Lecture 5
Library Complexity
Short Read Alignment (Mapping)

Foundations of Computational Systems Biology

David K. Gifford

Lecture 5 – Libraries and Indexing

- Library Complexity
  - How do we estimate the complexity of a sequencing library?

- Full-text Minute-size index (FM Index/BWT)
  - How do we convert a genome into an alternate representation that permits rapid matching of millions of sequence reads?

- Read Alignment
  - How can we use an FM index and BWT to rapidly align reads to a reference genome?

Library complexity is the number of unique molecules in the "library" that is sampled by finite sequencing

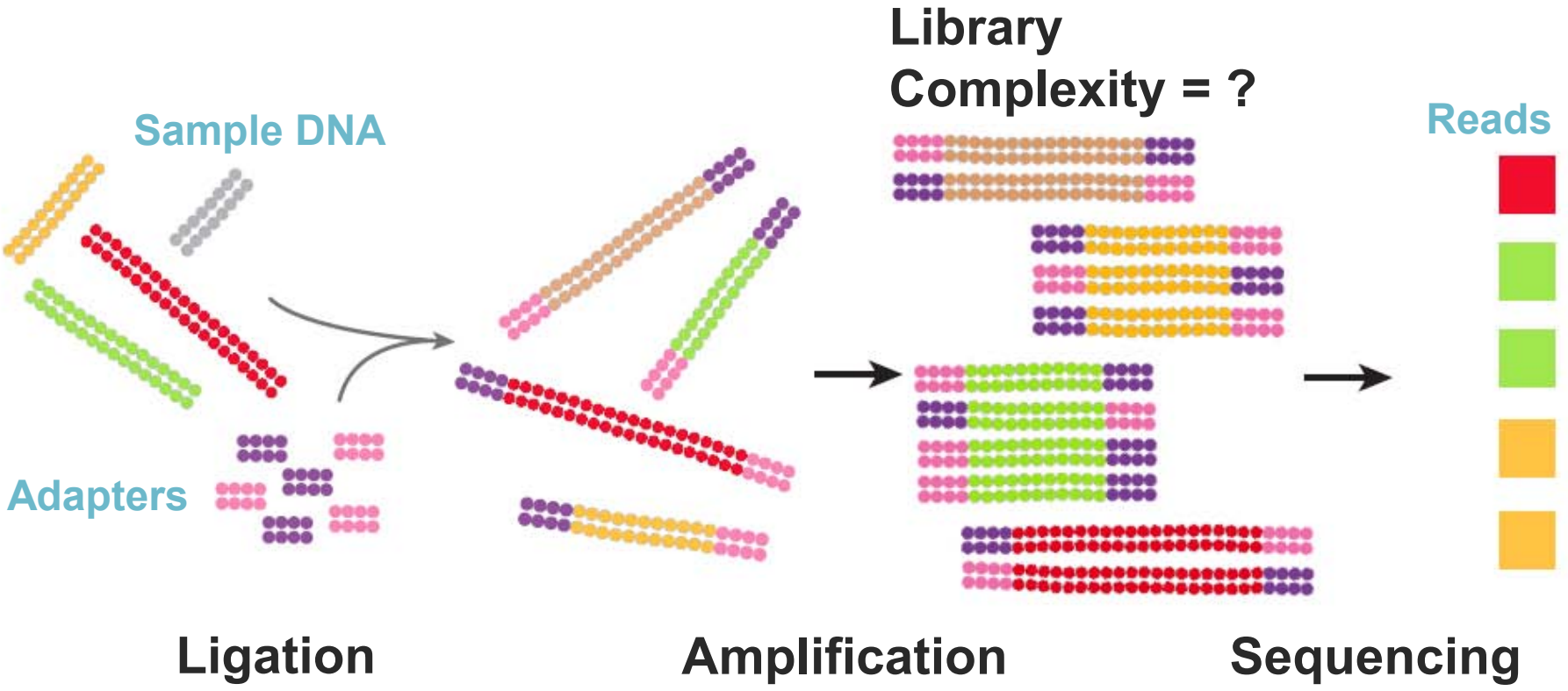
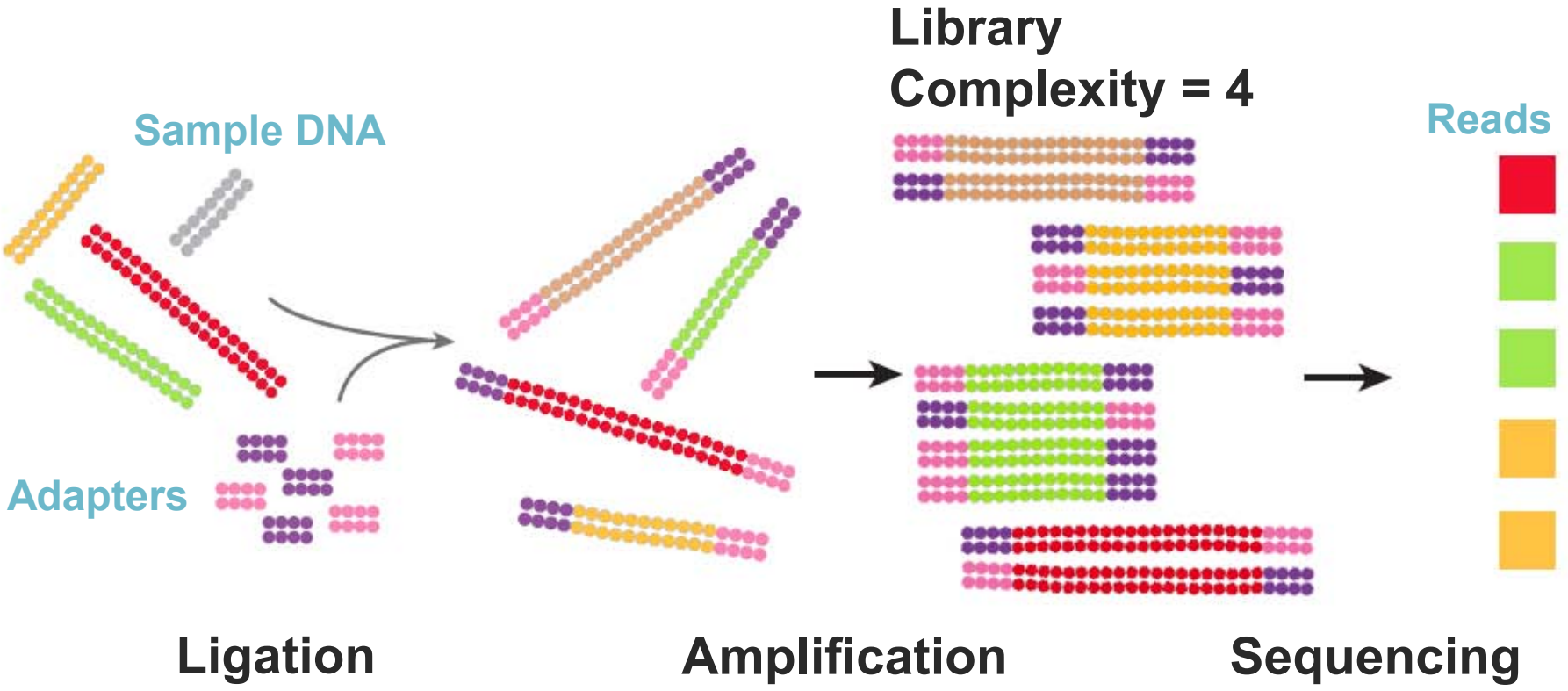


Image adapted from Mardis, *ARGHG* (2008)

Library complexity is the number of unique molecules in the "library" that is sampled by finite sequencing



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Modeling approach

- Assume we have *C* unique molecules in the library and we obtain *N* sequencing reads
- The probability distribution of the number of times we sequence a particular molecule is binomial (individual success probability $p=1/C$, *N* trials in total)
- Assume Poisson sampling as a tractable approximation (rate $\lambda = N/C$)
- Finally, truncate the Poisson process: we only see events that happened between *L* and *R* times (we don't know how many molecules were observed 0 times)

See e.g. Cohen, *JASA* (1954)

Estimating library complexity with a Poisson model

- For Poisson sampling, we can write the (truncated) distribution over x_i, the times we sequence the i^{th} molecule as:

$$\Pr(x_i|\lambda) = \frac{1}{K_{L,R}(\lambda)} \cdot \frac{e^{-\lambda}\lambda^{x_i}}{x_i!}$$

$$K_{L,R}(\lambda) = \sum_{x=L}^{R} \Pr(x_i|\lambda)$$

[The probability is 0 if x_i is less than L or greater than R]

- We can estimate the maximum likelihood rate parameter λ from a vector of observations **x**

Maximum likelihood library size

$$K_{L,R}(\lambda) = \sum_{x=L}^{R} \Pr(x_i|\lambda)$$

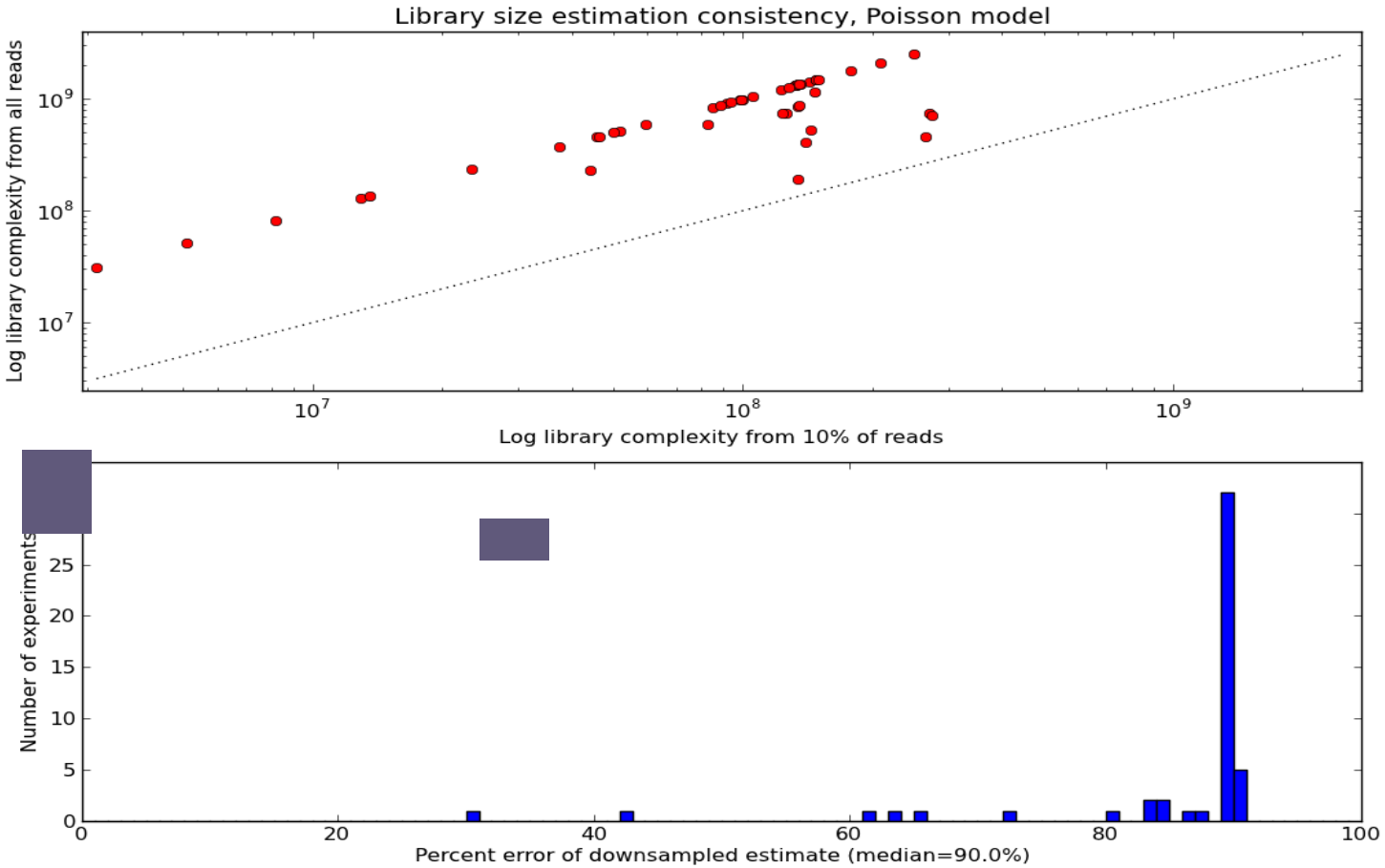
- M unique sequences observed, maximum likelihood library size is

