SBM 13

14-11-08

Cox RS III, Surette MG, Elowitz MB.
Programming gene expression with combinatorial promoters

(prok) transcriptional logic

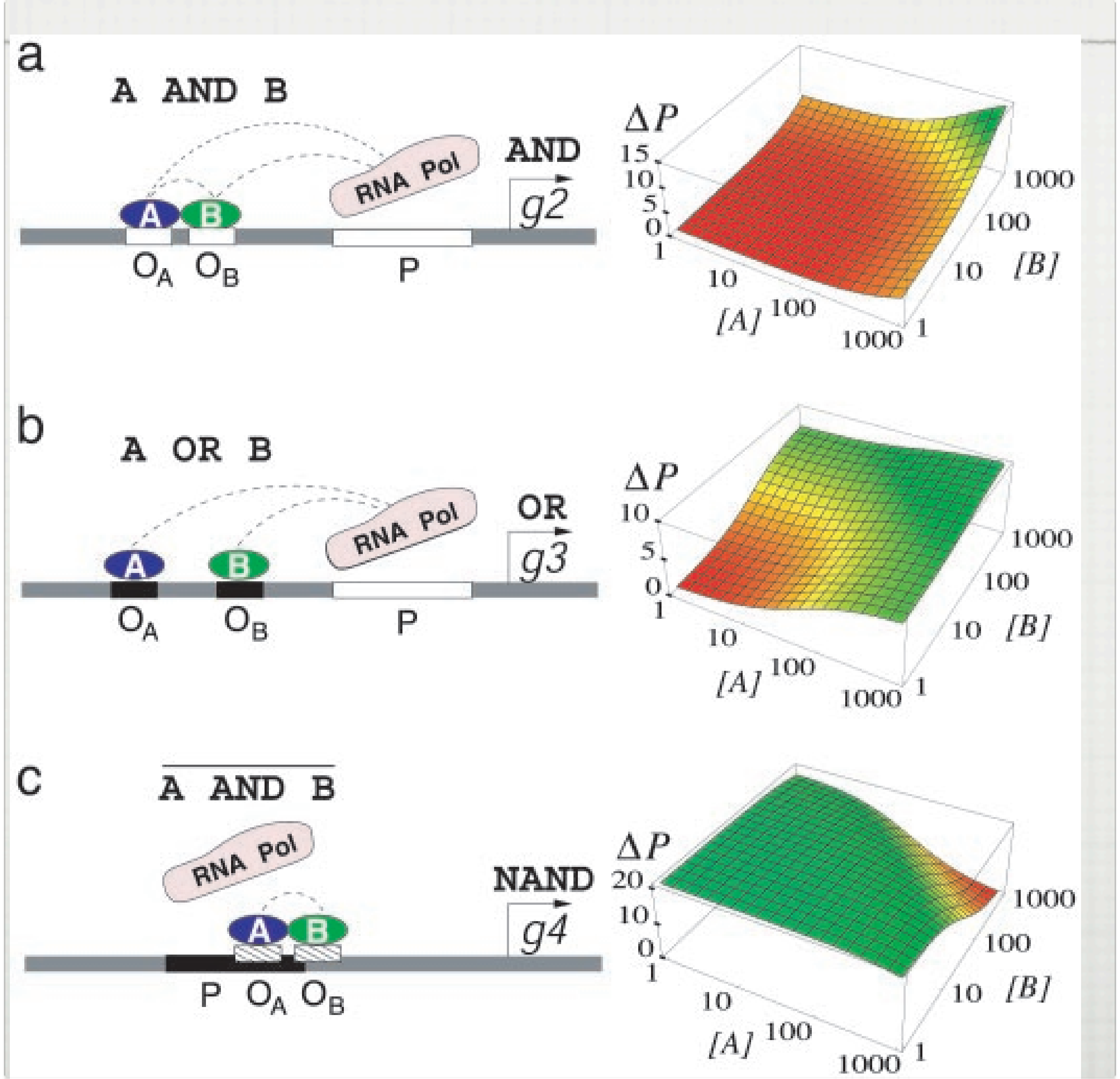
☐ The activity of a gene is regulated by other genes through the concentrations of their gene products, the transcription factors (TFs).

☐ This is accomplished mechanically by the interaction of the TFs with their respective DNA targets, with each other, and with the RNA polymerase (RNAP) complex in the regulatory region of the gene

☐ can be hugley complex in euk. (see Davidson's on sea urchin development)

Buchler et al, PNAS 100(9)

examples (theoretic)



deliberately simple promoters

Elowitz et al. Mol Syst Biol. 2007 3:145

- ☐ The paper reports the synthesis of about 200 promoters glued to a reporter gene;
- ☐ the obtained DNA constructs can be seen as binary functions (most have 2 operators so 2 TF they can interact with).
- ☐ The constructs are simple promoter architectures, a priori no TF-TF contacts and no operator overlap.
- ☐ Constructs are classified in an original way as real-valued binary functions and then sequenced (why?)

