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Genome-Wide DNA Read Mapping (or DNA Database Search)

Algorithms for Sequence Analysis

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Overview

Previously: Error Tolerant Pattern Matching

- Many online algorithms
- Index-based: **Error-tolerant backward search (FM-index + NFA-like table)**

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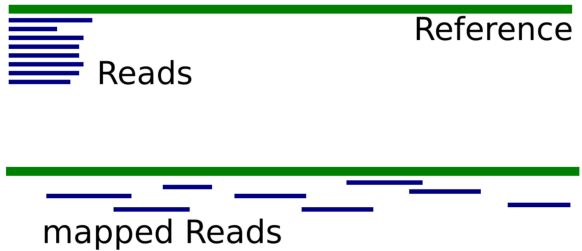
- Index-based: **Error-tolerant backward search (FM-index + NFA-like table)**

Today's Lecture: Genome-Wide DNA Sequence Search

- Read Mappers: bwa-sw, bwa-mem, bowtie2
- Seed-and-extend principle (anchors)

Read Mapping and DNA Database Search Problems



Read Mapping

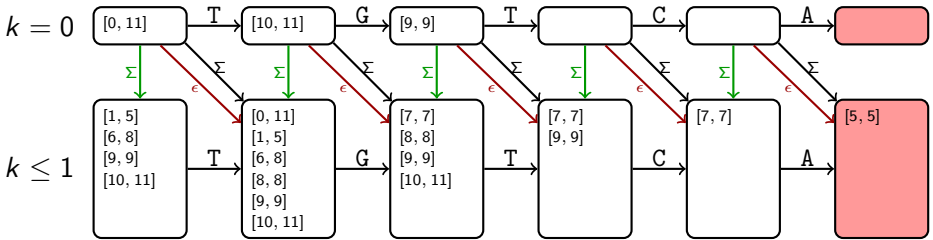
Find (possible) origin(s) of (short) piece of DNA ("read") within genome;
Find (possible) origin(s) of parts of (long) piece of DNA within genome.

DNA Database Search

Find (parts of) DNA query in huge DNA database (NCBI GenBank)

Reminder: Error Tolerant Backward Search

$T = \texttt{AAAACGTACCT\$}, \quad P = \texttt{ACTGT}, \quad \Sigma = \{\texttt{A}, \texttt{C}, \texttt{G}, \texttt{T}\}$:



- Green edges: insertions
- Red edges (ε): deletions
- Black edges: matches (horizontal) and mismatches (diagonal)
- Note: numbers for illustration only, not necessarily correct.

Alternative: Branch on FM Index (no storage in states)

(Example: `cta`, Hamming distance 1.)

	F		L
0	$	ctatata	t
1	a	t$ctata	t
2	a	tat$cta	t
3	a	tatat$c	t
4	c	tatatat	$
5	t	$ctatat	a
6	t	at$ctat	a
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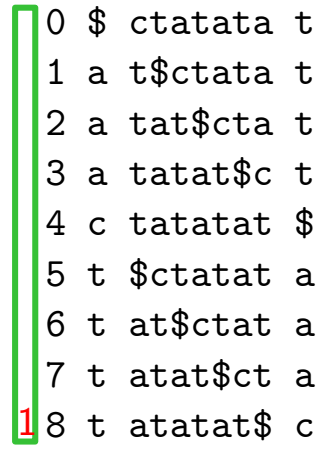

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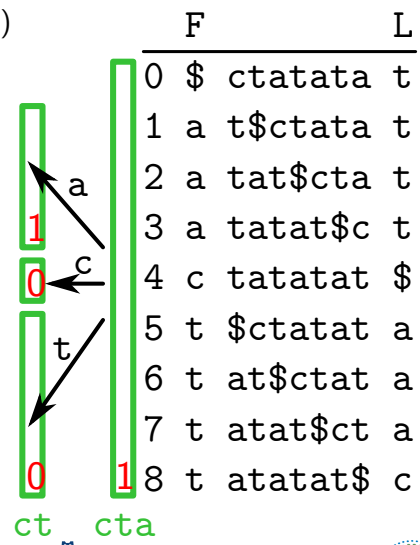

