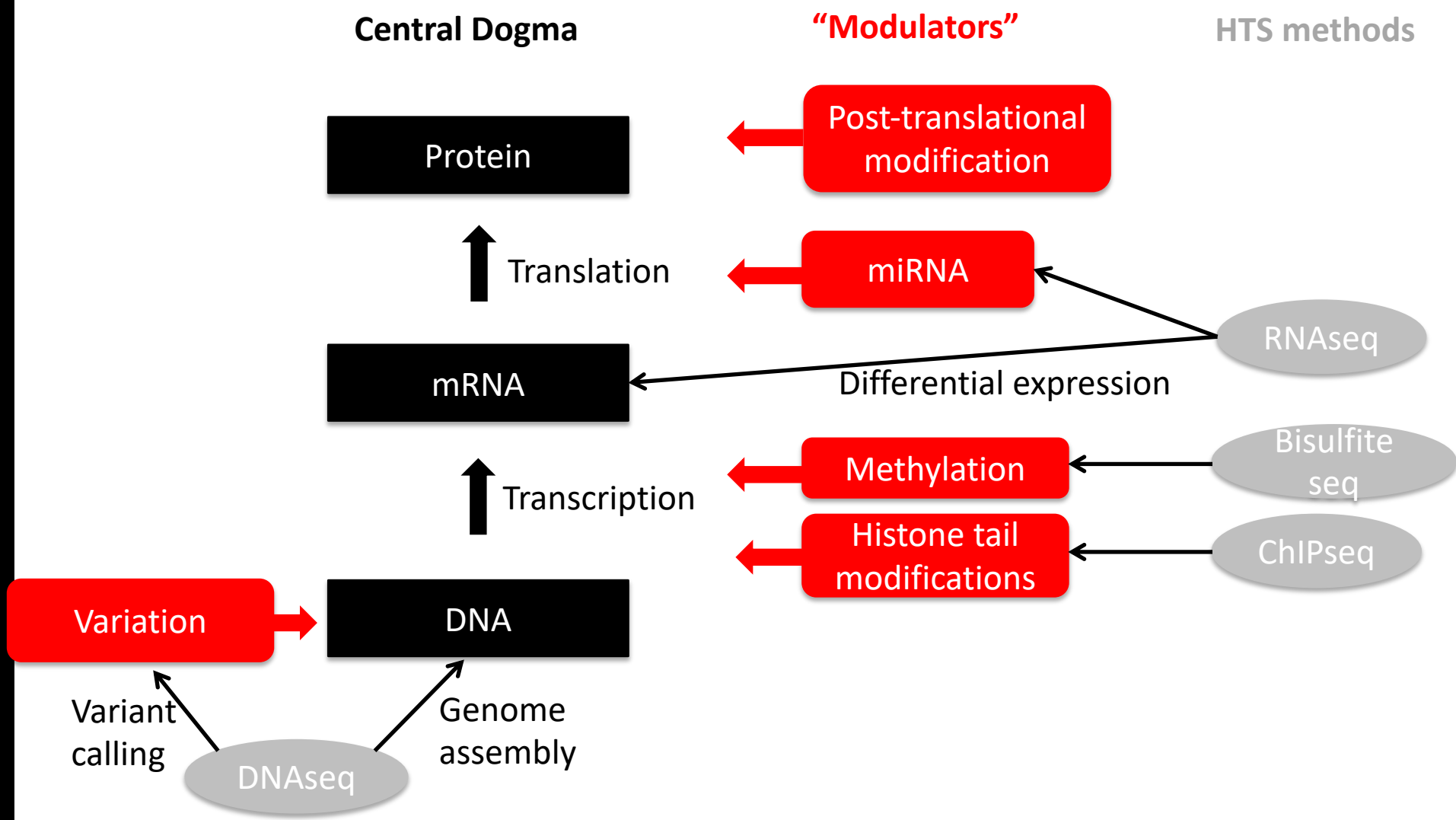
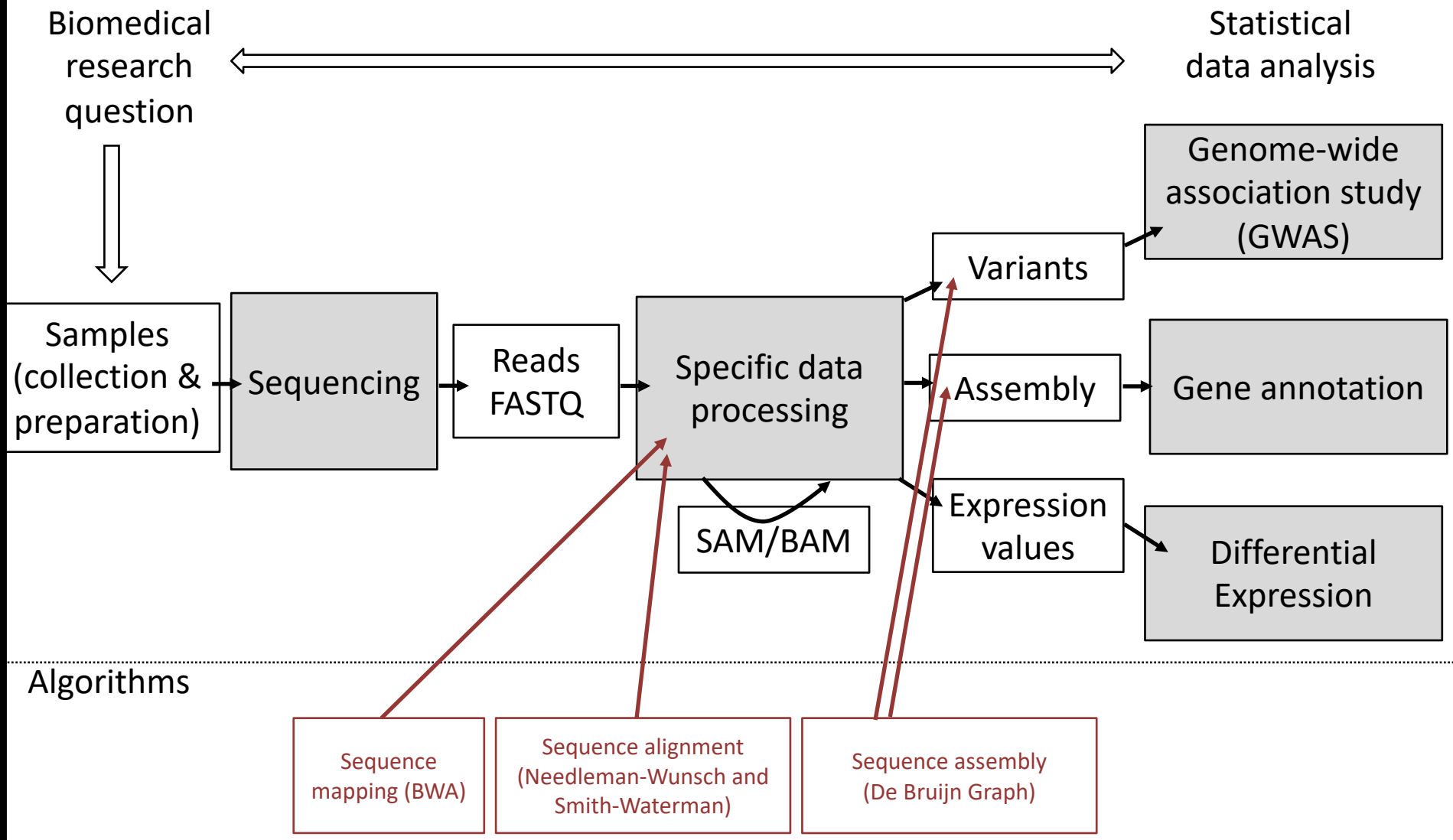
in-biosx000- Algorithms module
Fastq and alignment

