

# PATHWAYS IN CONTEXT

## EPIGENETIC BUFFERING OF GENOMIC VARIATION

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# Position effect and gene networks

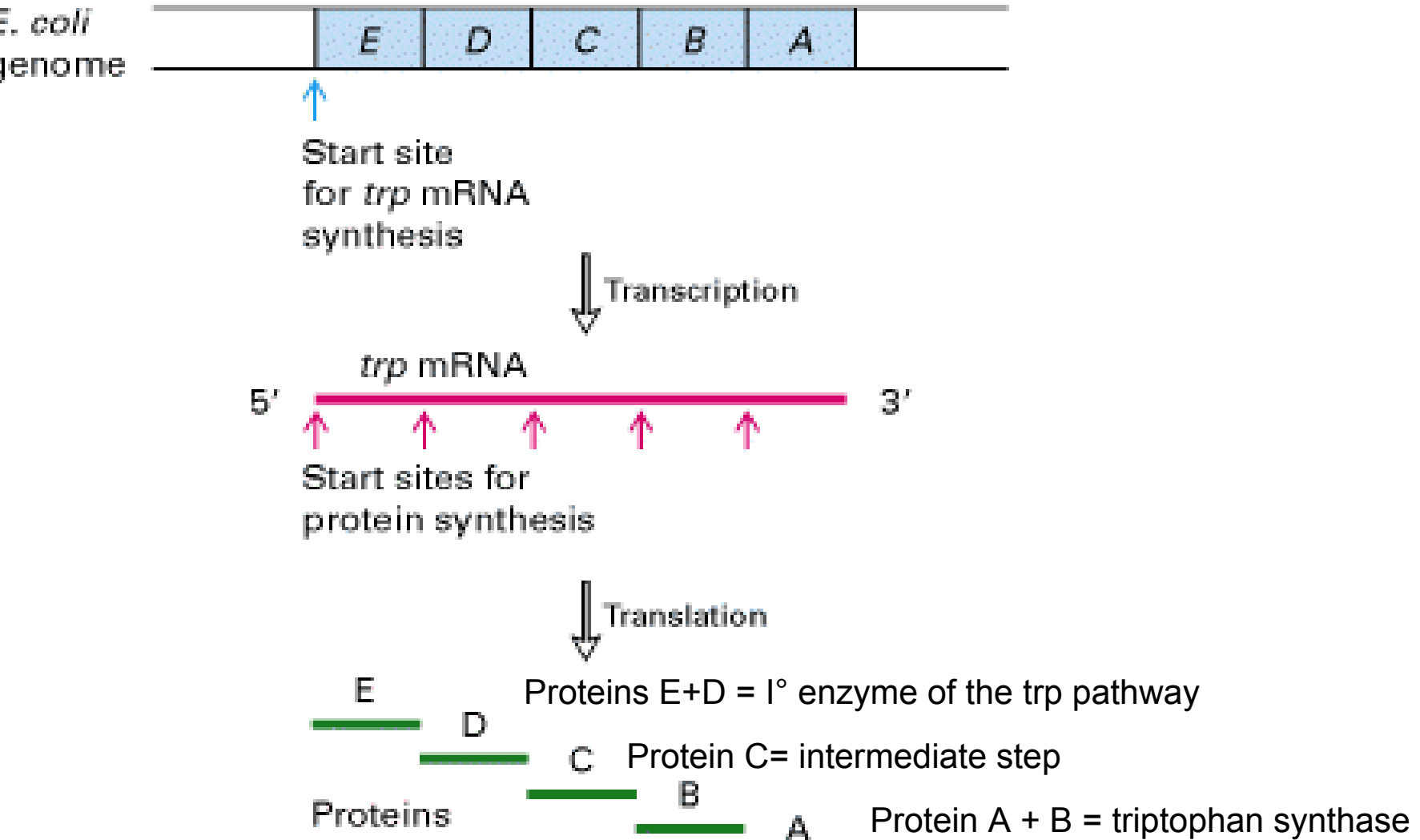
Geography of the genome and co-regulations of the genes

Chromosomal organization and regulation of gene expression.

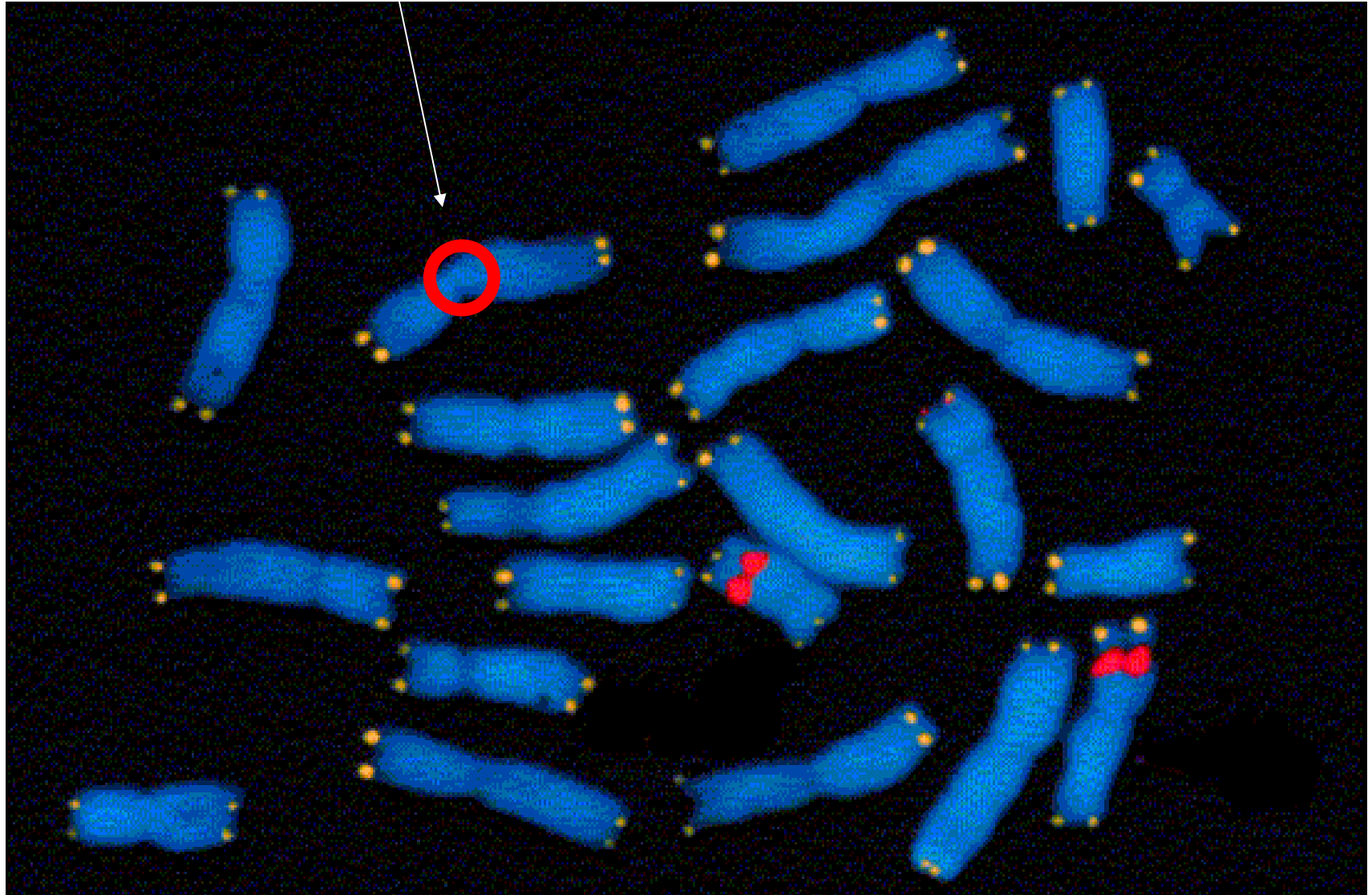
Chromatin structure and regulation of expression

# In bacteria co-regulated genes are organized in operons TRP operon *E. coli*

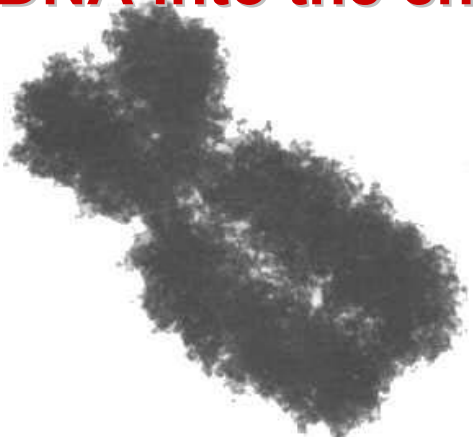
a) Prokaryotes



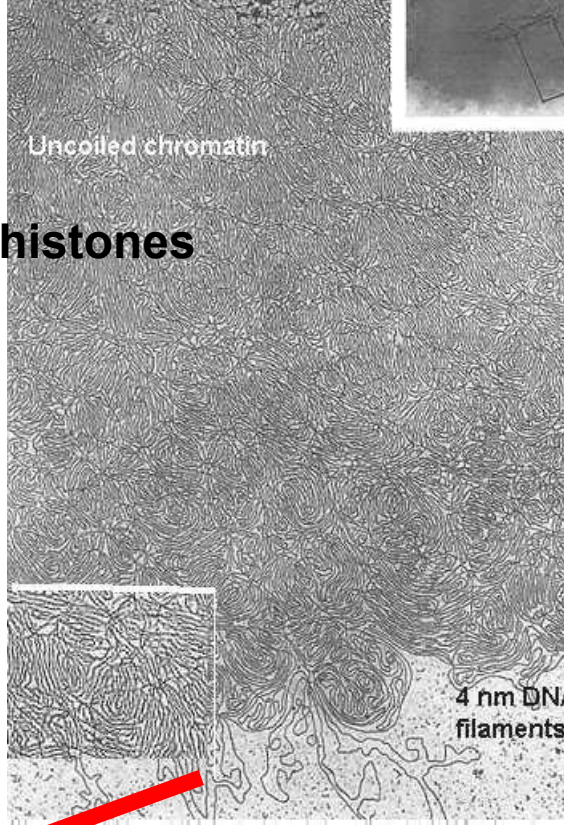
**The possibility for a gene to be expressed depends on its position on the chromosome- i.e. is off at telomeres or at centromeres**



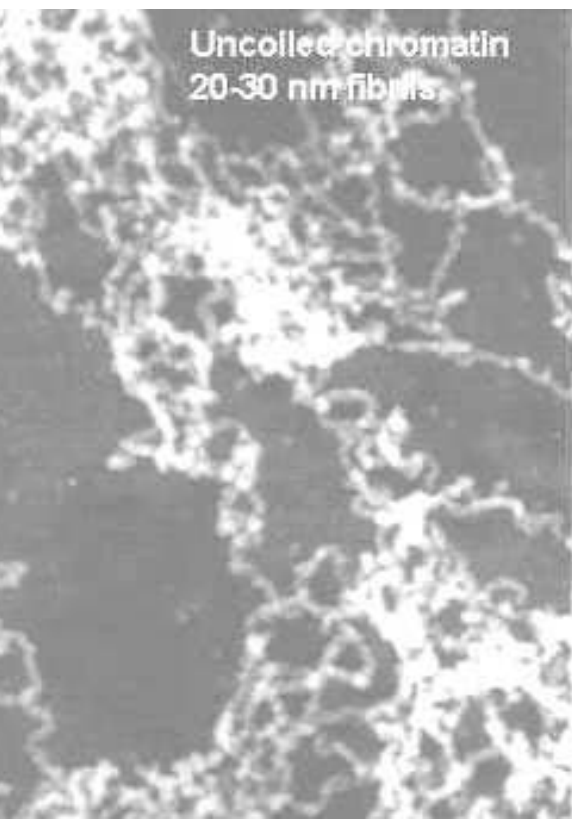
**Chromosomes are organized in histones,  
histones pack DNA into the chromosomes**



