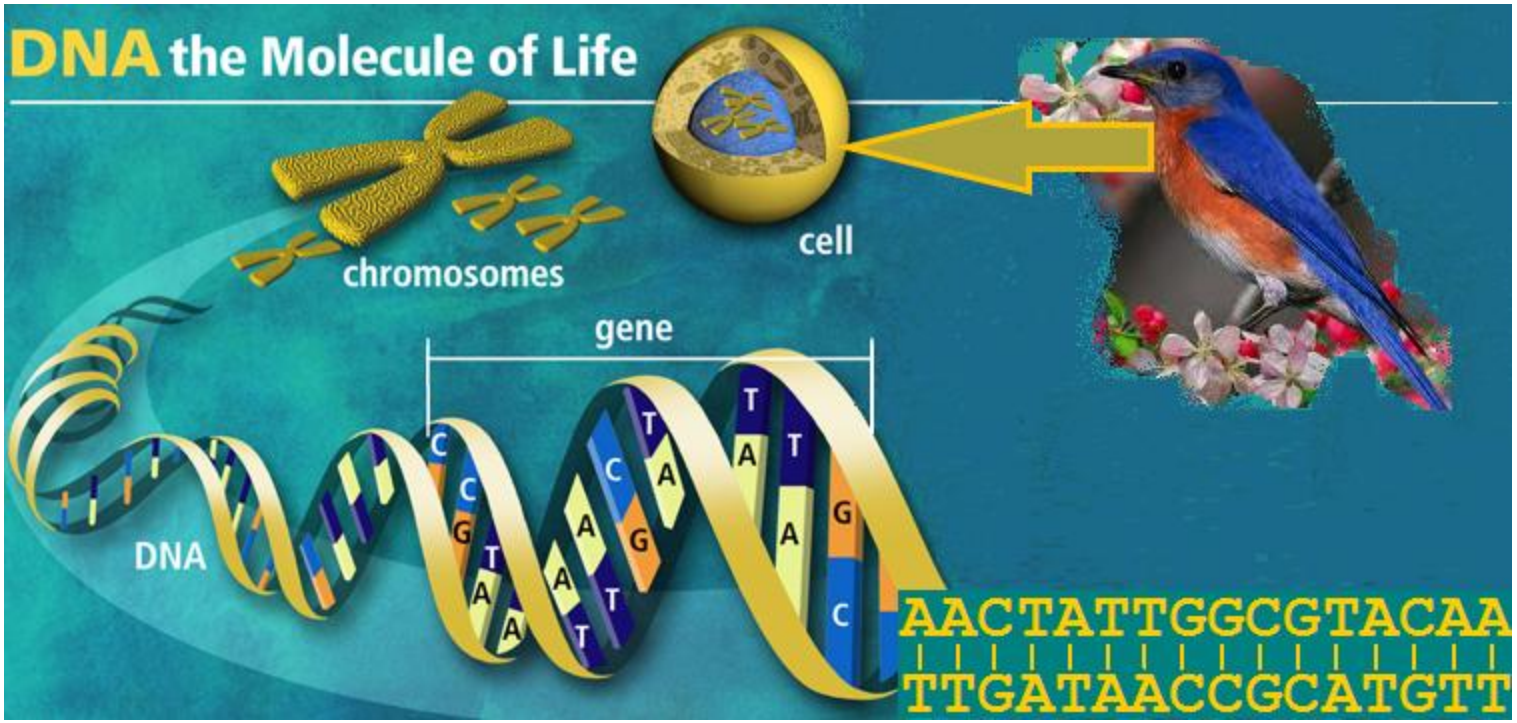
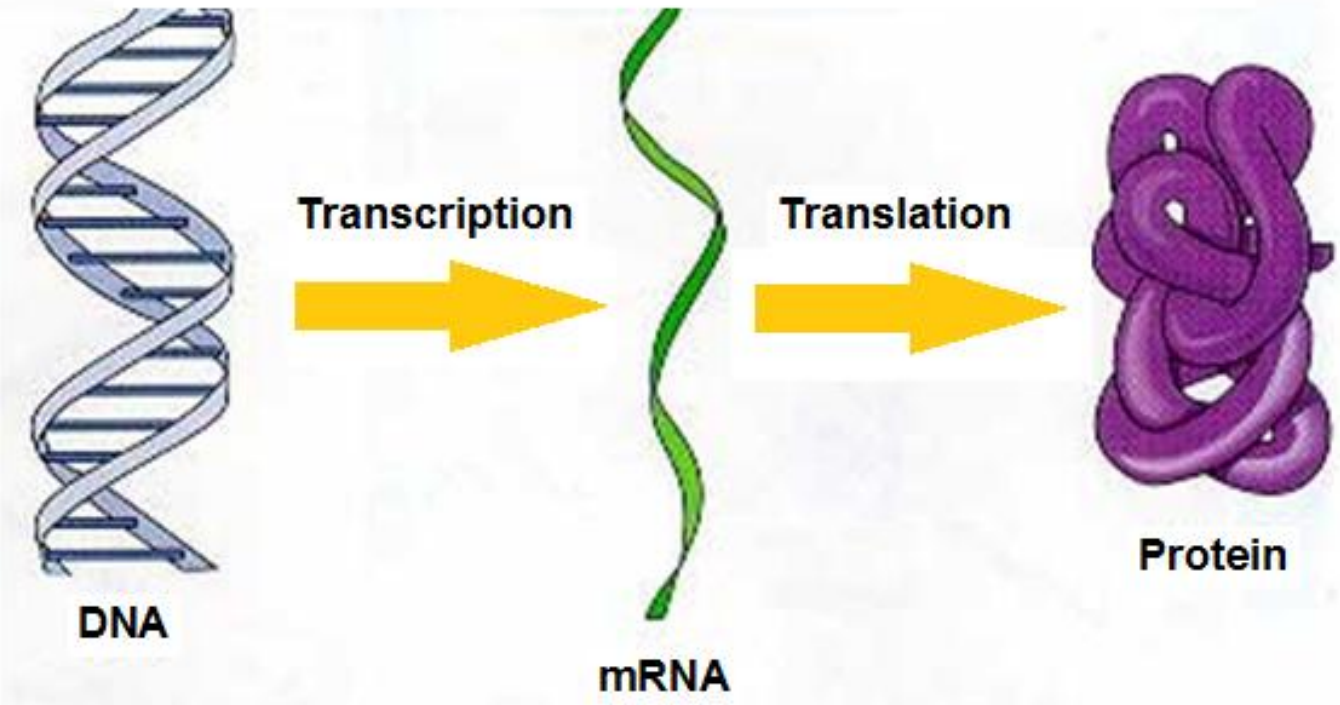
Mapping Reads to Reference Genome



