Differential Analysis of Whole-genome Shotgun Sequences

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Background

- metagenomics is the study of genetic material collected from environment
- each whole-genome shotgun (WGS) sample is a large ($\sim 10^7$) collection of short ($\sim$100 bp) DNA fragments, or reads from multiple ($\sim$100) species, e.g.,

$$\underbrace{\text{ATGCGTGA...GAACCGTAC}}_{\text{100 bp}}$$

- given two groups of metagenomic samples, differential analysis explores what attributes (compositional, functional) is over or under expressed

  e.g., Bacteroides sp. 20-3 is enriched in T2D patients

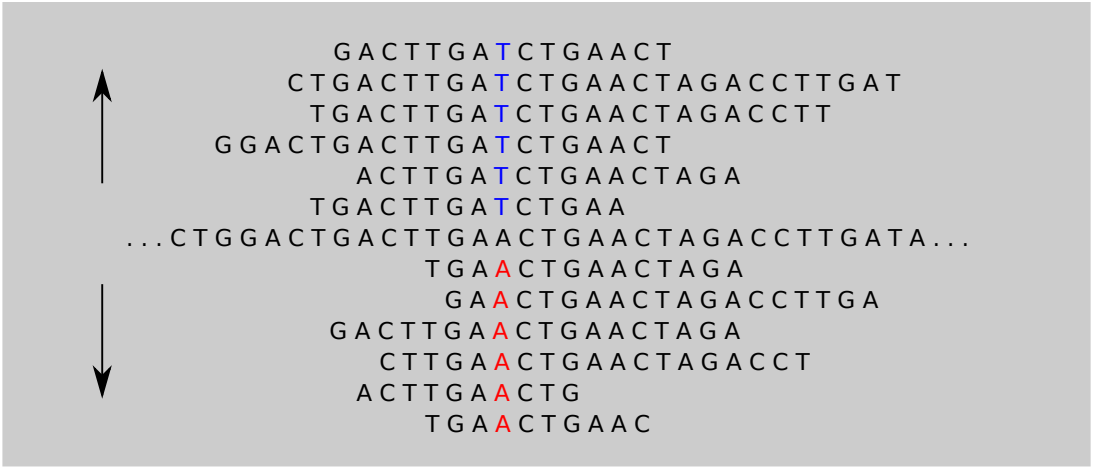
- differential analysis of metagenomic samples is usually done at the species or strains level,

  e.g., find relative abundances of taxa $\rightarrow$ do hypothesis testing

- our goal is to study local changes at the base pair level, e.g., mutations, insertion, deletion, duplication, etc., as well as global changes at the taxa level

Motivation

- longer k-mers are usually unique at the species and even strain level
- mutations, deletions etc. change the k-mer abundances locally, e.g.,



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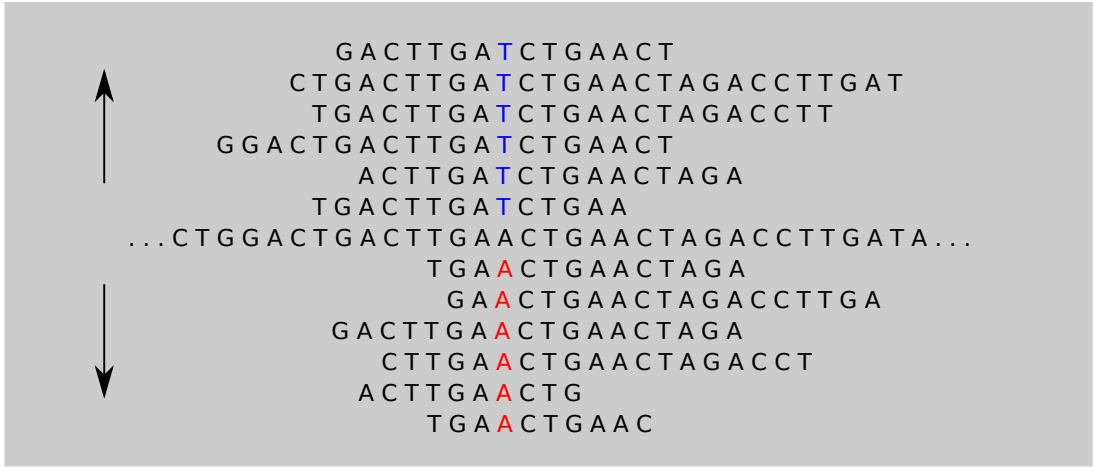

