Workshop in Computer Science

Algorithms for finding sequence motifs in HT-Selex data

Introduction

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Lab instructor: Yaron Orenstein

October 2013

Outline

0. Workshop goals and schedule
1. Basic biology (+ some stories)
2. Gene regulation and motif finding
3. PBM and HT-SELEX

0. Workshop goals and schedule

Motivation

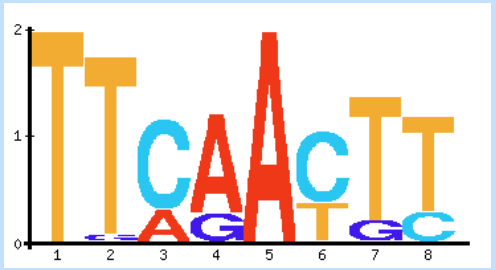
- Biological processes are regulated by genes (and other molecules)
- Understanding how this regulation works is a holy grail of biomedical research
- Many experimental and technological developments aim to achieve this understanding
- Our goal: analyze the data produced by the two newest technologies (HT-SELEX and PBM).

The workshop in a nutshell

1. Input 1: HTS experiment data

Cycle 0	Cycle 1	Cycle 2	Cycle 3
GTCAGTGTCAGTGACGTG	GTCAGTGTCAGTGACGTG	GTCAGTGTCAGTGACGTG	TTCACTCTCGAGGCTAGC
GCGTGACGTGAAGTCGTA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA
GTGCAACGTTGTTTGAGG	GTGCAACGTTGTTTGAGG	GTGCAACGTTGTTTGAGG	GTGCAACGTTGTTTGAGG
GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA
AAGGTGACCGTACGAGTC	AAGGTGACCGTACGAGTC	AAGGTGACCGTACGAGTC	AAGGTGACCGTACGAGTC
GTCCATTCACTGGTGAGT	GTCCATTCACTGGTGAGT	GTCCATTCACTGGTGAGT	GTCCATTCACTGGTGAGT
GTGCAGCGTGAACGTTGG	GTGCAGCGTGAACGTTGG	TTCACTCTCGAGGCTAGC	TTCACTCTCGAGGCTAGC
GTGATTTGGAACACCCAG	GTGATTTGGAACACCCAG	GTGATTTGGAACACCCAG	GTGATTTGGAACACCCAG
CCCCACCCACCGGCCTGC	CCCCACCCACCGGCCTGC	CCCCACCCACCGGCCTGC	TTCACTCTCGAGGCTAGC
ACAGTCAGCCCTAGCACG	TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT
CACATACGCTGACTCGTA	CACATACGCTGACTCGTA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA
TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT
CGATCGATCAGGCTAGCT	CGATCGATCAGGCTAGCT	CGATCGATCAGGCTAGCT	GCGATTAACTCTGTGAAA
...	...	...	...
...	...	...	...
...	...	...	...
TTCACTCTCGAGGCTAGC	TTCACTCTCGAGGCTAGC	TTCACTCTCGAGGCTAGC	TTCACTCTCGAGGCTAGC

2. Develop a method to build a model from the data



3. Input 2: Test data: PBM experiment

Sequence	Signal
CATGTAAGAGTTGACTCTGGTCTGTTCTAAT	28926
TTGCTCATCAGAGTCGCGTAACAGGCTTTC	1457
TCCAGTTTAGGTGGCGCCCGGAACCCTTAA	12972
……	……
CATGTAGCCCTTAACTGTGACTAAAGCCCC	33755

(hidden)

4. Output: prediction. Use the model to predict sequence ranks

Sequence	Rank
CATGTAAGAGTTGACTCTGGTCTGTTCTAAT	11
TTGCTCATCAGAGTCGCGTAACAGGCTTTC	5
TCCAGTTTAGGTGGCGCCCGGAACCCTTAA	200
……	……
CATGTAGCCCTTAACTGTGACTAAAGCCCC	4

5. Evaluate the prediction quality vs. the hidden signal

Administrata

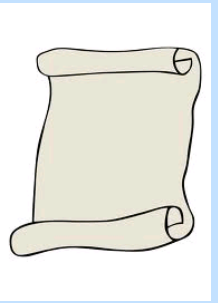
- Project should be written in Java/Linux
- Project can be done in pairs
- Pairs/singletons – please inform Yaron by next Monday who is on your team.
- Meetings – introductory lectures on week 1,2. Then individual meetings with groups (on the workshop time slot)
- Questions? Contact Yaron or me
- Website: www.cs.tau.ac.il/~rshamir/workshop/13

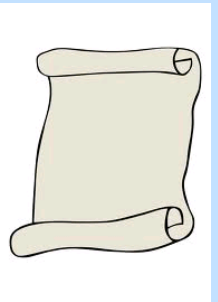
Grading

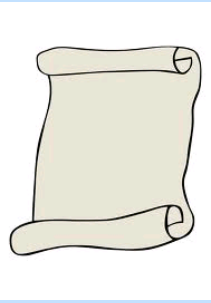
- 15% for the design
- 25% for the implementation (10% for modularity, clarity, documentation, f(r,k)* 15% for efficiency)
- 20% for the final report and presentation
- f(r,k)*50% for the accuracy of the test results
  - f(r,k)*15% for test 1
  - f(r,k)*20% for test 2
  - f(r,k)*15% for test 3
- Where
  - r = group's rank in test out of k groups (top rank r=k)
  - f(r,k) = 0.5+0.5*r/k
- So a uniformly top ranking group can get 110, and uniformly least ranking can still get 82.
- Ties will be scored לבית הילל

Schedule

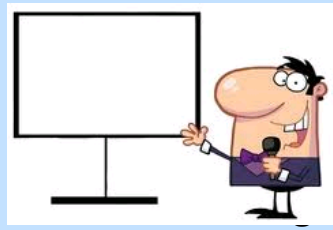
1. 19/11 Individual meetings and first progress report
2. 10/12 Submission of Test 1 results
3. 24/12 submission of Design document
4. 14/1 submission of Test 2 + executable
5. 18/2 Class meeting and final presentation







You are always welcome to meet us.
Contact us by email.