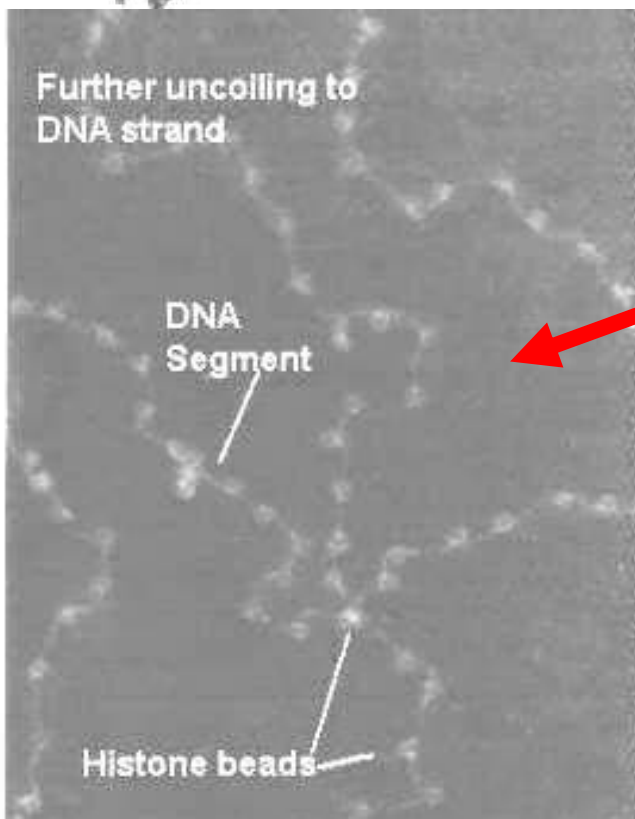
**Eliminating histones**



Uncoiled chromatin



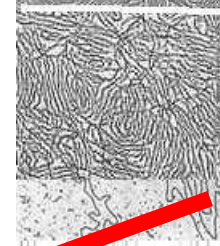
Uncoiled chromatin  
20-30 nm fibrils



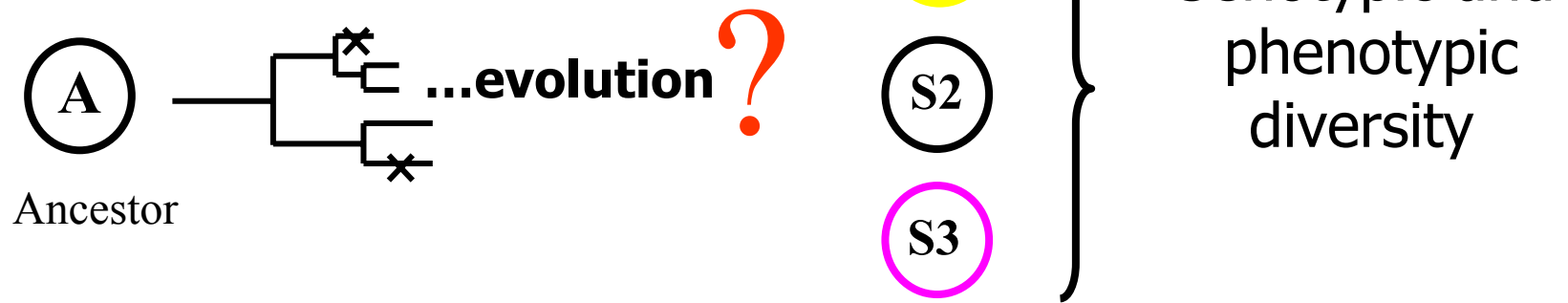
Further uncoiling to  
DNA strand

DNA  
Segment

Histone beads



4 nm DNA  
filaments



## **Genomic approaches to the discovery of the genetic basis of variation in populations**

**Phenotypic diversity is the result of the action of natural selection on genetic variation**

**Genetic variation is the result of mutation in the coding sequence or in the number of copies of the genes.**

**Selection acts on the phenotype**

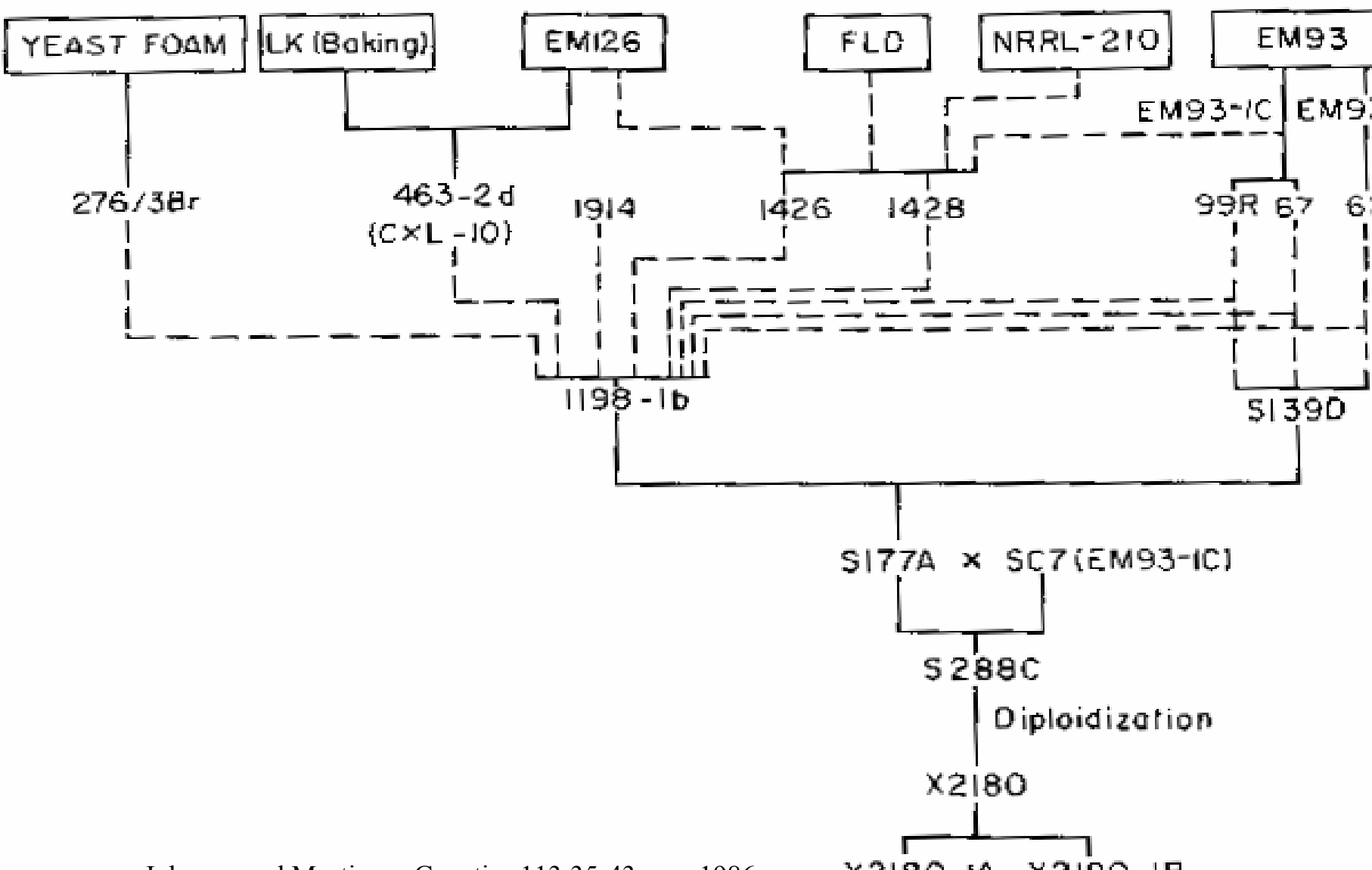
# Questions

- 1. Investigate genetic relatedness of the different strains and the extent at which they differ from the type strain**
- 2. Investigate whether there are parts of the genome that show different variation rates.**
- 3. Associate genetic diversity with Phenotypic diversity in the transcriptome.**

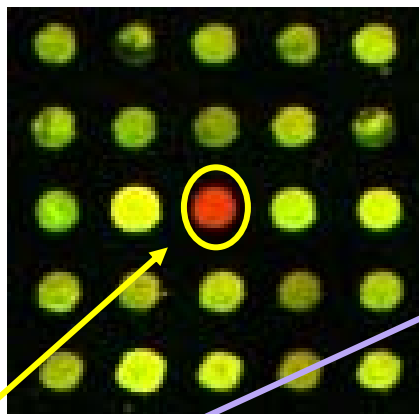
88% of the S288c gene pool derives from EM93, isolated from rotten figs in Mercedes, California by Emil Mrak in 1938.

38

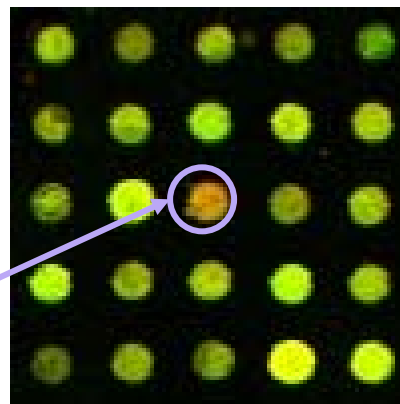
R. K. MORTIMER AND J. R. JOHNSTON



*ORF DNA arrays*



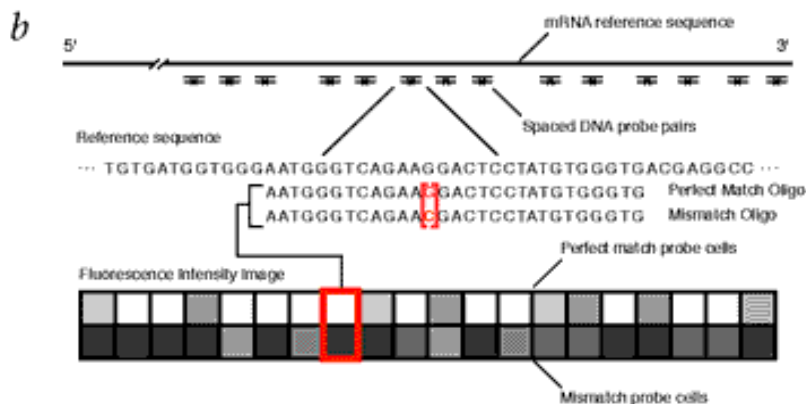
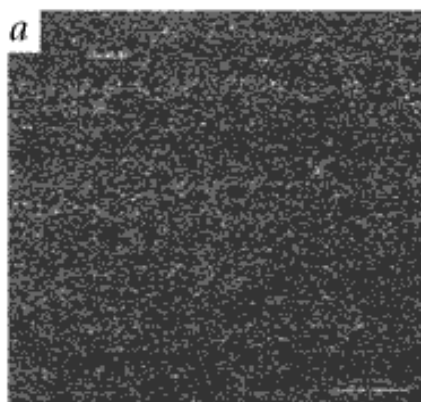
*Intergenic DNA arrays*