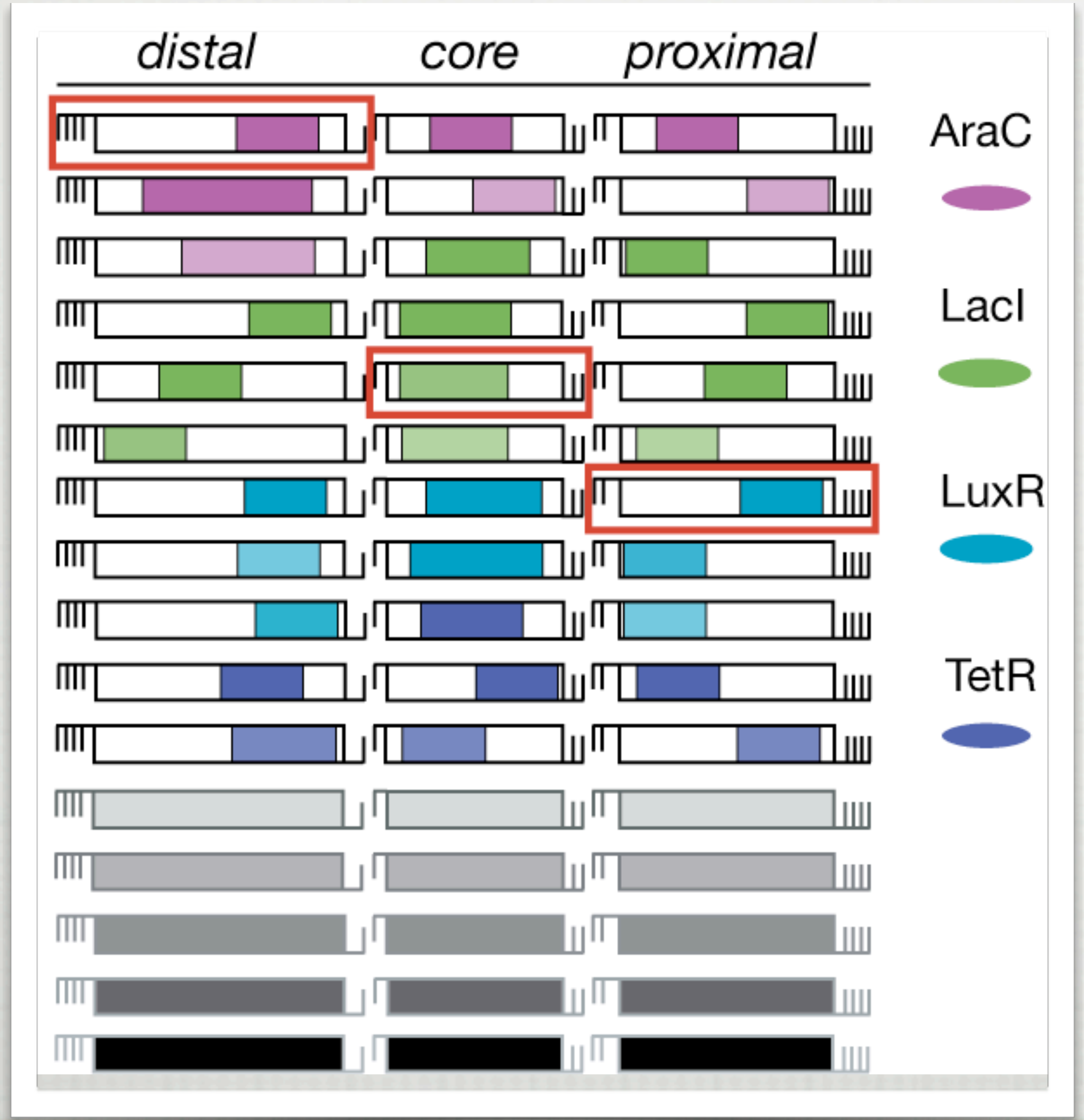
remarks

- A self-documenting automated bio-brick factory!
- yet output of a promoter::gene not a Boolean valued function of the concentrations of its TF/inputs (lac promoter has 4 output levels):
  - could take “low” value of a few molecules per bacterium (1 nM)
  - “high” value 1,000 molecules per bacterium (1M)