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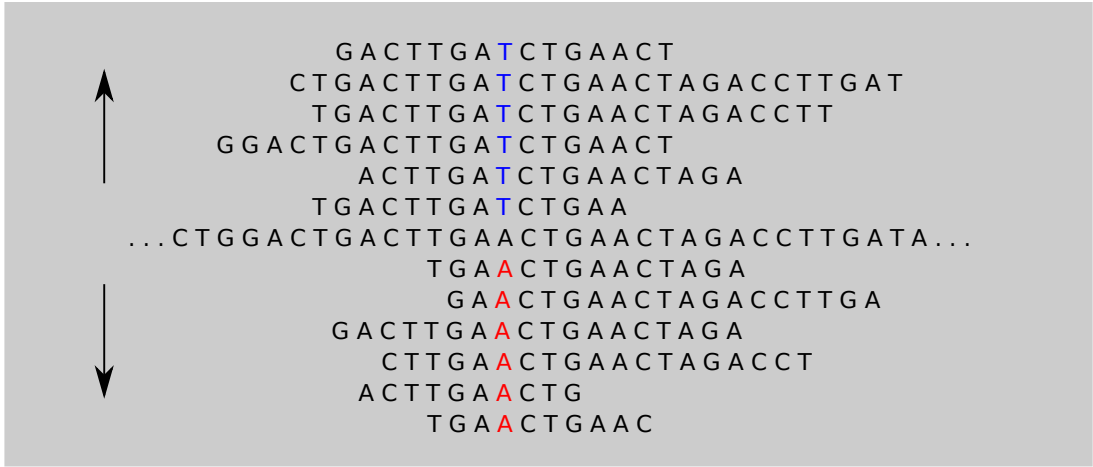
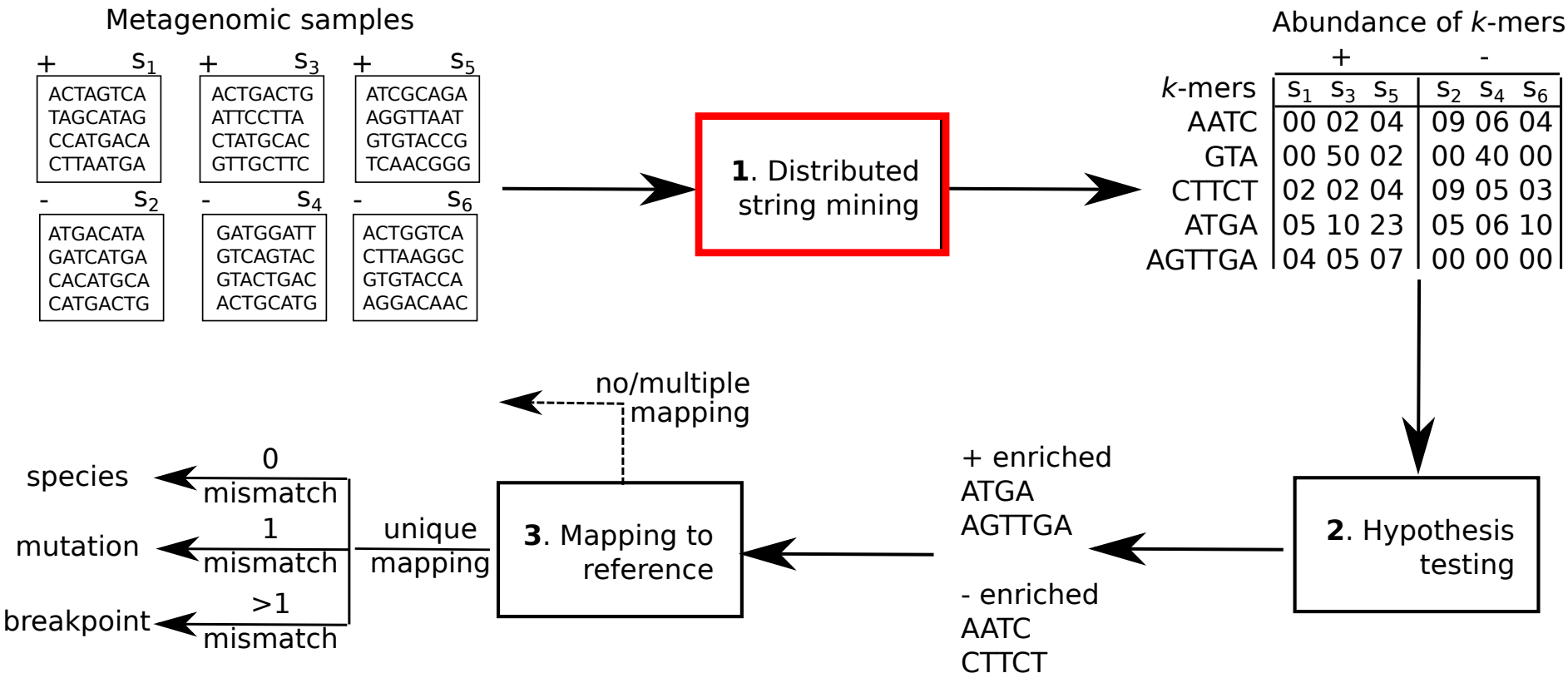


