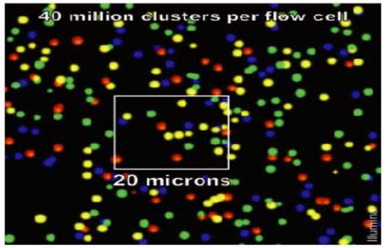
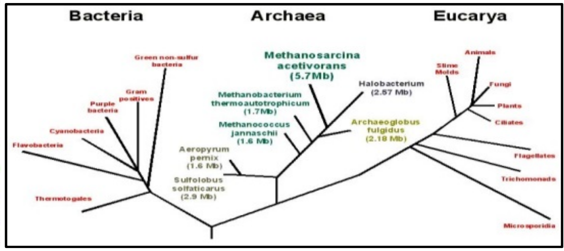
TAACCCTAACCCTAACCCTAACCCTAACCCTA
CCTAACCCTAACCCTAACCCTAACCCTAACCC
CCCTAACCCCTAACCCTAACCCTAACCCTAAC
AACCCTAACCCTAACCCTAACCCCTAACCCTA
ACCCTAACCCCAACCCCAACCCCAACCCCAAC
CTACCCTAACCCTAACCCTAACCCTAACCCTA
ACCCTAACCCTAACCCTAACCCCTAACCCTAA



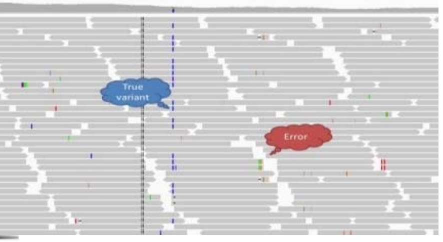

Bacteria
Archaea
Eucarya
Green non-sulfur bacteria
Gram positives
Purple bacteria
Cyanobacteria
Flavobacteria
Thermotogales
Methanosarcina acetivorans (5.7Mb)
Methanobacterium thermoautotrophicum (1.7Mb)
Methanococcus jannaschii (1.6 Mb)
Halobacterium (2.57 Mb)
Archaeoglobus fulgidus (2.18 Mb)
Aeropyrum pernix (1.6 Mb)
Sulfolobus solfataricus (2.9 Mb)
Animals
Slime Molds
Fungi
Plants
Ciliates
Flagellates
Trichomonads
Microsporidia

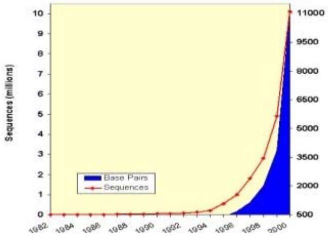
Next Generation Sequencing (NGS): Reads Mapping

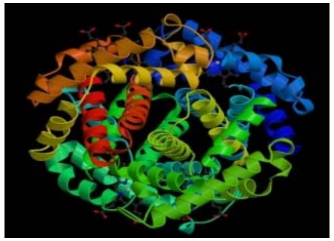
北京大学生物信息学中心 高歌

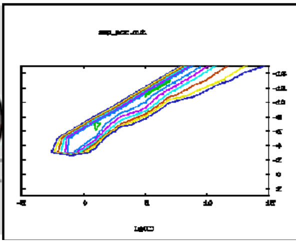
Ge Gao, Ph.D.

Center for Bioinformatics, Peking University









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TAACCCTAACCCTAACCCTAACCCTAACCCTA
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CCCTAACCCCTAACCCTAACCCTAACCCTAAC
AACCCTAACCCTAACCCTAACCCCTAACCCTA
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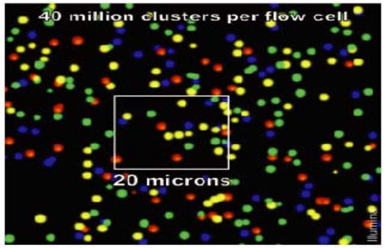

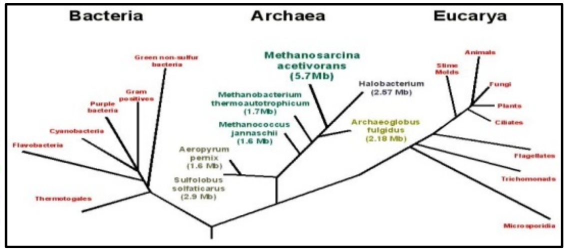
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Unit 2:
NGS: Reads Mapping

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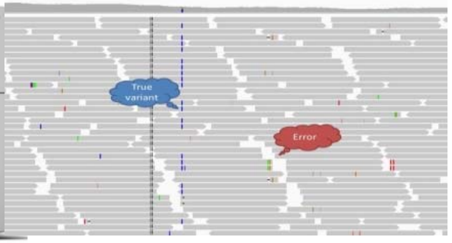
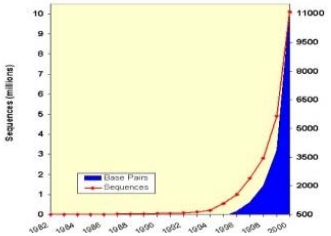
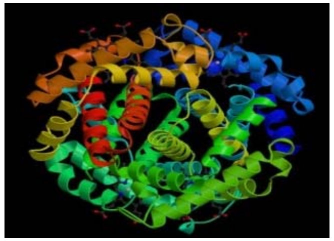
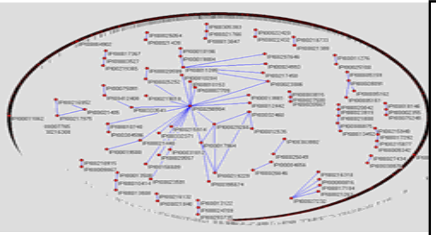
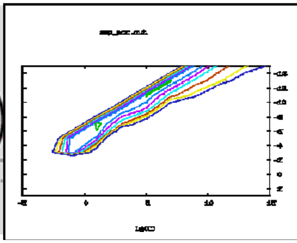