$$\hat{C} = \frac{M}{K_{L,R}(\lambda)}$$

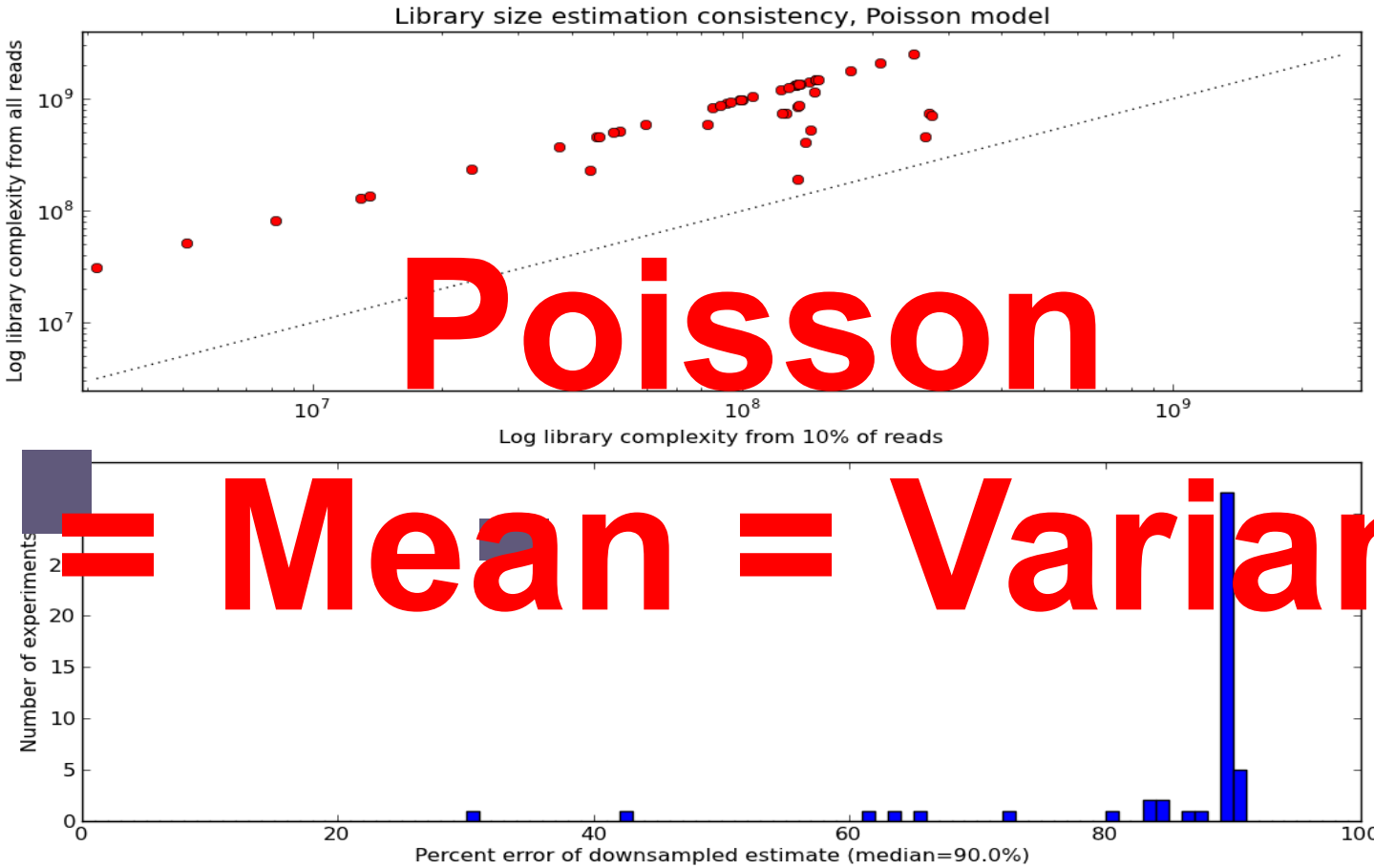
- Approximate solution

$$\hat{C} = \frac{M}{(1 - Poisson(0, \lambda))}$$

Poisson Library Complexity model 150 1000 Genome Datasets



Poisson Library Complexity model
150 1000 Genome Datasets



Library complexity is the number of unique molecules in the "library" that is sampled by finite sequencing

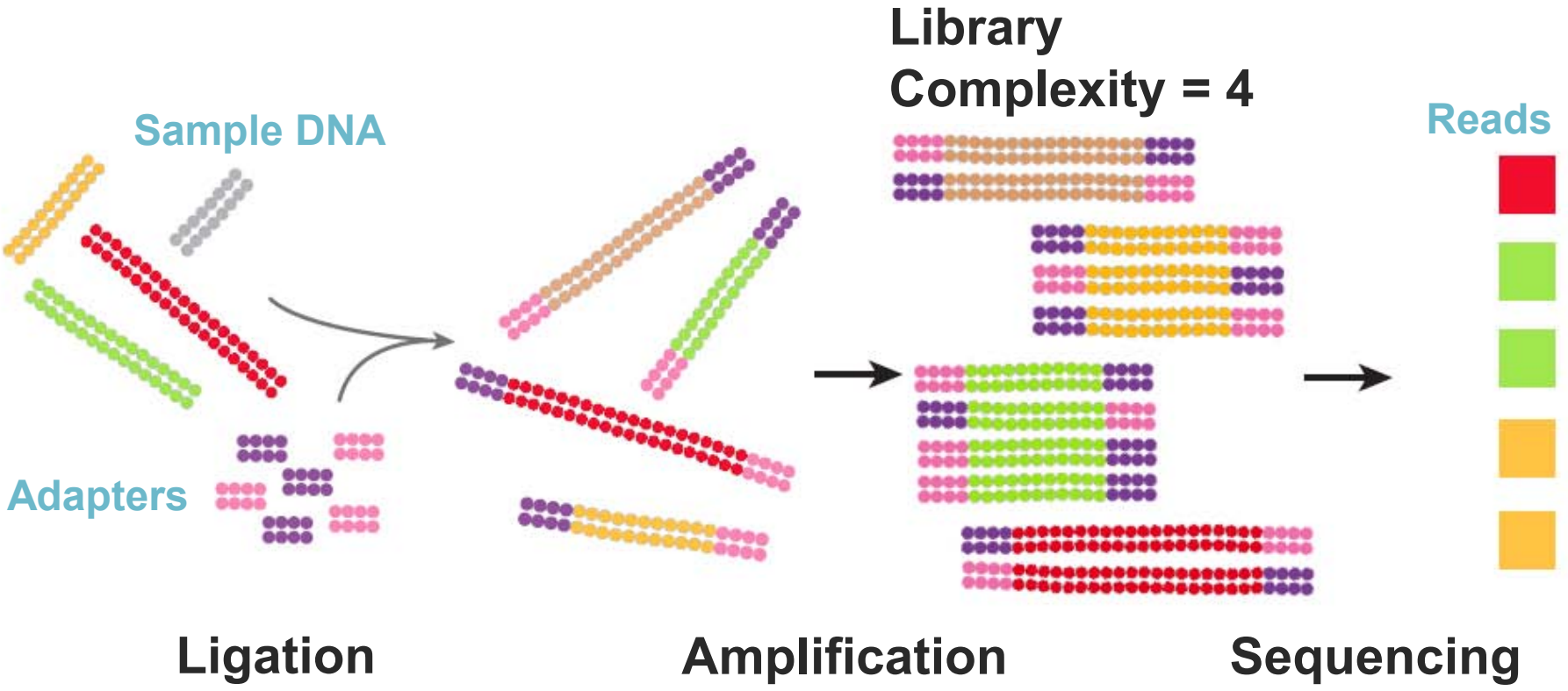
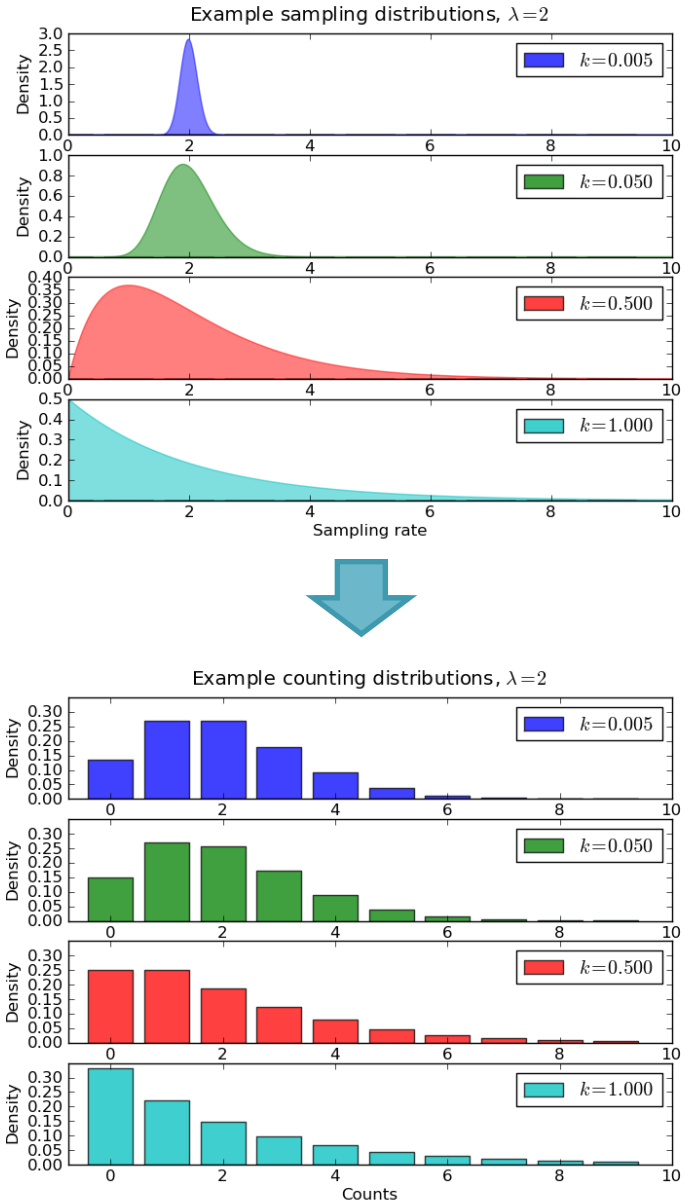


Image adapted from Mardis, *ARGHG* (2008)

Gamma sampling rates describe the entire population (library preparation)

Poisson sampling to form a smaller sample (*sequencing*)

Negative binomial distribution characterizes the resulting occurrence histogram



The gamma distribution is a "conjugate prior" for the Poission distribution

$$Poisson(x;\lambda) = \frac{\lambda^x e^{-\lambda}}{x!}$$

$$Gamma(x;\alpha,\beta) = \frac{\beta^\alpha x^{\alpha-1} e^{-\beta x}}{\Gamma(\alpha)}$$

$$NB(y;\alpha,\beta) = \int_0^\infty Poisson(y;x)\,Gamma(x;\alpha,\beta)\,dx$$

Negative Binomial model for sequence occurrences

C – library complexity (latent, fit to observed data)

N – number of reads

M – total number of unique sequences

$\lambda = N/C$

k - dispersion (latent, fit to observed data)