study all possible differentially expressed k-mers

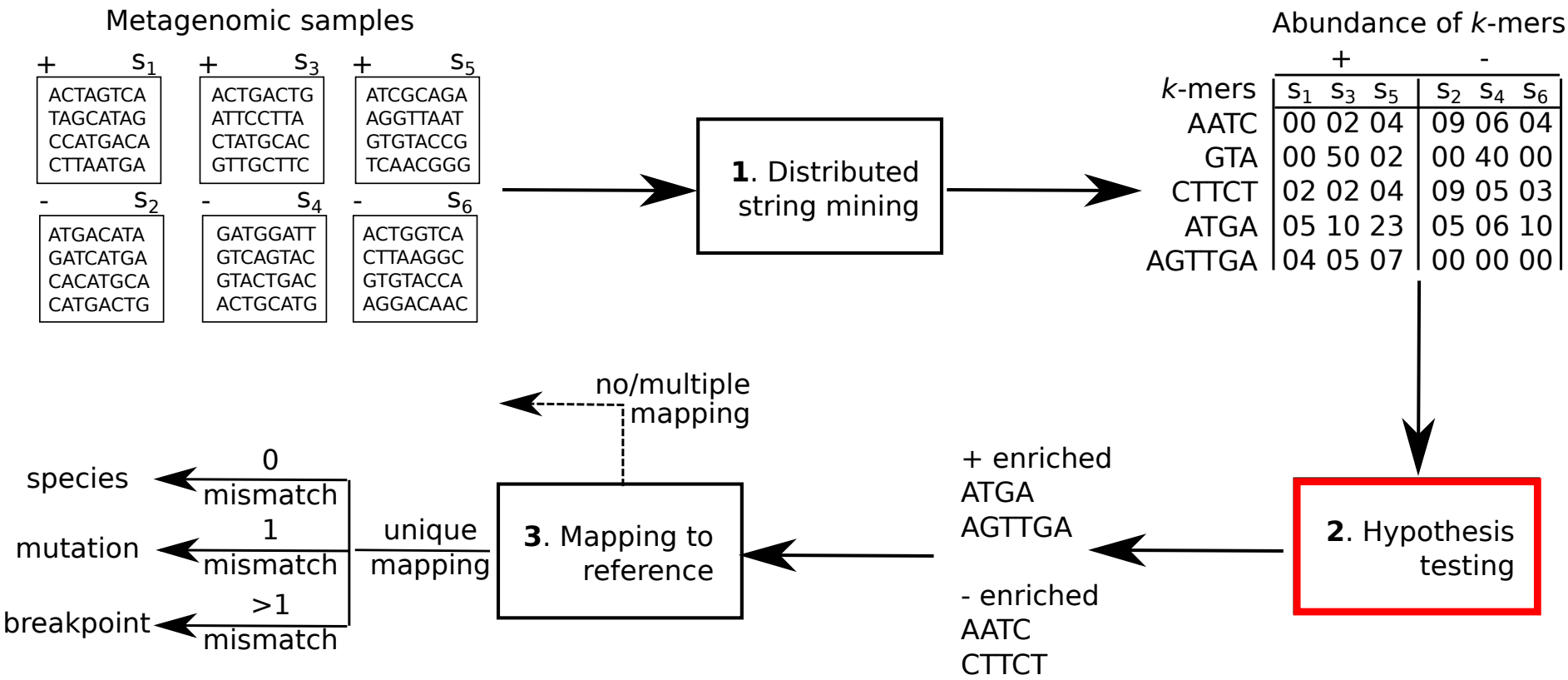
Pipeline



1 memory and time efficient computation of k-mer abundances,

- (minimal) sub-k-mers with same abundances as super-k-mers are ignored
- (robust) k-mers appearing only once in a sample are ignored
- (sequential) only enriched k-mers are stored for further analysis