1. Basic Biology

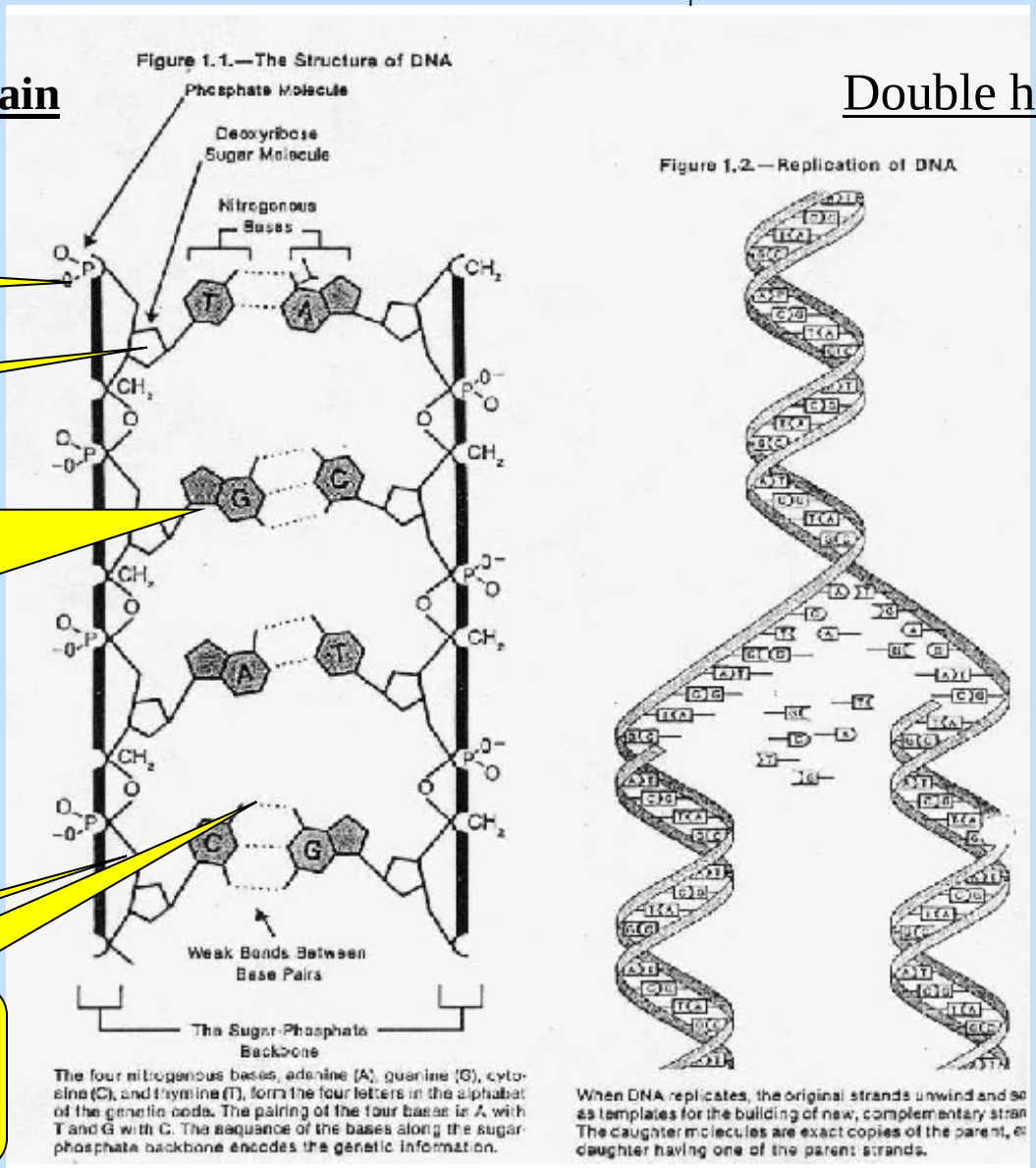
Slides with Adi Akavia

Gregor Mendel
laws of inheritance,"gene"
1866

Watson and Crick
DNA Discovery
1953

Nucleotides Chain

Double helix, 2-stranded helix



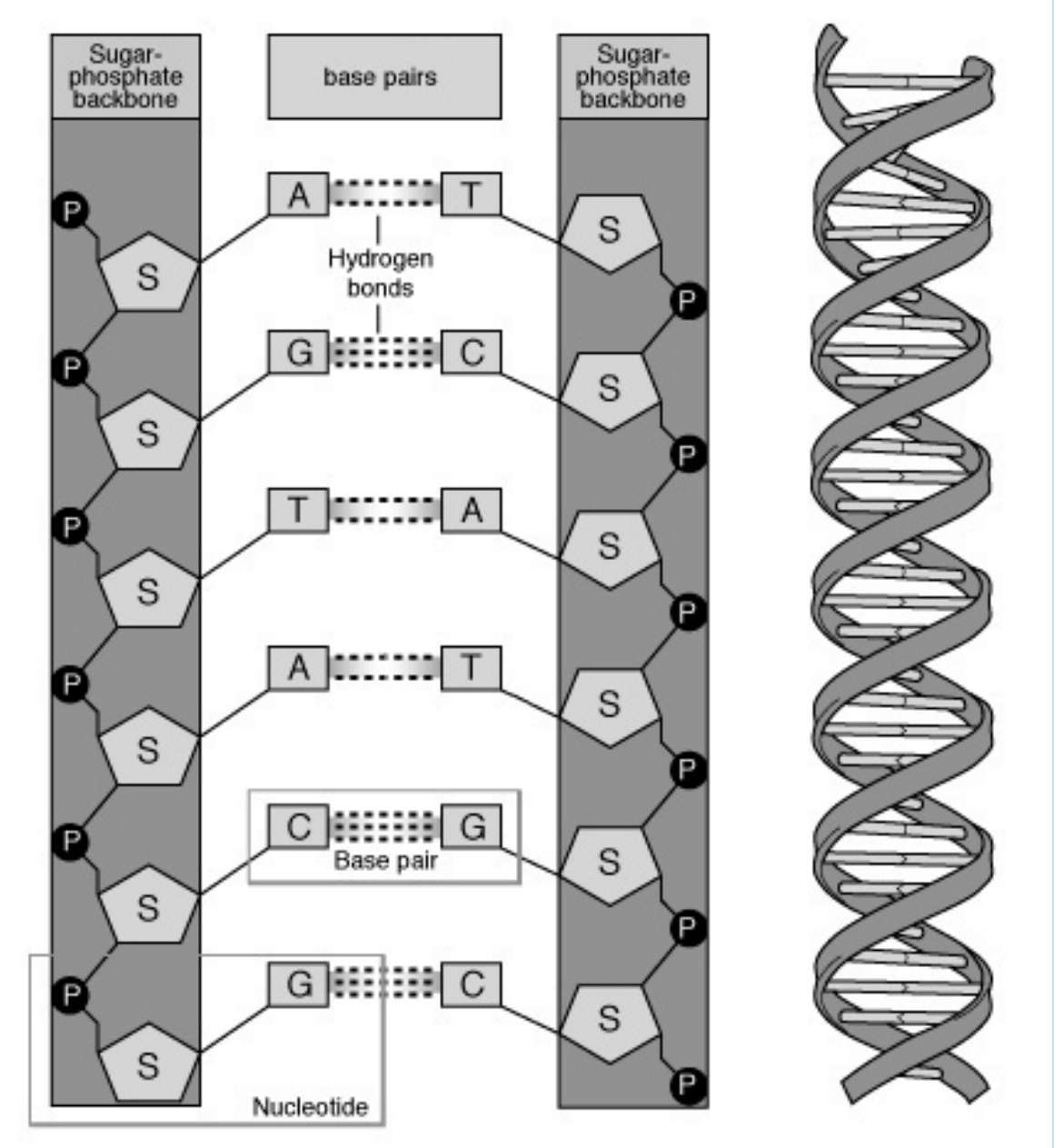
phosphate

sugar

Nucleotides/ Bases:
Adenine (A),
Guanine (G),
Cytosine (C),
Thymine (T).

Backbone

Weak hydrogen bonds between base pairs



http://www.cs.utexas.edu/users/almstrum/s2s/f98/10-5-kidzsoft/src/rnadnatutor.html

DNA (Deoxy-Ribonucleic acid)

- DNA is located in the cell nucleus
- Bases:
  - Adenine (A)
  - Guanine (G)
  - Cytosine (C)
  - Thymine (T)

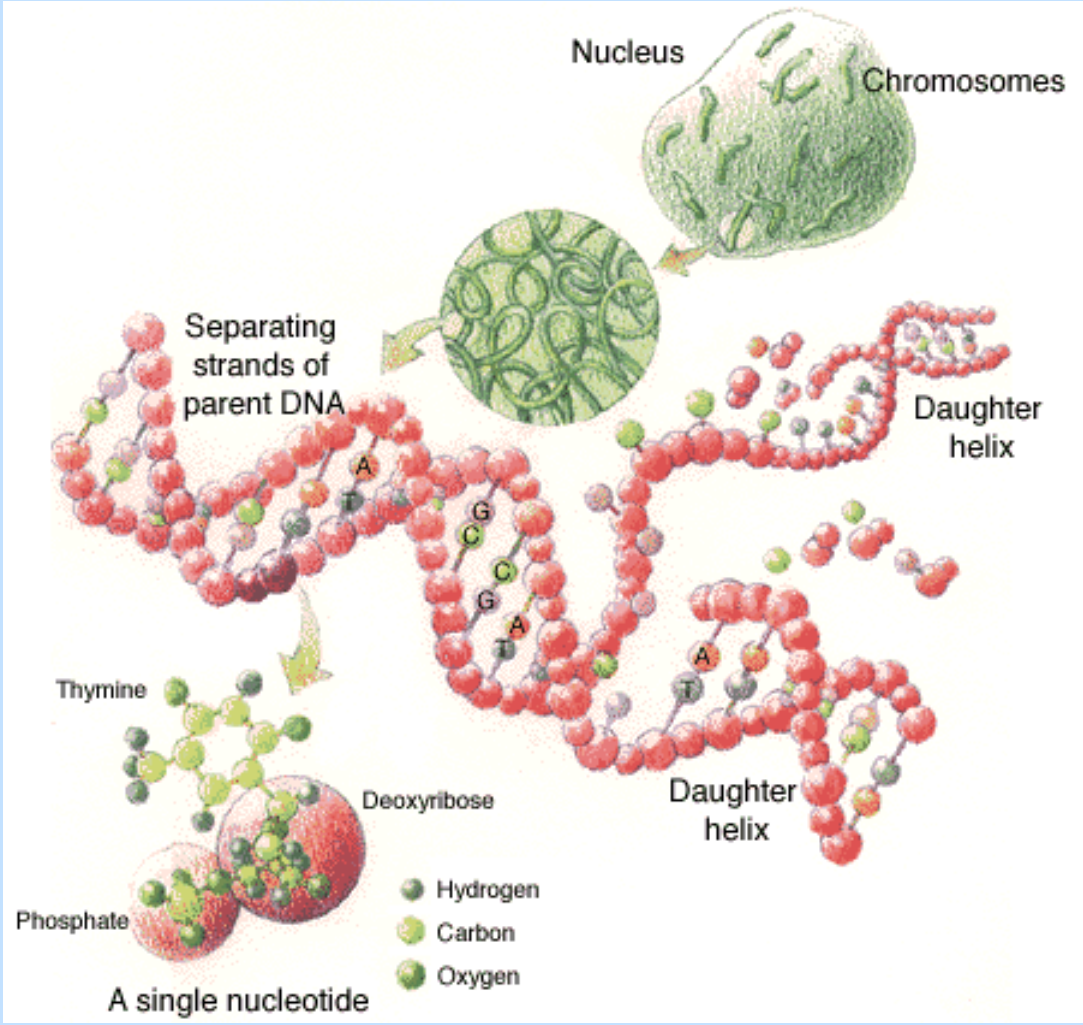
Purine base

pyrimidine base

- Bonds:
  - G - C
  - A - T
- Length of human DNA ~3×10^9 bp (=base pairs)

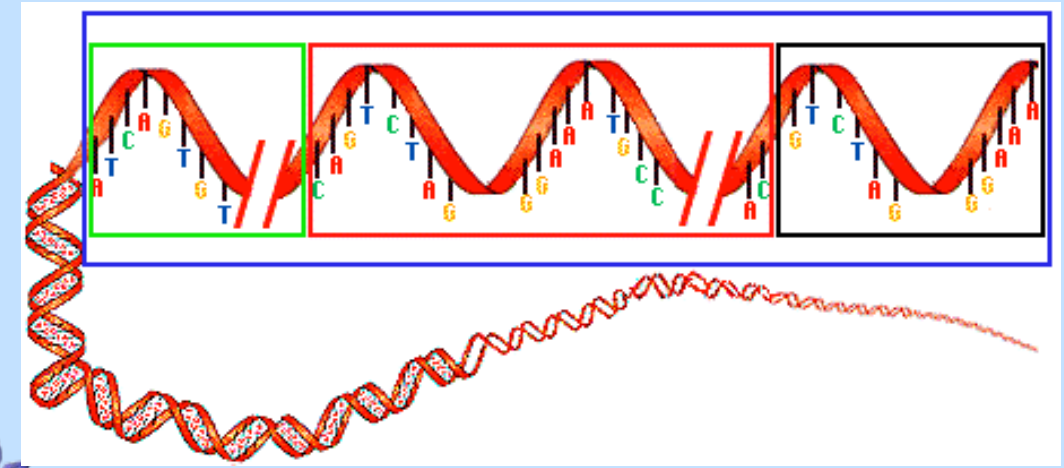
DNA and Chromosomes

- DNA: 4 bases molecule: ACGT
- Complementary strands: A-T; C-G
- Allows duplication
- Chromosome: contiguous stretch of DNA
- Genome: totality of DNA material