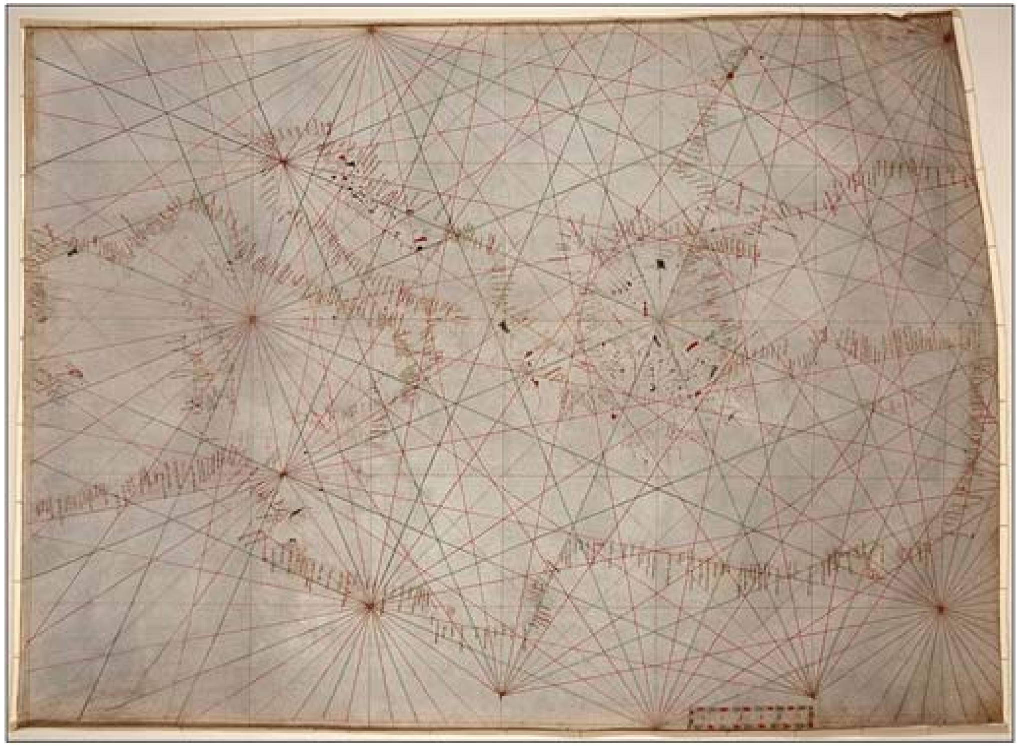








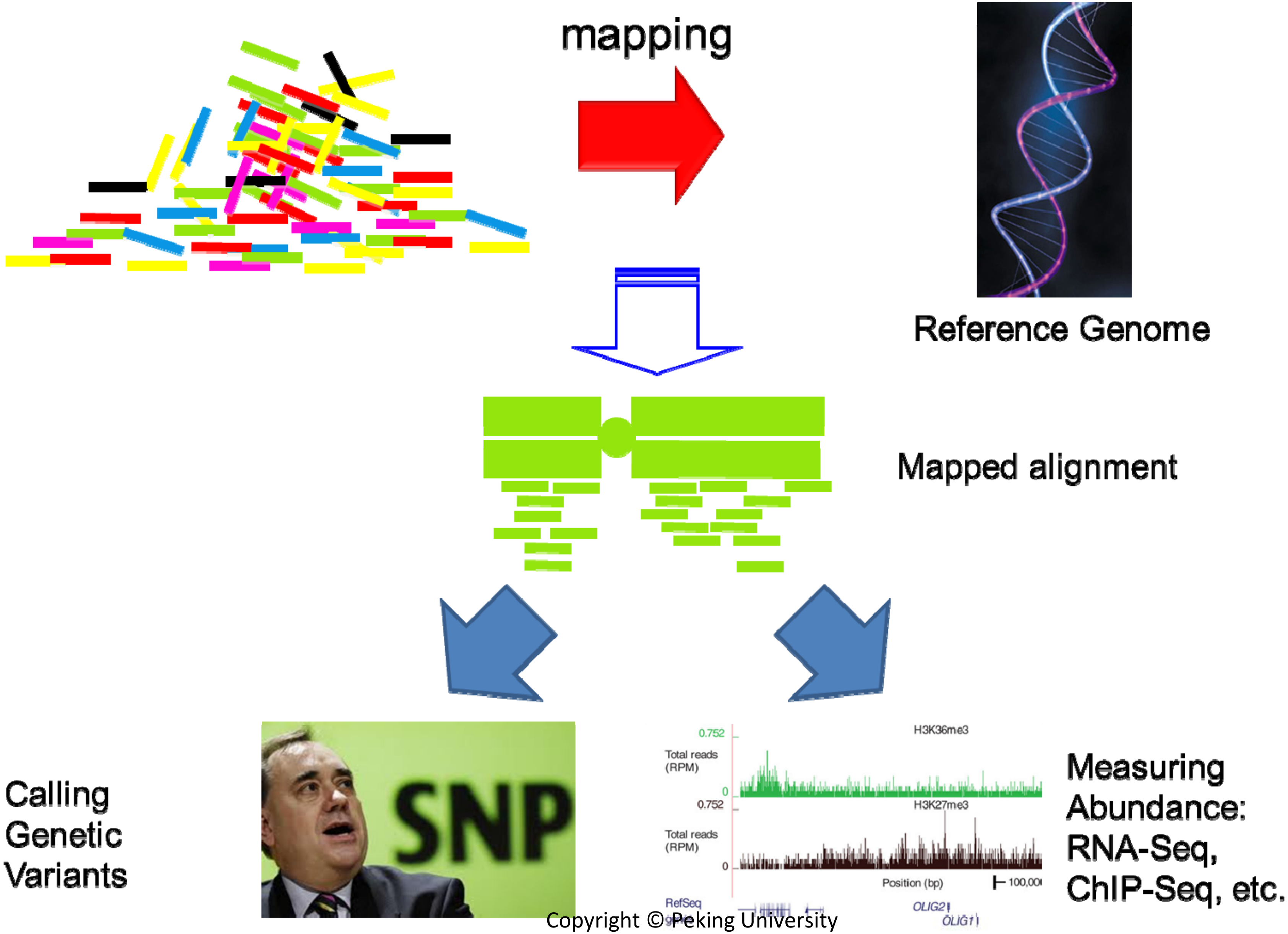
Reads Mapping



Map-Making / Cartography:
Establish relationship between locations
(http://en.wikipedia.org/wiki/Cartography)

<ins>Technological</ins>: Reads is usual too short to be used/assembled *de novo*

<ins>Scientific</ins>: Taking full usage of existing annotation/knowledge



mapping

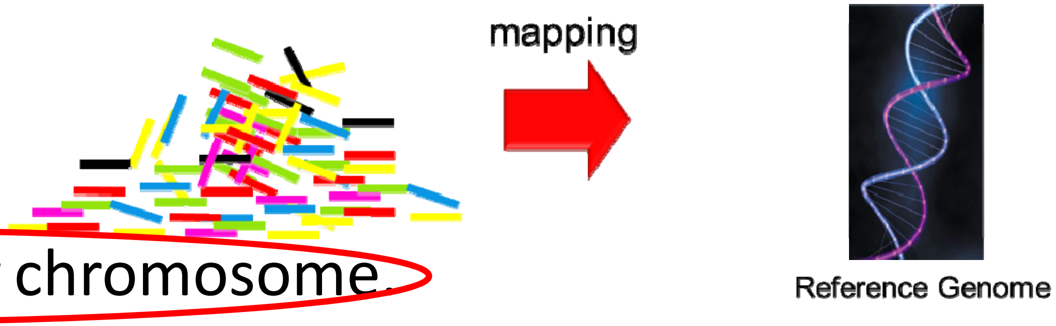
Reference Genome

Mapped alignment

Calling Genetic Variants

Measuring Abundance: RNA-Seq, ChIP-Seq, etc.

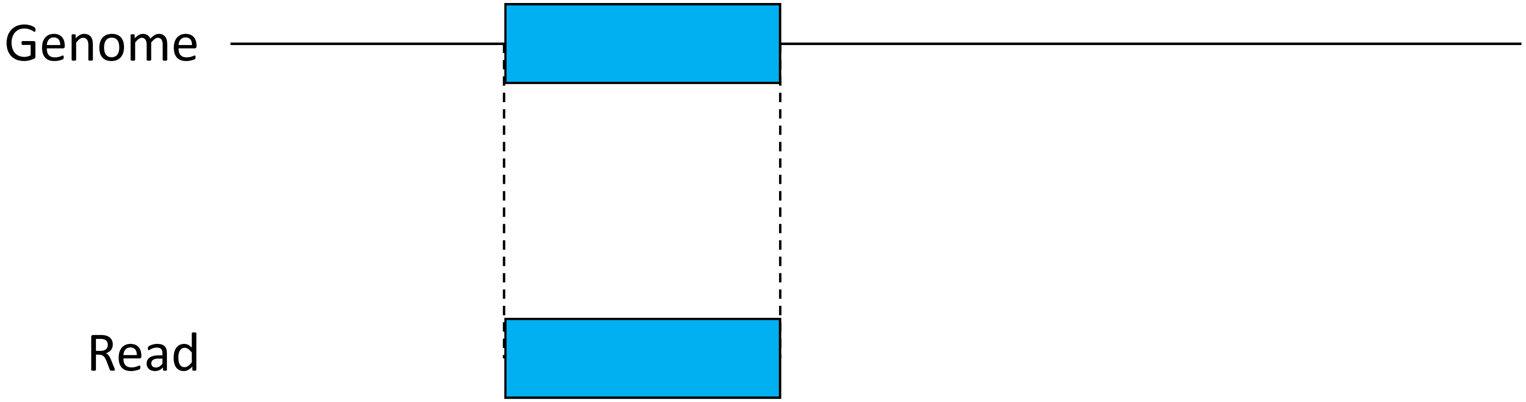
Mapping: Input Data



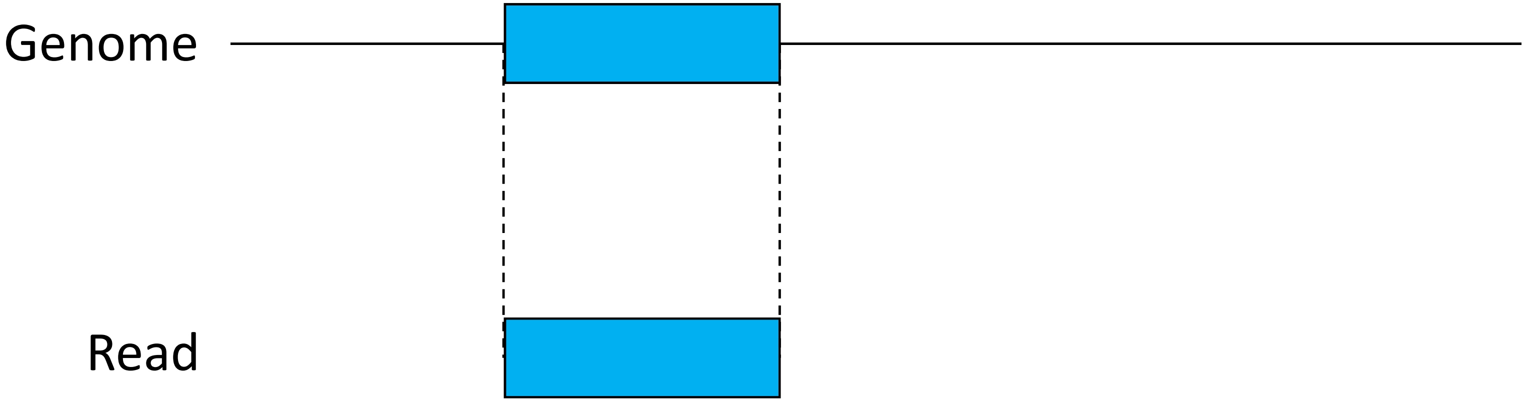
- Reference Genome
  - Nucleotide
  - **Length**: Hundreds of Mb *per* chromosome.
  - ~3 Gb in total (for human genome)