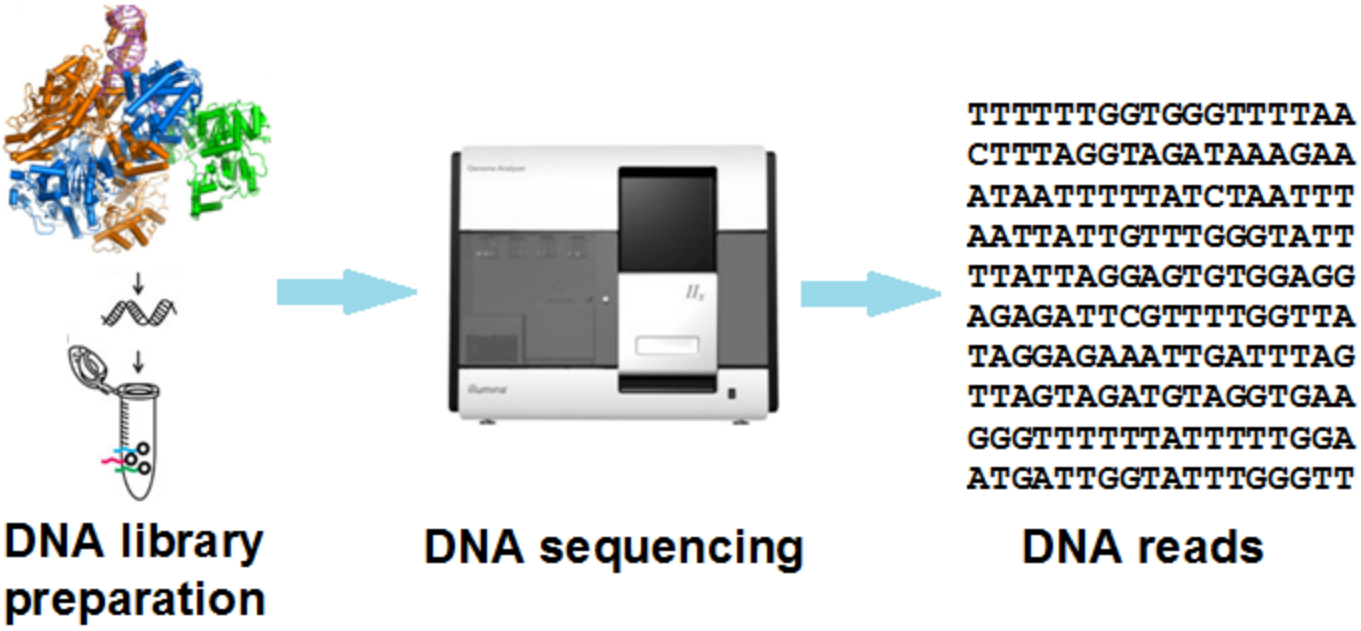
- DNA carries genetic information
- DNA is a double helix of two complementary strands formed by four nucleotides (bases):

  Adenine, **C**ytosine, **G**uanine and **T**hymine

The Central Dogma of Molecular Biology



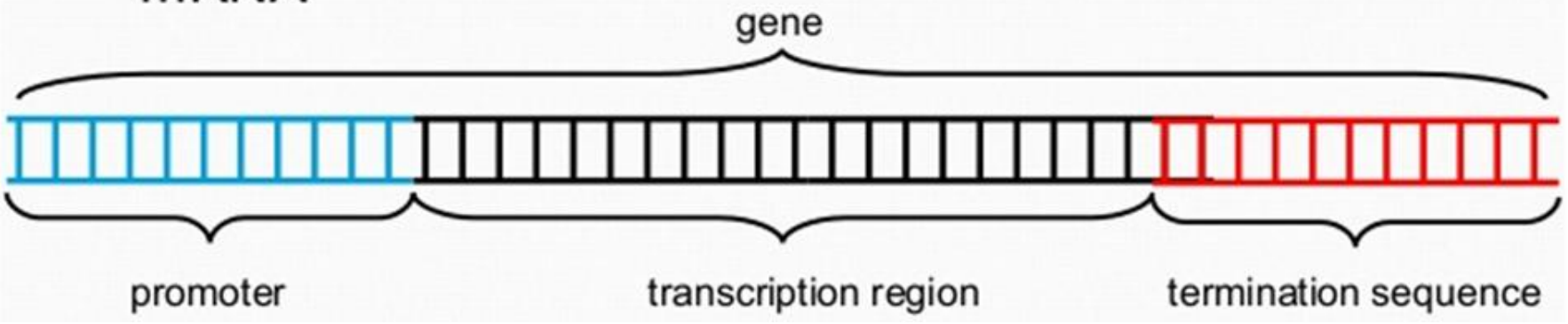
- **Gene expression** is the process by which DNA is transcribed into mRNA (eventually translated into proteins)
- Mechanisms controlling gene expression are not fully understood yet

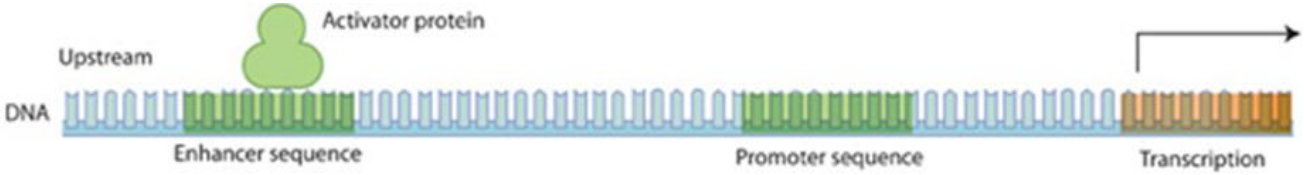


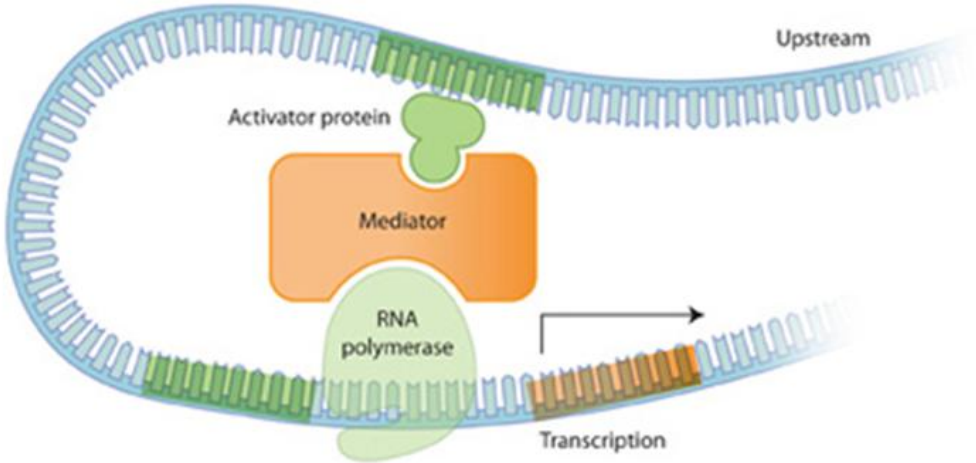
- New-generation sequencing technology allows fast and inexpensive DNA sequencing
- Helps biologists to study cellular processes