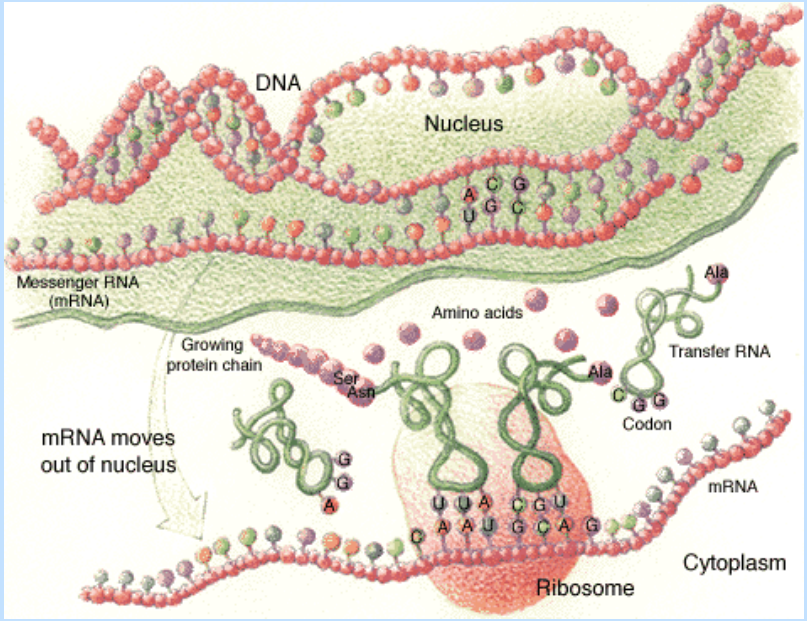
Genes

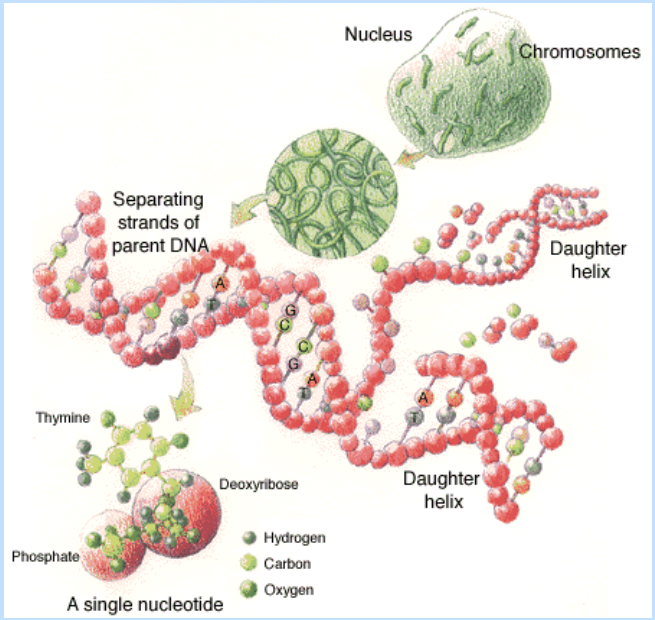
- Gene: a segment of DNA that specifies the sequence of a protein.
- Contains one or more regulatory sequences that either increase or decrease the rate of its transcription
- Genes are 2-3% of human DNA
- the rest - non-coding "junk DNA"



Red: a region that encodes a protein sequence
Black: a non-coding region (a single gene usually contains more than one)
Green: a regulatory sequence