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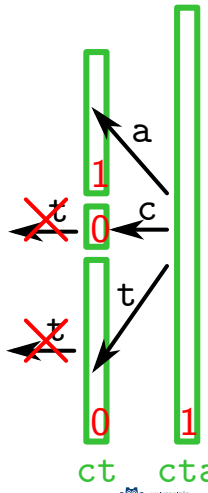

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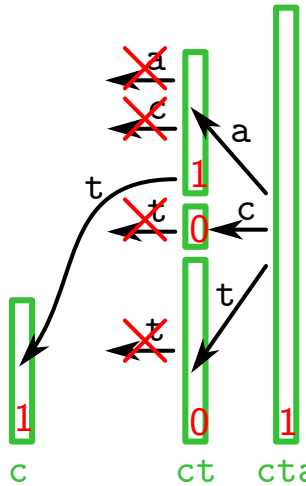

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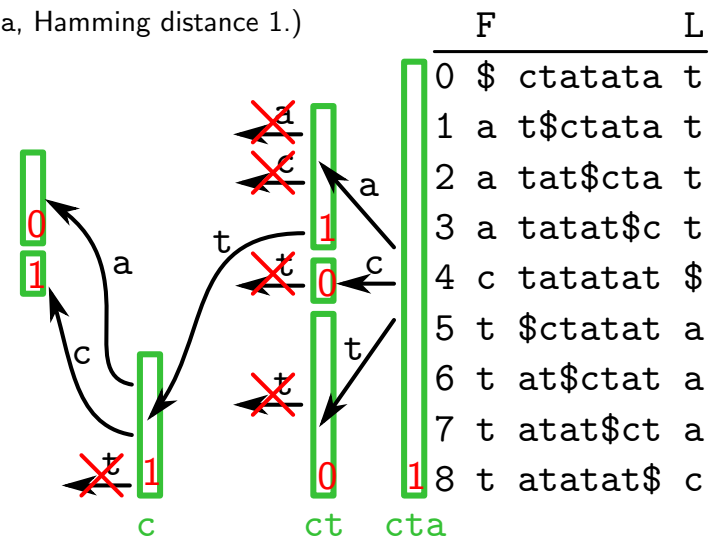

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Running time of Approximate Backward Search

(with NFA states or by branching on-the-fly)

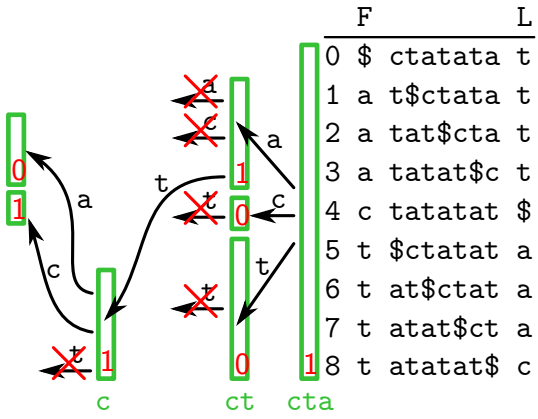
Worst case

- All strings in edit/Hamming neighborhood are enumerated, i.e. the set

$$\left\{P' \in \Sigma^* \,\middle|\, d(P, P') \leq k\right\},$$

  where $P \in \Sigma^m$ is the pattern and k a distance threshold.
- $\Rightarrow$ Running time is **exponential** in k in the worst case.
- In practice, it might be less bad, depending on the string that is searched.