Autumn 2020

Central dogma and HTS

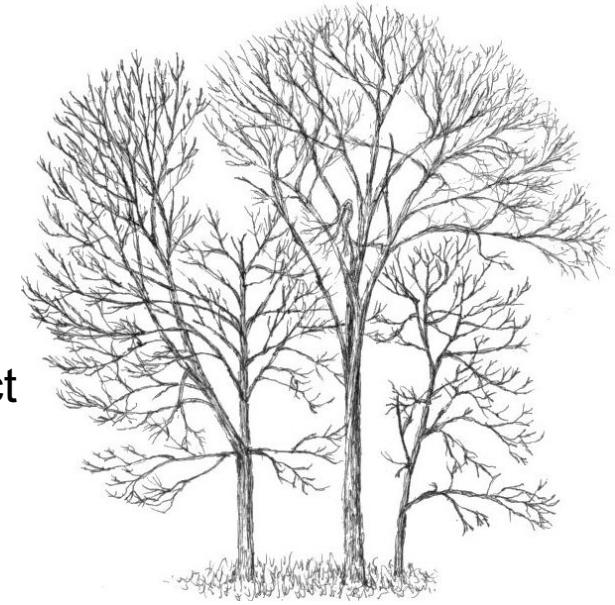


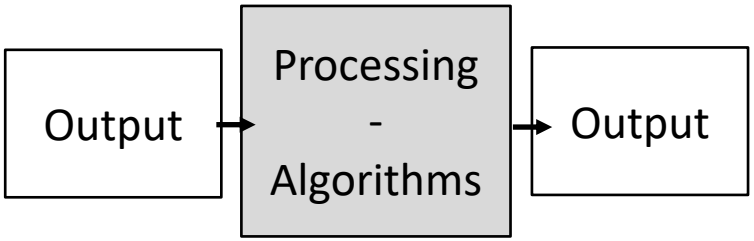
Key algorithms in the big picture?



Good practices for computational data processing and analysis

- Make sure you always know where you are!
- Be careful and structured where you put things:
  - file naming
  - directory structure
- Checking that the computer has done what you expect it to do, verifying outputs:
  - file size
  - timestamps
  - file contents
- A computer is a very complex device.
  - You don't need to understand all the details all the way down the stack
  - But you do need to understand what is happening at the level that is relevant for your work.

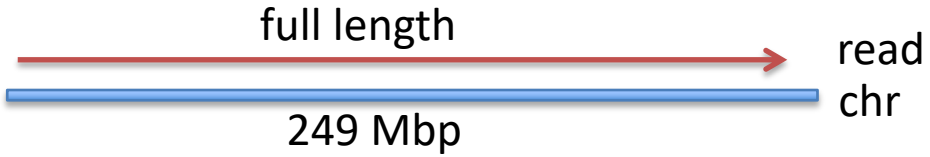


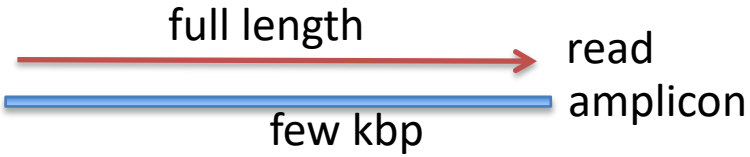


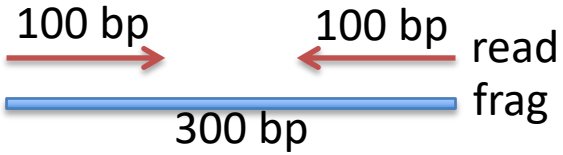
FASTQ: ERROR PROBABILITY, QUALITY SCORES AND ENCODINGS

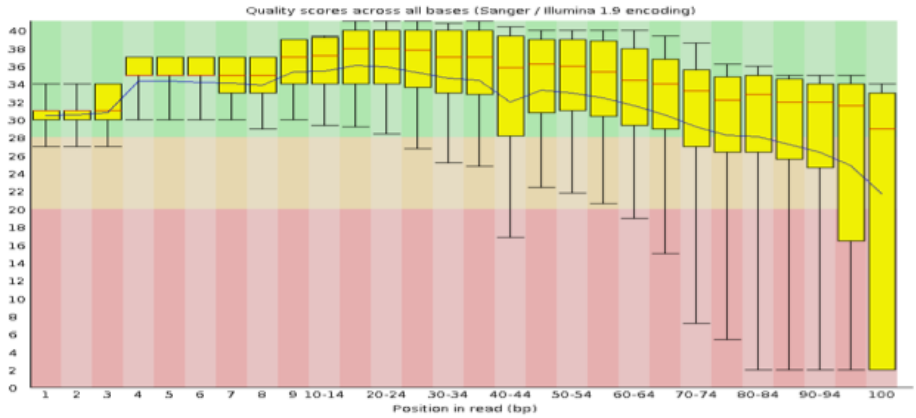
In a perfect world – Perfect sequencing

- Perfect sequencing:
  - single molecule (no PCR)
  - **full** length
  - no deterioration of quality
- While we are waiting:
  - Sanger
    - PCR
    - length: some kb
    - limited number of reads
    - high quality
  - HTS (Illumina)
    - PCR
    - 100 bp PE
    - billions of reads
    - high quality, but deteriorating along read

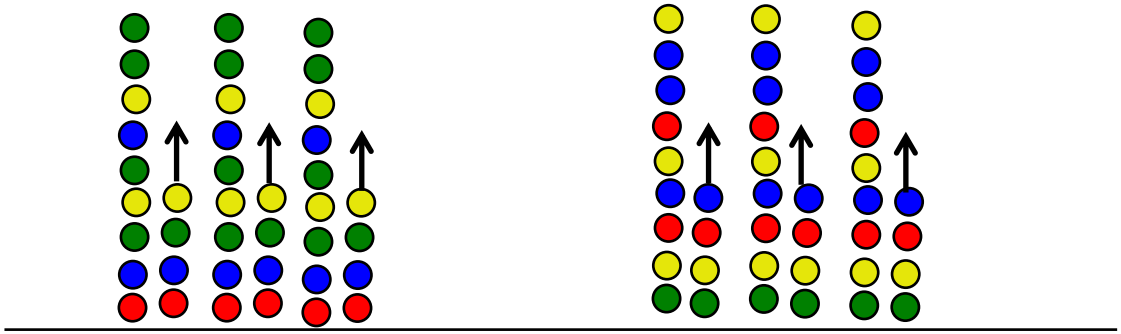




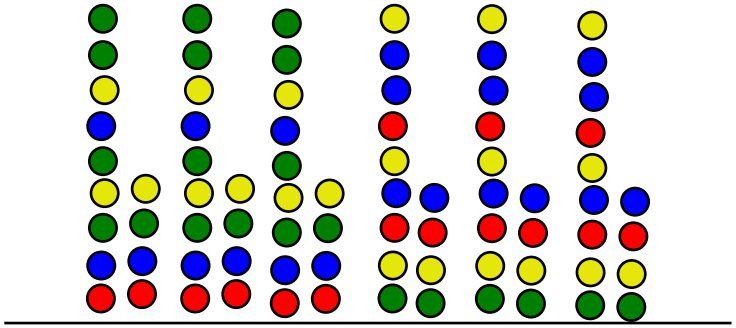




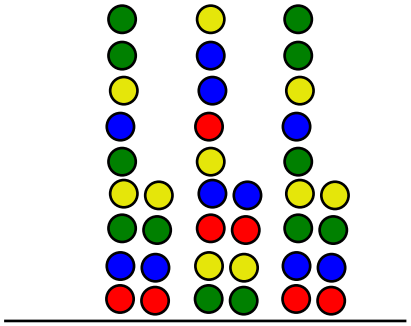
Explaining how/why sequence quality varies