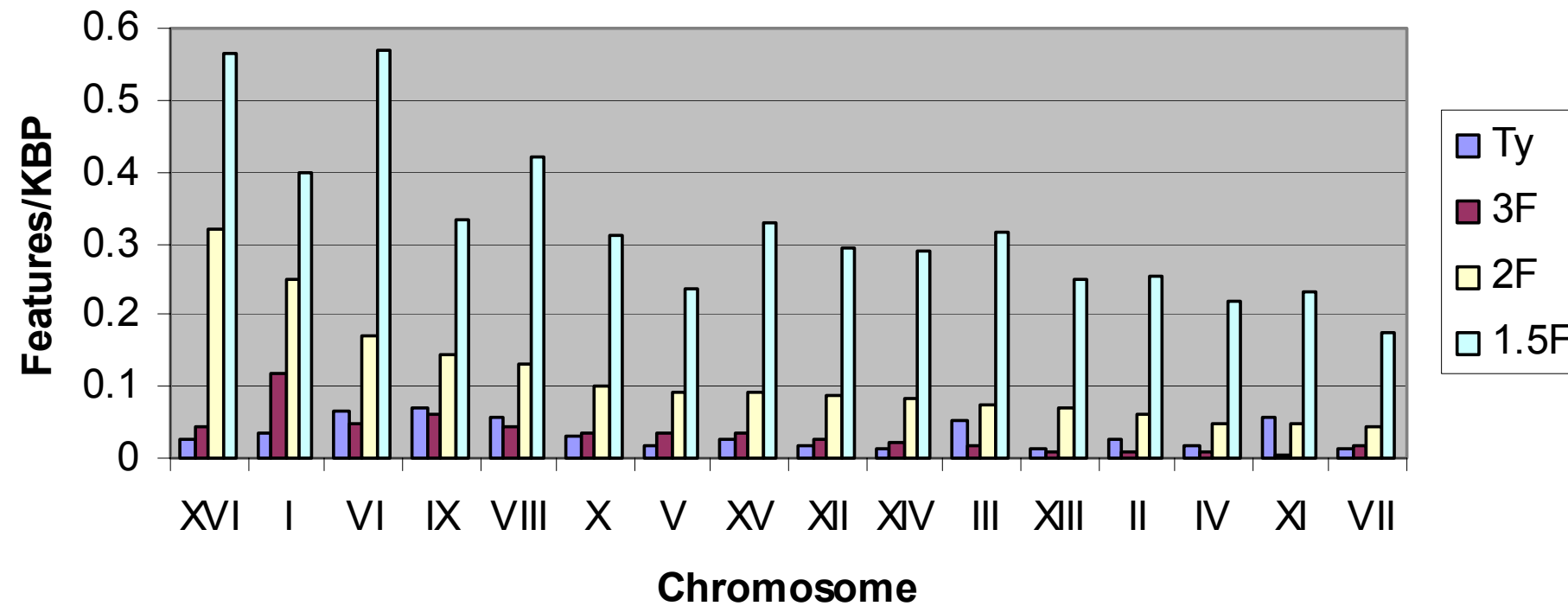
| ORF    | gene | CHR | 1    | 2    | 3    | 4    | 5   | 6   | 7    | 8    | 9    | 10   | 11   | 12   | 13  | 14   |
|--------|------|-----|------|------|------|------|-----|-----|------|------|------|------|------|------|-----|------|
| EL021W | URA3 | 5.0 | 12.9 | 15.8 | 24.5 | 46.8 | 9.3 | 8.3 | 20.0 | 11.2 | 19.1 | 20.0 | 21.9 | 25.1 | 1.1 | 12.9 |
| CL018W | LEU2 | 3.0 | 2.6  | 2.8  | 3.0  | 2.6  | 2.7 | 2.4 | 3.0  | 2.6  | 2.5  | 3.7  | 3.0  | 2.8  | 1.0 | 2.6  |

*Oligo (70mers) DNA arrays*

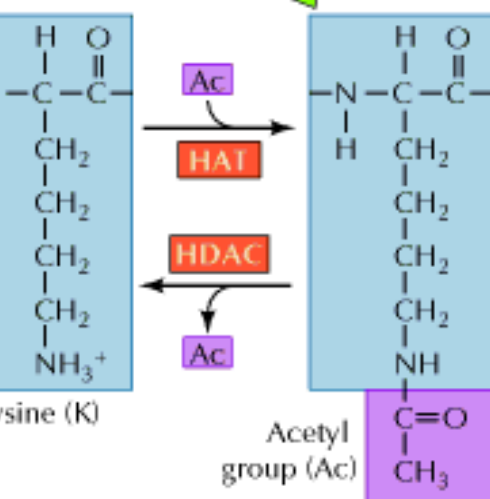
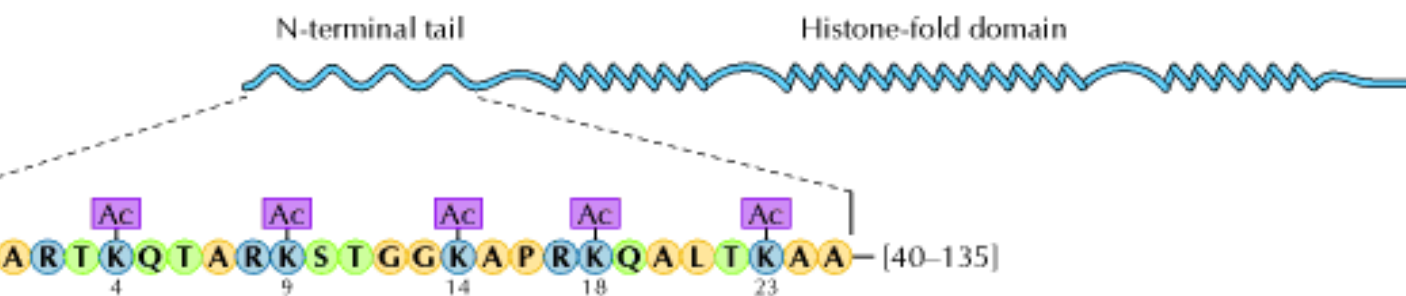
**Affymetrix oligoarrays**



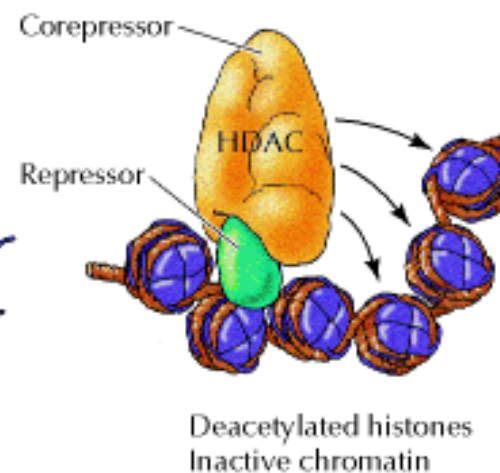
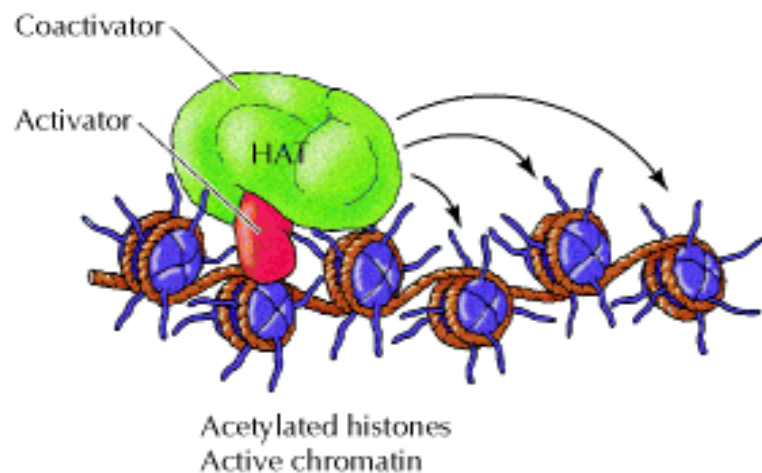
## ORF and INTERGENICS



| CATEGORY        | variable per category | tot variable elements | tot n of elements of cat. | exp surpris |
|-----------------|-----------------------|-----------------------|---------------------------|-------------|
| HDAC_list.dat   | 101                   | 475                   | 234                       | 0           |
| DAC intergenics | 118                   | 475                   | 284                       | 0           |
| HZAD            | 43                    | 475                   | 69                        | 0           |
| SDG_TY          | 32                    | 475                   | 127                       | 2.97E-10    |
| telo_10kb       | 107                   | 475                   | 163                       | 0           |
| telo_20kb       | 162                   | 475                   | 309                       | 0           |
| telo_30kb       | 190                   | 475                   | 464                       | 0           |
| Tfs             | 10                    | 475                   | 256                       | 0.00750031  |