Example: Identify Transcription Factors binding sites

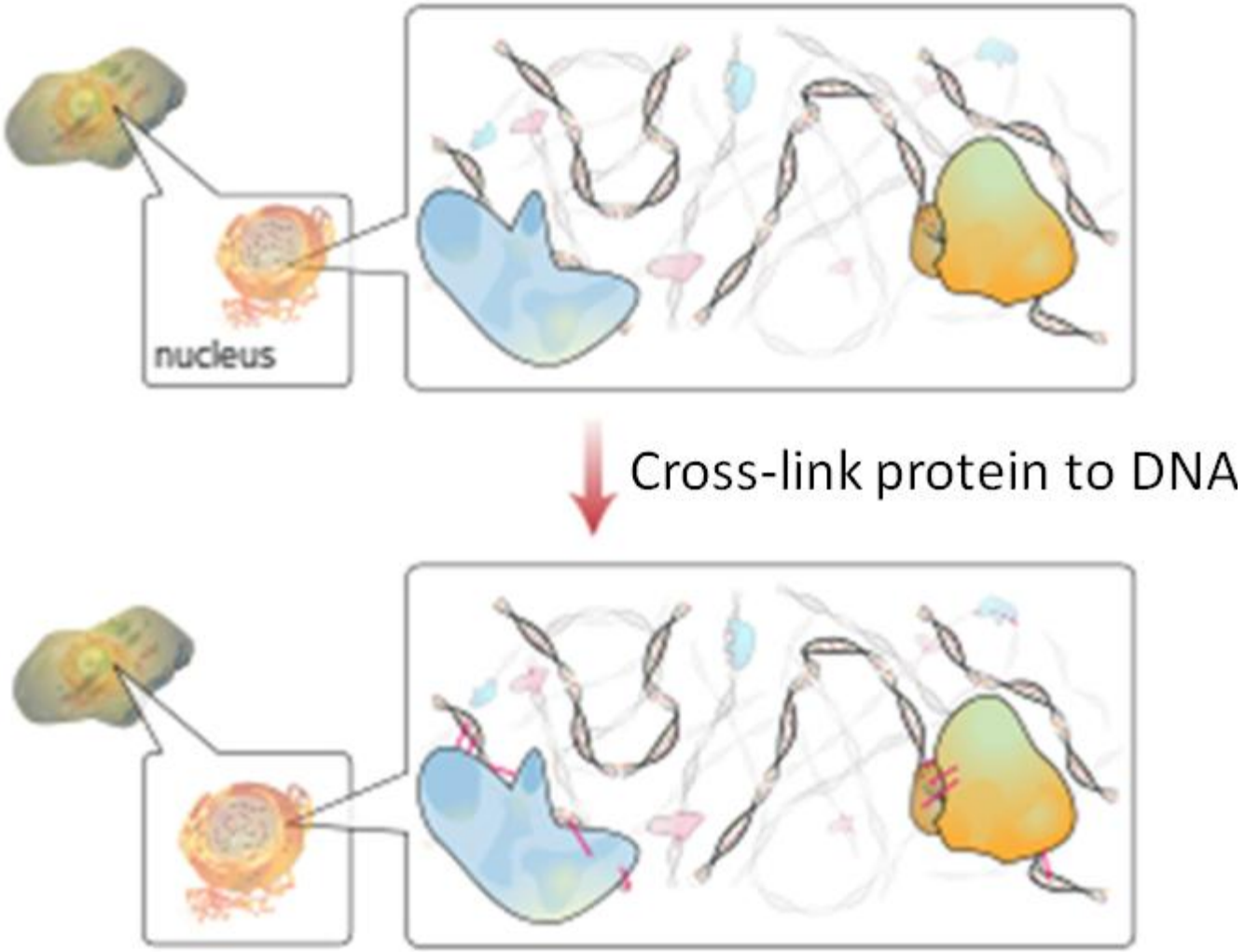
Promoters are located upstream from the DNA region that contains the information to be transcribed into mRNA