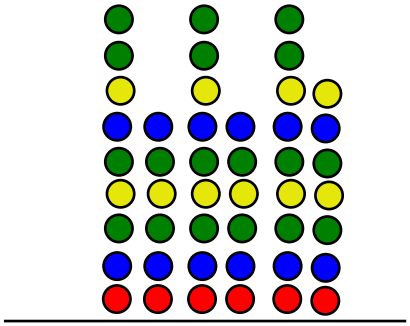
Normal situation: Well defined “pure” clusters



Neighbouring clusters



“Mixed” cluster



Unphased

The identity of the base in a given cluster in a given cycle is not known with certainty

Fastq format – fasta with qualities

```
@J00146:31:HJF5NBBXX:7:1101:2483:1121 1:N:0:NTTCAGAA+NTTCGCCT
NTTGTGAGGGAAAGGATTAGGAAGTTGAGTGTTCCTATTGAGTTTTGGATTGAAATGAGGGCAATTAAGAGTGGGA
+
#AAAFAFJJJJJA-JJAFJJ<7FJJJ---7-<F-7FA-<FJJ-<<FJ-F-AA7J-FFJ7A<JJJ--7FAJF7A<<A
```

- p = the probability that the corresponding base call is wrong
- Qualities $Q_{\text{sanger}} = -10 \log_{10} p$ $p = 10^{(Q/-10)}$
- Rule of thumb
  - p = 0.1 ➔ Q = 10
  - p = 0.01 ➔ Q = 20
  - P = 0.001 ➔ Q = 30

Quality scores

- Why use a quality score
  - More intuitive that a “better” characteristic gets a higher score
  - More usable representation: integer number of 1 or 2 digits rather than a decimal number

- Where are quality scores used:
  - base quality scores
  - mapping quality scores
  - variant quality scores

ASCII conversion

```
@J00146:31:HJF5NBBXX:7:1101:2483:1121 1:N:0:NTTCAGAA+NTTCGCCT
NTTGTGAGGGAAAGGATTAGGAAGTTGAGTGTTCCTATTGAGTTTTGGATTGAAATGAGGGCAATTAAGAGTGGGA'
+
#AAAFAFJJJJJA-JJAFJJ<7FJJJ---7-<F-7FA-<FJJ-<<FJ-F-AA7J-FFJ7A<JJJ--7FAJF7A<<A
```

ASCII encoding: Sanger/Phred format can encode a quality score from 0 to 93 using ASCII 33 to 126: Q + 33

Exercise:

- **Why do you think we add 33?**
- **What would be the ASCII code for a base that had a probability of error of 0.15**

-10log(0.15)=-10(-0.82)=8 8+33=41 >> ASCII character «)»

- **What is the probability of error of the first base in the above sequence?**

>> ASCII 35 >> 35 – 33 = 2 >> $p = 10^{(Q/-10)} = 10^{(2/-10)} = 0.63$

- **What is the probability of error of the 10th base in the above sequence?**

J >> ASCII 74 >> 74 – 33 = 41 >> $p = 10^{(Q/-10)} = 10^{(41/-10)} = 0.0001$

Dec	Hx	Oct	Html	Chr	Dec	Hx	Oct	Html	Chr
32	20	040		Space	64	40	100	@	@
33	21	041	!	!	65	41	101	A	A
34	22	042	"	"	66	42	102	B	B
35	23	043	#	#	67	43	103	C	C
36	24	044	$	$	68	44	104	D	D
37	25	045	%	%	69	45	105	E	E
38	26	046	&	&	70	46	106	F	F
39	27	047	'	'	71	47	107	G	G
40	28	050	(	(	72	48	110	H	H
41	29	051	)	)	73	49	111	I	I
42	2A	052	*	*	74	4A	112	J	J
43	2B	053	+	+	75	4B	113	K	K
44	2C	054	,	,	76	4C	114	L	L
45	2D	055	-	-	77	4D	115	M	M
46	2E	056	.	.	78	4E	116	N	N
47	2F	057	/	/	79	4F	117	O	O
48	30	060	0	0	80	50	120	P	P
49	31	061	1	1	81	51	121	Q	Q
50	32	062	2	2	82	52	122	R	R
51	33	063	3	3	83	53	123	S	S
52	34	064	4	4	84	54	124	T	T
53	35	065	5	5	85	55	125	U	U
54	36	066	6	6	86	56	126	V	V
55	37	067	7	7	87	57	127	W	W
56	38	070	8	8	88	58	130	X	X
57	39	071	9	9	89	59	131	Y	Y
58	3A	072	:	:	90	5A	132	Z	Z
59	3B	073	;	;	91	5B	133	[	[
60	3C	074	<	<	92	5C	134	\	\
61	3D	075	=	=	93	5D	135	]	]
62	3E	076	>	>	94	5E	136	^	^
63	3F	077	?	?	95	5F	137	_	_

http://www.unit-conversion.info/texttools/ascii/

Different quality formulas and encodings

- Beware of different versions

```
  SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
  ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX......................
  ..............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII..........................
  ...............................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.........................
  !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
  |                          |    |        |                              |                     |
 33                        59   64       73                            104                   126

 S - Sanger, Illumina 1.8+     Phred+33,  raw reads typically (0, 41)
 X - Solexa                    Solexa+64, raw reads typically (-5, 40)
 I - Illumina 1.3+             Phred+64,  raw reads typically (0, 40)
 J - Illumina 1.5+             Phred+64,  raw reads typically (3, 40)
```

Most recent

- With sequence data recently produced, you do not need to worry: **Phred + 33**
- For older data, be careful

Source: http://en.wikipedia.org/wiki/FASTQ_format

Illumina sequence identifiers

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

```
@EAS139:136:FC706VJ:2:2104:15343:197393 1:Y:18:ATCACG
```

EAS139	the unique instrument name
136	the run id
FC706VJ	the flowcell id
2	flowcell lane
2104	tile number within the flowcell lane
15343	'x'-coordinate of the cluster within the tile
197393	'y'-coordinate of the cluster within the tile
1	the member of a pair, 1 or 2 *(paired-end or mate-pair reads only)*
Y	Y if the read is filtered, N otherwise
18	0 when none of the control bits are on, otherwise it is an even number
ATCACG	index sequence

SEQUENCE MAPPING

Mapping and alignment

```
1         10        20        30        40        50
AGCTGTAGTAGCTGTAGCGTAGTTGCCGTACGTCTATGGTGAGGAGAGAGTTCCCCCTA
```