Getting the Actual Positions of Matches



Question

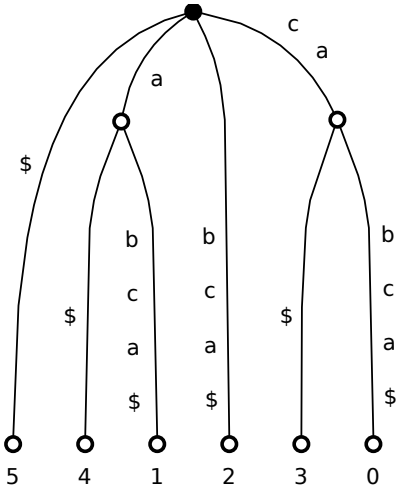
Given: interval on BWT
Sought: position of matches in original string

Answer

Use **(sparse) suffix array** pos

Alternative: Branch on Suffix Tree (Forward Search)

Examples (Hamming distance 1 for simplicity): (1) `acc`, (2) `bab`



Approaches in Practice

- Suffix tree (forward search) not used: memory footprint too large
- NFA states not used (memory overhead)

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- **bwa-sw**: approximate backward search with branching on-the-fly, intervals examined using depth-first search.
- **bwa-sw** starts at several locations in the read (local matches), allows for smaller edit distance thresholds
- **bwa-sw** collects a large number of intervals first, then performs batch lookup in suffix array

Literature: bwa-sw

Heng Li & Richard Durbin:
Fast and accurate short read alignment with Burrows–Wheeler transform.
Bioinformatics 25(14):1754–1760 (2009).

Seed and Extend Idea for Filter-based Approaches

Motivation

Branching on errors is computationally expensive

- Running time exponential in the number of errors
- Exact matches are inexpensive to compute
- **Idea:** Find exact matches
  - of fixed length q (q-grams) or k (k-mers)
  - of maximal length (MEMs, maximal exact matches) using FM-index
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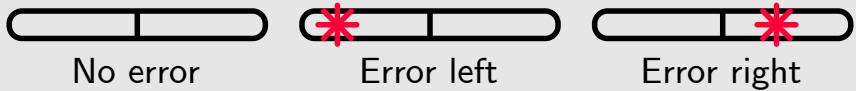
Literature: bwa-mem

Heng Li:
Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM
arXiv:1303.3997 [q-bio.GN]

Pigeon Hole Principle, Seed and Extend

Example

When we allow a read to contain up to 1 error, the following scenarios are possible:



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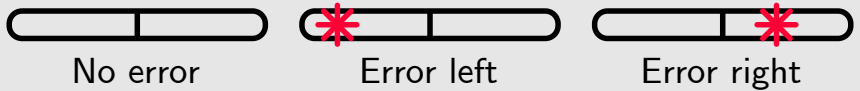

More than one error?

Pigeon hole principle holds for $d \geq 1$ errors:

- Cut pattern into $d + 1$ parts
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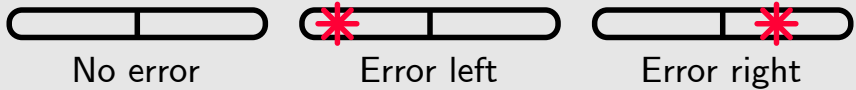

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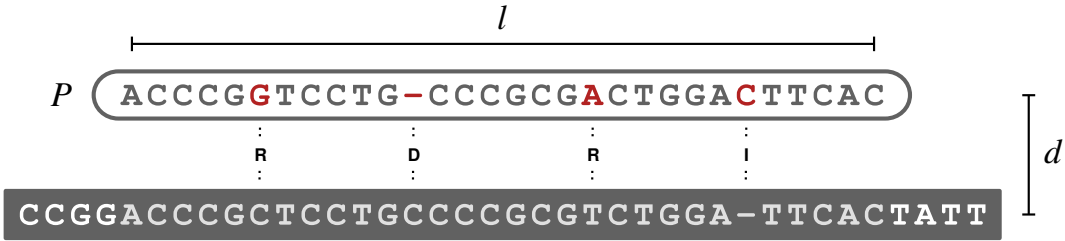
Seed and Extend

- **Seed:** Find origins of exact match
- **Extend:** Continue search **allowing errors** around exact match