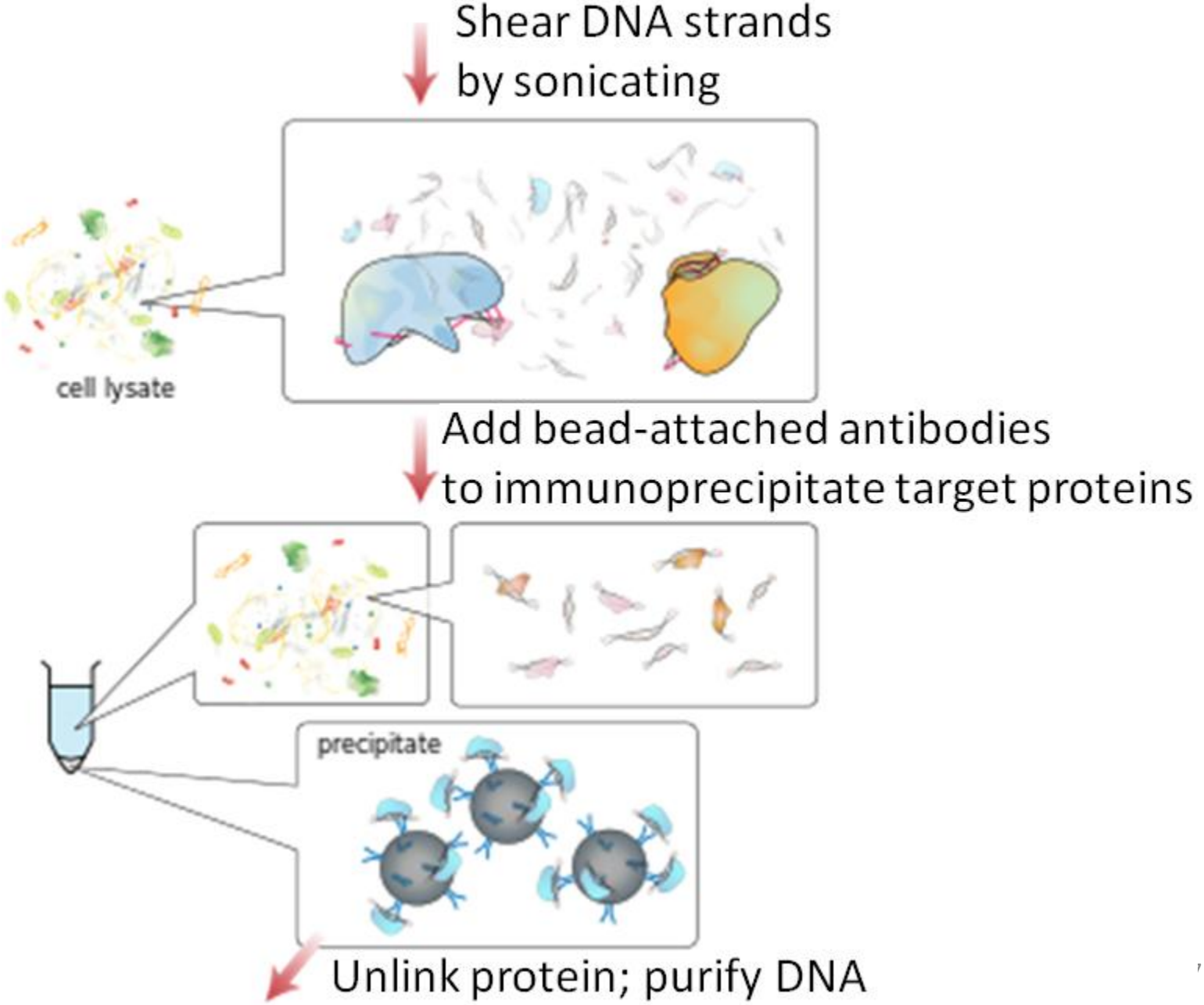


Example: Identify Transcription Factors binding sites

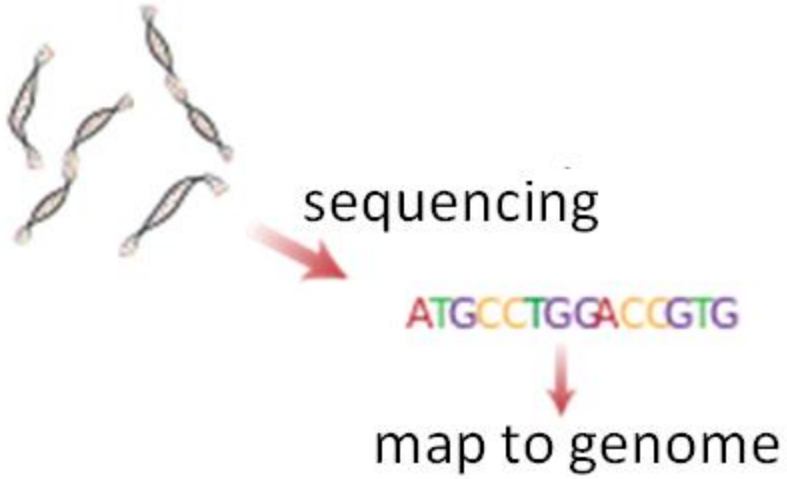


Cell diagram adapted from LadyOfHats' Animal Cell diagram. Wikipedia.

Example: Identify Transcription Factors binding sites



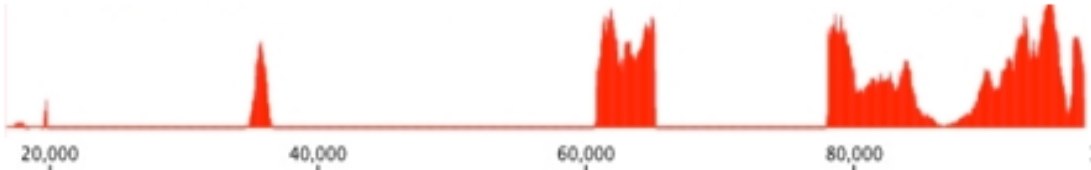
Example: Identify Transcription Factors binding sites



Reference genome

```
TATATTTATGCTATTCAGTTCTAAATATAGAAATTGAAACAGCTGTGTTTAGTGCCTTTGTTCA-----ACCCCCTTGCAACAACCTTGAGAACCCCAGGGAATTTGT
TATATT ATGCTATTCAGTTCTAAATATAGAAATTGAAACAG  GTGTTTAGTGCCTTTGTTCA-----ACCCCCTTGCAACAAC      aaccccagggaatttgt
tatatttatgctattcagttctaaatatagaaatt   acagctgtgtttagtgcctttgttca-----acccccttg aacaaccttgagaaccccagggaatttgt
TATAT TATGCTATTCAGTTCTAAATATAGAAATTGAAACA ctgtgtttagtgcctttgttca-----acccccttgcaac ACCTTGAGAACCCCAGGGAATTTGT
TATATTTA gctattcagttctaaatatagaaattgaaacagct  GTTTAGTGCCTTTGTTCACATAGACCCCCTTGCAA aaccttgagaaccccagggaatttgt
TATATTTATGCTATTCAGT          GAAATTGAAACAGCTGTGTTTAGTGCCTTTGTTCA       ccccttacaacaaccttgagaaccccagggaatttt
tatatttatgctattcagt                                        GCCTTTGTTCACATAGACCCCCTTGCAACAACCTT        cagggaatttgt
tatatttatgctattcagttcta                                              AG-----ACCCCCTTGCAACAACCTTGAGAACCCCAGGGA
TATATTTATGCTATTCAGTTCTAA                                              A-----ACCCCCTTGCAACAACCTTGAGAACCCCAGGGAA
TATATTTATGCTATTCAGTTCTAAA                                             A-----ACCCCCTTGCAACAACCTTGAGAACCCCAGGGAA
TATATTTATGCTATTCAGTTCTAAA                                                    TGCAACAACCTTGAGAACCCCAGGGAATTTGT
TATATTTATGCTATTCAGTTCTAAAT                                                   TGCAACAACCTTGAGAACCCCAGGGAATTTGT
TATATTTATGCTATTCAGTTCTAAAT                                                   TGCAACAACCTTGAGAACCCCAGGGAATTTGT
tatatttatgctattcagttctaaatatagaaatt                                          tgcaacaaccttgagaaccccagggaatttgt
tatatttatgctattcagttctaaatatagaaatt                                               CAACCTTGAGAACCCCAGGGAATTTGT
  TATTTATGCTATTCAGTTATAAATATAGAAATTGAAACAG                                           CCTTGAGAACCCCAGGGAATTTGT
   atttatgctattcagttctaaatatagaaattgaa                                               CTTGAGAACCCCAGGGAATTTGT
    tttacgctattcagtactaaatatagaaattgaaa                                              CTTGAGAACCCCAGGGAATTTGT
     ttatgctattcagttctaaatatagaaattgaaac                                                    gggaatttgt
```

reads



- Mapping DNA reads back to a reference genome is the first step in the data analysis
- Mapping short sequenced reads back to a reference genome is a string search problem: given a text and a query, find all (approximate) occurrences of the query in the text