Pipeline

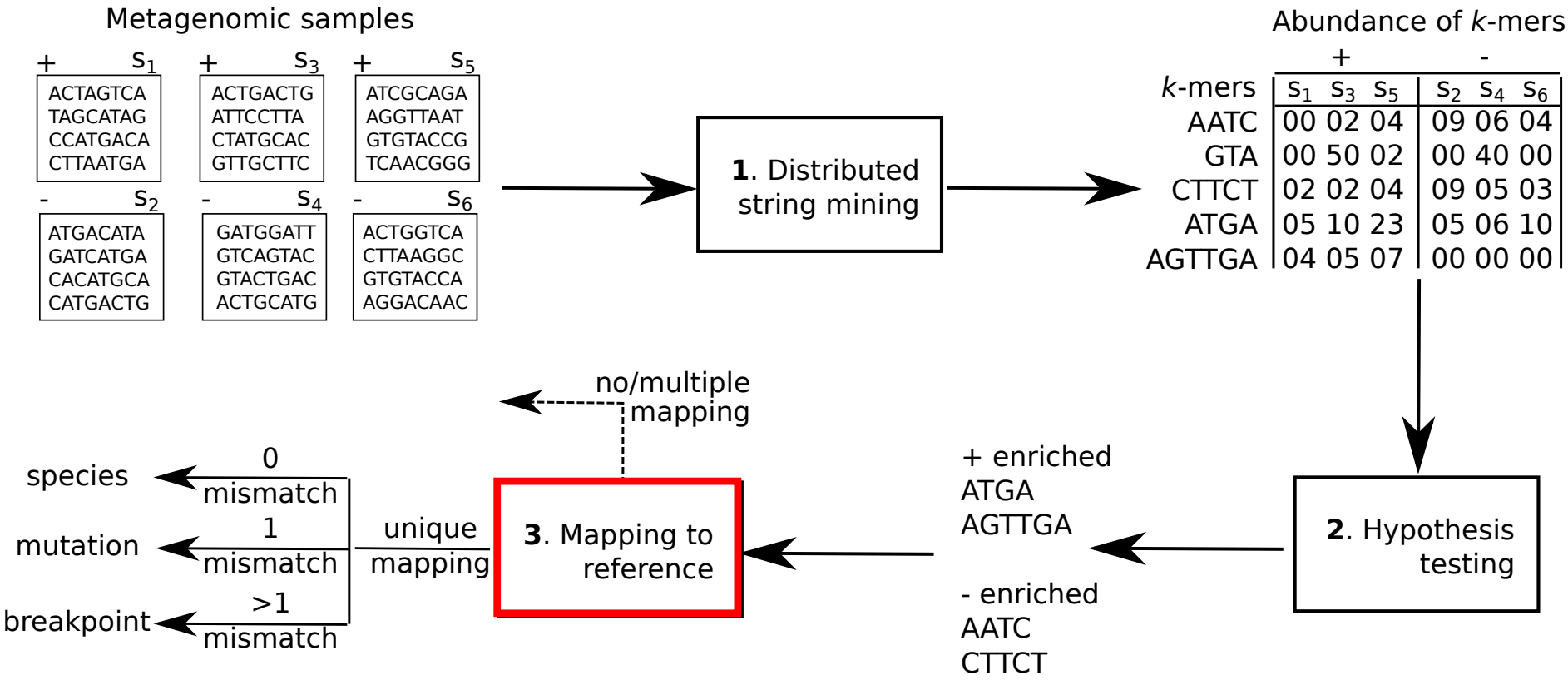


2 likelihood ratio test under model $n_j^c \sim \mathrm{NB}(s_j \mu^c, \alpha^c)$

sample j, group c, mean and dispersion (μ, α), and sequencing depth s_j

- Newton's method is used to find the maximum likelihood solution
- test is sensitive to change in both mean and dispersion $\Rightarrow$ simpler

