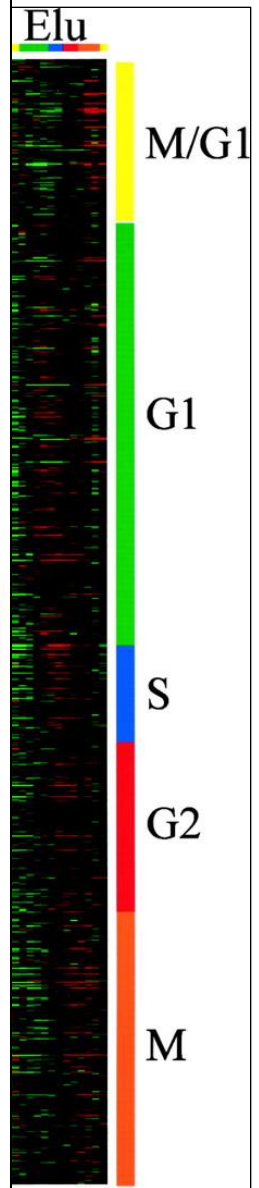
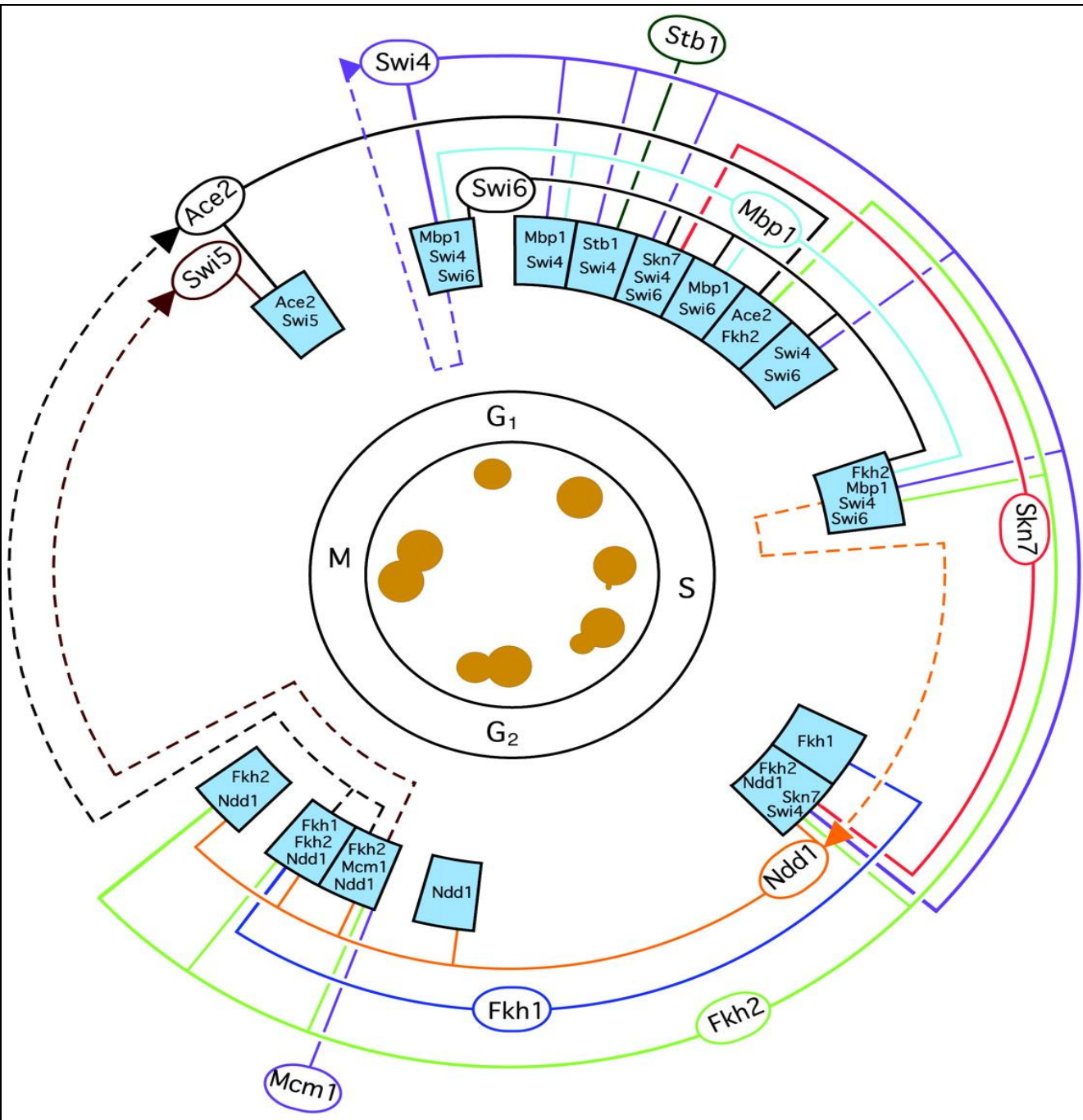
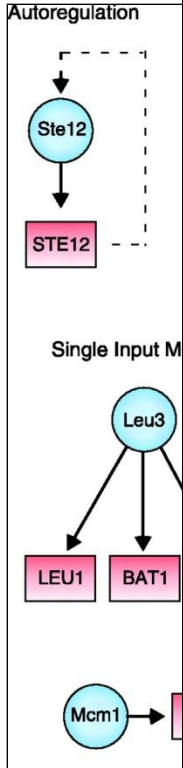
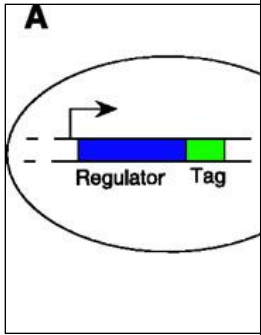