Approximate Pattern Matching



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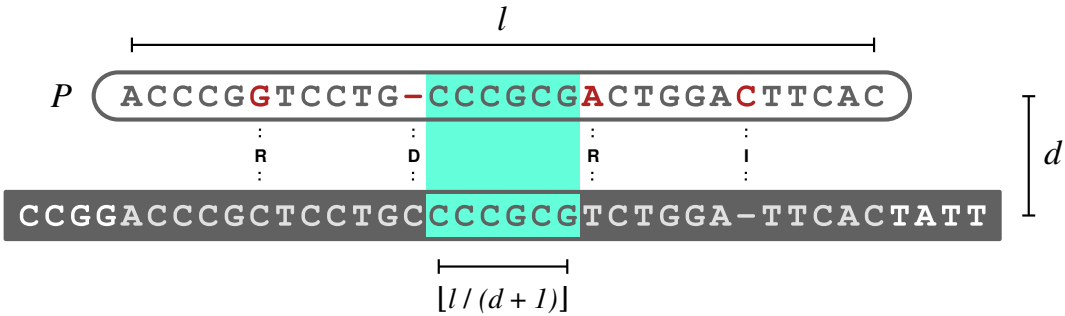

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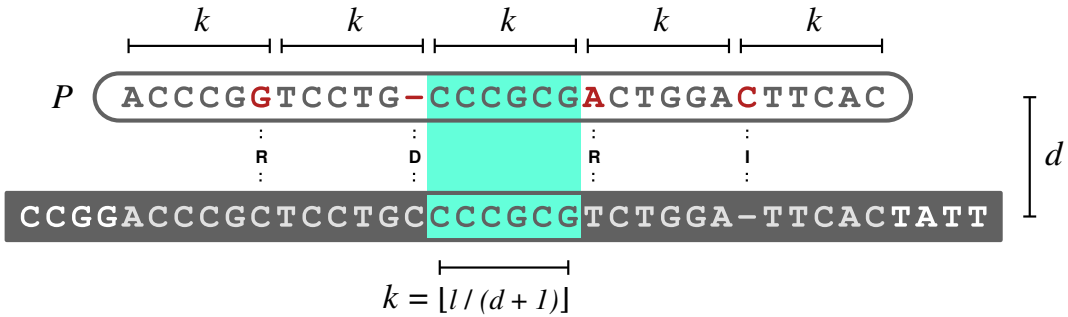

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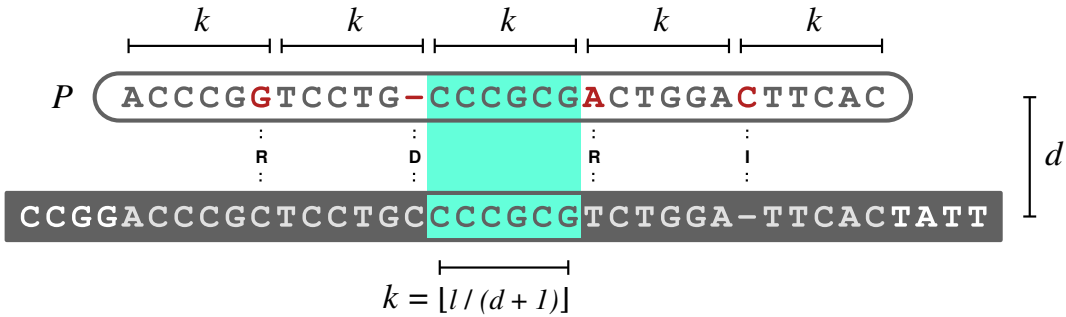

Seed-and-extend strategy

Bowtie

Implementation of seed-extend in bowtie

- Use two indexes: for searching forward/backward, respectively
- **Seed:** search for left/right half **without errors** using FM index
- **Extend:** continue search using branching in FM index