Group Work

- Assume that a human reference genome is given (a string of 3 billion characters long)
- Assume that you need to map 1 million 50bp reads to the genome
- Come up with a method to map fast the reads to the genome

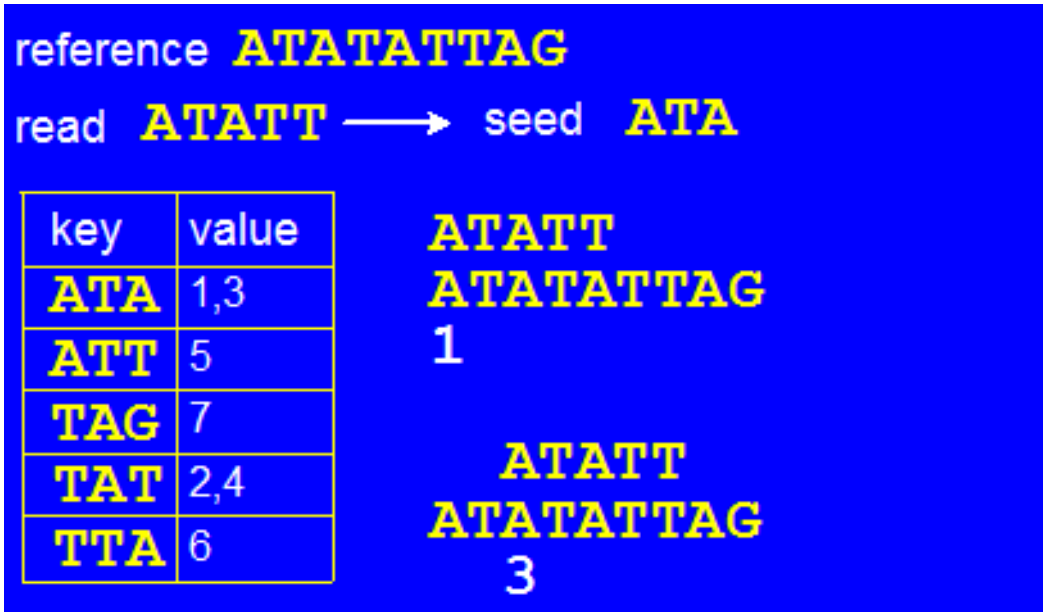
Problem statement:

Given a string S of length ***n*** and a short string P of length ***m*** (n >> m), find all locations where P occurs in S

Methods for Mapping Short Reads

- To speed up mapping, search space is reduced by focusing only on those regions of genome that share the same seed(s) with a read
- A seed, or *k*-mer (*q*-gram), is a substring of a read of length *k*
- Common data structures to index the data (genome) and speed-up the search:
  - hash tables
  - suffix trees
  - suffix arrays
  - Burrows-Wheeler transform (BWT) with Ferragina-Manzini (FM) index

Hash Indexing



- Hash all genome *k*-mers into a hash table using seeds of fixed length *k* as hash keys, and corresponding genomic positions as values
- Use the *k*-mers in a read as hash keys to retrieve locations that are potential hits
- Align the entire read to the potential locations and count the number of mismatches

Hash Indexing

Seed Size	Table Size	Space, GB
32	18,446,744,073,709,600,000	147,573,952,590
28	72,057,594,037,927,900	576,460,752
24	281,474,976,710,656	2,251,800
20	1,099,511,627,776	8,796
18	68,719,476,736	550
16	4,294,967,296	34
12	16,777,216	134 MB

Hash Indexing

Disadvantages:

1. The longer seeds, the more space demanding
2. The shorter seeds, the more time consuming

Group Work

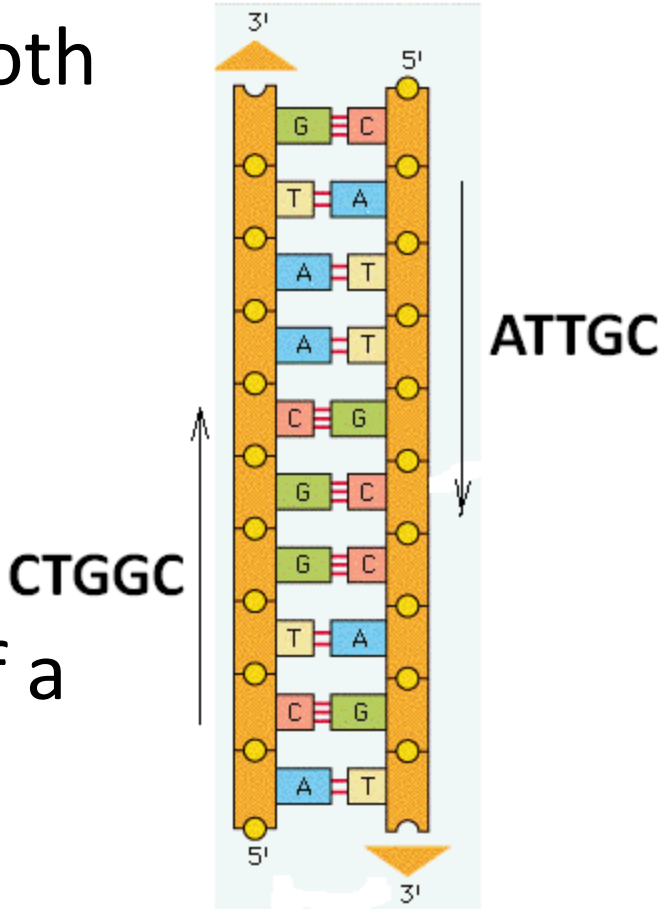
1. Build a hash table for the following sequence using seeds of length 2 and 3

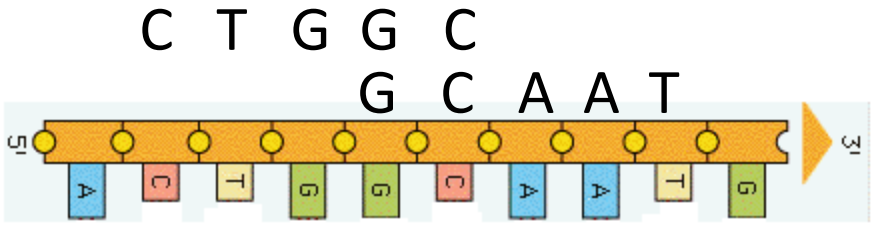
ATATGTTAGTCAAGTTAAGACCTATGTTAG

2. Map read TATG to the given sequence using the seed TA (TAT) and your hash tables. How many different alignments did you have to make?