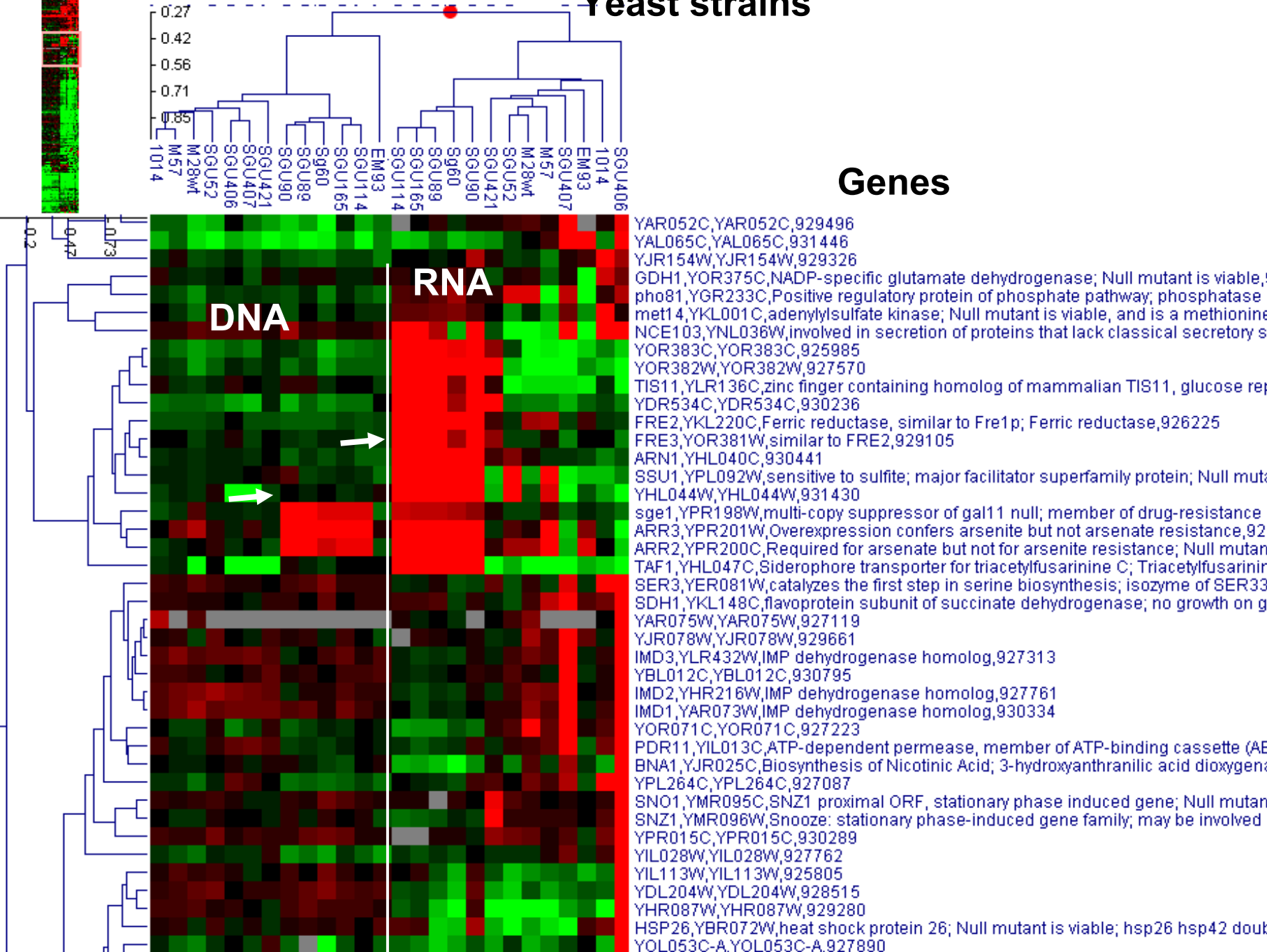


(B)



# Yeast strains

## Genes

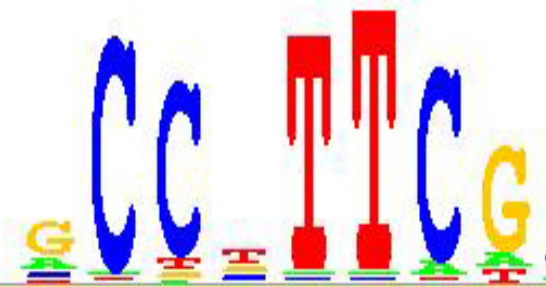




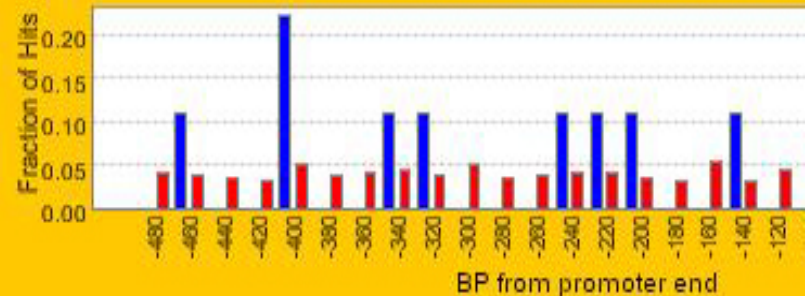
Fraction of Genes with Cluster 595



■ In-C ■ Out-C ■ 5% FPR - In-Cluster: 66.7%[8/12] Out-of-Cluster: 31.1%[1183/3801]  
 ■ 1% False Positive Rate - In-Cluster: 16.7%[2/12] Out-of-Cluster: 8.6%[328/3801]

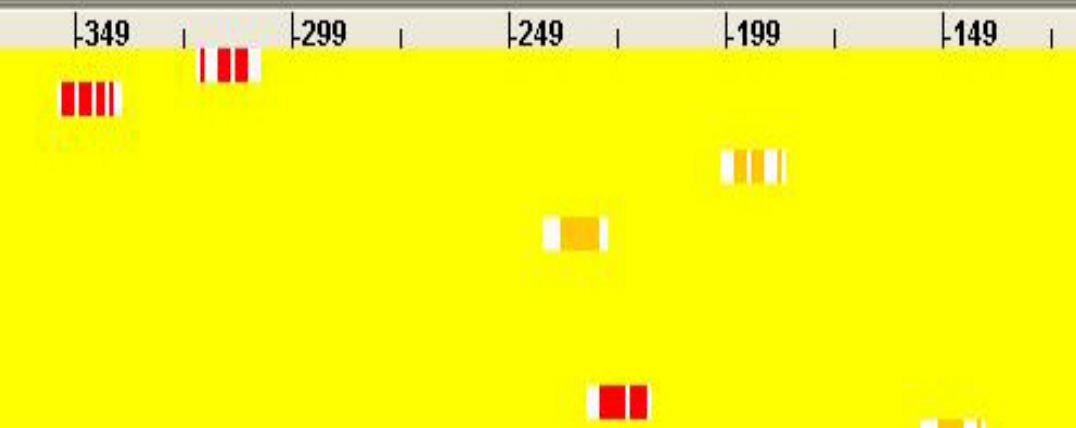


Positional Bias for Cluster 595

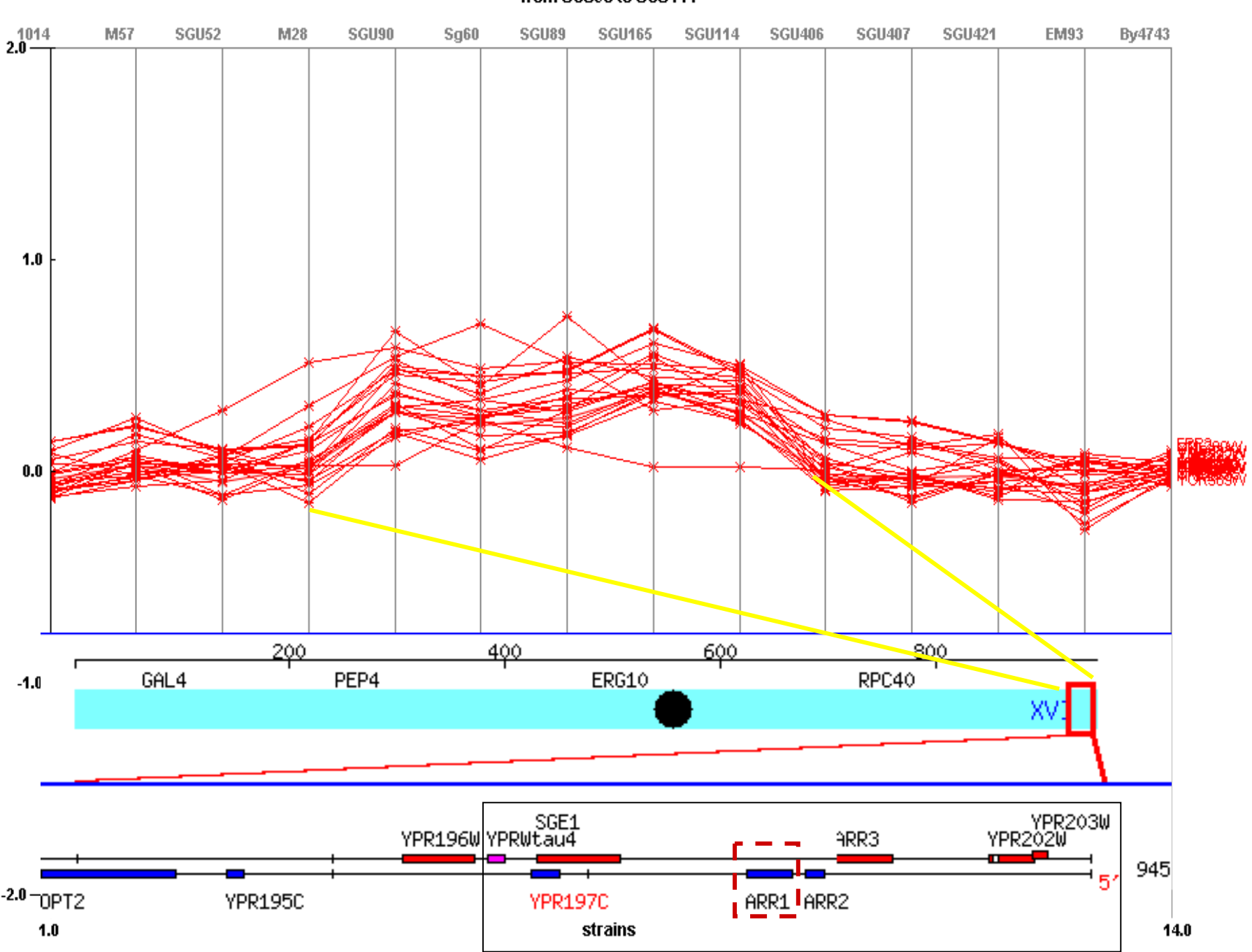


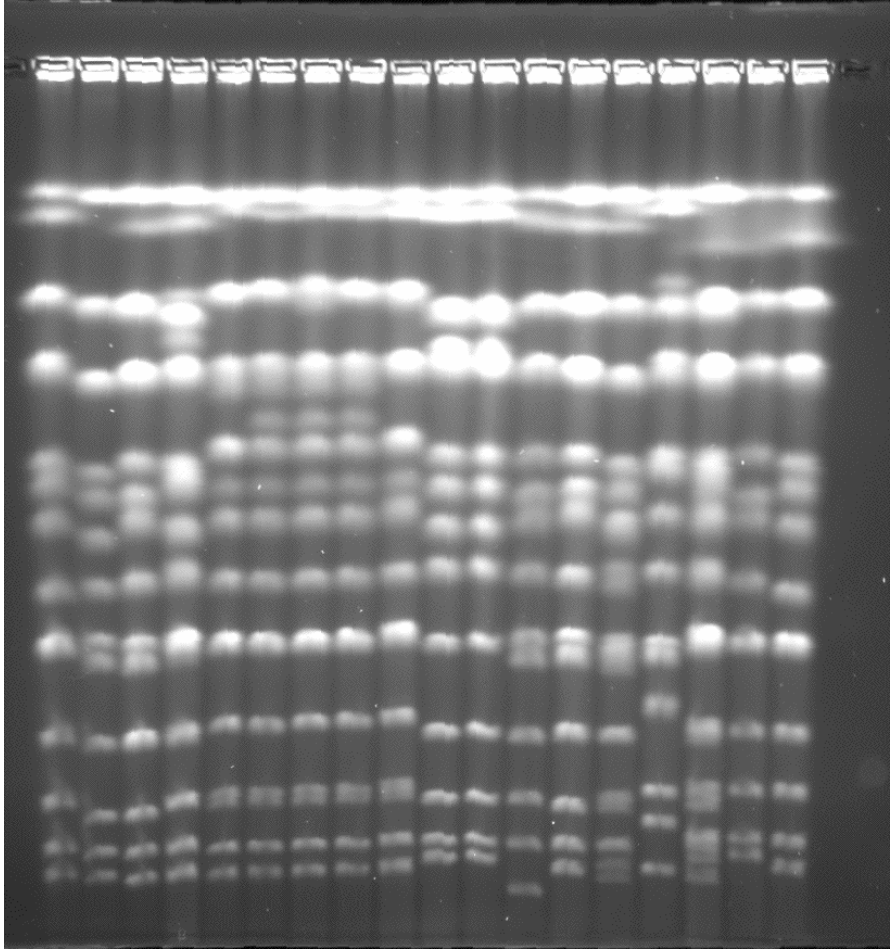
■ In-Cluster genes pval: 5.12E-2, b: -400 ■ Out-of-Cluster

