Selected Algorithms and formulas

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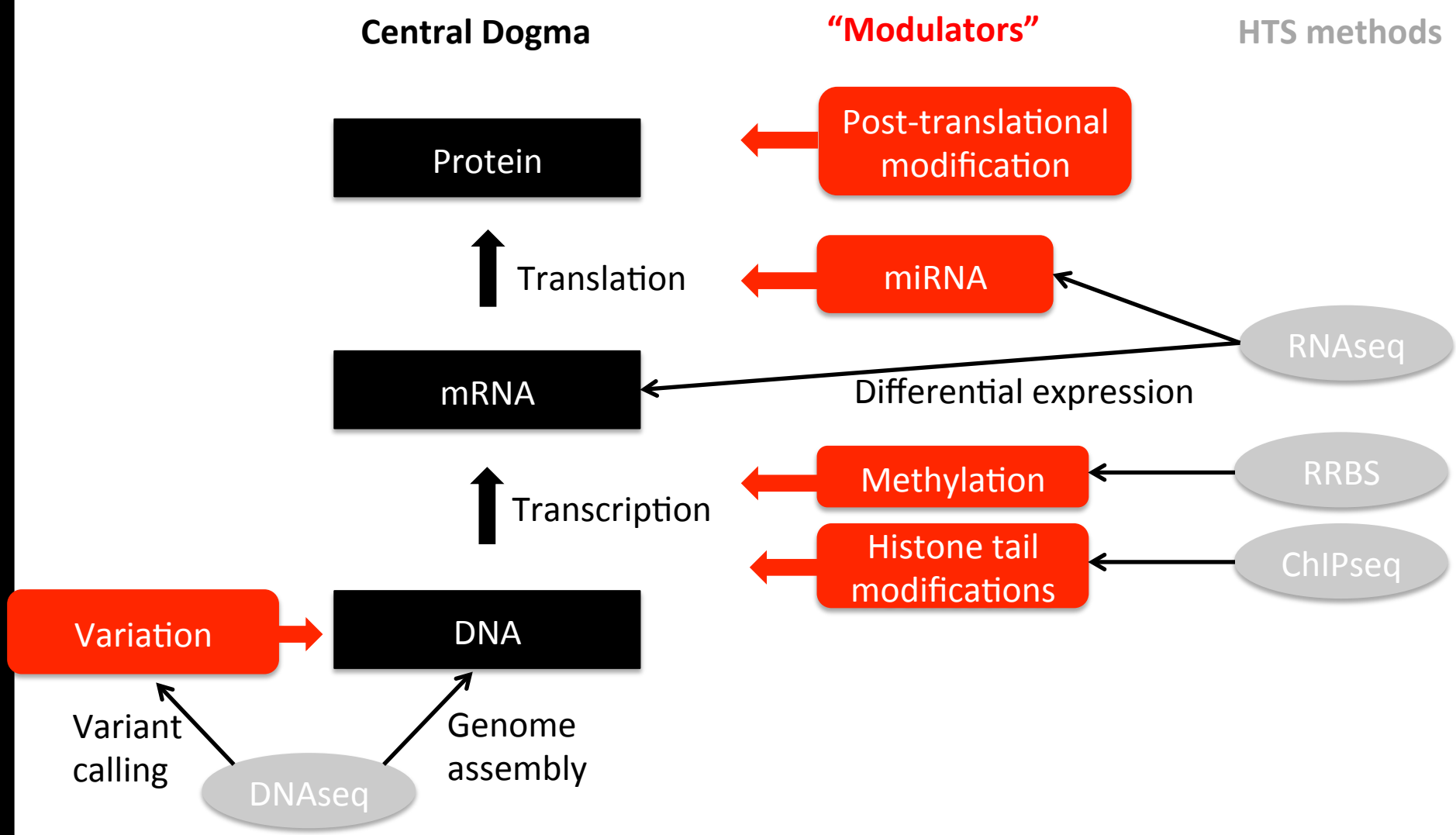
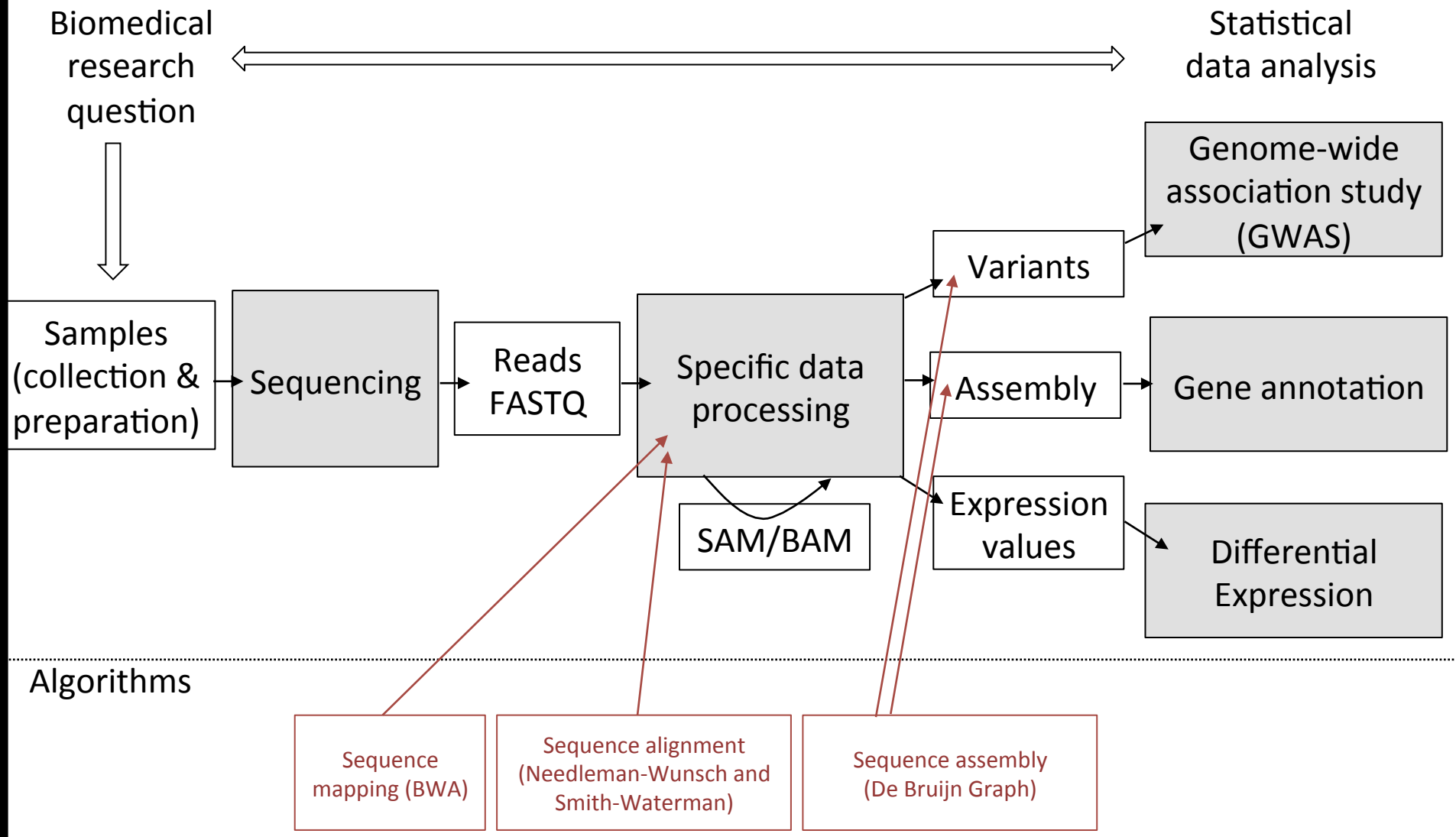
Dpmt of Medical Genetics

