Figures For CELL-SYSTEMS-D-22-00268R1

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Contents

Data

ANI bins

Load BAM counts

- For the neighbor reads, we look at neighbor correctly aligned, and neighbor incorrelctly aligned (on the species level).

Figure 4A: Reference Bias

```
##
##  Pearson's product-moment correlation
##
## data:  reference_bias_data$intra_species_ani and reference_bias_data$aligned_rate
## t = 32.276, df = 810, p-value < 0.00000000000000022
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.7183175 0.7786677
## sample estimates:
##       cor
## 0.7500498
```

Figure 6: MAPQ & MAPID Boxplot

Figure S6: Read Flow Chat

Cross Mapping

- neighbor-incorrect: neighbor reads cross mapped to on-target genome
- neighbor-correct: neighbor reads mapped to off-target genome

Figure S2: for Neighbor Genome

```
##
## Error: on_target_species
##                                        Df Sum Sq Mean Sq F value
## intra_species_ani                       1  0.799  0.7990  36.158
## between_species_ani                     1  2.533  2.5329 114.621
## intra_species_ani:between_species_ani   1  0.095  0.0954   4.318
## Residuals                             310  6.850  0.0221
##                                                     Pr(>F)
## intra_species_ani                            0.00000000511 ***
## between_species_ani                   < 0.0000000000000002 ***
## intra_species_ani:between_species_ani               0.0385 *
## Residuals
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##                                         Df Sum Sq Mean Sq F value
## intra_species_ani                        1  0.000   0.000    0.00
## between_species_ani                      1 15.774  15.774 3526.30
## intra_species_ani:between_species_ani    1  0.227   0.227   50.63
## Residuals                             3224 14.422   0.004
##                                                     Pr(>F)
## intra_species_ani                                        1
## between_species_ani                   < 0.0000000000000002 ***
## intra_species_ani:between_species_ani     0.00000000000136 ***
## Residuals
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure 5A: for on-target Genome

```
##
## Error: on_target_species
##                                         Df Sum Sq Mean Sq F value
## intra_species_ani                         1 2.2129  2.2129  339.17
## between_species_ani                       1 1.8280  1.8280  280.17
## intra_species_ani:between_species_ani     1 0.2466  0.2466   37.79
## Residuals                               310 2.0226  0.0065
##                                                       Pr(>F)
## intra_species_ani                       < 0.0000000000000002 ***
## between_species_ani                     < 0.0000000000000002 ***
## intra_species_ani:between_species_ani          0.00000000242 ***
## Residuals
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##                                         Df Sum Sq Mean Sq F value
## intra_species_ani                         1  1.922   1.922    1520
## between_species_ani                       1 10.630  10.630    8407
## intra_species_ani:between_species_ani     1  4.199   4.199    3321
## Residuals                              3224  4.076   0.001
##                                                     Pr(>F)
## intra_species_ani                      <0.0000000000000002 ***
## between_species_ani                    <0.0000000000000002 ***
## intra_species_ani:between_species_ani <0.0000000000000002 ***
## Residuals
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

MIDAS2 SNPs summary

Figure 5B: horizontal coverage

- Only one species in the reads and two species in the database.
- for off-target reads, there is no concept of percentage_aligned_reads because it is all from cross-mapping

```
##
## Error: on_target_species
##                 Df Sum Sq Mean Sq F value               Pr(>F)
## off_bin         18  9.485  0.5270  61.632 < 0.0000000000000002 ***
## on_bin           5  3.827  0.7654  89.525 < 0.0000000000000002 ***
## off_bin:on_bin  73  1.158  0.0159   1.855              0.00033 ***
## Residuals      217  1.855  0.0086
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## Error: Within
##                  Df Sum Sq Mean Sq F value             Pr(>F)    
## off_bin          17  34.11  2.0065 1224.08 <0.0000000000000002 ***
## on_bin            5   3.34  0.6674  407.15 <0.0000000000000002 ***
## off_bin:on_bin   84   8.50  0.1012   61.72 <0.0000000000000002 ***
## Residuals      3121   5.12  0.0016                               
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure S3: vertical coverage

```
## 
## Error: on_target_species
##                 Df Sum Sq Mean Sq F value         Pr(>F)    
## off_bin         18    822    45.6   1.296          0.192    
## on_bin           5   2018   403.7  11.459 0.000000000791 ***
## off_bin:on_bin  73   1431    19.6   0.556          0.998    
## Residuals      217   7645    35.2                           
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Error: Within
##                  Df Sum Sq Mean Sq F value             Pr(>F)    
## off_bin          17    833    49.0   1.193              0.261    
## on_bin            5   4211   842.2  20.498 <0.0000000000000002 ***
## off_bin:on_bin   84    866    10.3   0.251              1.000    
## Residuals      3121 128225    41.1                               
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure S4: Local Alignment

