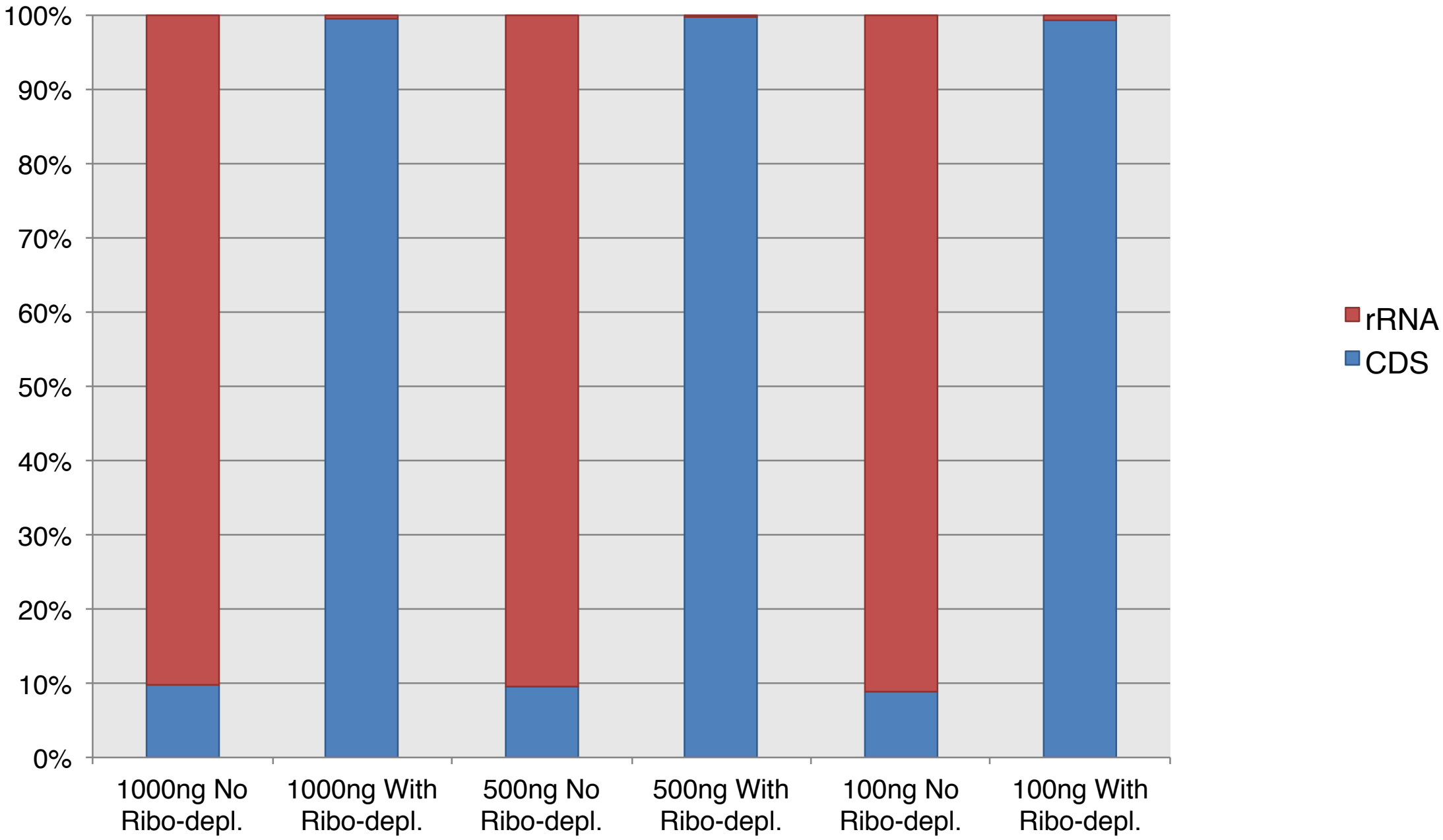
A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90%
80%
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