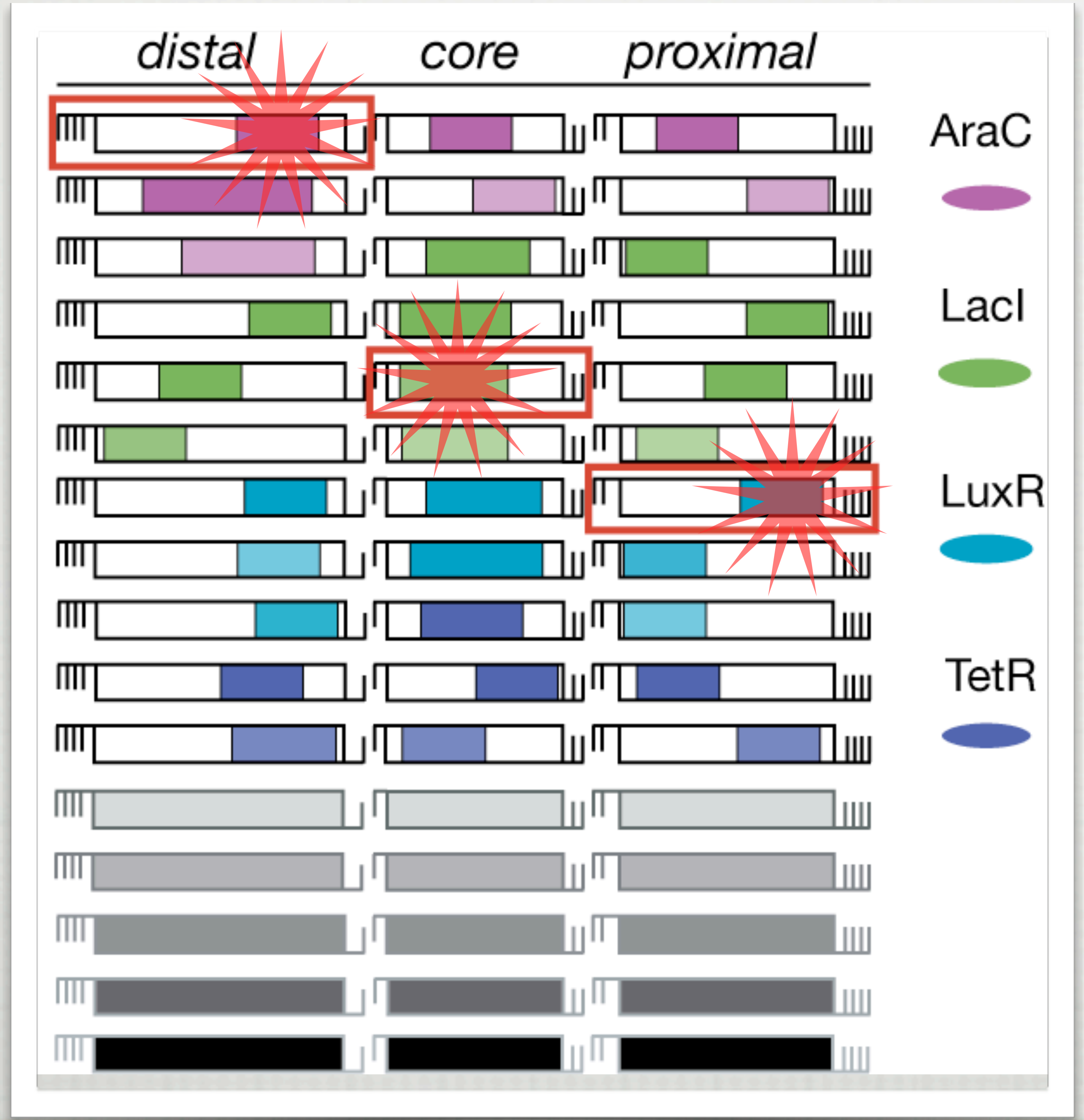
a combinatorial library of random promoter architectures

- ☐ 4096 of which 288 sequenced cassettes:
  - ☐ 217 unique
  - ☐ of which 27 binary (twofold response under 2 TFs)
- ☐ promoter = distal::core::proximal, device = promoter::G-luciferase
  - ☐ TFs= Arac, LuxR (activators) -activated by Lara, VAI
  - ☐ TetR, lacI (repressors) -inactivated by aTc, IPTG