A

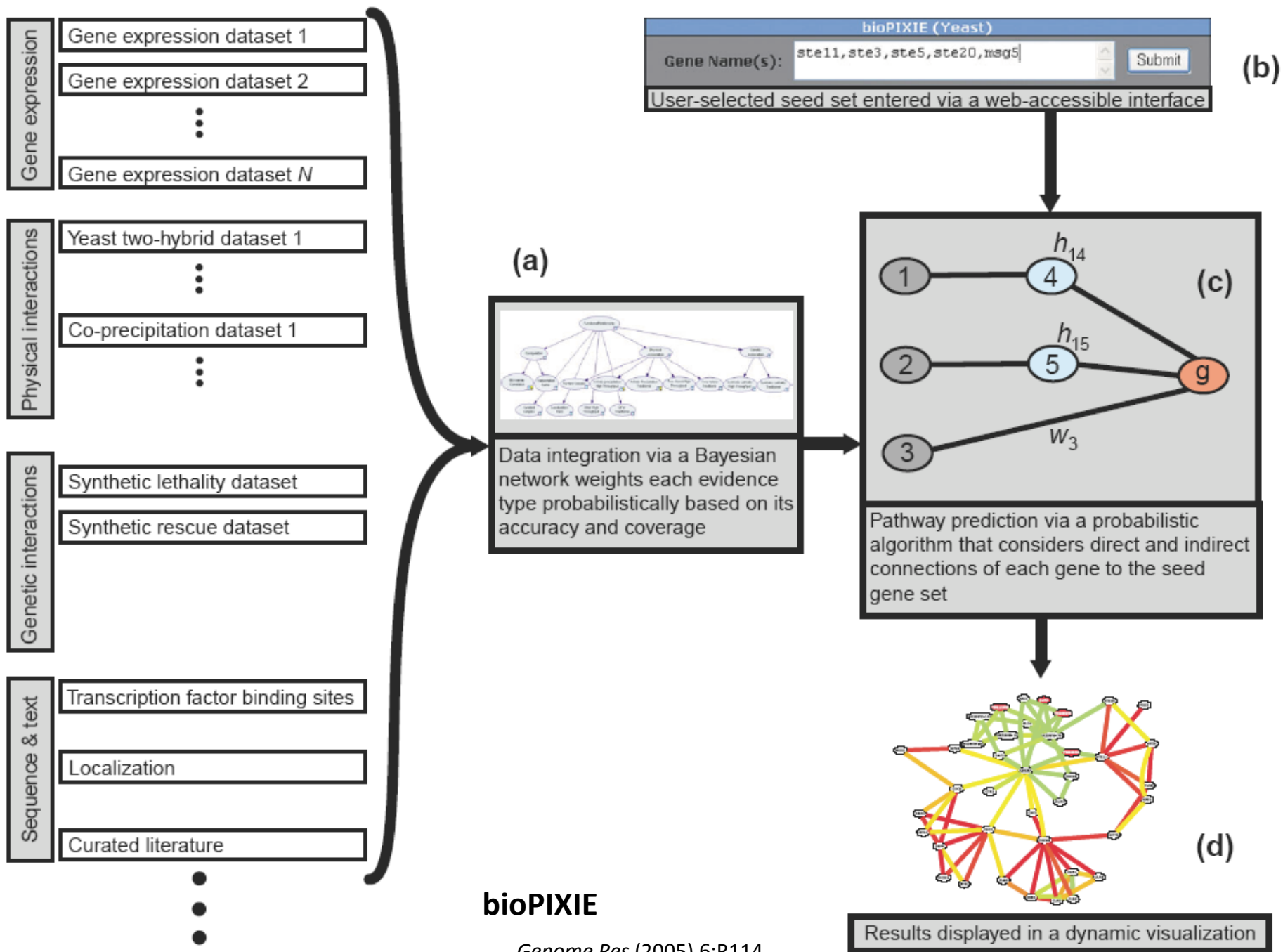


- Regulated target
- Transcription factor

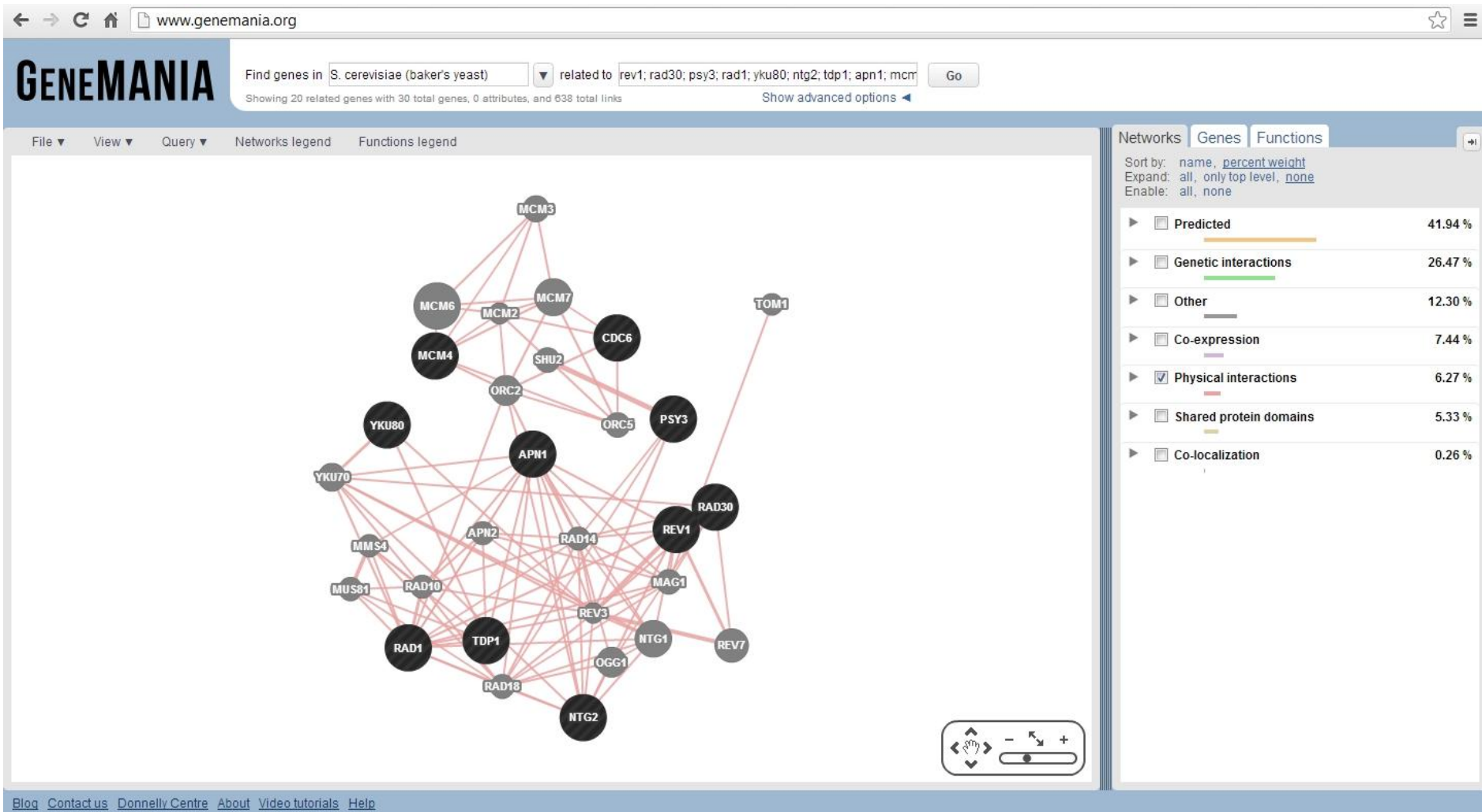
E



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Kombiniranje različnih tipov podatkov pri: *A. thaliana*, *C. elegans*, *D. melanogaster*, *H. sapiens*, *R. norvegicus*, *M. musculus* in *S. cerevisiae*



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Domača naloga!