Mapping

1. Reads are generated from both strands of DNA
2. Reads are always sequenced from 5’ to 3’
3. Mapping is performed to only (+) strand of DNA
4. Map reverse-complement of a read: ATTGC, rc: GCAAT

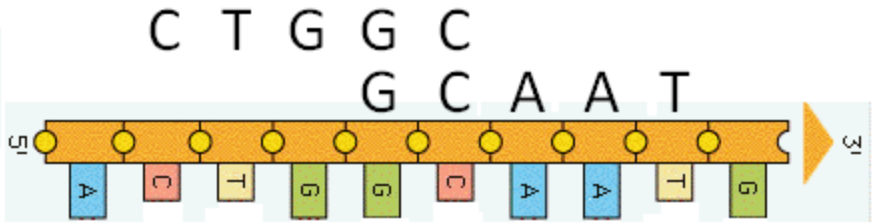




Mapping

Output format

Read_ID	Read	Chromosome	Position	Strand
1	CTGGC	1	1	+
2	ATTGC	1	4	-



Encoding of Reads

A = 00

C = 01

G = 10

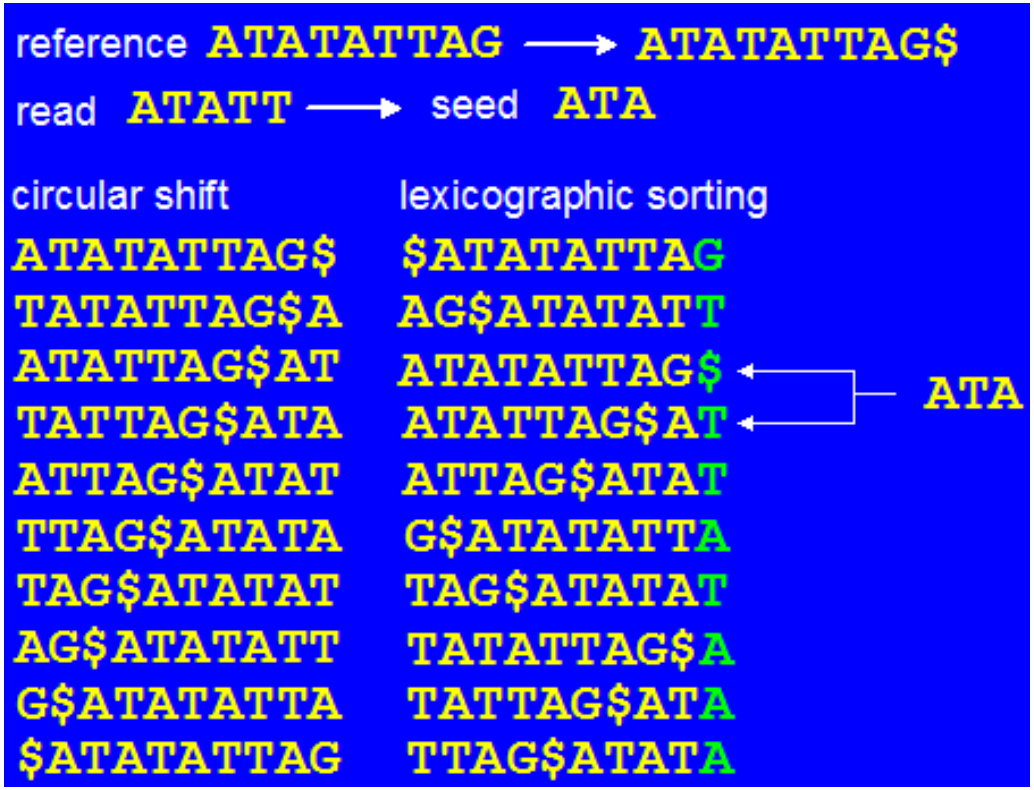
T = 11

ATTGC = 0011111001

Advantages:

1. Each character takes 2 bits instead of 8 bits
2. Retrieval of all positions where a seed occurs takes O(1) time (use encoding of a seed as an index for a hash table's bin)

Suffix Array



- Find all circular shifts of the reference genome
- Lexicographically sort the circular shifts
- All circular shifts that start with the same substring are consecutive
- Record the starting indices of the circular shifts

Suffix Array

10	8	1	3	5	9	7	2	4	6

For a given string S, Pos[i] = j, such that S[j…n] is a prefix of row i in M

To find a given pattern W of length m, we know that all rows having W as a prefix in M are contiguous; hence, positions of P in S are stored in contiguous range [L, R] in the suffix array Pos

Suffix Array

```
12345678910
ATATATTAG$
```

10	8	1	3	5	9	7	2	4	6

M

```
1  $ATATATTAG
2  AG$ATATATT
3  ATATATTAG$
4  ATATTAG$AT
5  ATTAG$ATAT
6  G$ATATATTA
7  TAG$ATATAT
8  TATATTAG$A
9  TATTAG$ATA
10 TTAG$ATATA
```

```
if W ≤_P A_Pos[0] then
    L_W ← 0
else if W >_P A_Pos[N−1] then
    L_W ← N
else
  { (L, R) ← (0, N−1)
    while R − L > 1 do
      { M ← (L + R)/2
        if W ≤_P A_Pos[M] then
            R ← M
        else
            L ← M
      }
    L_W ← R
  }
```

Suffix arrays: A new method for on-line string searches

Udi Manber Gene Myers

Suffix Array

```
12345678910
ATATATTAG$
```

10	8	1	3	5	9	7	2	4	6

M

```
1  $ATATATTAG
2  AG$ATATATT
3  ATATATTAG$
4  ATATTAG$AT
5  ATTAG$ATAT
6  G$ATATATTA
7  TAG$ATATAT
8  TATATTAG$A
9  TATTAG$ATA
10 TTAG$ATATA
```

Time to find all occurrences of W in S is O(|W|*log*(n)), where n = |S|
Space to store a suffix array is 4n

The authors also proposed algorithm with time O(|W| + log(n))

Suffix arrays: A new method for on-line string searches
Udi Manber Gene Myers

Input Format

Reference genome is usually given as a set of files, each file per chromosome.