$Pr(x_i \mid \lambda, k) = NegativeBinomial(x_i \mid \lambda, k)$

$= NegativeBinomial(x_i \mid n, p)$

$p = \lambda / (\lambda + 1/k)$

$n = 1/k$

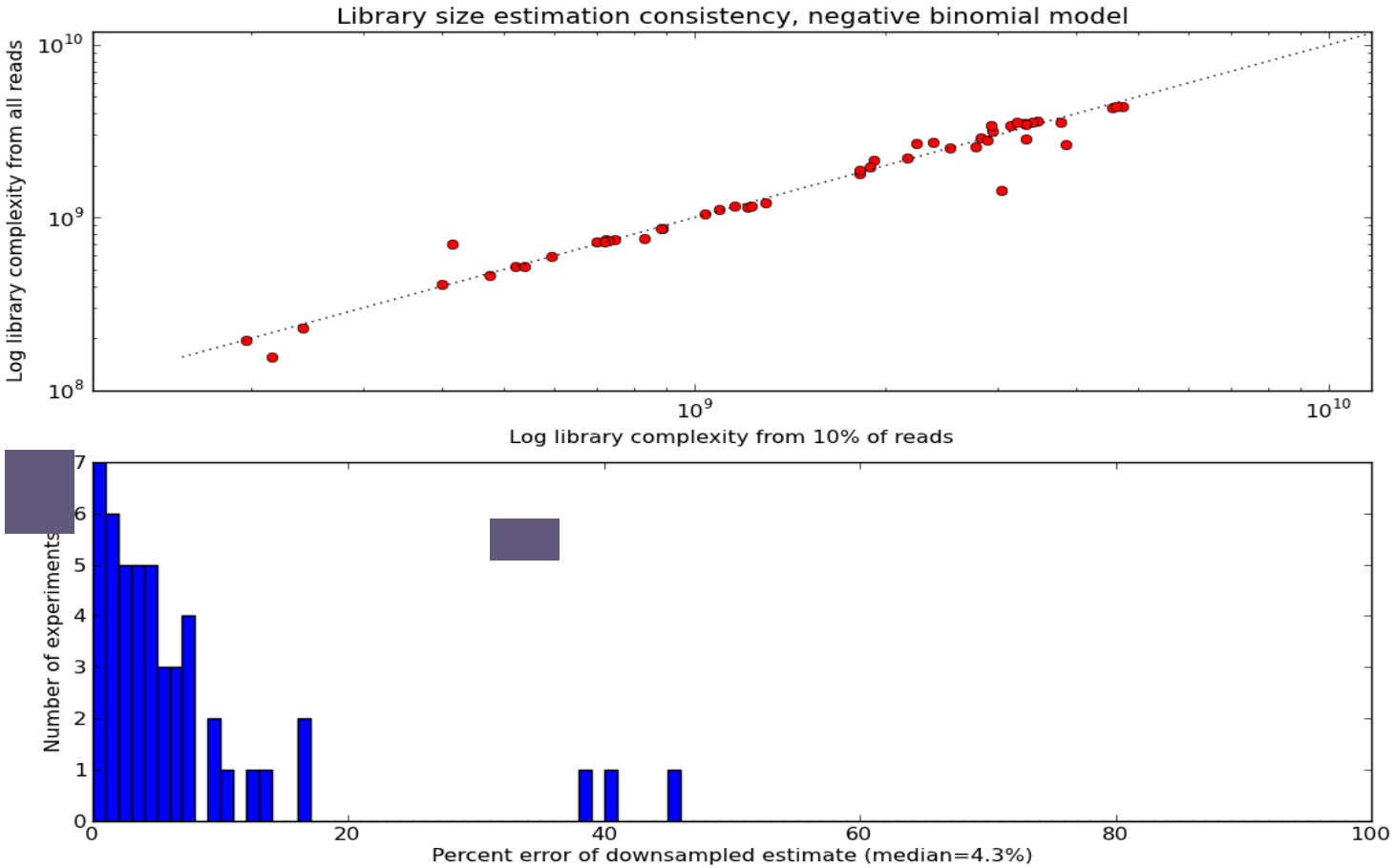
Simulation results show that the Gamma Possion works well for non-uniform libraries

- True library complexity: 1M unique molecules
- Vary *k* (controls sampling rate variance)

- Given 100K reads (λ=0.1), assess estimates from both models

–*k*=0.1	Poisson: 0.93M	GP: 0.96M	95% unique
–*k*=1	Poisson: 0.52M	GP: 1.01M	91% unique
–*k*=10	Poisson: 0.12M	GP: 1.10M	70% unique
–*k*=20	Poisson: 0.07M	GP: 0.68M	59% unique

Negative Binomial Library Complexity model
150 1000 Genome Datasets
Data are “overdispersed” (variance greater than mean)



Marginal value of additional sequencing

C – library complexity (latent – estimated)

N – number of reads

M – number of unique sequences

M can be estimated by $(1 - \text{Poisson}(0 \mid \lambda)) * C$

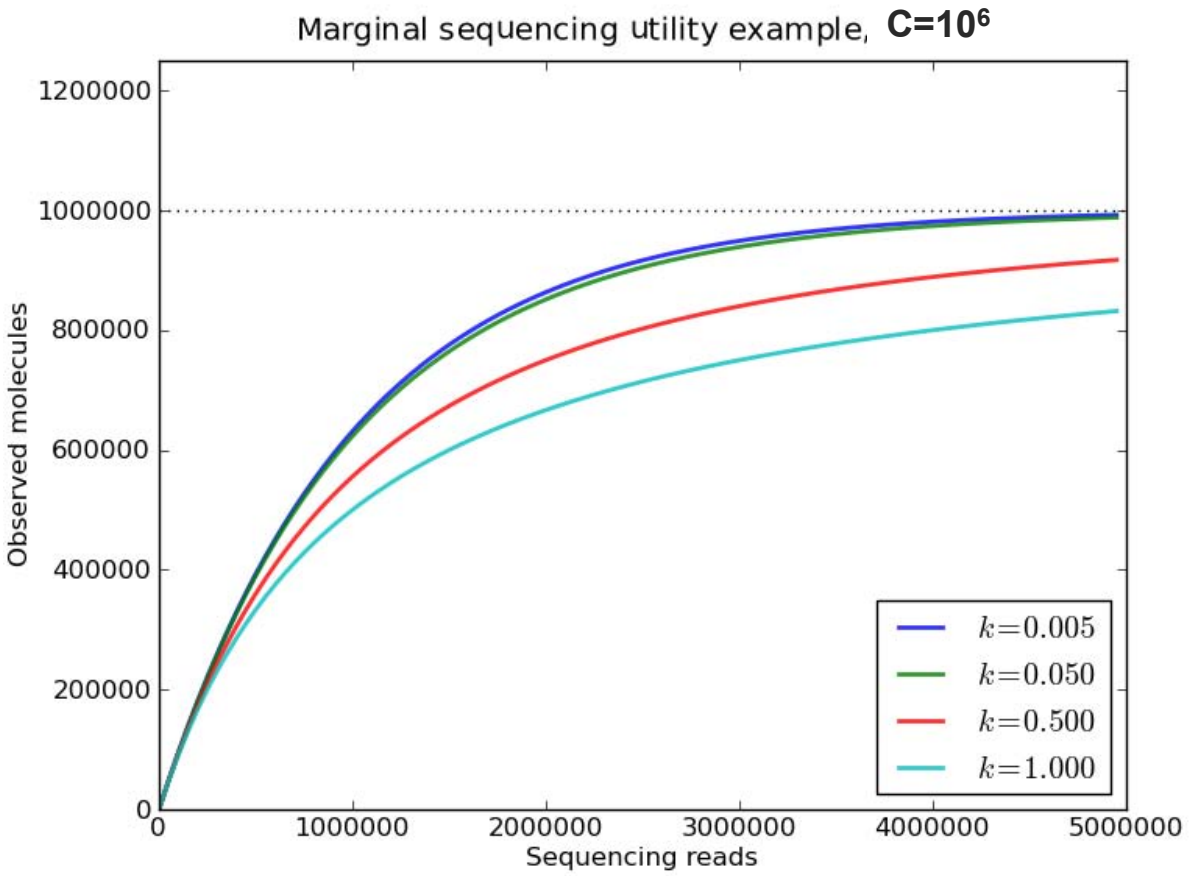
M can be estimated by $(1 - \text{NegativeBinomial}(0 \mid \lambda, k)) * C$

Assume we have r more reads

$$s = (N + r) / N$$

Replace λ by $s\lambda$ to estimate M' achieved with r more reads

Marginal utility of sequencing



Lecture 5 – Libraries and Indexing

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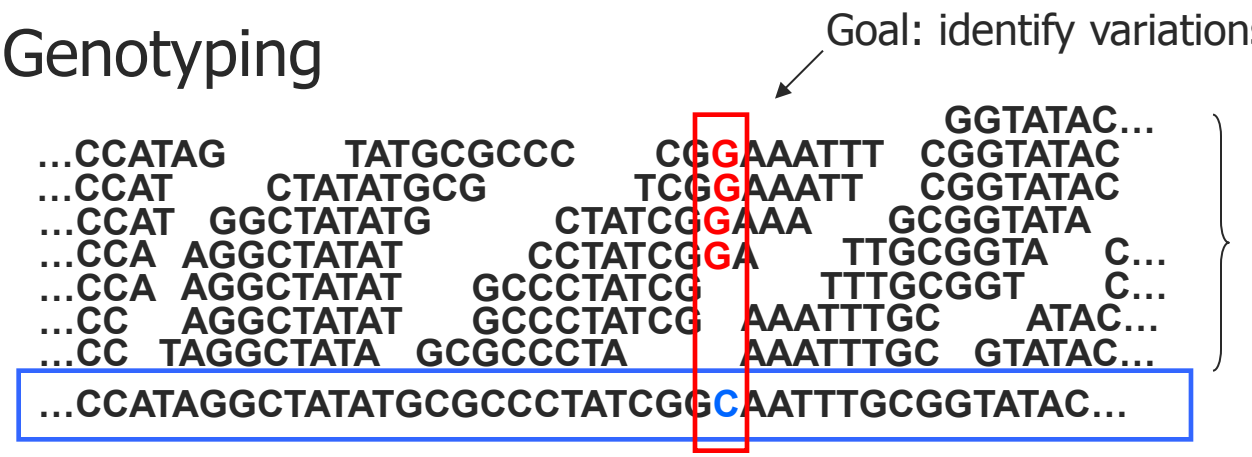
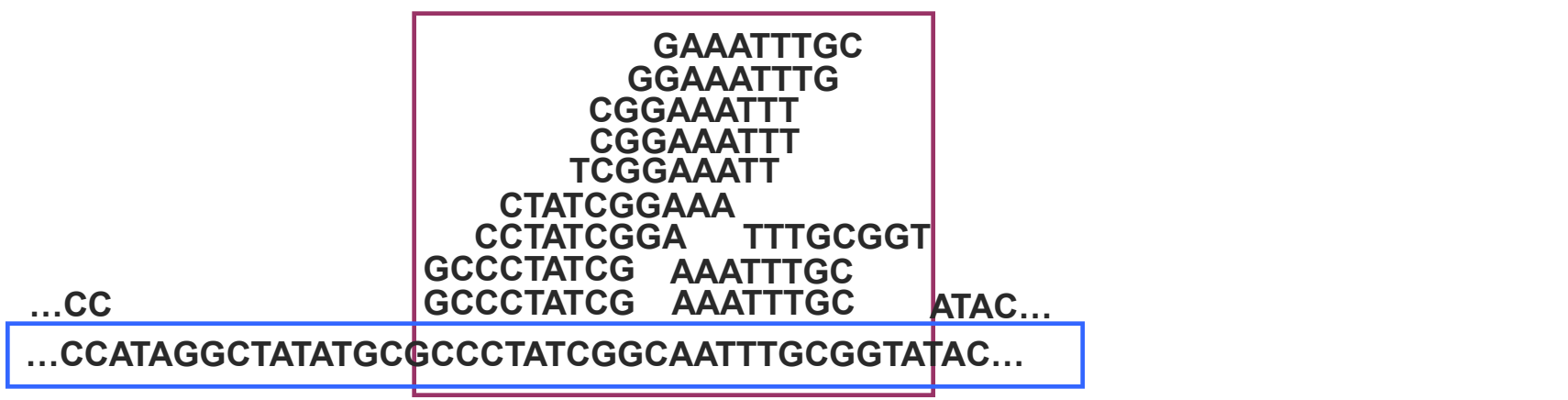
- Read Alignment
  - How can we use an FM index and BWT to rapidly align reads to a reference genome?

