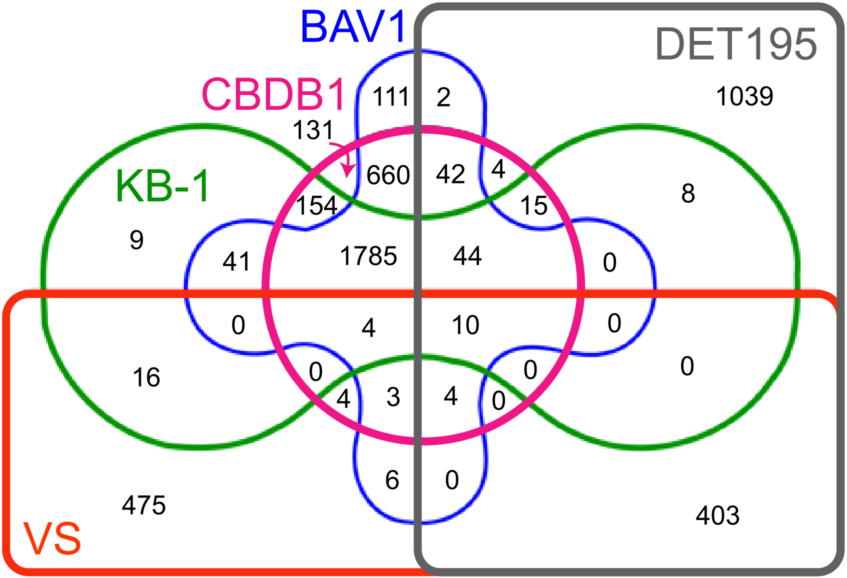
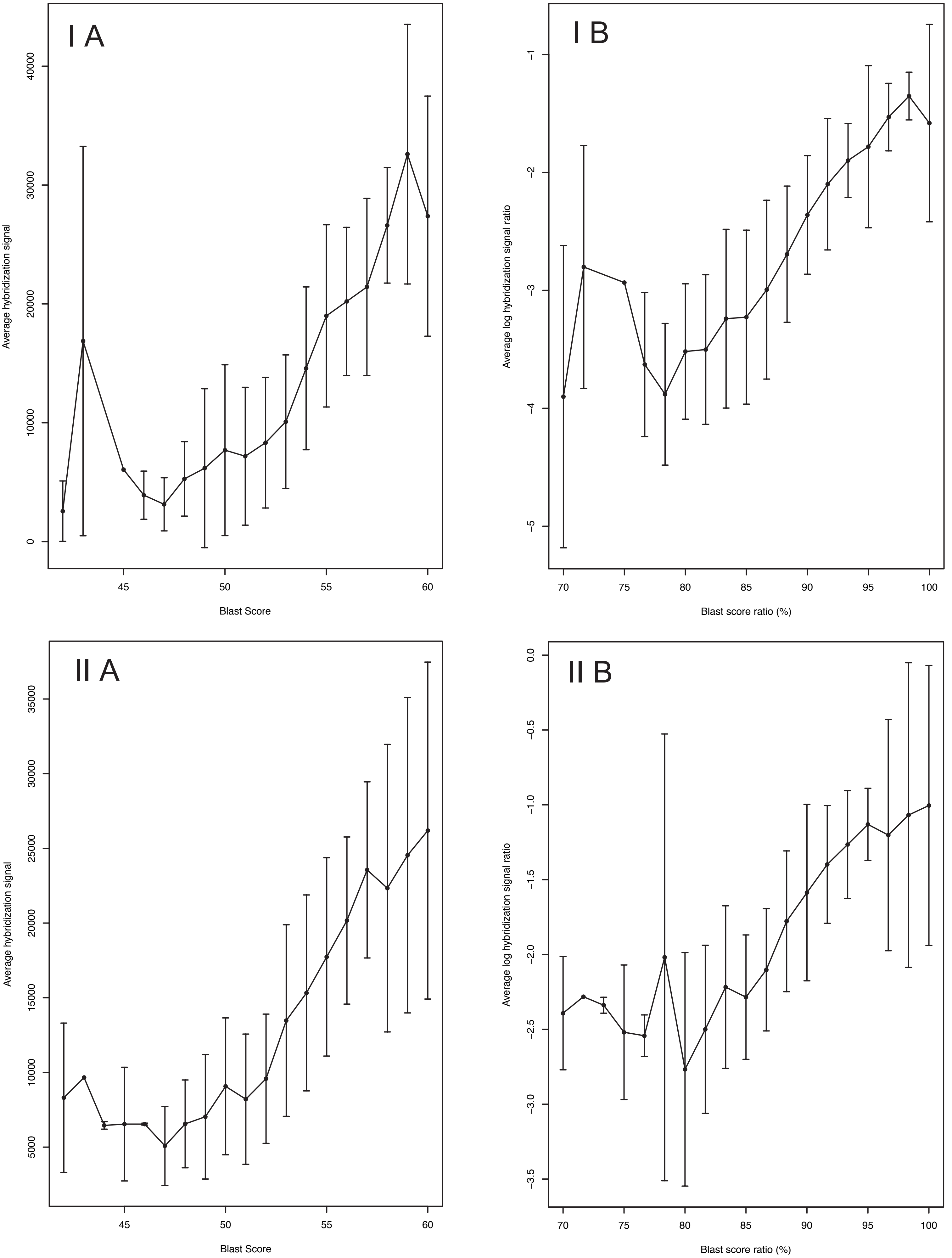
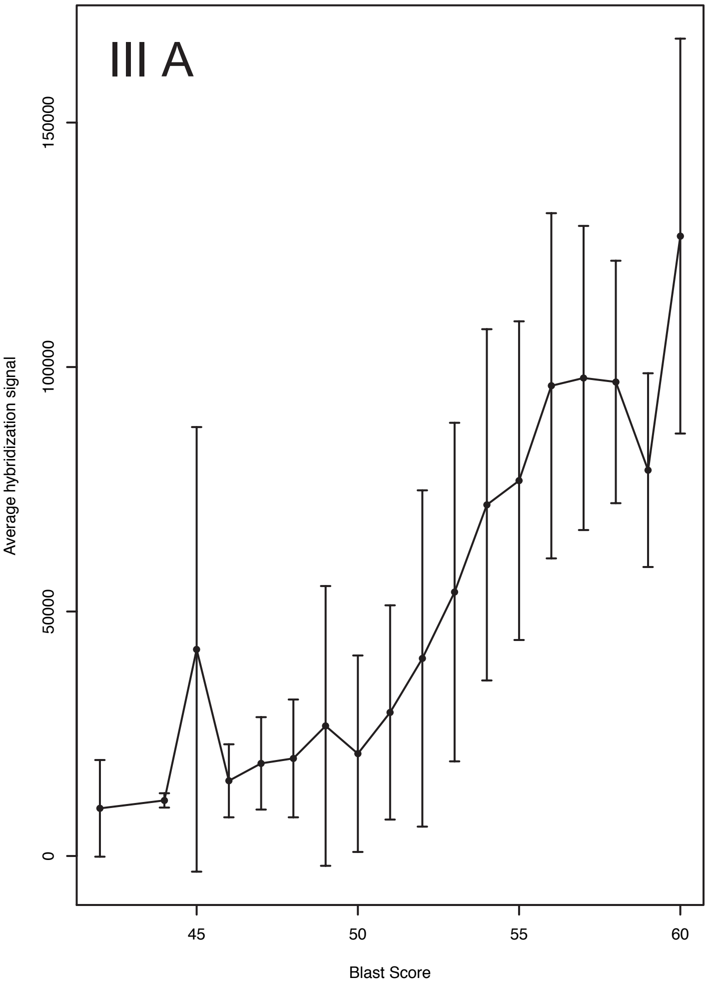
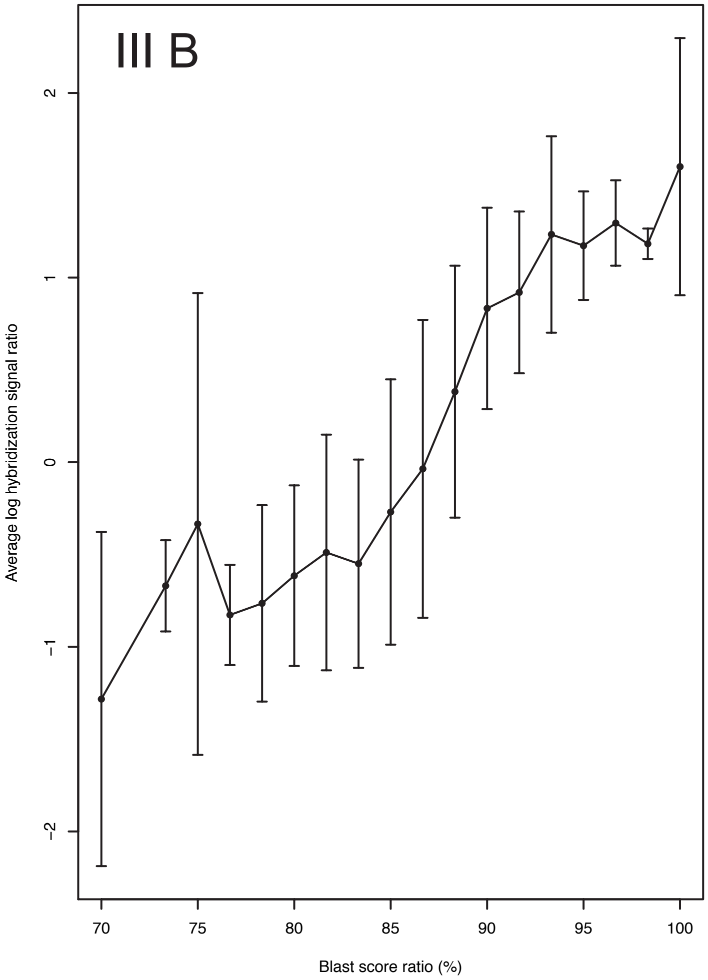
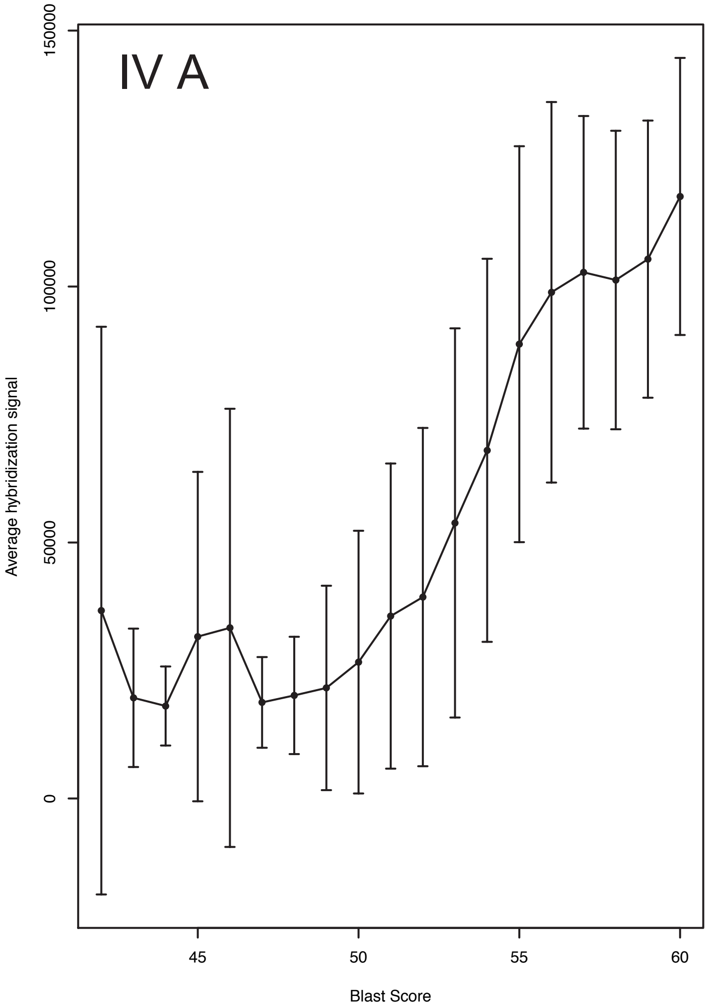