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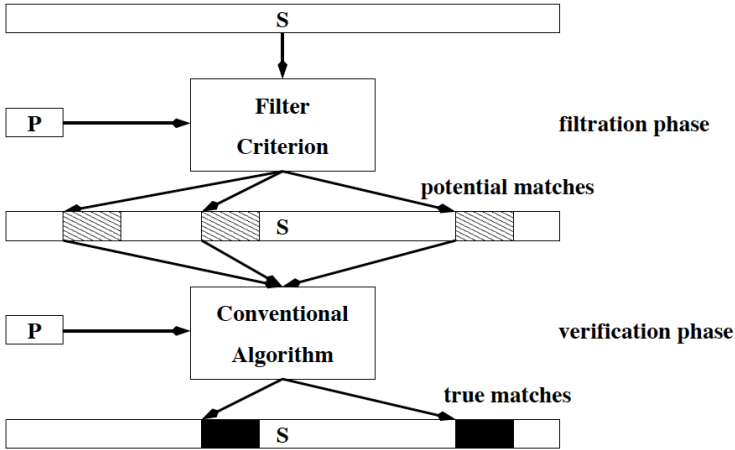
Variants used in other read mappers or search tools

- seed phase using index, extend phase using alignment
- multiple seeds, then alignment

Abstraction: Filter-based approximate search



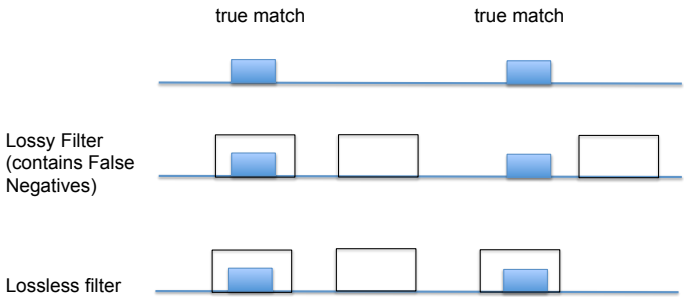
[Stefan Burkhardt, Ph.D. Thesis 2002]

Filter-based Search

A filter restricts the expensive verification to promising regions of the target sequence.

- A **lossy** filter may miss true matches in the target.
- A **lossless** filter contains all true matches.

A good filter always balances sensitivity and speed.



Definitions

q-gram

A **q-gram** of a string s is a substring of length q of s.

Matching Problem

Given a reference text T and a query string s,
the **approximate matching problem with d differences and window length w** consists of finding a pair of substrings $(s[i \ldots i+w-1], t)$ such that

1. $s[i, i+w-1]$ is a length-w substring of s, and t is a substring of T,
2. $d_{\text{edit}}(s[i \ldots i+w-1], t) \leq d$, i.e., edit distance is at most d.

Q-gram lemma

Lemma

Let an occurrence of $s[i, i + w - 1]$ with at most d differences end at position j in T. Then at least $w + 1 - (d + 1)q$ q-grams of $s[i, i + w - 1]$ occur in $T[j - w + 1, j]$.

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Proof

- each error destroys at most q q-grams (`Y` positions below)
- the last $q-1$ positions have no q-grams (`X` positions below)

$$\text{intact } q\text{-grams: } w - dq - (q-1) = w + 1 - (d+1)q$$

- Example: q=3 (valid positions of 3-grams are `M` below)

```
target   ATTGACAC
query    ATTCACAC
3-grams  MYYYMMXX
```

QUASAR algorithm

Task: Find approximate matches of s in T

Seed-and-extend based on q-gram lemma

1. Pre-compute the threshold α for a window of length w using the q-gram lemma.
2. Partition T into blocks (larger than window length w)
3. Count all q-grams in $s[1 \dots w]$ for each block along T
4. Each block that contains an approximate match has a counter of at least α. (The reverse is not true. We may have false positive blocks)
5. Advance the window in s from $s[i \dots j]$ to $s[i+1 \dots j+1]$

QUASAR - counting q-grams

In order to count the q-gram matches in a window, we need an efficient data structure.