Central dogma and HTS

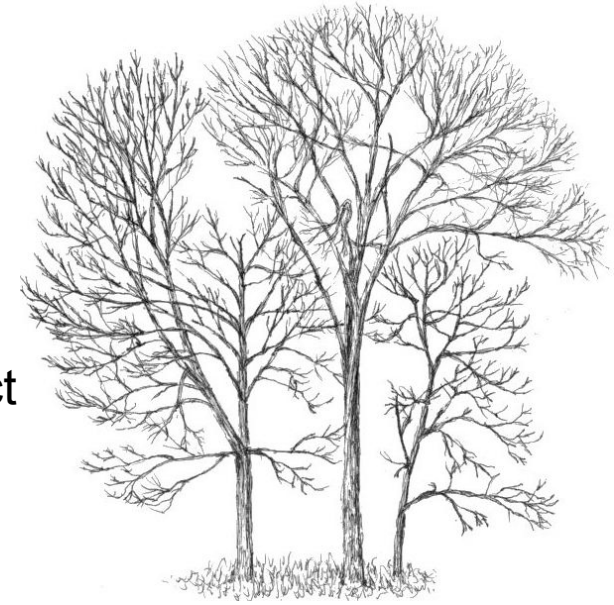


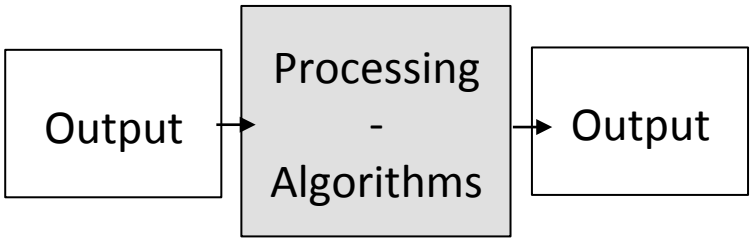
Key algorithms in the big picture?