http://morgan.rutgers.edu/MorganWebFrames/Level1/Page1/p1.html





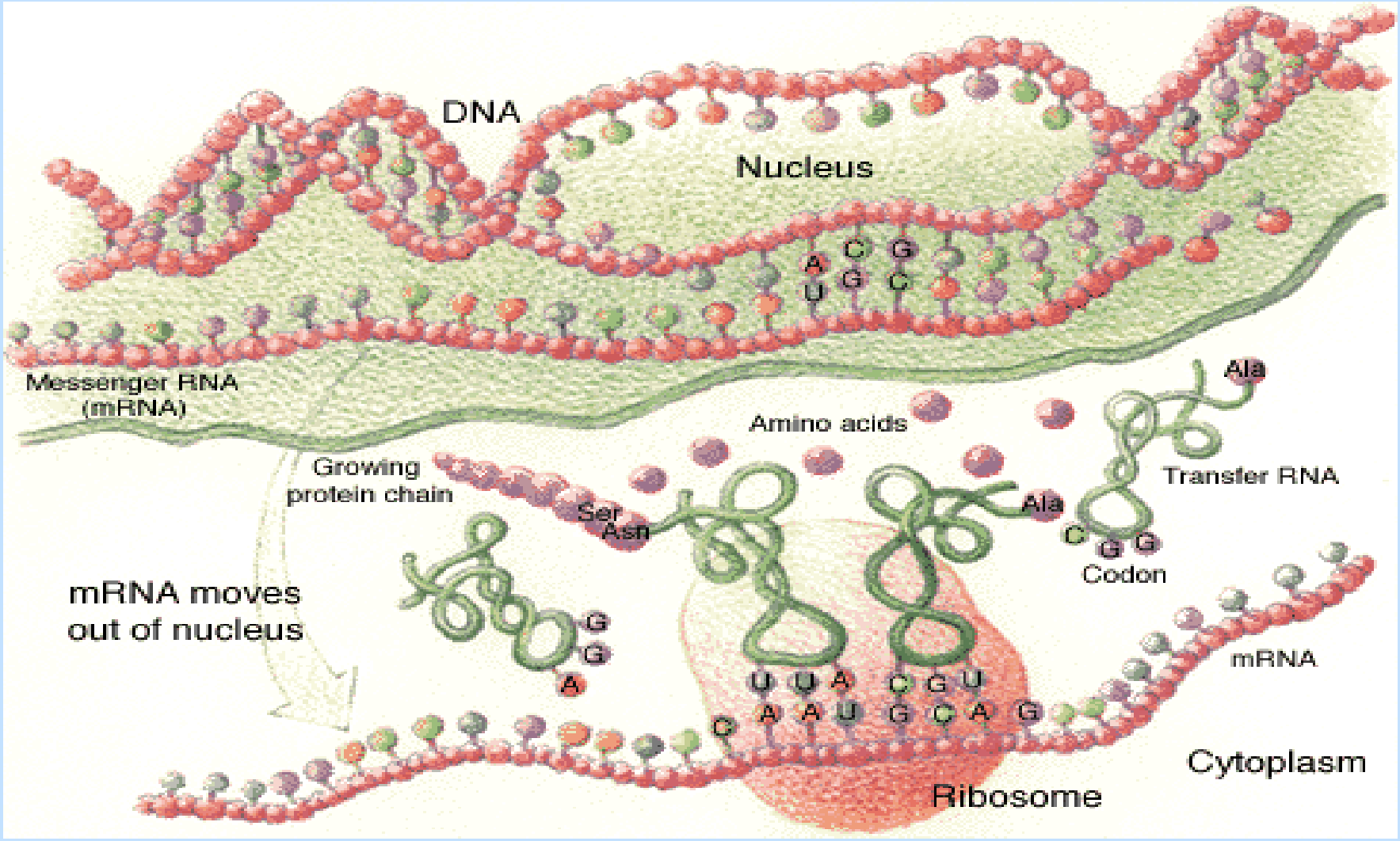
DNA → RNA → protein

The hard disk — **One program** — **Its output**

transcription

translation

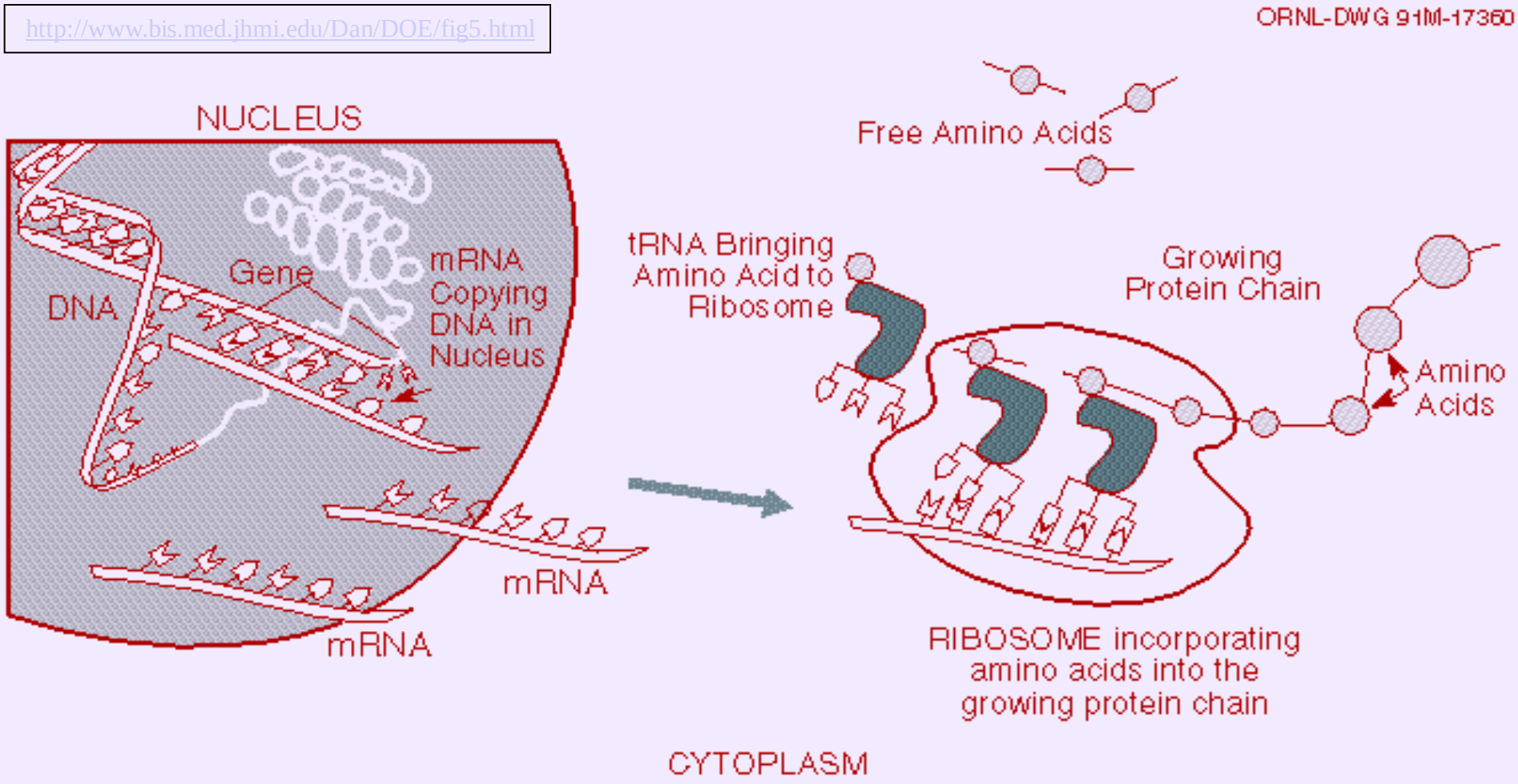
From Gene to Protein



http://www.ornl.gov/hgmis/publicat/tko/index.htm

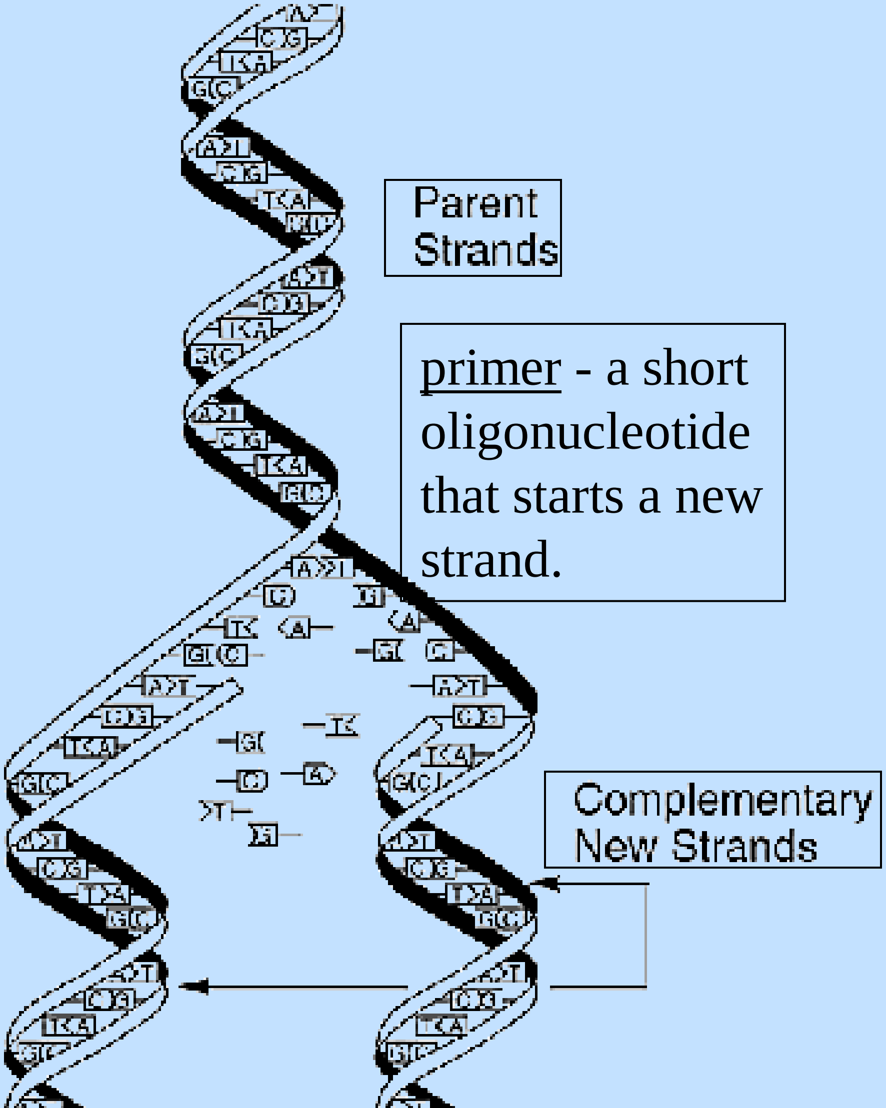
Translating DNA into Protein

http://www.bis.med.jhmi.edu/Dan/DOE/fig5.html



When genes are expressed, the genetic information (base sequence) on DNA is first transcribed (copied) to a molecule of messenger RNA in a process similar to DNA replication. The mRNA molecules then leave the cell nucleus and enter the cytoplasm, where triplets of bases (codons) forming the genetic code specify the particular amino acids that make up an individual protein. This process, called translation, is accomplished by ribosomes (cellular components composed of proteins and another class of RNA) that read the genetic code from the mRNA, and transfer RNAs (tRNAs) that transport amino acids to the ribosomes for attachment to the growing protein.

Replication



primer - a short oligonucleotide that starts a new strand.

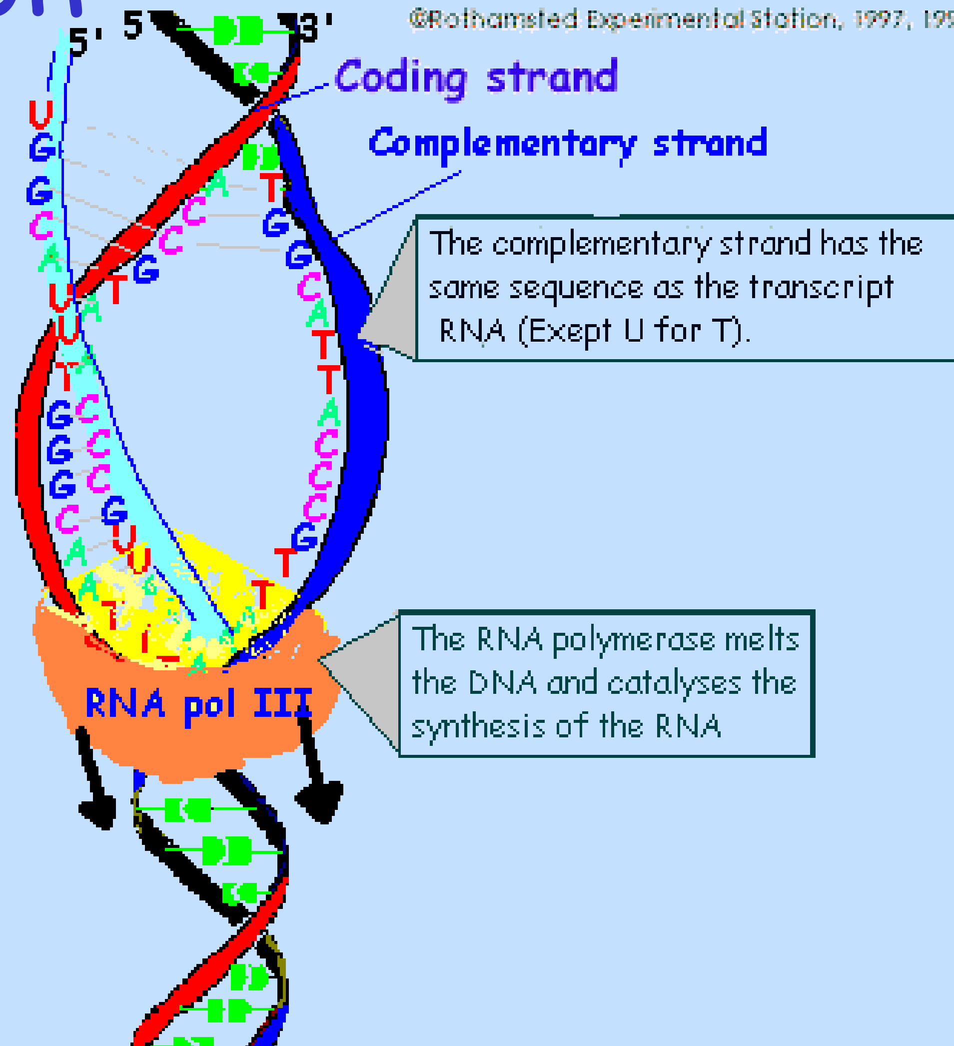


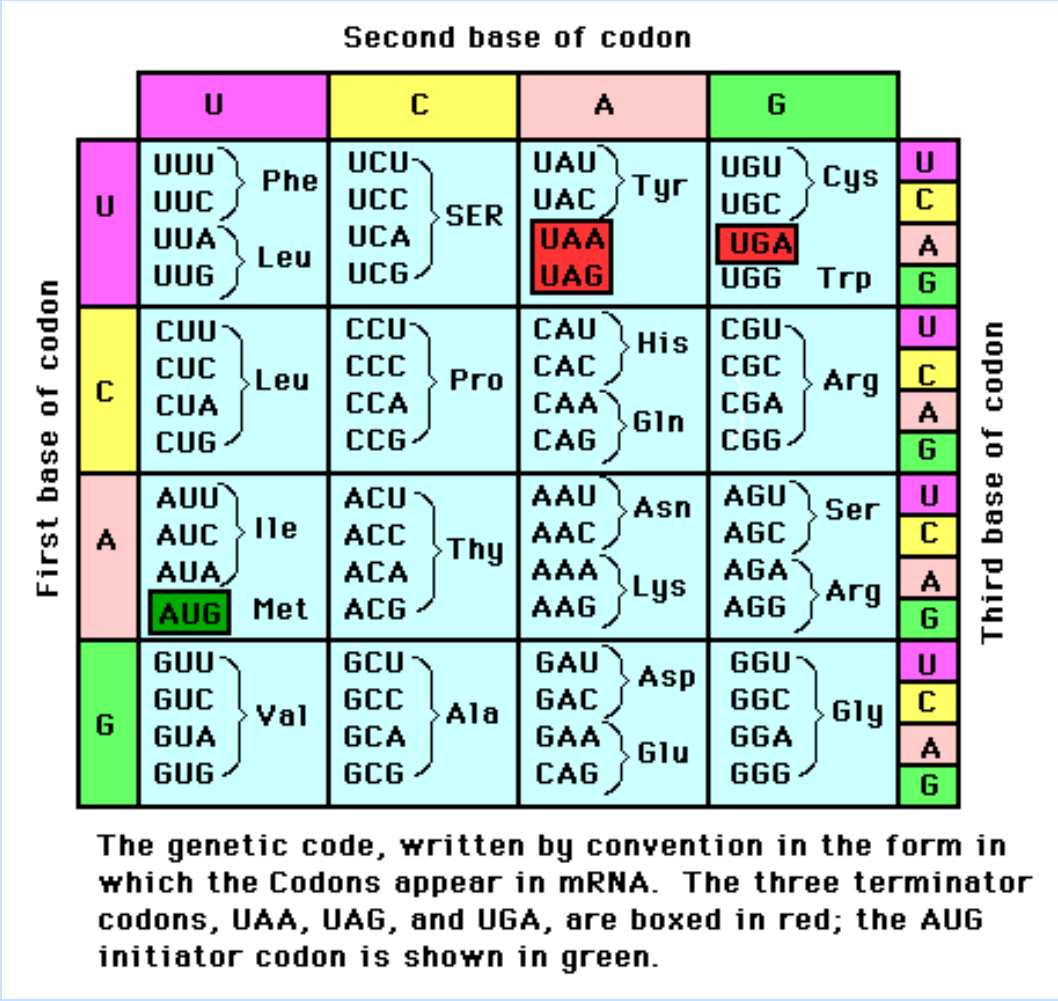
Figure 2:
Transcription of DNA into RNA

http://www.cs.utexas.edu/users/almstrum/cs2s/f98/10-5-kidzsoft/src/rnadnatutor.html

http://www.iacr.bbsrc.ac.uk/notebook/courses/guide/words/transcriptiongif.htm

The Genetic Code

- Codon - a triplet of bases, codes a specific amino acid (except the stop codons)
- Stop codons - signal termination of the protein synthesis process
- Different codons may code the same amino acid



The genetic code, written by convention in the form in which the Codons appear in mRNA. The three terminator codons, UAA, UAG, and UGA, are boxed in red; the AUG initiator codon is shown in green.

http://ntri.tamuk.edu/cell/ribosomes.html

The Human Genome: numbers

- 23 pairs of chromosomes
- ~3,200,000,000 bases
- ~25,000 genes
- Gene length: 1000-3000 bases, spanning 30-40,000 bases
- ~1,000,000 protein variants