Short Read Applications

- Genotyping



- RNA-seq, ChIP-seq, Methyl-seq



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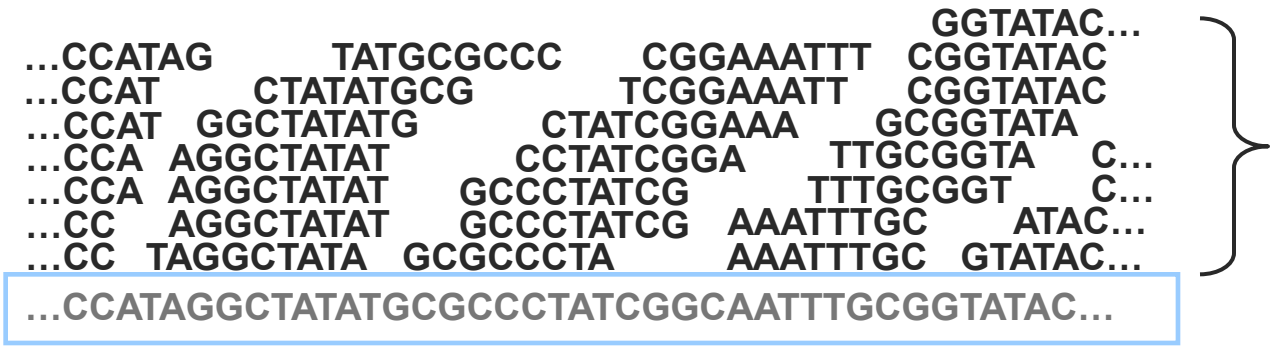
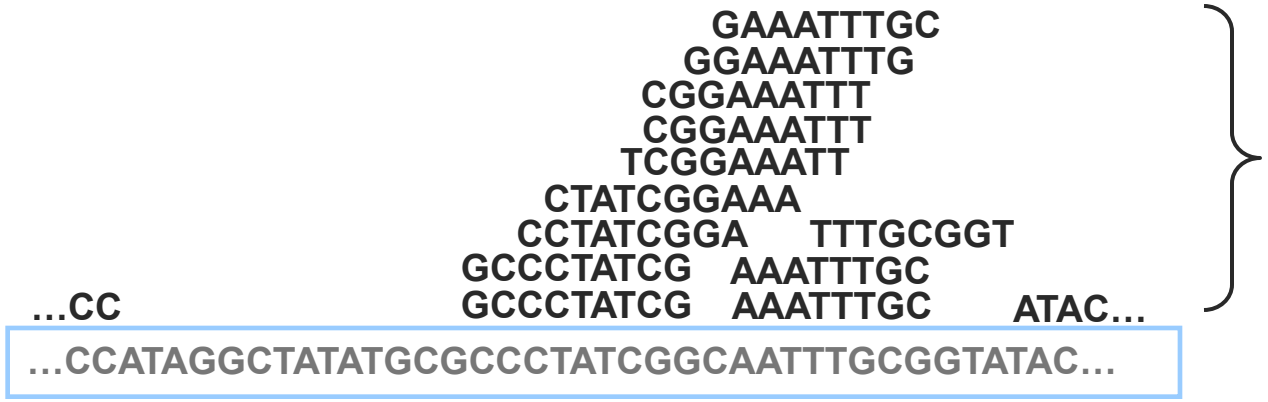
Short Read Applications





Finding the alignments is typically the performance bottleneck

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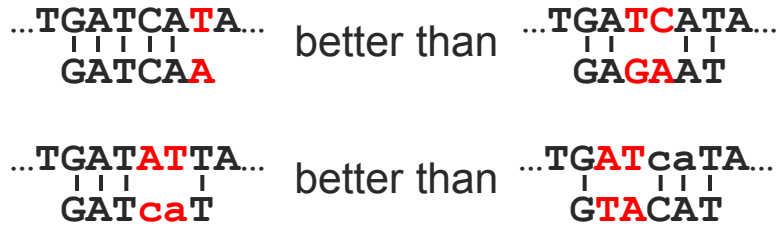
Short Read Alignment

- Given a reference and a set of reads, report at least one "good" local alignment for each read if one exists
  - Approximate answer to: where in genome did read originate?

- What is "good"? For now, we concentrate on:
  - Fewer mismatches are better
  - Failing to align a low-quality base is better than failing to align a high-quality base



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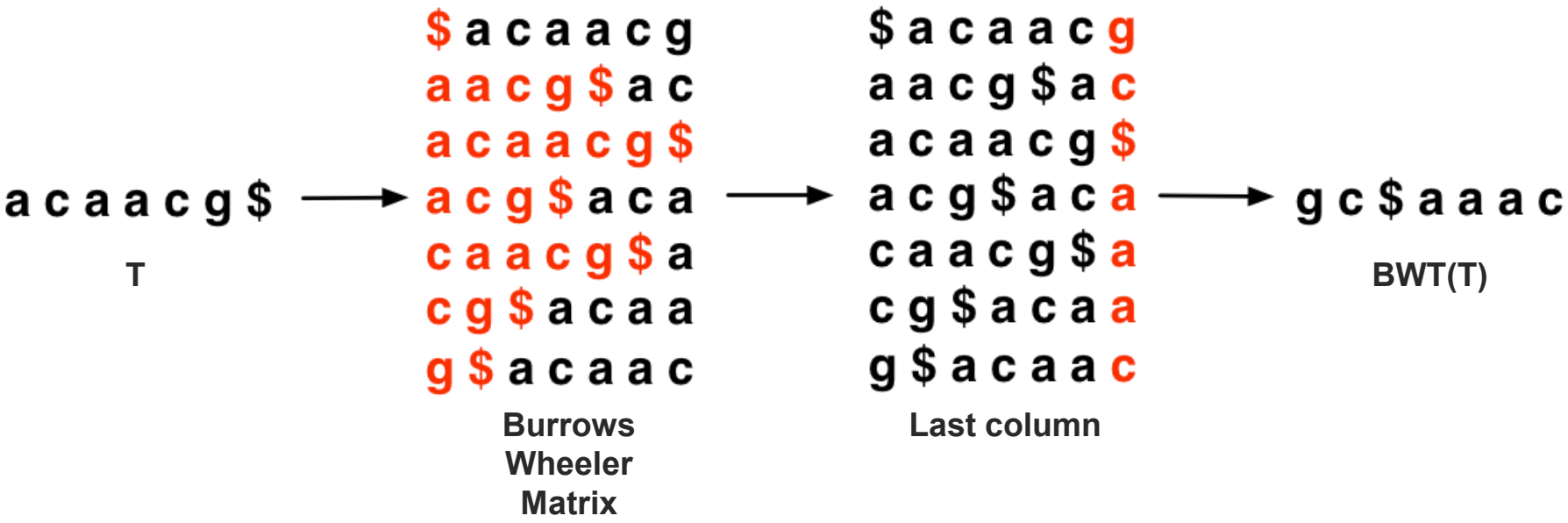
The Burrows-Wheeler Transform is a reversible representation with handy properties

- Sort all the possible rotations of original string

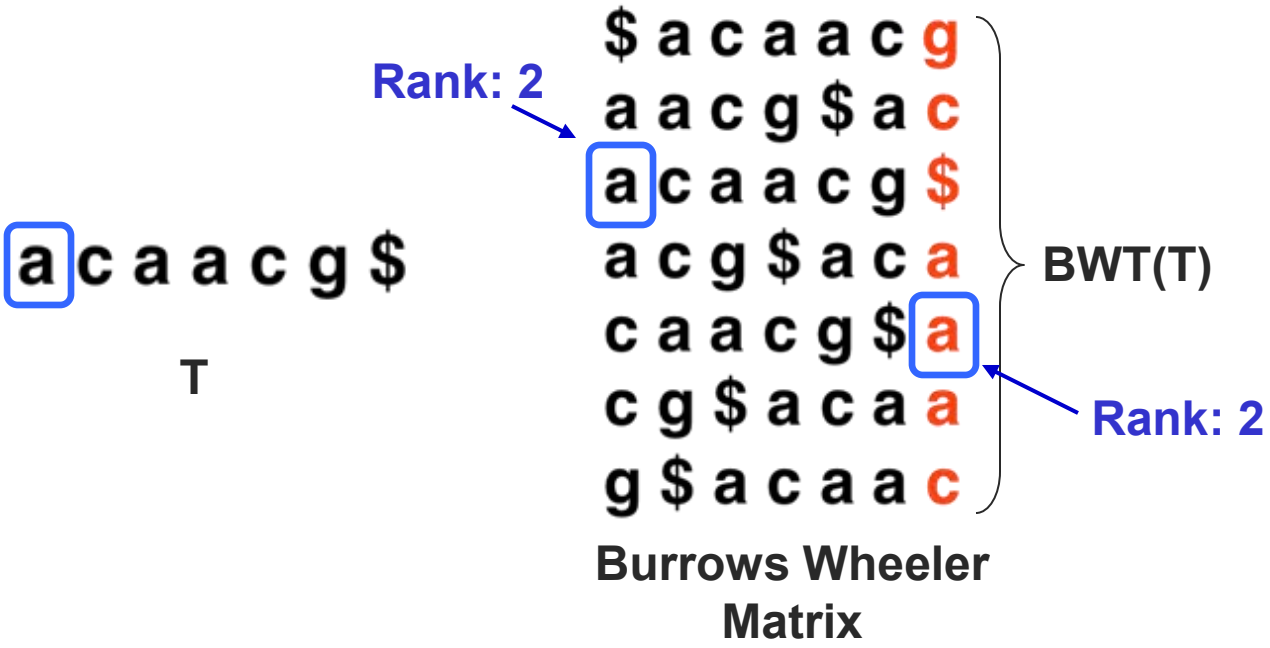


- Once BWT(T) is built, *all else shown here is discarded*
  - Matrix will be shown for illustration only

Burrows M, Wheeler DJ: **A block sorting lossless data compression algorithm**. *Digital Equipment Corporation, Palo Alto, CA* 1994, Technical Report 124; 1994

A text occurrence has the same rank in the first and last columns

- When we rotate left and sort, the first character retains its rank. Thus the same text occurrence of a character has the same rank in the **L**ast and **F**irst columns.