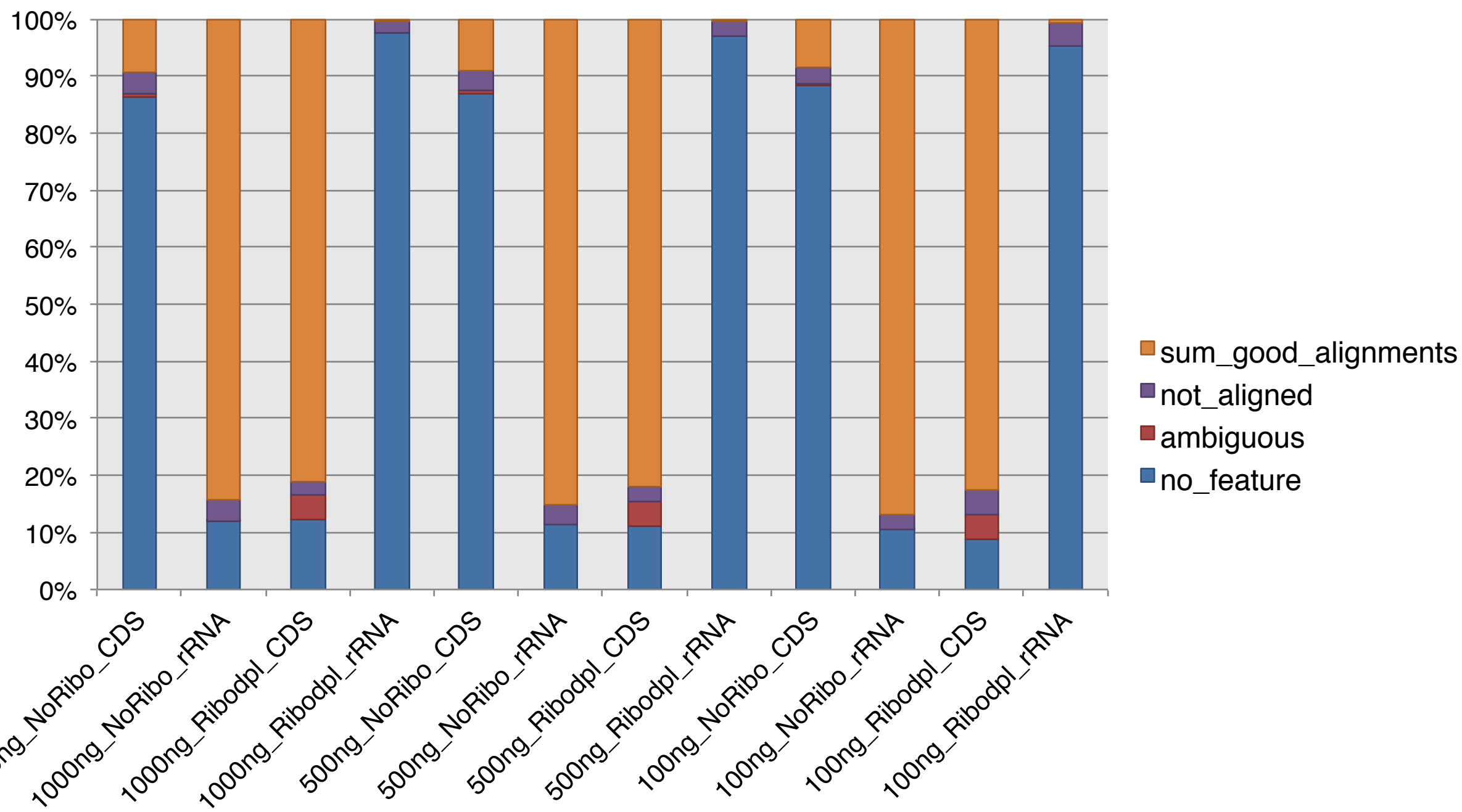
60%
50%
40%
30%
20%
10%
0%
1000ng No Ribo-depl.
1000ng With Ribo-depl.
500ng No Ribo-depl.
500ng With Ribo-depl.
