Supplementary Materials

Tables

1. Tool Configuration Details

For full version and build information see the git repo at [code.jgi.doe.gov/spacersdb/spacer*matching*bench](code.jgi.doe.gov/spacersdb/spacermatchingbench)

Tool	Version	Command
bbmapskimmer	39.13	`bbmapskimmer.sh sam=1.4 maxindel=0 tipsearch=0 midpad=100 in={spacers_file} ref=./{contigs_file} outm={output_dir}/bbmap_skimmer_output.sam t={threads} minid=0.85 path={output_dir}`
blastn	2.16.0+, Dec 14 2024	`makeblastdb -in {contigs_file} -dbtype nucl -out {output_dir}/contigs_blastdb``blastn -query {spacers_file} -db {output_dir}/contigs_blastdb -max_target_seqs 1000000 -out {output_dir}/blastn_output.tsv -evalue 1e-5 -num_threads {threads} -task blastn-short -outfmt 6 qaccver saccver nident length mismatch qlen gapopen qstart qend sstart send evalue bitscore""`
bowtie1	bowtie-align-s version 1.3.1	`bowtie-build --threads {threads} {contigs_file} {results_dir}/simulated_data/bt1_contigs_index``bowtie --threads {threads} -f --all -v 3 -x {results_dir}/simulated_data/bt1_contigs_index {spacers_file} -S {output_dir}/bowtie1_output.sam`
bowtie2	bowtie2-align-s 2.5.4	`bowtie2-build --large-index --threads {threads} {contigs_file} {results_dir}/simulated_data/contigs_bt2indx``bowtie2 --all --xeq --very-sensitive -x {results_dir}/simulated_data/contigs_bt2indx -f {spacers_file} -S {output_dir}/bowtie2_output.sam --threads {threads}`
hisat2	hisat2-align-s 2.2.1	`hisat2-build -p {threads} {contigs_file} {output_dir}/hisat2_idx``hisat2 -a --no-spliced-alignment --no-unal --no-softclip --secondary -p {threads} -x {output_dir}/hisat2_idx -f {spacers_file} -S {output_dir}/hisat2_output.sam`
lexicmap	v0.5.0 (06741c8)	`mkdir -p {output_dir}/tmp_lexicmap_contigs {output_dir}/tmp_lexicmap_spacers``cp {contigs_file} {output_dir}/tmp_lexicmap_contigs/simulated_contigs.fa``cp {spacers_file} {output_dir}/tmp_lexicmap_spacers/simulated_spacers.fa``lexicmap index -k 15 -m 40000 --seed-max-desert 200 --seed-in-desert-dist 50 -I {output_dir}/tmp_lexicmap_contigs -O {output_dir}/tmp_lexicmap.lmi``lexicmap search -d {output_dir}/tmp_lexicmap.lmi {output_dir}/tmp_lexicmap_spacers/simulated_spacers.fa -o {output_dir}/lexicmap_output.tsv --threads {threads} --align-min-match-len 17 --align-min-match-pident 85 --seed-min-prefix 15 --seed-min-single-prefix 15 --seed-max-dist 100 --seed-max-gap 100 --align-max-gap 20 --align-band 100 --top-n-genomes 0 -a`
minimap2	2.28-r1209	`minimap2 -N 100 --eqx -t {threads} -a {contigs_file} {spacers_file} -o {output_dir}/minimap2_output.sam`
mmseqs2	db8ad2d14d0a285ce0ad62bbefd0dce927663315	`mkdir -p {output_dir}/tmp_spacers {output_dir}/tmp_contigs {output_dir}/tmp_mmseqs {output_dir}/tmp_mmseqs_outputs``mmseqs createdb {spacers_file} {output_dir}/tmp_spacers/mmdb``mmseqs createdb {contigs_file} {output_dir}/tmp_contigs/mmdb``mmseqs search {output_dir}/tmp_spacers/mmdb {output_dir}/tmp_contigs/mmdb {output_dir}/tmp_mmseqs_outputs/mmseqs_output {output_dir}/tmp_mmseqs --min-seq-id 0.85 --min-aln-len 17 --threads {threads} -a --search-type 3 -v`

Tool	Version	Command
		1``mmseqs convertalis {output_dir}/tmp_spacers/mmdb {output_dir}/tmp_contigs/mmdb {output_dir}/tmp_mmseqs_outputs/mmseqs_output {output_dir}/mmseqs_output.tsv --format-mode 0 --search-type 4 --format-output query,target,nident,alnlen,mismatch,qlen,gapopen,qstart,qend,tstart,tend,evalue,bits
mummer4	4.1.0-r1304 // 4.1	nucmer --maxmatch --nosimplify --batch=10000000 --threads {threads} --sam-long={output_dir}/mummer4_output.sam -c 1 {contigs_file} {spacers_file}
spacer_containment	0.1.0	spacer-containment --n-threads {threads} {contigs_file} {spacers_file} > {output_dir}/spacer_containment_output.tsv
strobealign	0.15.0	strobealign --eqx -k 15 -N 1000 -t {threads} {contigs_file} {spacers_file} -o {output_dir}/strobealign_output.sam

2. Recall values for each tool at different mismatch thresholds

A. IMG/VR4 dataset

Every row lists the results for a specific mismatch threshold and tool.
The values are for exact mismatches (not max), and represent the total number of unique spacer-contig pairs (aligning at that mismatch threshold).
The fraction is tool*matches divided by total*possible.

mismatches	tool	total_possible	tool_matches	fraction
0	mummer4	16866829	16866546	0.9999832
0	mmseqs2	16866829	15803286	0.9369447
0	bowtie1	16866829	16866784	0.9999973
0	lexicmap	16866829	6033115	0.3576911
0	minimap2	16866829	5110	0.000303
0	strobealign	16866829	4233236	0.25098
0	blastn	16866829	16850968	0.9990596
0	bbmap_skimmer	16866829	14978104	0.8880213
0	bowtie2	16866829	16866667	0.9999904
1	mummer4	12197007	8032640	0.6585747
1	mmseqs2	12197007	9805301	0.8039104
1	bowtie1	12197007	11992384	0.9832235
1	lexicmap	12197007	835330	0.0684865
1	minimap2	12197007	1086	0.000089038237
1	strobealign	12197007	1311189	0.1075009
1	blastn	12197007	7318398	0.6000159
1	bbmap_skimmer	12197007	2413864	0.1979063
1	bowtie2	12197007	12112313	0.9930562
2	mummer4	12359867	3129926	0.253233
2	mmseqs2	12359867	6756086	0.5466148
2	bowtie1	12359867	11173197	0.9039901
2	lexicmap	12359867	426554	0.0345112
2	minimap2	12359867	667	0.000053964982
2	strobealign	12359867	375251	0.0303604
2	blastn	12359867	3217012	0.2602789
2	bbmap_skimmer	12359867	1047205	0.0847262
2	bowtie2	12359867	5243454	0.4242322
3	mummer4	16472212	673444	0.0408836
3	mmseqs2	16472212	3560155	0.216131
3	bowtie1	16472212	14523177	0.8816774
3	lexicmap	16472212	430208	0.0261172
3	minimap2	16472212	392	0.000023797654

mismatches	tool	total_possible	tool_matches	fraction
3	strobealign	16472212	88246	0.0053573
3	blastn	16472212	1972148	0.1197258
3	bbmap_skimmer	16472212	630914	0.0383017
3	bowtie2	16472212	2998722	0.1820473