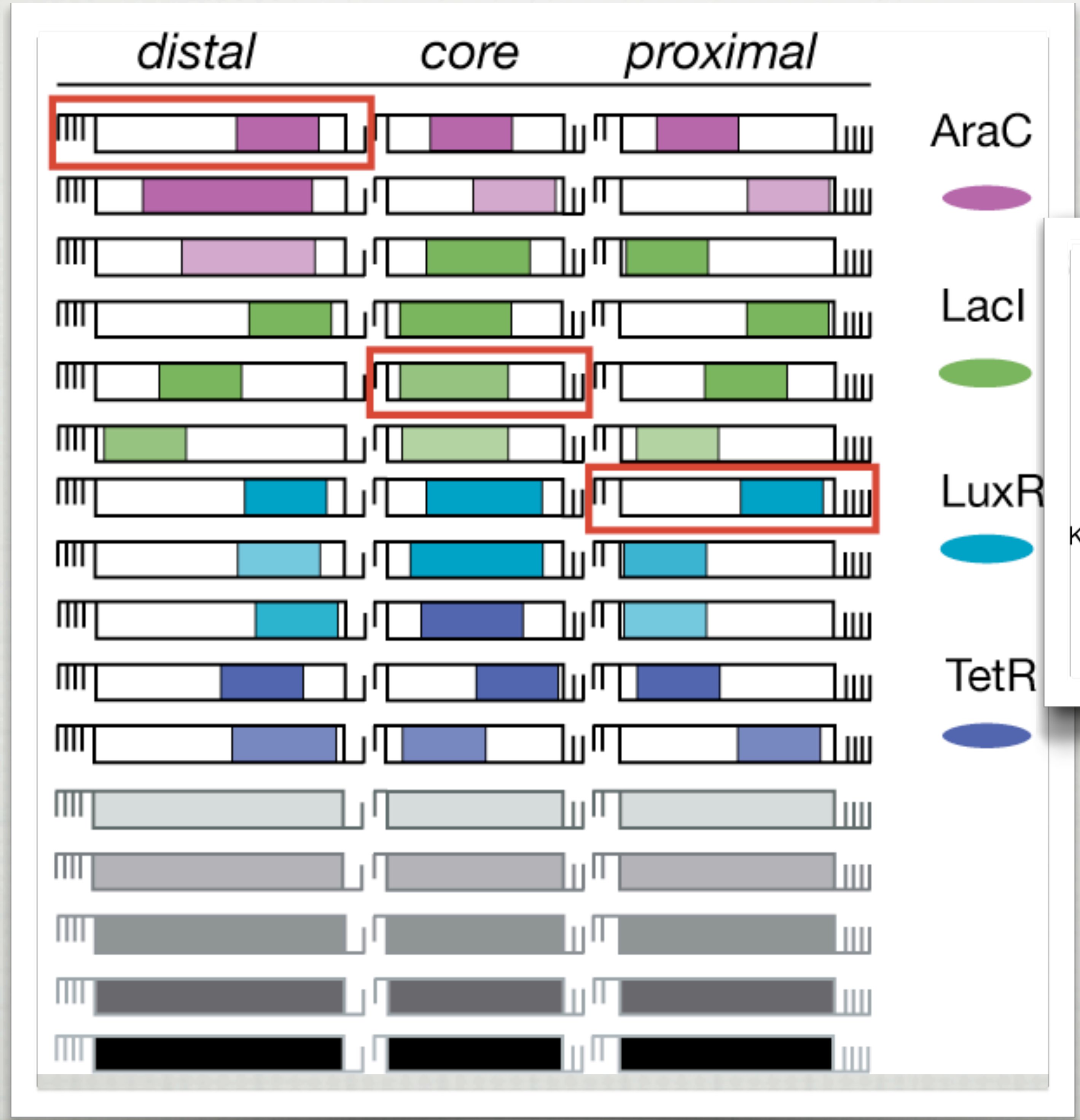
The library - sequence work

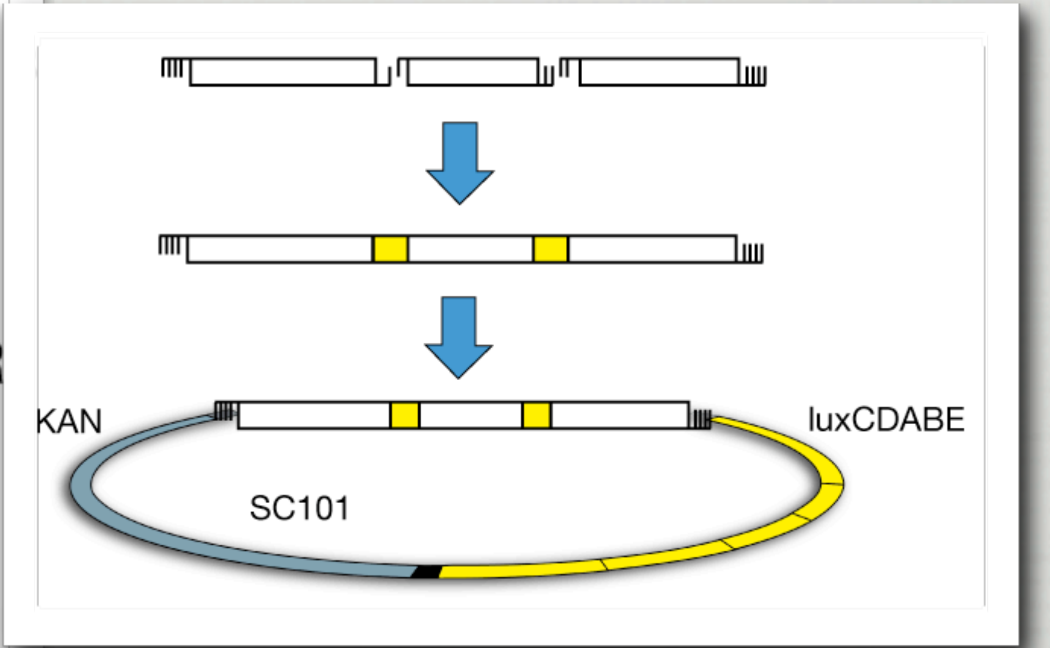


The library - sequence work