Human genome is 3G bases spread over 23 chromosomes

1. Can you locate GTTGCCGTA?

>> this is the sequence mapping problem

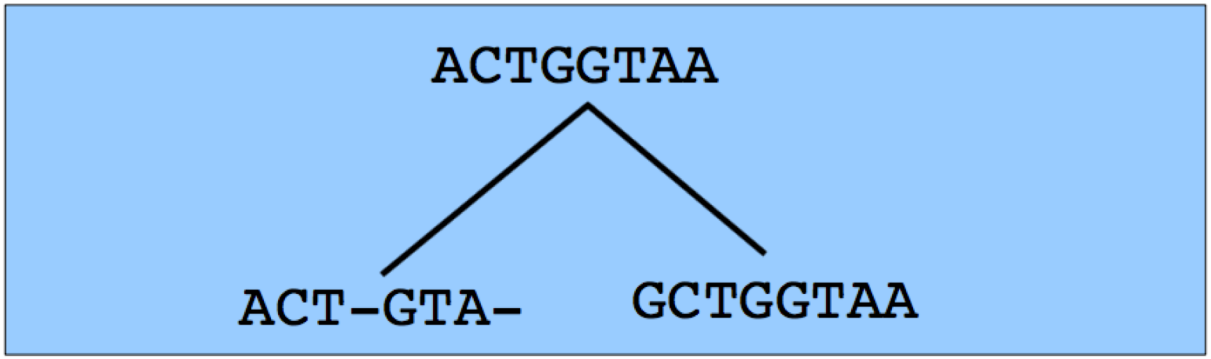
2. Assume that you now have a slightly different sequence GTTGCGTT, how does this compare to the sequence in 1?

>> this is the sequence alignment problem

SEQUENCE ALIGNMENT

Sequence divergence

Evolution



Observation

ACTGTA
GCTGGTAA

2 unaligned sequences

Goal

ACT-GTA-
GCTGGTAA

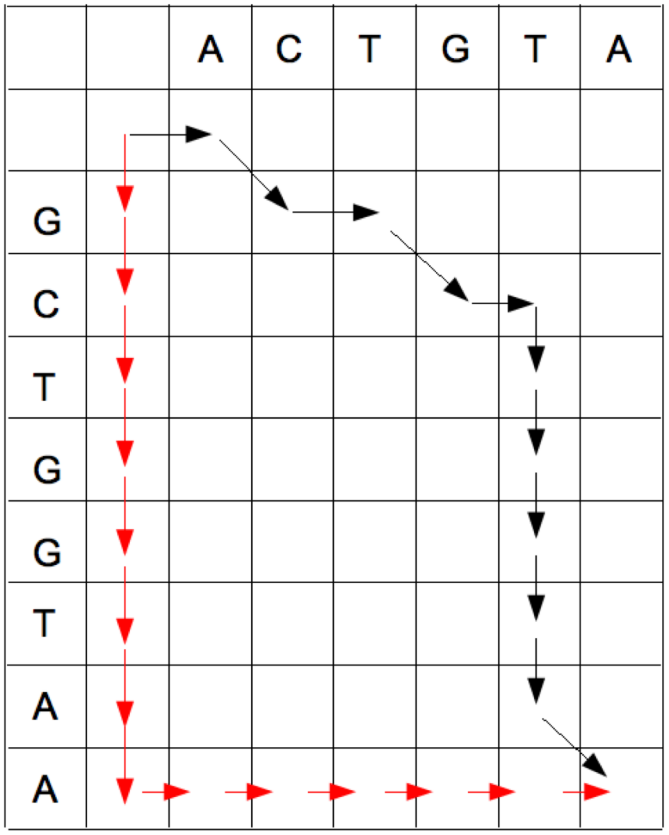
2 aligned sequences

What is a global alignment?

- A global alignment of 2 sequences q and d must satisfy:
  - All symbols in q and d have to be in the alignment, and in the same order as they appear in q and d
  - We can align one symbol from q with one from d
  - A symbol can be aligned with a blank (gap)
  - Two blanks cannot be aligned

- In the case of DNA, the symbols are A, G, C, and T

A global alignment is a path in a matrix




```
ACTGT-----A
-G-C-TGGTAA
```

Alignment produced by the black path

```
--------ACTGTA
GCTGGTAA------
```

Alignment produced by the red path

There are a large number of paths through the matrix

We need:

- a way of scoring different paths
- a way of finding the best scoring path

Important: Paths through the matrix have to incorporate a new base from at least one of the sequences i.e. arrows moving "back up" the matrix do not correspond to an alignment.

Scoring schemes

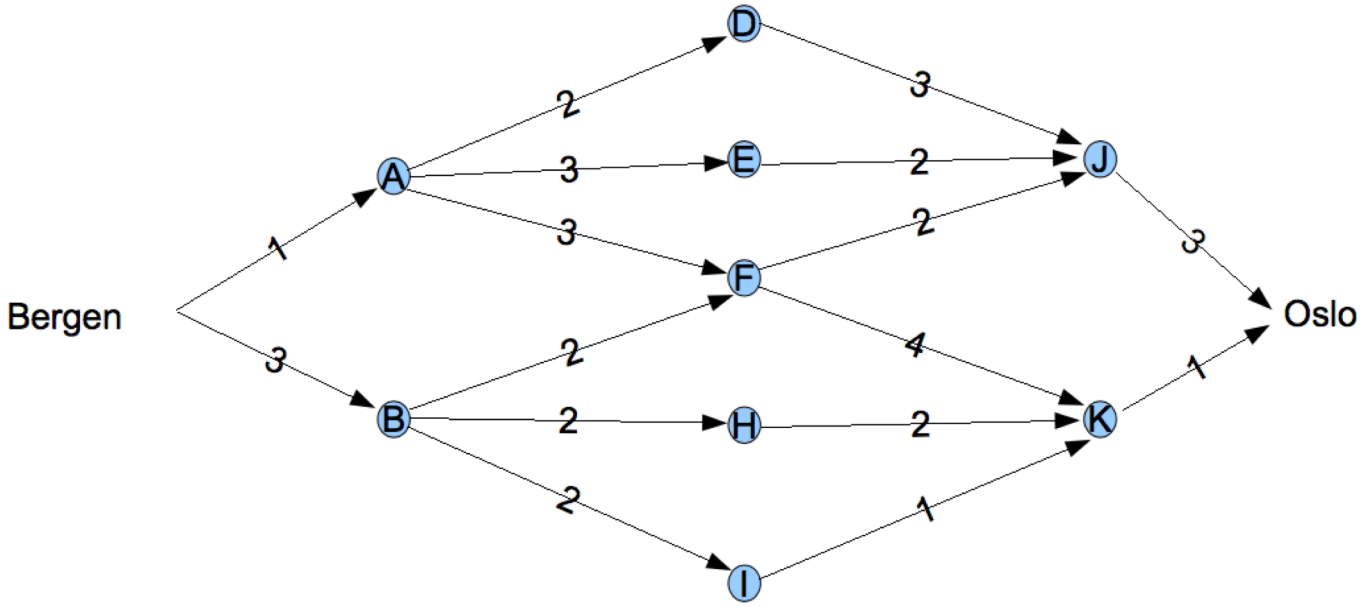
- A scoring scheme
  - Each column in the alignment can be given a score, independently of the other columns
    - Need score for alignment of two residues
    - Need score for alignment of residue with gap
  - The score of the alignment can be found as the sum of the score of all columns

- Scoring scheme is critical to the alignment produced (different scoring schemes will most likely produce different alignments)

- Computation time intensive to enumerate all alignments and score them all to find the best scoring one