100ng No Ribo-depl.
100ng With Ribo-depl.
rRNA
CDS

B


100%
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1000ng_NoRibo_CDS
1000ng_NoRibo_rRNA
1000ng_Ribodpl_CDS
1000ng_Ribodpl_rRNA
500ng_NoRibo_CDS
500ng_NoRibo_rRNA
500ng_Ribodpl_CDS
500ng_Ribodpl_rRNA
100ng_NoRibo_CDS
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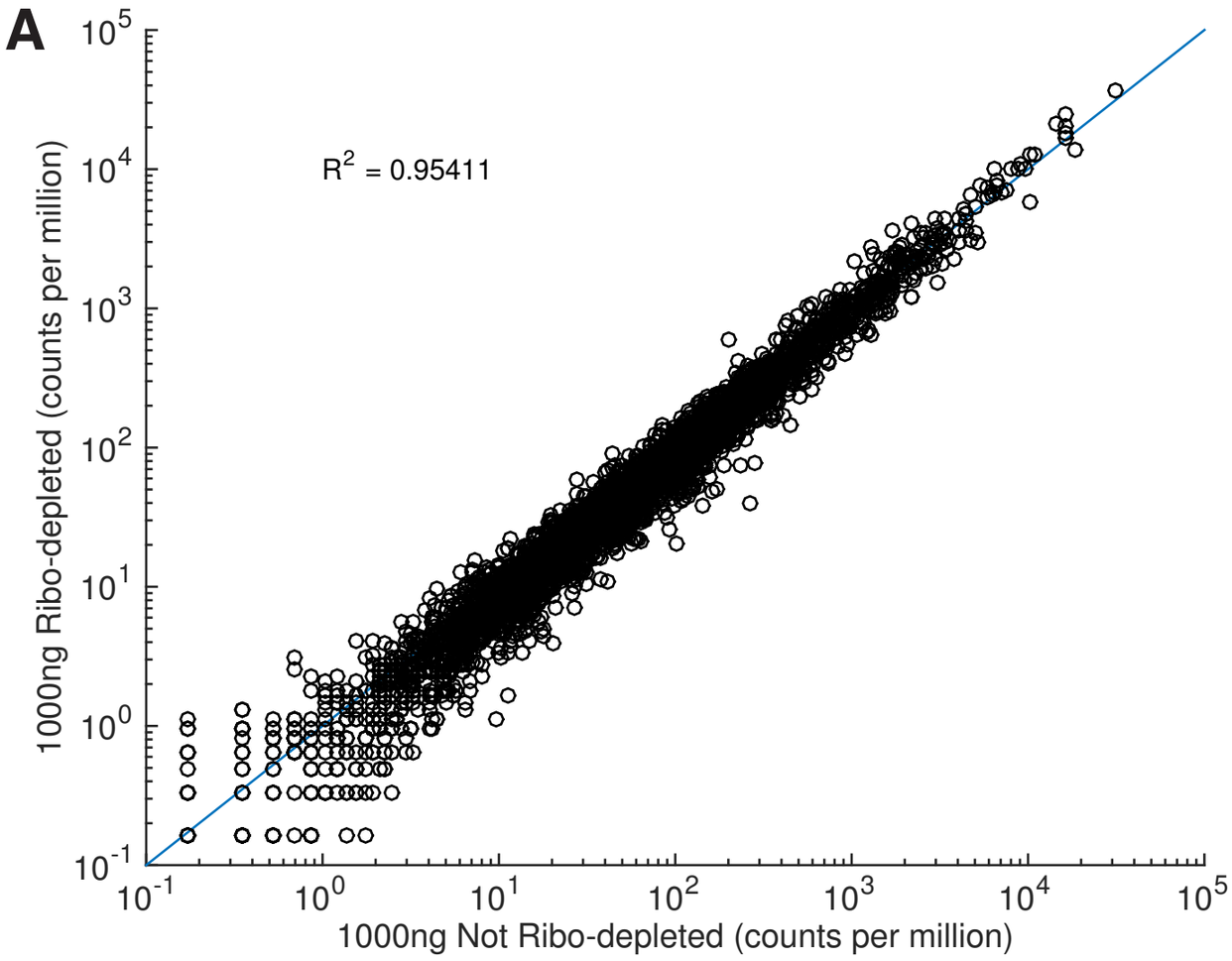
100ng_Ribodpl_CDS
100ng_Ribodpl_rRNA
sum_good_alignments
not_aligned
ambiguous