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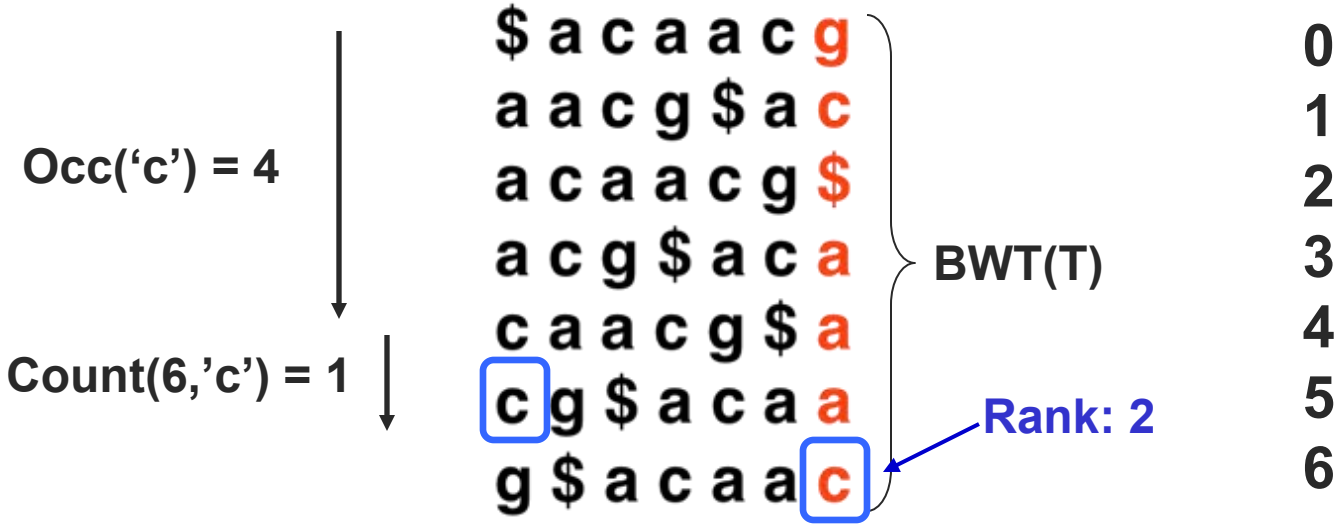
The Last to First (LF) function matches character and rank

LF(6, ‘c’) = Occ(‘c’) + Count(6,’c’) = 5



Occ(qc) – Number of characters lexically smaller than qc in BWT(T)

Count(idx, qc) – Number of qc characters before position idx in BWT(T)

The Walk Left Algorithm inverts the BWT

```
i = 0
t = ""
while bwt[i] != '$':
    t = bwt[i] + t
    i = LF(i, bwt[i])
```

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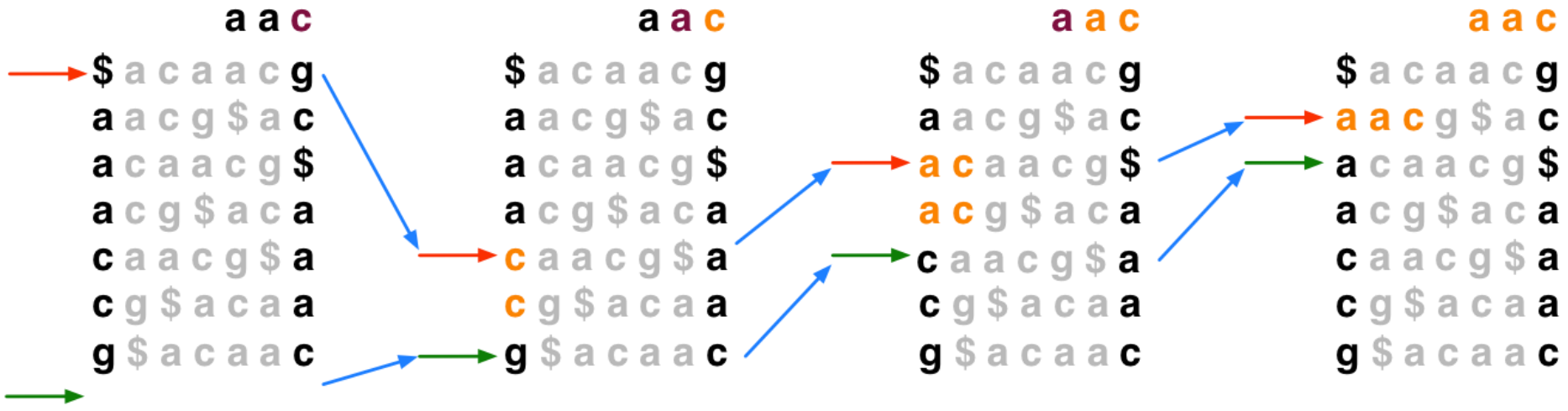
Exact Matching with FM Index

```
q = "aac"
top = 0
bot = len(bwt)
for qc in reverse(q):
   top = LF(top, qc)
   bot = LF(bot, qc)
```

In each iteration top & bot delimit the range of rows beginning with progressively longer suffixes of q



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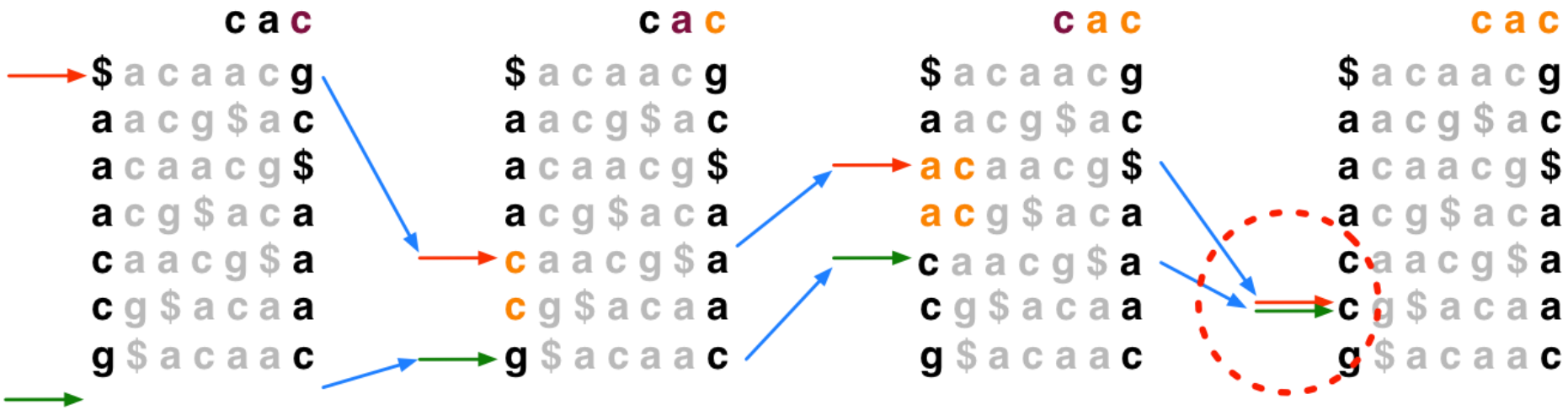
Exact Matching with FM Index



- If range becomes empty (**top** = **bot**) the query suffix (and therefore the query) does not occur in the text

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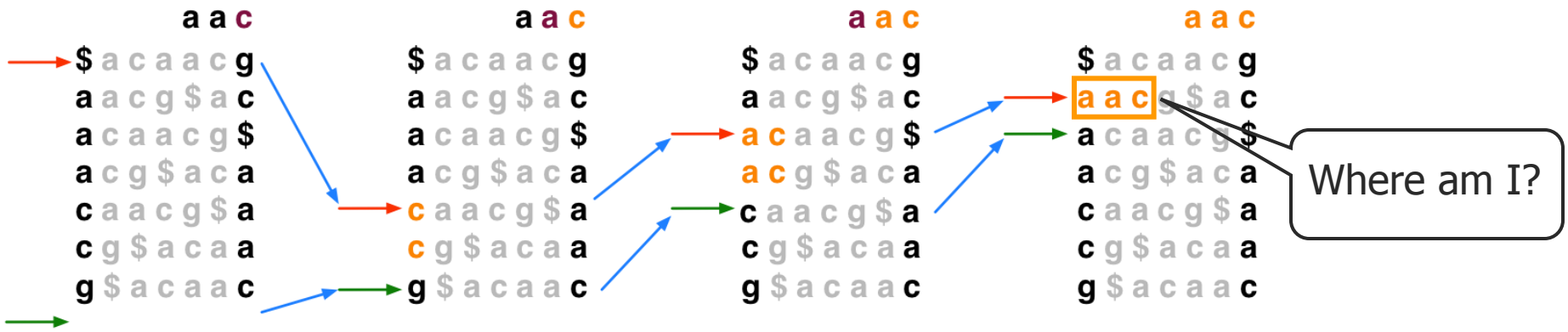
Rows to Reference Positions

- Once we know a row contains a legal alignment, how do we determine its position in the reference?



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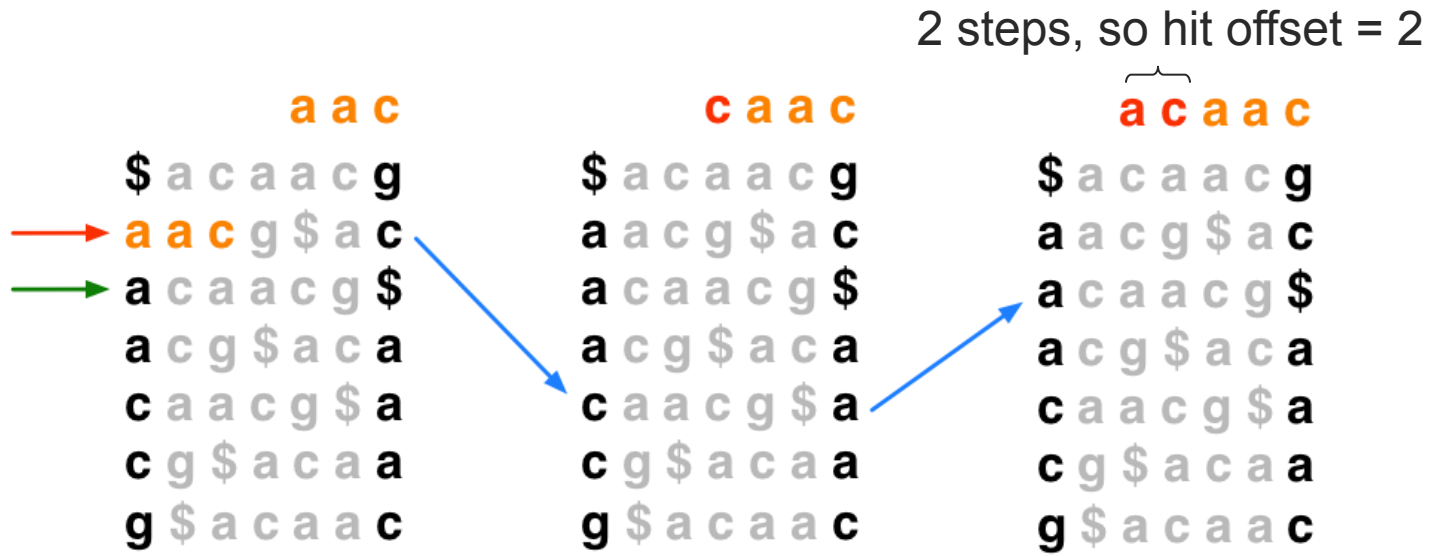
Rows to Reference Positions

- Naïve solution 1: Use "walk-left" to walk back to the beginning of the text; number of steps = offset of hit



- Linear in length of text in general – too slow

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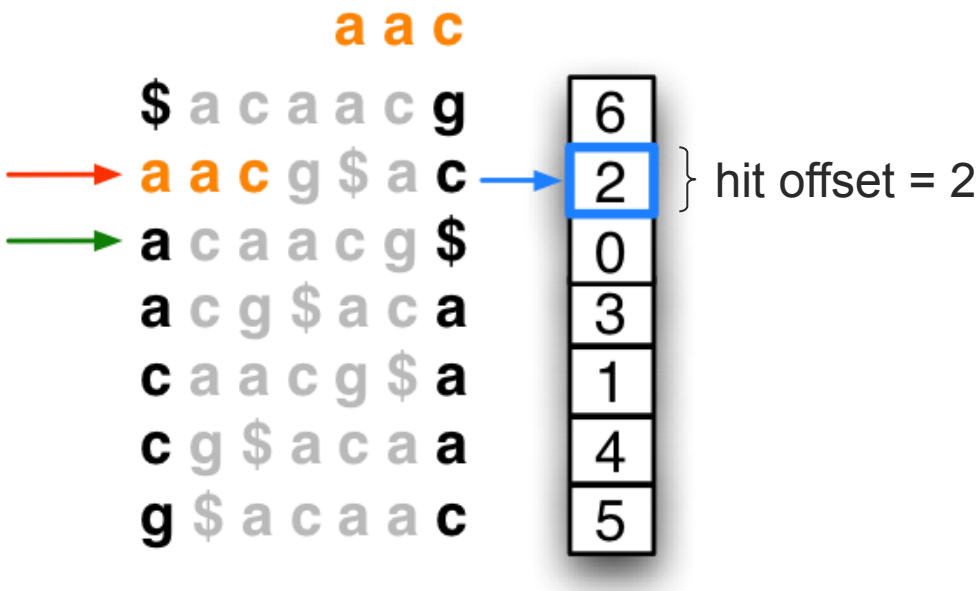
Rows to Reference Positions

- Naïve solution 2: Keep whole suffix array in memory. Finding reference position is a lookup in the array.