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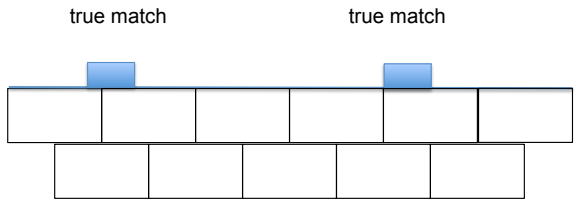
The **q-gram index**:

1. Construct the suffix array pos of T
2. Create a table of size $|\Sigma|^q$ that maps a q-gram to its starting rank in pos, to allow constant time lookup.
3. q-grams are integer-encoded as numbers $0 \dots |\Sigma|^q - 1$, e.g. DNA: $\texttt{TTGCCA} = (332110)_4 = 3988$ (base-4 number)

QUASAR - Blockwise counting

- Keeping a counter for each possible window of length w of the text would lead to a huge array of size $\mathcal{O}(|T|)$
- To save space, we define two arrays of blocks of size $b \geq 2w$. The first block array is shifted by $b/2$ positions against the second.



QUASAR - Efficient counting and shifting

When the query window $s[i \ldots j]$ is moved to $s[i+1 \ldots j+1]$,
the only difference for the block counters are two q-grams,
one that leaves (blue) and one that enters the window (red).

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So for each shift update, change the block counters as follows:

- subtract the count of the leading q-gram (blue) from blocks,
  unless the block counter is $\geq \alpha$ (lock-in)
- add a count to all blocks that contain the trailing q-gram (red)

Gapped q-Grams

Definition

Gapped q-grams are specified with a **mask**, a string of

- # characters denote required matches
- . characters denote "don't care" positions

For example ##.#.# denotes a gapped 4-gram of **span** (length) 6.

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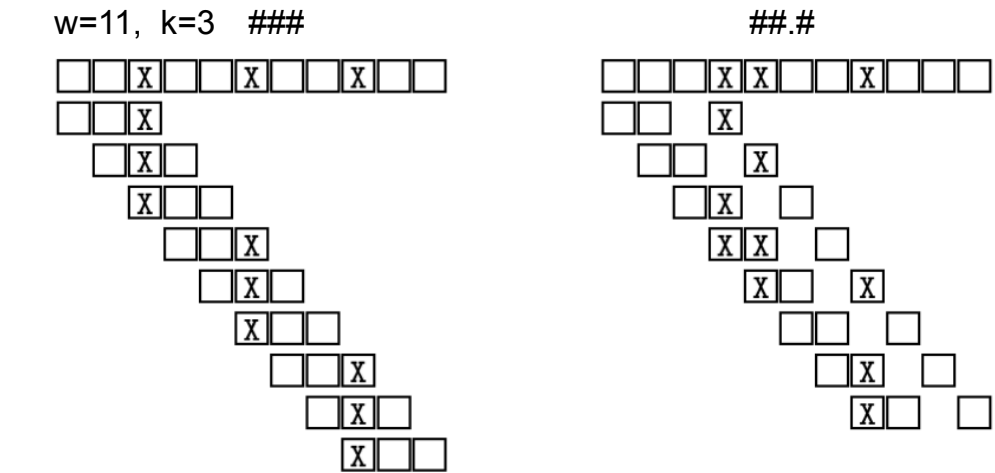
- Gapped q-grams can improve the filter efficiency by orders of magnitude.
- The q-gram lemma is not tight for gapped q-grams and there is no closed formula for computing the threshold for a given edit distance d.