- Reads
  - Nucleotide, with **various qualities** (relatively **high error rate**: 1e-2 ~ 1e-5)
  - **Length**: 36~80 bp *per* read
  - Hundreds of Gbs *per* run

"Embedded" Alignment

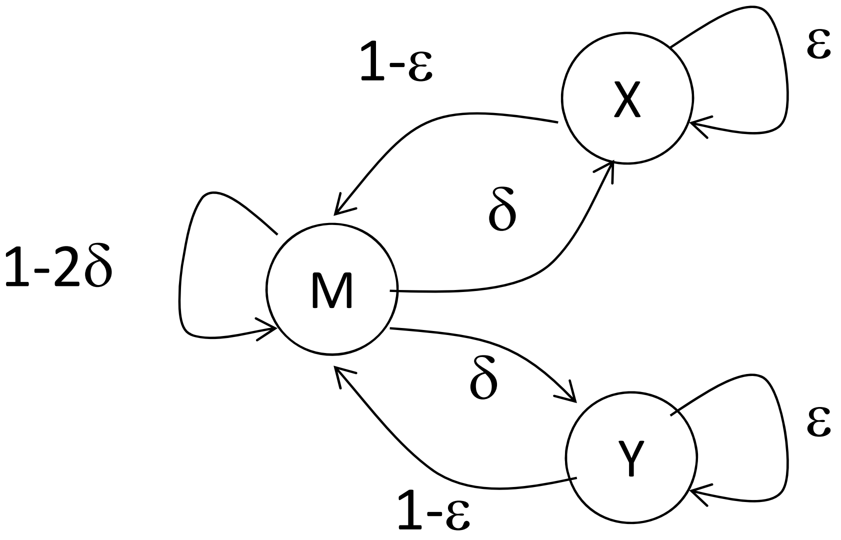


One sequence is "*embedded*" in the other sequence (NGS Reads, PCR primer, *etc.*)



What we need here is actually a hybrid "global-local" alignment

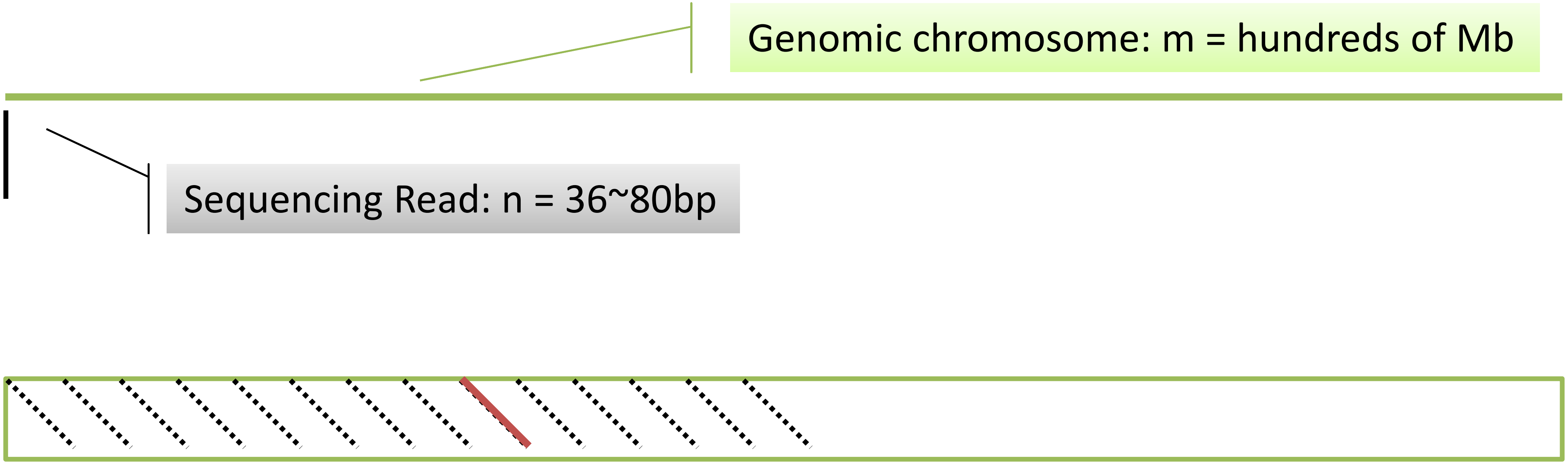
- ✓ "Global" for short sequence (i.e. NGS Read)
- ✓ But "Local" for long sequence (i.e. Reference Genome)
- ✓ In particular, the surrounding "overhang" gaps should be not penalized.



δ	Gap open
ε	Gap Extension

M	*Match*
X	*Insert* at sequence X (delete at sequence Y)
Y	~~*Insert* at sequence Y~~ ~~(delete at sequence X)~~

	M	X	Y
M	1-2δ	δ	~~δ~~
X	1-ε	ε	~~0~~
Y	~~1-ε~~	~~0~~	~~0~~



Genomic chromosome: m = hundreds of Mb

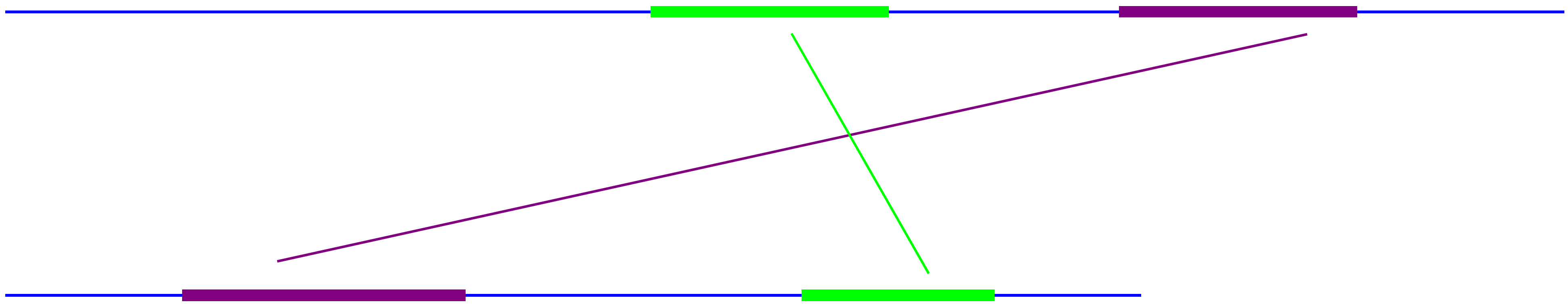
Sequencing Read: n = 36~80bp

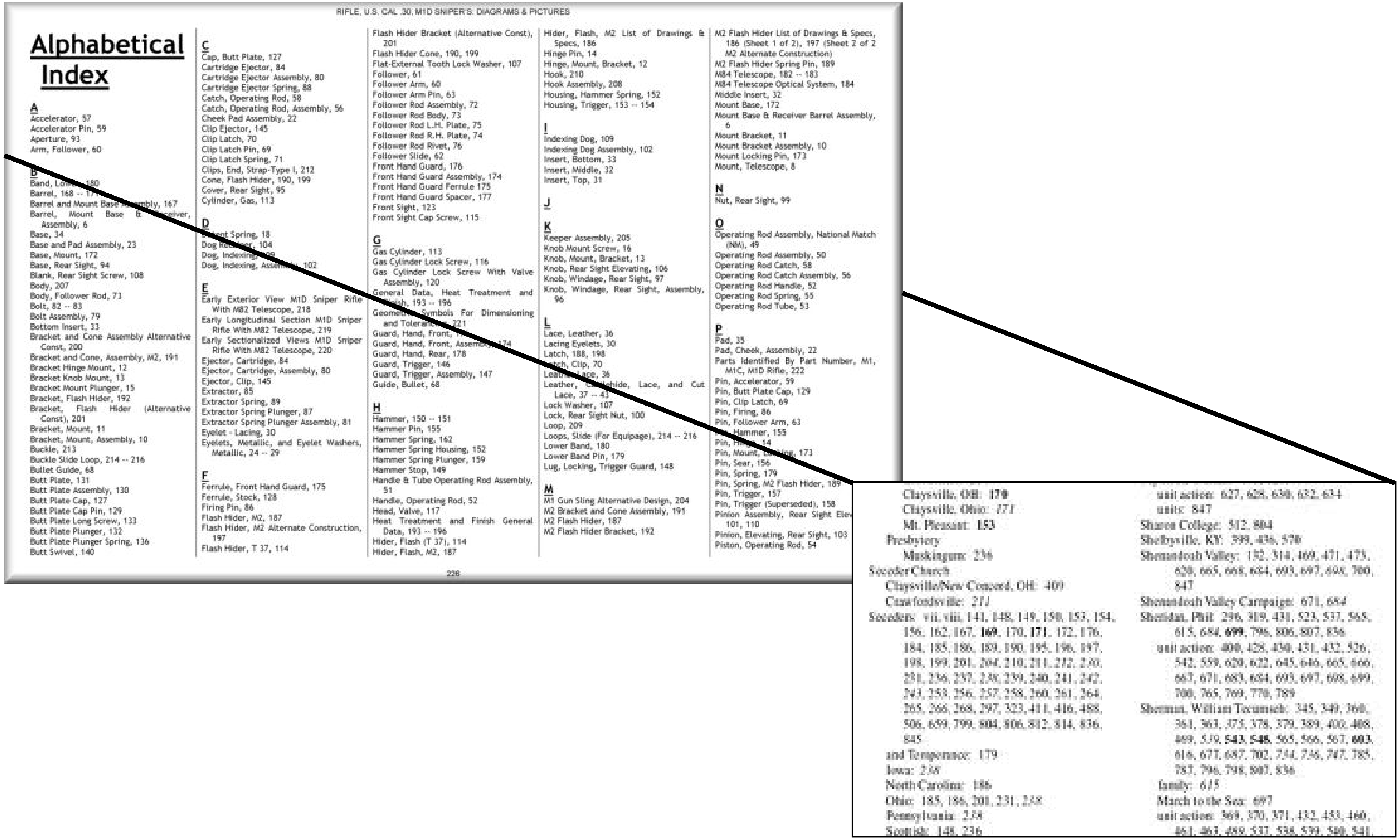
Most of paths will just fail eventually!