Each file is in **Fasta** format:

```
>Gene 4582 Conserved Region
GACTAGCATGCAGCATGCAGAGCTAGCAGCGAGCAGCAGC
ATGCATGCAGCAGCAGCATCGAGCAGCAGCATGCATGCAG
CAGCAGCAGCAGCAGCAGCATGAGAGCAGCACGCAGCTAG
CTAGCATGCATGCATGCATGCAGCATGCATGCATGCATGC
ATGCTAGCTAGCTAGTCGATGCATGCATGCAGCGACGATAT
CGAATACAGCGACGATGCATGCATGCATGCAGCATGCATG
CATGATGATATAT
```

```
>Region_1 taxid=9606|spec=Homo sapiens|chr=1|ctg=NC_000001|str=(+)|start=1655789|end=1655989|len=201
GATGATATAATAGCCGACCTCTGGCCCAGAACTCAAGACGACAGGGGCTCGCTCTGTGCGGCACTTCCTGTGTCTGCGCG
GGATGATGACGCATAAAACAGCGCTTGCTCAGGTCCAGGACTCCAAAAGAAACTGCGCCGTGAGCTGCACTTCCGACTTC
GGCGCGGGCCGGGGCGCCGAGCAGAGCGACGCCGACTTTTG

>Region_2 taxid=9606|spec=Homo sapiens|chr=1|ctg=NC_000001|str=(-)|start=8014301|end=8014551|len=251
GCCCTGACATTTGAGGCGGCGCTGGTGCAAGGGGGGAAATTCTGACACCGAGTTCTGTGAGGGGCCCTGGGAACGGCTTC
GCCTCTGCGCCCACCAGTGTGAAGAGCCCCCCCCCCAGCGTGGGCCACCAGACACAACTCCCACGGGGCGCTGGGCGCCGG
AAACCTACCGACTGACCTGCATGACGTAGGTCCACTTCCGGCGGCCGCAGCATGCGCCACCCGGGATTGGCCGAGTCAGG
TCGCAGTGGGC
```

Input Format

Reads are usually given in **FASTQ** format:

```
@ERR030887.1 HWI-BRUNOP16X_0001:8:1:7336:1073#0/1
TNTCGATTACATGTGGATCAGGTTGATTTAATAATGGCGATAGGGNNNCT
+
5#145555555A;A84455555555>>>>.=@####################
@ERR030887.2 HWI-BRUNOP16X_0001:8:1:10288:1073#0/1
TNAGTCTTTCCCAGCCTAACAAAGAAAGCAAGAATAATTGGGCACNNNGA
+
5#156+43&4(0*55CFDAF##############################
@ERR030887.3 HWI-BRUNOP16X_0001:8:1:13787:1073#0/1
ANGTTGCTATTCCCGGCCGTCTAAACCAAACCACTTTCACCGCTANNNGA
+
5#55555554GGGG?FFFFFGGGGEGGGGGGGEGGCC>C############
@ERR030887.4 HWI-BRUNOP16X_0001:8:1:15389:1074#0/1
CNGTTCAAGCAGAAGACGTTCTGGGCGTCTGTATGGACACTGATCNNNAG
+
5#555525555545EGGGGGGGGA@;>A>A<A>A################
@ERR030887.5 HWI-BRUNOP16X_0001:8:1:16693:1073#0/1
CNAGTCCGTCACTCCATCCTACCCTTATGGGCCAGGTAAGCCAACNNNCC
+
5#555))665=<H<F@1=E:88<(=55441A?AADCBFB############
```

Read ID

Sequenced Read

Ignore

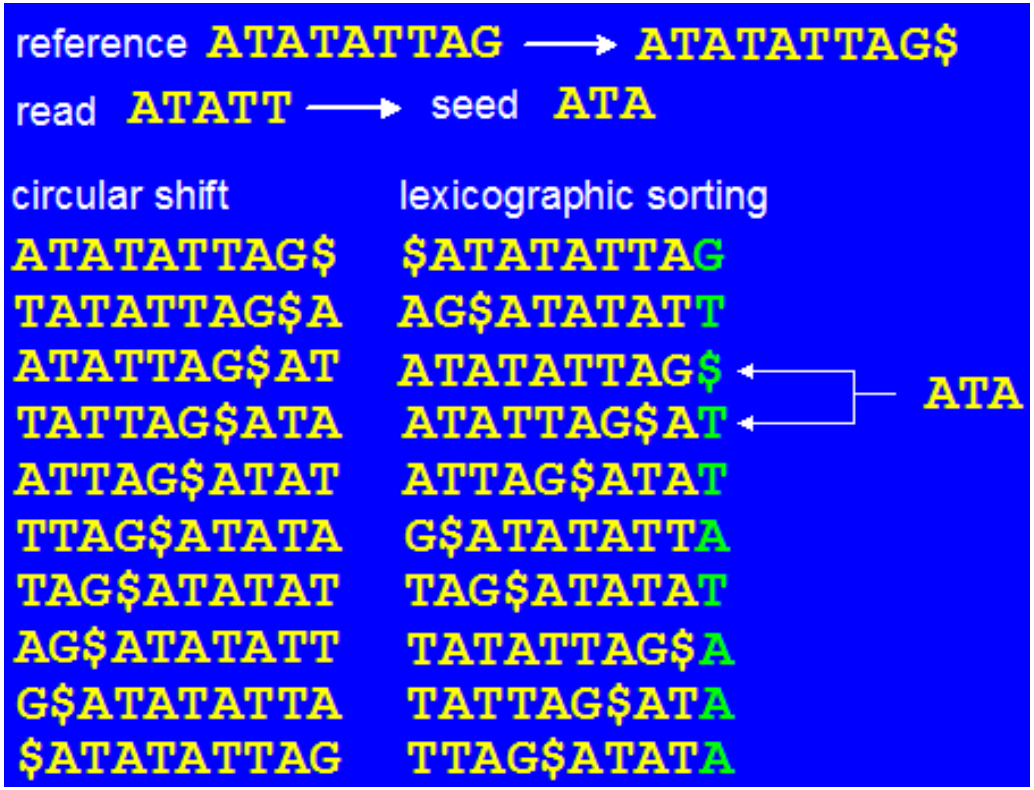
Quality Info

Homework 1 discussion

Next Lecture

- Approximate string search
- Smith-Waterman algorithm
- Hash table, suffix array for approximate string search