The library - sequence work





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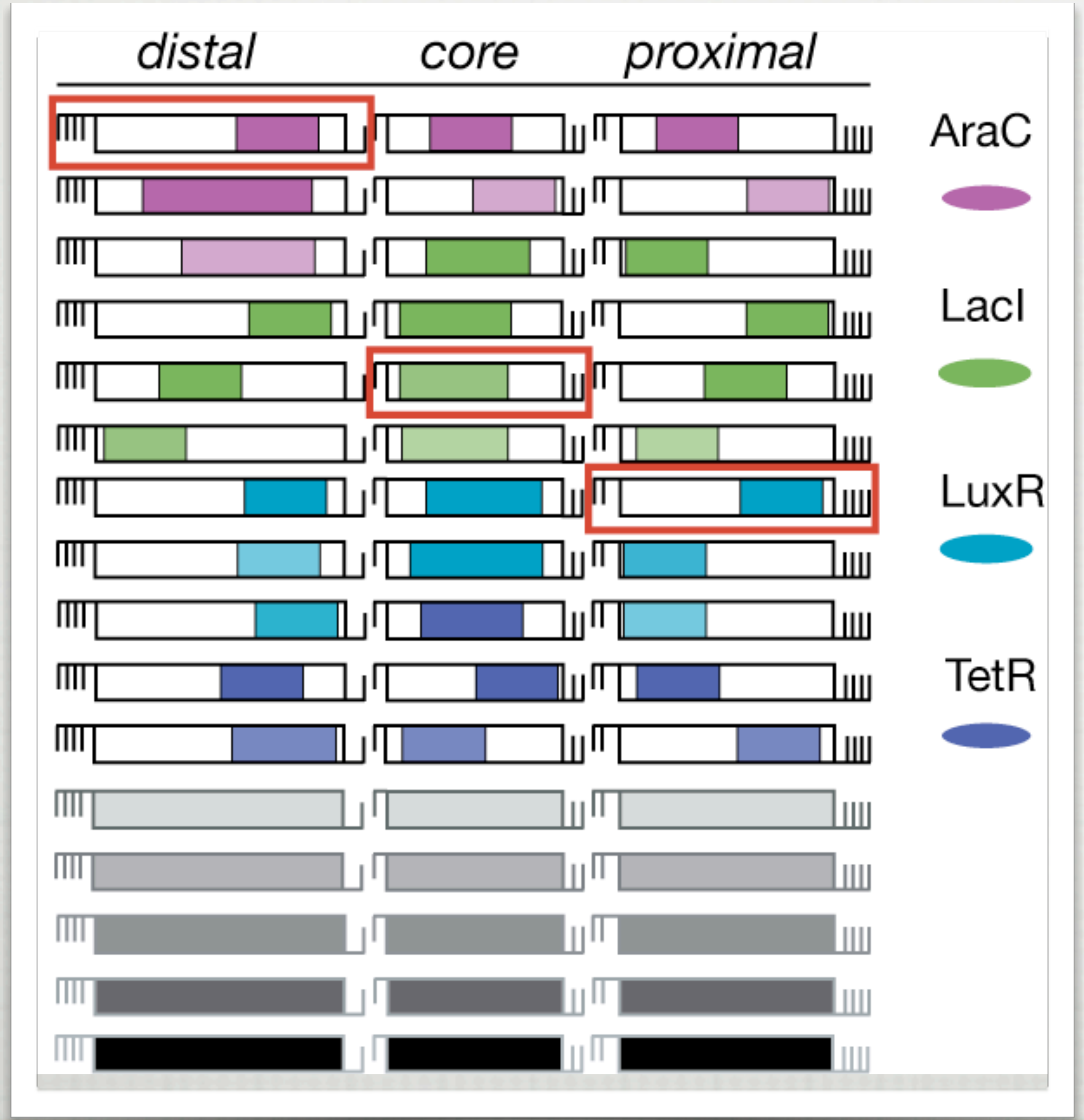