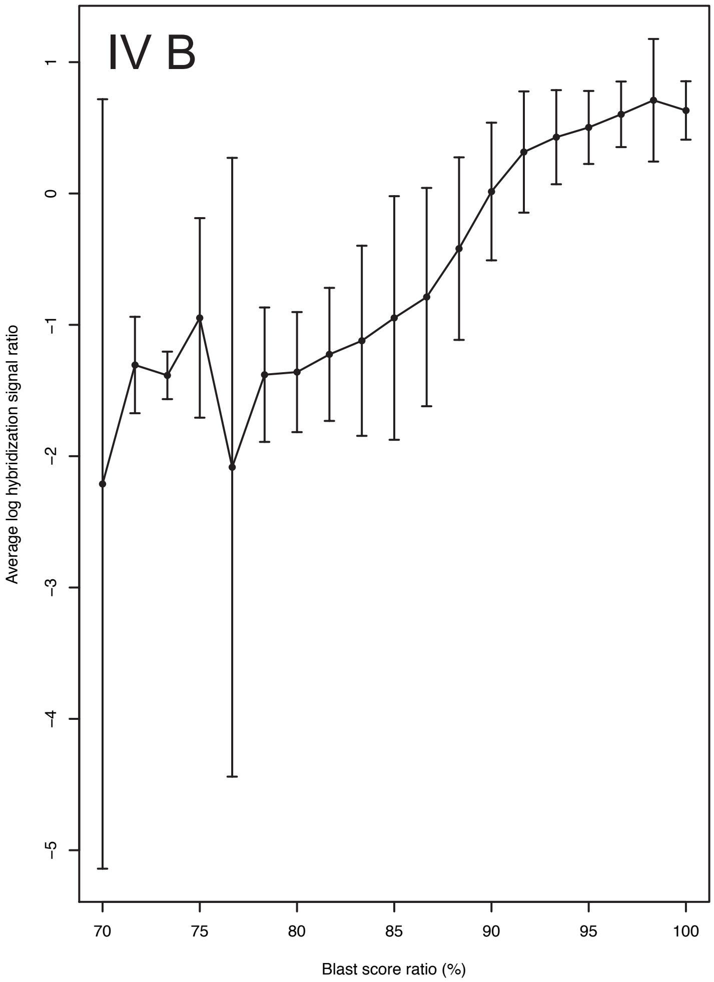
Figure S1: Venn diagram depicting the number of genes covered by the pan-genome probe set for specific genome combinations.