- Suffix array is ~12 gigabytes for human – too big

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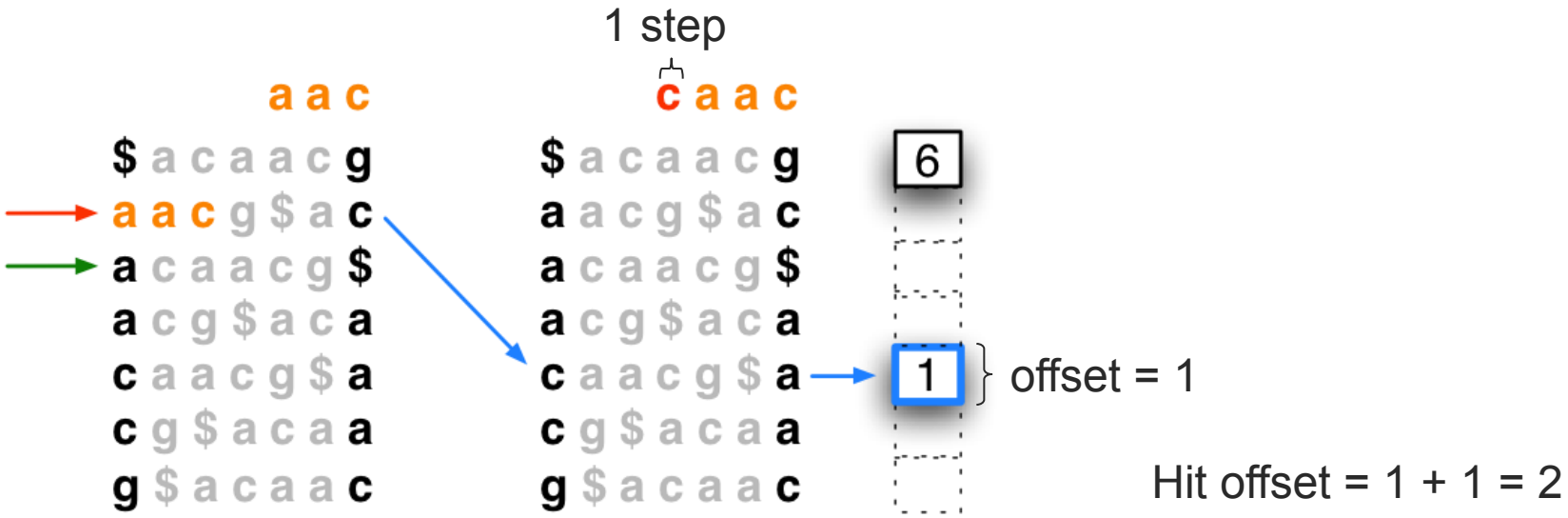
Rows to Reference Positions

- Hybrid solution: Store *sample* of suffix array; "walk left" to next sampled ("marked") row to the left
  - Due to Ferragina and Manzini



- Bowtie marks every 32nd row by default (configurable)

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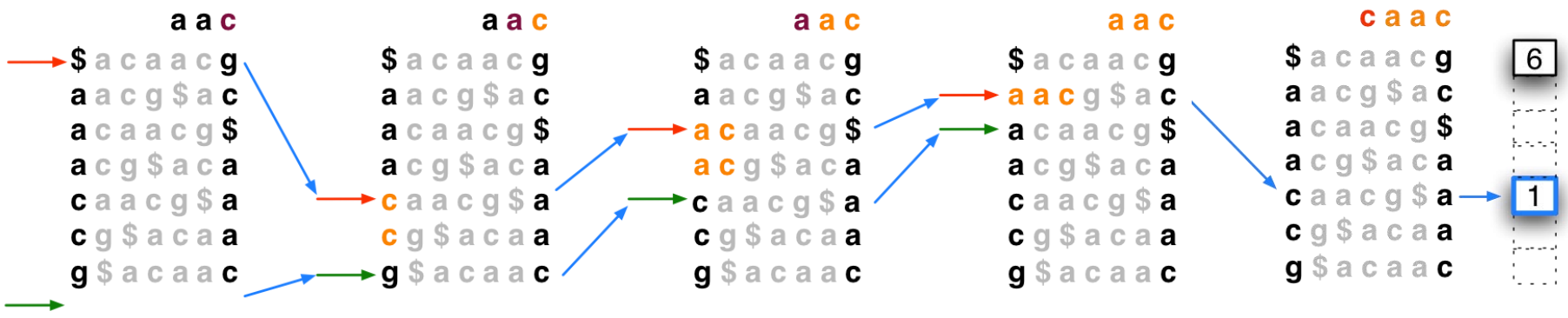
Put It All Together



- Algorithm concludes: “aac” occurs at offset 2 in “acaacg”

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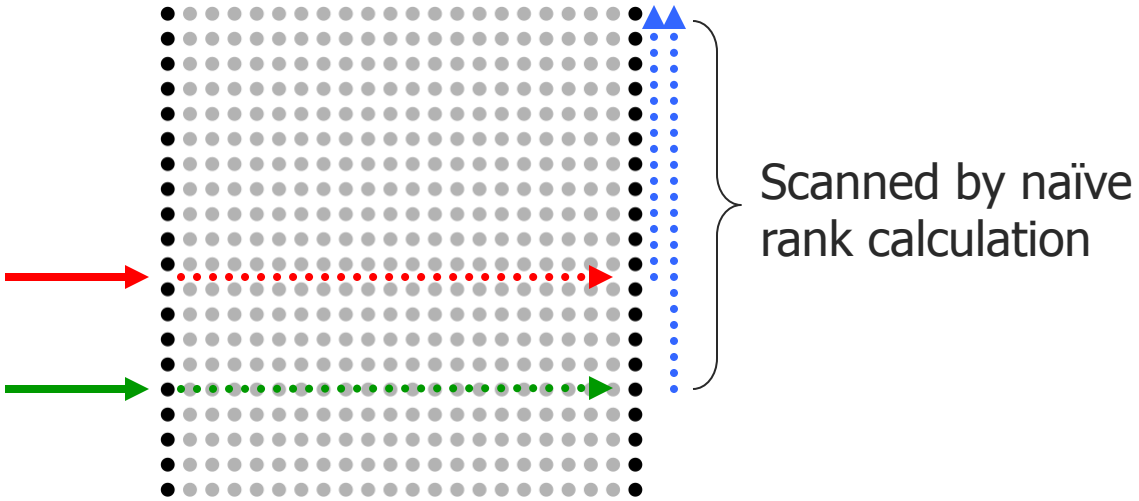
The FM index makes LF fast

- **LF**(i, **qc**) must determine the *rank* of **qc** in row i
- Naïve way: count occurrences of **qc** in all previous rows
  - This **LF**(i, **qc**) is linear in length of text – too slow



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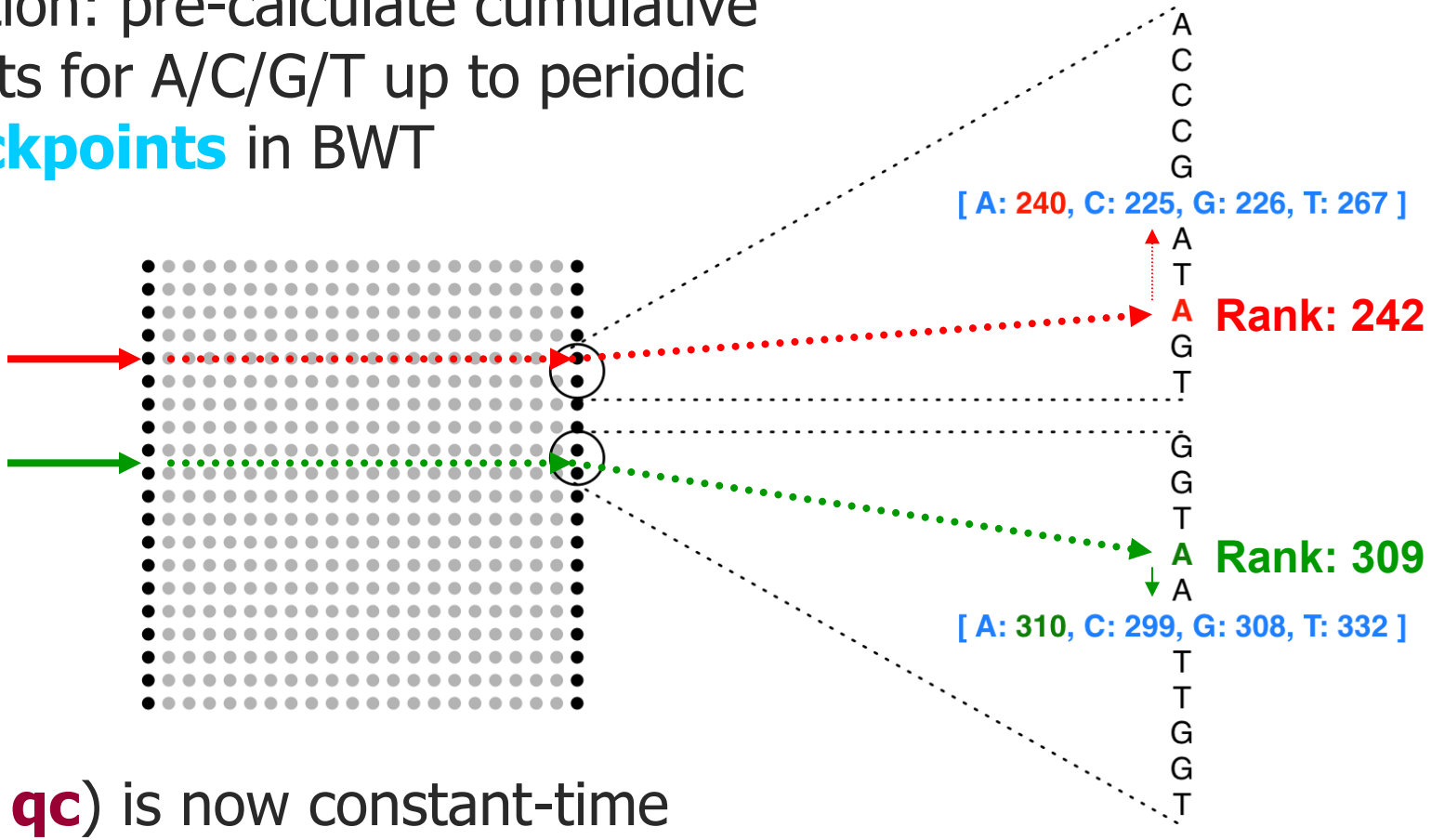
A Full-text Minute-size (FM) index makes LF constant time

- Solution: pre-calculate cumulative counts for A/C/G/T up to periodic **checkpoints** in BWT



- **LF**(i, **qc**) is now constant-time
  (if space between checkpoints is considered constant)

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http://www.cbcb.umd.edu/~langmead/NCBI_Nov2008.ppt

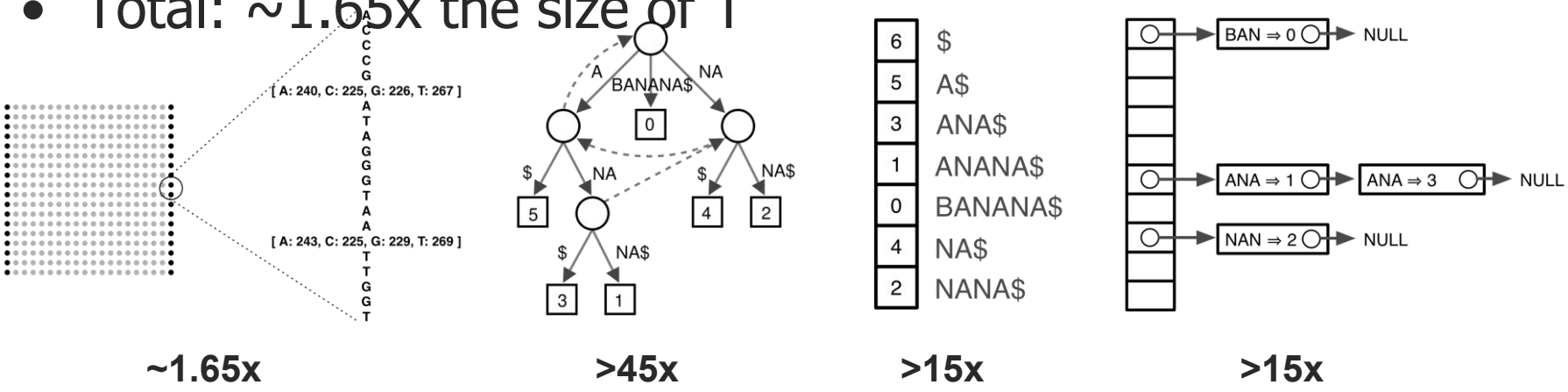
An FM Index is Small

- Entire FM Index on DNA reference consists of:
  - BWT (same size as T)
  - Checkpoints (~15% size of T)
  - Suffix array sample (~50% size of T)
- Total: ~1.65x the size of T

Assuming 2-bit-per-base encoding and no compression, as in Bowtie

Assuming a 16-byte checkpoint every 448 characters, as in Bowtie

Assuming Bowtie defaults for suffix-array sampling rate, etc



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FM Index in Bioinformatics

- Oligomer counting
  - Healy J *et al*: Annotating large genomes with exact word matches. *Genome Res* 2003, 13(10):2306-2315.
- Whole-genome alignment
  - Li H et al: Fast and accurate short read alignment with Burrows-Wheeler transform *Bioinformatics* 2009, 25(14):1754-1760.