Hybridization

- DNA double strands form by "gluing" of complementary single strands
- Complementarity rule: A-T, G-C

TGAGGC
| | | | | |
ACTCCG

Use probe to identify if target contains a particular sequence

The Human Genome Project

- Project planned for 15 years, initiated 1990
- US budget: 3 billion Dollars
- Main players: US, Europe, Japan
- Over 50 participating laboratories

2. Introduction to Promoter Analysis

Slides with Chaim Linhart

Regulation of Expression

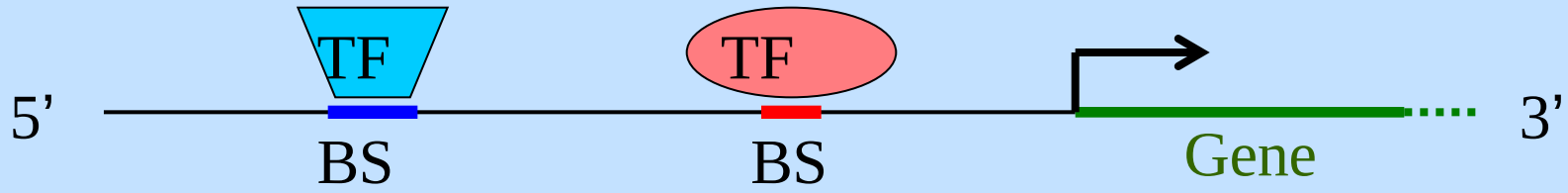
- Each cell contains a copy of the whole genome - but utilizes only a subset of the genes
- Most genes are highly regulated – their expression is limited to specific tissues, developmental stages, physiological condition

How is the expression of genes regulated?

One way is through *transcriptional regulation*

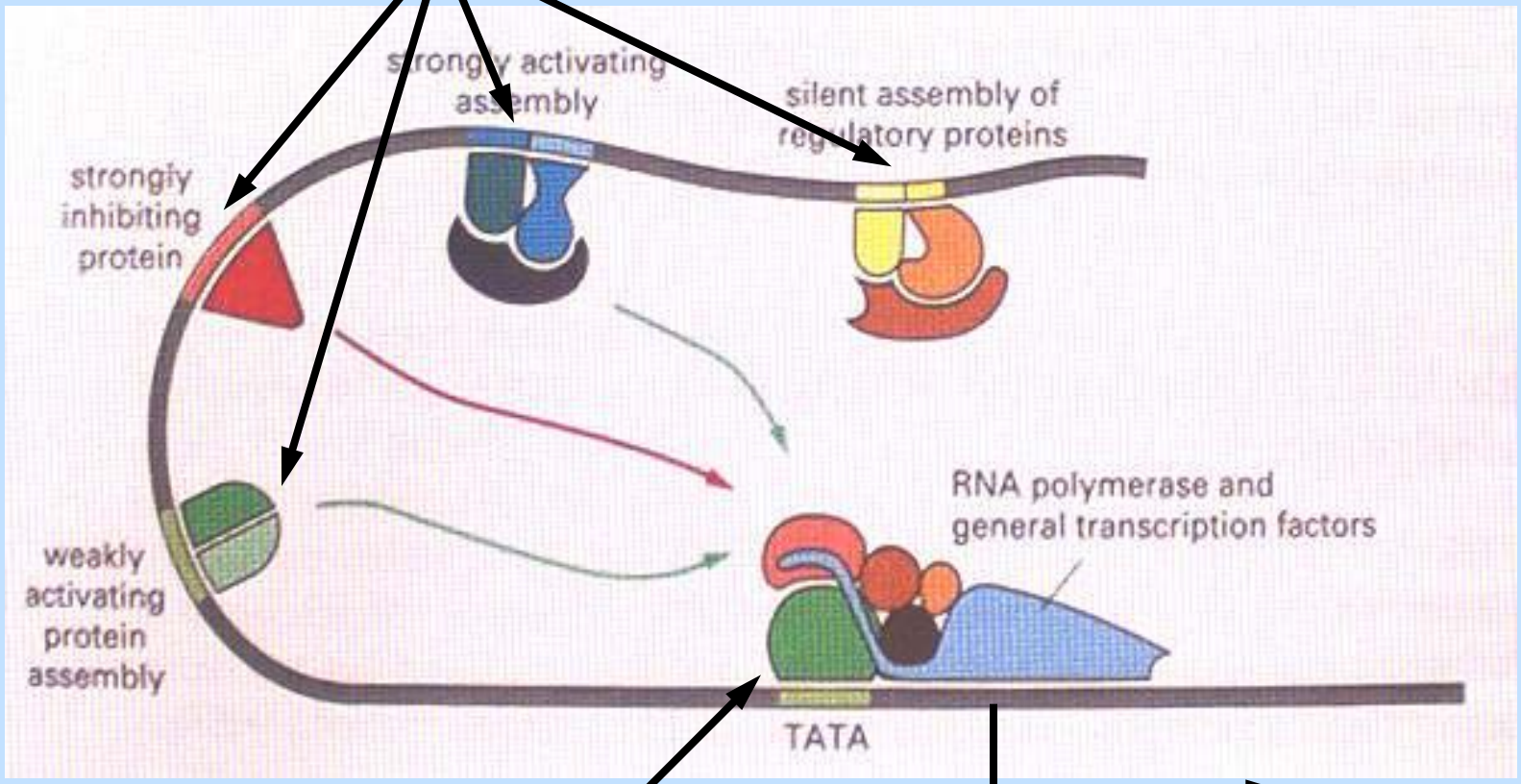
Regulation of Transcription

- A gene's ranscription regulation is mainly encoded in the DNA in a region called the promoter
- Each promoter contains several short DNA subsequences, called binding sites (BSs) that are bound by specific proteins called transcription factors (TFs)



Regulation of Transcription (II)

TFs bound to their BSs

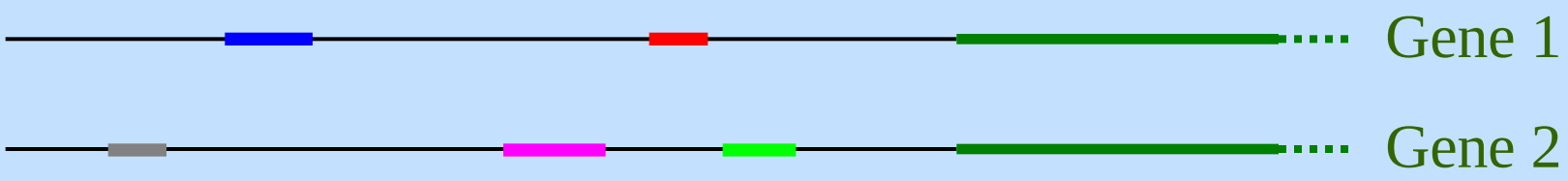


Transcription machinery

Gene start

Regulation of Transcription (III)

- By binding to a gene's promoter, TFs *promote* or *repress* the recruitment of the transcription machinery
- A gene's transcription is determined by the specific combination of BSs in its promoter



WH-questions

- √ Why are we looking for common BSs?
- *What* exactly are we trying to find?
- *Where* should we look for it?
- *How* can we find it?

Models for Binding Sites

(I) Exact string(s)

Example:

BS = TACACC , TACGGC

CAATGCAGGATACACCGATCGGTA

GGAGTACGGCAAGTCCCCATGTGA

AGGCTGGACCAGACTCTACACCTA

(II) String with mismatches

Example:

BS = TACACC + 1 mismatch

CAATGCAGGATTCACCGATCGGTA

GGAGTACAGCAAGTCCCCATGTGA

AGGCTGGACCAGACTCTACACCTA

(III) Degenerate string

a.k.a consensus

Example:

BS = TASDAC (S={C,G} D={A,G,T})

CAATGCAGGATACAACGATCGGTA

GGAGTAGTACAAGTCCCCATGTGA

AGGCTGGACCAGACTCTACGACTA

(IV) Position Weight Matrix (PWM)

a.k.a Position Specific Scoring Matrix (PSSM)

Example:

Need to set score threshold

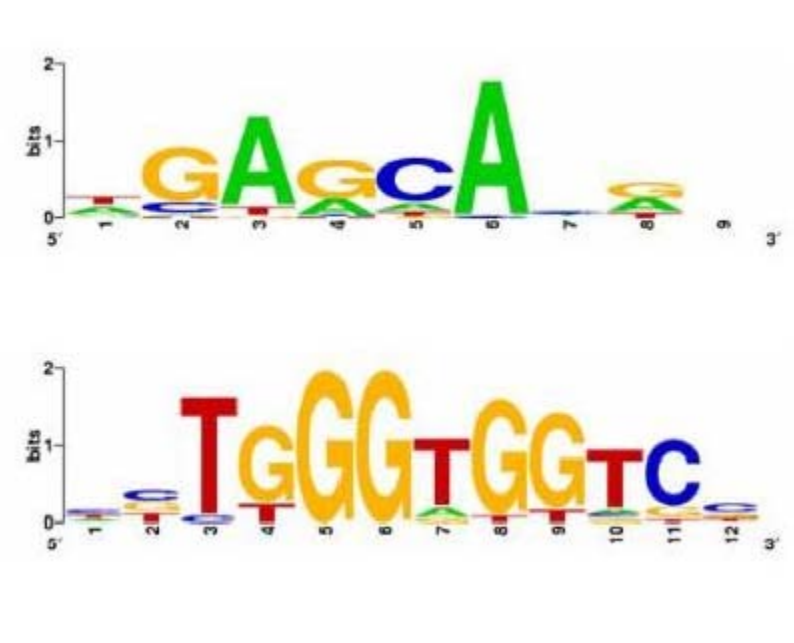
A	0.1	0.8	0	0.7	0.2	0
C	0	0.1	0.5	0.1	0.4	0.6
G	0	0	0.5	0.1	0.4	0.1
T	0.9	0.1	0	0.1	0	0.3

