ITHAKA: A TAXONOMIC CLASSIFIER BASED ON BLOOM FILTERS, AND SPACED SEEDS

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METAGENOMICS & NGS

Metagenomics is a powerful approach to study genetic material contained in environmental samples, which is revolutionized by high-throughput sequencing technologies. Taxonomic classification of metagenomics data sets is a common step in analysis, a step for which computational cost becomes prohibitive with the growth of metagenomic datasets.

Approaches using sequence alignment algorithms, often based on the Burrows-Wheeler transform, such as Kaiju[1], Centrifuge[2], compete successfully with k-mer based alignment-free comparison methods such as Kraken[3], Clark[4]. Improvements to k-mer based approaches include: extending contiguous k-mers with spaced seeds [5][4], using Bloom filters as an underlying data structure for storing k-mers.[6]

SPACED SEEDS

A **spaced seed** is a pattern over alphabet $A = \{\#, -\}$, where
$\#$ matching position, $-$ don't care position.

```
A A C A T T C T
  # # - # - #
A A C C T T C T
```

Previously, we demonstrated that spaced seeds allow for a better classification of NGS reads coming from a genome G between two other genomes G_1 and G_2 of the same genus.[5]
Coverage is a number of aligned pairs covered by # from a spaced seed matches while sliding over an alignment (=4 above).
Coverage pattern is the pattern of matches (hits) of a seed over an alignment.
The NP-HARD PROBLEM: Assume a read f comes from genome g. For a given seed, computing $e_{f,g}$ – a minimal number of errors(mutations) in fragment f, given the *coverage pattern* of the read f with hits to g is NP-hard. In other words, answering what is the minimal number of errors in a fragment f which guarantees a certain coverage pattern (eg. found by Ithaka query) turns out to be an instance of 0-1 integer programming (an NP-complete problem). We programmed our solution using *coin-Cbc* integer programming library. In practice, it works fast on reads with high coverage, and slows down significantly on reads with low coverage (then heuristic is preferred).

REFERENCES

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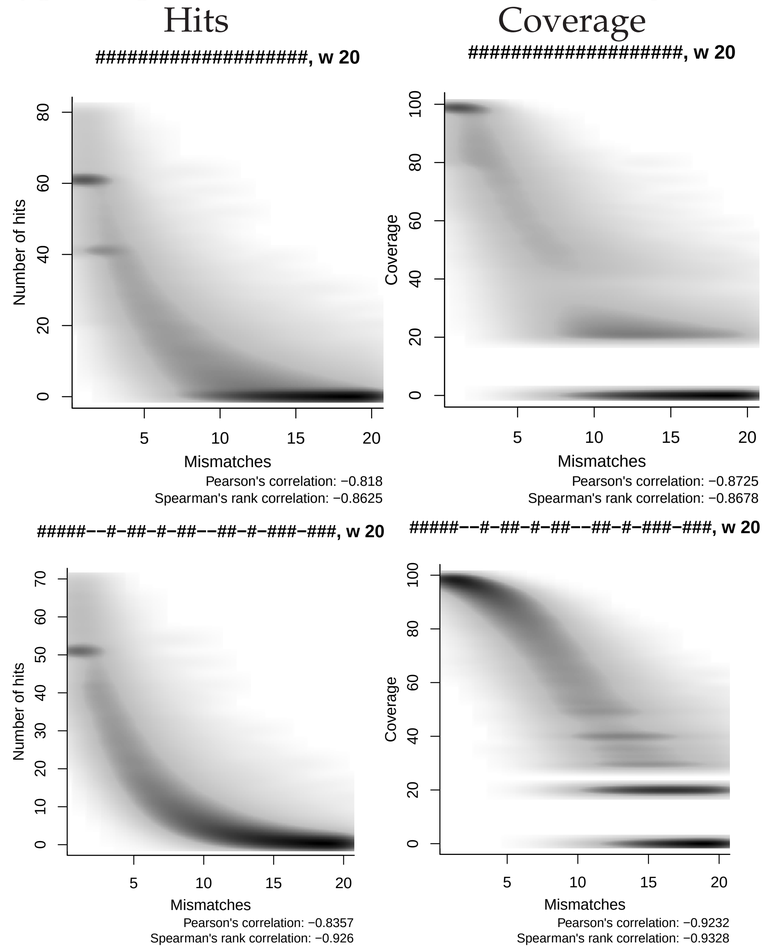
https://github.com/gregorykucherov/ithaka
http://seed-kraken.readthedocs.org