Pipeline

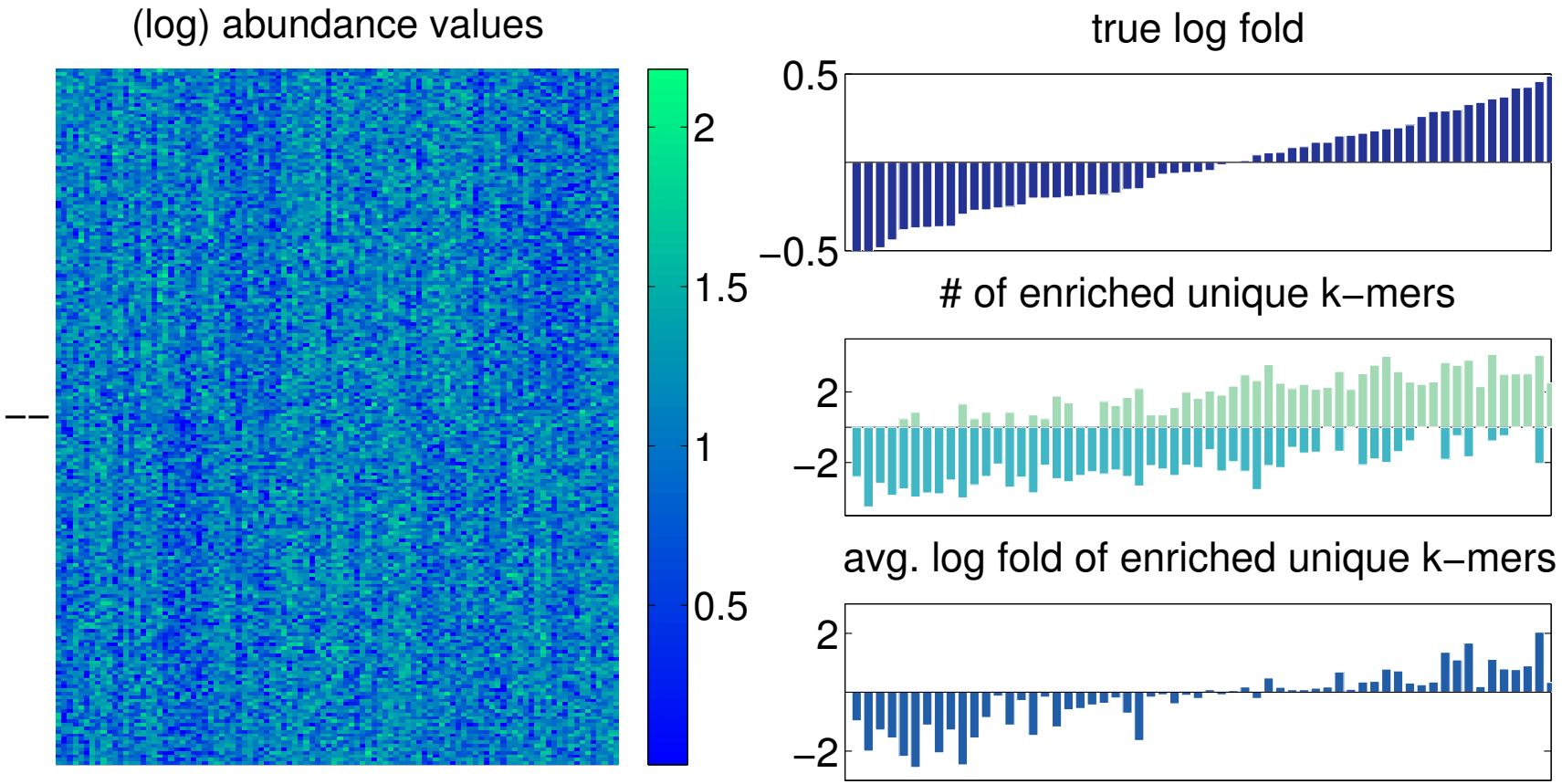


3 mapping to suitable reference database with standard tools, e.g., BWA

- none hits and multiple best hits are (so far) ignored
- unique mappings (single best hit) are stored and investigated