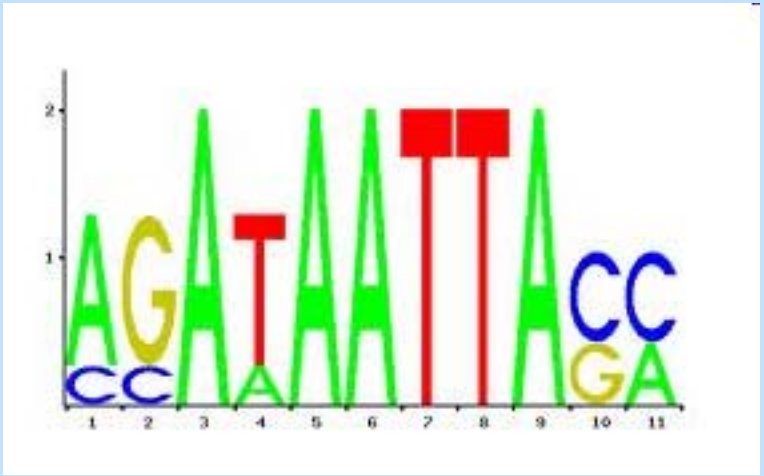
ATGCAGGATACACCGATCGGTA 0.0605

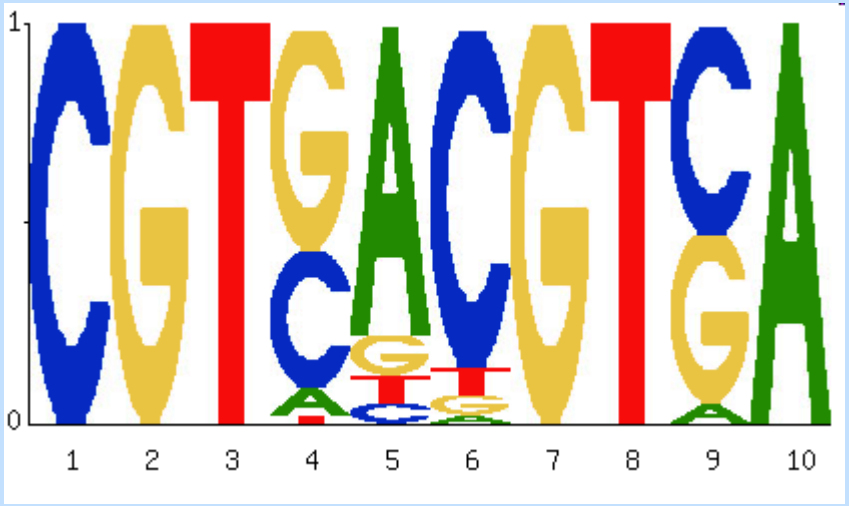
GGAGTAGAGCAAGTCCCGTGA 0.0605

AAGACTCTACAATTATGGCGT 0.0151

Motif Logo representations

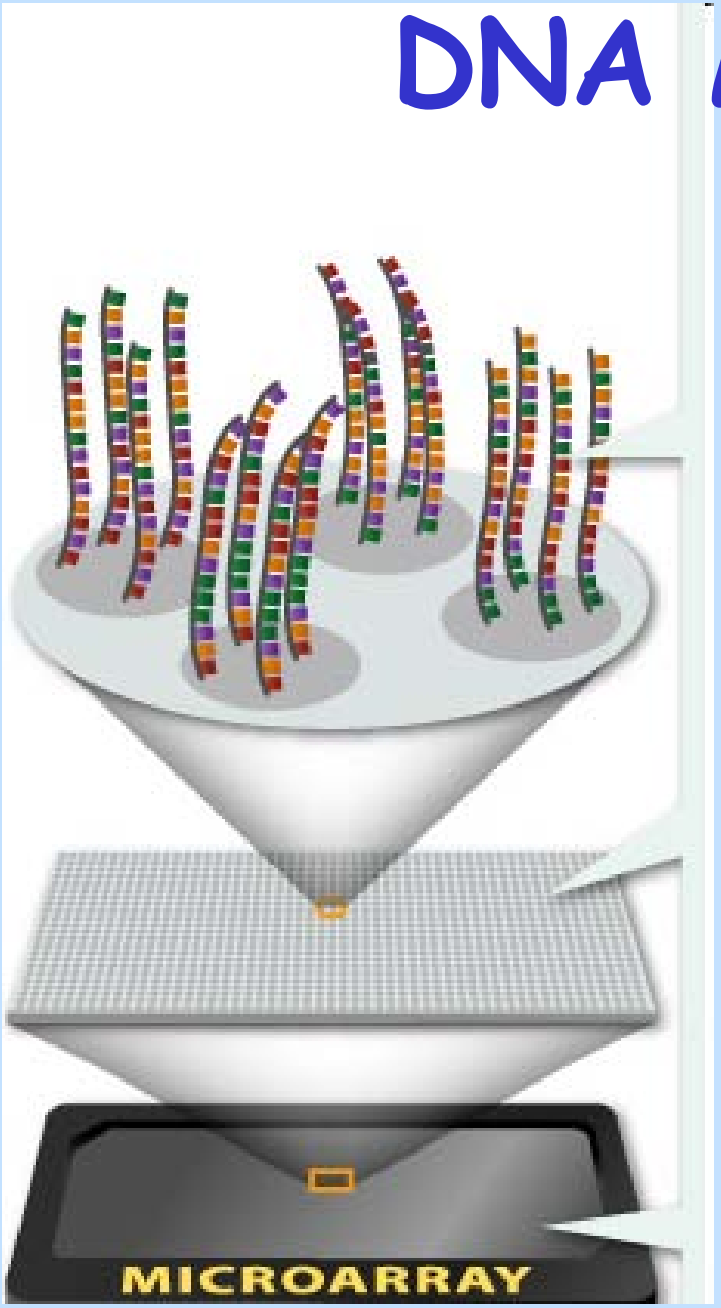






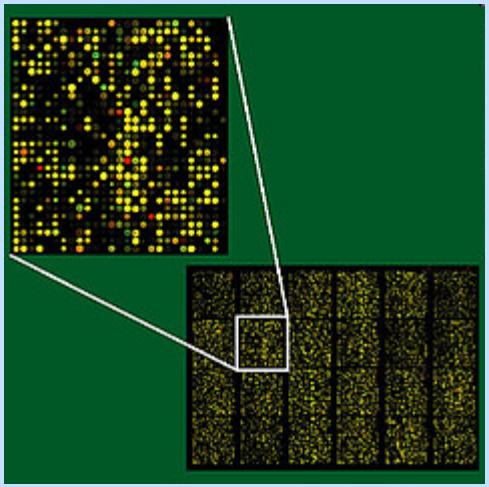
3. PBM and HT-SELEX

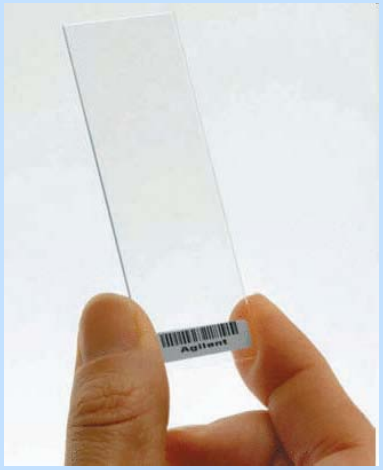
DNA Microarrays



- A DNA microarray allows scientists to perform an experiment on thousands of genes at the same time.
- Each spot on a microarray contains multiple identical strands of DNA.
- The DNA sequence on each spot is unique.
- Each spot represents one gene.
- Thousands of spots are arrayed in orderly rows and columns on a solid surface (usually glass).
- The precise location and sequence of each spot is recorded in a computer database.
- Microarrays can be the size of a microscope slide, or even smaller.