no_feature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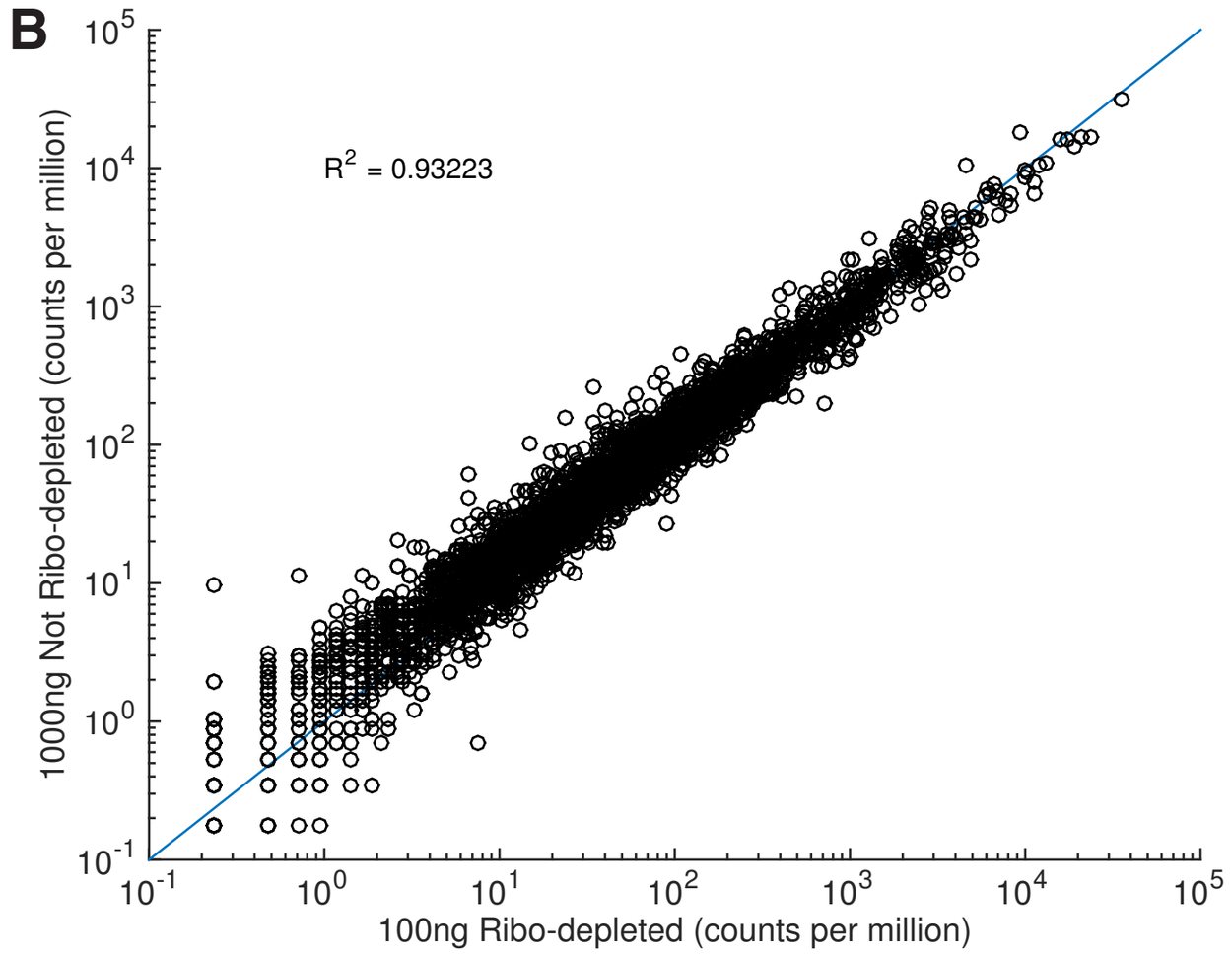
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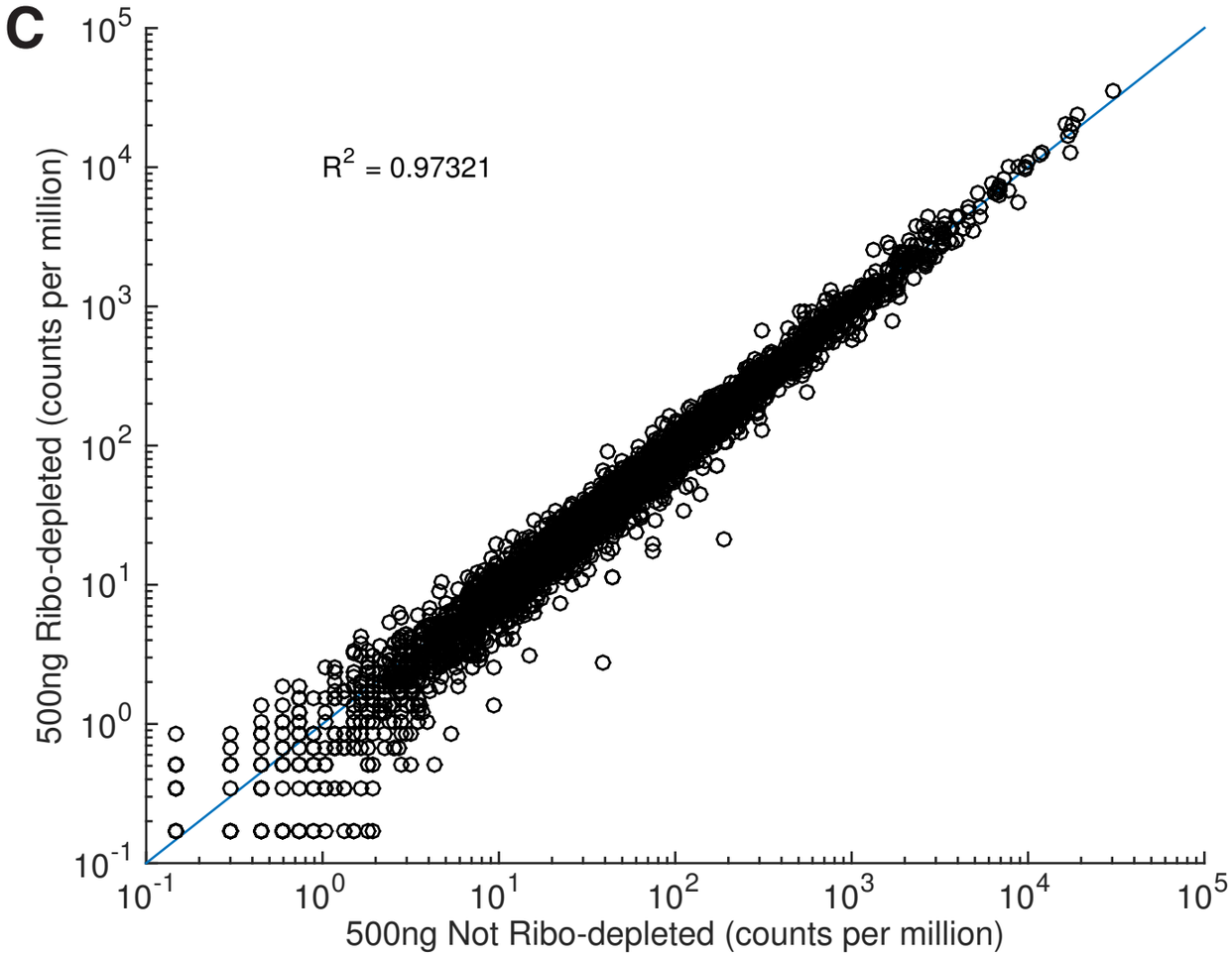