Figure S5: BWA

86 NCBI strains

Figure 7A

```
## 
## Error: accession
##                     Df Sum Sq Mean Sq F value        Pr(>F)    
## intra_species_ani    1 0.3018  0.3018   26.55 0.00000180988 ***
## between_species_ani  1 0.5409  0.5409   47.58 0.00000000112 ***
## Residuals           80 0.9095  0.0114                          
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## 
## Error: accession
##                     Df Sum Sq Mean Sq F value          Pr(>F)    
## intra_species_ani    1 0.1839 0.18388   57.13 0.0000000000589 ***
## between_species_ani  1 0.1205 0.12048   37.43 0.0000000330236 ***
## Residuals           80 0.2575 0.00322
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

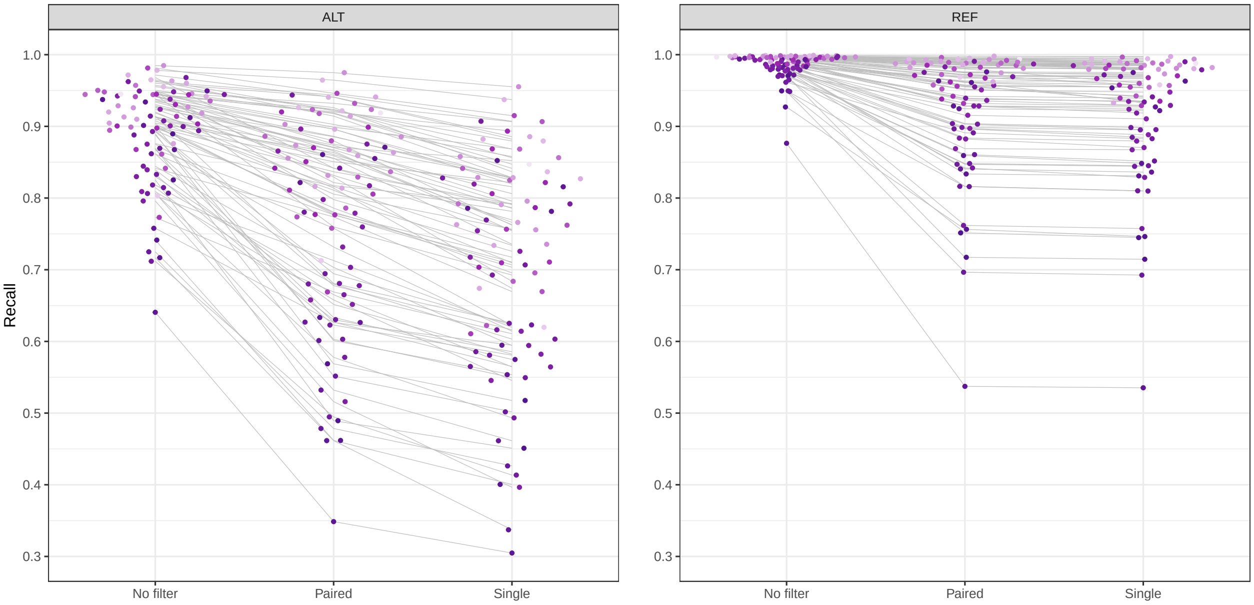
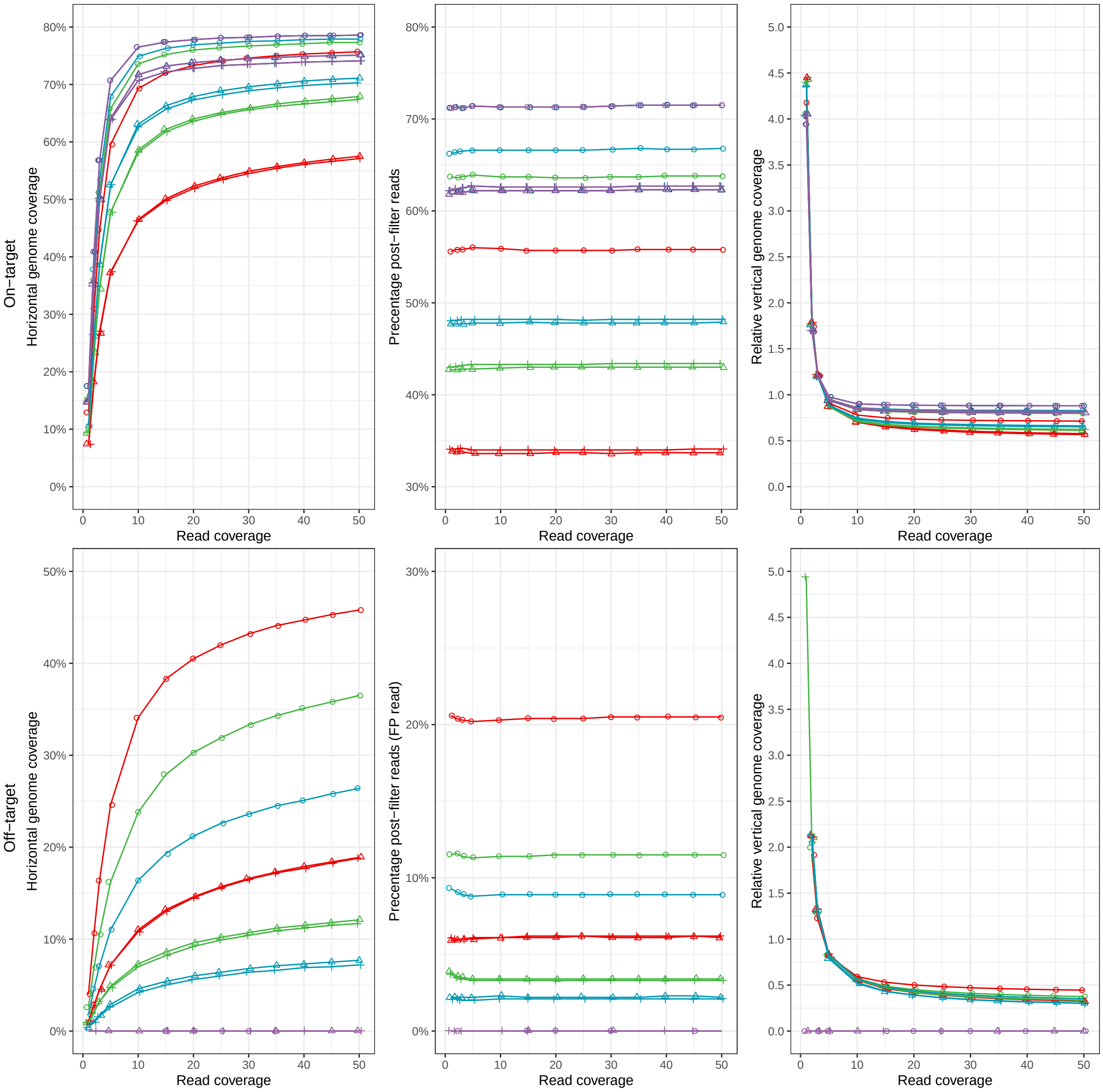


Figure 7B

Figure 7C

Figure 4B & 4C

Figure S1



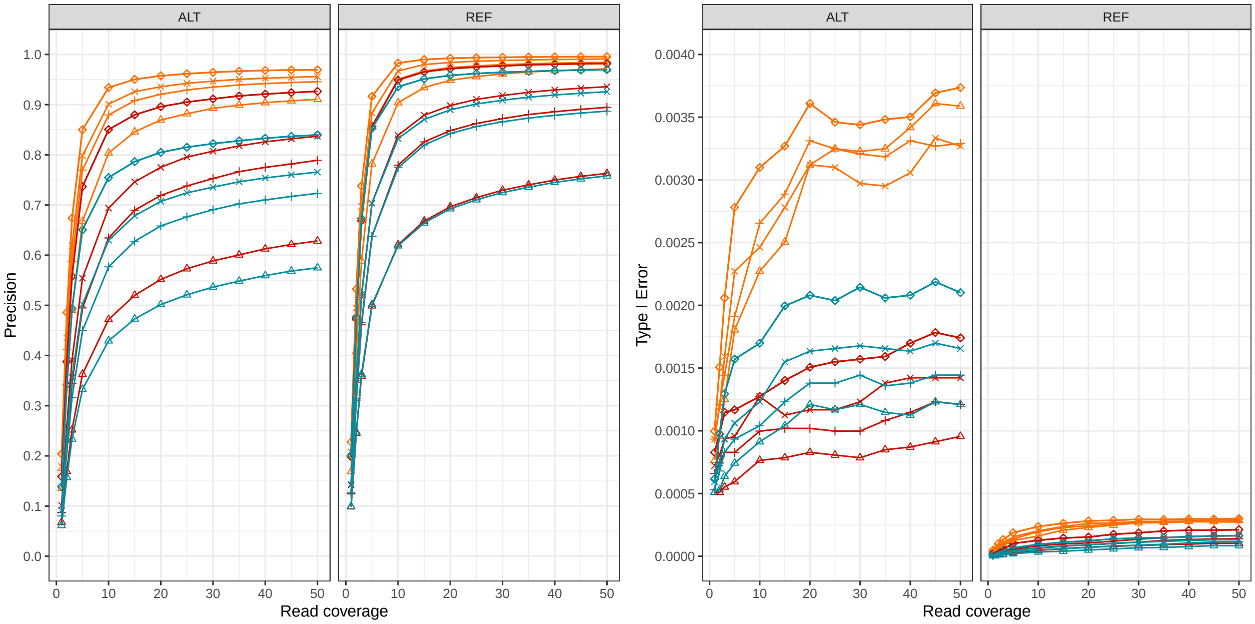
