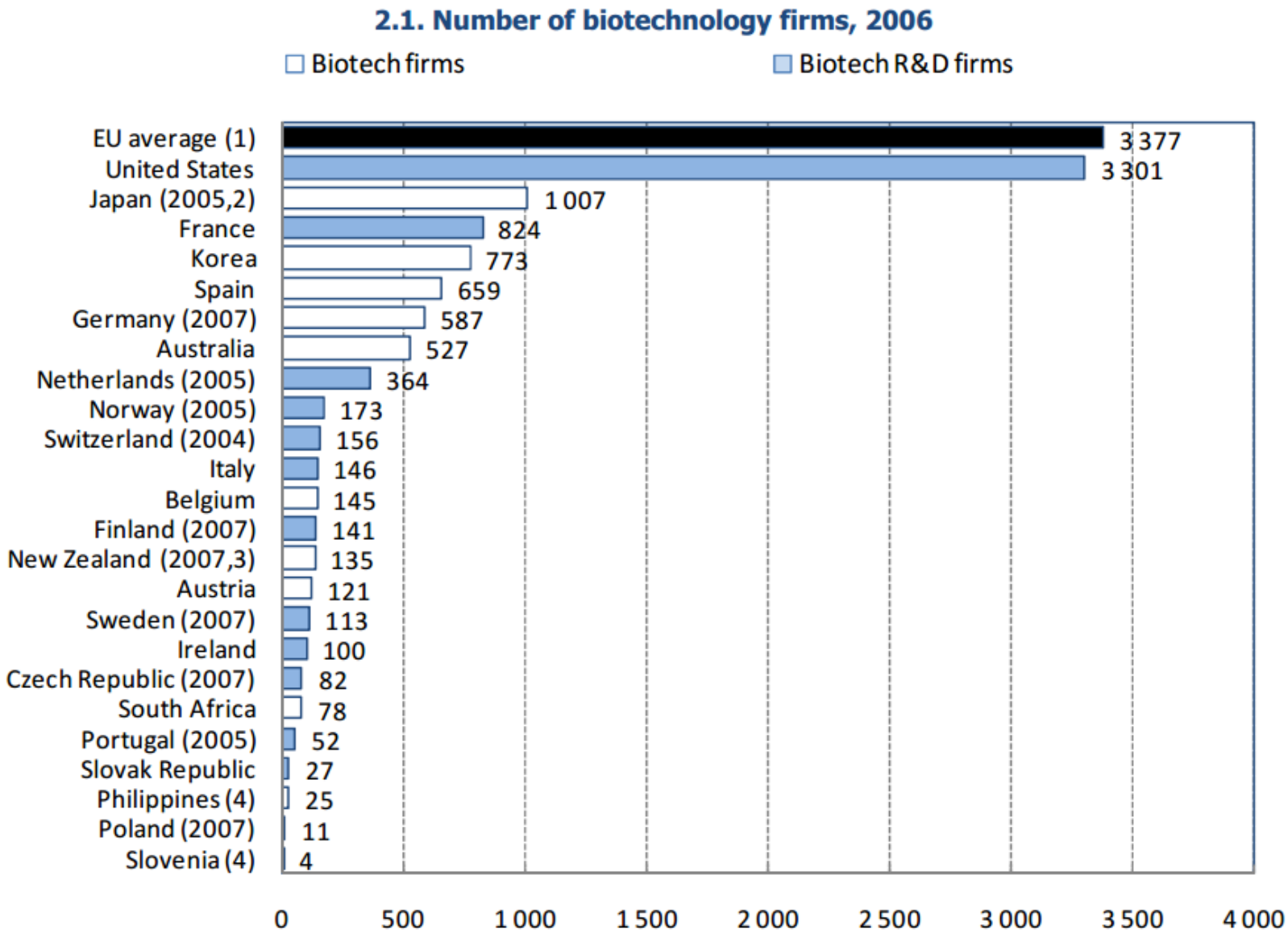
Zakaj smo tu?



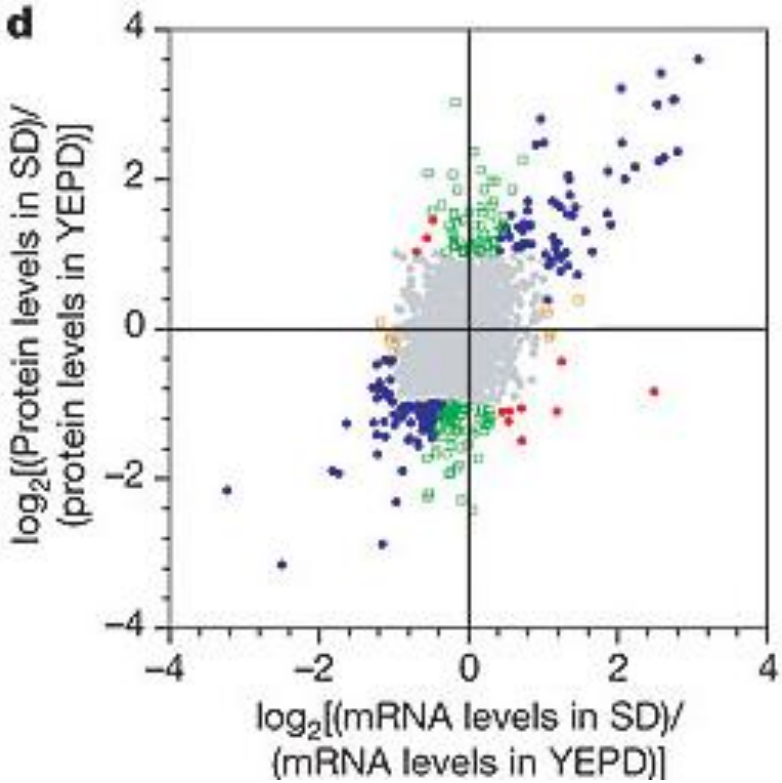
OECD Biotechnology Statistics 2009 (http://www.oecd.org/sti/42833898.pdf)

Proteomika

“Kot da geni in mRNA kaj pomenijo...”

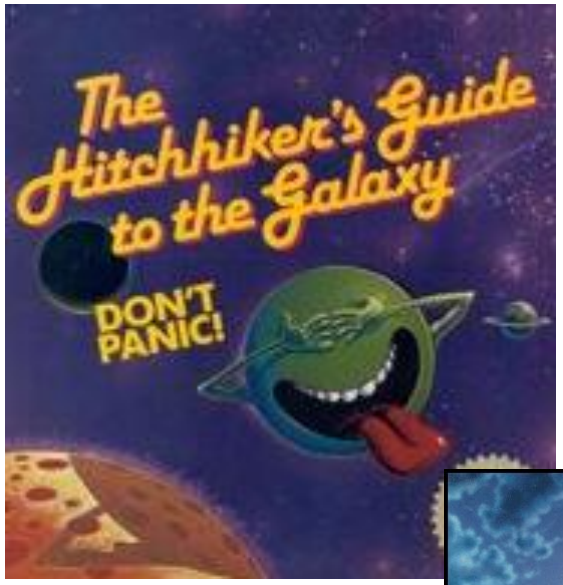
PROTEOMIKA

Single-cell proteomics
(flow cytometry)



DNA microarray

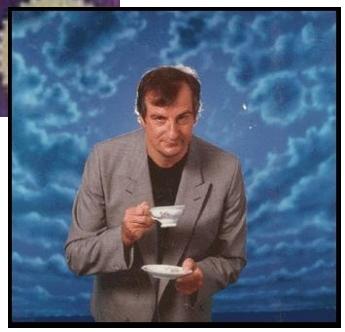
Nature (2006) 441:840



Life?

Universe?

Everything?



Douglas **N**. **A**dams

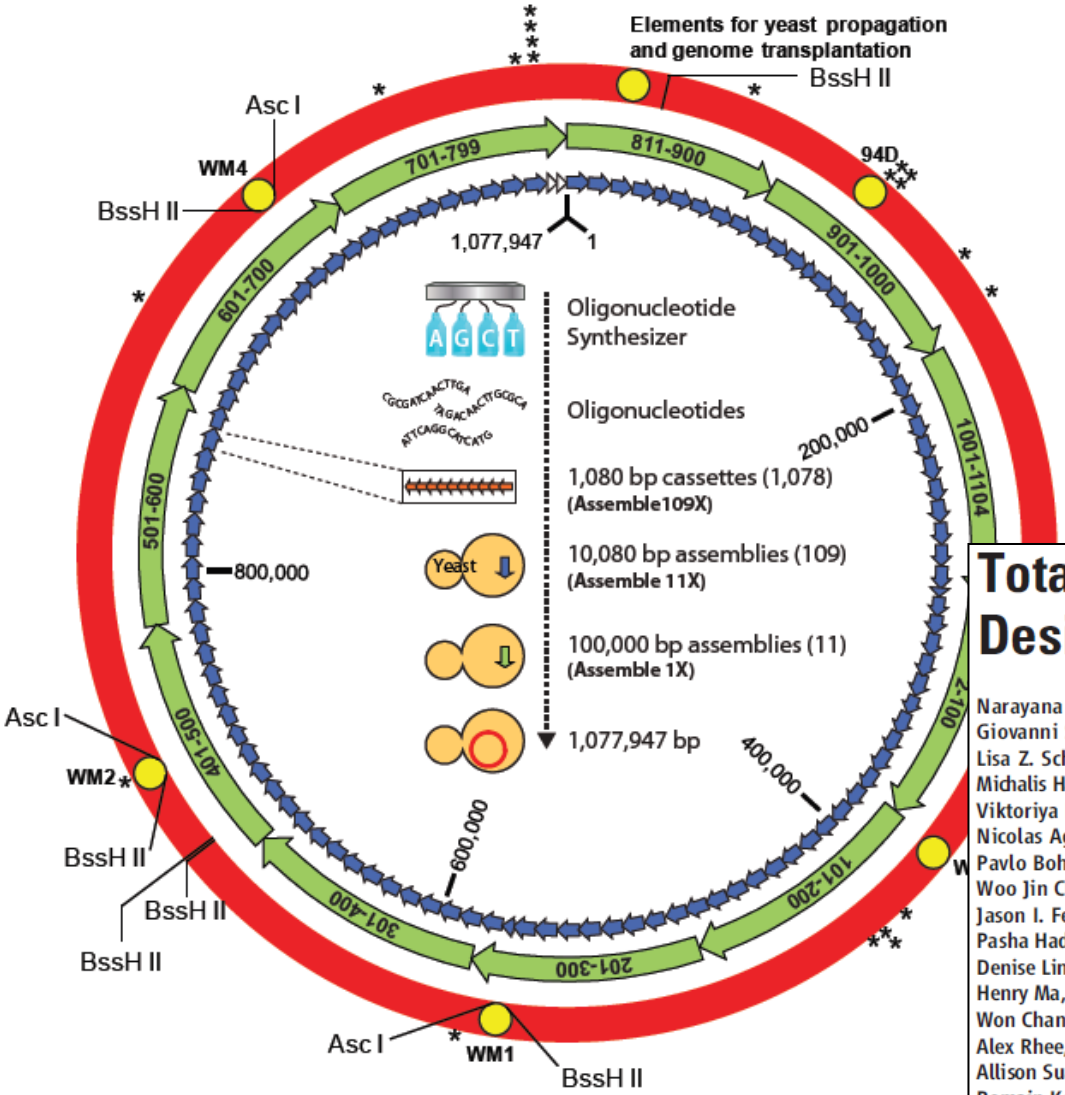
1952-2001

"I think the problem, to be quite honest with you, is that you've never actually known what the question is."

Eksperimenti brez a priori hipoteze

Fishing expeditions vs. Postavljanje pravih vprašanj

The assembly of a synthetic *M. mycoides* genome in yeast



Human Proteome Organisation

Total Synthesis of a Functional Designer Eukaryotic Chromosome

Narayana Annaluru,[1]* Héloïse Muller,[1,2,3,4]* Leslie A. Mitchell,[2,5] Sivaprakash Ramalingam,[1] Giovanni Stracquadanio,[2,6] Sarah M. Richardson,[6] Jessica S. Dymond,[2,7] Zheng Kuang,[2] Lisa Z. Scheifele,[2,8] Eric M. Cooper,[2] Yizhi Cai,[2,9] Karen Zeller,[2] Neta Agmon,[2,5] Jeffrey S. Han,[10] Michalis Hadjithomas,[11] Jennifer Tullman,[6] Katrina Caravelli,[2,12] Kimberly Cirelli,[1,12] Zheyuan Guo,[1,13] Viktoriya London,[1,13] Apurva Yeluru,[1,13] Sindurathy Murugan,[6] Karthikeyan Kandavelou,[1,14] Nicolas Agier,[15,16] Gilles Fischer,[15,16] Kun Yang,[2,6] J. Andrew Martin,[2,6] Murat Bilgel,[13] Pavlo Bohutski,[13] Kristin M. Boulier,[12] Brian J. Capaldo,[13] Joy Chang,[13] Kristie Charoen,[13] Woo Jin Choi,[13] Peter Deng,[11] James E. DiCarlo,[13] Judy Doong,[13] Jessilyn Dunn,[13] Jason I. Feinberg,[12] Christopher Fernandez,[12] Charlotte E. Floria,[12] David Gladowski,[12] Pasha Hadidi,[13] Isabel Ishizuka,[12] Javaneh Jabbari,[12] Calvin Y. L. Lau,[13] Pablo A. Lee,[13] Sean Li,[13] Denise Lin,[12] Matthias E. Linder,[12] Jonathan Ling,[13] Jaime Liu,[13] Jonathan Liu,[13] Mariya London,[12] Henry Ma,[13] Jessica Mao,[13] Jessica E. McDade,[13] Alexandra McMillan,[12] Aaron M. Moore,[12] Won Chan Oh,[13] Yu Ouyang,[13] Ruchi Patel,[13] Marina Paul,[12] Laura C. Paulsen,[13] Judy Qiu,[13] Alex Rhee,[13] Matthew G. Rubashkin,[13] Ina Y. Soh,[12] Nathaniel E. Sotuyo,[12] Venkatesh Srinivas,[13] Allison Suarez,[13] Andy Wong,[13] Remus Wong,[13] Wei Rose Xie,[12] Yijie Xu,[13] Allen T. Yu,[12] Romain Koszul,[3,4] Joel S. Bader,[2,6] Jef D. Boeke,[2,11,5]† Srinivasan Chandrasegaran[1]†