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R^2 = 0.97321
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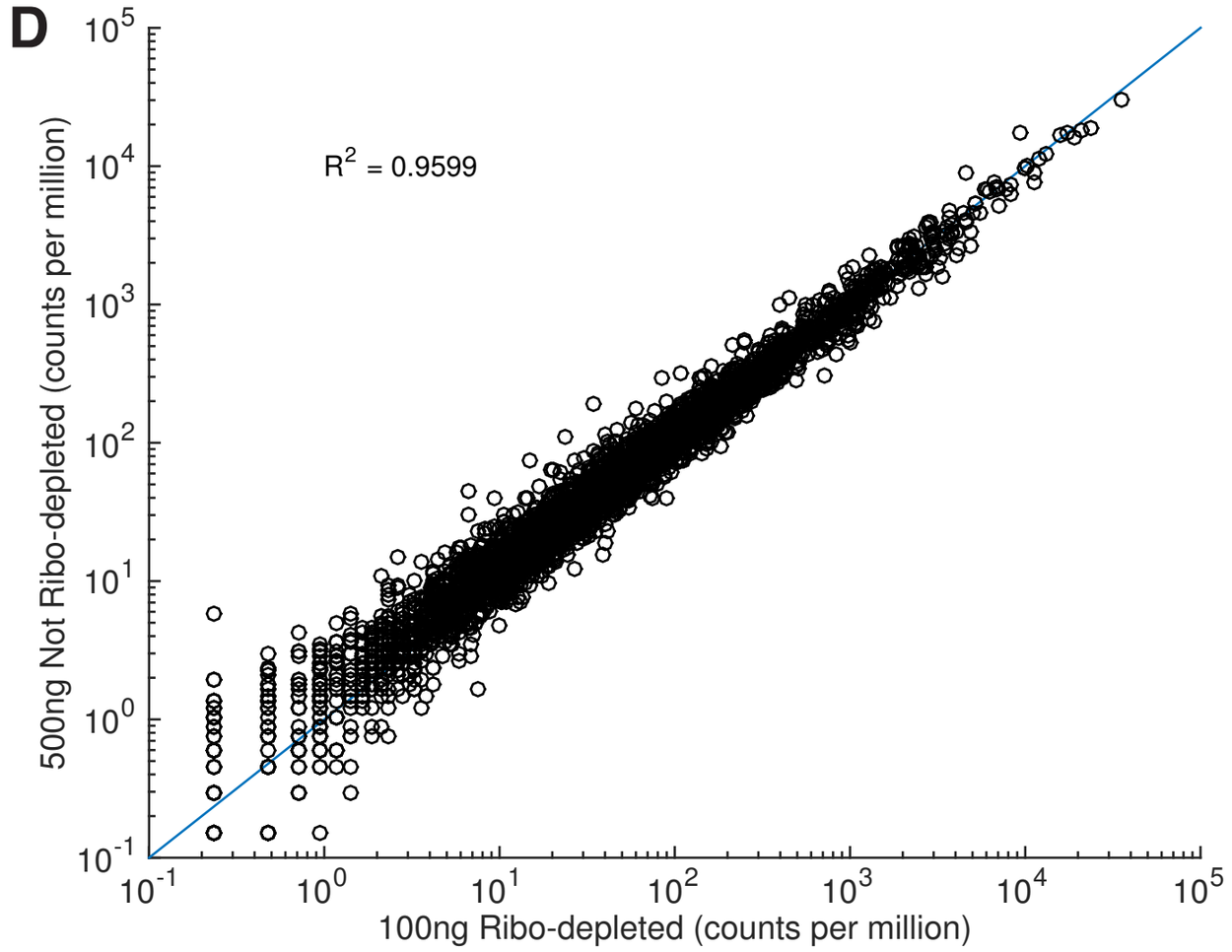
500ng Not Ribo-depleted (counts per million)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