In real world, the speed will be a BIG problem!

BLAST Ideas: Seeding-and-extending

1. Find matches (seed) between the query and subject
2. Extend seed into High Scoring Segment Pairs (HSPs)
   - Run Smith-Waterman algorithm on the specified region only.
3. Assess the reliability of the alignment.





RIFLE, U.S. CAL .30, M1D SNIPER'S: DIAGRAMS & PICTURES

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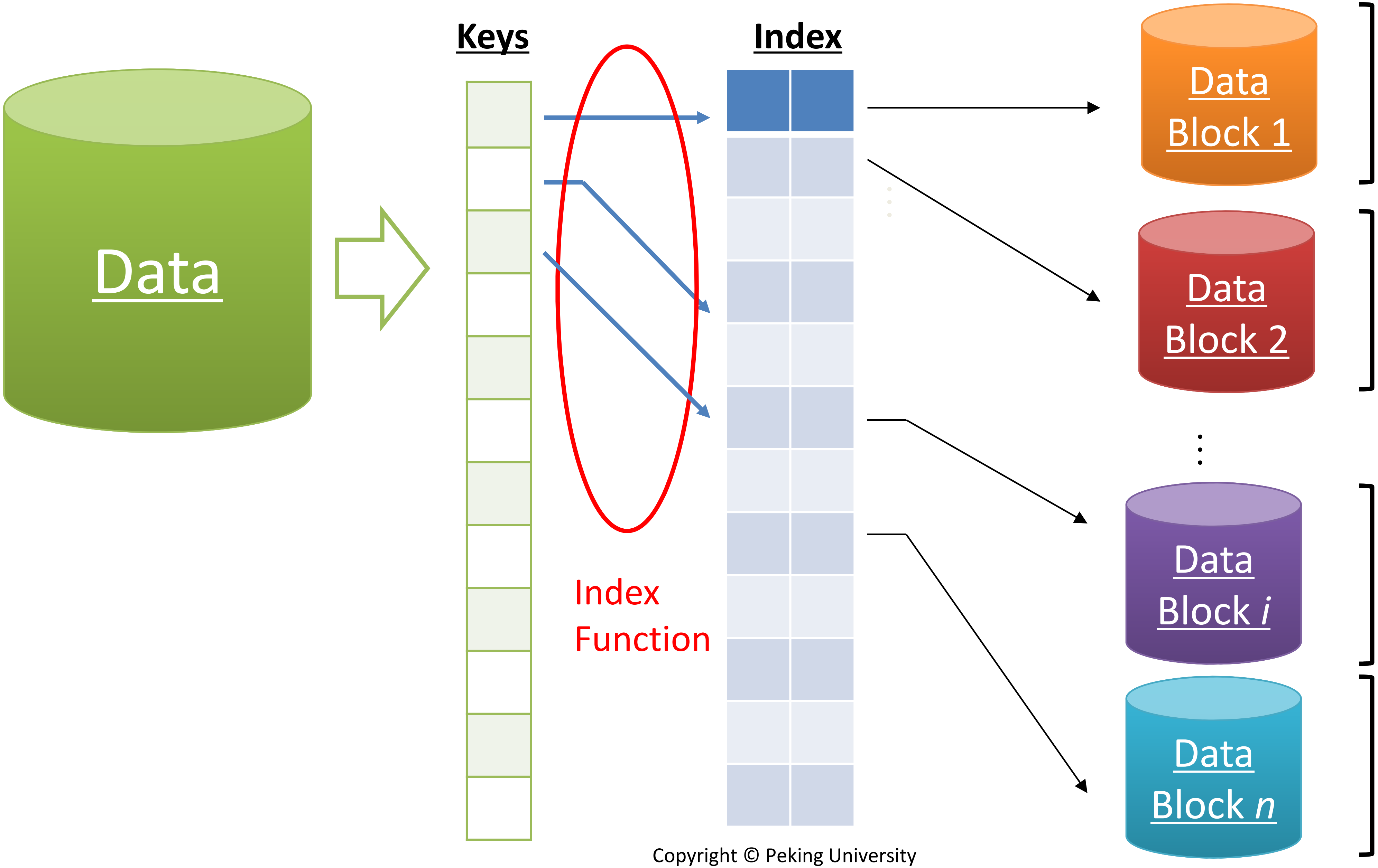
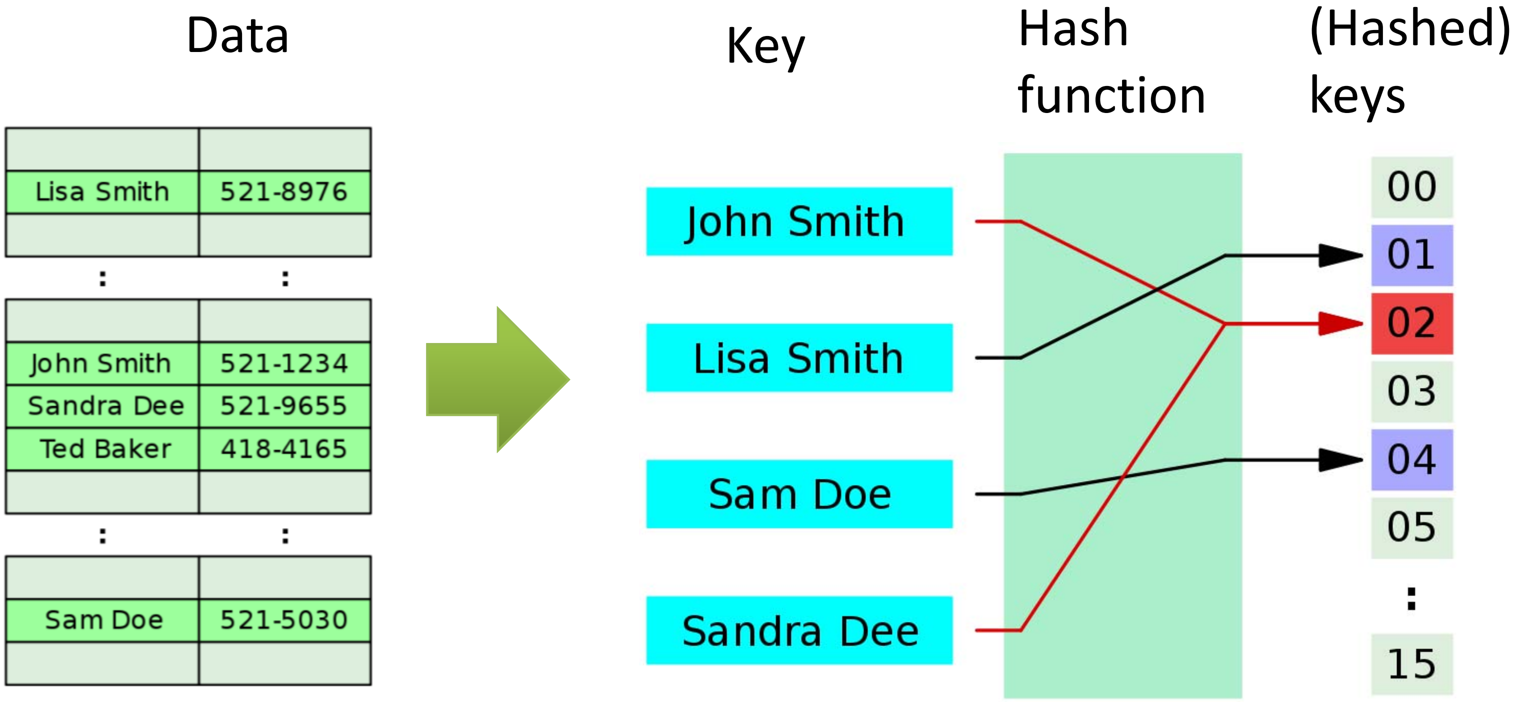