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.

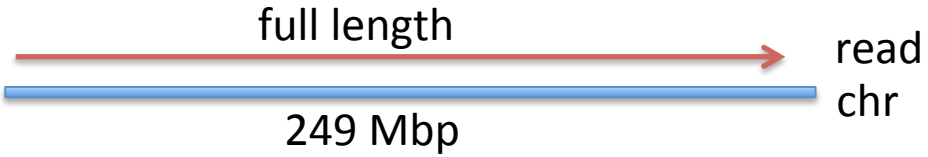


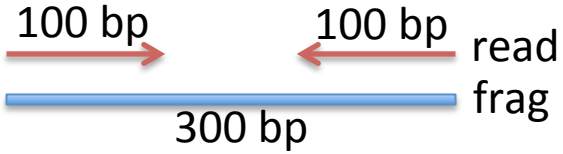


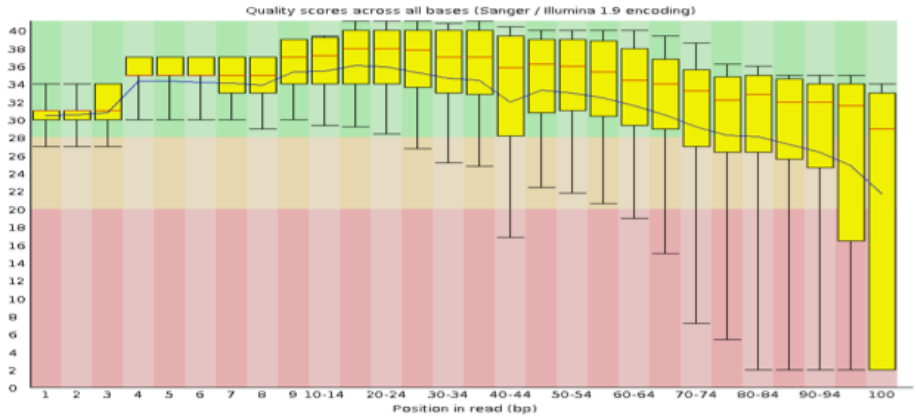
FASTQ: ERROR PROBABILITY, QUALITY SCORES AND ENCODINGS

In a perfect world – Perfect sequencing

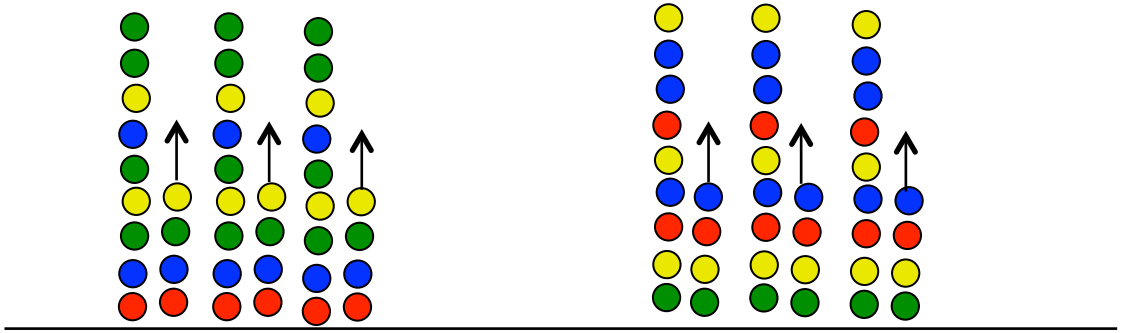
- Perfect sequencing:
  - single molecule (no PCR)
  - **full** length
  - no deterioration of quality
- While we are waiting:
  - Sanger
    - PCR
    - length: some kb
    - limited number of reads
    - high quality
  - HTS (Illumina)
    - PCR
    - 100 bp PE
    - billions of reads
    - high quality,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



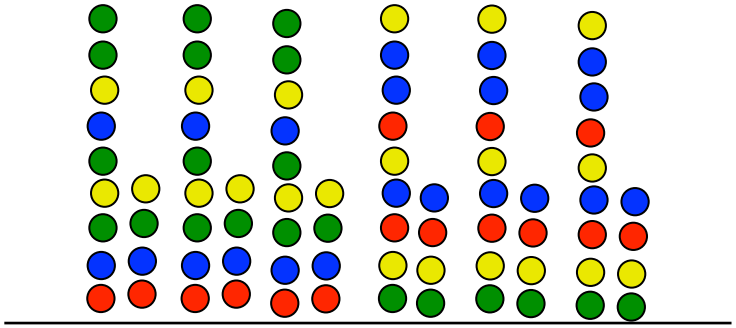




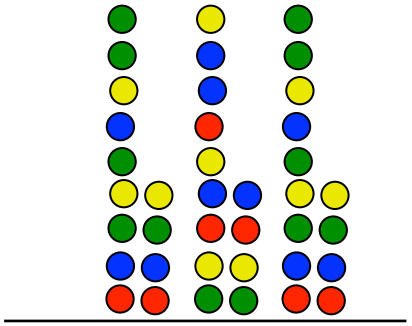
Explaining how/why sequence quality varies