http://learn.genetics.utah.edu/content/labs/microarray/analysis/



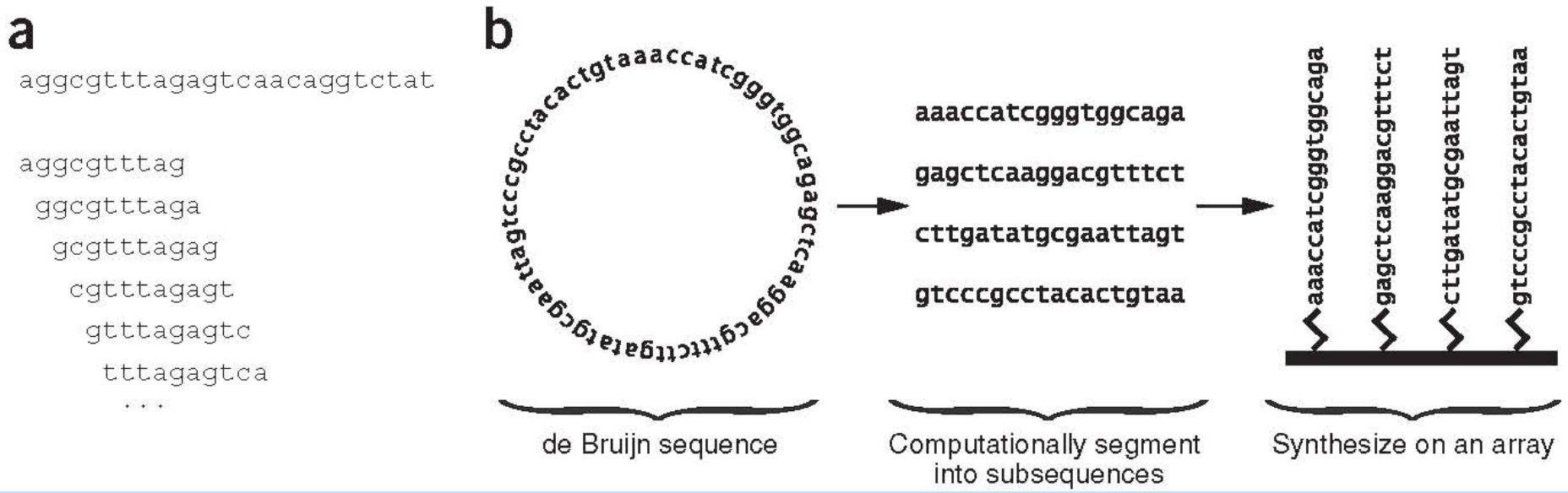

Agilent


GeneChip®

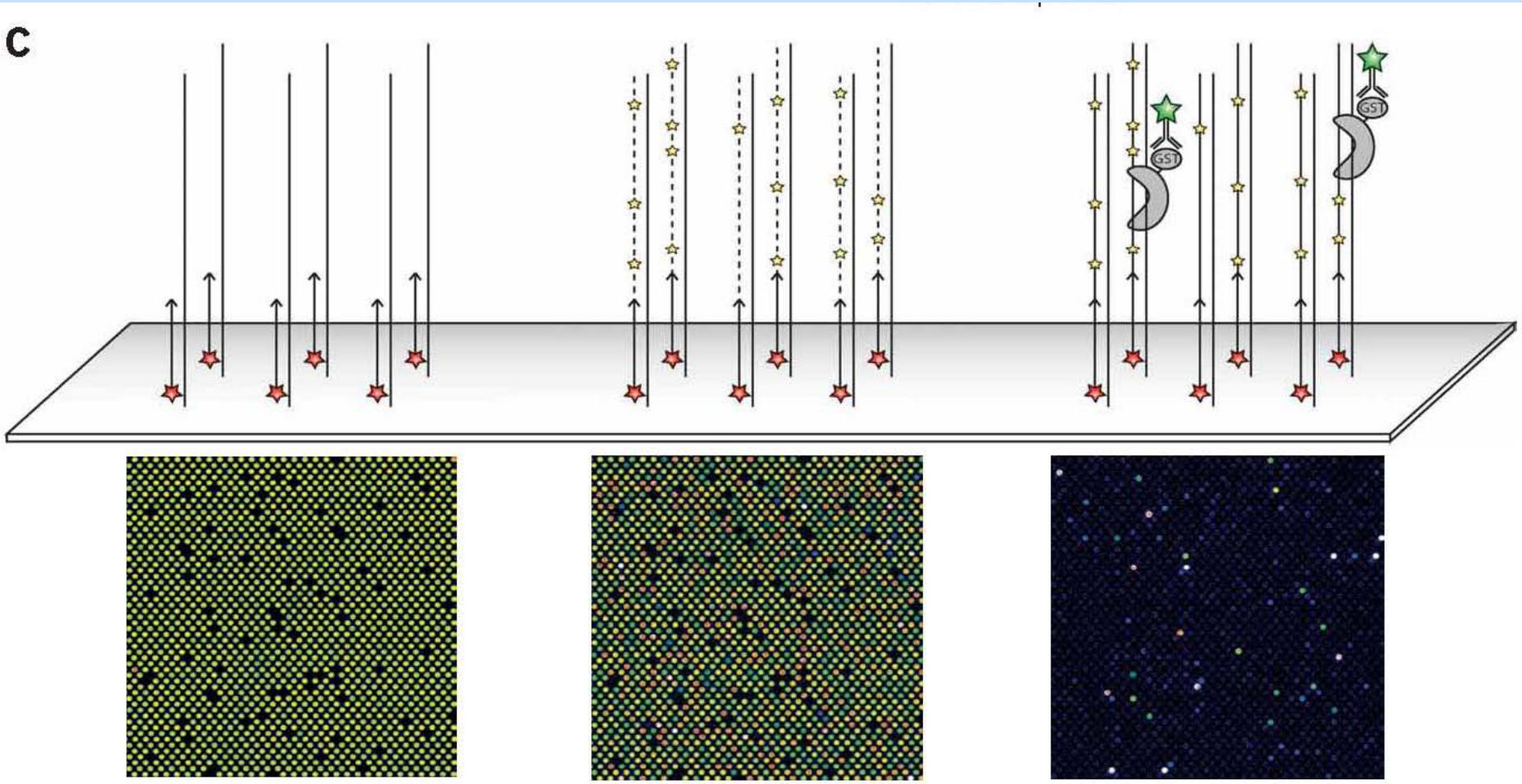
Protein Binding Microarrays

Berger et al, Nat. Biotech 2006

- Generate an array of double-stranded DNA with all possible 10-mers



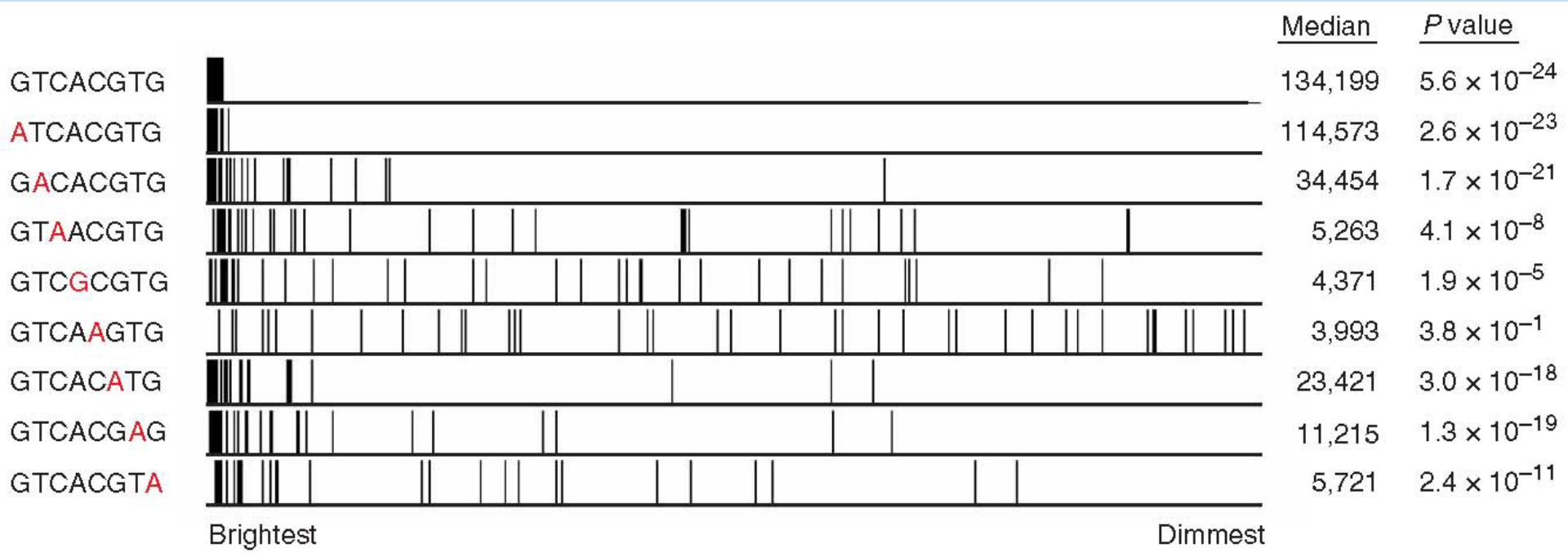
PBM (2)



PBM - implementation

- ~41K probes, each 35nt long
- Probes contain all possible 10-mers
- Experiment gives binding intensity of the TF to each probe

- For each 8-mer, can combine signals from all probes that contain it (or differ in 1nt) to obtain signal


Median
P value
GTCACGTG
134,199
5.6 × 10−24
ATCACGTG
114,573
2.6 × 10−23
GACACGTG
34,454
1.7 × 10−21
GTAACGTG
5,263
4.1 × 10−8
GTCGCGTG
4,371
1.9 × 10−5
GTCAAGTG
3,993
3.8 × 10−1
GTCACATG
23,421
3.0 × 10−18
GTCACGAG
11,215
1.3 × 10−19
GTCACGTA
5,721
2.4 × 10−11
Brightest
Dimmest

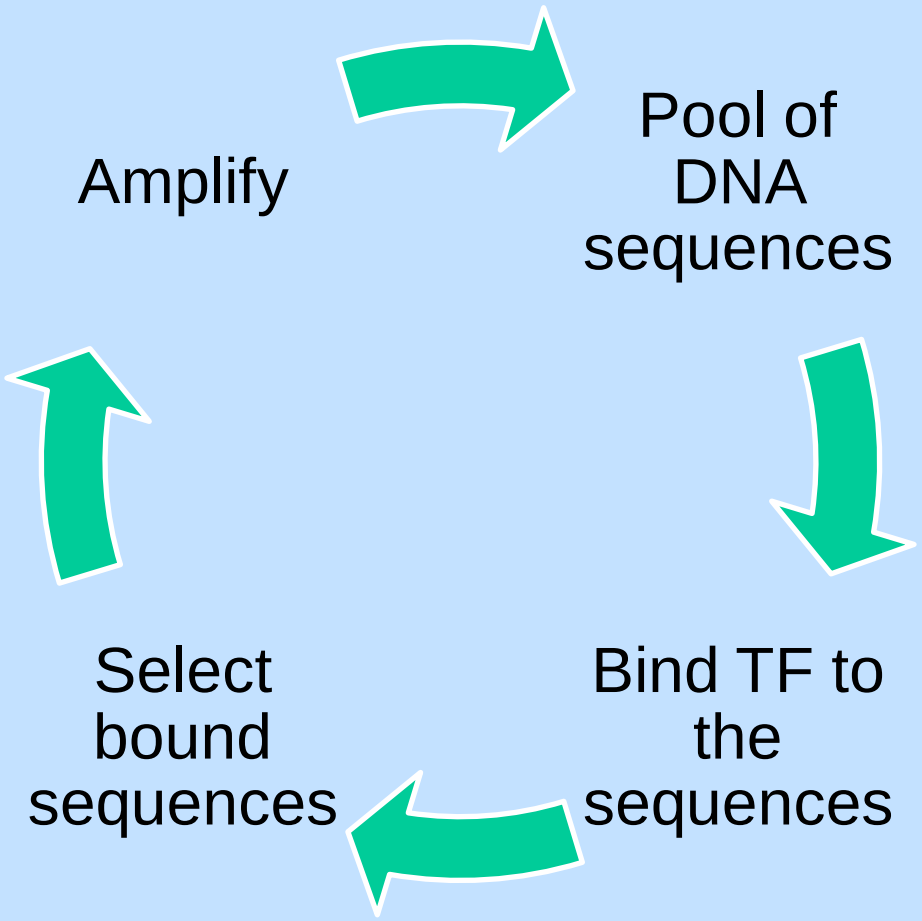
SELEX

Systematic Evolution of Ligands by EXponential enrichment

Start with a random pool of double-stranded DNA sequence of a fixed length (20-50nt)