www.sciencemag.org SCIENCE VOL 344 4 APRIL 2014

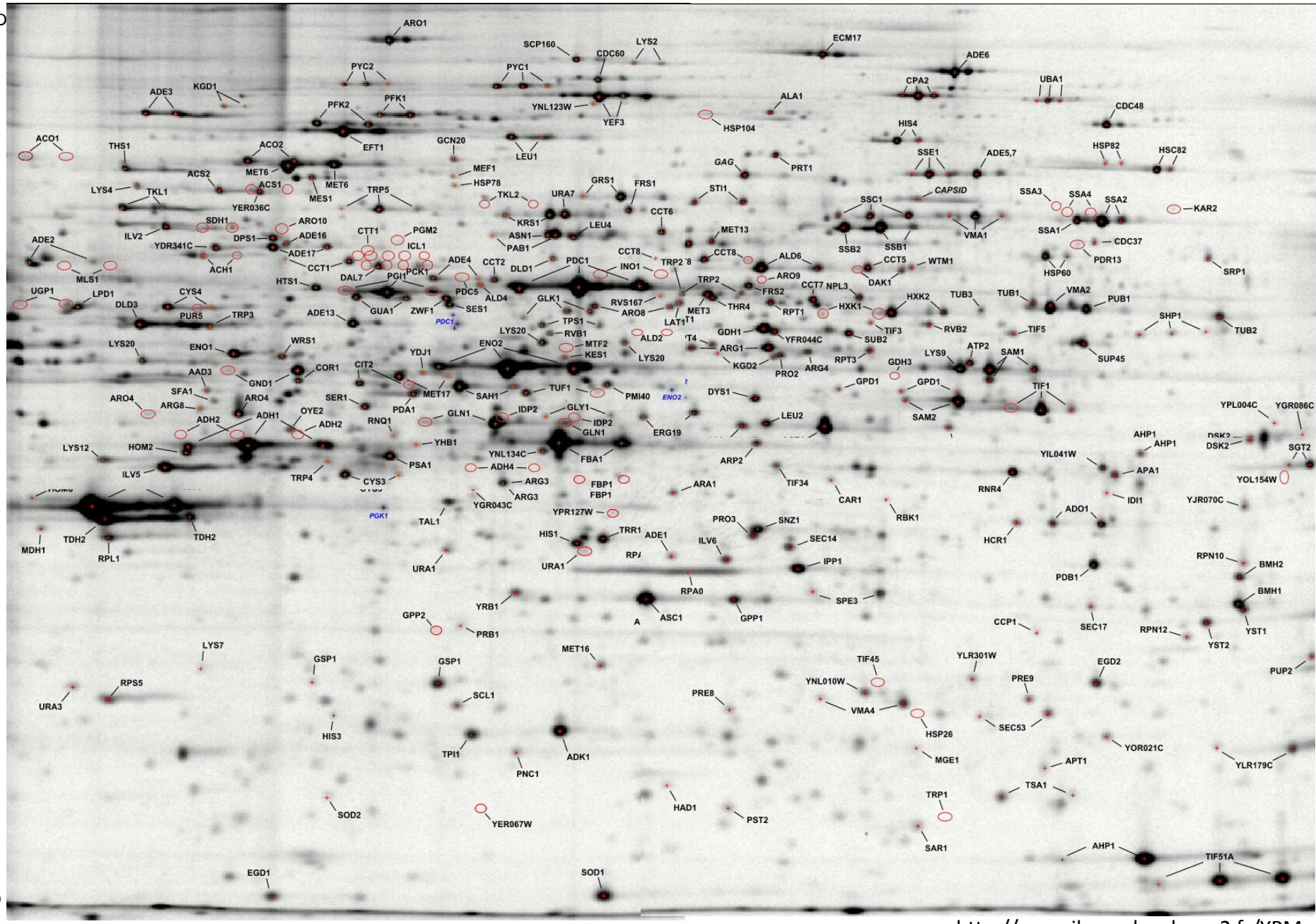
Gibson, D. G., J. I. Glass, C. Lartigue, V. N. Noskov, R.-Y. Chuang, M. A. Algire, G. A. Benders, M. G. Montague, L. Ma, M. M. Moodie, C. Merryman, S. Vashee, R. Krishnakumar, N. Assad-Garcia, C. Andrews-Pfannkoch, E. A. Denisova, L. Young, Z.-Q. Qi, T. H. Segall-Shapiro, C. H. Calvey, P. P. Parmar, C. A. Hutchison III, H. O. Smith, and J. C. Venter. 2010. Creation of a bacterial cell controlled by a chemically synthesized genome. Science, Published online May 20 2010.

J. Craig Venter™ INSTITUTE

6,8 **pI** 4,2

180kD

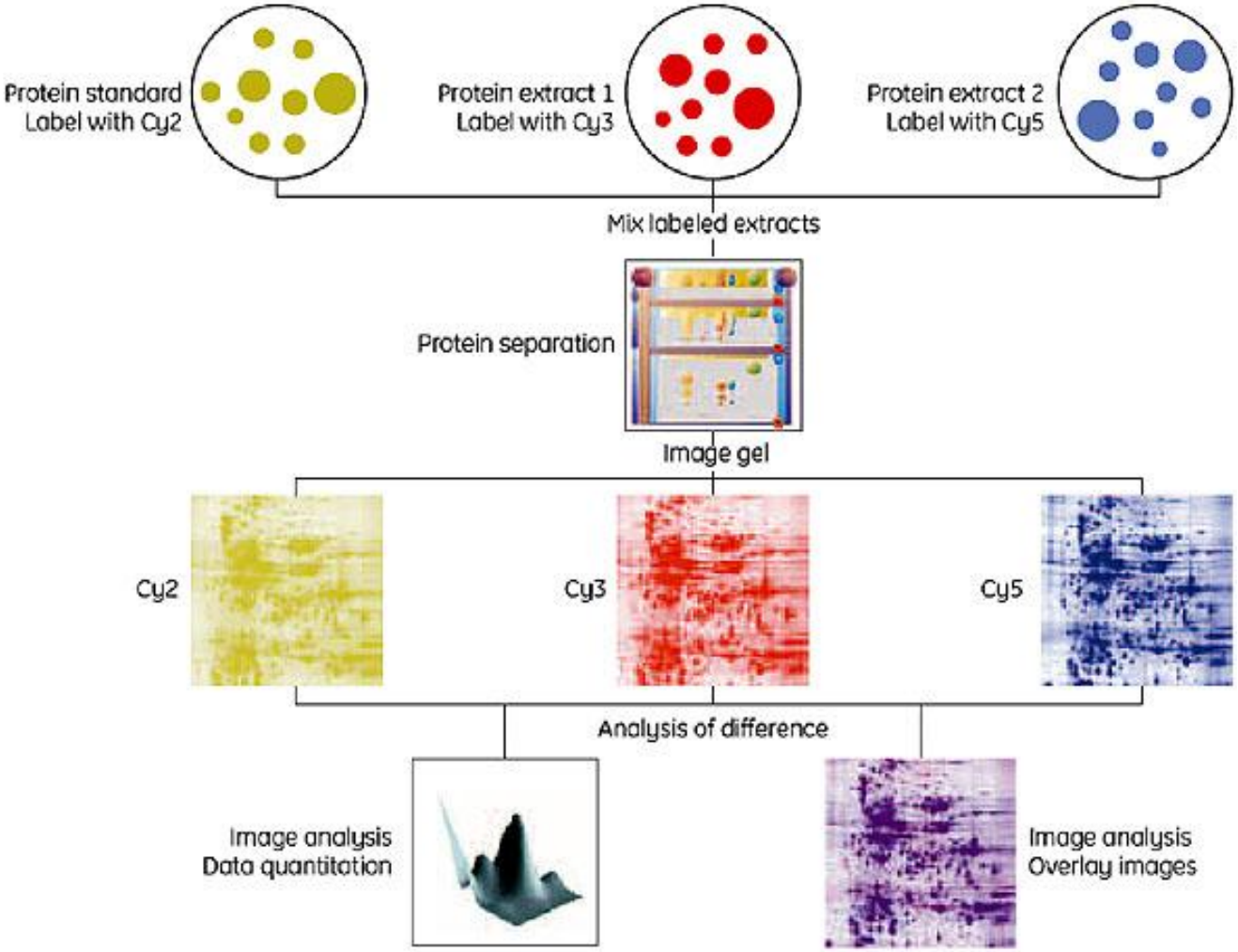
Mw



15kD

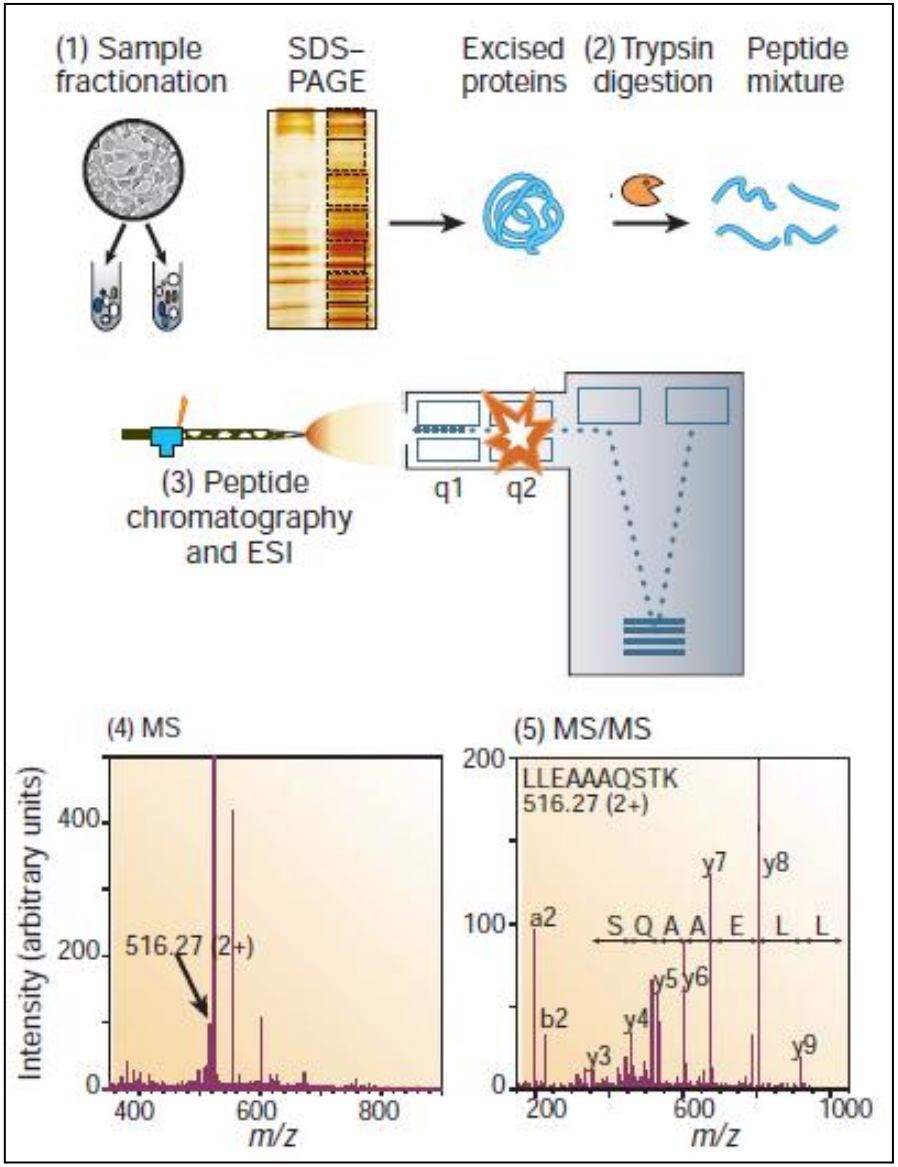
http://www.ibgc.u-bordeaux2.fr/YPM

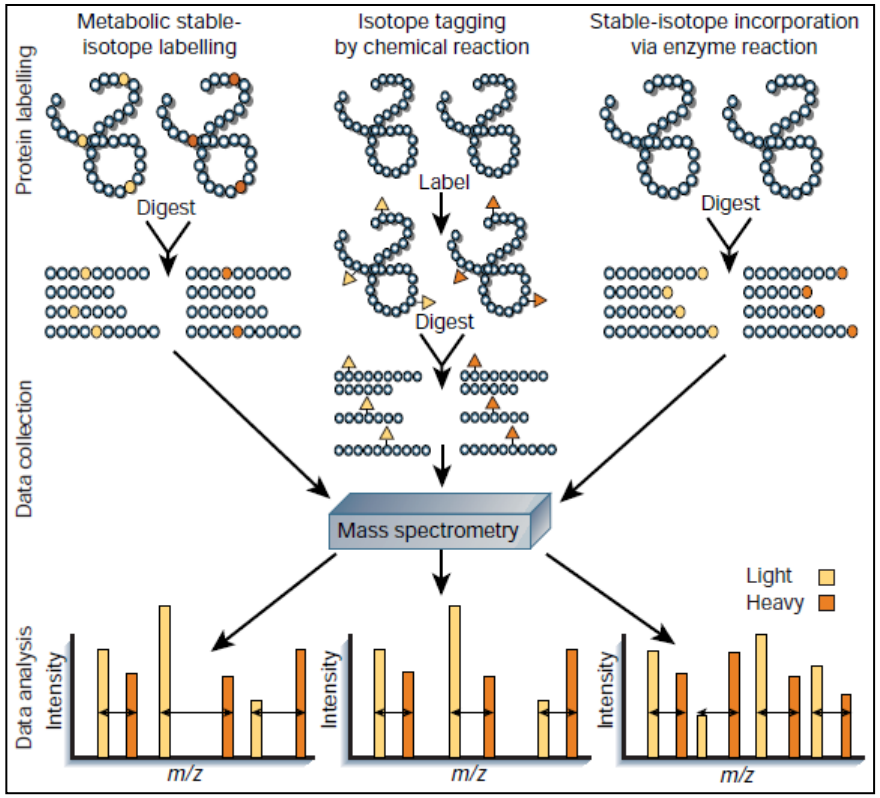
Merjenje razlik v izražanju proteinov: ‘**Di**fference **G**el **E**lectrophoresis (DiGE)’