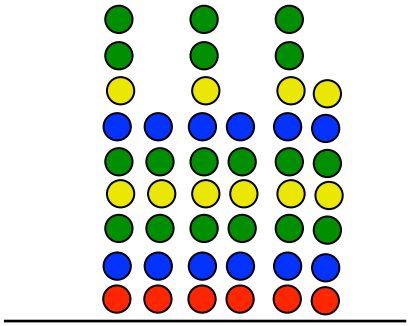
Normal situation: Well defined “pure” clusters



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 $$Q_{\text{sanger}} = -10 \log_{10} p$$
- 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 for:
  - 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
- What is the probability of error of the first base in the above sequence?
- What is the probability of error of the 10th base in the above sequence?

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.....................
  ..............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.....................
  ..............................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
  !"#$%&'()*+,-./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)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
```

- With sequence data recently produced, you do not need to worry
- 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

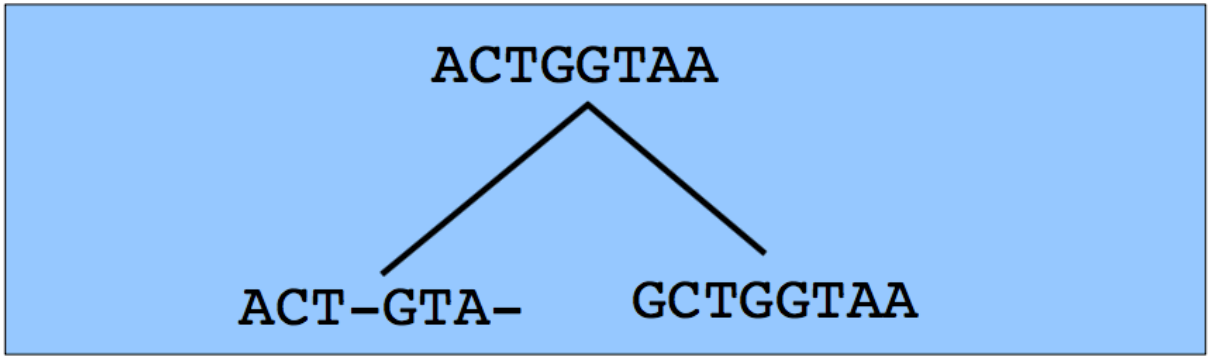
1. Can you locate GTTGCCGTA?
>> sequence mapping

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

SEQUENCE ALIGNMENT

Sequence divergence

Evolution



Observation