B. Synthetic dataset

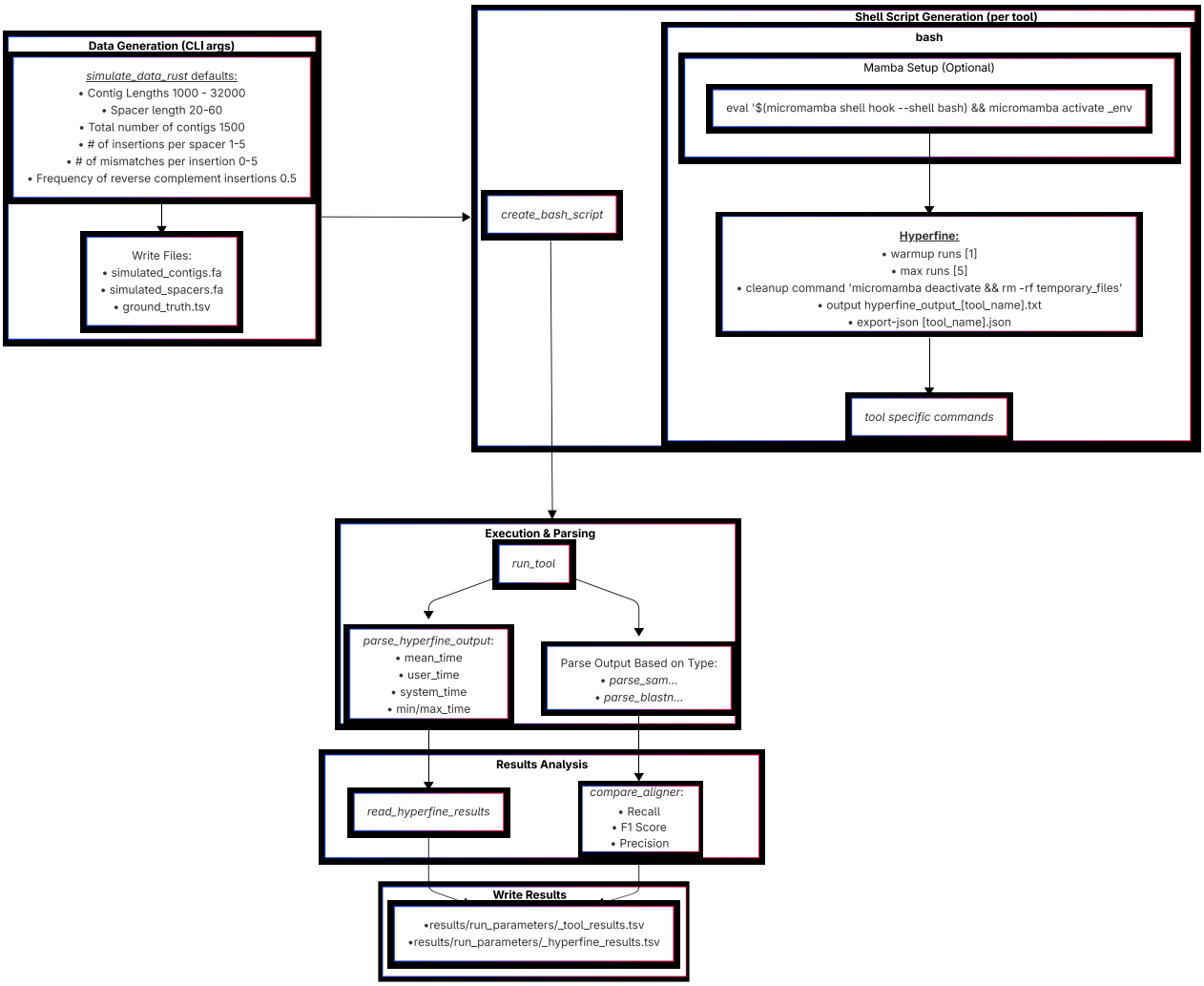
mismatches	tool	total_possible	tool_matches	recall	false_positives	false_negatives	precision	f1_score
0	minimap2	1899024	176	0.00009267918678	49	1898848	0.7822222222	0.0001853364146
0	bbmapskimmer	1899024	377426	0.1987473565	1010	1521598	0.9973311207	0.3314446796
0	mmseqs2map	1899024	1021533	0.5379252711	57809	877491	0.9464405165	0.6859687493
0	mmseqs2	1899024	1021533	0.5379252711	134608	877491	0.8835712945	0.6687252571
0	minimap2_mod	1899024	176	0.00009267918678	49	1898848	0.7822222222	0.0001853364146
0	bowtie1	1899024	1899024	1	0	0	1	1
0	bbmapskimmermod	1899024	1899024	1	1130	0	0.9994053114	0.9997025672
0	bwa_mem	1899024	683227	0.3597779702	15626	1215797	0.9776405052	0.5259887208
0	lexicmap	1899024	1427612	0.7517609045	469844	471412	0.7523821369	0.7520713924
0	hisat2	1899024	1875654	0.9876936784	3	23370	0.9999984006	0.9938079536
0	minimap2_og	1899024	48	0.00002527614185	6	1898976	0.8888888889	0.00005055084625
0	spacer_containment	1899024	1899024	1	0	0	1	1
0	bowtie2	1899024	1899024	1	3	0	0.9999984202	0.9999992101
0	blastn	1899024	1745238	0.919018401	596453	153786	0.7452896219	0.8230866729
0	mummer4	1899024	1860393	0.9796574451	250245	38631	0.881436324	0.9279550246
0	strobealign	1899024	42016	0.0221250495	193	1857008	0.9954275155	0.04328795152
1	minimap2	1992339	86	0.00004316534485	49	1992253	0.637037037	0.00008632484037
1	bbmapskimmer	1992339	814246	0.4086884812	1010	1178093	0.9987611253	0.5800309518
1	mmseqs2map	1992339	982267	0.4930220209	57809	1010072	0.9444184848	0.6478447046
1	mmseqs2	1992339	971696	0.4877161969	134608	1020643	0.8783263913	0.627175186
1	minimap2_mod	1992339	86	0.00004316534485	49	1992253	0.637037037	0.00008632484037
1	bowtie1	1992339	1924529	0.9659646275	0	67810	1	0.9826876984
1	bbmapskimmermod	1992339	1903036	0.9551768048	1130	89303	0.9994065643	0.9767912527
1	bwa_mem	1992339	569097	0.2856426542	15626	1423242	0.9732762351	0.4416634136
1	lexicmap	1992339	848885	0.4260745787	469844	1143454	0.643714516	0.512756005
1	hisat2	1992339	1638156	0.8222275426	3	354183	0.9999981687	0.9024414832
1	minimap2_og	1992339	12	0.00000602	6	1992327	0.6666666667	0.00001204603392
1	spacer_containment	0	0	0	0	0	0	0
1	bowtie2	1992339	1924529	0.9659646275	3	67810	0.9999984412	0.9826869458
1	blastn	1992339	1534121	0.7700100234	596453	458218	0.7200505591	0.7441927589
1	mummer4	1992339	1605268	0.8057203117	250245	387071	0.8651343321	0.8343709685
1	strobealign	1992339	39698	0.01992532395	193	1952641	0.9951618159	0.039068412533
2	minimap2	2137067	91	0.00004258172533	49	2136976	0.65	0.00008515787193
2	bbmapskimmer	2137067	804812	0.3765965222	1010	1332255	0.9987466215	0.5469536907
2	mmseqs2map	2137067	912682	0.4270722443	57809	1224385	0.9404332446	0.5873949899
2	mmseqs2	2137067	901193	0.4216961845	134608	1235874	0.8700445356	0.5680620814
2	minimap2_mod	2137067	91	0.00004258172533	49	2136976	0.65	0.00008515787193
2	bowtie1	2137067	1934586	0.9052528536	0	202481	1	0.9502705658
2		2137067	1734297	0.811531412	1130	402770	0.9993488634	0.8957002903

mismatches	tool	total_possible	tool_matches	recall	false_positives	false_negatives	precision	f1_score
	bbmapskimmermod							
2	bwa_mem	2137067	515856	0.2413850385	15626	1621211	0.9705991924	0.3866190952
2	lexicmap	2137067	562385	0.2631574022	469844	1574682	0.544825809	0.3548958507
2	hisat2	2137067	1115625	0.5220355749	3	1021442	0.9999973109	0.6859696344
2	minimap2_og	2137067	26	0.0000121662 0724	6	2137041	0.8125	0.0000243320 5013
2	spacer_containment	0	0	0	0	0	0	0
2	bowtie2	2137067	1573821	0.7364397092	3	563246	0.9999980938	0.8482173149
2	blastn	2137067	1355458	0.6342608819	596453	781609	0.6944261291	0.6629813122
2	mummer4	2137067	1432751	0.6704286763	250245	704316	0.851309807	0.7501190425
2	strobealign	2137067	34729	0.0162507773 5	193	2102338	0.9944733979	0.0319789833 2
3	minimap2	2609997	220	0.0000842912 8463	49	2609777	0.8178438662	0.0001685651 96
3	bbmapskimmer	2609997	760618	0.291424856	1010	1849379	0.9986738933	0.4511877804
3	mmseqs2map	2609997	860977	0.3298766244	57809	1749020	0.9370811048	0.4879738992
3	mmseqs2	2609997	862278	0.3303750924	134608	1747719	0.8649715213	0.4781291769
3	minimap2_mod	2609997	228	0.0000873564 2225	49	2609769	0.8231046931	0.0001746943 041
3	bowtie1	2609997	2238309	0.8575906409	0	371688	1	0.9233365221
3	bbmapskimmermod	2609997	1534102	0.5877792197	1130	1075895	0.9992639549	0.7401772013
3	bwa_mem	2609997	497712	0.1906944721	15626	2112285	0.969560017	0.3187054863
3	lexicmap	2609997	443019	0.1697392756	469844	2166978	0.4853072148	0.2515109882
3	hisat2	2609997	564049	0.2161109764	3	2045948	0.9999946813	0.3554129127
3	minimap2_og	2609997	35	0.0000134099 771	6	2609962	0.8536585366	0.0000268195 329
3	spacer_containment	0	0	0	0	0	0	0
3	bowtie2	2609997	1415300	0.5422611597	3	1194697	0.9999978803	0.7032022458
3	blastn	2609997	1270806	0.4868994102	596453	1339191	0.6805729682	0.5676718061
3	mummer4	2609997	1338301	0.5127595932	250245	1271696	0.842469151	0.6375073448
3	strobealign	2609997	30913	0.0118440749 2	193	2579084	0.9937954092	0.0234091589 8