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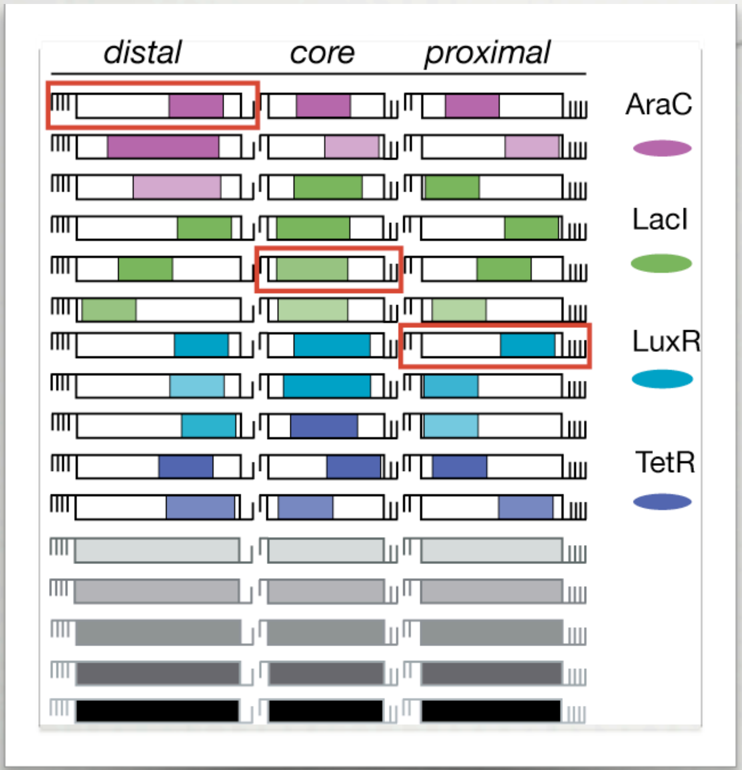

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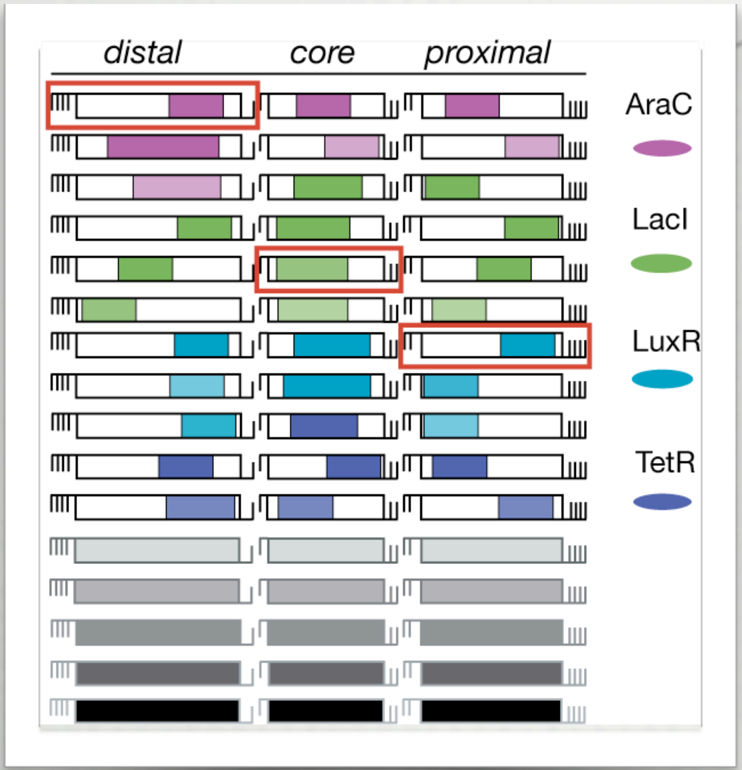