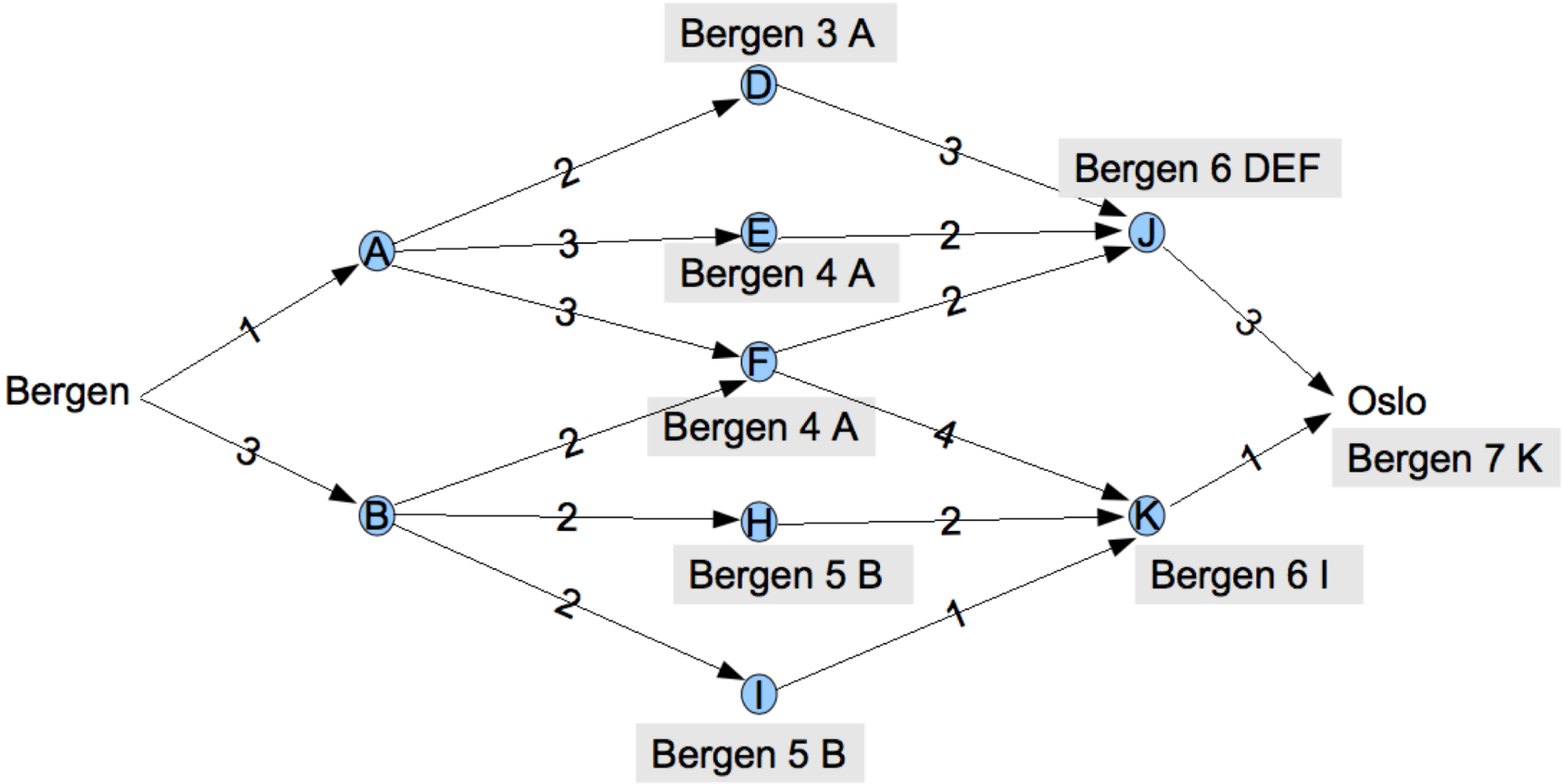
- Dynamic programming to find the best scoring alignments (Needleman and Wunsch 1970)

An analogy



- The shortest path is not obvious
- Enumerating all paths is not a viable solution for anything but a trivial case (like the above)
- Greedy algorithm is inappropriate
- Solution: dynamic programming

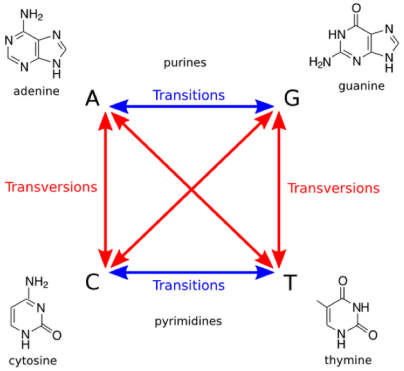
Illustration of dynamic programming



1. Scoring moving forward
2. Followed by backtracking >> Oslo > K > I > B > Bergen

The best alignment

Example scoring scheme
- gap: −1
- match: 2
- transition: 1
- transversion: −2



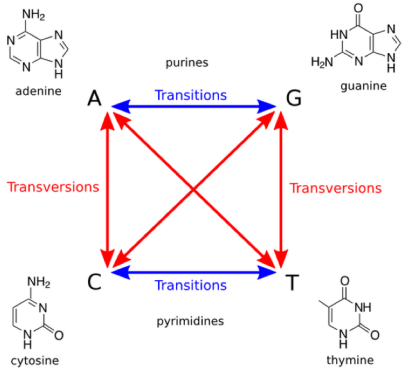
Sequence 1

Sequence 2

		A	C	T	G	T	A
	0 →	-1 →	-2 →	-3 →	-4 →	-5 →	-6
G	-1						
C	-2						
T	-3						
G	-4						
G	-5						
T	-6						
A	-7						
A	-8						

The best alignment

Example scoring scheme
- gap: -1
- match: 2
- transition: 1
- transversion: -2



Sequence 1

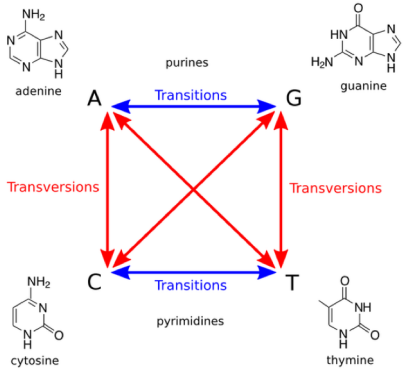
Sequence 2

		A	C	T	G	T	A
	0	-1	-2	-3	-4	-5	-6
G	-1	1	?	?	?	?	?
C	-2	?	?	?	?	?	?
T	-3	?	?	?	?	?	?
G	-4	?	?	?	?	?	?
G	-5	?	?	?	?	?	?
T	-6	?	?	?	?	?	?
A	-7	?	?	?	?	?	?
A	-8	?	?	?	?	?	?

	Option1	Option2	Option3
Sequence 1	-	A	A
Sequence 2	G	G	-
	Gap	Transition	Gap
	-1-2	0+1	-1-2

The best alignment

Example scoring scheme
- gap: –1
- match: 2
- transition: 1
- transversion: –2



Sequence 1

Sequence 2

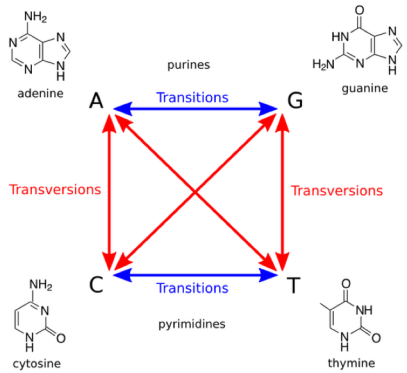
		A	C	T	G	T	A
	0	-1	-2	-3	-4	-5	-6
G	-1	1	0	?	?	?	?
C	-2	?	?	?	?	?	?
T	-3	?	?	?	?	?	?
G	-4	?	?	?	?	?	?
G	-5	?	?	?	?	?	?
T	-6	?	?	?	?	?	?
A	-7	?	?	?	?	?	?
A	-8	?	?	?	?	?	?