Figures

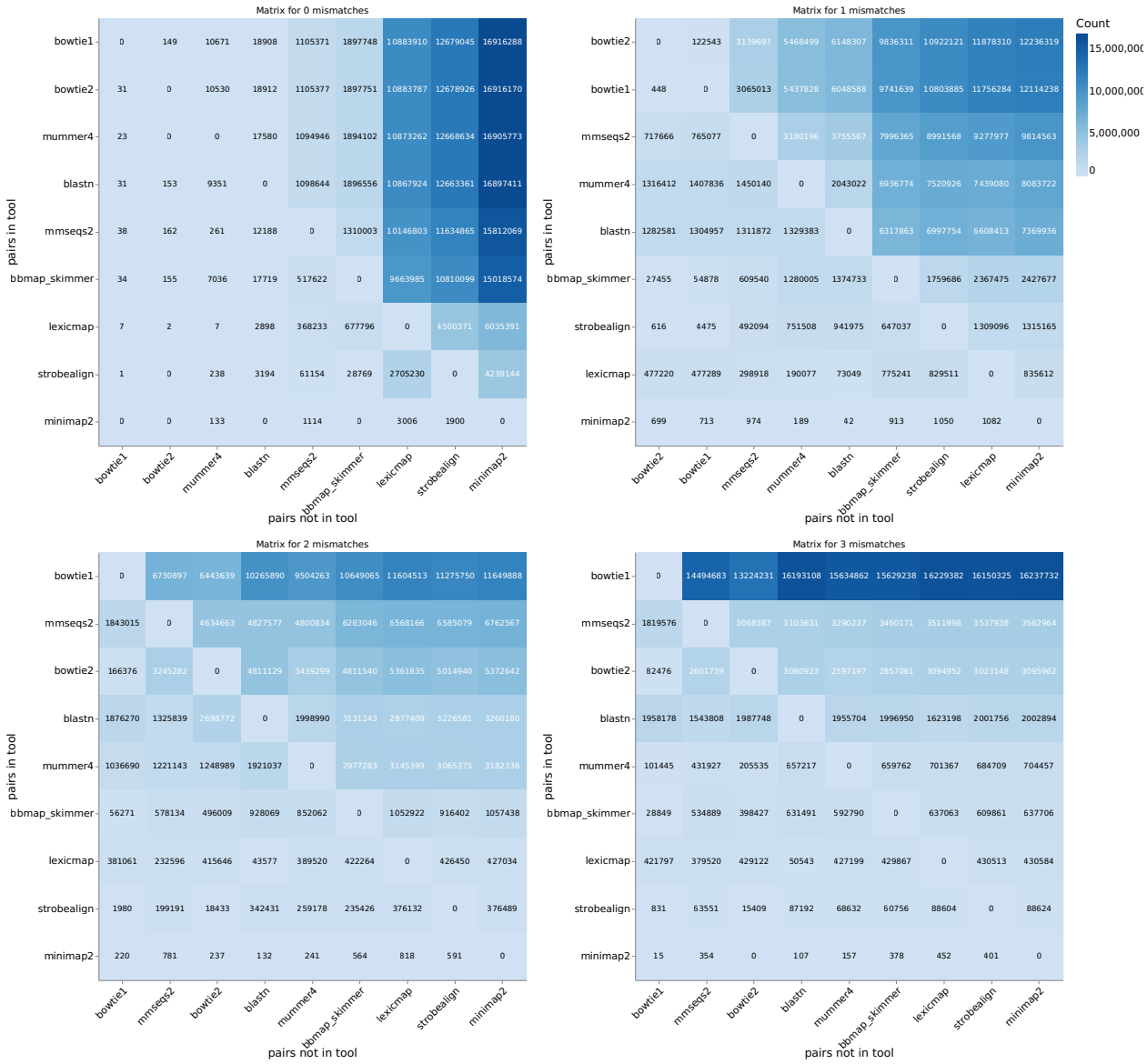
Supplementary figure 1.

Benchmarking framework pipeline overview.



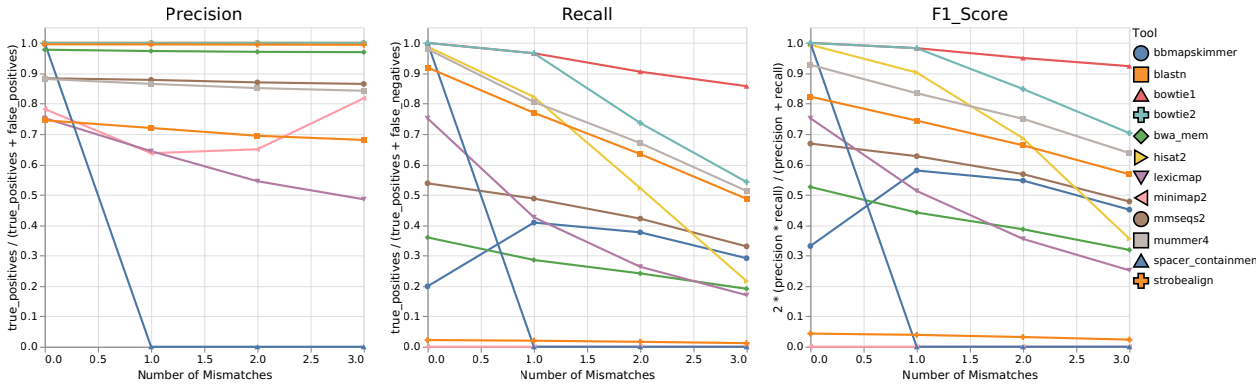
Supplementary figure 2.

IMG/VR v4 based results in a tool vs tool comparison. Unlike the similar matrix in the main text(figure 1) which showcases the values for up to 1 and 3 mismatches, here the matrixes are separated for each mismatch threshold at an **exact** mismatch value. From top left to bottom right, the mismatch threshold is 0, 1, 2, 3. Like the main text figure, the value of a cell(i,j) is the fraction of spacer-contig pairs identified by the tool listed in row i, which were not identified by the tool listed in the j column.



Supplementary figure 3

Simulated dataset performance metrics.
Percision = True Positives / (True Positives + False Positives)
Recall = True Positives / (True Positives + False Negatives)
F1 = 2 * (Percision * Recall) / (Percision + Recall)
Note: Because of the various prefiltering steps, the number of False Negatives and False Positives may not be indicative of the actual raw tool-reported results. As such, we recommend focusing on the recall rate between tools, which is equivalent to the fraction of detected spacer-protospacer pairs out of all the spacer-protospacer pairs in the reference file.



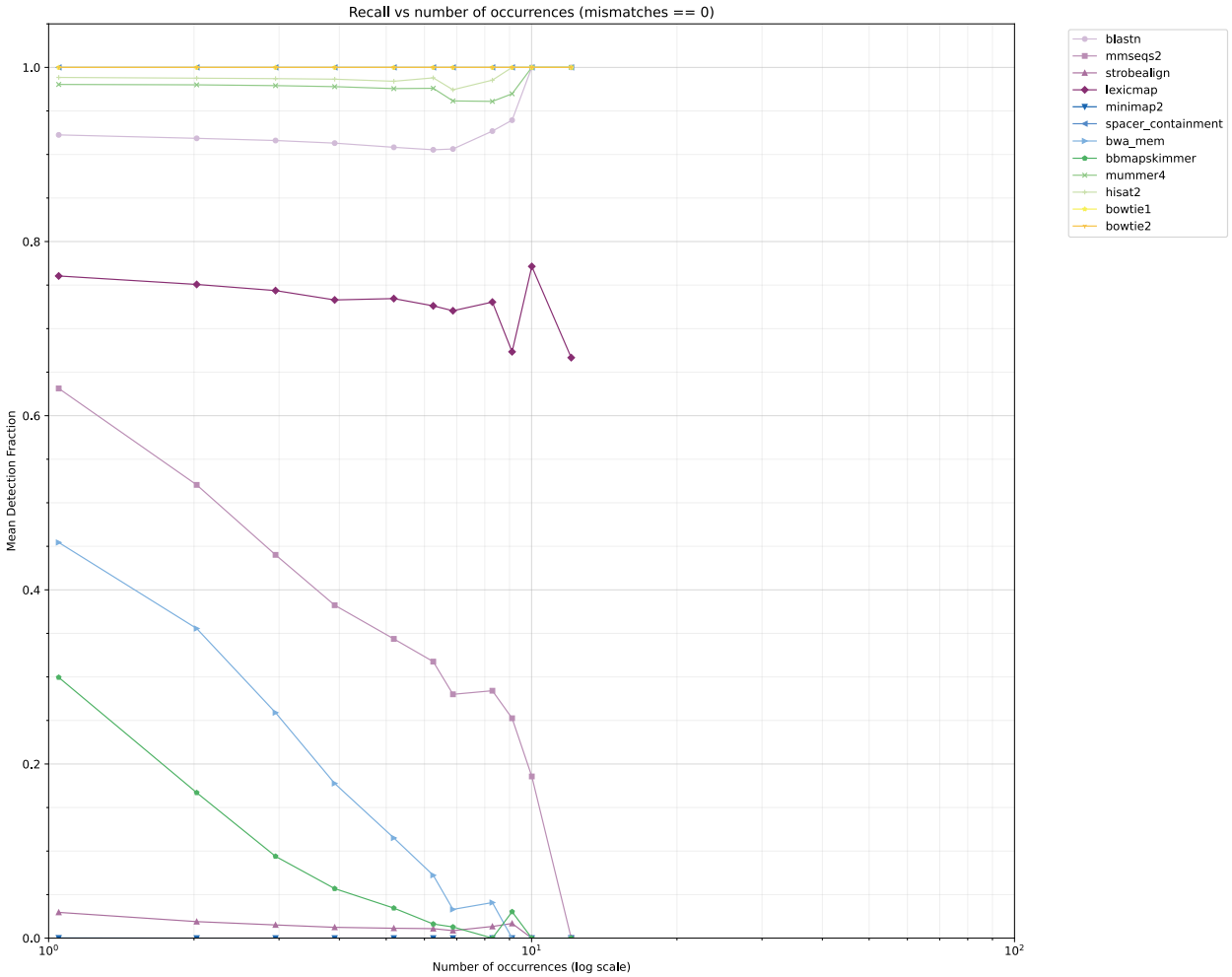
Supplementary figure 4.