Data
Keys
Index
Index Function
Data Block 1
Data Block 2
Data Block *i*
Data Block *n*

Hash

Hash function maps (partial) data into (hashed) keys for following-up indexing



Data	
Lisa Smith	521-8976
:	:
John Smith	521-1234
Sandra Dee	521-9655
Ted Baker	418-4165
:	:
Sam Doe	521-5030

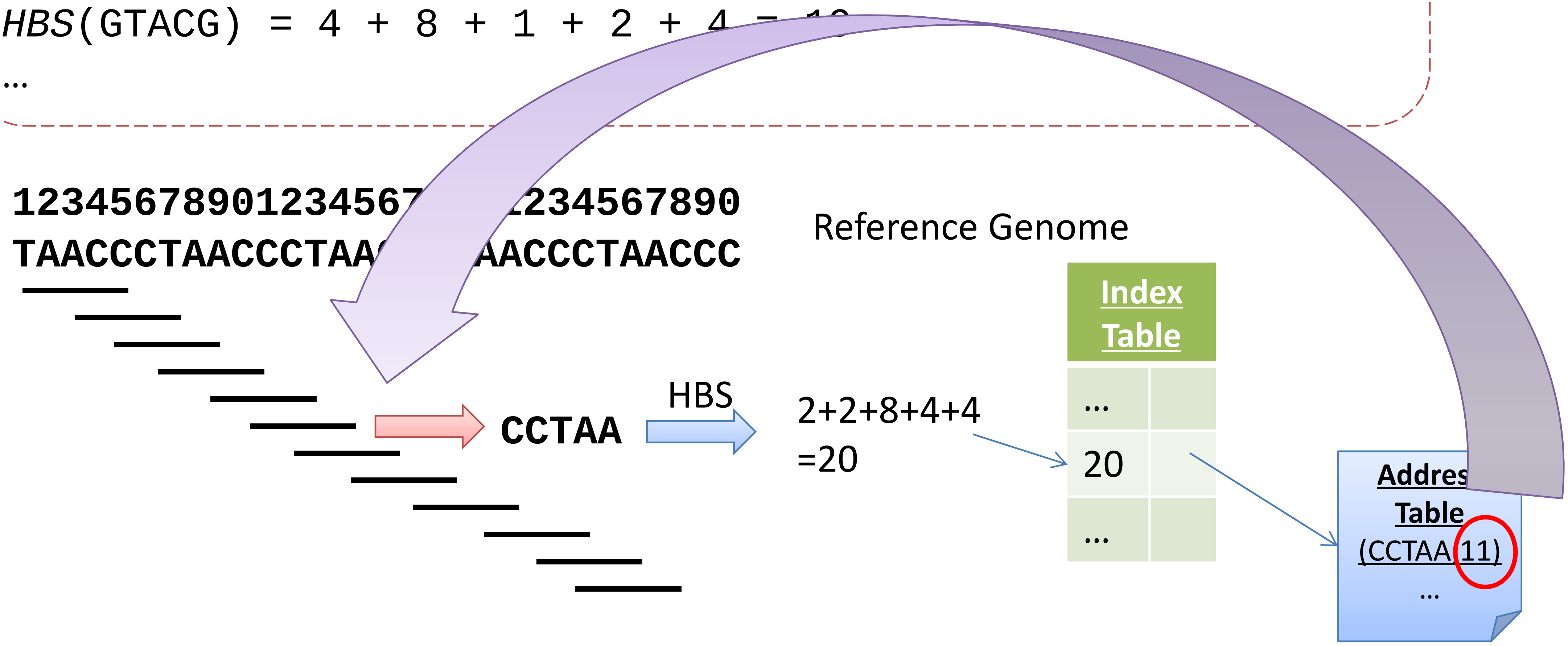
HBS: A naive hash function

Let’s assume: A = 1, C = 2, G = 4, T = 8, then: $HBS(S) = \sum_i HBS(S_i)$, e.g:

HBS(AAAAA) = 1 + 1 + 1 + 1 + 1 = 5

HBS(GTACG) = 4 + 8 + 1 + 2 + 4 = 19

…

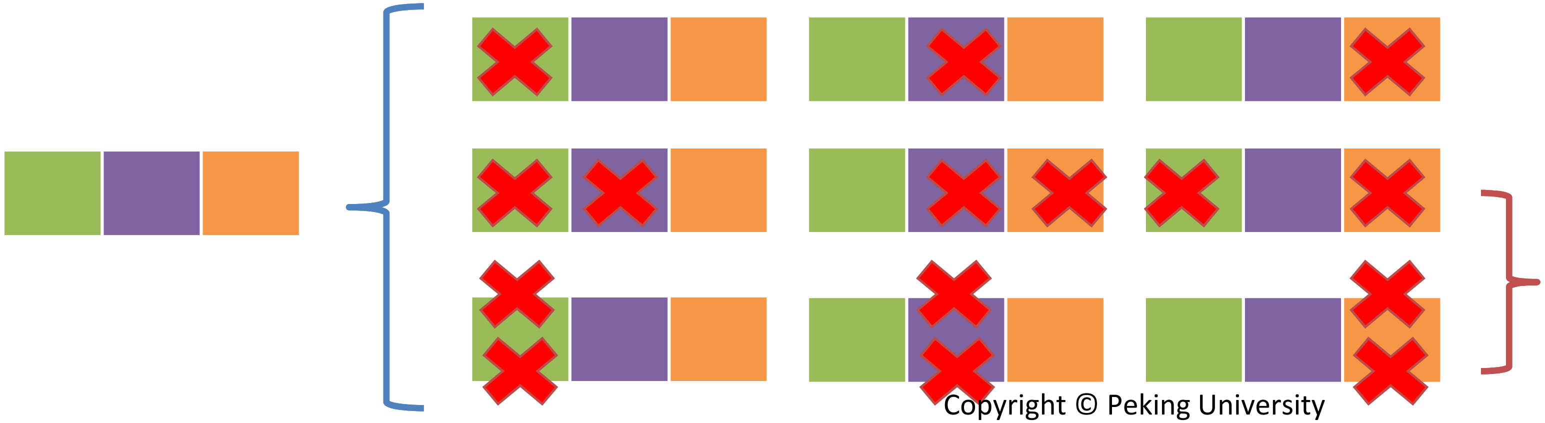


Pigeonhole principle (抽屉原理)

"In mathematics, the pigeonhole principle states that if *n* items are put into *m* pigeonholes with *n* > *m*, then at least one pigeonhole must contain more than one item. "

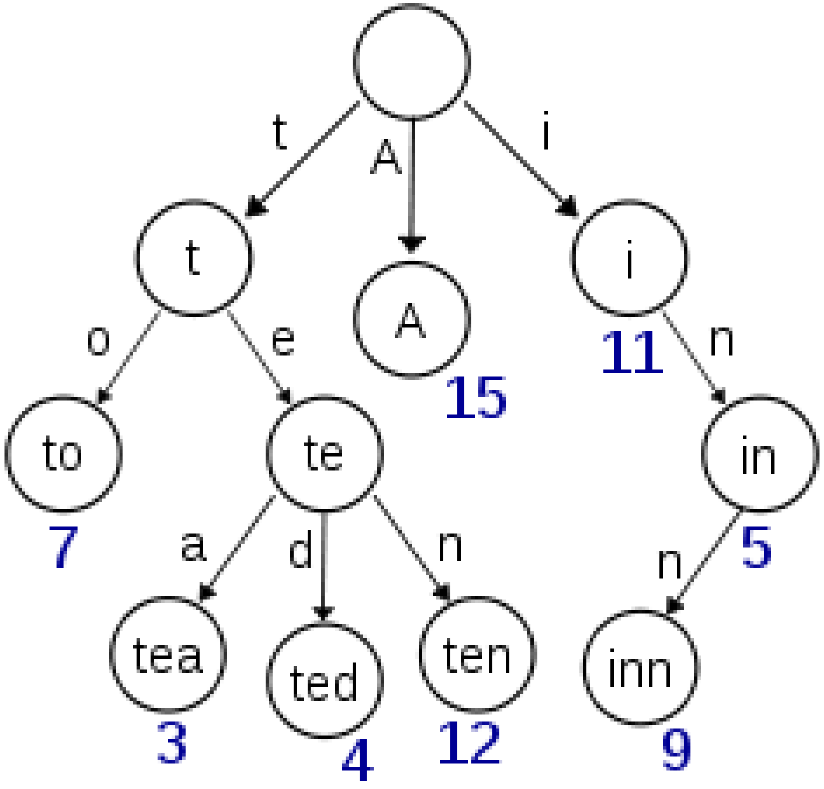
http://en.wikipedia.org/wiki/Pigeonhole_principle

After splitting the read into *n* (non-overlapped) blocks, there will be at least *n*-*m* perfectly-matched blocks (i.e. without any mismatch with in the block) by allowing up-to-*m* mismatches.