	Option1	Option2	Option3
Sequence 1	-	C	C
Sequence 2	G	G	-
	Gap	**Transvers.**	**Gap**
	-2-1	**-1-2**	**1-1**

Needleman-Wunsch 1970

Example scoring scheme

- gap: $W_1 = -1$
- match: $a_i = b_j$, $s(a_i, b_j) = 2$
- mismatch: $a_i \neq b_j$
  - transition: $s(a_i, b_j) = 1$
  - transversion: $s(a_i, b_j) = -2$



		A	C	T	G	T	A
	0	-1	-2	-3	-4	-5	-6
G	-1	1	0	-1	-1	-2	-3
C	-2	0	3	2	1	0	-1
T	-3	-1	1	5	0	3	2
G	-4	-2	0	4	7	6	5
G	-5	-3	-1	3	6	5	7
T	-6	-4	-2	2	5	8	7
A	-7	-4	-3	1	4	7	10
A	-8	-5	-4	0	3	6	9

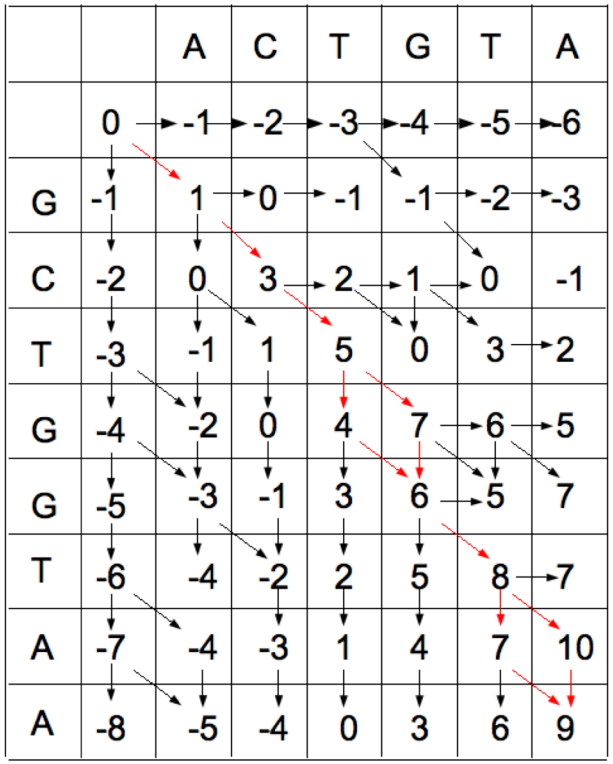
$H_{i-1,j-1}$	$H_{i-1,j}$
$H_{i,j-1}$	$H_{i,j}$

$$H_{ij} = \max \begin{matrix} H_{i-1,j-1} + s(a_i, b_j), \\ H_{i-1,j} + W_1, \\ H_{i,j-1} + W_1, \end{matrix}$$

The best alignments by traceback

Remember: dynamic programming involves two phases:

- First scoring (which we just did)
- Then traceback



		A	C	T	G	T	A
	0	-1	-2	-3	-4	-5	-6
G	-1	1	0	-1	-1	-2	-3
C	-2	0	3	2	1	0	-1
T	-3	-1	1	5	0	3	2
G	-4	-2	0	4	7	6	5
G	-5	-3	-1	3	6	5	7
T	-6	-4	-2	2	5	8	7
A	-7	-4	-3	1	4	7	10
A	-8	-5	-4	0	3	6	9

```
ACT-GTA-
GCTGGTAA
```

Truth

```
ACT-GT-A
GCTGGTAA
```

```
ACTG-TA-
GCTGGTAA
```

```
ACTG-T-A
GCTGGTAA
```

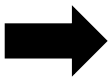
$$H_{ij} = \max \begin{cases} H_{i-1,j-1} + s(a_i, b_j), \\ H_{i-1,j} + W_1, \\ H_{i,j-1} + W_1, \end{cases}$$

Time complexity is O(mn)
Space complexity is same, but can be made linear

Scoring matrix

- We chose a simple scoring matrix
- More generally:
  - the score of the alignment of two residues should reflect the probability that they are homologous
  - or in other words that one residue is the result of one or several mutations of the other
- Gap penalties
  - we used a linear gap penalty
  - a more general form would be an affine gap penalty, this means that all blanks in the alignment do NOT carry the same penalty

gap cost = opening cost + length x extension cost



Affine gap penalty leads to a minor modification to the scoring of the matrix, but principle remains the same

The importance of scoring

http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html

Two homologous sequences

AGTAAAATTATATATGTA

GGTAAAA-------ATATGT**T**

Needleman-Wunsch
Gap open 5 and ext 1 (typical defaults)
Notice the affine gap

```
AGTAAAATTATATATGTA      18
.||||||     |||||||.
GGTAAAA----ATATGTT      14
```

The importance of scoring

http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html

AGTAAAATTATATATGTA
GGTAAAA-------ATATGTT

Gap open 1 and ext 0.001
("cheap" gap)

```
A-GTAAAATTATATATG-TA     18
  ||||||     ||||| |
-GGTAAAA----ATATGTT-     14
```

>> 8 gaps and 0 substitutions

Gap open 50 and ext 1
("expensive" gap)

```
AGTAAAATTATATATGTA       18
.|||||||.|||.|.
GGTAAAAATATGTT----       14
```