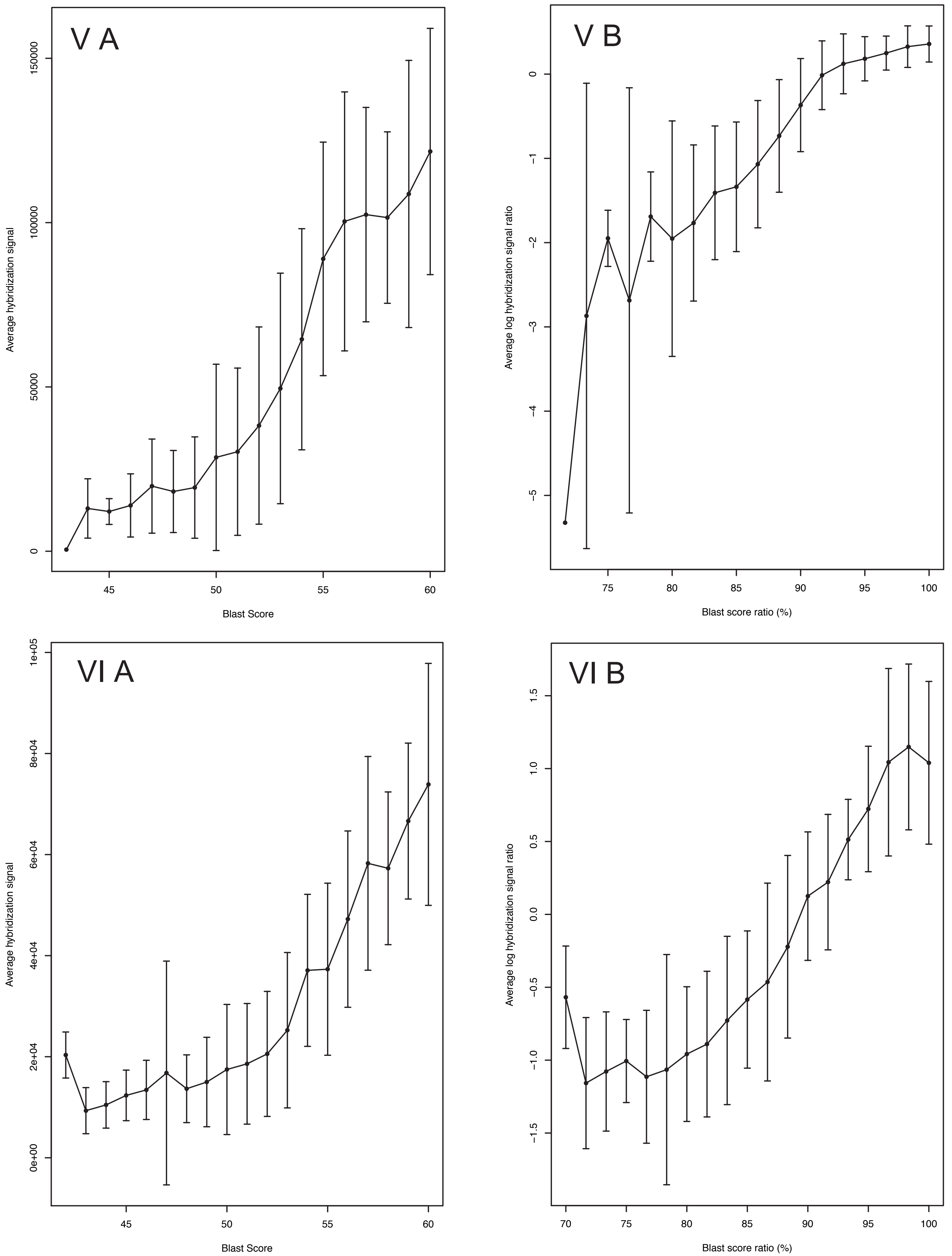

I A
Average hybridization signal
0
10000
20000
30000
40000
45
50
55
60
Blast Score
I B
Average log hybridization signal ratio
-1
-2
-3
-4
-5
70
75
80
85
90
95
100
Blast score ratio (%)
II A
Average hybridization signal
5000
10000
15000
20000
25000
30000
35000
45
50
55
60
Blast Score
II B
Average log hybridization signal ratio
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
70
75
80
85
90
95
100
Blast score ratio (%)