```
ACTGTA
GCTGGTAA
```

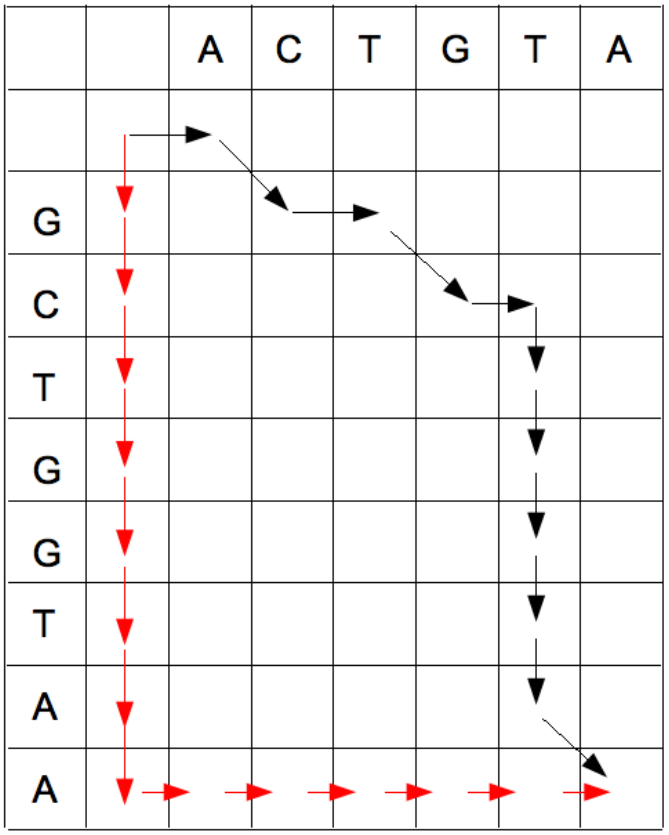
Goal

```
ACT-GTA-
GCTGGTAA
```

What is a global alignment?

- A global alignment of 2 sequences q (query) and d (database) 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
  - Two blanks cannot be aligned

A global alignment is a path in a matrix




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

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

There are a large number of paths through the matrix

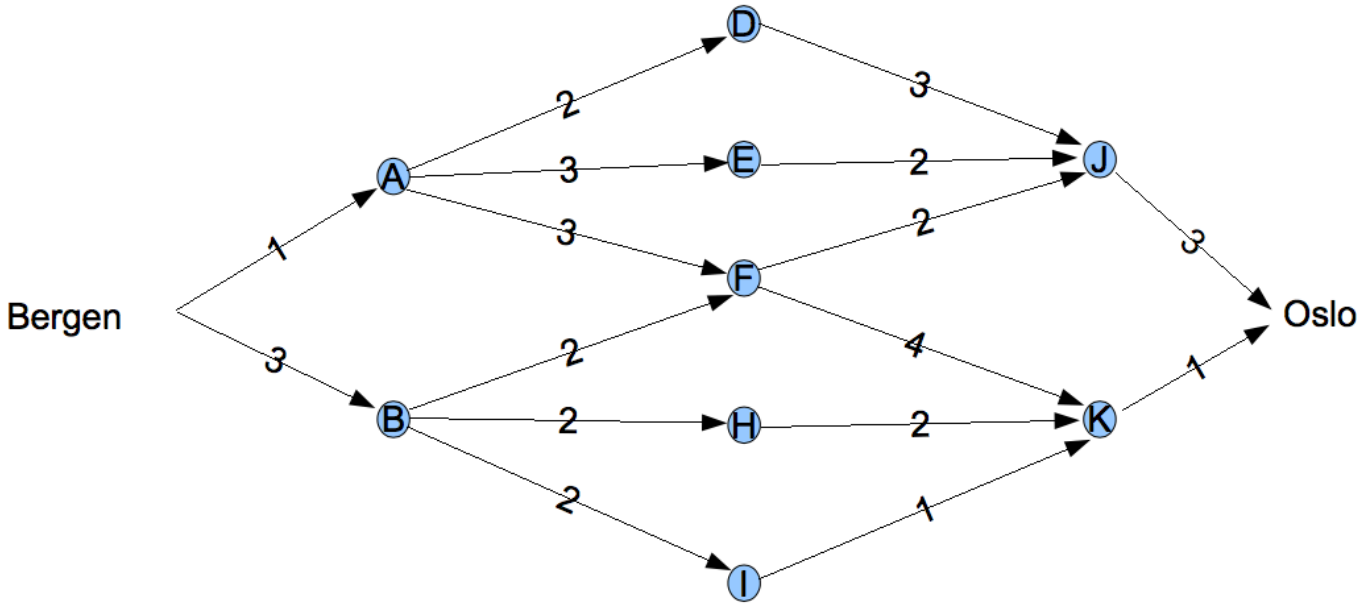
We need:

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

Scoring schemes

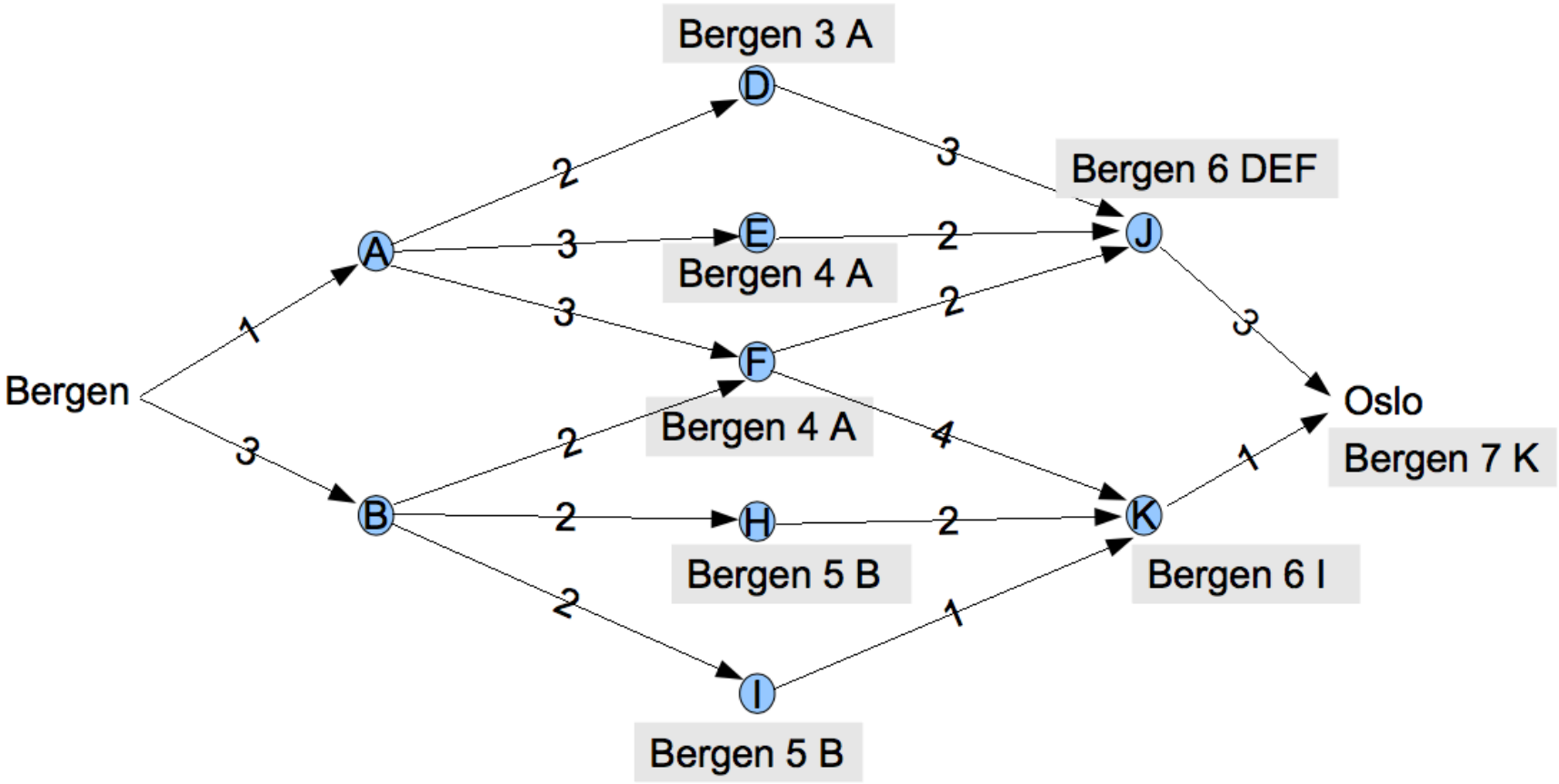
- A scoring scheme
  - each column can be given a score, independently of the other columns, meaning that mutations are single mutations
    - need score for alignment of two residues