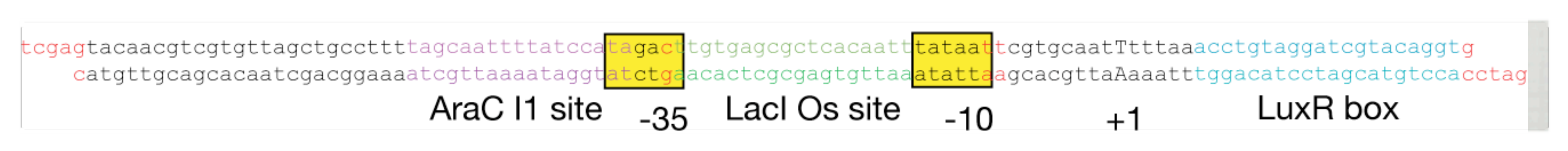

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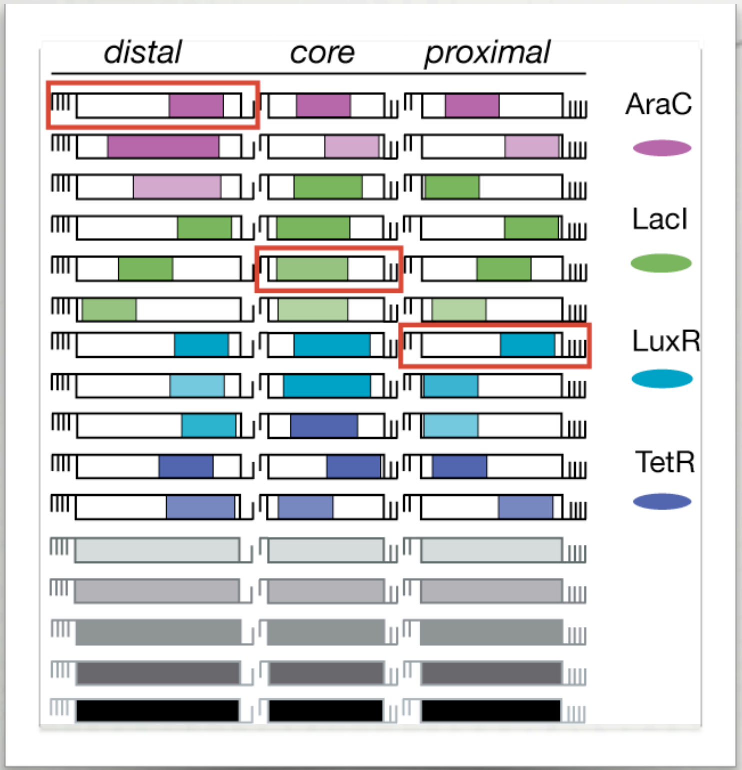