>> 4 gaps and 4 substitutions

Practical with Needleman-Wunsch

$H_{i-1,j-1}$	$H_{i-1,j}$
$H_{i,j-1}$	$H_{i,j}$

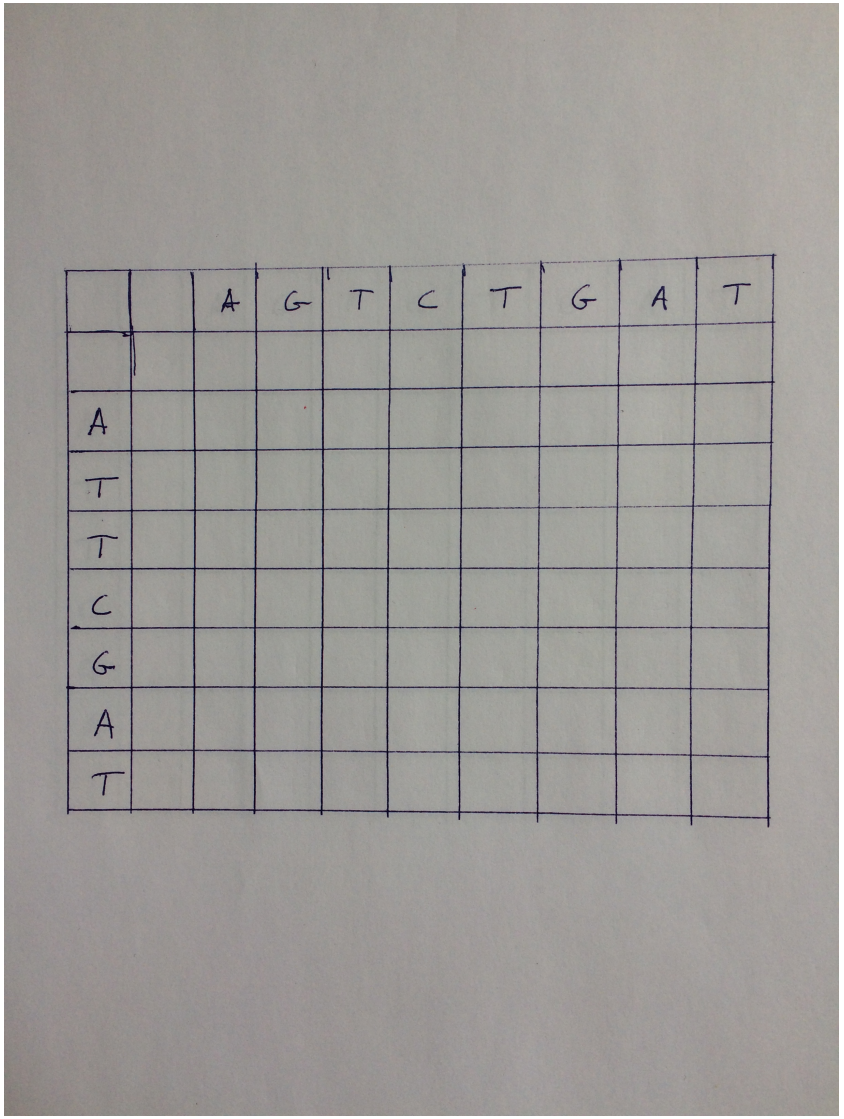
Example scoring scheme

- gap: $W_1 = -1$
- match: $a_i = b_j$, $s(a_i, b_j) = 2$
- mismatch: $a_i \neq b_j$, $s(a_i, b_j) = -1$

1. **Initialise**
2. **Score**

$$H_{ij} = \max \begin{cases} H_{i-1,j-1} + s(a_i, b_j), \\ H_{i-1,j} + W_1, \\ H_{i,j-1} + W_1, \end{cases}$$

3. **Traceback**



Practical with Needleman-Wunsch

$H_{i-1,j-1}$	$H_{i-1,j}$
$H_{i,j-1}$	$H_{i,j}$

Example scoring scheme

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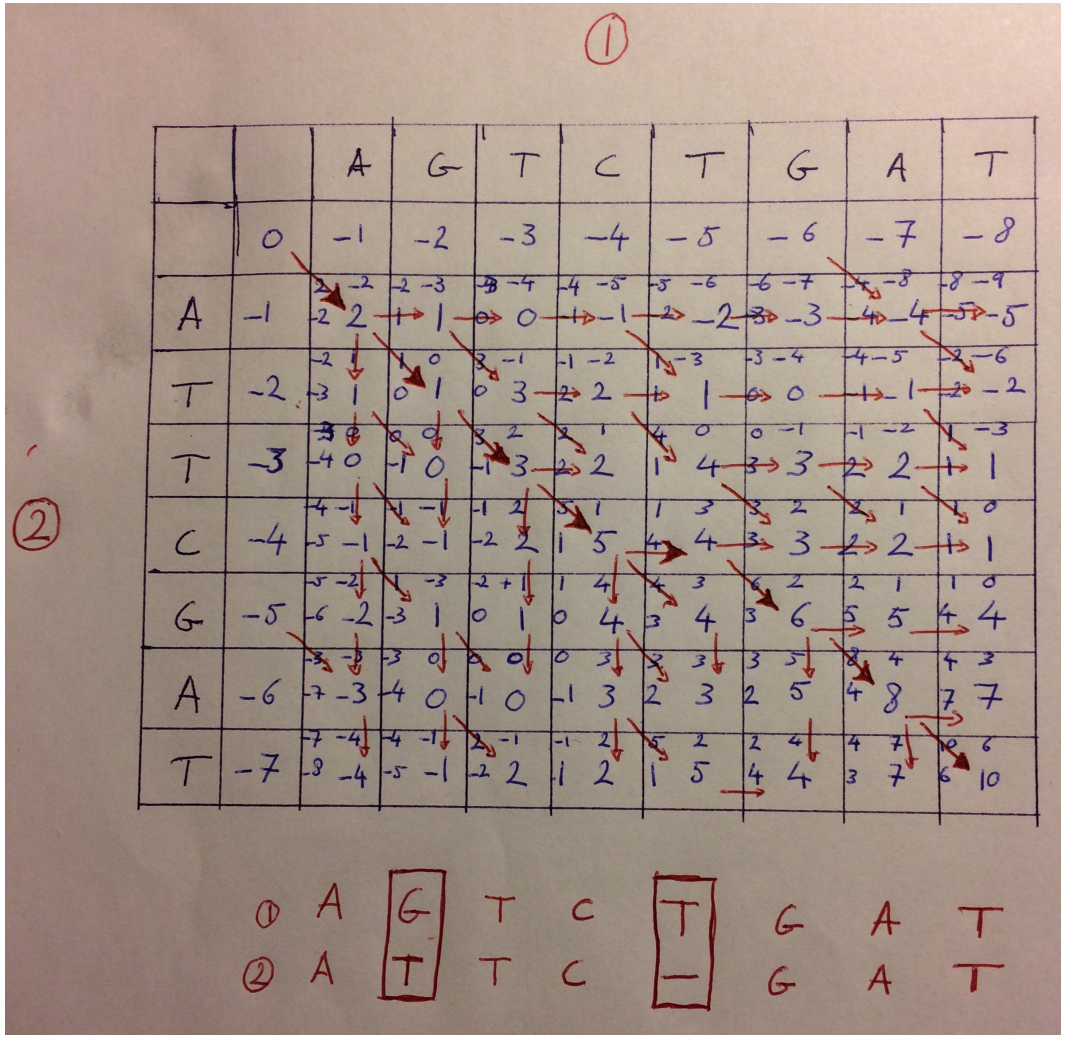
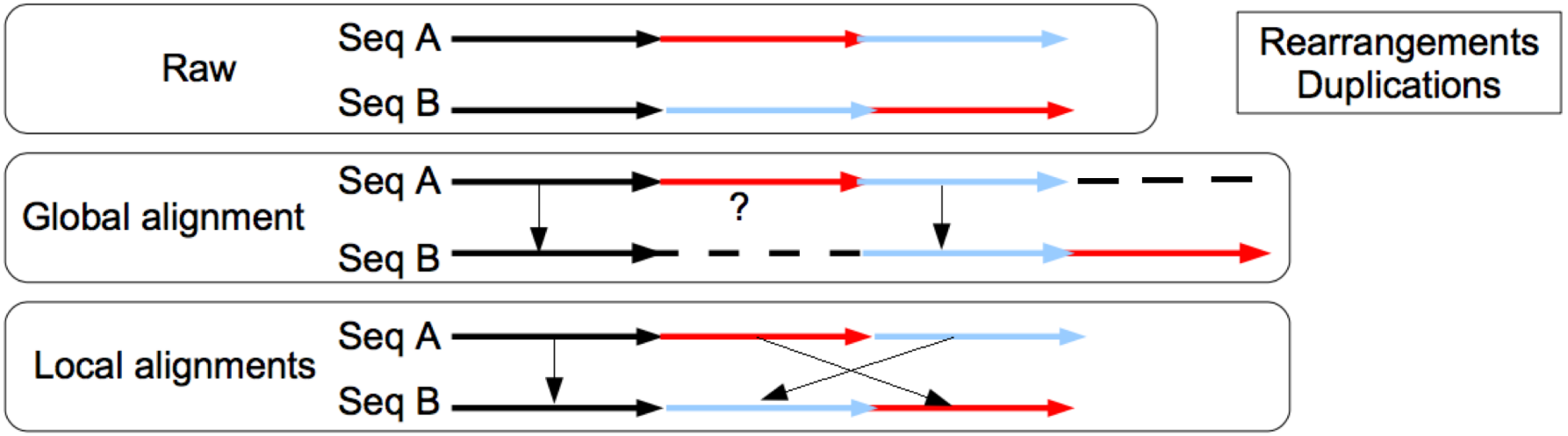
3. **Traceback**