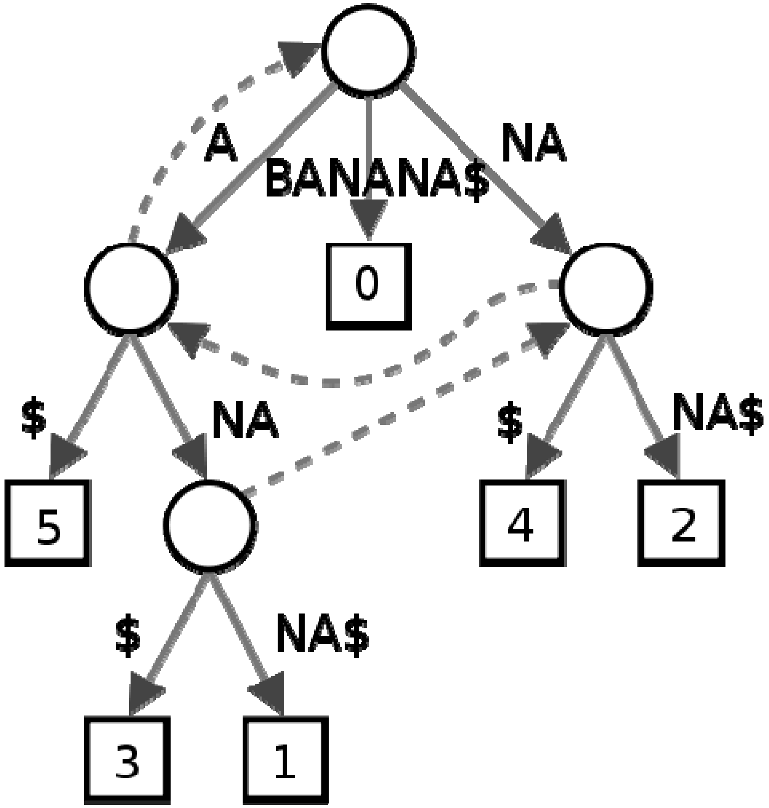
ELAND
MAQ
SOAP1
…

Prefix Tree



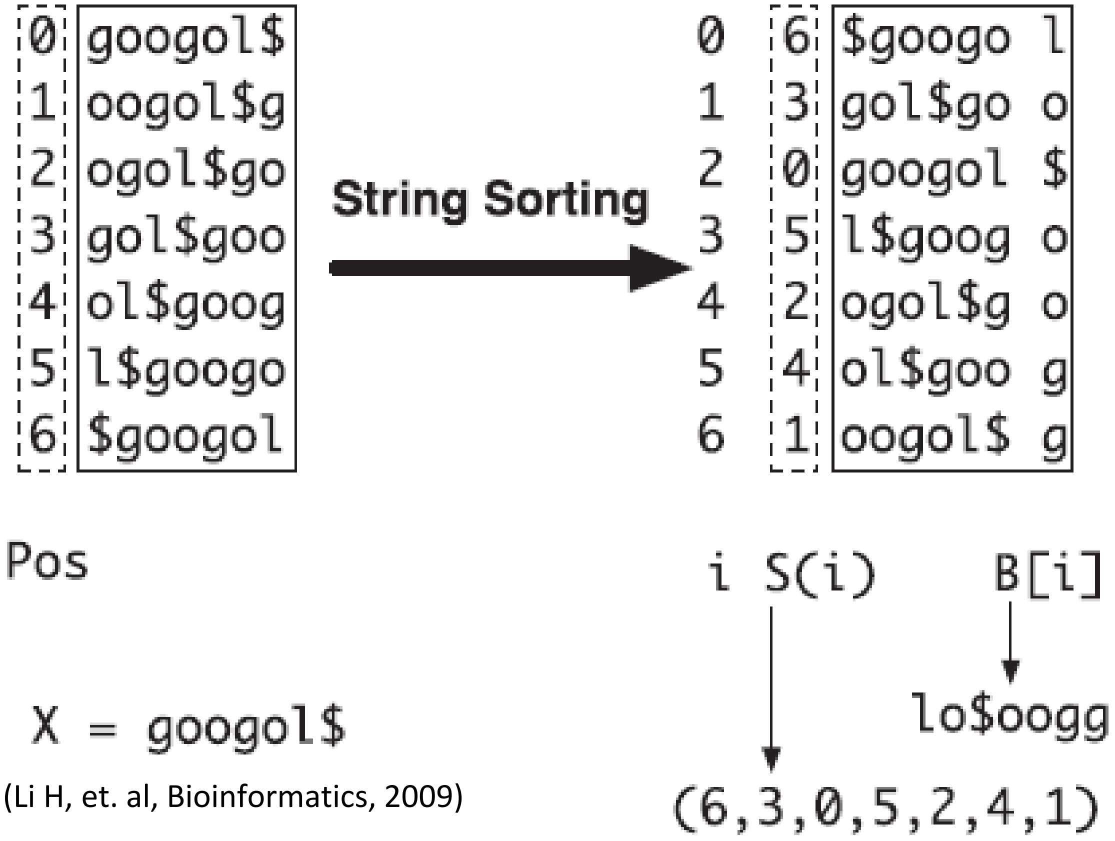
http://en.wikipedia.org/wiki/Trie

Suffix Tree



http://en.wikipedia.org/wiki/Suffix_tree

Burrows–Wheeler transform (BWT)



Pos	
0	googol$
1	oogol$g
2	ogol$go
3	gol$goo
4	ol$goog
5	l$googo
6	$googol

String Sorting

i	S(i)		B[i]
0	6	$googo	l
1	3	gol$go	o
2	0	googol	$
3	5	l$goog	o
4	2	ogol$g	o
5	4	ol$goo	g
6	1	oogol$	g

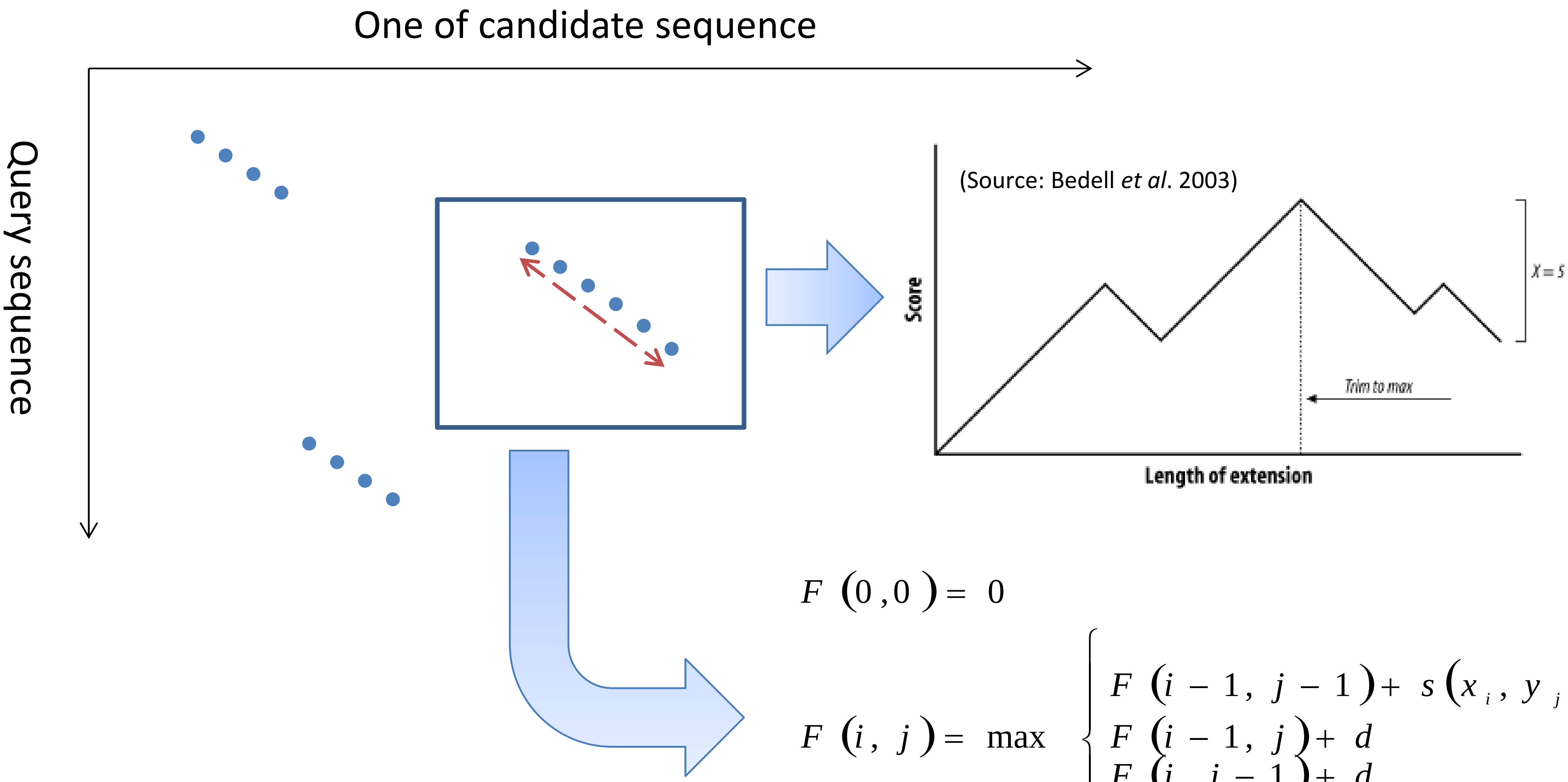
X = googol$

lo$oogg

(6,3,0,5,2,4,1)