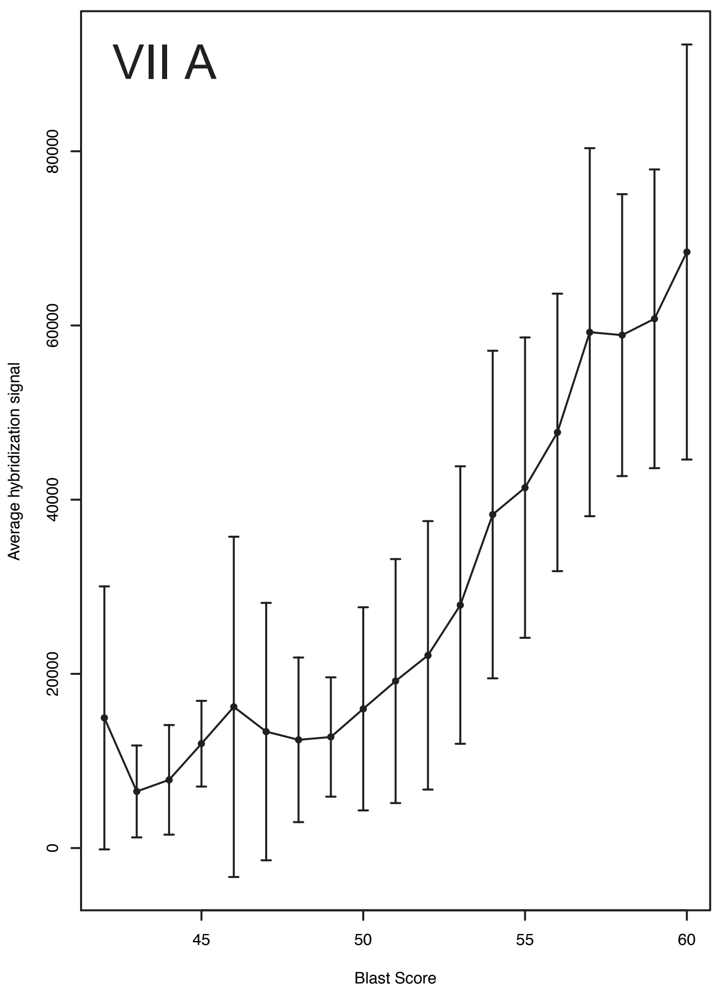

III A
Average hybridization signal
0
50000
100000
150000
45
50
55
60
Blast Score

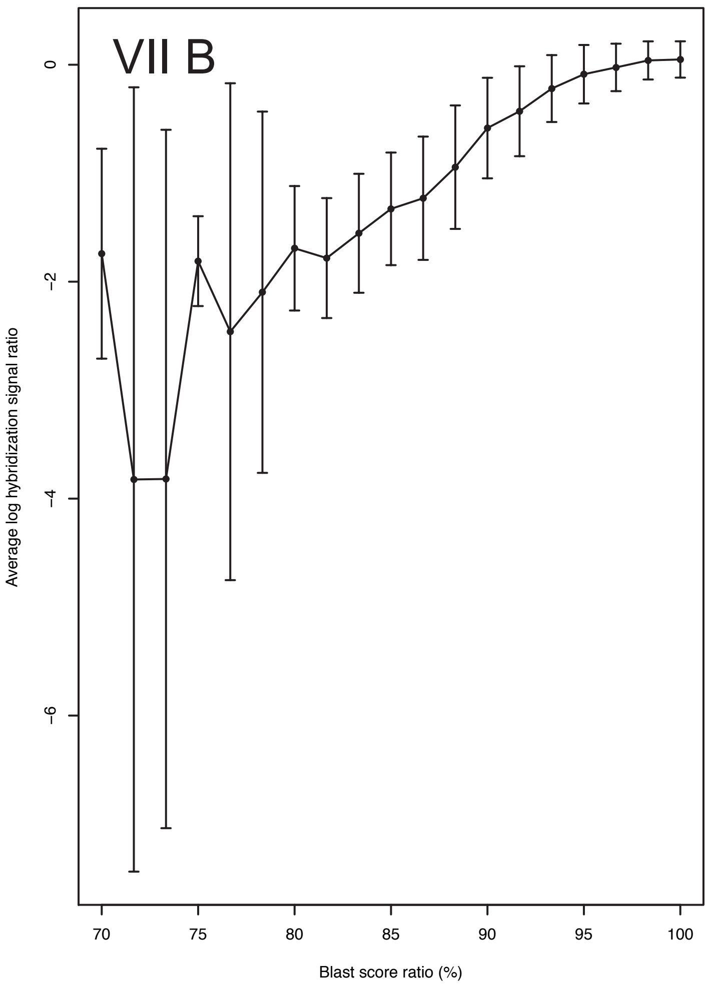

III B
Average log hybridization signal ratio
−2
−1
0
1
2
70
75
80
85
90
95
100
Blast score ratio (%)