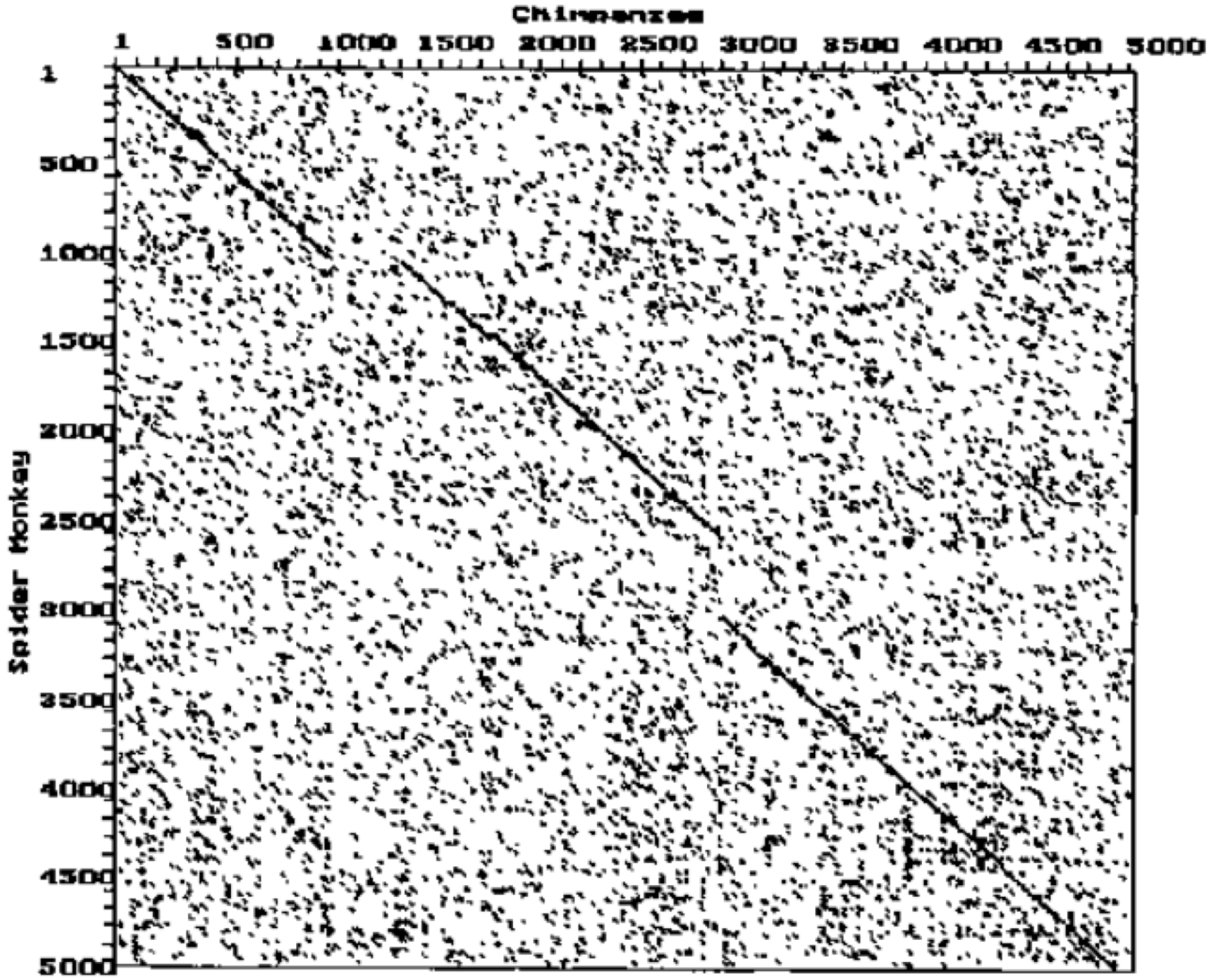


Pairwise local alignment

- Up to this point we were looking at global alignments: all bases in both sequences had to be in the alignment.
- But, sometimes a local alignment is desirable



A visual technique – The dot-plot



Identities of length 6bp- Chimpanzee hemoglobin intergenic DNA against spider monkey. From helix.biology.mcmaster.ca

DP – Smith Waterman 1981

Protein sequences

Needleman-Wunsch scoring

q\d			R	E	D	C	E	D	K	L
	i\j	0	1	2	3	4	5	6	7	8
	0	0	−0.5	−1	−1.5	−2	−2.5	−3		
A	1	−0.5	−0.3	−0.8	−1.3	−1.8	−2.3	−2.8		
C	2	−1	−0.8	−0.6	−1.1	−0.8	−1.3	−1.8		
E	3	−1.5	−1.3	−0.3	−0.8	−1.3	−0.3	−0.8		
D	4	−2	−1.8	−0.8	0.2	−0.3	−0.8	0.2		
E	5	−2.5	−2.3	−1.3	−0.3	−0.1	−0.2	−0.7		
C	6	−3	−2.8	−1.8	−0.8	0.2	−0.3	−0.5		
A	7	−3.5	−3.3	−2.3	−1.3	−0.3	−0.1	−0.6		
D	8	−4	−3.8	−2.8	−1.8	−0.8	−0.6	0.4		
E	9									

Smith-Waterman scoring

q\d			R	E	D	C	E	D	K	L
	i\j	0	1	2	3	4	5	6	7	8
	0	0	0	0	0	0	0	0	0	0
A	1	0	0	0	0	0	0	0	0	0
C	2	0	0	0	0	0.5	0	0	0	0
E	3	0	0	0.5	0	0	1	0.5	0	0
D	4	0	0	0	1	0.5	0.5	1.5	1	0.5
E	5	0	0	0.5	0.5	0.7	1.0	1.0	1.2	0.7
C	6	0	0	0	0.2	1	0.5	0.7	0.7	0.9
A	7	0	0	0	0	0.5	0.7	0.2	0.4	0.4
D	8	0	0	0	0.5	0	0.2	1.2	0.7	0.2
E	9	0	0	0.5	0	0.2	0.5	0.7	0.9	0.4

From Eidhammer et al. 2004

Smith-Waterman is an adaptation of Needleman-Wunsch:

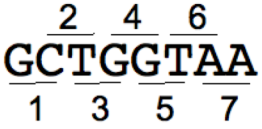
	Needleman-Wunsch	Smith-Waterman
Initialisation	First row and first column are subject to gap penalty	First row and first column set to 0
Scoring	Score can be negative	Negative score is set to 0
Traceback	Begin with cell at bottom right and end at the top left	Begin with the highest score and end when reach score 0

APPENDIX

Local alignment in practice – BLAST 1990

- Smith-Waterman is time expensive, especially for searching large databases >> use heuristics
- BLAST is the most popular local alignment heuristic
  - find short segments of equal length that score > T (preprocessing and scanning)
  - extend the matches formed by such segment pairs without introducing gaps as long as the score does not fall below threshold (~90% execution time)

Example with segment length 2
(in practice longer)



4^2 words

GA 4:3 7:3
GC 1:4 5:3
GG
GT
CA
CC
CG
. .
. .

- match: 2
- transition: 1
- transversion: -2

This basic BLAST method was further refined in 1997 with the two hit method