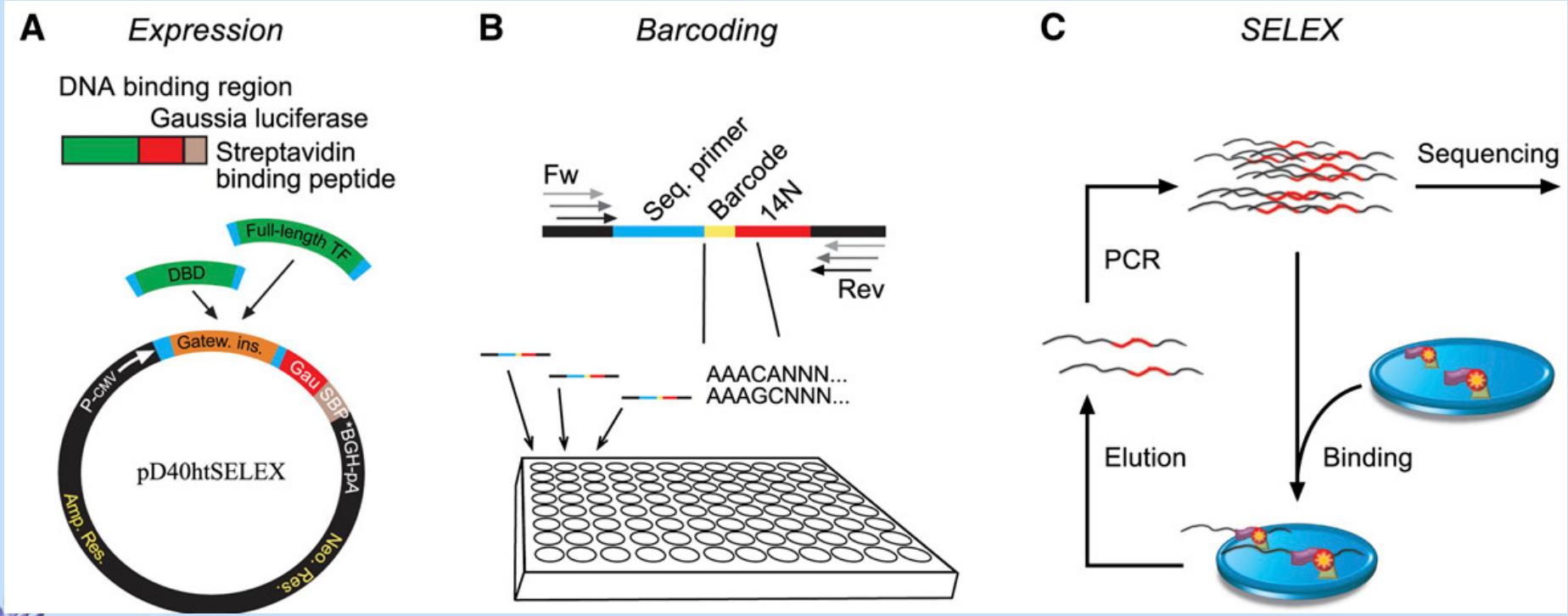
Each cycle contains higher fraction of bound sequences

After each cycle – sequence a sample from the pool



High-Throughput SELEX (HT-SELEX)

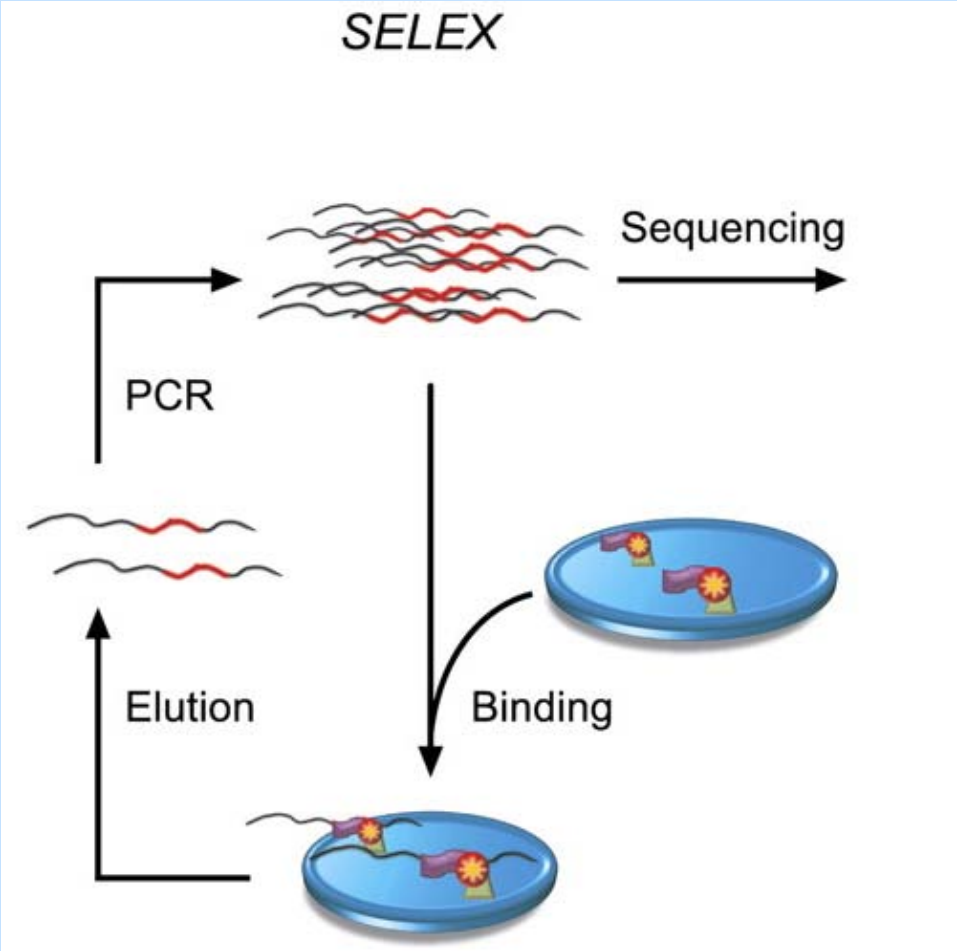
- Same – but using high throughput technologies (in particular ultra cheap and fast deep sequencing techniques)



Jolma et al, Genome Res 2010)

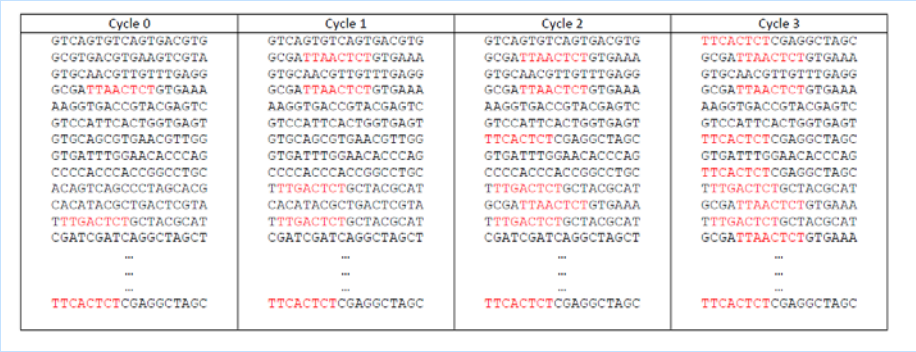
HT-SELEX (2)

- Output: for each of cycles 0,1,..4/5 , a sample of the sequences present.
- Sequence length: 14-50
- Sample size: thousands to millions per cycle



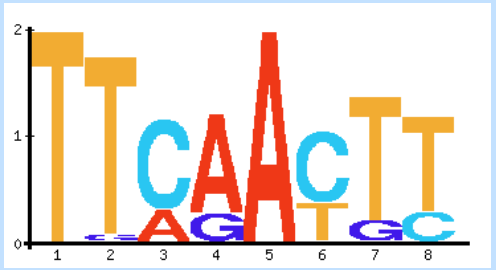
The workshop in a nutshell (again)

1. Input 1: HTS experiment data



Cycle 0	Cycle 1	Cycle 2	Cycle 3
GTCAGTGTCAGTGACGTG	GTCAGTGTCAGTGACGTG	GTCAGTGTCAGTGACGTG	TTCACTCTCGAGGCTAGC
GCGTGACGTGAAGTCGTA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA
GTGCAACGTTGTTTGAGG	GTGCAACGTTGTTTGAGG	GTGCAACGTTGTTTGAGG	GTGCAACGTTGTTTGAGG
GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA
AAGGTGACCGTACGAGTC	AAGGTGACCGTACGAGTC	AAGGTGACCGTACGAGTC	AAGGTGACCGTACGAGTC
GTCCATTCACTGGTGAGT	GTCCATTCACTGGTGAGT	GTCCATTCACTGGTGAGT	GTCCATTCACTGGTGAGT
GTGCAGCGTGAACGTTGG	GTGCAGCGTGAACGTTGG	TTCACTCTCGAGGCTAGC	TTCACTCTCGAGGCTAGC
GTGATTTGGAACACCCAG	GTGATTTGGAACACCCAG	GTGATTTGGAACACCCAG	GTGATTTGGAACACCCAG
CCCCACCCACCGGCCTGC	CCCCACCCACCGGCCTGC	CCCCACCCACCGGCCTGC	TTCACTCTCGAGGCTAGC
ACAGTCAGCCCTAGCACG	TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT
CACATACGCTGACTCGTA	CACATACGCTGACTCGTA	GCGATTAACTCTGTGAAA	GCGATTAACTCTGTGAAA
TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT	TTTGACTCTGCTACGCAT
CGATCGATCAGGCTAGCT	CGATCGATCAGGCTAGCT	CGATCGATCAGGCTAGCT	GCGATTAACTCTGTGAAA
...	...	...	...
...	...	...	...
...	...	...	...
TTCACTCTCGAGGCTAGC	TTCACTCTCGAGGCTAGC	TTCACTCTCGAGGCTAGC	TTCACTCTCGAGGCTAGC

2. Develop a method to build a model from the data



3. Input 2: Test data: PBM experiment

Sequence	Signal
CATGTAAGAGTTGACTCTGGTCTGTTCTAAT	28926
TTGCTCATCAGAGTCGCGTAACAGGCTTTC	1457
TCCAGTTTAGGTGGCGCCCGGAACCCTTAA	12972
……	……
CATGTAGCCCTTAACTGTGACTAAAGCCCC	33755

(hidden)

4. Output: prediction. Use the model to predict sequence ranks

Sequence	Rank
CATGTAAGAGTTGACTCTGGTCTGTTCTAAT	11
TTGCTCATCAGAGTCGCGTAACAGGCTTTC	5
TCCAGTTTAGGTGGCGCCCGGAACCCTTAA	200
……	……
CATGTAGCCCTTAACTGTGACTAAAGCCCC	4

5. Evaluate the prediction quality vs. the hidden signal

References

HT-SELEX:

- Zhao Y, Granas D and Stormo GD. **Inferring binding energies from selected binding sites**. PLoS Computational Biology. 2009;5(12):e1000590.
- Jolma A, Kivioja T, Toivonen J, Cheng L, Wei GH, Enge M, Taipale M, Vaquerizas JM, Yan J, Sillanpaa MJ, Bonke M, Palin K, Talukder S, Hughes TR, Luscombe NM, Ukkonen E and Taipale J. **Multiplexed massively parallel SELEX for characterization of human transcription factor binding specificities**. Genome Research. 2010;20:861-873
- Slattery M, Riley T, Liu P, Abe N, Gomez-Alcala P, Dror I, Zhou T, Rohs R, Honig B, Bussemaker HJ and Mann RS. **Cofactor binding evokes differences in DNA binding specificity between Hox proteins**. Cell. 2011;147:1270-1282.

PBM:

- Berger MF, Philippakis AA, Qureshi AM, He FS, EstepIII PW, Bulyk ML. **Compact, universal DNA microarrays to comprehensively determine transcription-factor binding site specificities**. Nature biotechnology. 2006;338:1429-1435.

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