2-D Electrophoresis. Principles and Methods; GE Healthcare

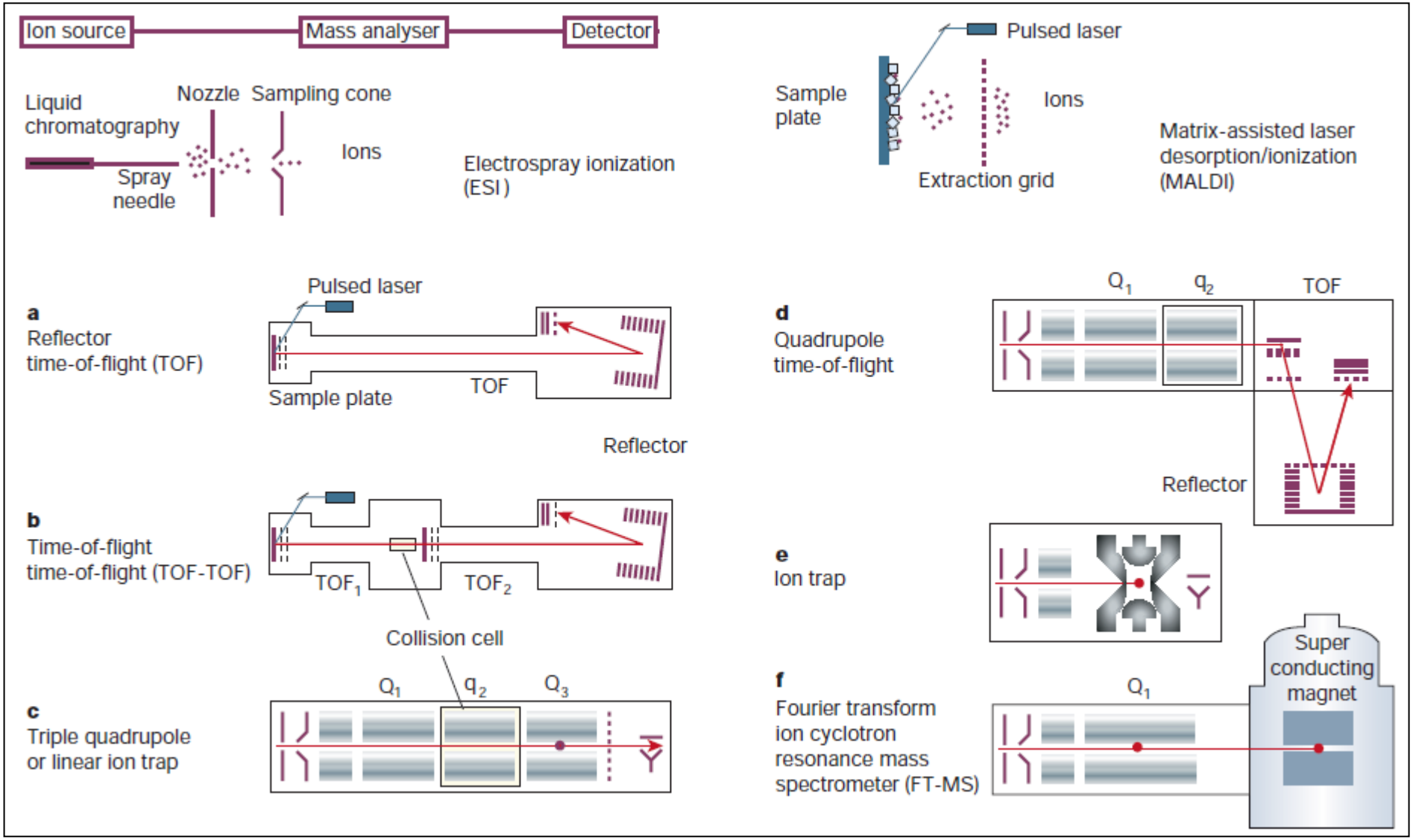
Masna spektromerija v proteomiki





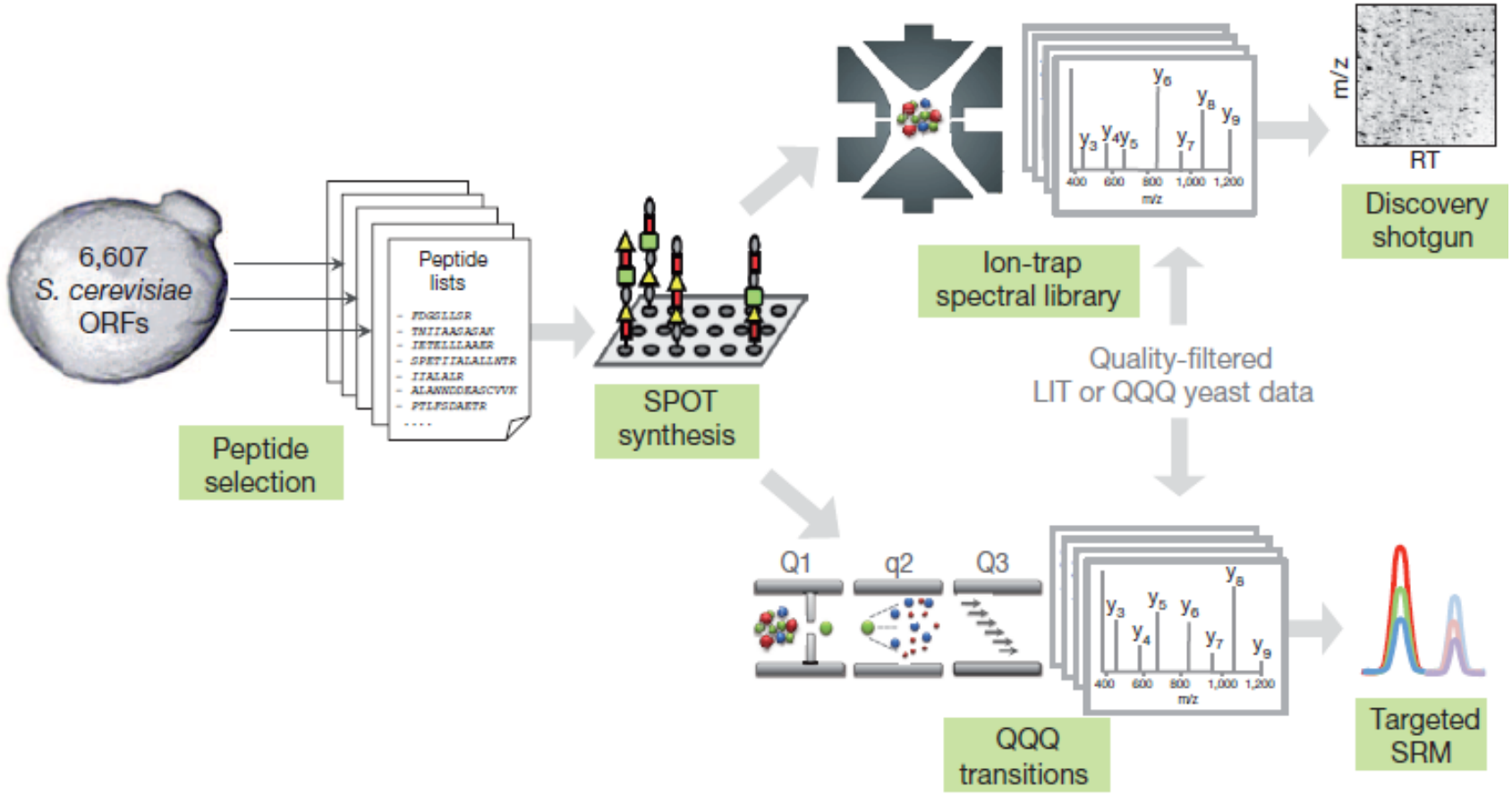
Nature (2003) 422:198

Masna spektromerija v proteomiki



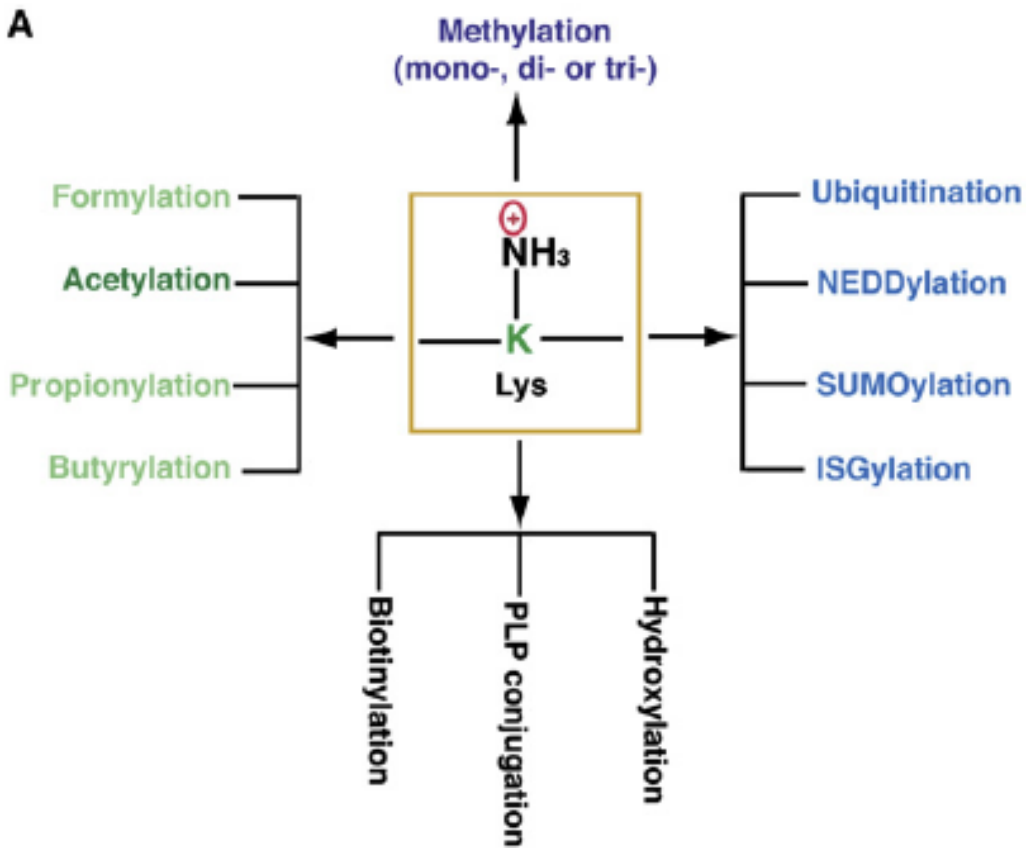
Nature (2003) 422:198

Proteom kot kvantitivna lastnost (fenotip)