Burrows-Wheeler Transform



- Build Burrows-Wheeler transform (BWT) for the reference genome
- Find positions within BWT corresponding to suffixes whose prefix is a seed of the read
- Calculate from these positions genomic positions
- Align the entire read to the potential locations and count the number of mismatches

Methods for Mapping Short Reads

	Hash Indexing	Burrows-Wheeler transform
Seed length for a read	fixed	variable
Time to find genomic positions for a *k*-mer	O(1)	O($k + Occ \cdot \log N$)
Time to map entire read of length *P* to *Occ* genomic positions, where the *k*-mer occurs	O($Occ \cdot P$)	O($Occ \cdot P$)

- The length of the seed used in hashing is fixed and usually shorter than the seed for BWT
- Hence, *Occ* with BWT is smaller than *Occ* with hash indexing
- We need to check a smaller number of full-length read alignments with BWT; thus, mapping of short reads with BWT is more time-efficient

Find positions within BWT corresponding to suffixes whose prefix is a seed of the read

M

1	$ATATATTAG
2	AG$ATATATT
3	ATATATTAG$
4	ATATTAG$AT
5	ATTAG$ATAT
6	G$ATATATTA
7	TAG$ATATAT
8	TATATTAG$A
9	TATTAG$ATA
10	TTAG$ATATA

F

$	0
A	1
C	5
G	5
T	6
	10

Given: P, a pattern of length p

BW_Search(P[1,p])

c = P[p], i=p

sp = F[c] + 1, ep = F[c+1]

while sp < ep and i > 1

    c = P[i-1], i = i - 1

    sp = F[c] + Occ(c, 1, sp-1) + 1

    ep = F[c] + Occ(c, 1, ep)

print sp and ep

Example: *BW_Search*(ATA)

c = A, **i** = 3

sp = F[A] + 1 = 2, **ep** = F[A + 1] = F[C] = 5

i=3: **c** = T, **sp** = 6 + 0 + 1 = 7, **ep** = 6 + 3 = 9

i=2: **c** = A, **sp**= 1 + 1 + 1 = 3, **ep** = 1 + 3 = 4

At each iteration *i*, sp points to the first row of M prefixed by P[i,p], and ep points to the last row of M prefixed by P[i,p]

Calculate Genomic Positions from [*sp,ep*]

1 2 3 4 5 6 7 8 9 10
A T A T A T T A G $

GI

10	1	7	4

M

1	$ATATATTAG	1
2	AG$ATATATT	0
3	ATATATTAG$	1
4	ATATTAG$AT	0
5	ATTAG$ATAT	0
6	G$ATATATTA	0
7	TAG$ATATAT	1
8	TATATTAG$A	0
9	TATTAG$ATA	1
10	TTAG$ATATA	0

F

$	0
A	1
C	5
G	5
T	6
	10

- Mark row of M corresponding to each 3-d genomic position
- Store explicitly these positions in array GI
- If i-th position in BWT is marked, Occ(1, 1, i) is index for genomic position in GI (e.g., GI[Occ(1,1,3)]=GI[2]=1)
- If i-th position in BWT is not marked, do LF_mapping until encounter marked position j, BWT[j] = 1, marked
- Pos(sp) = Number_of_LF_mappings + GI[Occ(1,1,j)]

LF_mapping(sp)

c = get_BWT_char(sp)

sp = F[c] + Occ(c, 1, sp)

Example: *1. LF_mapping*(4)

c = get_BWT_char(4) = T

sp = F[T] + Occ(T, 1, 4) = 6 + 2 = 8 (not marked)

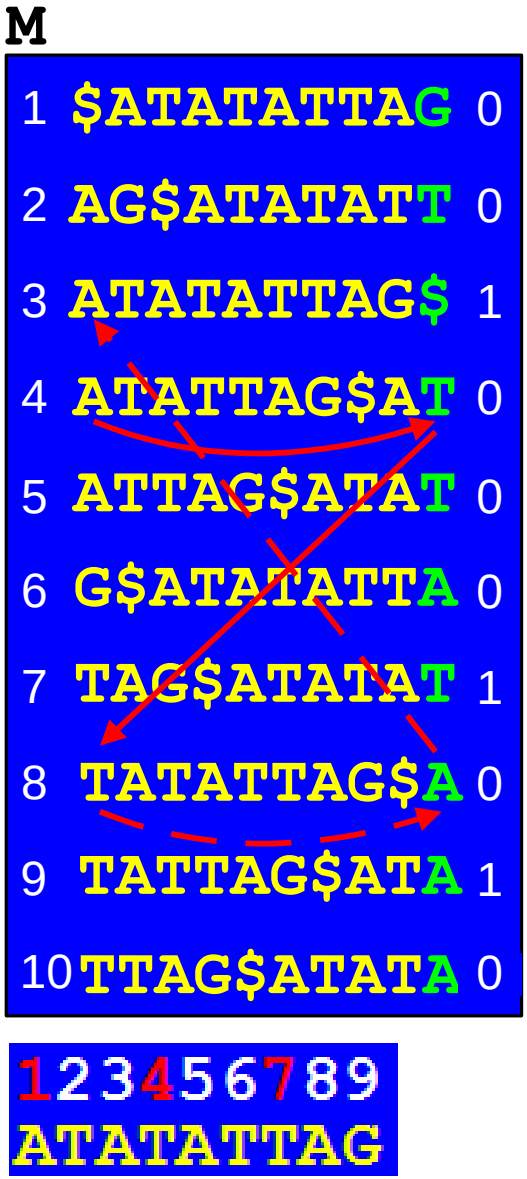
2. LF_mapping(8)

c = get_BWT_char(8) = A

sp = F[A] + Occ(A, 1, 8) = 1 + 2 = 3 (marked)

Pos(sp) = 2 + 1 = 3 (total of 2 LF_mappings and GI[Occ(1,1,3)])

Why LF_mapping works?



- Why do we identify correctly the genomic position for unmarked BWT[i]?
- Given row M[i] starting with prefix P, we find the closest marked preceding genomic position
- Since the rows of M are the circular shifts of T$, the last character of i-th row, L[i], precedes the first character F[i]
- Let L[i] = c and ri be the rank of the row M[i] among all rows ending with c. Then F[j] = c is the corresponding character to L[i] in T, where M[j] is the ri-th row of M starting with c
- Define LF_mapping as

$$LF[i] = F[\ L[i]\ ] + ri$$