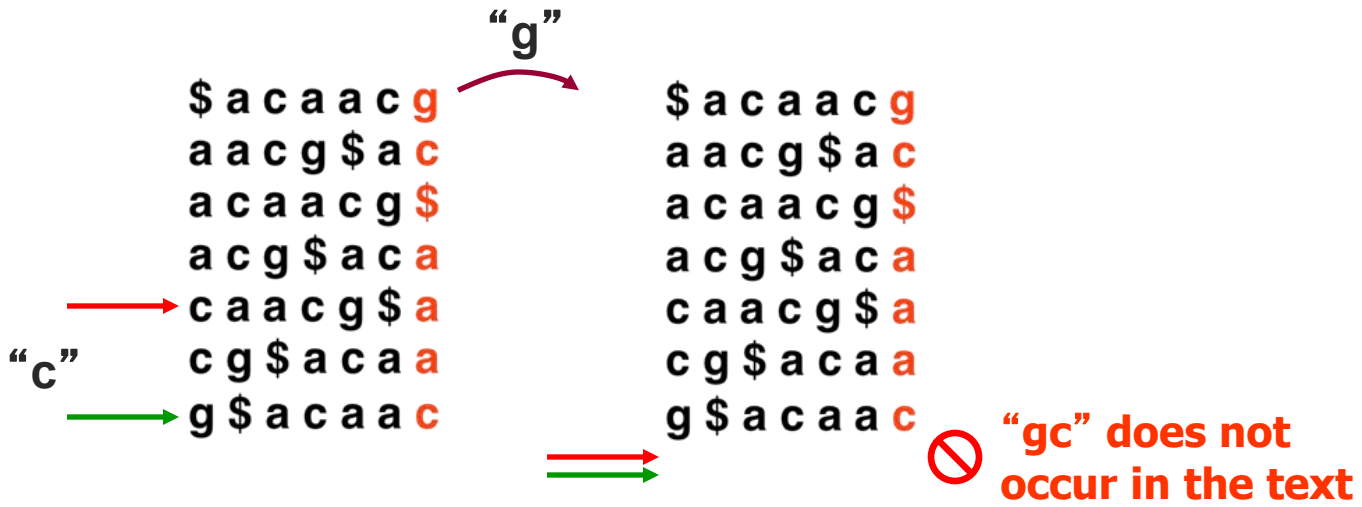
    BWA Aligner
  - Lippert RA: Space-efficient whole genome comparisons with Burrows-Wheeler transforms. *J Comp Bio* 2005, 12(4):407-415.
- Smith-Waterman alignment to large reference
  - Lam TW *et al*: Compressed indexing and local alignment of DNA. *Bioinformatics* 2008, 24(6):791-797.

Short Read Alignment

- FM Index finds exact sequence matches quickly in small memory, but short read alignment demands more:
  - Allowances for mismatches
  - Consideration of quality values
- Bowtie's solution: *backtracking quality-aware search*

Backtracking

- Consider an attempt to find Q = “agc” in T = “acaacg”:



- Instead of giving up, try to “backtrack” to a previous position and try a different base

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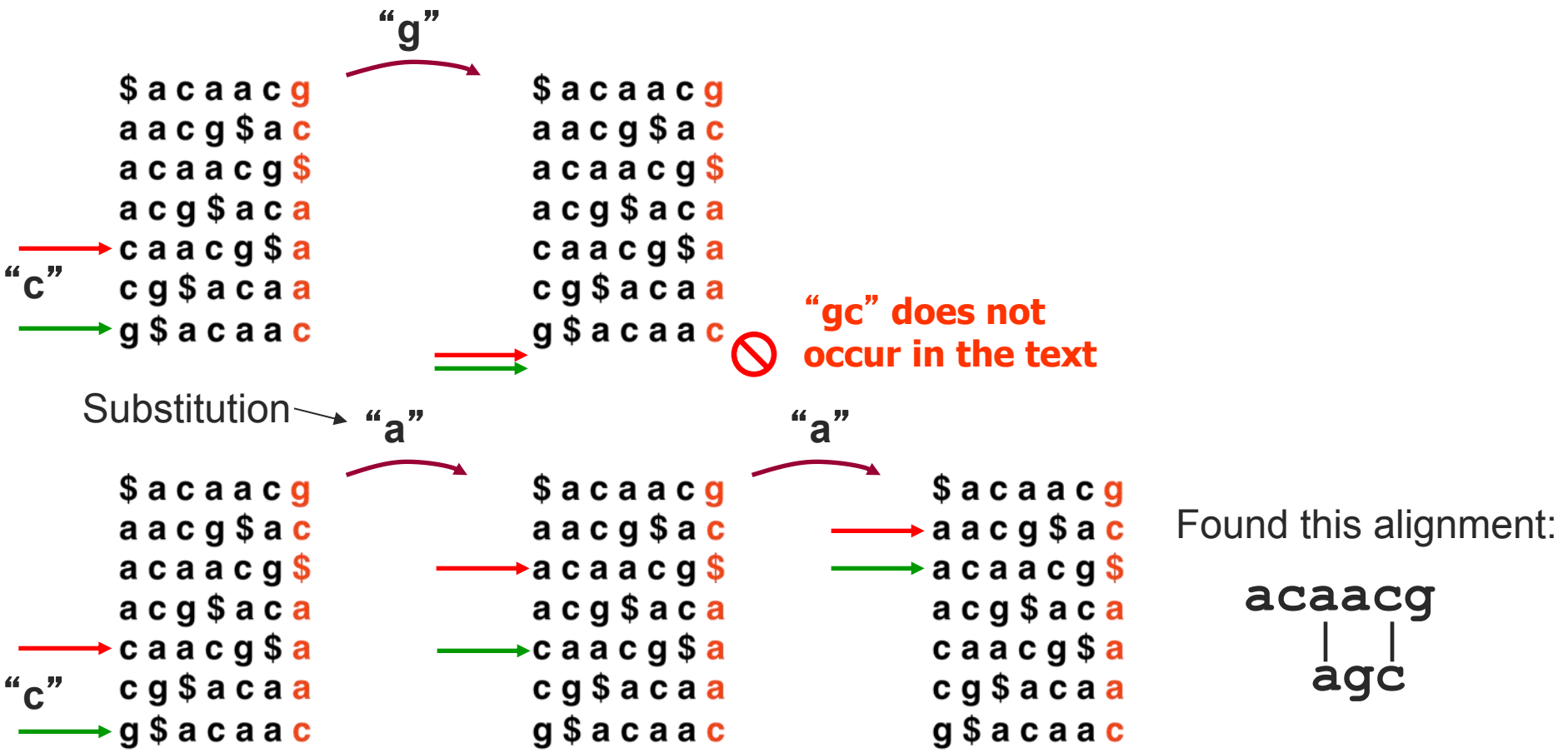
Backtracking

- Backtracking attempt for Q = “agc”, T = “acaacg”:



Found this alignment:

```
acaacg
| |
agc
```

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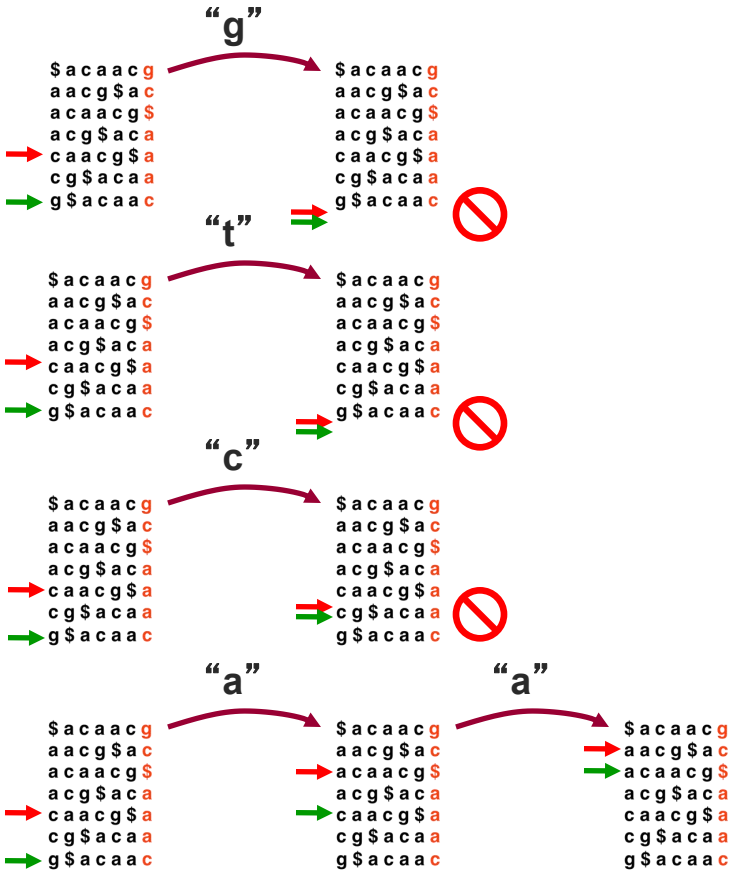
Backtracking

- May not be so lucky



Found this alignment (eventually):

```
acaacg
 |  |
agc
```

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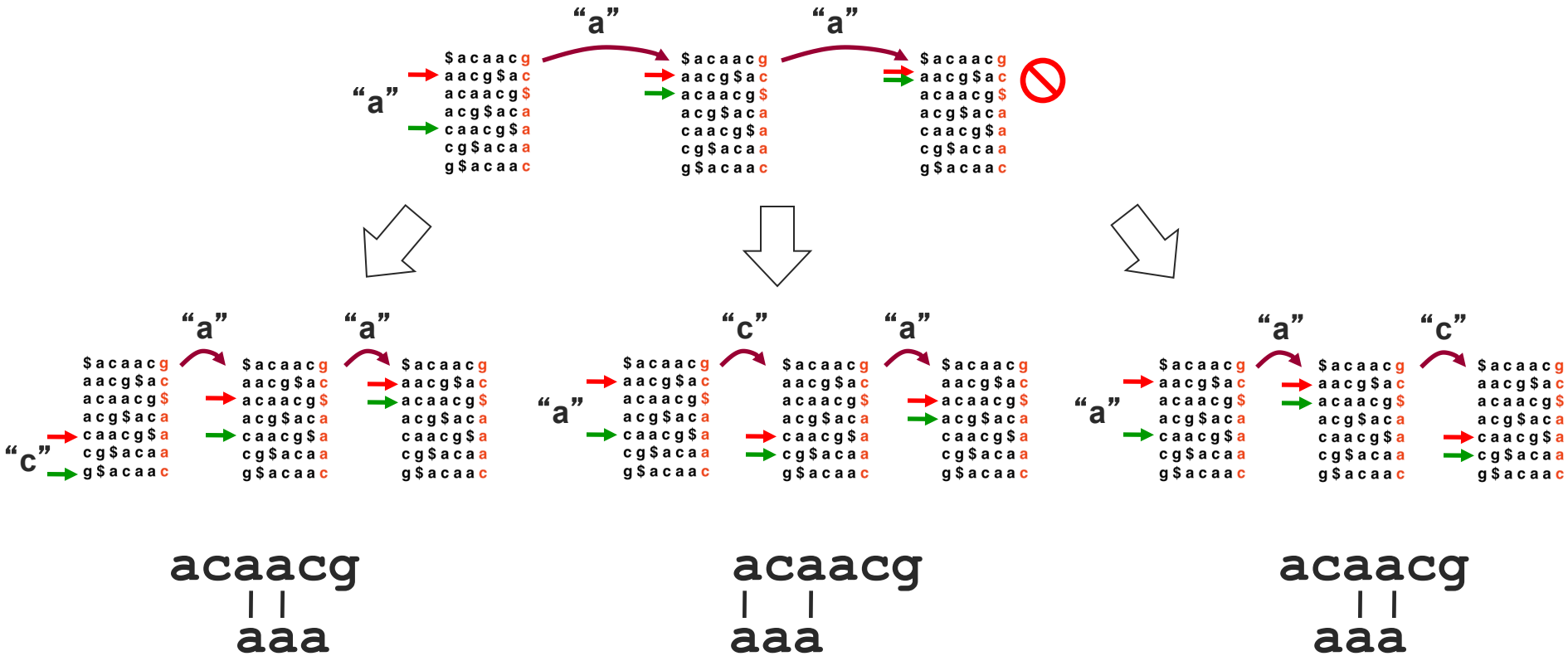
Backtracking