(Li H, et. al, Bioinformatics, 2009)

BOWTIE
BWA
SOAP3
…



(Source: Bedell *et al*. 2003)

$$F(0,0) = 0$$

$$F(i,j) = \max \begin{cases} F(i-1, j-1) + s(x_i, y_j) \\ F(i-1, j) + d \\ F(i, j-1) + d \\ 0 \end{cases}$$

Quality: Given *p* = the probability of a base calling is *wrong*, its Quality Score can be written as

$$Q = -10 * log_{10}(p)$$

p	Q
0.1	10
0.01	20
0.001	30
0.0001	40

```
0         10        20        30        40
|         |         |         |         |
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHI
|         |         |         |         |
0         10        20        30        40
```

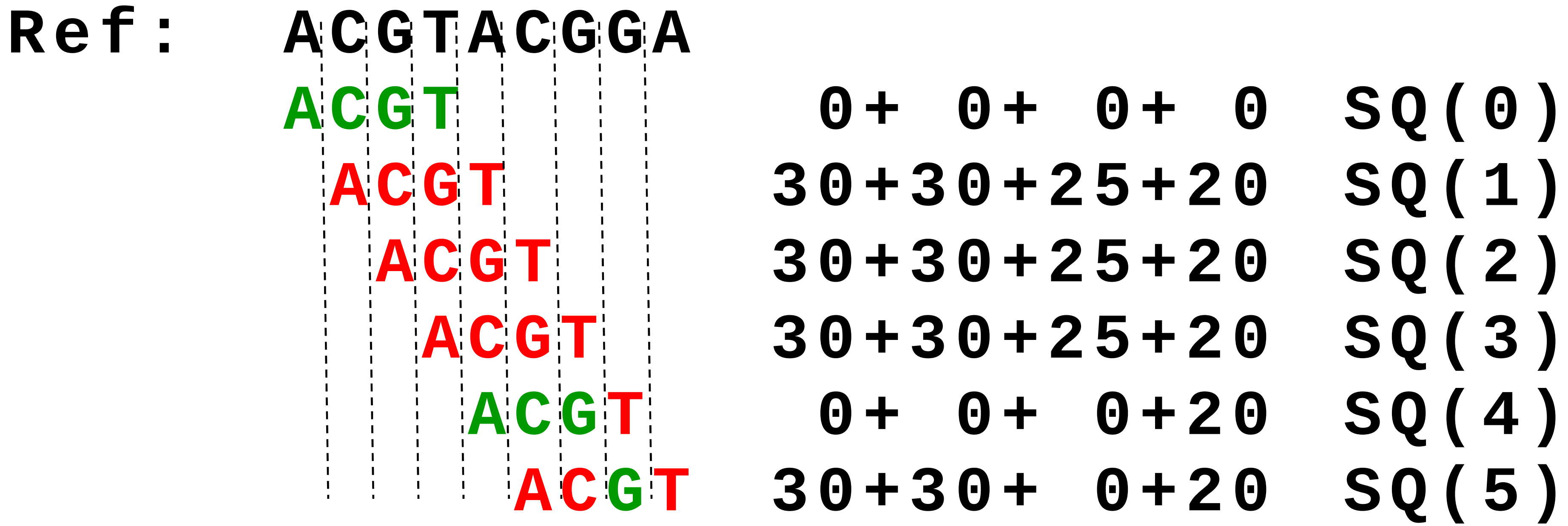
Mapping Quality

Given reference sequence *z* (length *L*), a read sequence *x* (length *l*), *u* is the alignment position of *x* on *z*, the probability that *z* actually coming from the position *u* is ***p(z|x,u)***

(*Genome Res*. 2008 Nov;18(11):1851.)

$$p(z\,|\,x,u)=\prod_{mismatch} p(z_i) \qquad SQ(u)=\log(p(z\,|\,x,u))=\sum_{mismatch} p(z_i)=\sum_{mismatch} Q(z_i)$$

```
Read:  ACGT  (Quality: 30 30 25 20)
```



```
Ref:   ACGTACGGA
       ACGT             0+ 0+ 0+ 0   SQ(0)
        ACGT           30+30+25+20   SQ(1)
         ACGT          30+30+25+20   SQ(2)
          ACGT         30+30+25+20   SQ(3)
           ACGT         0+ 0+ 0+20   SQ(4)
            ACGT       30+30+ 0+20   SQ(5)
```

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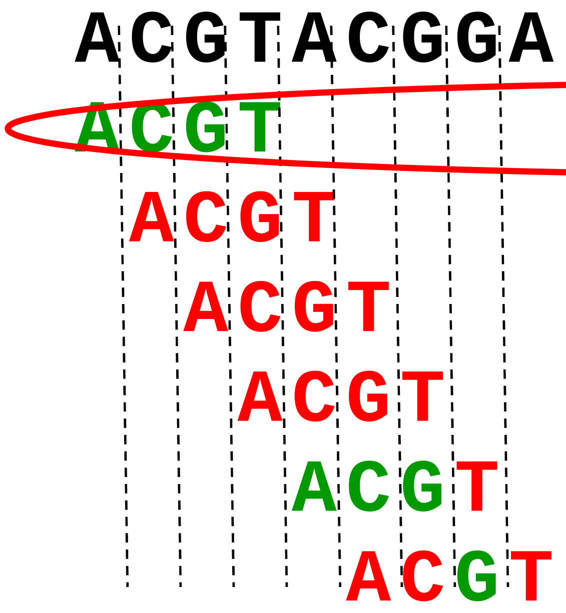
Mapping Quality

If we assume that a uniform NULL model, i.e. the read can randomly come from all possible positions with equal probability, then the error of mapping to a specified position *u* could be written as

(*Genome Res*. 2008 Nov;18(11):1851.)

$$E(u) = \frac{SQ(u)}{\sum_i SQ(i)}$$

Read: ACGT (Quality: 30 30 25 20)

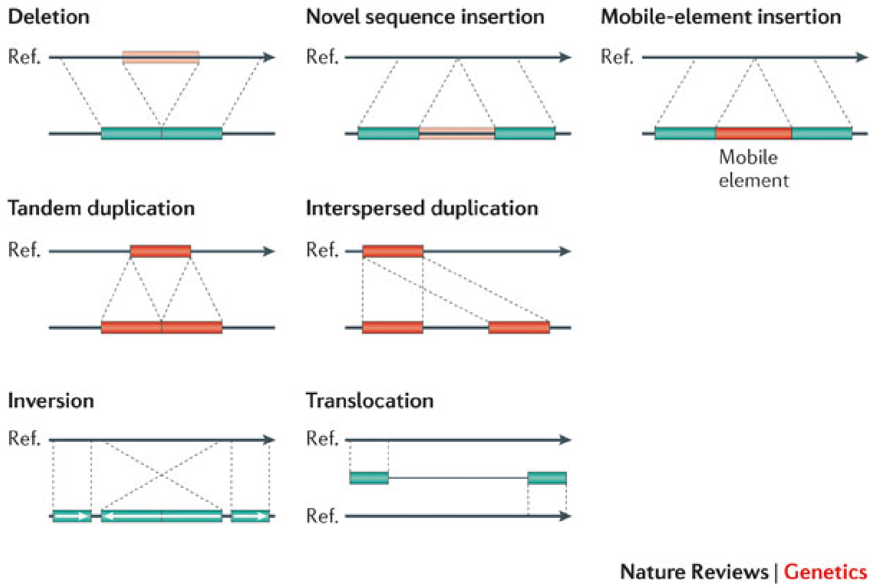


Ref:	ACGTACGGA	SQ(*u*)	E(*u*)
	ACGT	0+ 0+ 0+ 0	0/415
	ACGT	30+30+25+20	105/415
	ACGT	30+30+25+20	105/415
	ACGT	30+30+25+20	105/415
	ACGT	0+ 0+ 0+20	20/415
	ACGT	30+30+ 0+20	80/415