    - need scoring for alignment of residue with gap
  - The score of the alignment can be found as the sum of the score of all columns
- Scoring scheme is critical to the alignment produced
- Computation time intensive to enumerate all alignments and score them all to find the best scoring
- Dynamic programming to find the best scoring alignments (Needleman and Wunsch 1970)

Shortest path



- 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



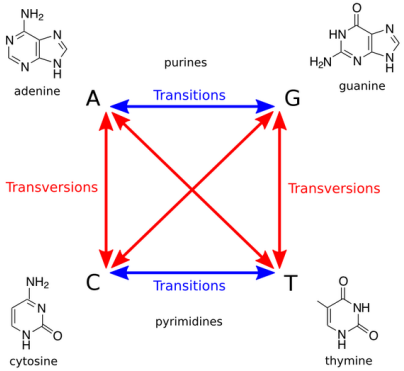
The best alignment

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

Needleman-Wunsch 1970

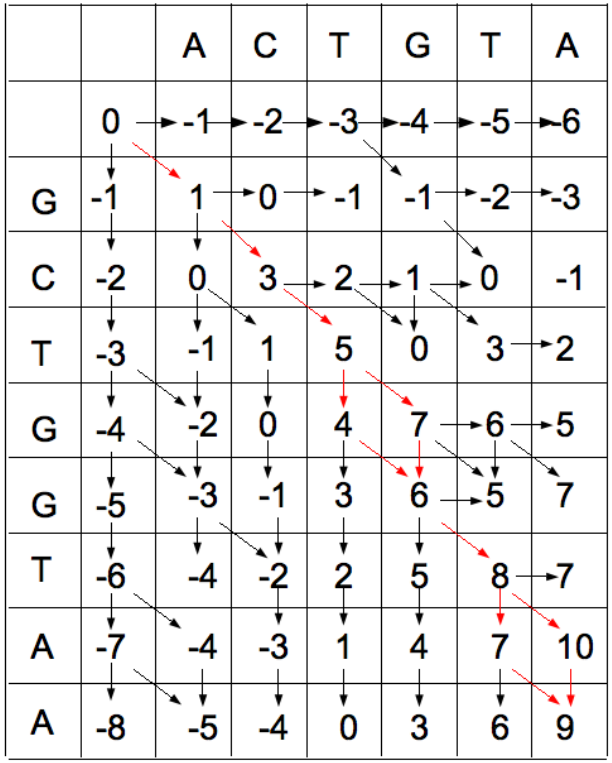
Example scoring scheme
- gap: -1
- match: 2
- transition: 1
- transversion: -2



		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

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

The best alignments