Simulated dataset recall (detection fraction) for different values of spacer occurrences.

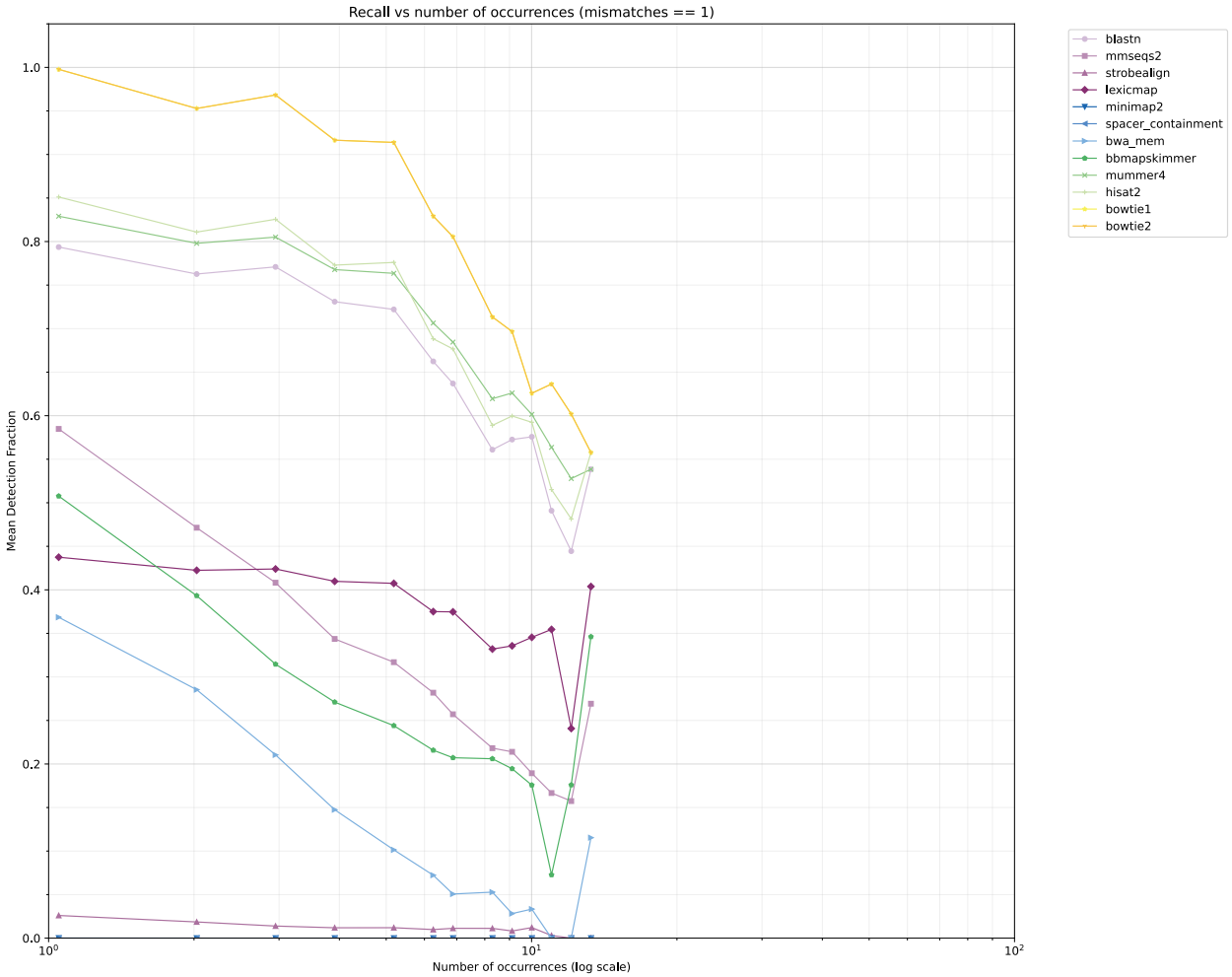
A. for exact mismatches, in a contig dependent manner

Per contigs manner means the that the recall measure is the fraction of occurences each tool identified out of the total number of spacer-contig pairs, identified by all tools.