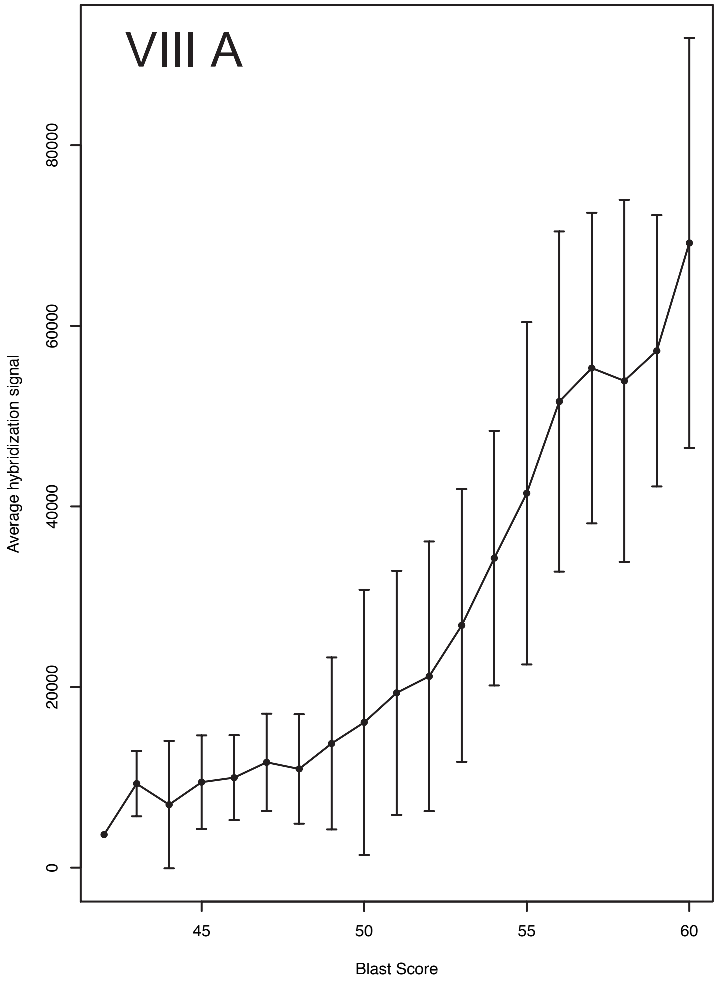

IV A
Average hybridization signal
0
50000
100000
150000
45
50
55
60
Blast Score

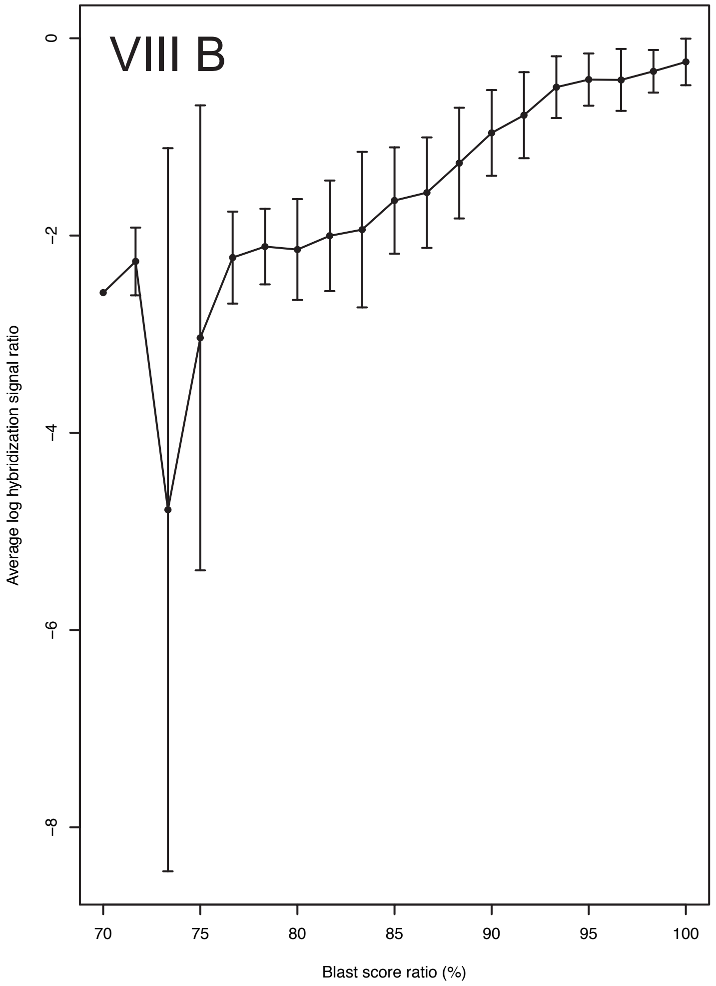

IV B
Average log hybridization signal ratio
−5
−4
−3
−2
−1
0
1
70
75
80
85
90
95
100
Blast score ratio (%)

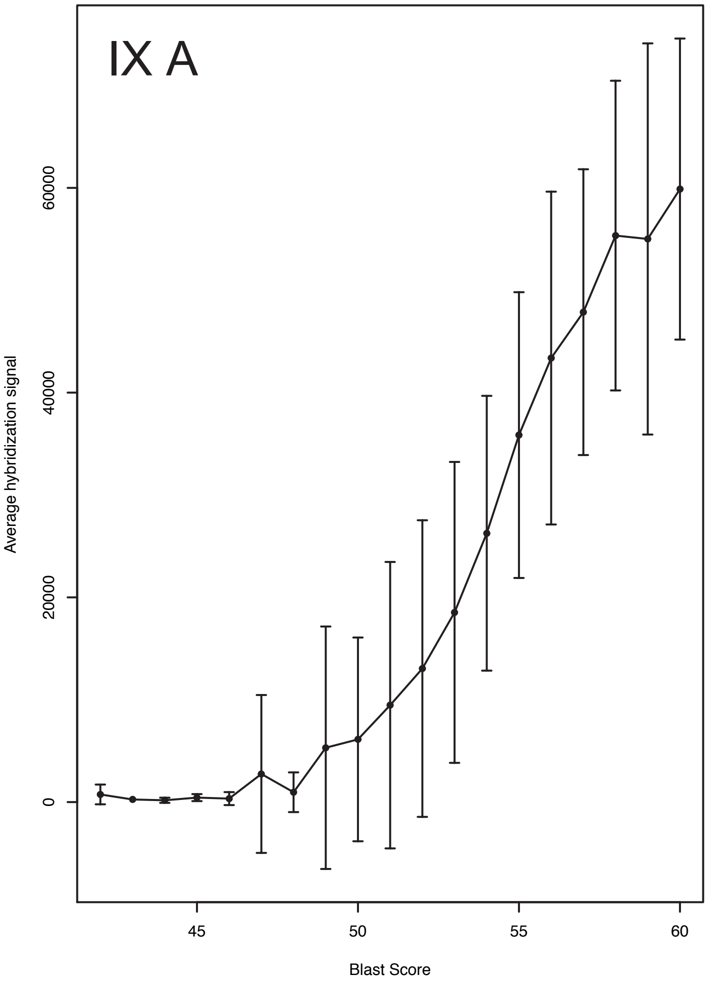

V A
Average hybridization signal
0
50000
100000
150000
Blast Score
45
50
55
60
V B
Average log hybridization signal ratio
0
−1
−2
−3
−4
−5
Blast score ratio (%)
75
80
85
90
95
100
VI A
Average hybridization signal
0e+00
2e+04
4e+04
6e+04
8e+04
1e+05
Blast Score
45
50
55
60
VI B
Average log hybridization signal ratio
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
Blast score ratio (%)
70
75
80
85
90
95
100

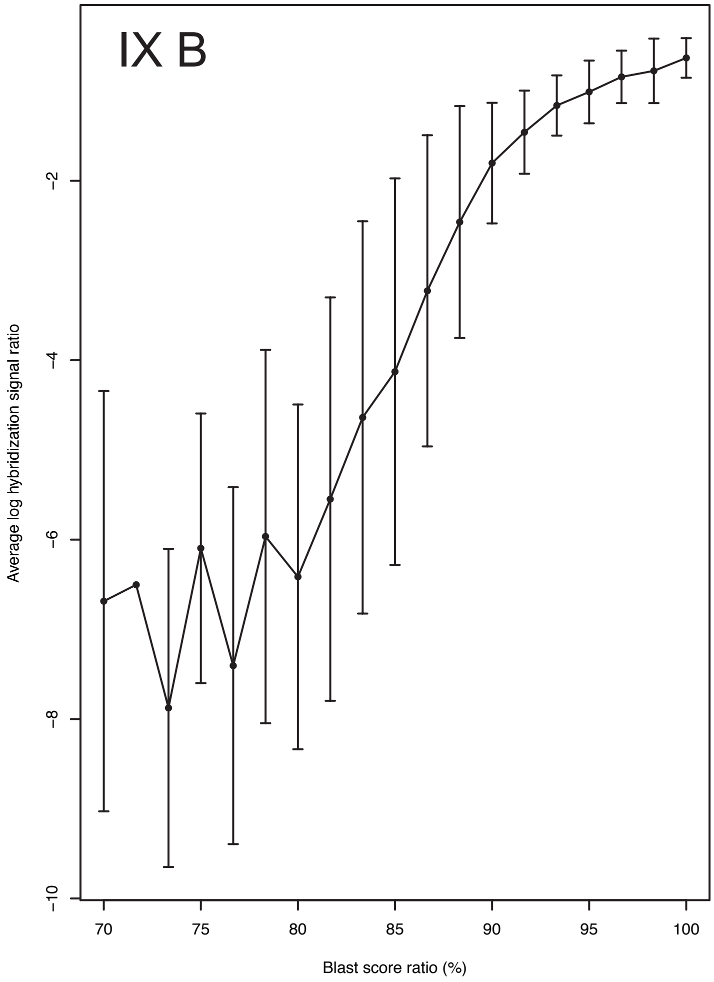

VII A
Average hybridization signal
0
20000
40000
60000
80000
45
50
55
60
Blast Score