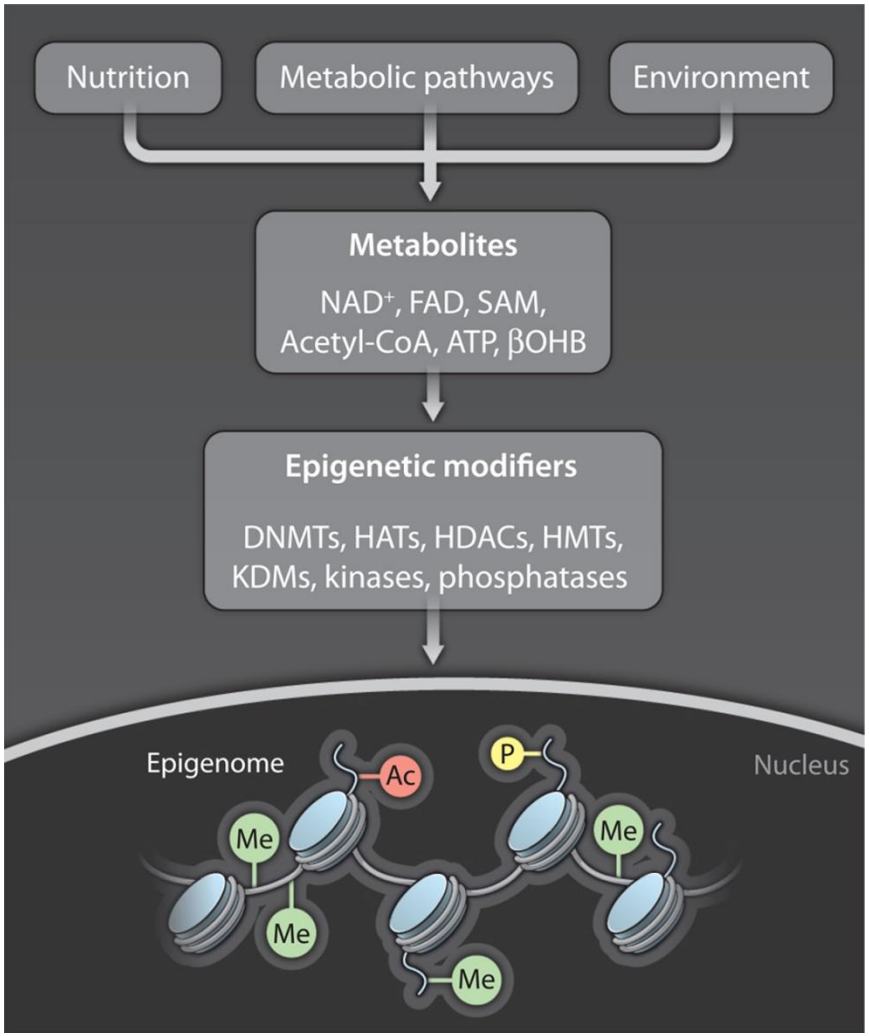
Nature (2013) 494:266

Post-translacijske modifikacije proteinov

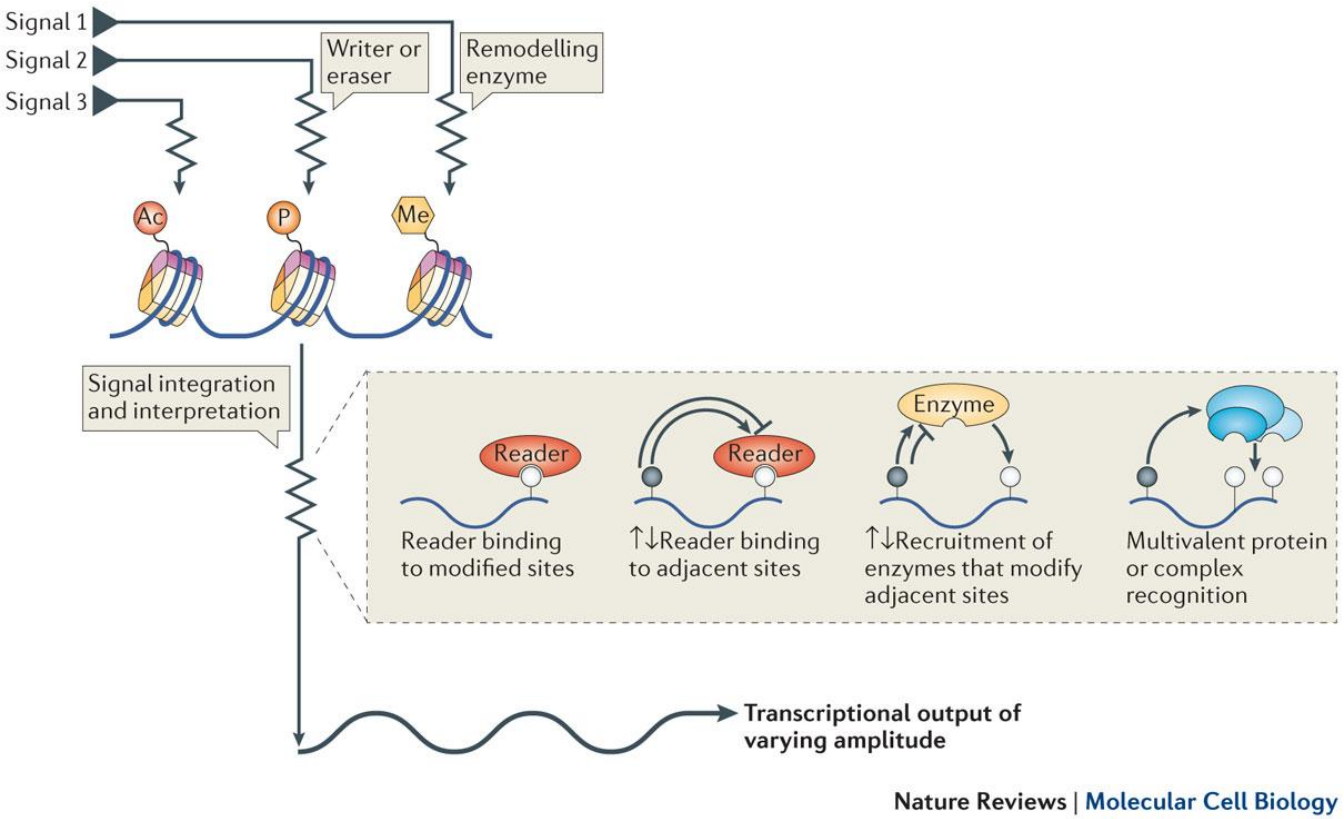


Molecular Cell (2008) 31:449

A plastic epigenome. DNA and histones are targets of multiple modifications that convey flexibility to the genome.

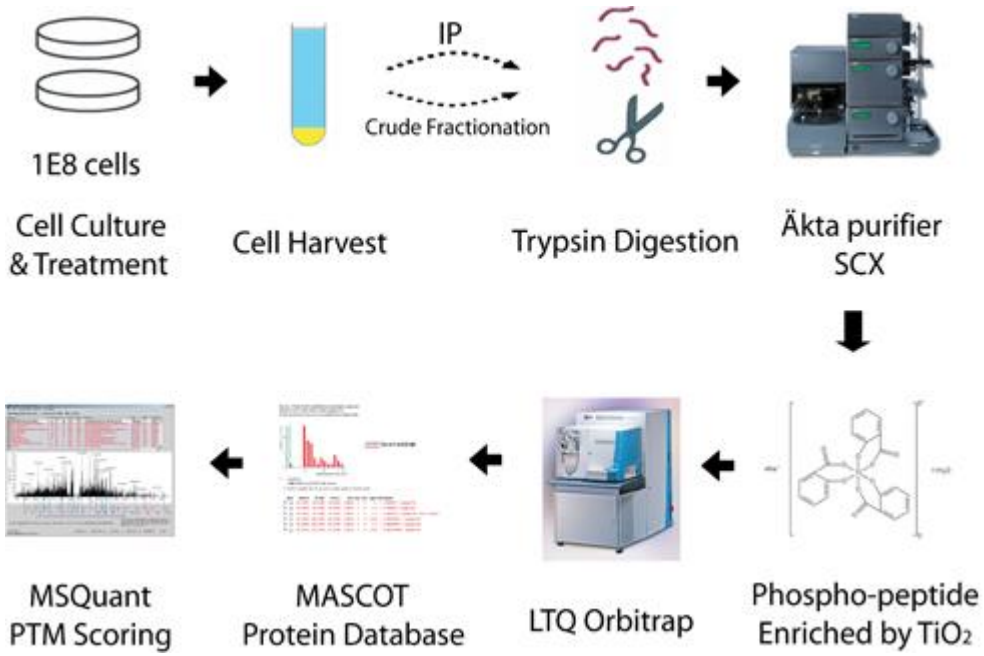


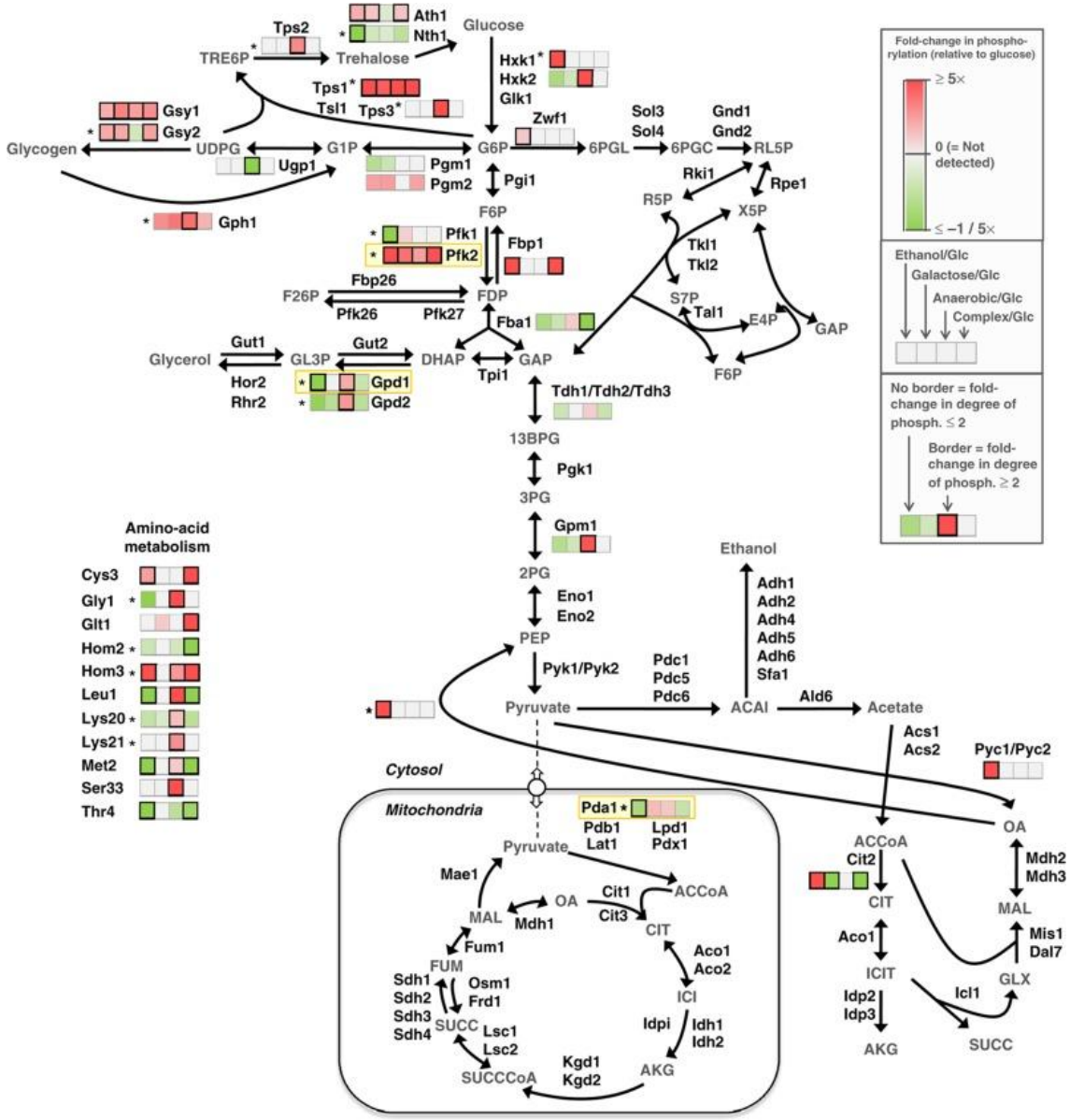
P Sassone-Corsi Science 2013;339:148-150

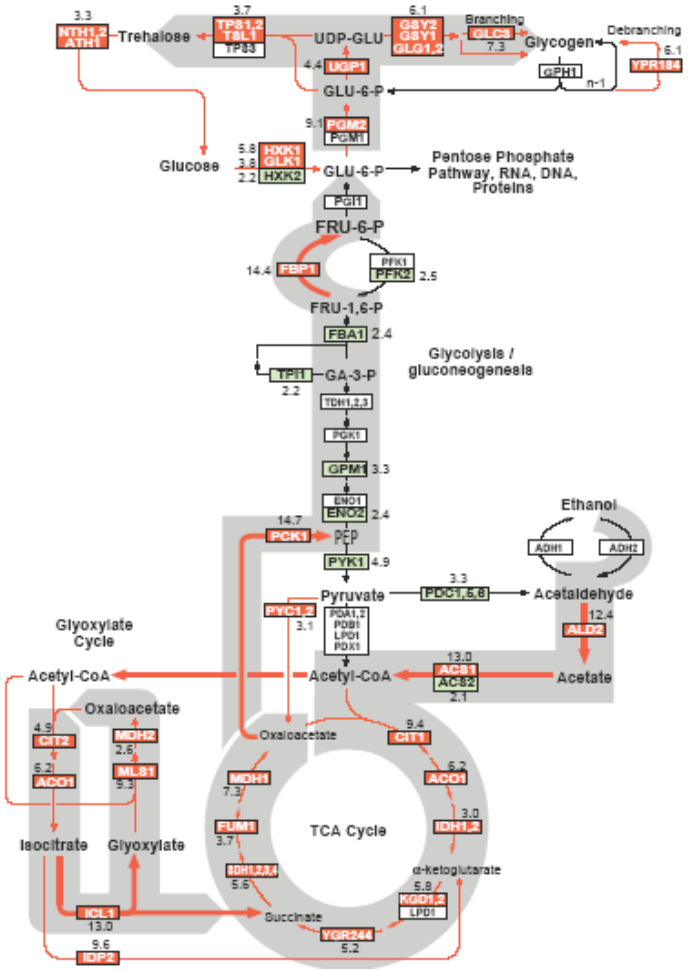


Nature Reviews Mol Cell Biol (2013) 14:211

Primer analize ‘fosfoproteoma’