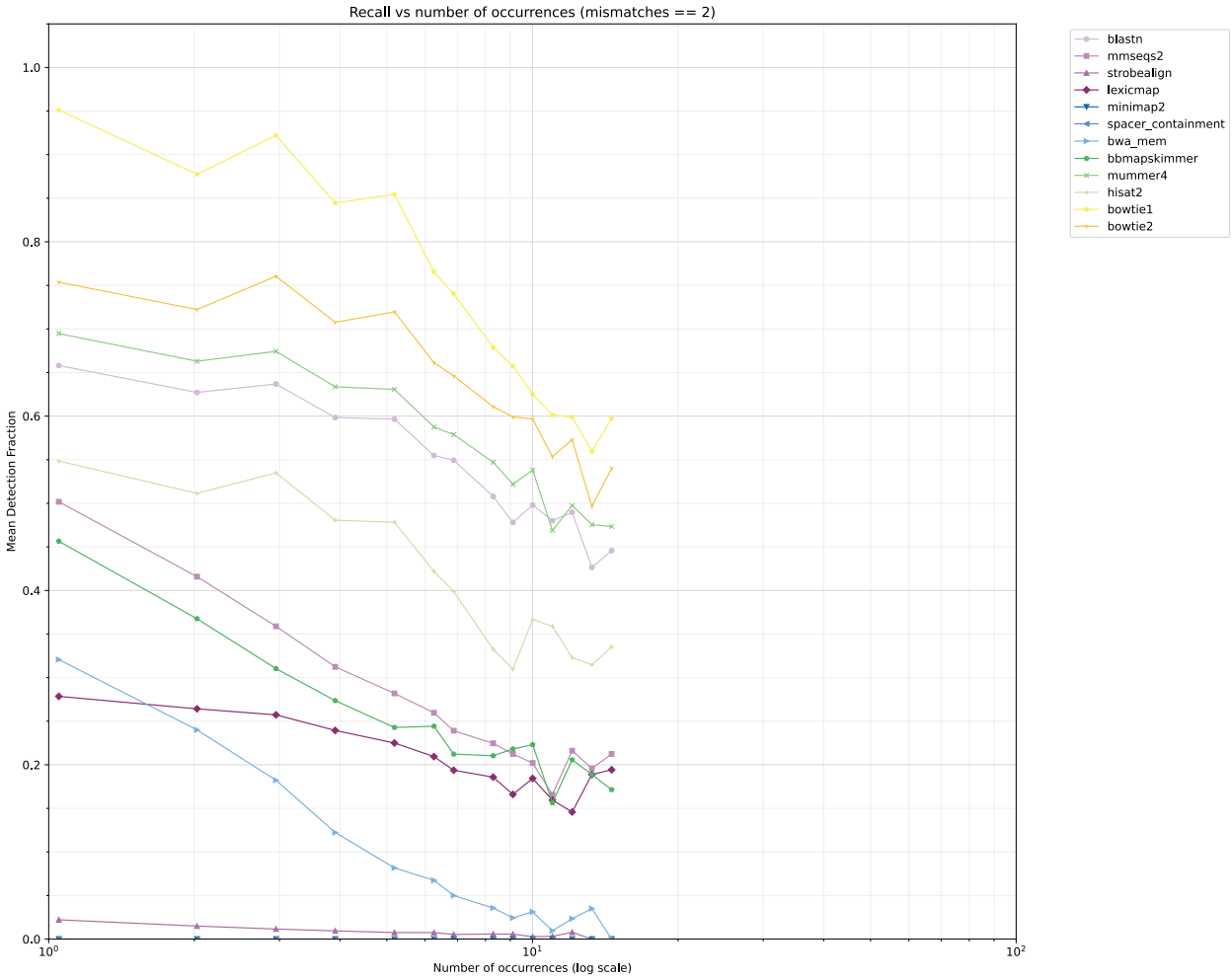
1. 0 mismatches



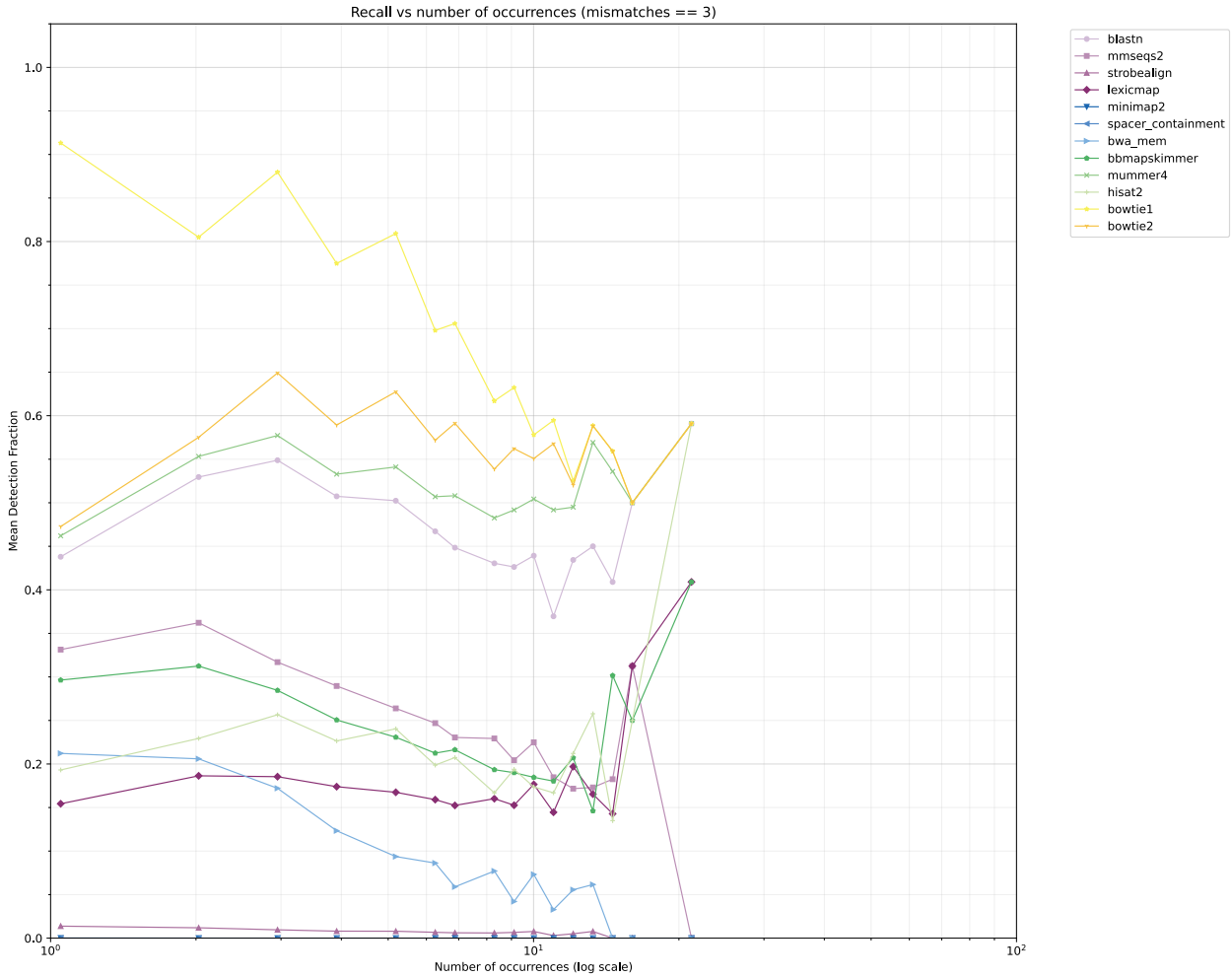
1. exactly 1 mismatch



1. exactly 2 mismatches



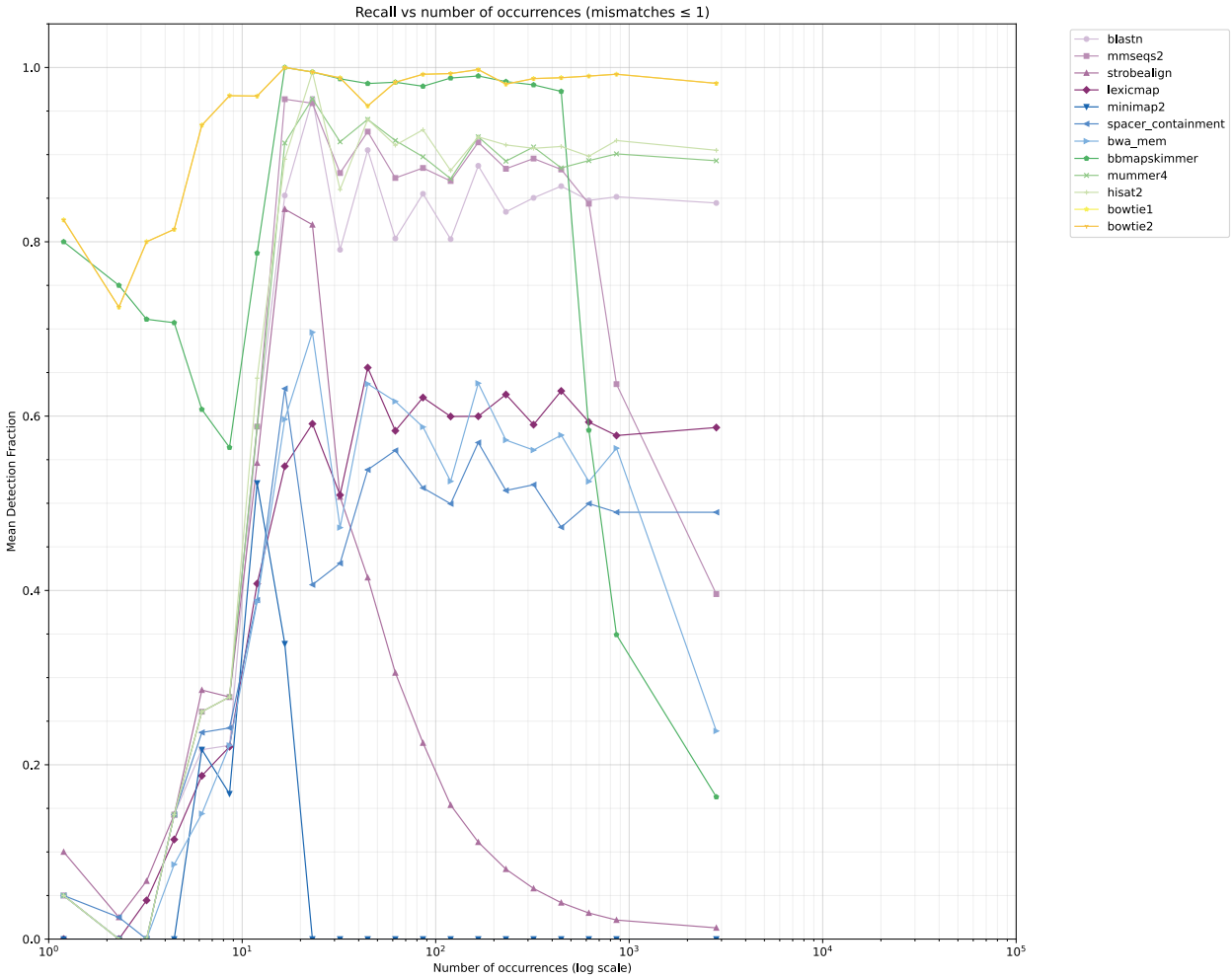
1. exactly 3 mismatches



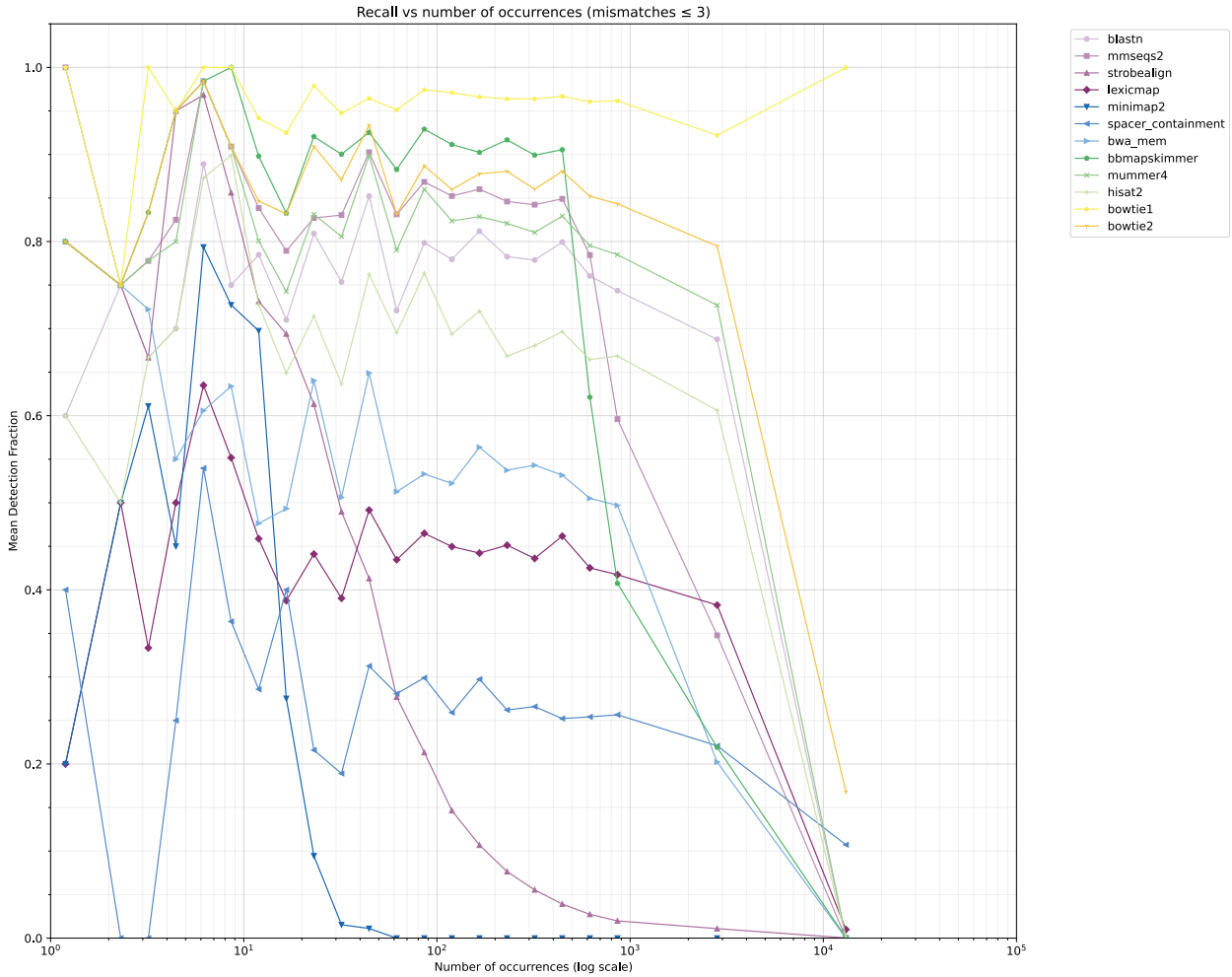
B. up to 1 and 3 mismatches, in a contig independent manner

Per contig independent manner means that the recall measure is the fraction of occurences each tool identified out of the total number of times the spacer occurs in the reference file (regardless of in which contig).