Simulation: species level change

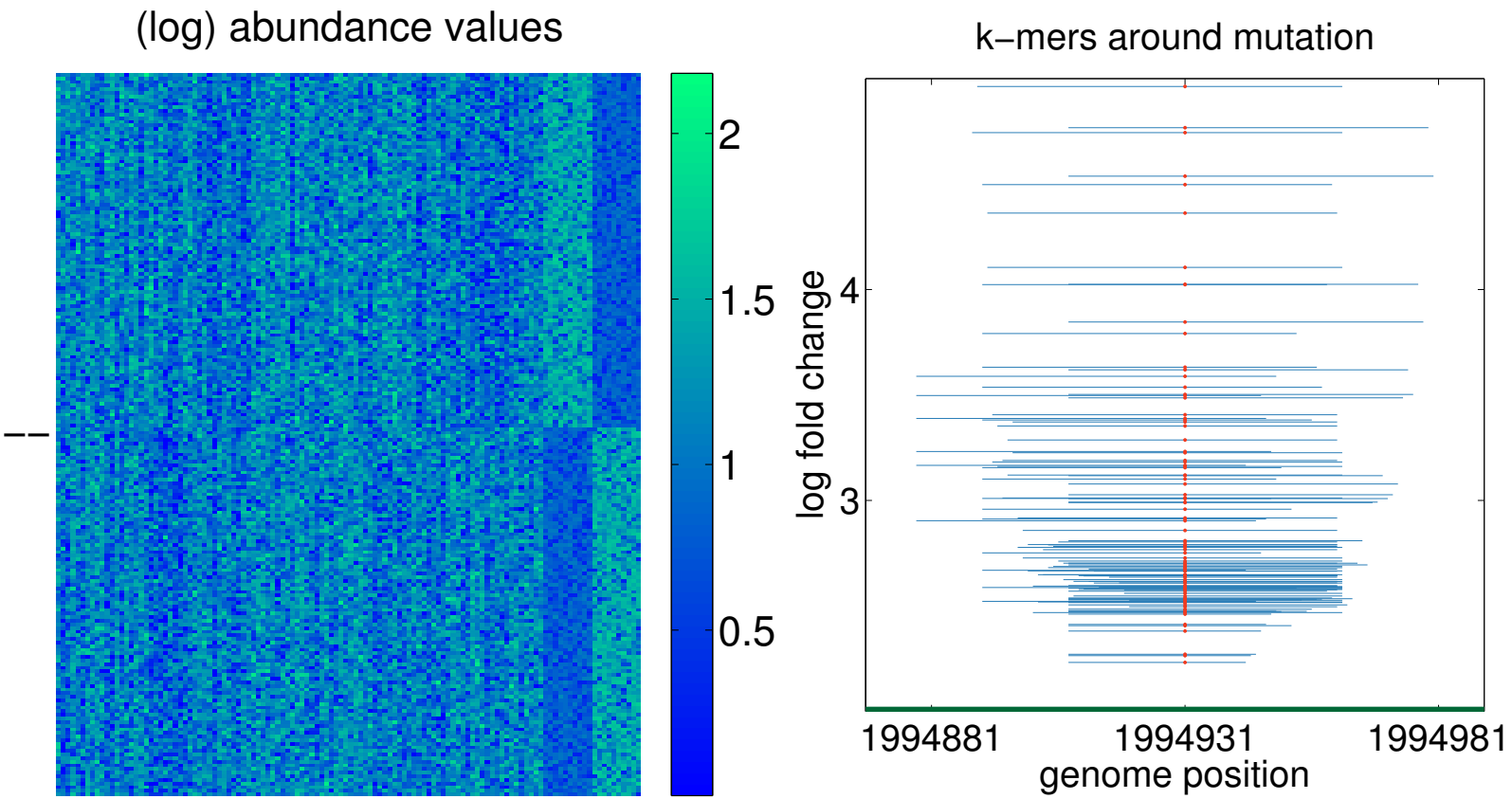
- proportion of exactly mapped enriched *k*-mers in two groups provides evidence toward enrichment at taxa level
- mock metagenomic samples: 100 species/strains, 100 samples in each group