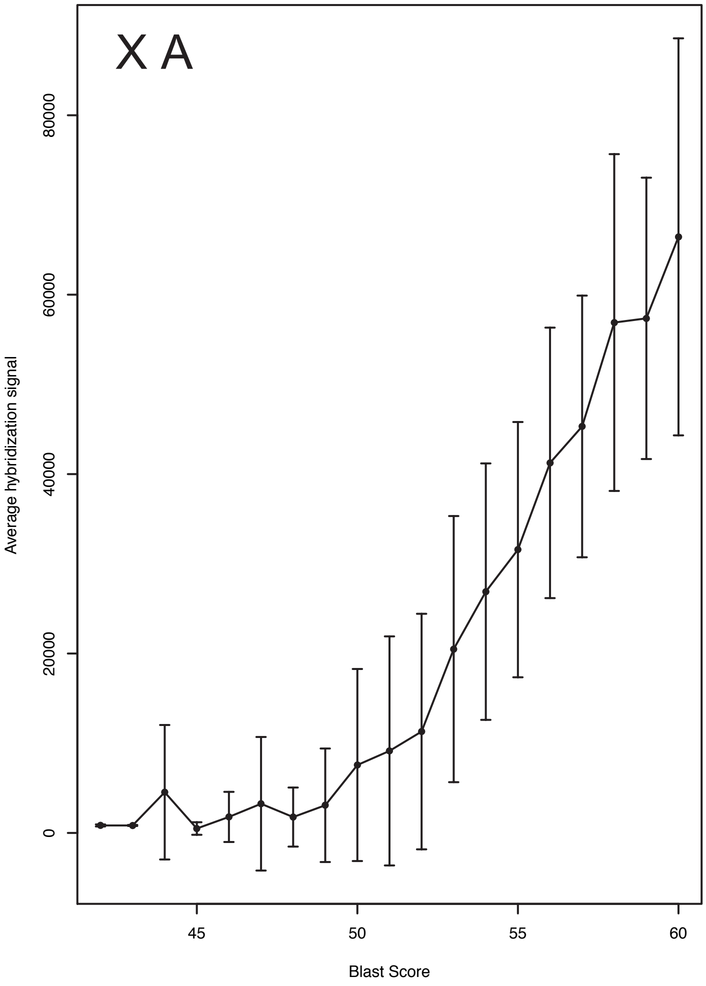

VII B
Average log hybridization signal ratio
0
−2
−4
−6
70
75
80
85
90
95
100
Blast score ratio (%)

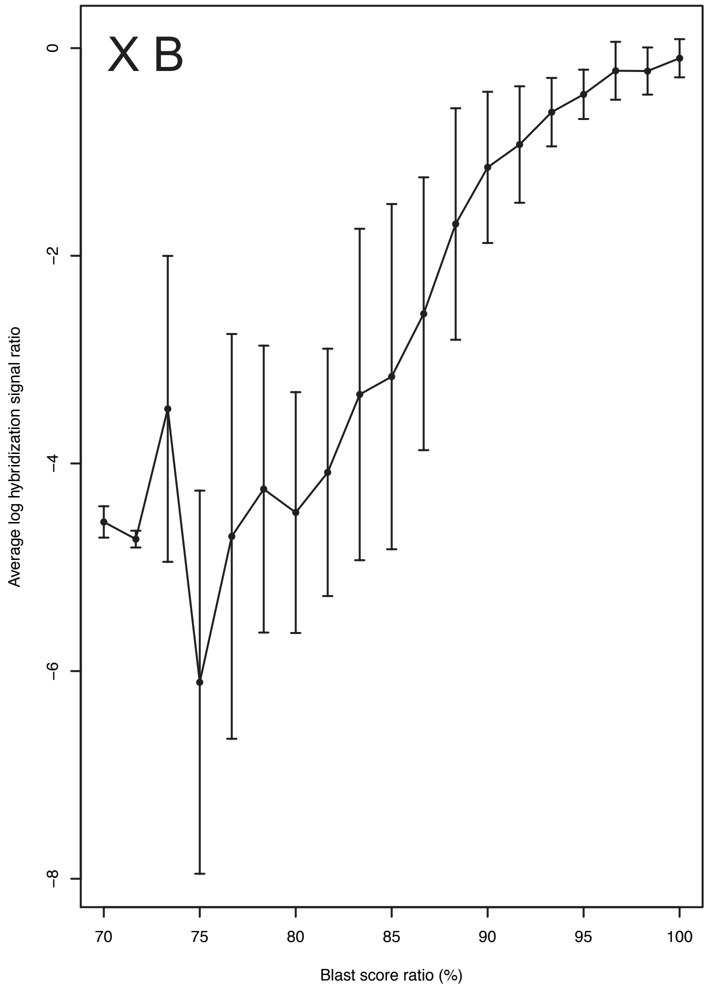

VIII A
Average hybridization signal
0
20000
40000
60000
80000
45
50
55
60
Blast Score

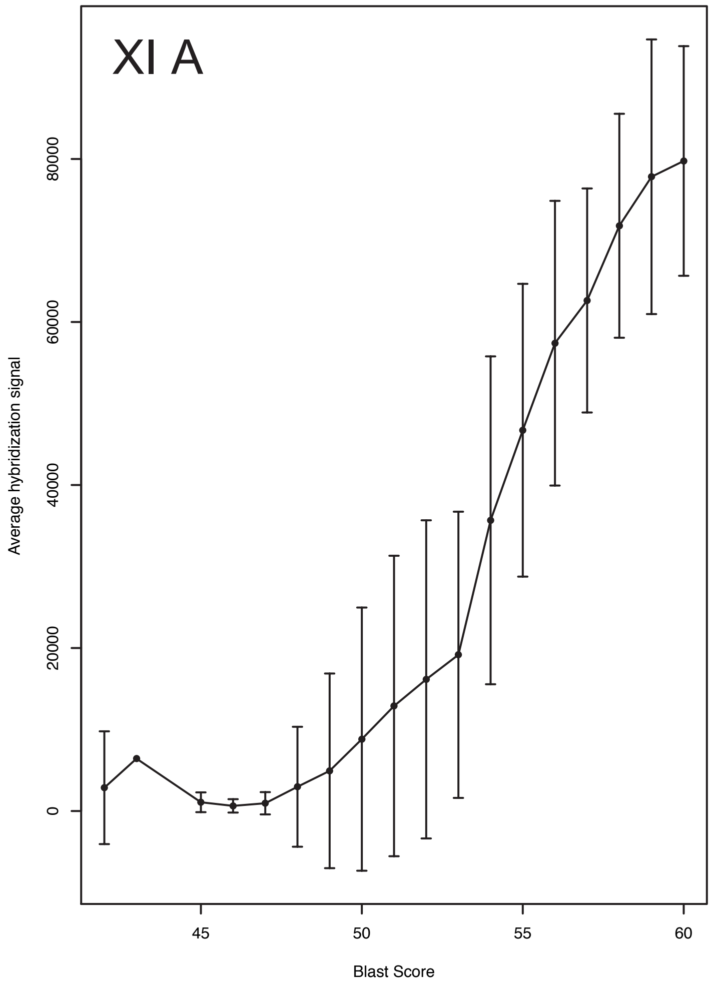

VIII B
Average log hybridization signal ratio
0
−2
−4
−6
−8
70
75
80
85
90
95
100
Blast score ratio (%)