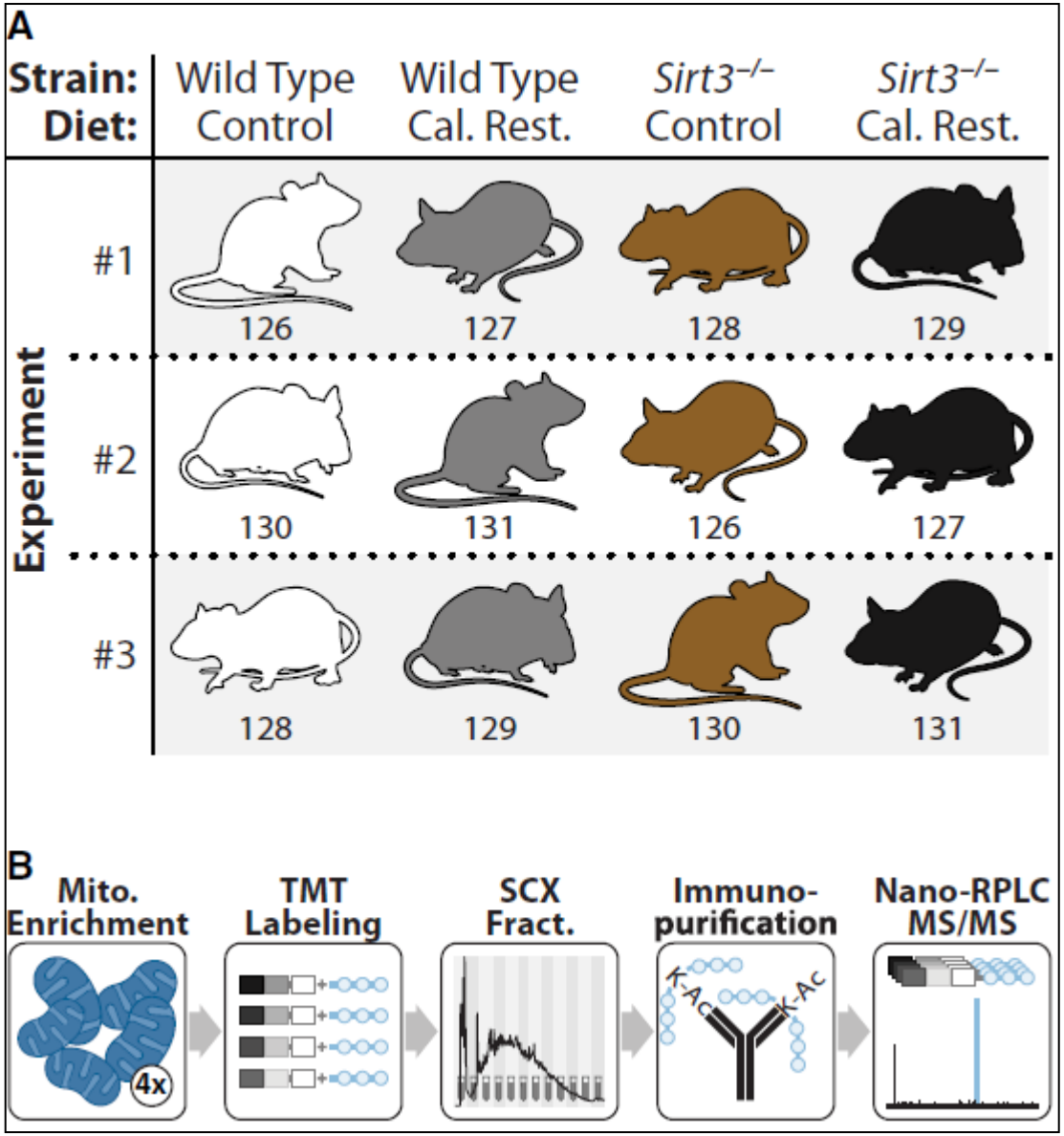


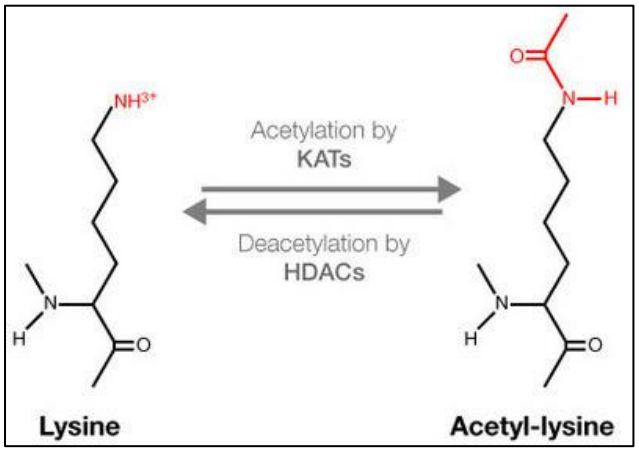


Science (1997) 278:680

Molecular Systems Biology (2012) 8:623

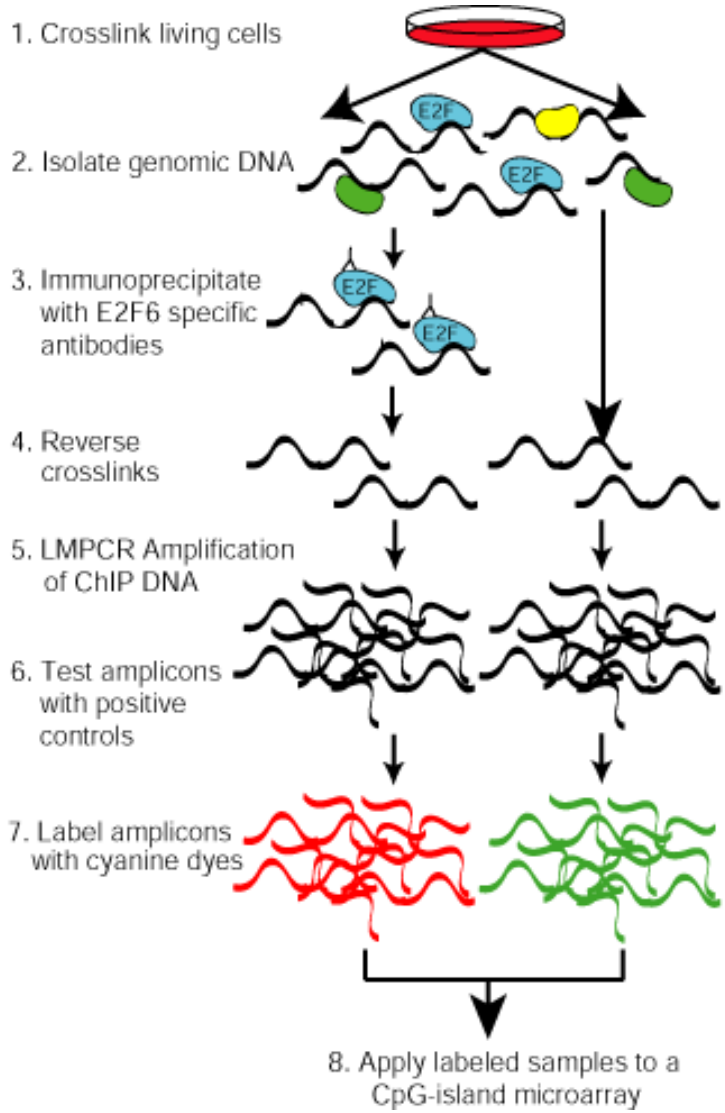
Acetilacija proteinov / ‘acetilom’





Molecular Cell (2013) 49:1

Interakcije protein-DNA (ChIP-chip, ChIP-seq)

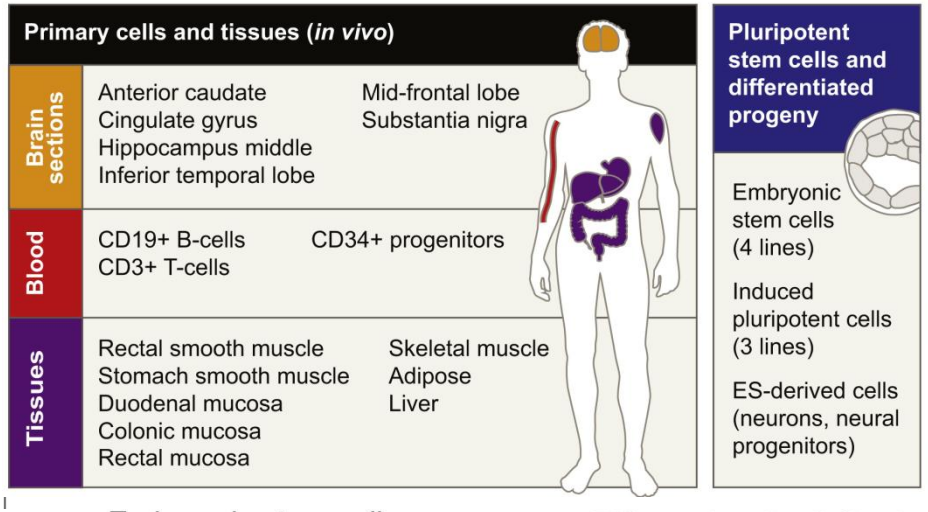


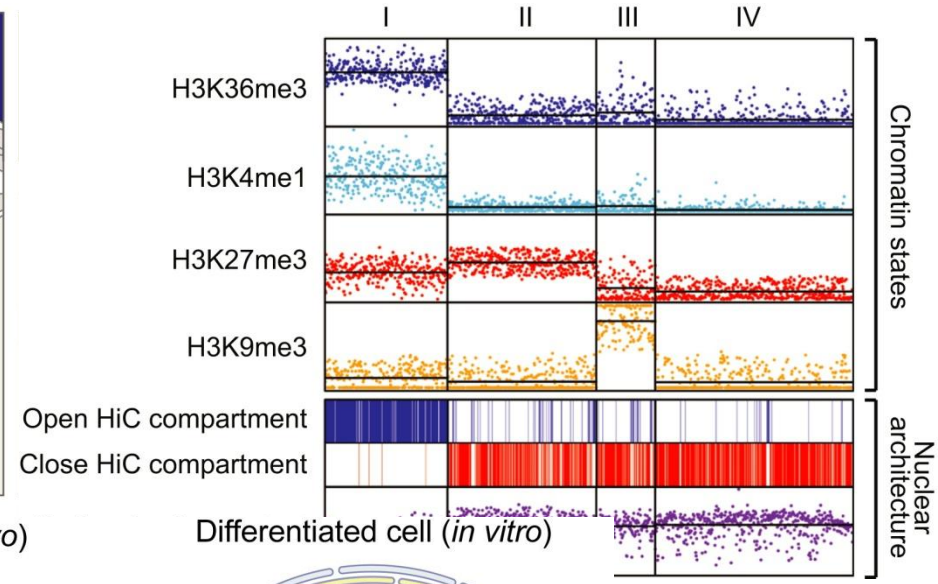
ENCODE - NGS in proteomika

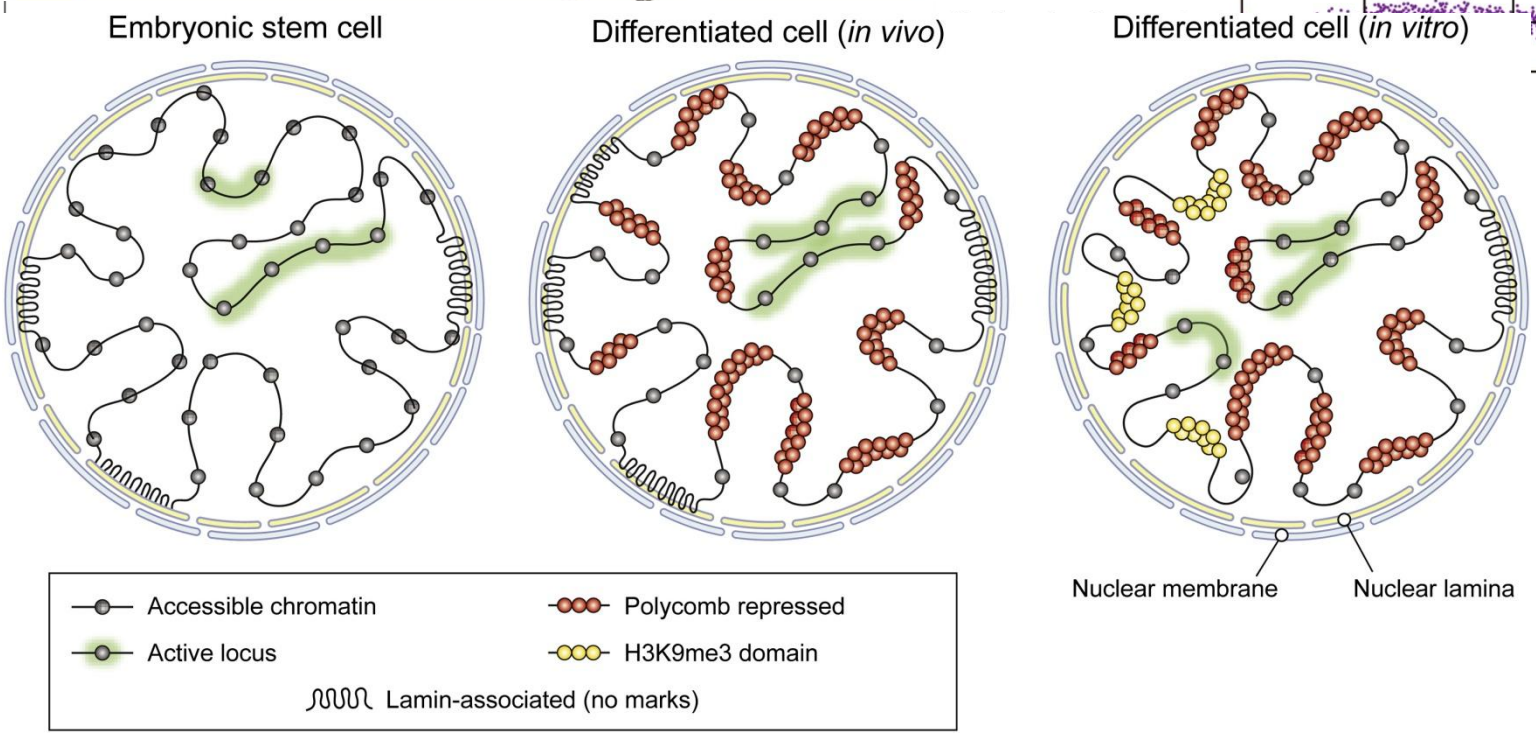
Table 1 The various NGS assays employed in the ENCODE project to annotate the human genome