This is a new transcriptional motif, that is probably recognized by **ARR1**, member of a yeast-specific family of basic leucine zipper (bZIP) proteins: Yap1-3-5-6-7-p, Cad1p-Cir1p



YOR384W FRE5 similar to FRE2 (YOR384W)  
 YDR534C YDR534C (YDR534C)  
 YLR136CT IS11 zinc finger containing homolog of mammalian  
 YOR383C YOR383C (YOR383C)  
 YOR382W YOR382W (YOR382W)  
 YDR269C YDR269C (YDR269C)  
 YDR270W CCC2 Copper-transporting P-type ATPase with six  
 YEL065W SIT1 Siderophore Iron Transport, Ferrioxamine B binding  
 YHL040C ARN1 (YHL040C)  
 YEL067C YEL067C (YEL067C)  
 YOR381W FRE3 similar to FRE2 (YOR381W)  
 YLR136CT IS11 zinc finger containing homolog of mammalian





S288c

M12

1014

SG 60

SGU89

SGU90

SGU114

SGU406

SGU407

SGU421

M28

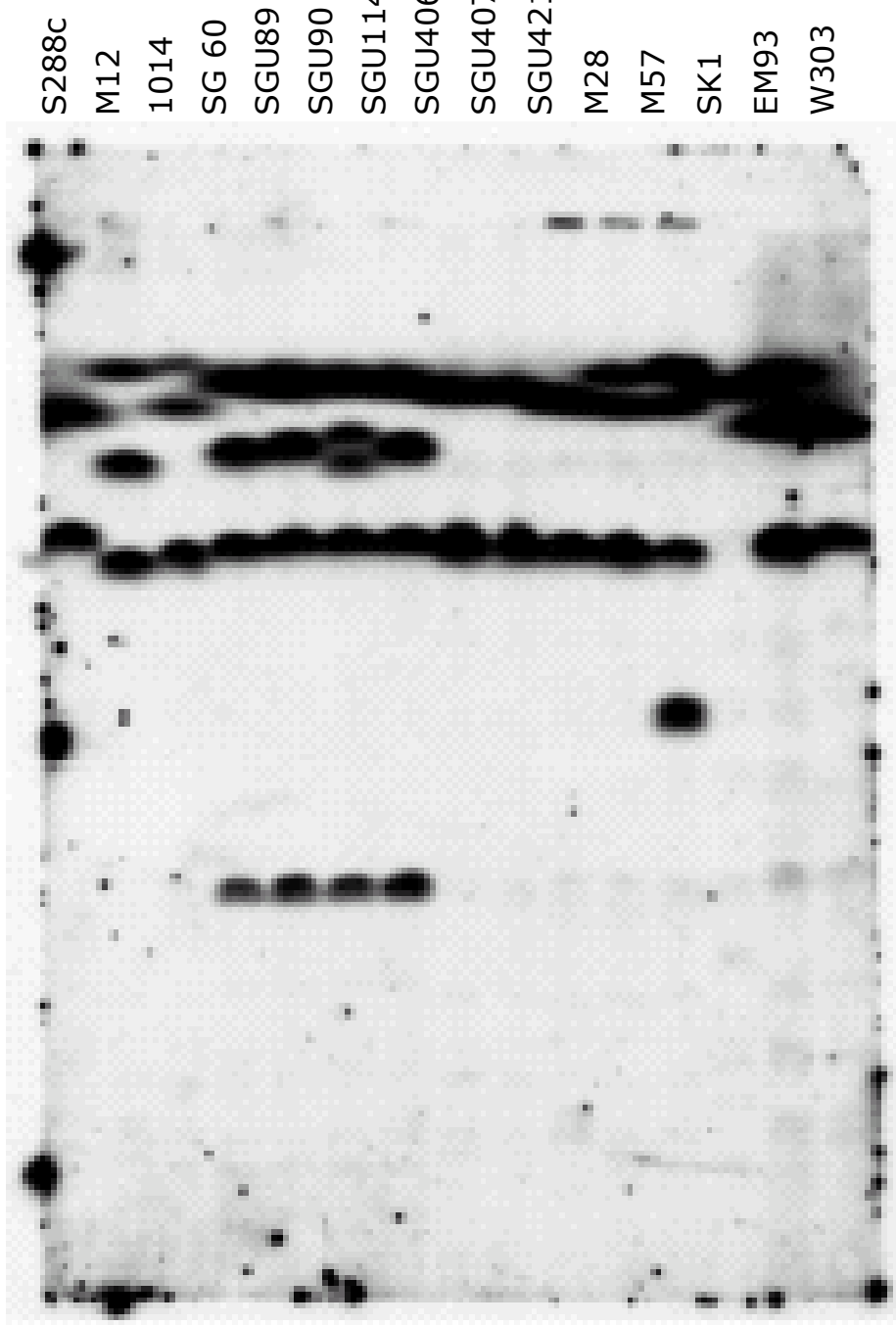
M57

SK1

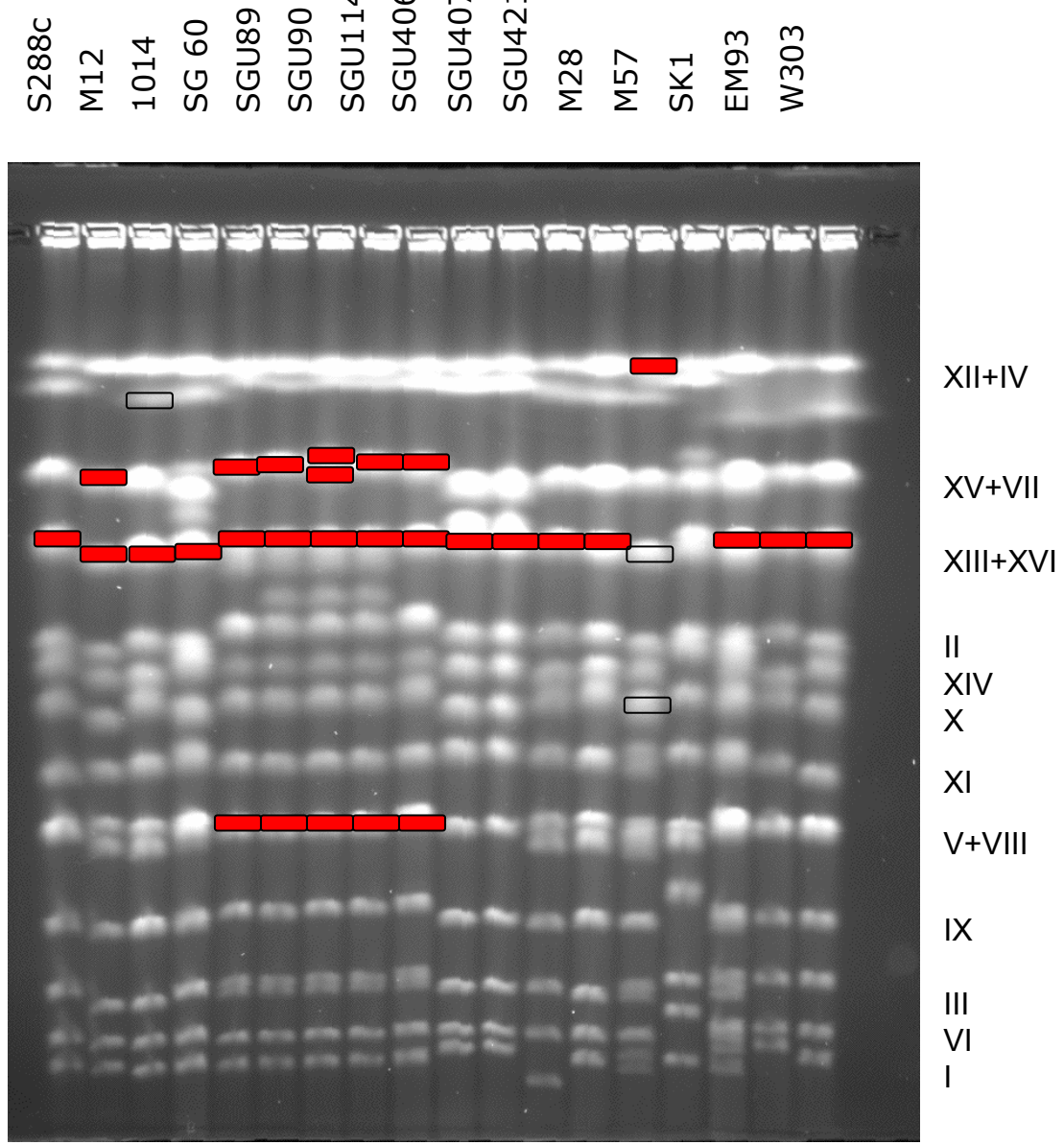
EM93

W303

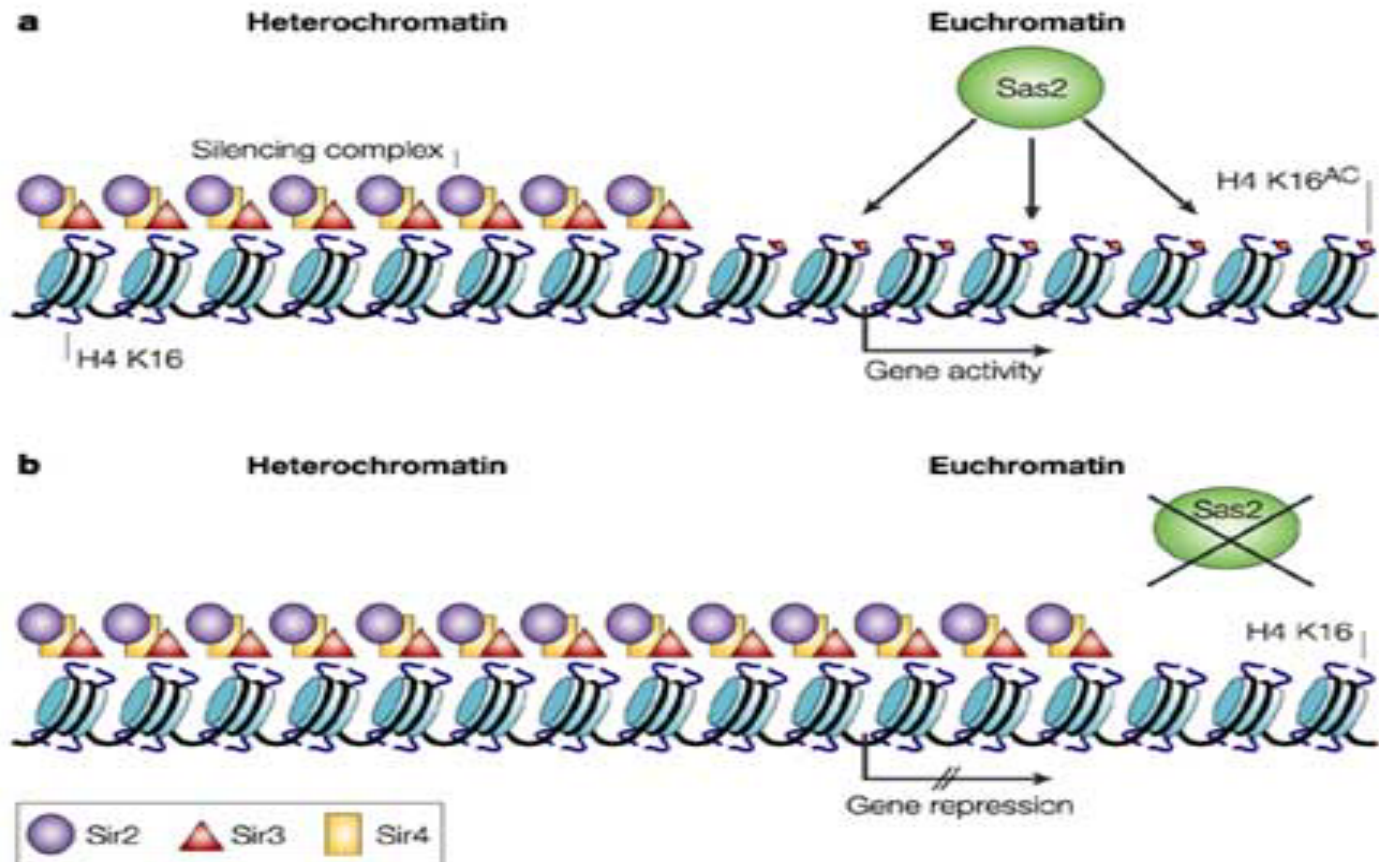
ARR3



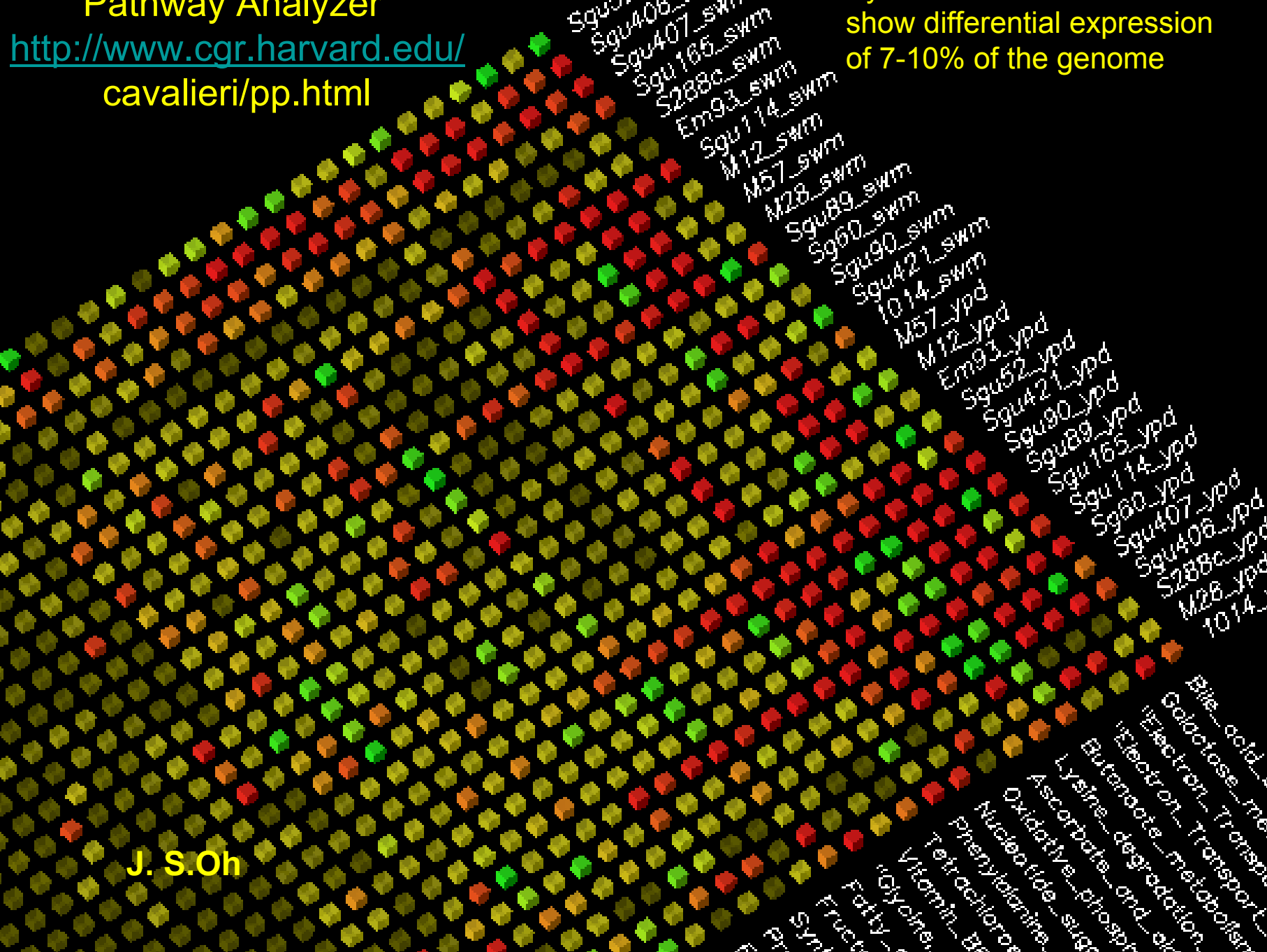
# ARR3



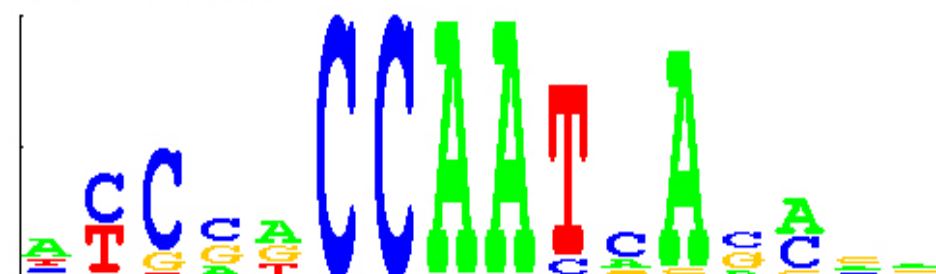
# Translocation or inversion events can affect histone deacetylase based control of gene expression



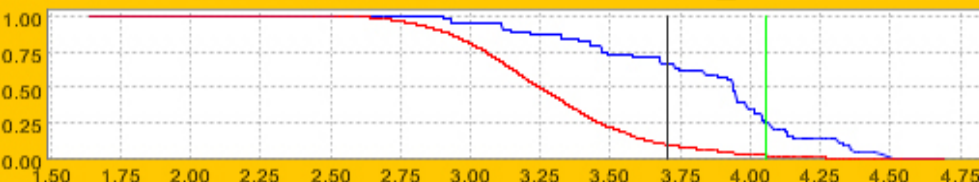
Boundaries between eu- e etero-chromatin are variable  
gene duplication can alter gene expression moving the  
gene in a non silenced location



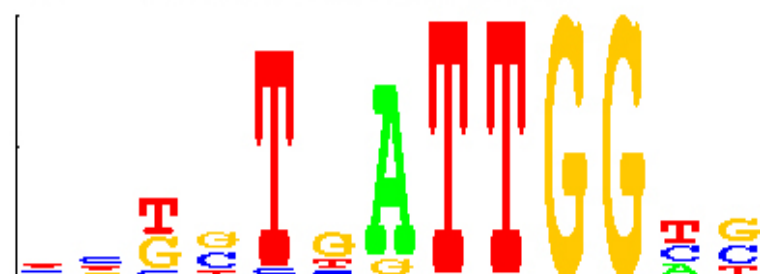
Motif 1: HAP234\_01



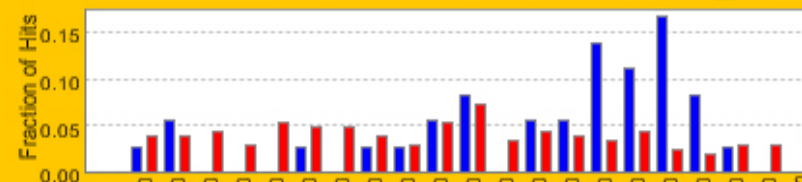
Fraction of Genes with HAP234\_01



Motif 1: HAP234\_01 (reverse complement)