SCORES ON REAL GENOMES

We generated a set of ILLUMINA-like single-end reads: we've selected random substrings of *M.tuberculosis* genome of length $L = 100$ and introduced k mismatch errors, with k random between 1 and 20. For each read, we computed: **number of hits** and **coverage** to the genome under a given seed. A typical plot error vs score (seed weight 20).



Spaced seeds exhibit a better correlation between errors and score, while *contiguous seeds* plots are more blurred.

EM ABUNDANCE ESTIMATION

EM solves two problems at once: abundance estimation, and assignment. EM maximizes likelihood as a function of two types of variables:

1. abundances α_g such that $\sum_{g \in G} \alpha_g = 1$ correspond to proportion of reads from the whole sample which belong to genome g. Here G is the set of all genomes plus U category of unknown/unclassified reads.
2. categorical assignment indicator 0-1 variables $y_{f,g}$âĂŃ which if equal to 1 mean that read f comes from a genome(or category) g. Matrix of these variables is sparse âĂŞ most of them are 0 âĂŞ since we only consider $y_{f,g}$ non zero if there are some hits from genome g in fragment f. $\sum_{g \in G} y_{f,g} = 1$

The **total likelihood** is proportional to

$$\left(\prod_{f \in F} \prod_{g \in G} P(f\|g)^{y_{f,g}} \alpha_g^{y_{f,g}} \right) \times P_{prior}(\alpha)$$

$P_{prior}(\alpha)$ is the conjugate prior distribution to categorical distribution, which is Dirichlet distribution.
Expectation step goes over $y_{f,g}$âĂŃ variables (with α_g set)
Maximization step goes over α_g variables (with $y_{f,g}$âĂŃ set).

$P(f\|g)$ is given, it's probability of obtaining fragment f assuming it comes from genome g, it depends on $\frac{1}{\text{kmer\_richness\_of\_g}}$. One proposal for it depends also on $e_{f,g}$ – a minimal number of errors/mutations in fragment f, given the coverage pattern (see NP-HARD, left panel).

ITHAKA TOOLCHAIN AND PERFORMANCE

Ithaka tool chain:

- ithaka-build.py – indexing fasta files, tree binarization, creating of **2outof3 index**.
- ithaka-query.py – querying the index with a set of reads.
- ithaka-assigment.py – 2 steps:
  - samtoerr – computing error counts from coverage (NP-hard),
  - Expectation Maximization(EM) – taxonomic abundance estimation, and read assignment (blazing fast: 1000 iterations of 50K reads (>2 million alignments) in <10 seconds)

Possible improvements to ithaka-query.py: In development implementation (partially in C++) is still slow: 700 reads (of 220-250len) per second. This can be easily sped up 4x by moving totaly to C++. Further speed up is not possible without cache optimization because 25% of time is spend on querying Bloom filter bitmap: it's the time to access memory with cache misses, the access is random. Effective implementation using *"cache blocking"* is possible (10x, total 40x speedup).
Performances of classification of SEED-KRAKEN[5] and ITHAKA (with spaced seeds), and original KRAKEN, were computed on simulated metagenomes (primarily used in [3]): MiSeq (10 bacterial genomes, average error rate), Charted are genus precision (positive predictive value) against genus sensitivity (rate of correct assignments). Varying are *k-mer length*, and its spaced seed equivalent *seed weight*, while the *seed span* (not indicated) varies from 31 to 40. Ithaka marks *black*: only leaves(genomes) fed to EM; *blue*: also inner nodes fed to EM (precision at cost of sensitivity).