Feature	Method	Description	Reference
Transcripts, small RNA and transcribed regions	RNA-seq	Isolate RNA followed by HT sequencing	(Waern *et al*, 2011)
	CAGE	HT sequencing of 5'-methylated RNA	(Kodzius *et al*, 2006)
	RNA-PET	CAGE combined with HT sequencing of poly-A tail	(Fullwood *et al*, 2009c)
	ChIRP-Seq	Antibody-based pull down of DNA bound to lncRNAs followed by HT sequencing	(Chu *et al*, 2011)
	GRO-Seq	HT sequencing of bromouridinated RNA to identify transcriptionally engaged PolII and determine direction of transcription	(Core *et al*, 2008)
	NET-seq	Deep sequencing of 3′ ends of nascent transcripts associated with RNA polymerase, to monitor transcription at nucleotide resolution	(Churchman and Weissman, 2011)
	Ribo-Seq	Quantification of ribosome-bound regions revealed uORFs and non-ATG codons	(Ingolia *et al*, 2009)
Transcriptional machinery and protein–DNA interactions	ChIP-seq	Antibody-based pull down of DNA bound to protein followed by HT sequencing	(Robertson *et al*, 2007)
	DNAse footprinting	HT sequencing of regions protected from DNAse1 by presence of proteins on the DNA	(Hesselberth *et al*, 2009)
	DNAse-seq	HT sequencing of hypersensitive non-methylated regions cut by DNAse1	(Crawford *et al*, 2006)
	FAIRE	Open regions of chromatin that is sensitive to formaldehyde is isolated and sequenced	(Giresi *et al*, 2007)
	Histone modification	ChIP-seq to identify various methylation marks	(Wang *et al*, 2009a)
DNA methylation	RRBS	Bisulfite treatment creates C to U modification that is a marker for methylation	(Smith *et al*, 2009)
Chromosome-interacting sites	5C	HT sequencing of ligated chromosomal regions	(Dostie *et al*, 2006)
	ChIA-PET	Chromatin-IP of formaldehyde cross-linked chromosomal regions, followed by HT sequencing	(Fullwood *et al*, 2009a)

Molecular Systems Biology (2013) 9:640



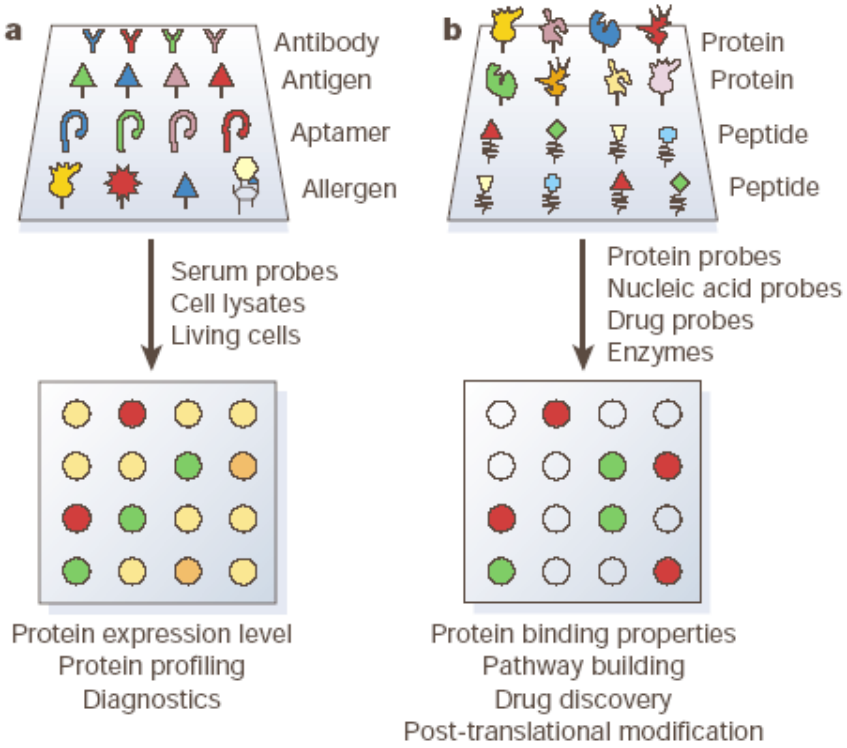




Cell (2013) 152:642

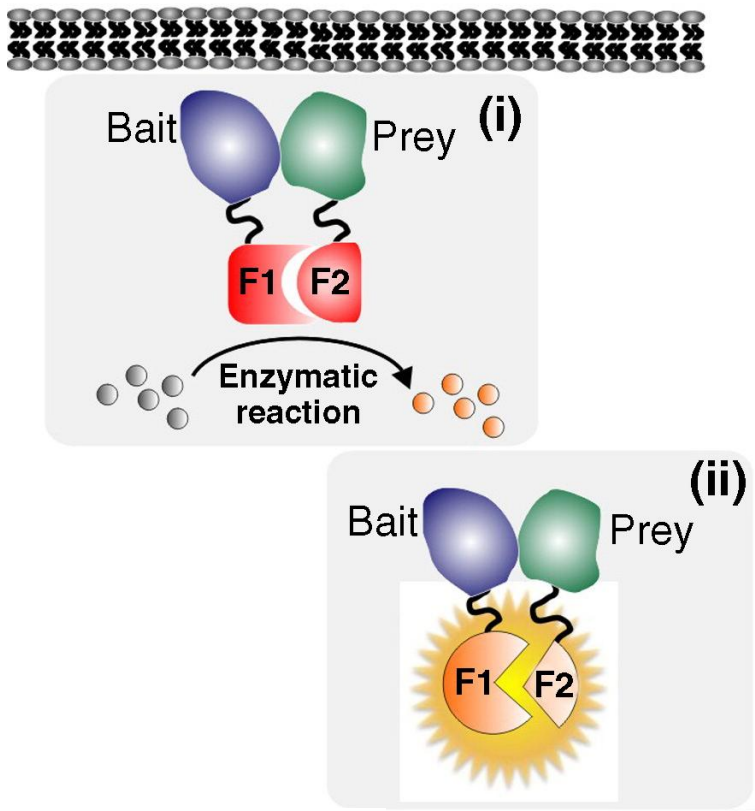
Določanje prisotnosti/količine : določanje funkcije

- imunoprecipitacija s pomočjo TAP označevalca/MS (proteinski kompleksi)
- dvohibridni sistem kvasovke (Y2H, protein-protein fizične interakcije)
- proteinske mikromreže

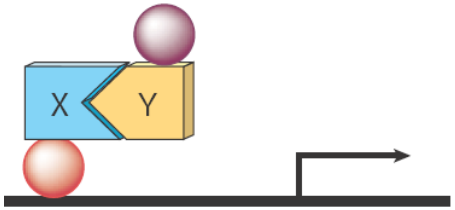


Nature (2003) 422:208

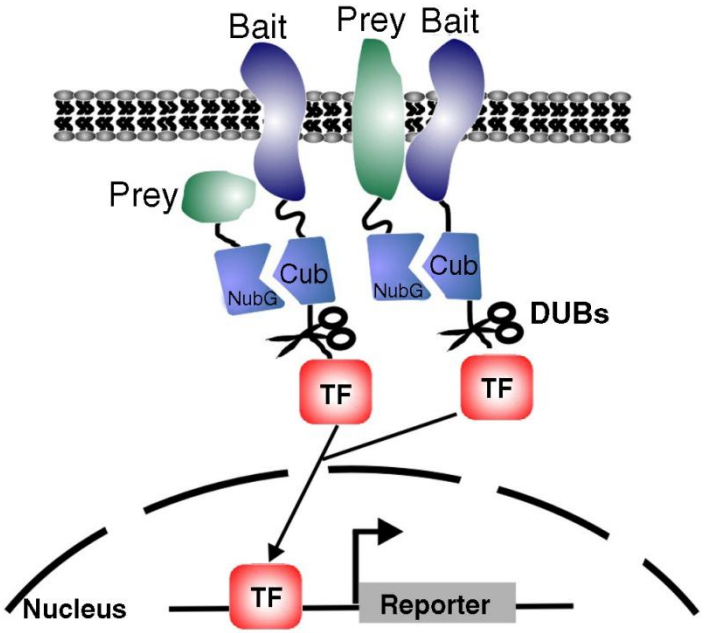
Test s komplementacijo fragmentov proteina
(Protein-fragment complementation assay; PCA)

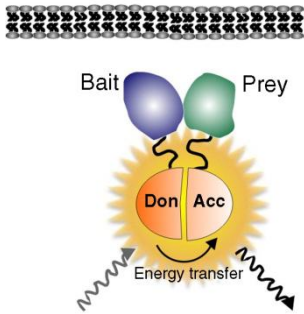


Kvasni dvohibridni sistem



Membranski kvasni dvohibridni sistem

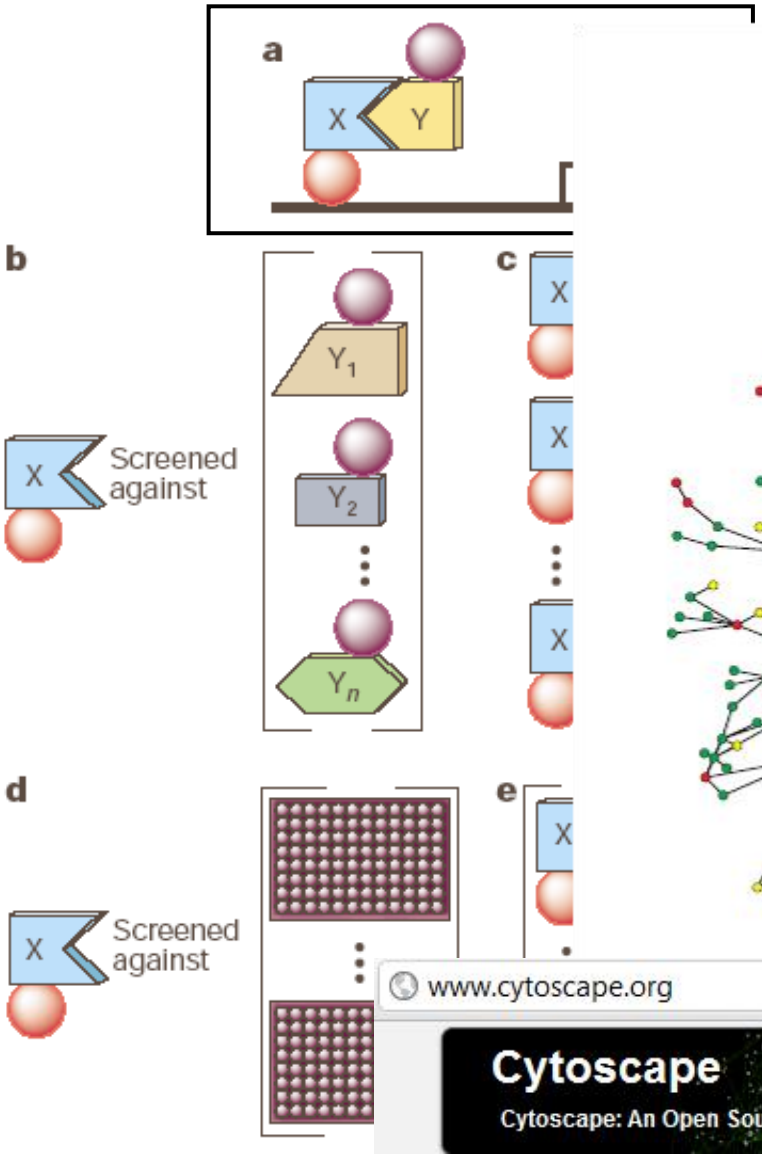


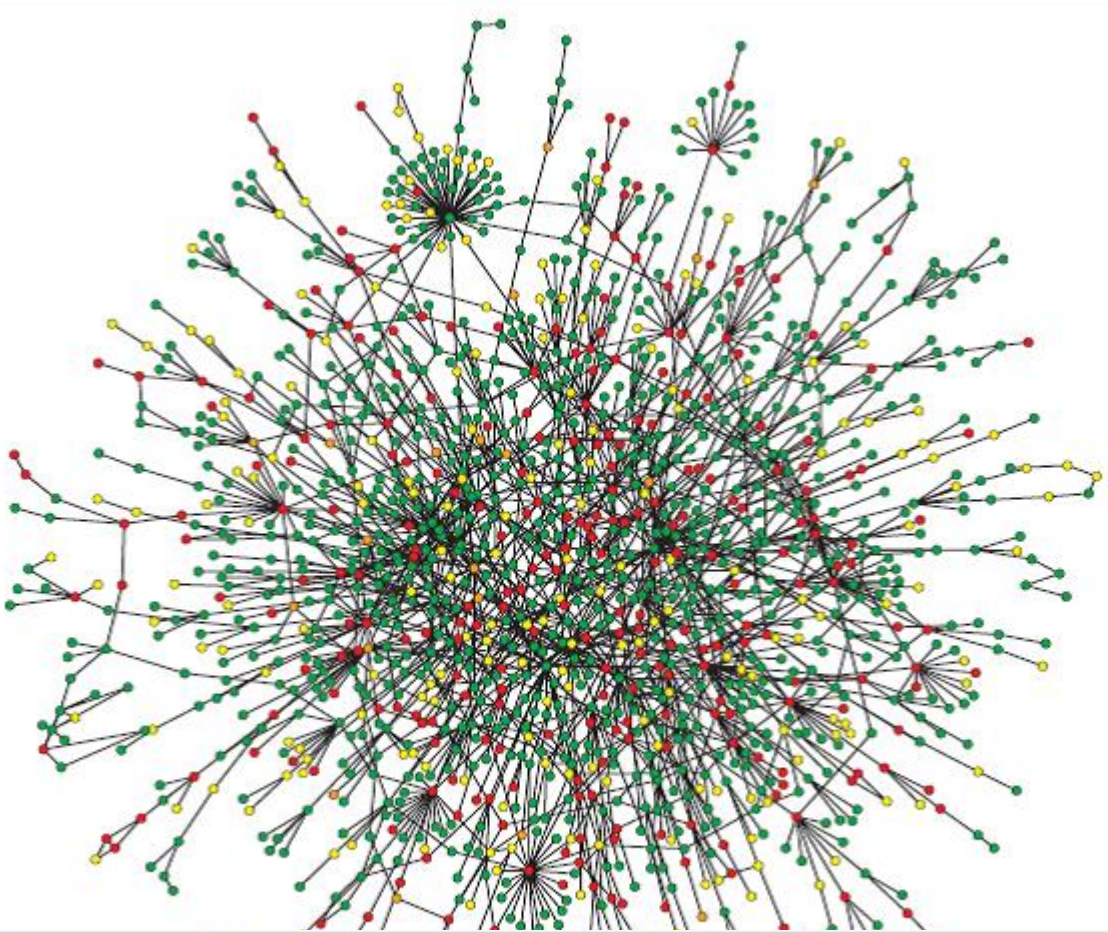


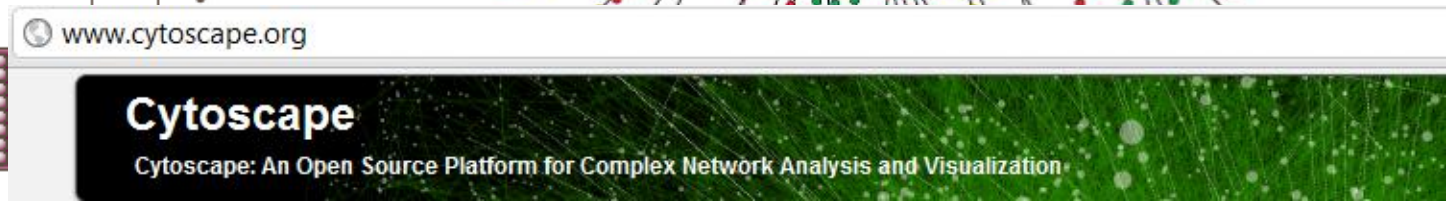
FRET/BRET
(Fluorescence/bioluminescence resonance-energy transfer)