1. up to 1 mismatches



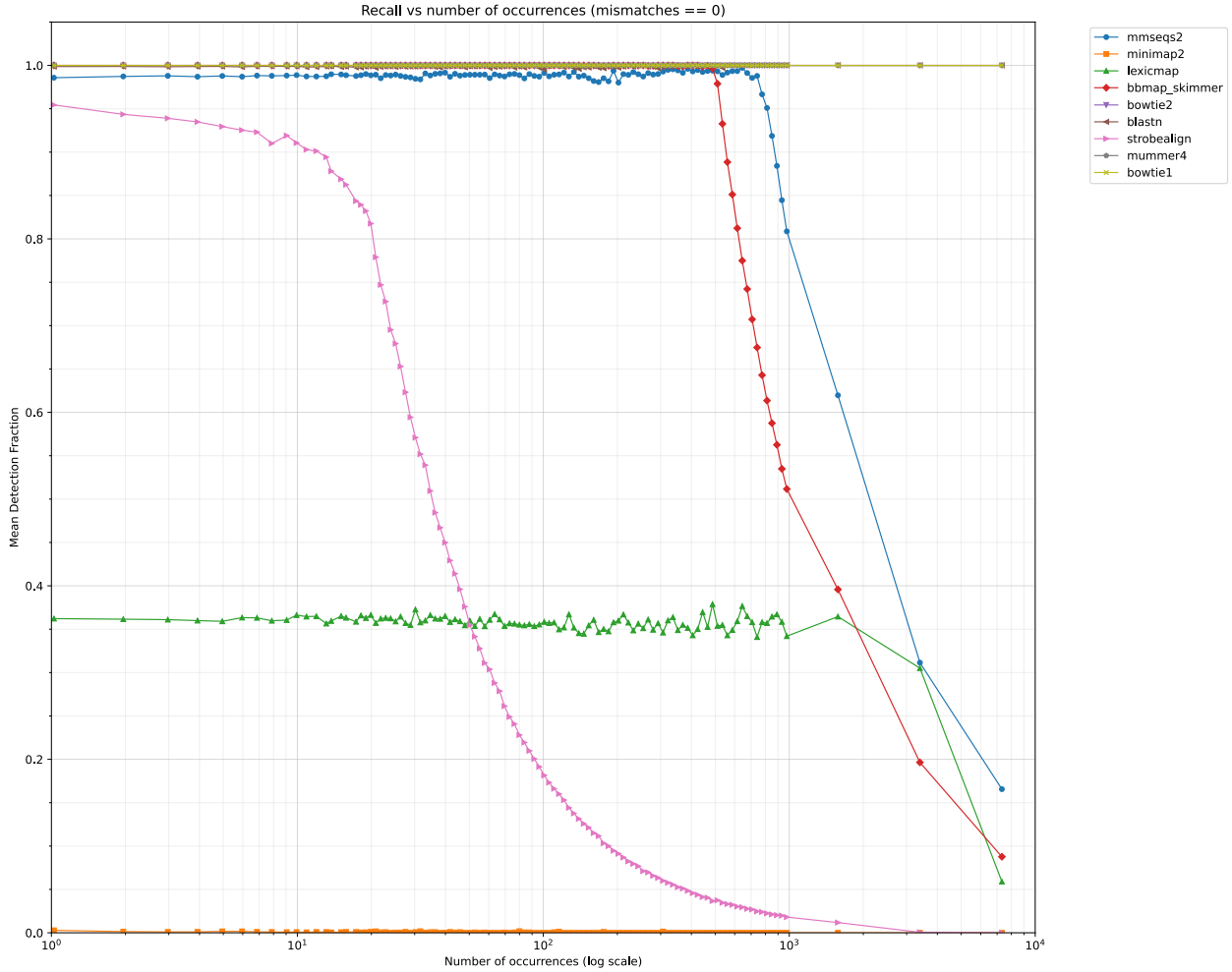
1. up to 3 mismatches



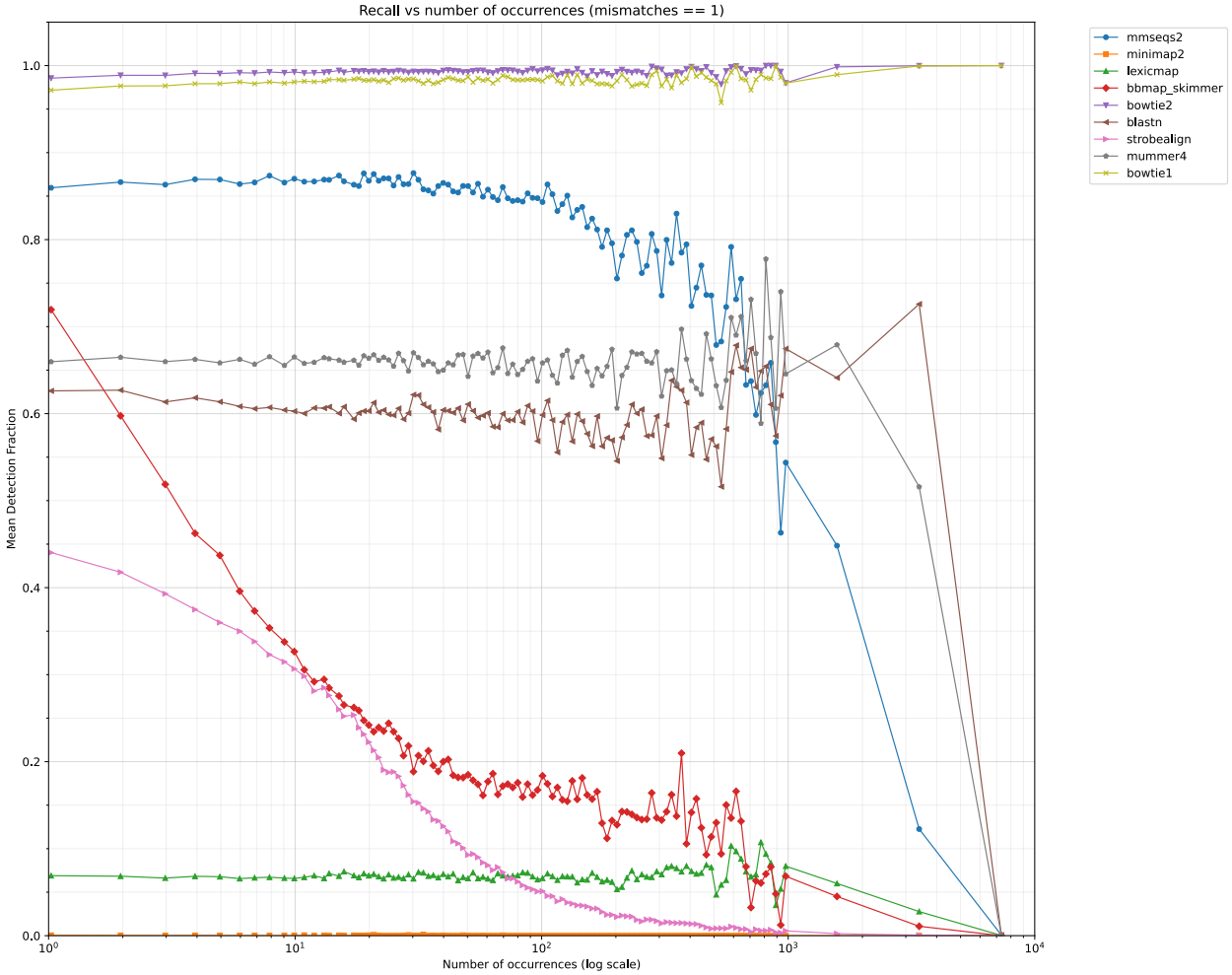
Supplementary figure 5.

Tool recall versus occurrence frequency for **IMG/VR4 dataset**.
Similar to main text figure, except for exact values for different mismatch thresholds (0-3).