Positional Bias for HAP234\_01



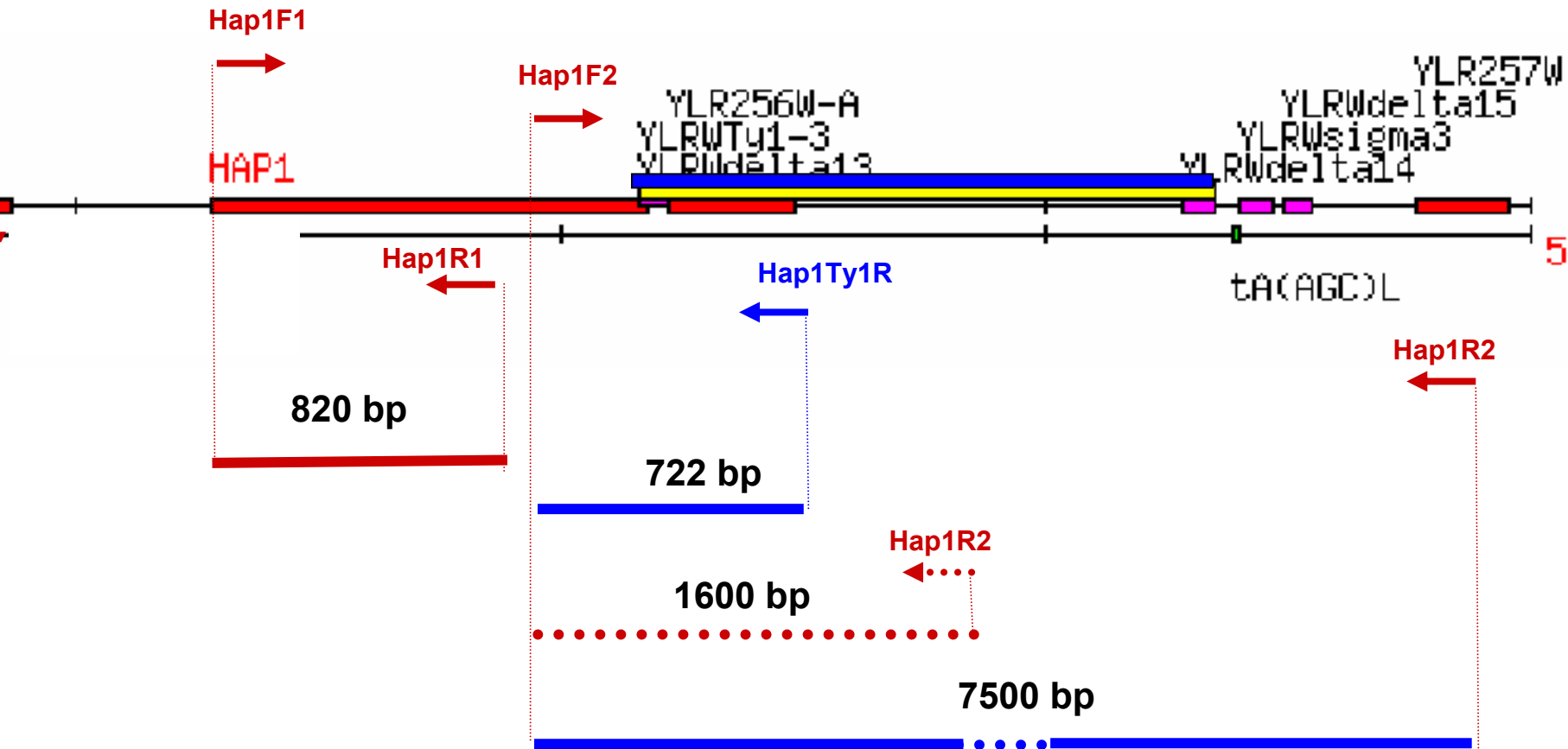
3 | 333 | 283 | 233 | 183 | 13

<http://genexpress.stanford.edu/>

YDL067C@COX9@Plays role in cytochrome c oxidase holoenzyme assembly or stability  
 YKL016C@ATP7@ATP synthase d subunit; glycerol minus phenotype; mitochondria ha  
 YDR377W@ATP17@ATP synthase subunit f; No growth on glycerol (YDR377W)  
 \*YNL052W@COX5A@One of two genes (COX5A and COX5B, both nuclear-encoded) c  
 \*YLR395C@COX8@Cytochrome-c oxidase chain VIII; Null mutant is viable, deficient in  
 YGL187C@COX4@subunit IV of cytochrome c oxidase; Null mutant is viable and unab  
 \*YGR183C@qcr9@7.3 kDa subunit 9 of the ubiquinol cytochrome c oxidoreductase com  
 YJL166W@QCR8@Ubiquinol cytochrome-c reductase subunit 8 (11 kDa protein); Null m  
 \*YMR256C@COX7@subunit VII of cytochrome c oxidase; Null mutant is viable, lacks cy  
 YPR020W@ATP20@Protein associated with mitochondrial ATP Synthase; essential for  
 \*YLR038C@COX12@essential during assembly for full cytochrome c oxidase activity; su  
 \*YHR001W-A@QCR10@8.5 kDa subunit of the ubiquinol-cytochrome c oxidoreductase c  
 \*YDR529C@QCR7@ubiquinol-cytochrome c oxidoreductase subunit 7 (14 kDa); Null m  
 \*YGL191W@COX13@modulates cytochrome c oxidase activity; subunit VIa of cytochr  
 YJR048W@cyc1@iso-1-cytochrome c; Cytochrome c deficiency (YJR048W)  
 YDL181W@INH1@ATPase inhibitor; Null mutant is viable; exhibits marked ATP hydroly  
 \*YDR298C@ATP5@involved in the assembly and function of the mitochondrial ATP sy  
 \*YPL078C@ATP4@Encodes subunit 4 of F0 sector of ATP synthase and is analogous to  
 YDL004W@ATP16@ATP synthase delta subunit; cells are entirely cytoplasmic petite (Y  
 YLR295C@ATP14@ATP synthase subunit h; unable to grow on glycerol medium; no de  
 YML120C@NDI1@NADH dehydrogenase (ubiquinone) (YML120C)  
 YBL015W@ACH1@Mannose-containing glycoprotein which binds concanavalin A; ace  
 YJL102W@MEF2@mitochondrial elongation factor G-like protein (YJL102W)  
 \*YBL030C@pet9@the major mitochondrial ADP/ATP translocator; highly homologous t  
 YBL045C@COR1@44 kDa core protein of yeast coenzyme QH2 cytochrome c reductas  
 YOR065W@CYT1@Cytochrome c1 (YOR065W)  
 \*YEL024W@RIP1@oxidizes ubiquinol at center P in the protonmotive Q cycle mechan  
 YBR097W@vps15@myristoylated protein kinase involved in vacuolar protein sorting; My  
 YBL099W@ATP1@mitochondrial F1F0-ATPase alpha subunit; null mutant is viable; gr  
 \*YJR077C@MIR1@Null mutant is viable on glucose containing media, but is unable to

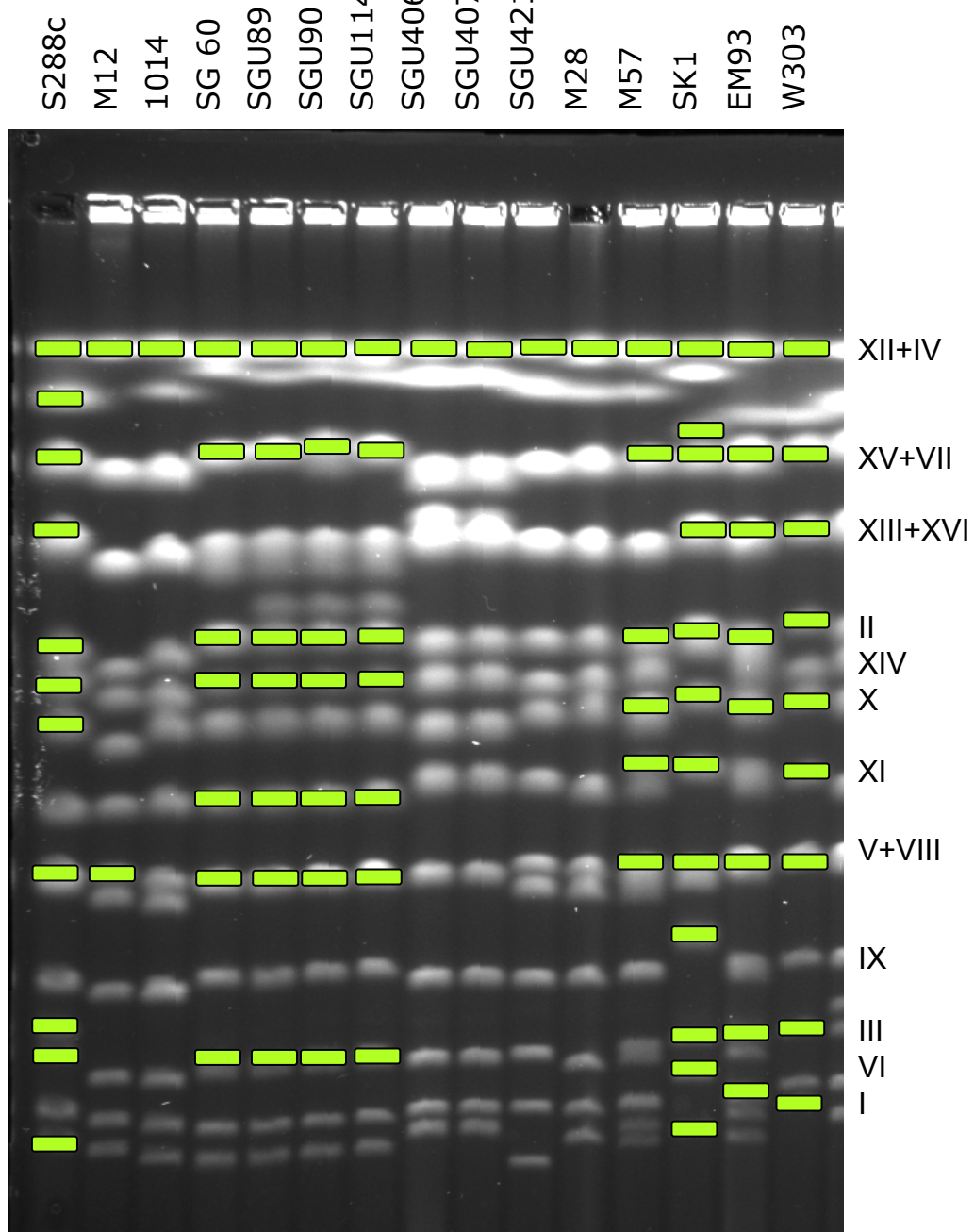
The change in expression is due to a Ty1 insertion in the 3' end of Hap1.

This insertion causes: a deletion of 13 AA, addition of 32 residues, and a reduction of Hap1 activity as a transcriptional activator.