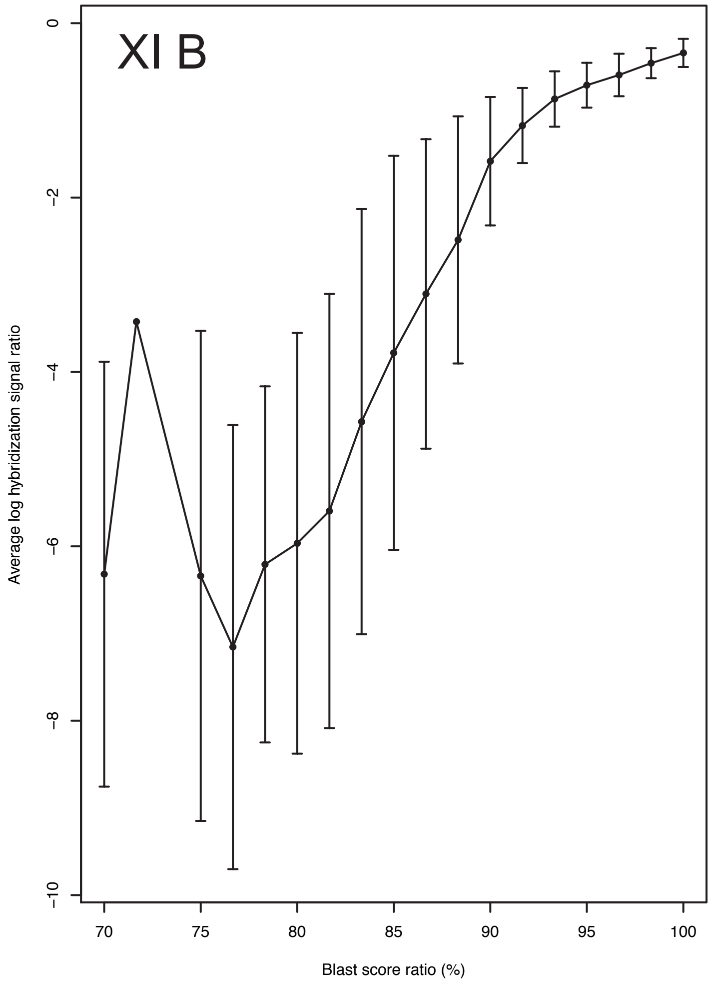

IX A
Average hybridization signal
0
20000
40000
60000
45
50
55
60
Blast Score

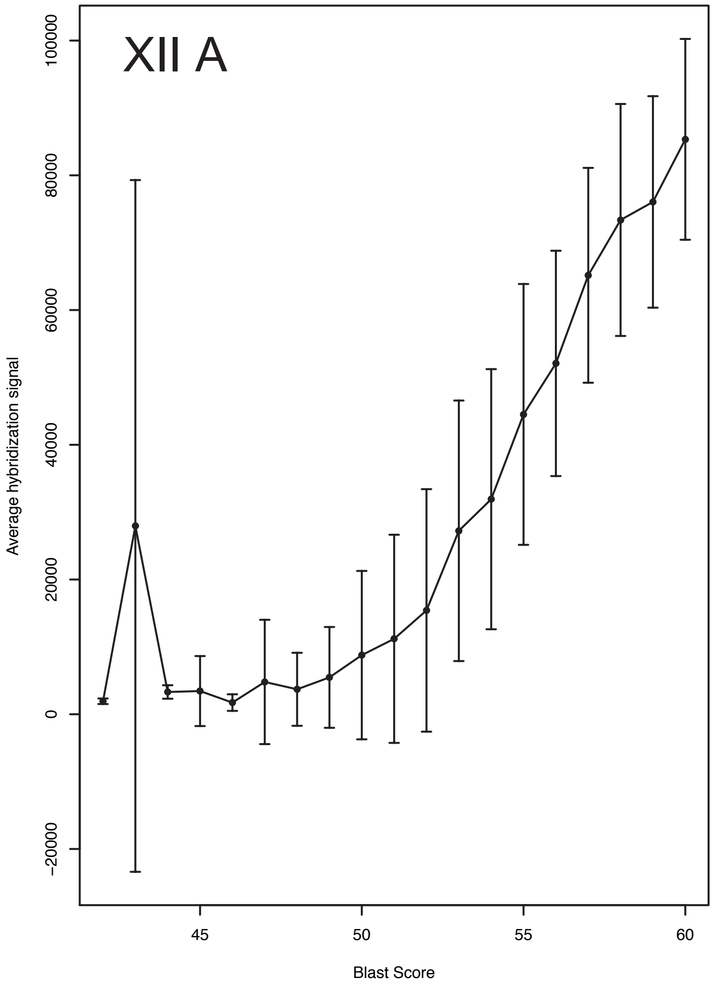

IX B
Average log hybridization signal ratio
−10
−8
−6
−4
−2
70
75
80
85
90
95
100
Blast score ratio (%)


X A
Average hybridization signal
0
20000
40000
60000
80000
45
50
55
60
Blast Score


X B
Average log hybridization signal ratio
−8
−6
−4
−2
0
70
75
80
85
90
95
100
Blast score ratio (%)


XI A
Average hybridization signal
0
20000
40000
60000
80000
45
50
55
60
Blast Score


XI B
Average log hybridization signal ratio
0
-2
-4
-6
-8
-10
70
75
80
85
90
95
100
Blast score ratio (%)


XII A
Average hybridization signal
-20000
0
20000
40000
60000
80000
100000
45
50
55
60
Blast Score