		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-------ATATGTT

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

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

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

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

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

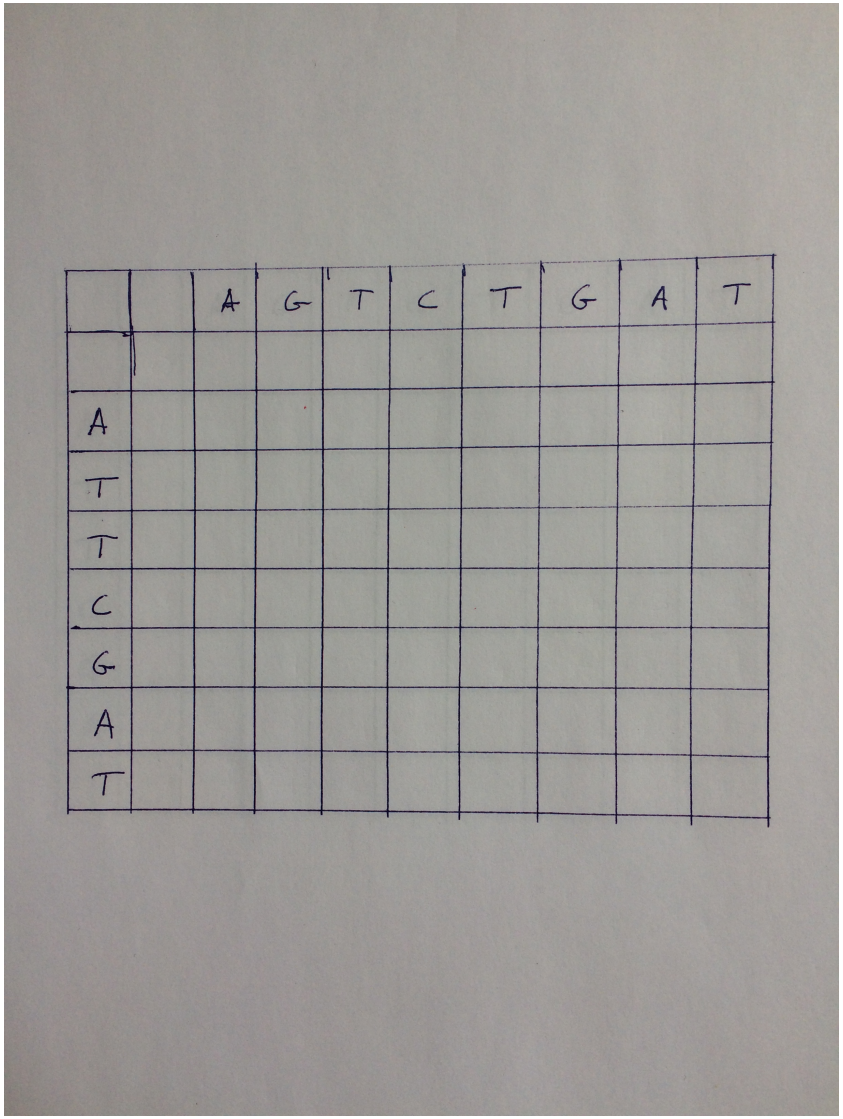
Practical with Needleman-Wunsch

Match scores +2
Mismatch scores -1
Gap scores -1

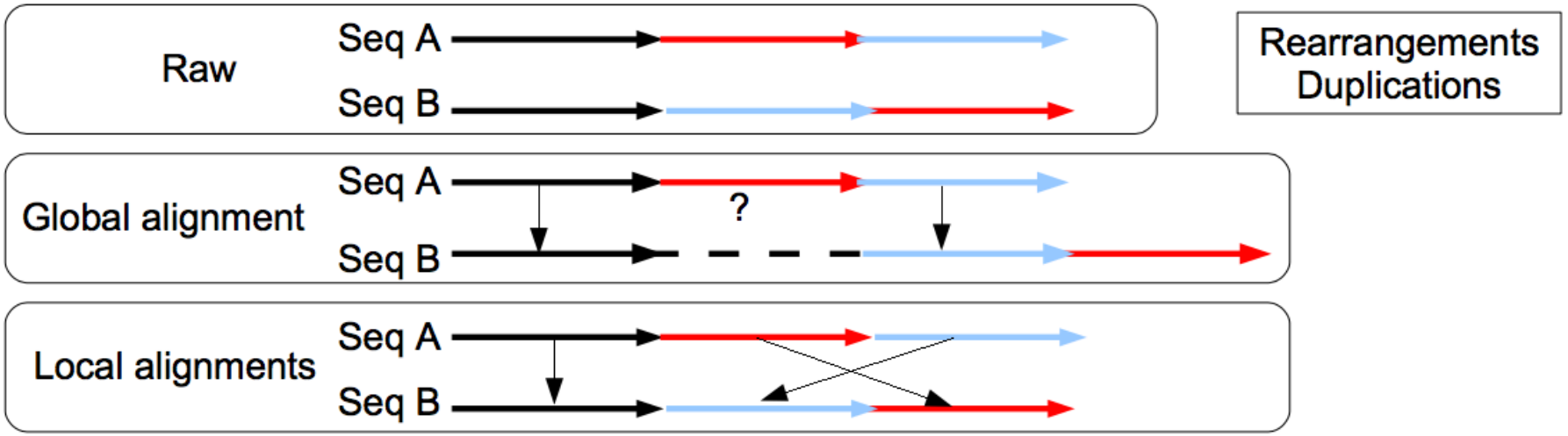
1. Initialise
2. Score

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

3. Traceback

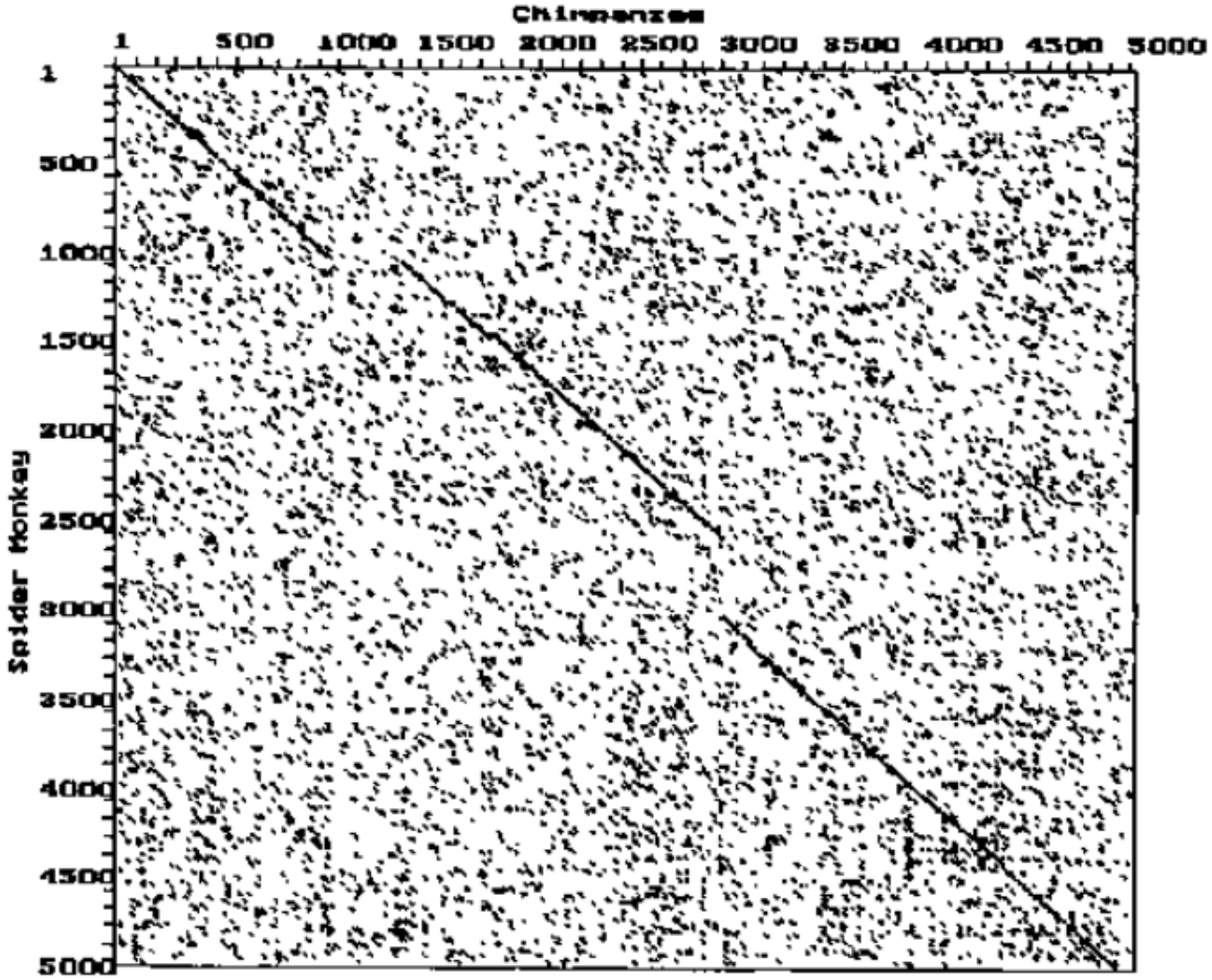


Pairwise local alignment



- A segment is a substring of q or d (it does not contain gaps)
- A segment pair is a pair with one segment from each of q and d (they need not be of equal length)
- A local alignment is an alignment of a segment pair

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

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									

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:

- Initialise with 0 and allow min value to be 0
- The best local alignments: backtrack arrows from the cells with maximum value, until a cell with value 0 is reached

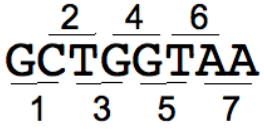
	Smith–Waterman algorithm	Needleman–Wunsch algorithm
Initialization	First row and first column are set to 0	First row and first column are subject to gap penalty
Scoring	Negative score is set to 0	Score can be negative
Traceback	Begin with the highest score, end when 0 is encountered	Begin with the cell at the lower right of the matrix, end at top left cell

From Wikipedia

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