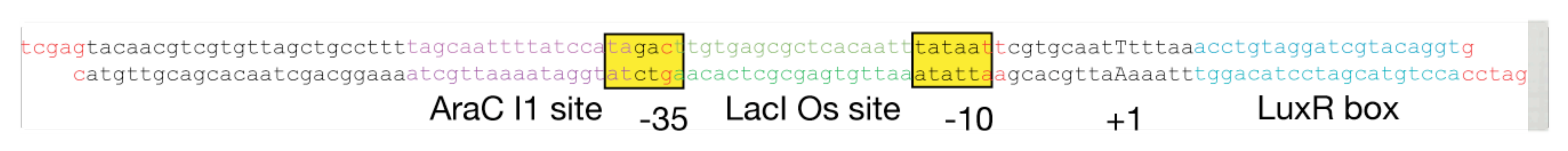

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    catgttgcagcacaatcgacggaaaatcgttaaaataggtatctgaacactcgcgagtgttaaatattaagcacgttaAaaatttggacatcctagcatgtccacctag
```

AraC I1 site -35 LacI Os site -10 +1 LuxR box

288/4096 SEQUENCED

remarks

- ☐ The search is limited to neighbourhood of existing operators; it is really variation
- ☐ The observation is discretized (how robust is that?); who is listening to the outputs intervals;
  - ☐ lacks a composition/impedance study; endogenousness?
- ☐ specificity, name space: possible to engineer chemical/TF specificity? wrt what is this complete?

typology (construction of the phenotype)

- ☐ regulatory range: exp-on/EXP-off [caveat: this is always >1 by def]
- ☐ logic type: from or l=0, to and l=1
- ☐ symmetry: from a=0 (complete symmetry) to A=1 (dependency in only 1 input) [works only for binary functions]

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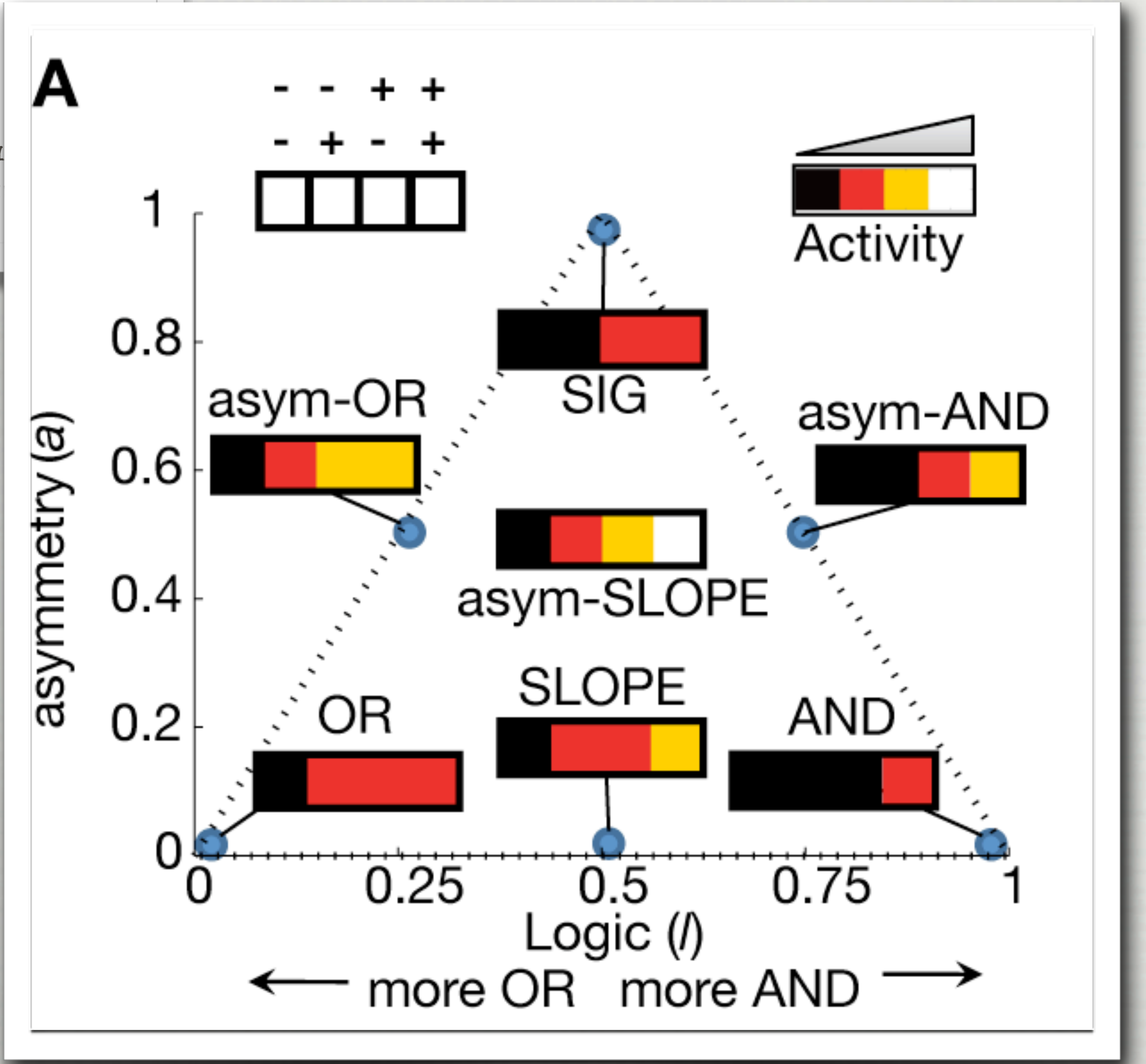

typology (2)

- ☐ The level of TF is controlled undirectly by chemicals, and repressors are repressed, while activators are activated.
- ☐ So whatever the construct is, the attached function is monotonic increasing.
- ☐ The classification scheme -writing $b1<b2<b3<b4$ for the increasing sequence of responses (by monotony b1, and b4 are obtained for 00 and 11 inputs)- is:
  - ☐ - the dynamic range $r= \log(b4/b1)$ in log scale
  - ☐ - the asymmetry $a=\log(b3/b2)/r$ the b3 to b2 gap normalised to r so in 0 (fully symmetric) to 1 (unary function)
  - ☐ - the and-ity $l= (\log(b4) -1/2(\log(b3) + \log(b2)))/r$ which is 0 if $b4=b3=b2$, 1 (an OR) if $b3=b2=b1$ (an AND)

model of RR promoter activity under dual repression (Bintu)

- The r, a, and l trinity above can be defined in terms of the micro-trinity c1, c2, omega measuring the joint activity of a pair of repressors
- P(R1,R2)=A/(c1 R1+c2 R2+omega c1 c2 R1 R2)
  - A max promoter activity
  - c1, c2 TF efficiencies (at excluding RNApol)
  - omega=coooperation (>1)

Looking for Mr Nice component

☐ computational models of transcription (eg "Transcriptional regulation by the numbers" Curr Opin Genet Dev -2005)

☐ evolution driven design (eg "Directed evolution of a genetic circuit" PNAS 2002)

☐ combinatorial approach (this paper)