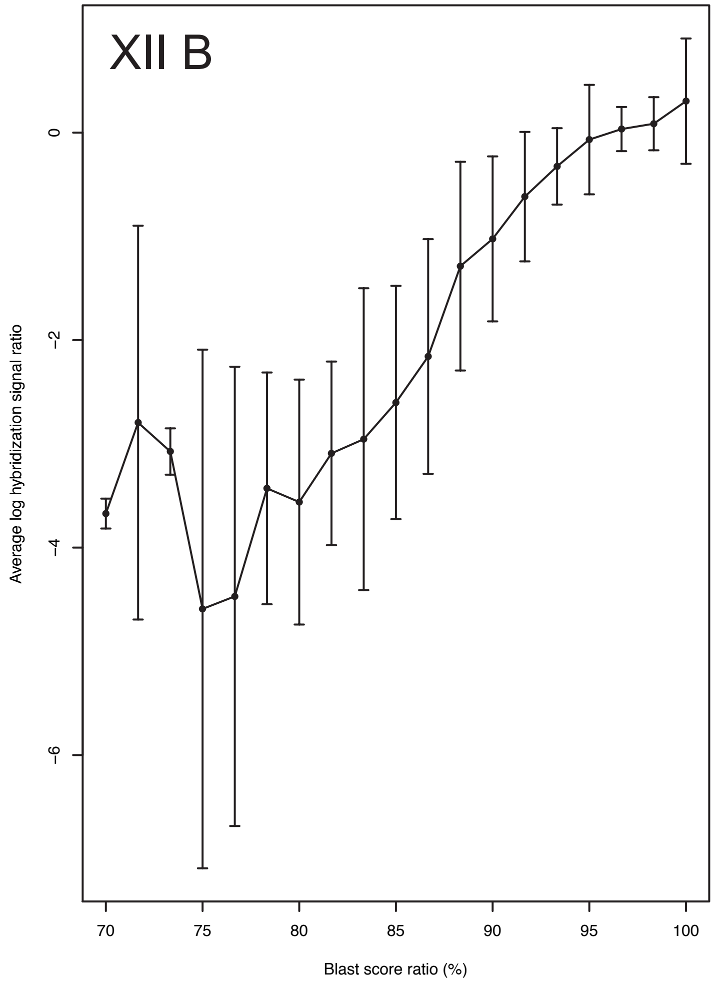

XII B
Average log hybridization signal ratio
0
-2
-4
-6
70
75
80
85
90
95
100
Blast score ratio (%)

Figure S2: Determination of fluorescence intensity signal threshold using blast score ratio statistics adapted from Oh *et al.* Appl Environ Microbiol 76:2980-2988, 2010, for the 12 possible genome pairwise comparisons given an ANI of 90% or lower. For each tester:reference genome pair, the plots indicate the following: A: Blast score of probes against non-target genome sequences vs. average fluorescence intensity signal. B: Blast score ratio of probes against non-target genomes over probes against reference genome vs the average log(signal intensity ratio for non-target over reference genomes). Error bars represent mean ± 3x the standard deviation of intensity.

Tester vs. reference genome pairs are as follows:

I - KB1 vs. VS
II - KB1 vs. DET195
III - VS vs. KB1
IV - VS vs. CBDB1
V - VS vs. BAV1
VI - DET195 vs. KB1
VII - DET195 vs. CBDB1
VIII - DET195 vs. BAV1
IX - CBDB1 vs. VS
X - CBDB1 vs. DET195
XI - BAV1 vs. VS
XII - BAV1 vs. DET195

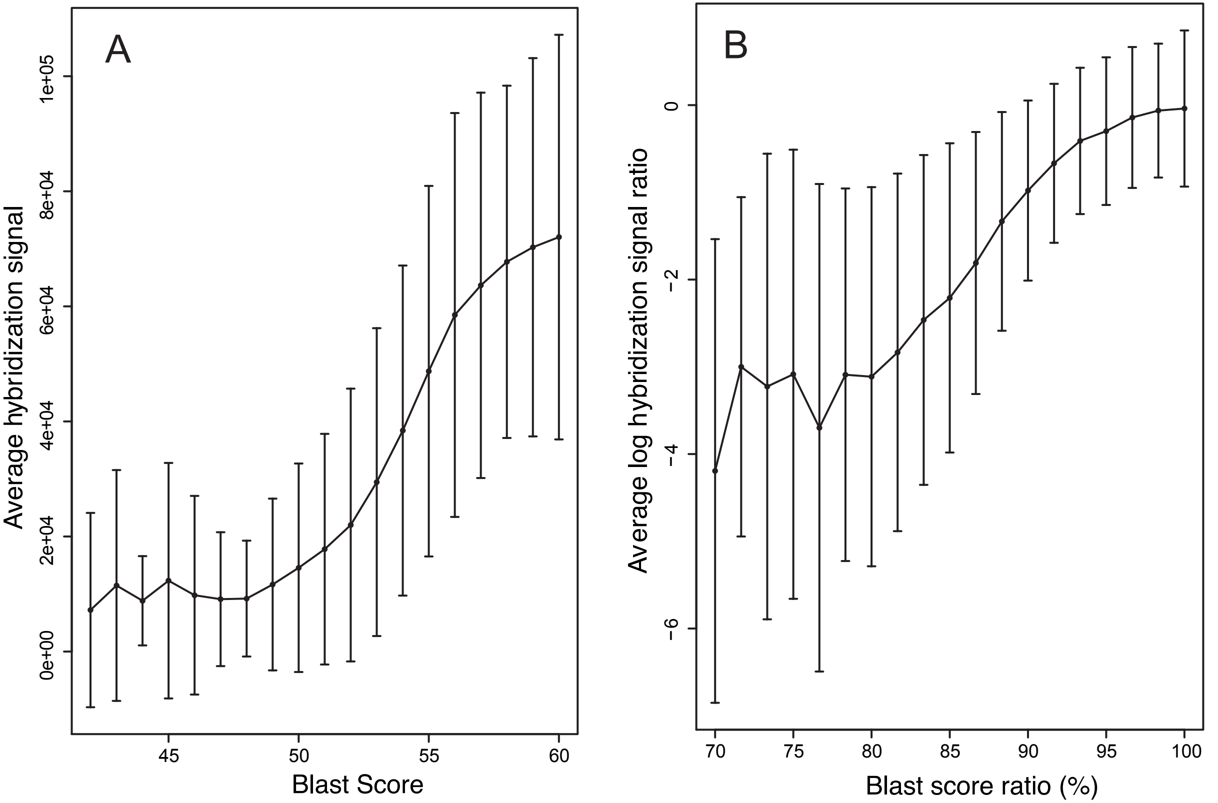


Figure S3: Determination of fluorescence intensity signal threshold using blast score ratio statistics adapted from Oh *et al.* Appl Environ Microbiol 76:2980-2988, 2010, for the 12 possible genome pairwise comparisons given an ANI of 90% or lower (Table S3). All individual pairs were examined and the data merged to provide average values across the 12 permutations. A: Blast score of probes against non-target genome sequences vs. average fluorescence intensity signal. B: Blast score ratio of probes against non-target genomes over probes against reference genome vs the average log(signal intensity ratio for non-target over reference genomes). Error bars represent mean ± 3x the standard deviation of intensity.