proportion of over/under expressed *k*-mers are related to true fold change

Simulation: *k*-mer level change

- multiple enriched unique *k*-mers with single mismatch at a common genome position provides evidence toward mutation
- additional mock species with mutations (5), deletion (5), etc.



many *k*-mers of arbitrary lengths and locations overlap a common mutated base

T2D P2: Species level change

3.2 million k-mers with p-value < 0.000005

Organism	# of unique k-mers total	T2D	CON	k-mer len mean ± std	log-fold mean ± std
Bacteroides coprocola	27870	71	27799	47.35±17.90	-4.18±2.51
Clostridium bartlettii	31104	44	31060	44.64±17.09	-3.75±0.87
* Roseburia intestinalis	2259855	471	2259384	42.62±16.86	-3.34±1.19
* Faecalibacterium prausnitzii	61406	90	61316	31.91±12.85	-2.22±0.78
* Roseburia inulinivorans	18056	259	17797	33.58±16.00	-1.95±1.38
Bacteroides pectinophilus	19427	91	19336	40.36±18.36	-1.91±0.82
* Faecalibacterium prausnitzii	18882	383	18499	29.02±10.67	-1.80±1.09
* Eubacterium rectale	181315	108	181207	38.04±13.96	-1.74±0.87
* Eubacterium eligens	81902	72	81830	46.14±19.74	-1.53±0.46
Ruminococcus obeum	10667	843	9824	28.12±10.29	-1.33±1.34
Ruminococcus sp. 5-1-39	16475	952	15523	28.39±11.53	-1.25±1.05
Clostridium methylpentosum	10828	10485	343	43.07±14.08	2.04±1.14
Eubacterium hallii	17282	11784	5498	25.40±8.93	2.63±3.44
* Bacteroides sp. 20-3	23884	23710	174	46.87±18.12	3.05±1.53
* Clostridium bolteae	15877	14631	1246	29.51±13.27	3.80±2.82
* Bacteroides caccae	46884	46389	495	65.27±12.89	3.82±1.73
* Bacteroides cellulosilyticus	27089	26705	384	50.01±17.95	4.19±2.02

enrichment directions tally with previously reported findings $*$

T2D P2: k-mer level change

mutation locations covered by at least two k-mers

Organism	mutations
Faecalibacterium prausnitzii M21/2	1107
Faecalibacterium prausnitzii A2	892
Roseburia intestinalis L1 82	857
Eubacterium rectale ATCC 33656	825
Bacteroides coprocola DSM 17136	574
Roseburia inulinivorans DSM 16841	291
Eubacterium eligens ATCC 27750	266

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- understanding differential abundance is a crucial problem, e.g., understanding the difference between patients and healthy microbiome
- efficiently exploring local changes opens up new avenues
- our future goal is to draw viable conclusion without the reference database

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Thank You! Question?