Curr Opinion Biotech (2011) 22:50

Strategije Y2H

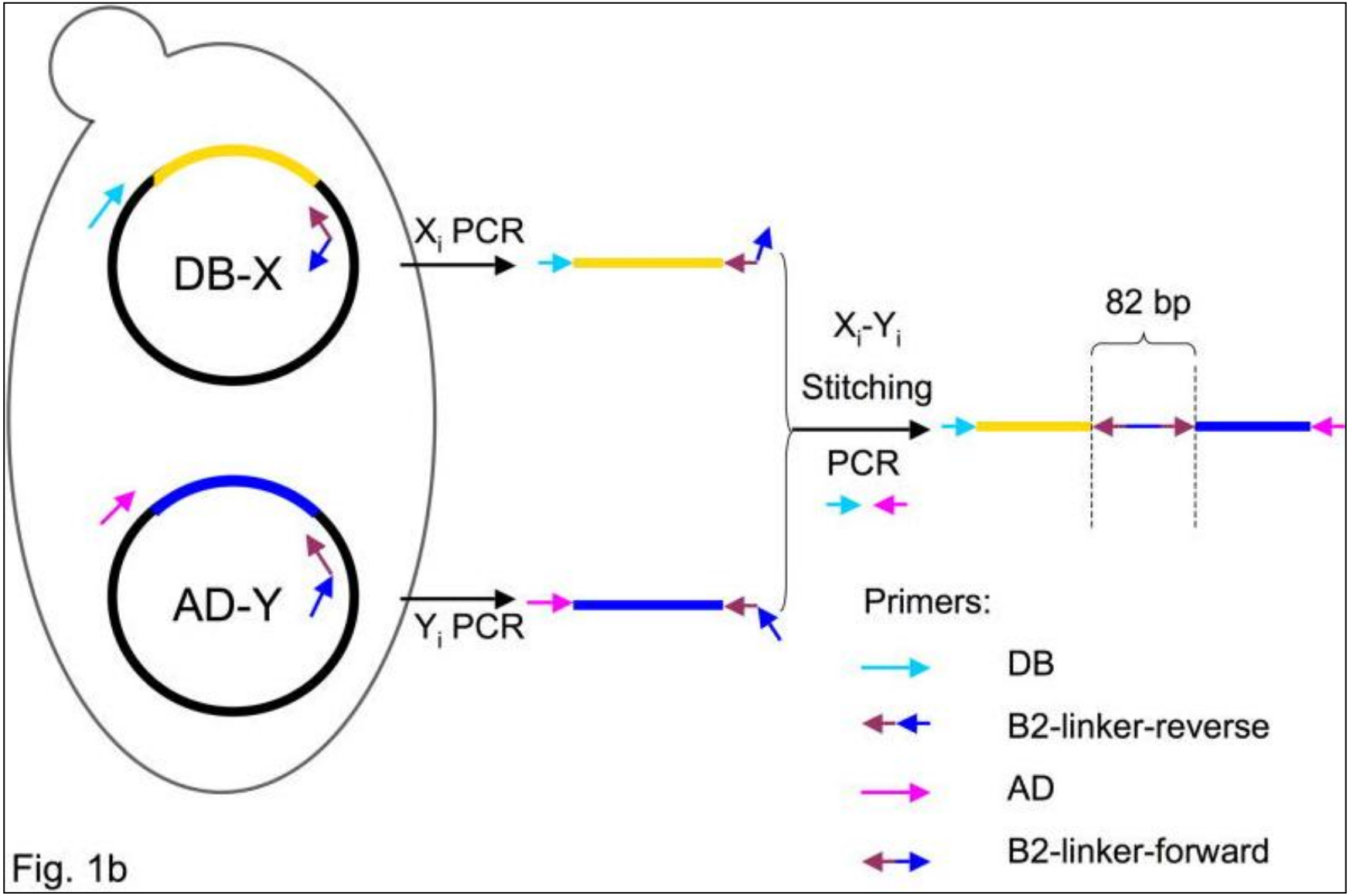




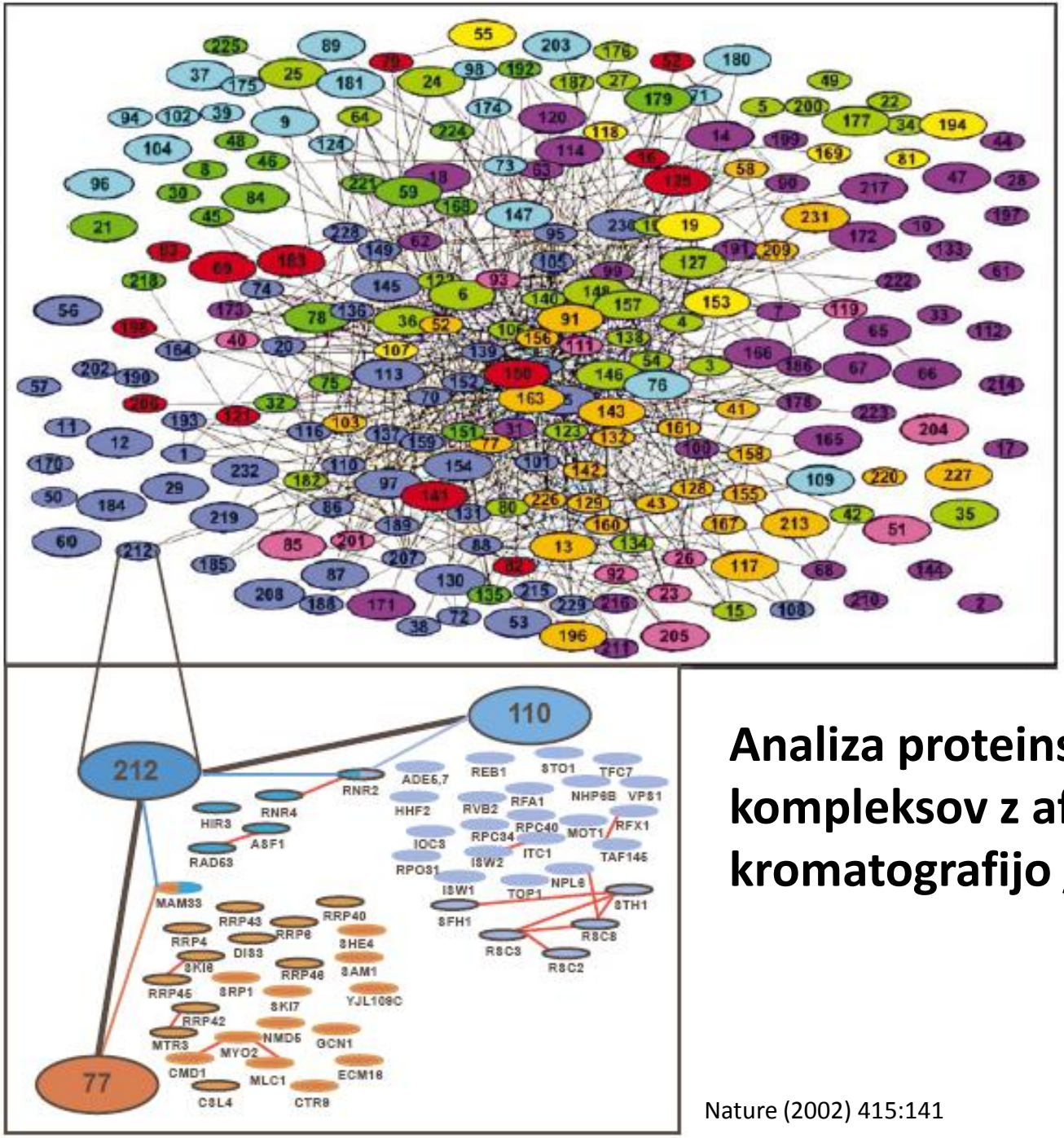


Nature (2003) 422:208

Y2H & NGS



Nature Methods (2011) 8:478



Analiza proteinskih kompleksov z afinitetno kromatografijo / MS

Nature (2002) 415:141

Yeast GFP Fusion Localization Database

yeastgfp.yeastgenome.org

CDC19 YAL038W

molecules/cell: 291000 ±24100

loc cytoplasm

comments

add

order

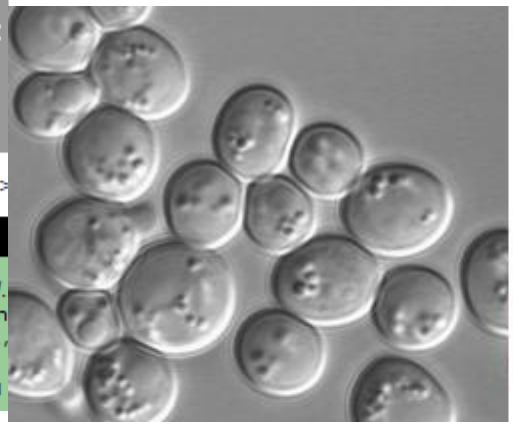
GFP

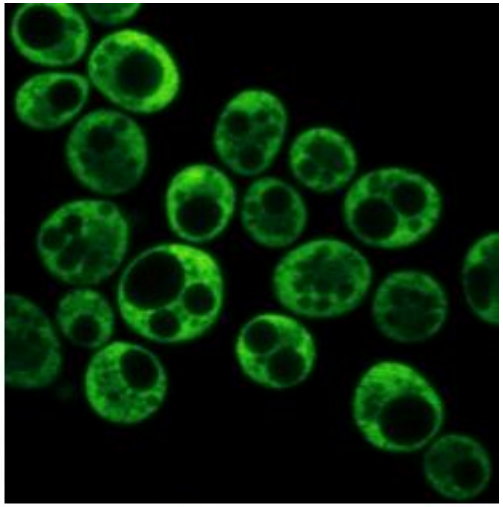
TAP

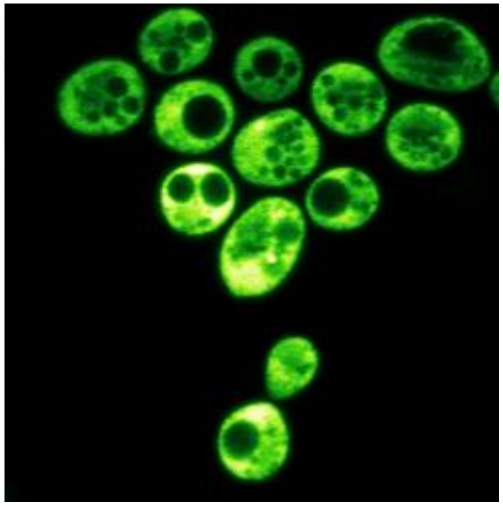
INITIAL

COLOC

FINAL





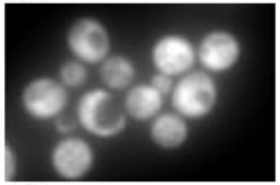
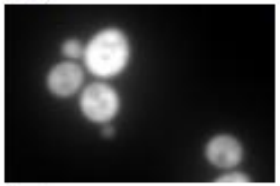
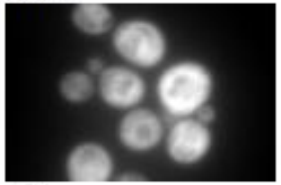
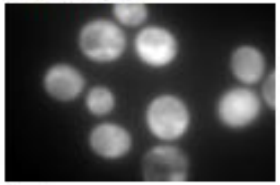


the localization data presented here is published in Huh, *et al.*
the quantitation data presented here is published in Ghaemmagham
detailed collection construction methods can be found in Howson *et al.*,

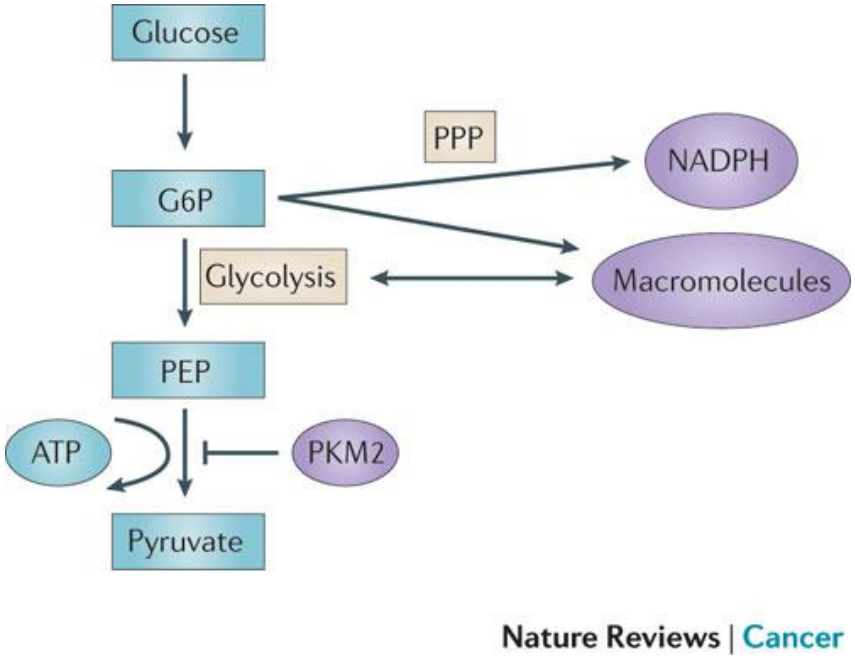
please direct comments, concerns, and questions regarding