Genetic Variants

- SNV: Single Nucleotide Variant
  - Substitution (SNP)
  - Indel: insertion/deletion

- Structural Variation (SV)
  - Large-scale insertion/deletion
  - Inversion
  - Translocation
  - Copy Number Variation (CNV)



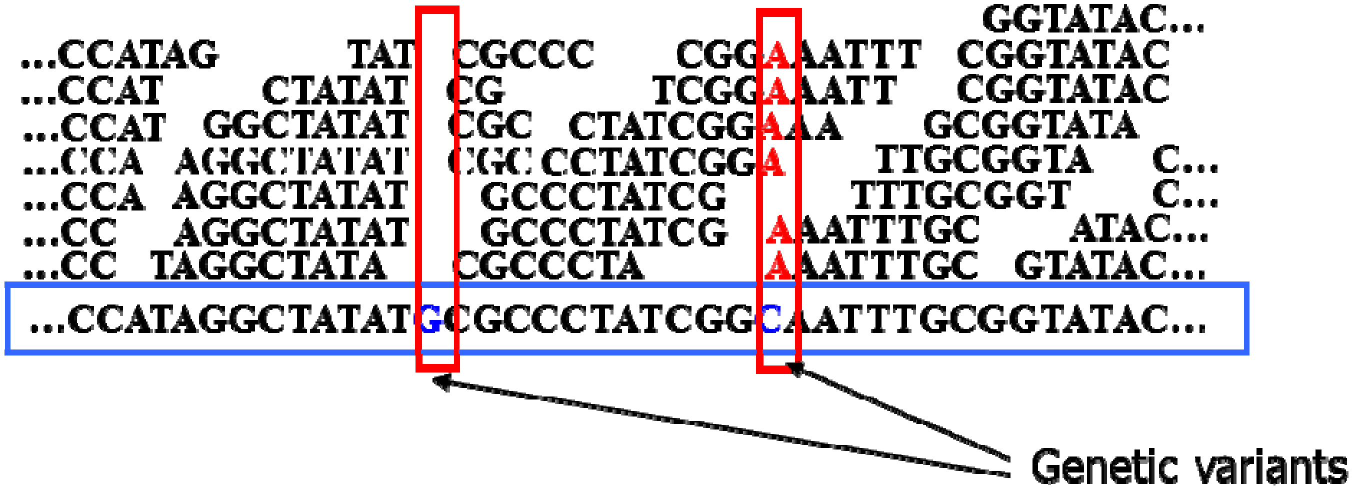
Nature Reviews | Genetics

SNP Calling is NOT Genotyping

- “SNP calling aims to determine in which positions there are polymorphisms or in which positions at least one of the bases differs from a reference sequence”
- “Genotype calling is the process of determining the genotype for each individual and is typically only done for positions in which a SNP or a 'variant' has already been called.”

(Source: *Nature Reviews Genetics* 12, 443-451)

Counting: an intuitive (and naïve) approach



GGTATAC...
...CCATAG TAT CGCCC CGGAAATTT CGGTATAC
...CCAT CTATAT CG TCGGAAATT CGGTATAC
...CCAT GGCTATAT CGC CTATCGGAAA GCGGTATA
...CCA AGGCTATAT CGC CCTATCGGA TTGCGGTA C...
...CCA AGGCTATAT GCCCTATCG TTTGCGGT C...
...CC AGGCTATAT GCCCTATCG AAATTTGC ATAC...
...CC TAGGCTATA CGCCCTA AAATTTGC GTATAC...
...CCATAGGCTATATGCGCCCTATCGGCAATTTGCGGTATAC...
Genetic variants

- Counting high-confident , non-reference allele (i.e. Quality >= 20)
  - Freq <20% or > 80%: homozygous genotype
  - Otherwise: heterozygous

- Works well for “deeply sequenced regions” (DSR), i.e. depth > 25x
  - But suffer from under-calling of heterozygous genotypes for low-coverage regions
  - And can’t give an objective measurement for reliability

(Source: *Nature Reviews Genetics* 12, 443-451)

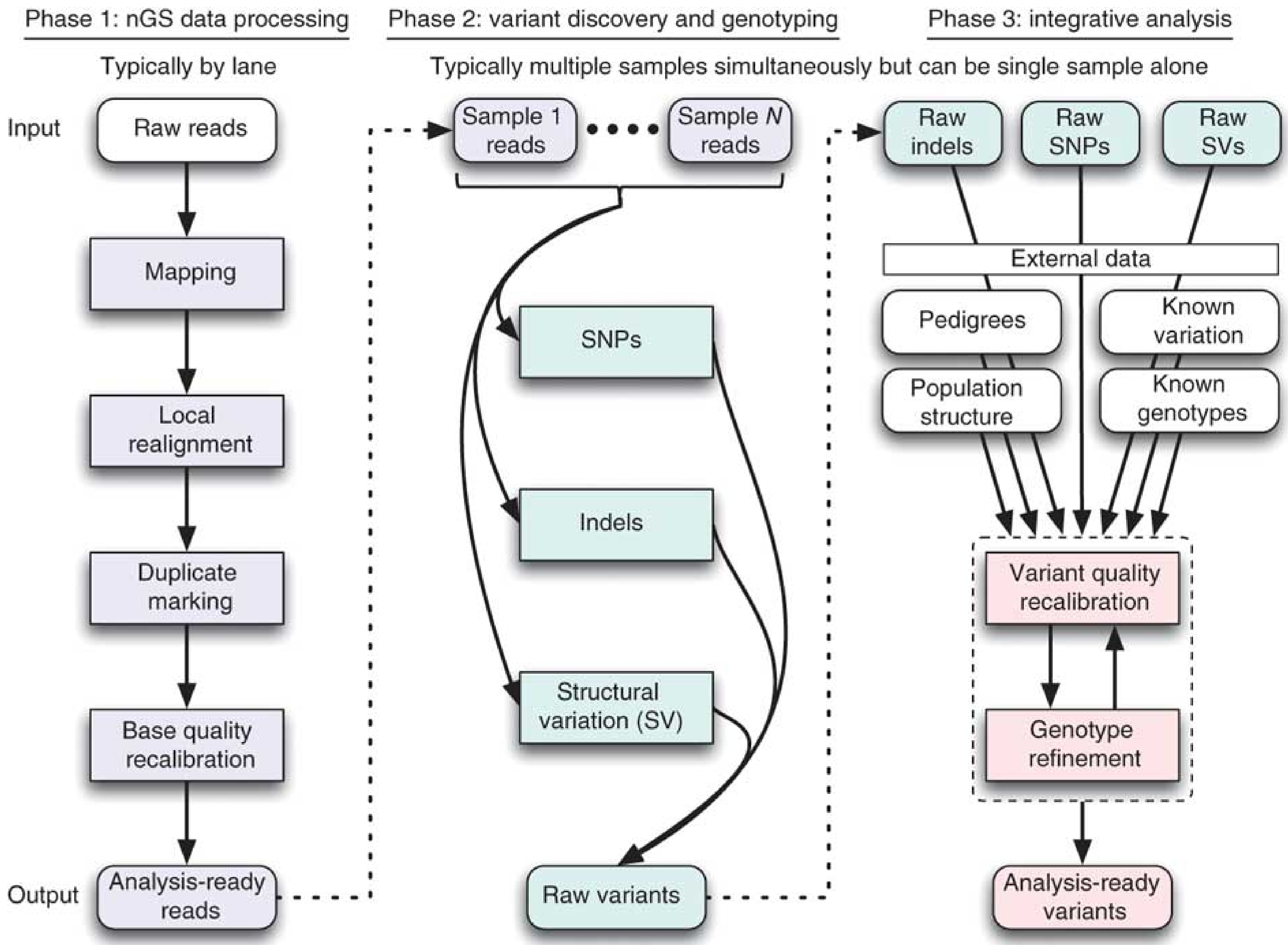
A Simple Probabilistic Model for Genotyping

1. For a diploid genome, there will be at most two different alleles (A and a) observed at a given site:
   - 3 possible genotypes: <A,A>, <A,a>, <a,a>
   - Number of A: k; Number of a: n-k

2. Then, the probability for each genotypes is
   - P(D|<A,A>) = the probability that we have (n-k) sequencing errors at this site $\prod_{n-k} P(x_i)$
   - Similarly, we can see the P(D|<a,a>) =$\prod_{k} P(x_i)$
   - P(D|<A,a>) = 1 – (P(D|<A,A>) + P(D|<a,a>))

3. Bayes Formula can be further employed to calculate posterior probabilities, i.e. P(<A,A>|D), P(<a,a>|D), and P(<A,a>|D) if we can estimate the prior probabilities P(<A,A>), P(<a,a>) and P(<A,a>)

<u>G</u>enome <u>A</u>nalysis <u>T</u>ool<u>K</u>it (GATK)



Nature Genetics 43, 491–498 (2011)

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