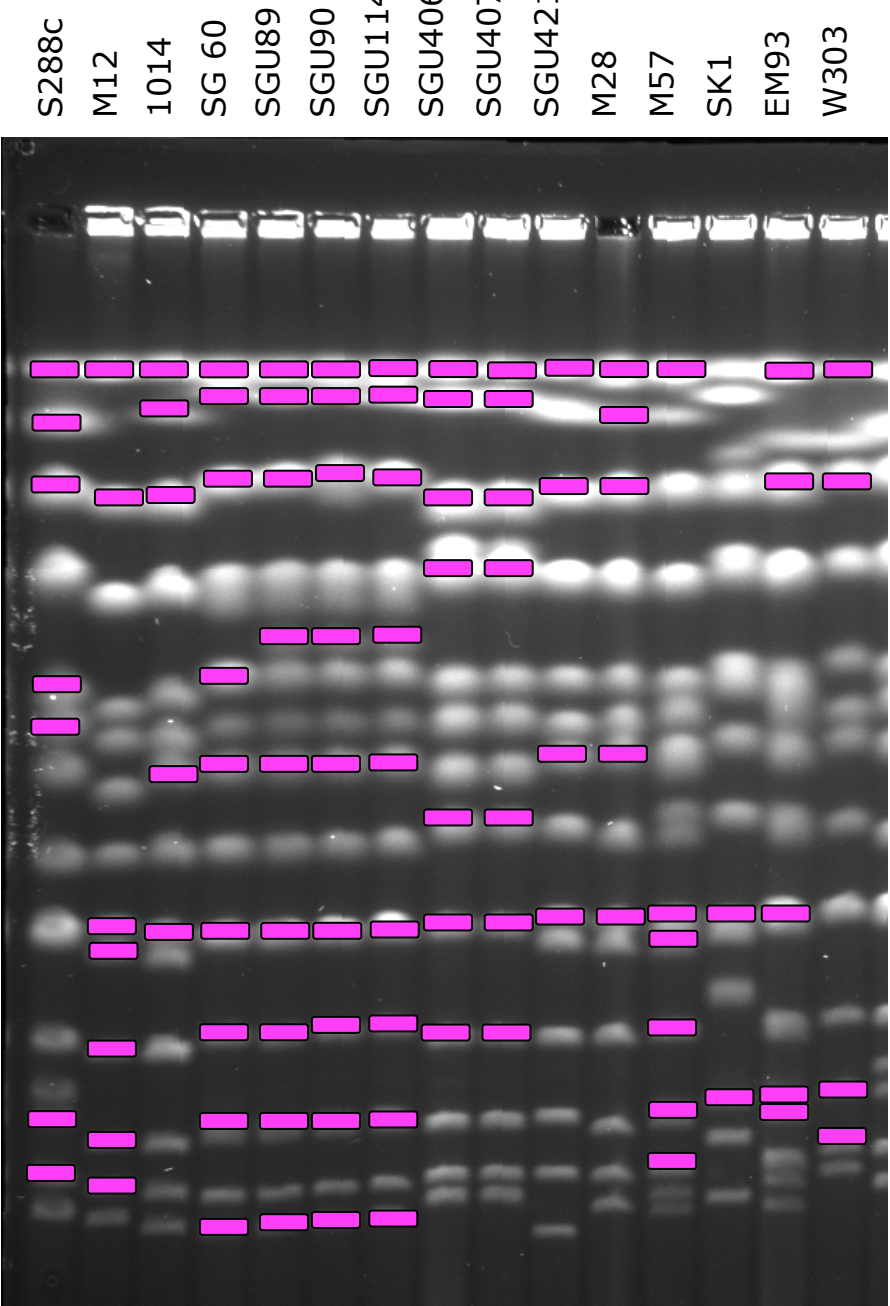


| Primary Seq | dc ty a  | 1014  | SGU52 | SG60  | SGU89 | SGU90 | SGU114 | SGU165 | SGU406 | SGU407 | SGU421 | M28  | M57   | SK1   | EM93 | W303 | By4743 |
|-------------|----------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|------|-------|-------|------|------|--------|
| YAR009C     | TY1B     | -5.1  | -10.3 | -2.3  | -2.0  | 1.2   | 1.1    | -1.2   | 1.2    | -1.1   | 1.1    | -2.5 | -1.2  | -1.2  | 1.0  |      |        |
| YAR010C     | TY1A     | -11.6 | -9.9  | -5.6  | -2.2  | -2.0  | -2.2   | -1.9   | -8.5   | -10.2  | -10.0  | -3.2 | -6.2  | -1.5  | -1.6 | 1.1  | -1.0   |
| YBL005W-A   | TY1A     | -4.0  | -5.2  | -2.2  | -1.6  | -2.0  | -1.6   | -1.6   | -2.3   | -2.4   | -3.1   | -2.1 | -2.1  | -1.7  | -1.3 | -1.0 | 1.1    |
| YBL005W-B   | TY1B     | -2.6  | -1.9  | -1.4  | -1.1  | 1.1   | -1.5   | -1.1   | -1.2   | -1.6   | -2.0   | -2.2 | -1.3  |       | 1.0  |      | 1.0    |
| YBL101W-A   | TY1A     | -1.6  | -1.5  | -1.5  | -1.1  | -1.5  | -1.2   | 1.2    | 1.0    | 1.1    | -1.1   | 1.1  | -1.1  | -2.0  | -1.3 | 1.3  | 1.2    |
| YBL101W-B   | TY1B     | -1.2  | -1.2  | -1.2  | -1.1  | -1.0  | -1.2   | 1.1    | 1.1    | -1.1   | -1.1   | -1.5 | -1.4  | -1.5  | 1.0  | 1.0  | -1.2   |
| YBR012W-A   | TY1A     | -6.6  | -5.3  | -1.8  | -2.0  | -2.3  | -1.8   | -1.9   | -6.7   | -9.4   | -5.9   | -3.4 | -6.8  | -1.4  | -1.5 | 1.2  | -1.0   |
| YBR012W-B   | TY1B     | -1.3  | -1.0  | -1.4  | -1.5  | -1.2  | -1.9   | 1.2    | -1.0   | 1.1    | -1.6   | -1.3 | -1.4  | -1.1  | 1.6  |      | 1.0    |
| YCL019W     | TY2B     | -1.3  | -1.1  | -1.0  | -1.1  | -1.0  | -1.2   | 1.1    | 1.2    | 1.1    | -1.1   | -1.1 | -1.0  | -1.2  | -1.0 | 1.2  | 1.3    |
| YCL020W     | TY2A     | -1.8  | -1.4  | -1.6  | -1.1  | -1.2  | -1.1   | 1.2    | -1.1   | -1.0   | 1.2    | 1.1  | -1.1  | -1.5  | -1.1 | 1.6  | -1.0   |
| YCL074W     | TY5      | 1.7   | 1.9   | 2.1   | 1.9   | 2.0   | 1.7    | 1.8    | 2.2    | 1.5    | -1.1   | 2.1  | 2.4   | -1.0  | 1.7  | -1.3 | 1.0    |
| YCL075W     | TY5      | 1.6   | 1.6   | 1.4   | 1.5   | 1.3   | 1.9    | 1.8    | 1.6    | 1.3    | 1.0    | 2.0  | 1.7   | 1.0   | 1.2  | -1.1 | 1.1    |
| YCL076W     | TY5      | 1.7   | 1.8   | 2.3   | 1.8   | 2.0   | 1.8    | 1.7    | 1.7    | 1.8    | -1.1   | 2.0  | 2.9   | -1.1  | 1.7  | -1.2 | -1.0   |
| YER138C     | RCdelta  | -1.3  | -1.7  | -1.4  | -1.1  | -1.1  | -1.4   | -1.1   | -1.3   | -1.2   | -1.2   | -1.4 | -1.5  | -1.1  | -1.1 | 1.0  | -1.0   |
| YER160C     | Ty1B     | -1.4  | -1.2  | -1.2  | -1.1  | -1.1  | -1.3   | -1.1   | -1.2   | -1.3   | -1.2   | -1.5 | -1.4  | -1.2  | 1.0  | 1.0  | -1.0   |
| YHR214C-B   | RCdelta  | -1.3  | -1.2  | -1.4  | -1.4  | -1.2  | -1.3   | 1.3    | 1.1    | -1.0   | -1.3   | 1.0  | -1.2  | -1.2  | -1.0 | -1.0 | 1.1    |
| YIL080W     | Ty3      | -7.8  | -6.0  | 1.0   | 1.1   | 1.1   | 1.2    | 1.1    | -3.6   | -4.6   | -3.6   | -2.1 | -4.5  | -1.4  | -2.8 | 1.6  | -1.1   |
| YIL082W     | TY3      | -70.3 | -54.9 | 1.3   | 1.4   | 1.3   | 1.3    | 1.1    | -8.4   | -10.0  | -53.3  | -3.8 | -29.9 | -1.8  | -3.8 | 1.7  | 1.1    |
| YIL082W-A   | Ty3      | -6.9  | -3.5  | 1.3   | 1.4   | 1.4   | -1.0   | -1.1   | -1.8   | -2.4   | -2.9   | -2.2 | -2.0  | -1.7  | -1.8 | 1.3  | 1.1    |
| YJL113W     | Ty4B     | -1.2  | -1.3  | -1.4  | -1.3  | -1.1  | -1.5   | -1.0   | -1.0   | -1.1   | 1.2    | -1.6 | -1.4  | -1.4  | -1.1 | -1.1 | 1.1    |
| YJL114W     | TY4B     | -49.8 | -36.3 | -23.1 | -36.2 | -54.3 | -48.9  | -23.6  | -34.1  | -31.7  | -37.5  | -4.9 | -78.0 | -61.1 | -2.3 | -1.5 | 1.0    |
| YJR026W     | TY1A     | -21.6 | -16.8 | -2.6  | -2.3  | -2.4  | -2.3   | -1.9   | -11.8  | -13.9  | -19.9  | -4.3 | -14.9 | -1.6  | -1.7 | 1.1  | 1.0    |
| YJR027W     | TY1B     | -2.2  | -1.8  | -1.8  | -1.3  | -1.4  | -1.5   | -1.4   | -1.2   | -1.3   | -1.4   | -1.4 | -1.2  | -1.5  | 1.0  | -1.2 | 1.3    |
| YJR028W     | TY1A     | -25.1 | -21.4 | -2.6  | -2.4  | -3.1  | -2.0   | -2.1   | -18.5  | -25.6  | -22.8  | -4.6 | -24.5 | -1.6  | -1.6 | 1.0  | 1.0    |
| YJR029W     | TY1B     | -4.6  | -3.4  | -2.4  | -1.9  | -1.8  | -2.2   | -1.1   | -2.0   | -2.0   | -2.3   | -2.3 | -2.0  | -1.9  | -1.1 | 1.1  | -1.4   |
| YML039W     | Ty1A     | -4.2  | -3.9  | -2.0  | -1.5  | -1.5  | -2.2   | -1.6   | -2.0   | -1.9   | -2.9   | -2.2 | -2.0  | -1.6  | -1.2 | -1.0 | -1.0   |
| YML040W     | TY1A     | -20.2 | -12.9 | -2.4  | -2.2  | -2.3  | -1.9   | -2.2   | -9.5   | -9.1   | -12.0  | -3.9 | -10.4 | -1.7  | -1.8 | 1.0  | -1.4   |
| YML045W     | TY1A     | -56.9 | -31.8 | -2.6  | -2.7  | -2.8  | -2.4   | -2.5   | -41.9  | -29.8  | -38.2  | -4.9 | -42.1 | -1.5  | -1.7 | 1.2  | 1.1    |
| YMR045C     | IRCdelta | -1.3  | -1.2  | -1.1  | 1.2   | -1.3  | -1.2   | 1.1    | 1.0    | 1.1    | 1.0    | -1.0 | 1.1   | -1.1  | 1.3  | -1.3 | -1.0   |
| YMR046C     | IRCdelta | -9.9  | -8.8  | -2.1  | -2.3  | -2.2  | -1.8   | -2.0   | -9.1   | -10.5  | -7.2   | -3.9 | -8.6  | -1.5  | -1.5 | 1.2  | 1.2    |
| YMR050C     | Ty1A     | -1.4  | -1.6  | -1.4  | 1.1   | 1.1   | -1.5   | -1.2   | 1.1    | 1.1    | -1.1   | -1.3 | -1.3  | -1.3  | 1.0  | -1.1 | -1.0   |
| YMR051C     | TY1A     | -62.3 | -30.2 | -2.5  | -2.7  | -2.9  | -2.2   | -2.7   | -23.0  | -13.0  | -39.9  | -4.2 | -25.8 | -1.6  | -1.6 | 1.0  | 1.1    |

Ty1



Ty2



# Conclusions-1

HDAC and SIR-SAS gene expression control provides the cell with a mechanism of epigenetic buffering of variation.

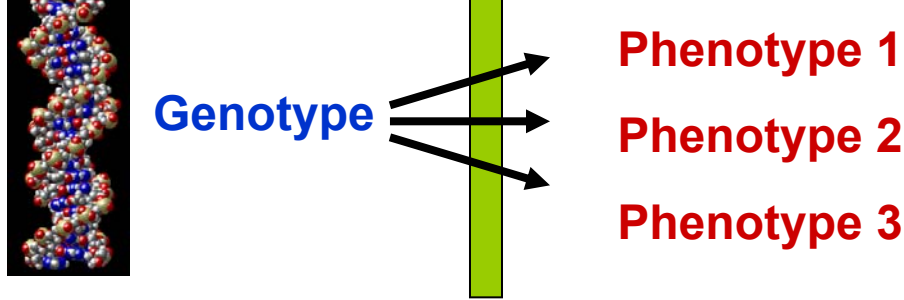
These regions could be used to acquire new genes through horizontal gene transfer, accelerate evolution.

Genomic variation could be accumulated and kept silent, expression in HDAC domains is stochastic and can be selectively uncovered in permissive or extreme conditions.

Gene duplications, moving genes from a silenced to a non silenced region, could give advantage under selective conditions, and be then fixed in the population.

# Perspectives

- CHIP genomic DNA from selected wine strains with HDAC, SAS, SIR2 to selectively discover “new” genes of environmental interest.
- Study regulation of HDAC, SAS, SIR2 in wine strains and the evolution of these regulatory systems in different strains and species.
- Control of modulation of expression of Genes in HDAC regions is important to Understand the **connections** between **genetic networks** involved in **production**, **adaptation** and **resistance** to stressors



**A single genotype may produce many  
different phenotypes environment  
dependent**

**A single phenotype may be produced  
by many different genotypes  
environment dependent**