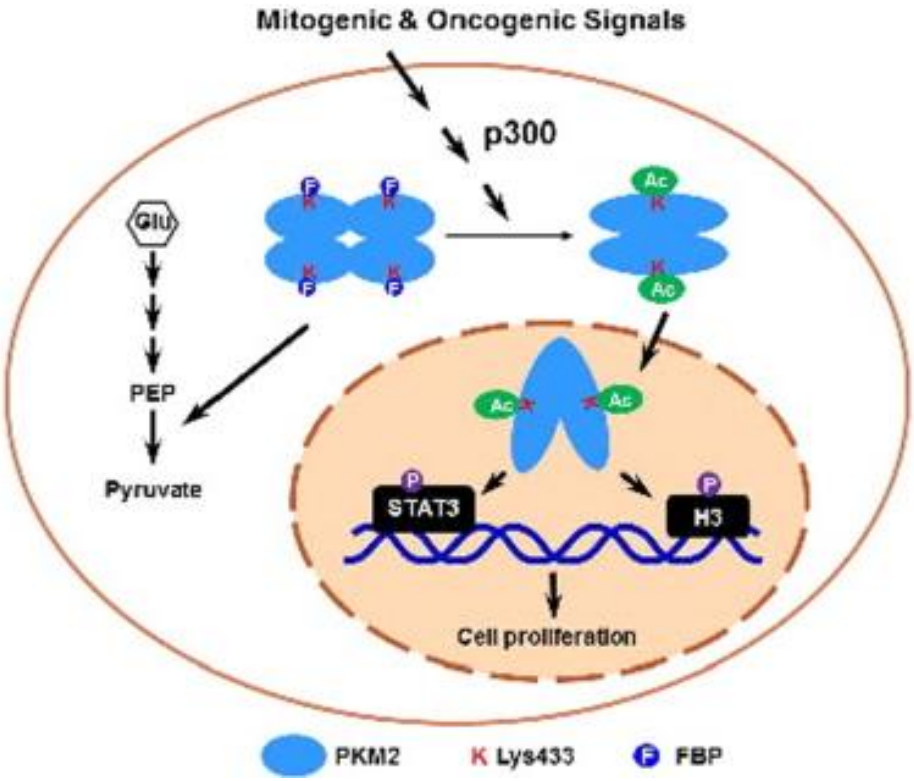
Cdc19-GFP

▸ View description

	SD	DTT	H_2O_2	Starvation
				
	☐ Show in Bright-field	☐ Show in Bright-field	☐ Show in Bright-field	☐ Show in Bright-field
Localization:	Cytosol	Cytosol	Cytosol	Cytosol
Intensity:	1884.27	1738.67	1738.67	1569.63
Fold Change:		0.92	0.92	0.83
Significance:		No	No	No

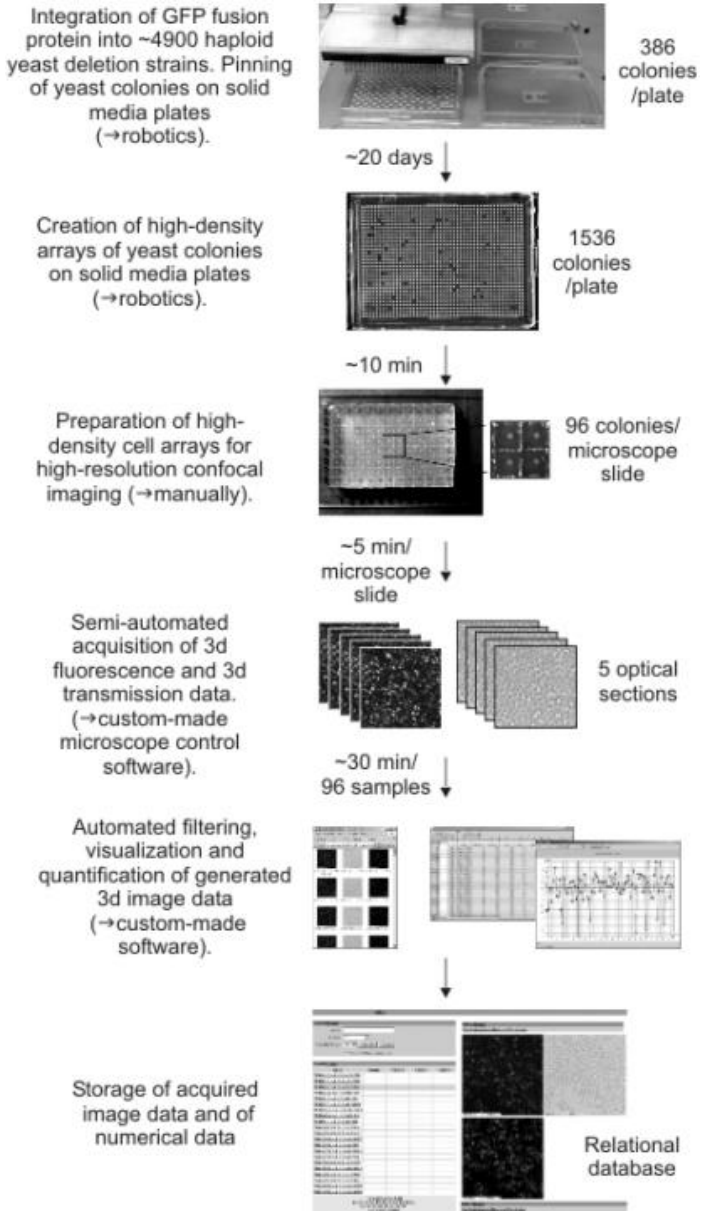
Primer: **Piruvat kinaza – lokalizacija in acetilacija**

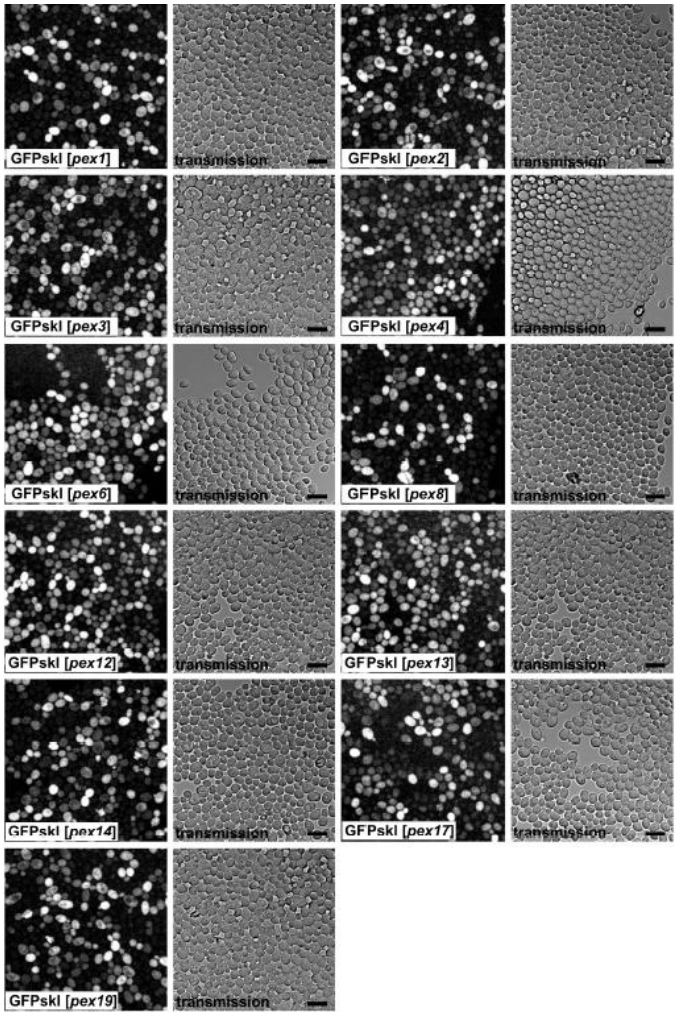


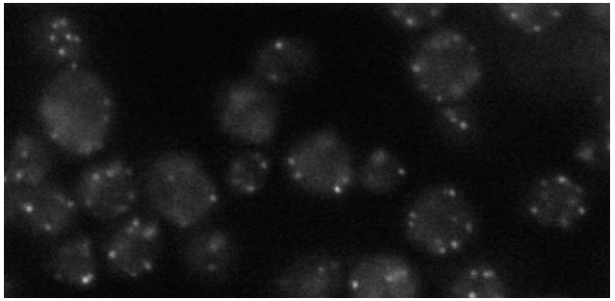


Mol Cell (20013) 52:340

High content microscopy



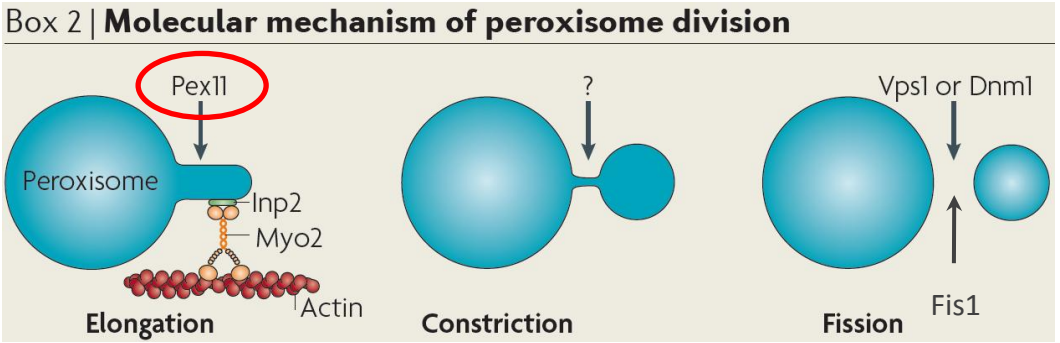




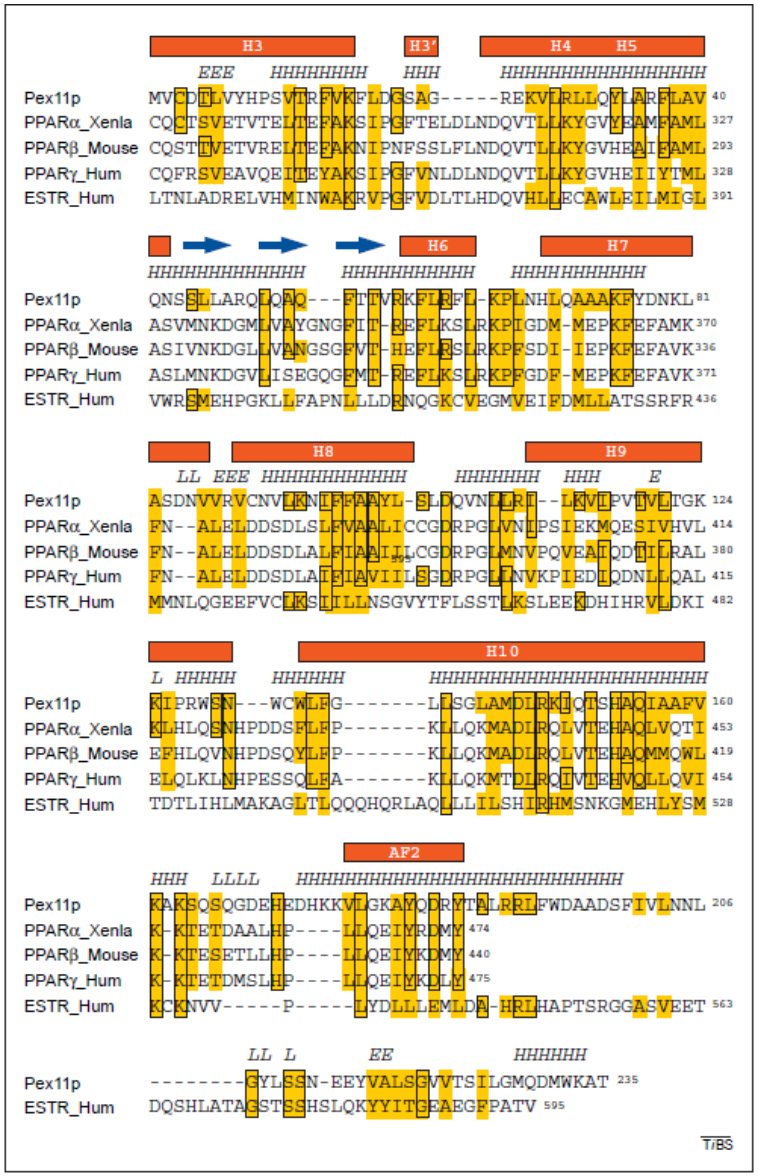
J Proteom Res (2009) 8:20

Pex11 represents an ancestral module from which multidomain nuclear receptors arose

Pex11 is required for peroxisome proliferation



Nature Rev Mol Cell Biol (2010) 11:644



TiBS (2000) 25:227

Pex11

- the most abundant protein in the px membrane
- deletion prevents and over-expression induces px proliferation
- recruits, but does not necessarily binds to, (mt) fission machinery proteins

How is the signal (from Pex11) transmitted?