- Relevant alignments may lie along multiple paths
  - E.g., Q = “aaa”, T = “acaacg”



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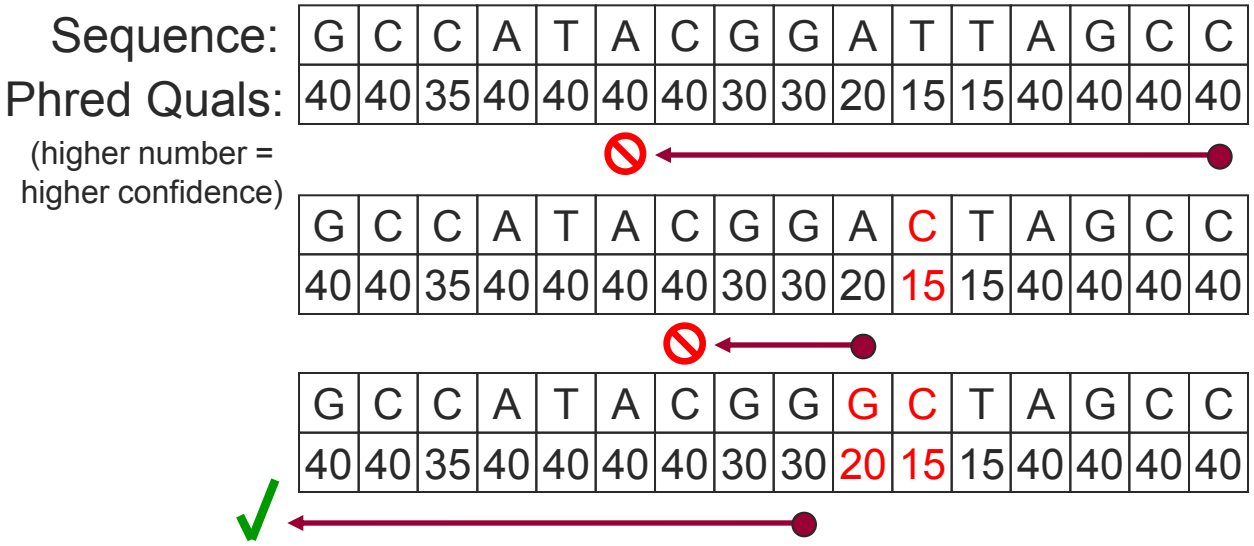
Bowtie backtracks to leftmost just-visited position with minimal quality

- PHRED score = -10log(p) Where p is probability of error



- Greedy, depth-first, not optimal, but simple

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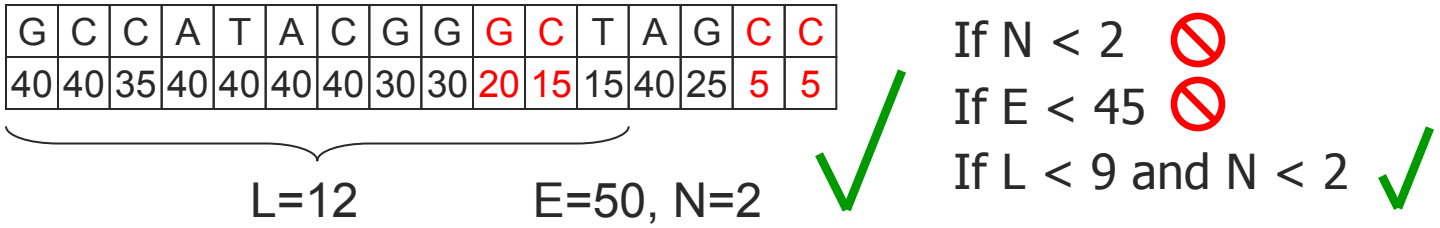
Specifying match quality

- Bowtie supports a Maq*-like alignment policy
  - ≤ N mismatches allowed in first L bases on left end
  - Sum of mismatch qualities may not exceed E
  - N, L and E configured with **`-n`**, **`-l`**, **`-e`**
  - E.g.:

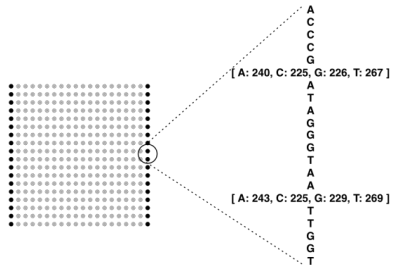


- PHRED score = -10log(p) Where p is probability of error

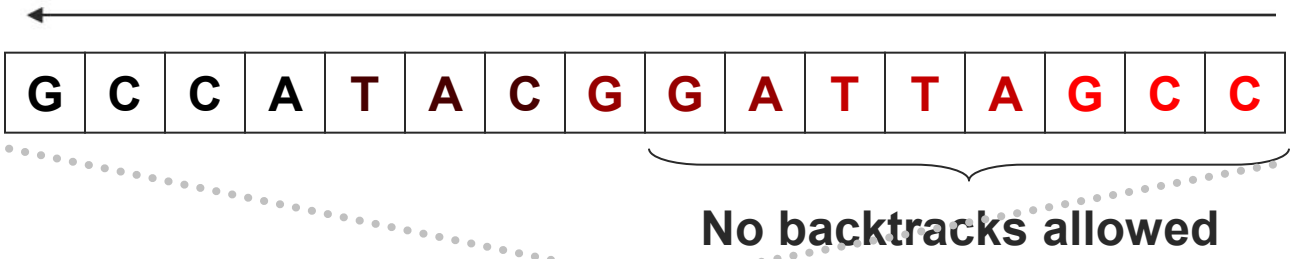
* Li H, Ruan J, Durbin R: Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res* 2008.

Bowtie can match starting from the left to limit backtracking

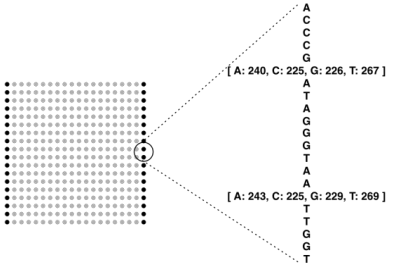
- But how to match left-to-right?
- Double indexing:
  - Reverse read and use “mirror index”: index for reference with sequence reversed



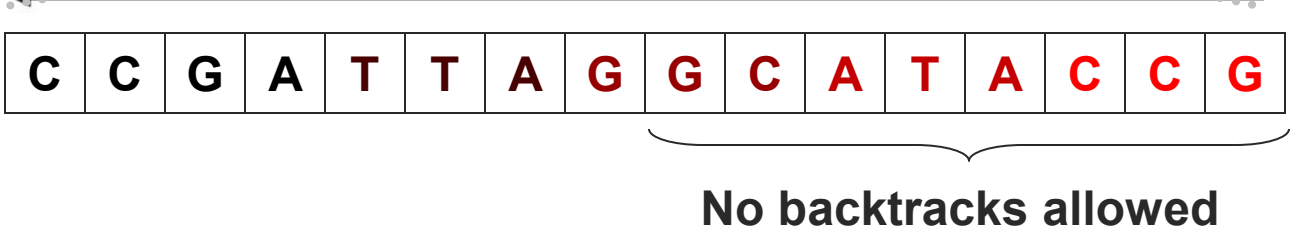
Forward Index



No backtracks allowed



Mirror Index



No backtracks allowed

Courtesy of Ben Langmead. Used with permission.

Time to build a BWT/FM index

- Bowtie employs a indexing algorithm* that can trade flexibly between memory usage and running time
- For human genome (NCBI 36.3) on 2.4 GHz AMD Opteron:

Physical memory Target	Actual peak memory footprint	Wall clock time
16 GB	14.4 GB	4h:36m
8 GB	5.84 GB	5h:05m
4 GB	3.39 GB	7h:40m
2 GB	1.39 GB	21h:30m

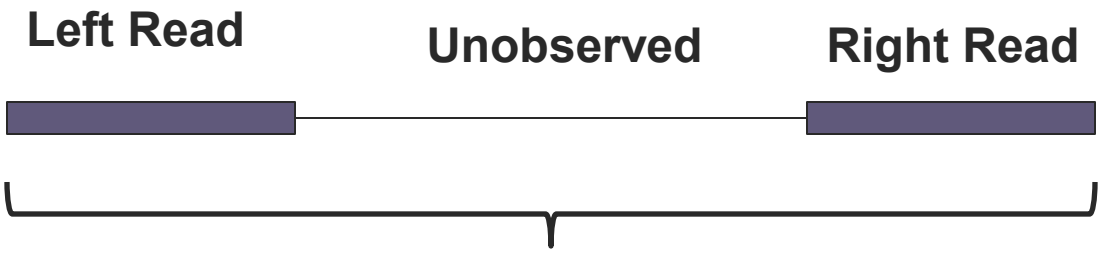
* Kärkkäinen J: Fast BWT in small space by blockwise suffix sorting. *Theor Comput Sci* 2007, 387(3):249-257.

35bp read alignment performance

	CPU time	Wall clock time	Reads per hour	Peak virtual memory footprint	Speedup
Bowtie, 1 thread (server)	18m:19s	18m:46s	28.3 M	**1,353 MB**	-
Bowtie, 2 threads (server)	20m:34s	10m:35s	**50.1 M**	**1,363 MB**	**1.77x**
Bowtie, 4 threads (server)	23m:09s	6m:01s	**88.1 M**	**1,384 MB**	**3.12x**

- Bowtie uses POSIX threads to exploit multi-processor computers
  - Reads are distributed across parallel threads
  - Threads synchronize when fetching reads, outputting results, etc.
  - Index is shared by all threads, so footprint does not increase substantially as # threads increases
- Table shows performance results for Bowtie v0.9.6 on 4-core Server with 1, 2, 4 threads

Paired read alignment in BWA

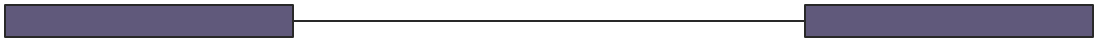


Sequencing instrument identifies read pairs (also called mate pairs) in its output file

First, align Left and Right Reads (they can only be oriented with respect to a genome sequence)

If one read fails to align uniquely, use Smith-Waterman for the unaligned read in proximal sequence to the aligned read

Considerations for read alignment



Uniquely aligning reads vs. “multimaped” reads in output

Desired mismatch tolerance

Desired processing for paired reads

FIN

7.91J / 20.490J / 20.390J / 7.36J / 6.802J / 6.874J / HST.506J Foundations of Computational and Systems Biology

Spring 2014