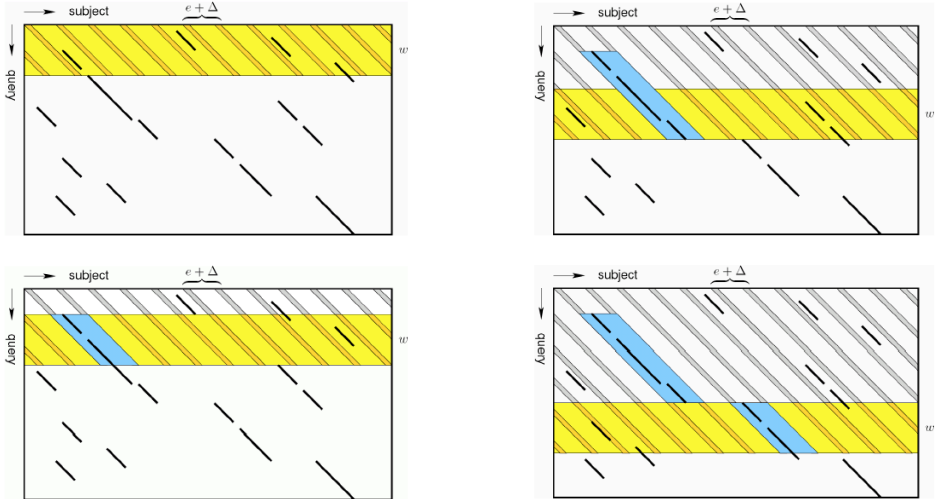
Gapped Q-Grams: Example



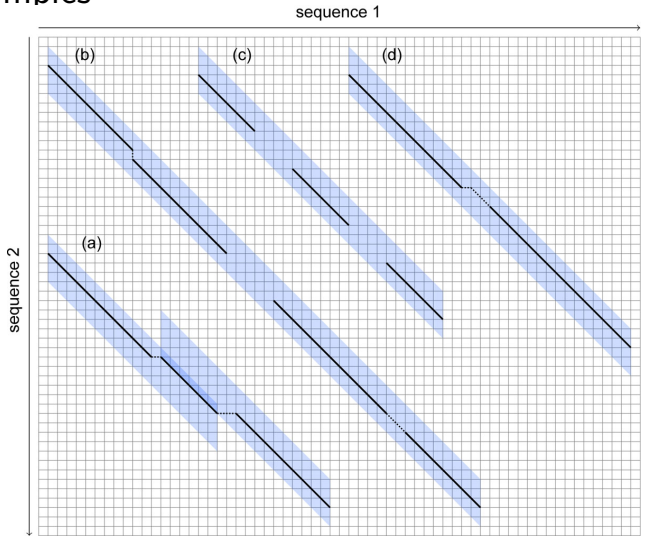
[Stefan Burkhardt and Juha Kärkkäinen, Fundamenta Informaticae, 2003]

SWIFT algorithm: Parallelograms instead of blocks

Slide length-w window over query, count q-grams in diagonals



SWIFT - Examples



from Kehr, Weese, Reinert, BMC Bioinformatics 2011

Other Tools

There are a number of read mappers that were made for the analysis of next-generation sequencing reads against large genomes and use variants of filter algorithms with q-grams

- RMAP
- RazerS and RazerS3
- Hobbes
- GEM
- Stellar
- and many more

Literature

- Q-gram Based Database Searching Using a Suffix Array (QUASAR). Burkhardt et al., RECOMB 1999
- Efficient q-Gram Filters for Finding All ϵ-Matches over a Given Length. Rasmussen, Stoye, Myers, Journal of Computational Biology, 2006
- Better filtering with gapped q-grams. Stefan Burkhardt and Juha Kärkkäinen, Fundamenta Informaticae, 2003

Summary

Approximatie pattern matching **with index**

- NFA or branching on FM index
- Branching on suffix tree
- Examples: bwa-sw
- Seed-extend principle: avoid branching by using exact matches
- Examples: bwa-mem, bowtie
- q-gram lemma
- Fast q-gram access: q-gram index
- Filtration approaches (QUASAR, SWIFT)
- Idea of gapped q-grams

Possible Exam Questions

- How can a suffix tree be used to search for approximate pattern occurrences?
- What is the resulting running time?
- What is the benefit of using an FM index instead of a suffix tree?
- Explain approximate search on an FM index by means of an example.
- What's the main idea behind the "seed and extend" paradigm?
- What is the purpose of a filter?
- What is a lossy vs. a lossless filter?
- Explain the q-gram lemma.
- What is a q-gram index? How is it related to the suffix array?
- What is the idea of gapped q-grams?