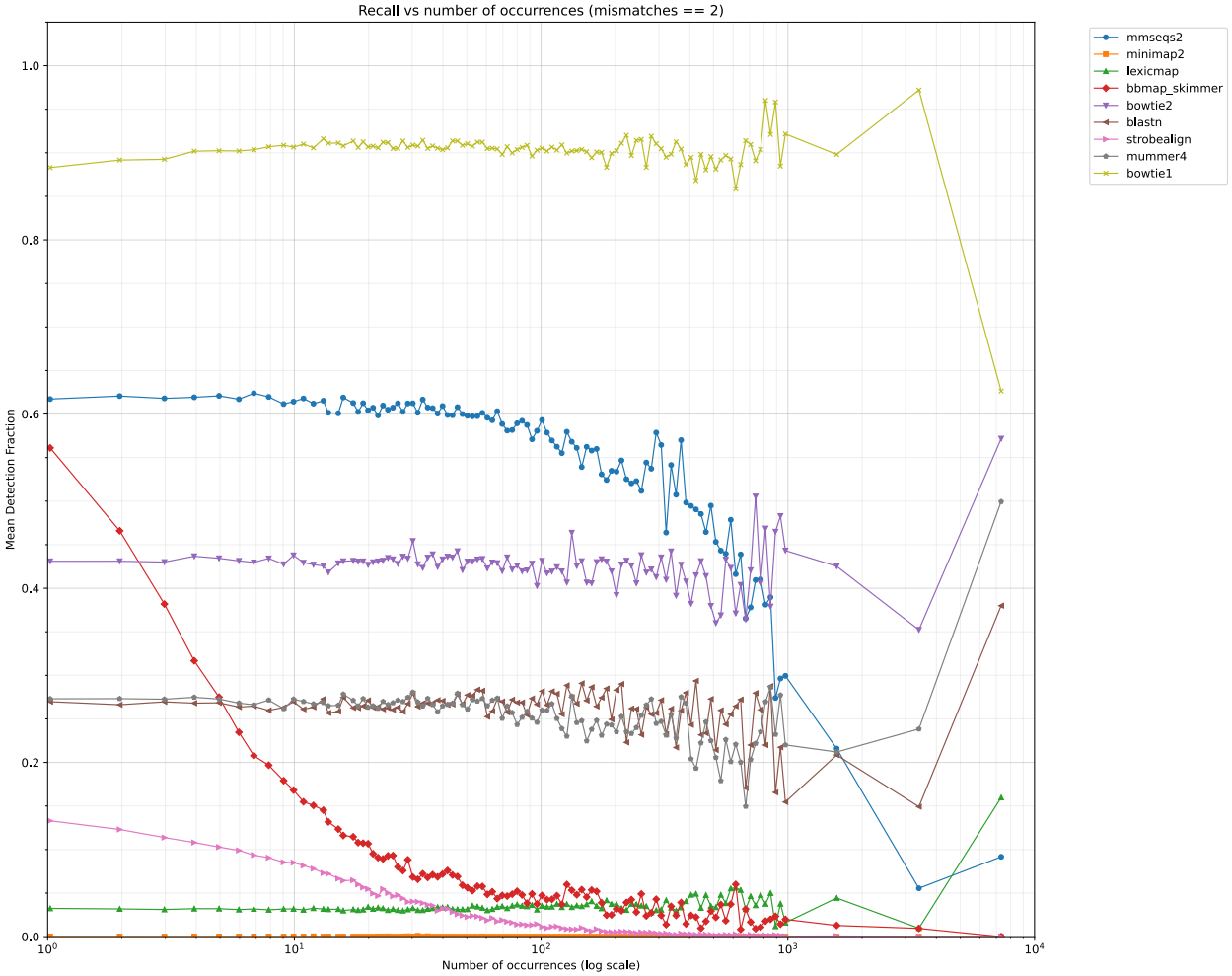
A. 0 mismatches



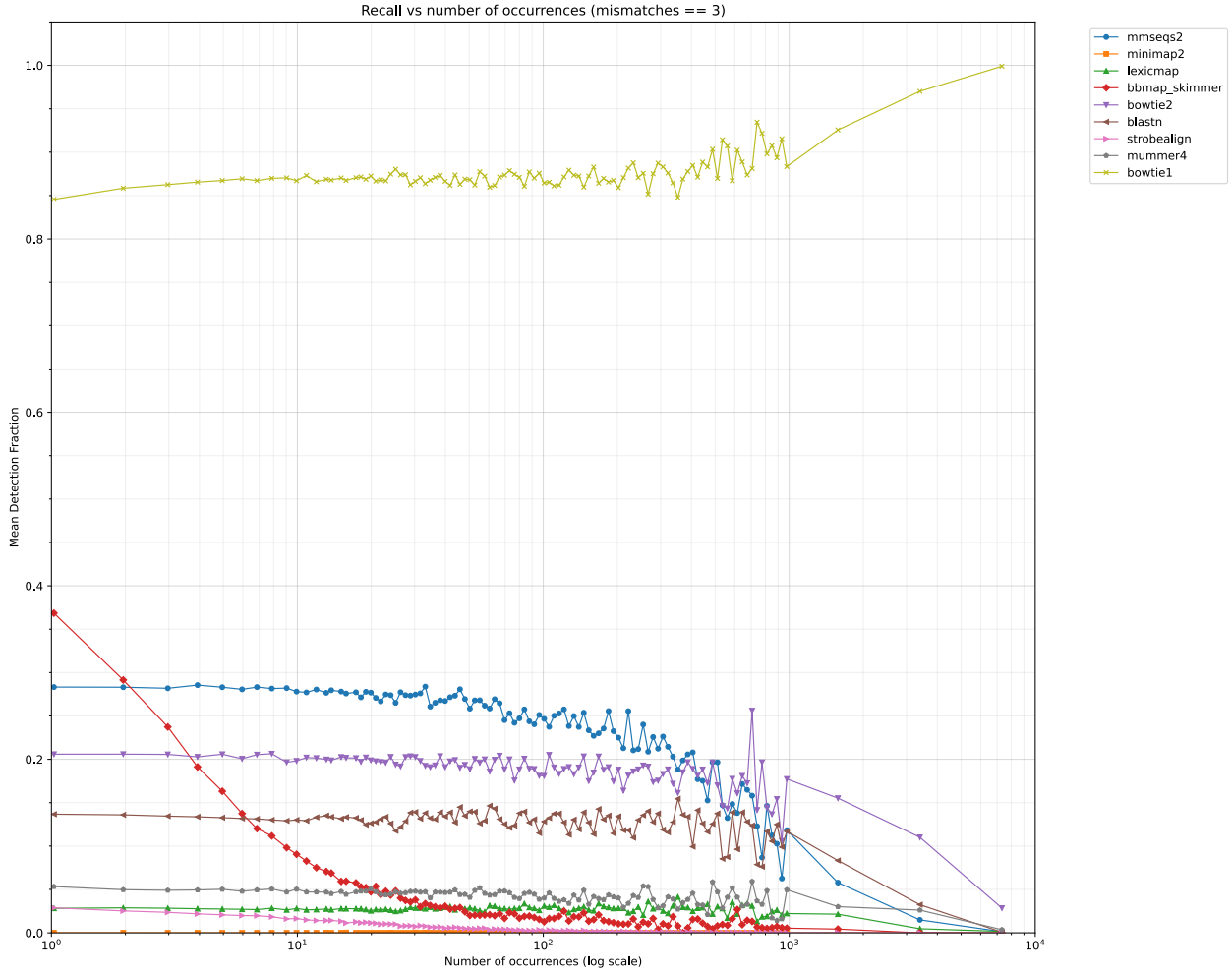
B. 1 mismatch


Recall vs number of occurrences (mismatches == 1)
Mean Detection Fraction
Number of occurrences (log scale)
0.0
0.2
0.4
0.6
0.8
1.0
10^0
10^1
10^2
10^3
10^4
mmseqs2
minimap2
lexicmap
bbmap_skimmer
bowtie2
blastn
strobealign
mummer4
bowtie1

C. 2 mismatches



D. 3 mismatches

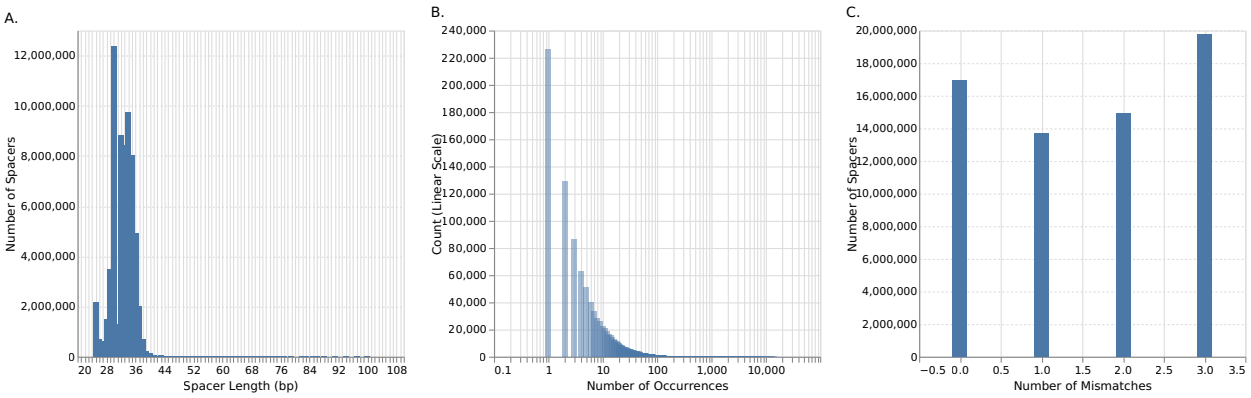


Supplementary figure 6.

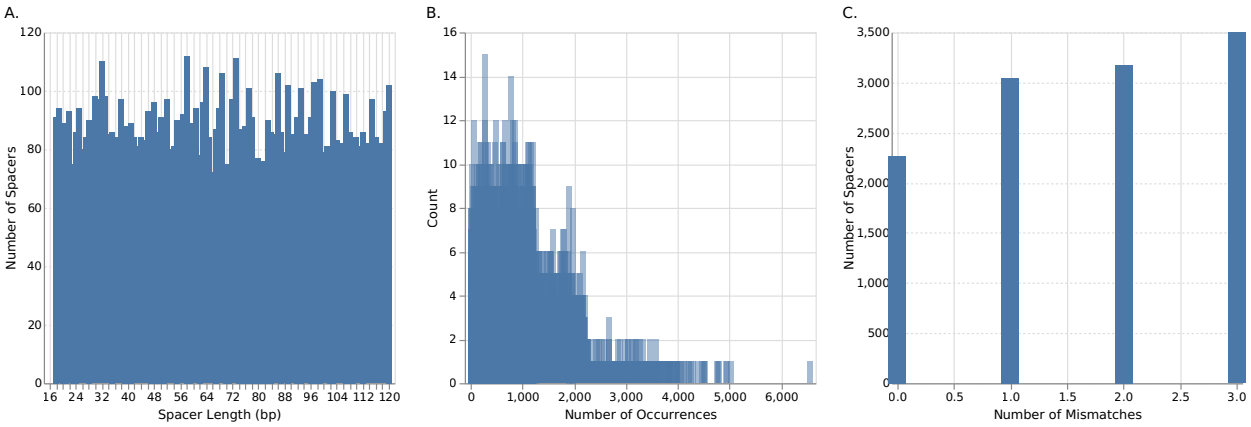
Distributions of spacers characteristics.
(A) Spacer size (length in bp)
(B) Mismatches observed in spacer alignments
(C) Spacer occurrence rate.

1. IMG/VR4 dataset

Note: the horizontal axis is logarithmic.



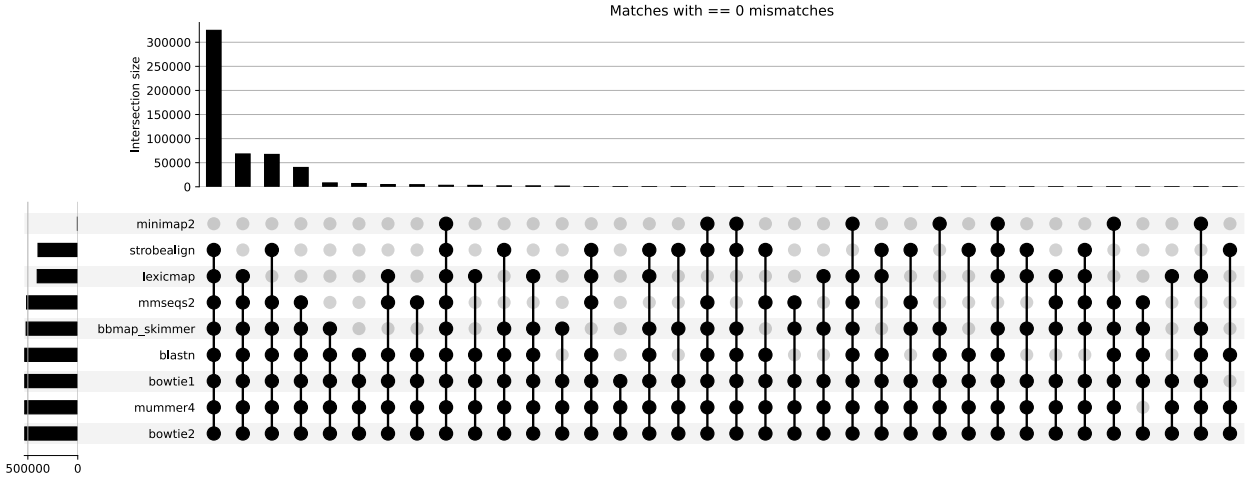
2. Synthetic dataset



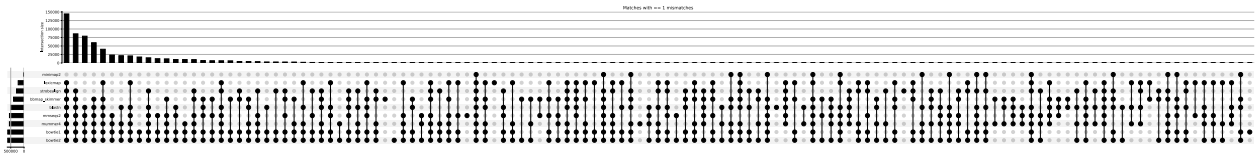
Supplementary figure 7.

Upset plot of tool performance for IMG/VR4 dataset. The panels (from top to bottom) are sorted by number of mismatches. The sets in each panel are sorted from left to right by the set size. Each row in each panel represents a tool, and the vertical lines connecting the dots indicate the intersection of the connected tool results'.

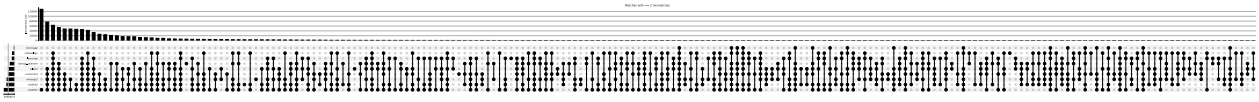
A. 0 mismatches



B. 1 mismatch



C. 2 mismatches



D. 3 mismatches