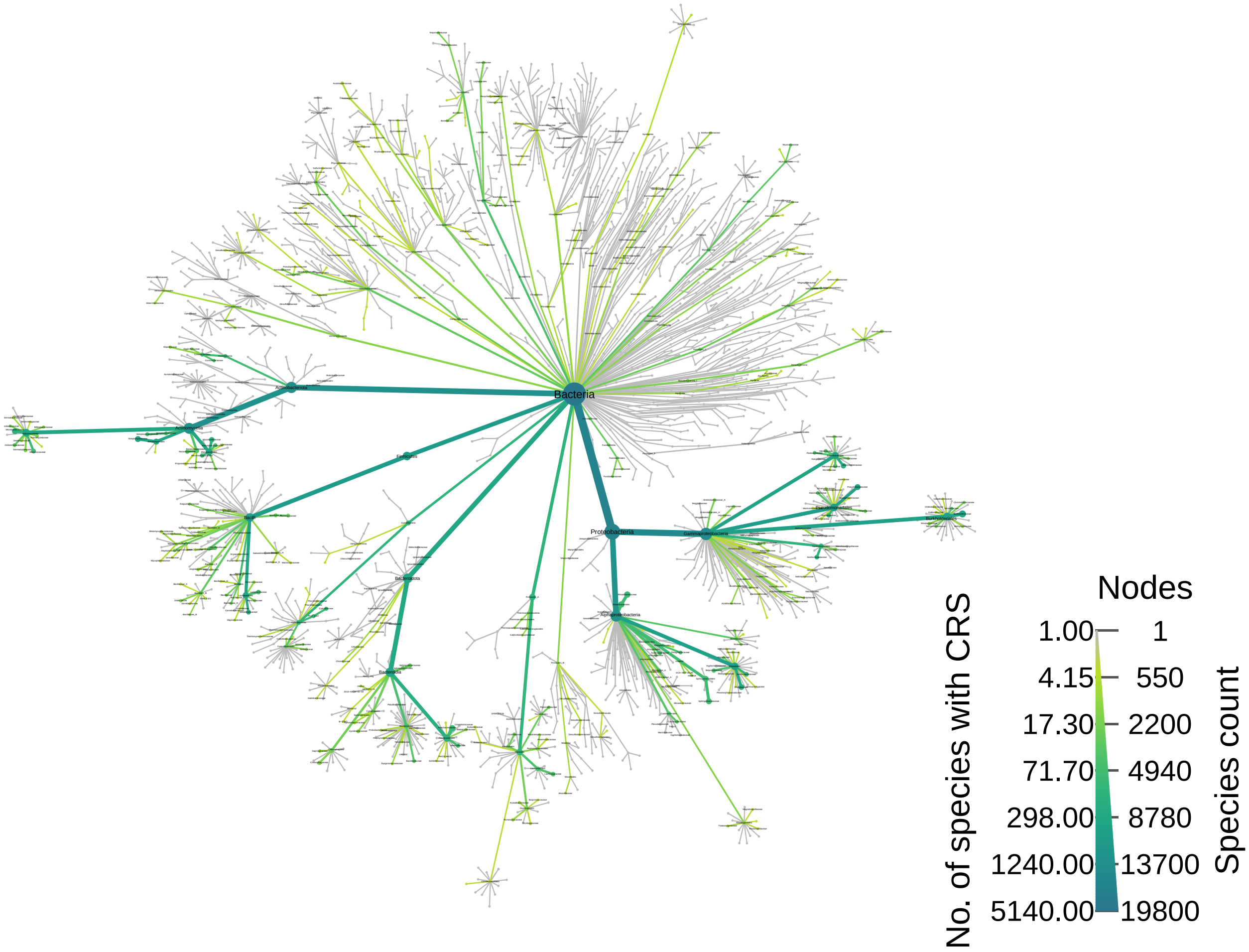

ALT
REF
Precision
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
Read coverage
ALT
REF
Type I Error
0.0040
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
0
10
20
30
40
50
Read coverage

Figure S7 Runtime

Figure 3


Bacteria
Actinobacteriota
Firmicutes
Bacilli
Proteobacteria
Gammaproteobacteria
Alphaproteobacteria
Bacteroidota
Bacteroidia
Nodes
No. of species with CRS
Species count