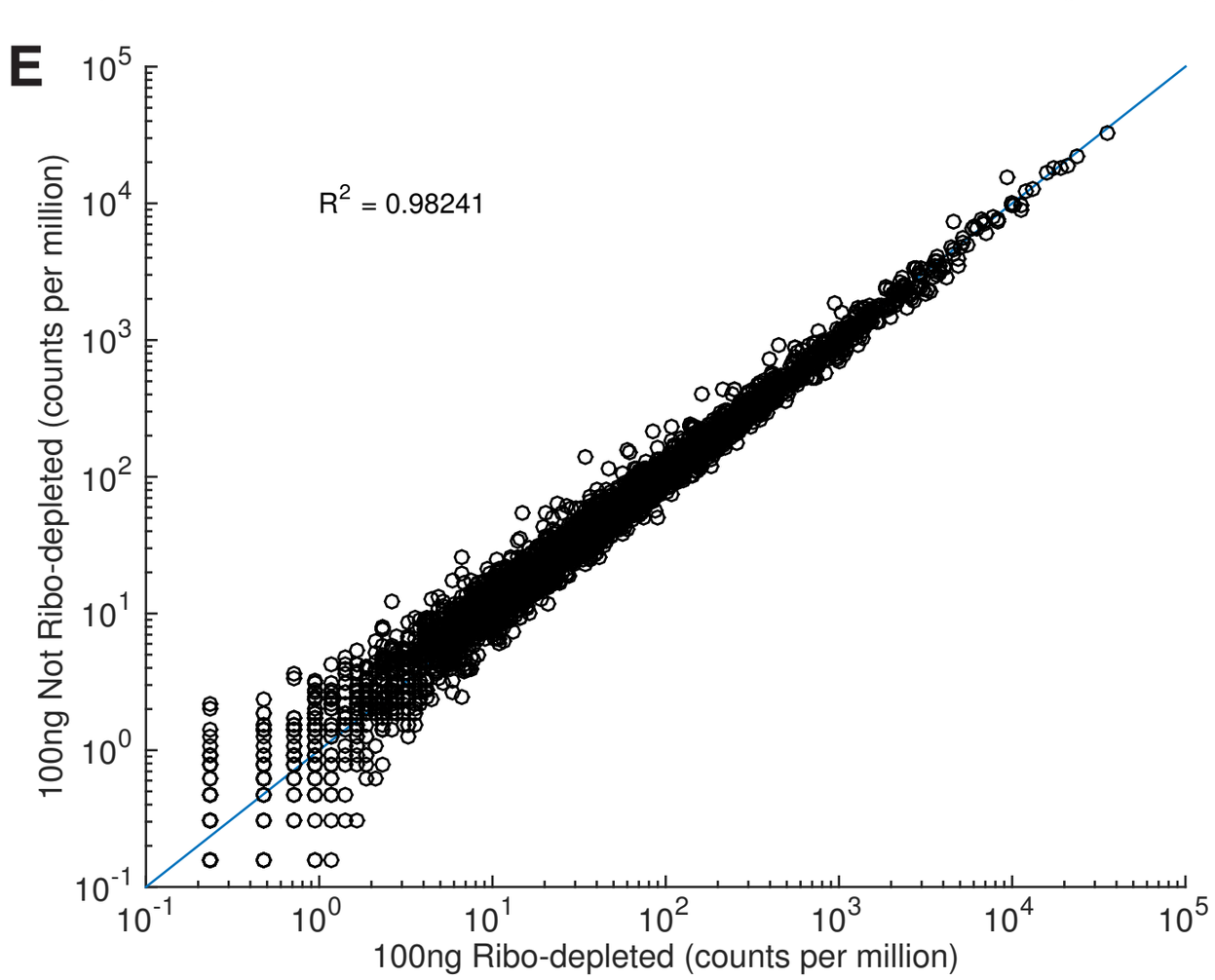
R^2 = 0.9599
500ng Not Ribo-depleted (counts per million)
100ng Ribo-depleted (counts per million)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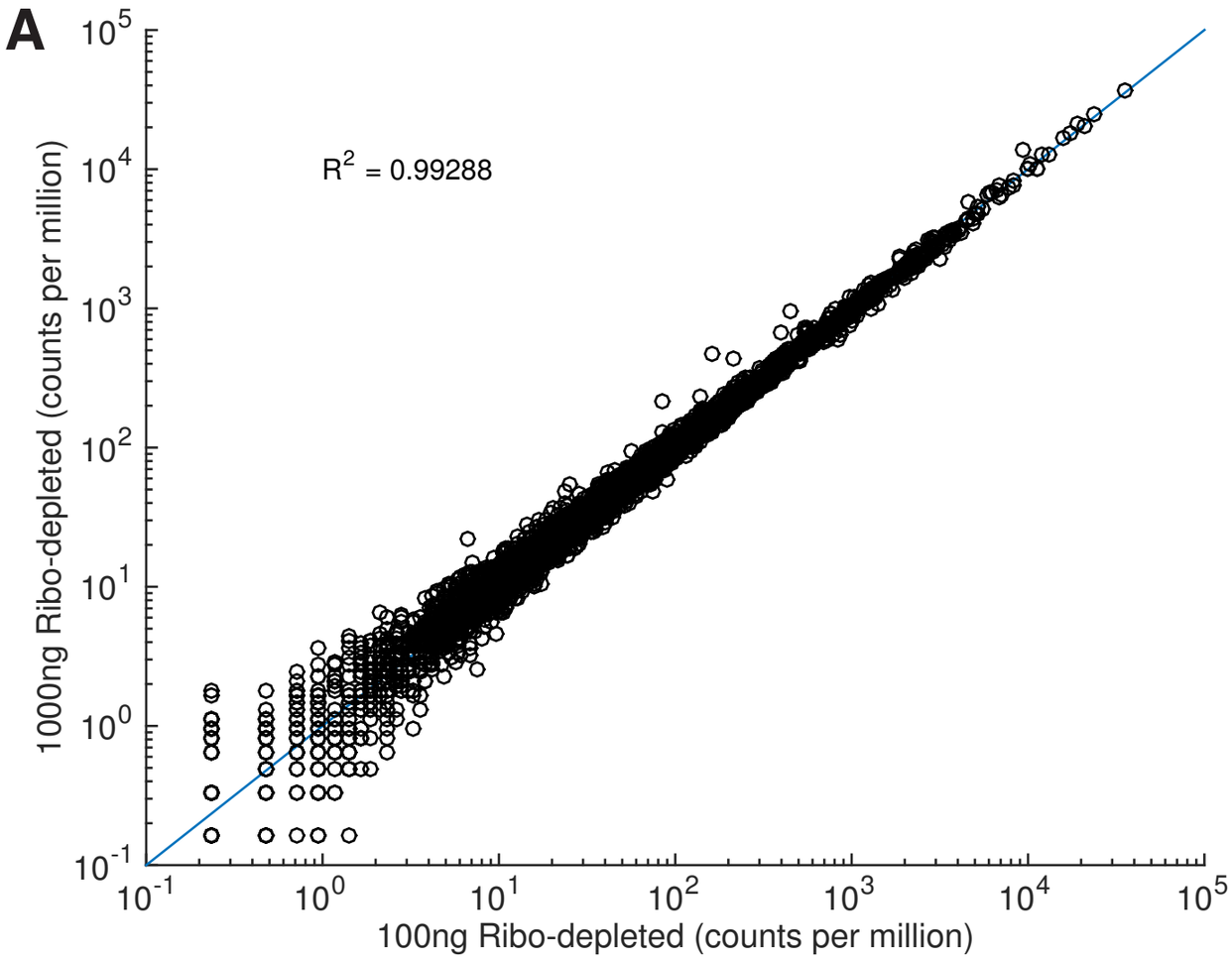
100ng Not Ribo-depleted (counts per million)
100ng Ribo-depleted (counts per million)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R^2 = 0.99288
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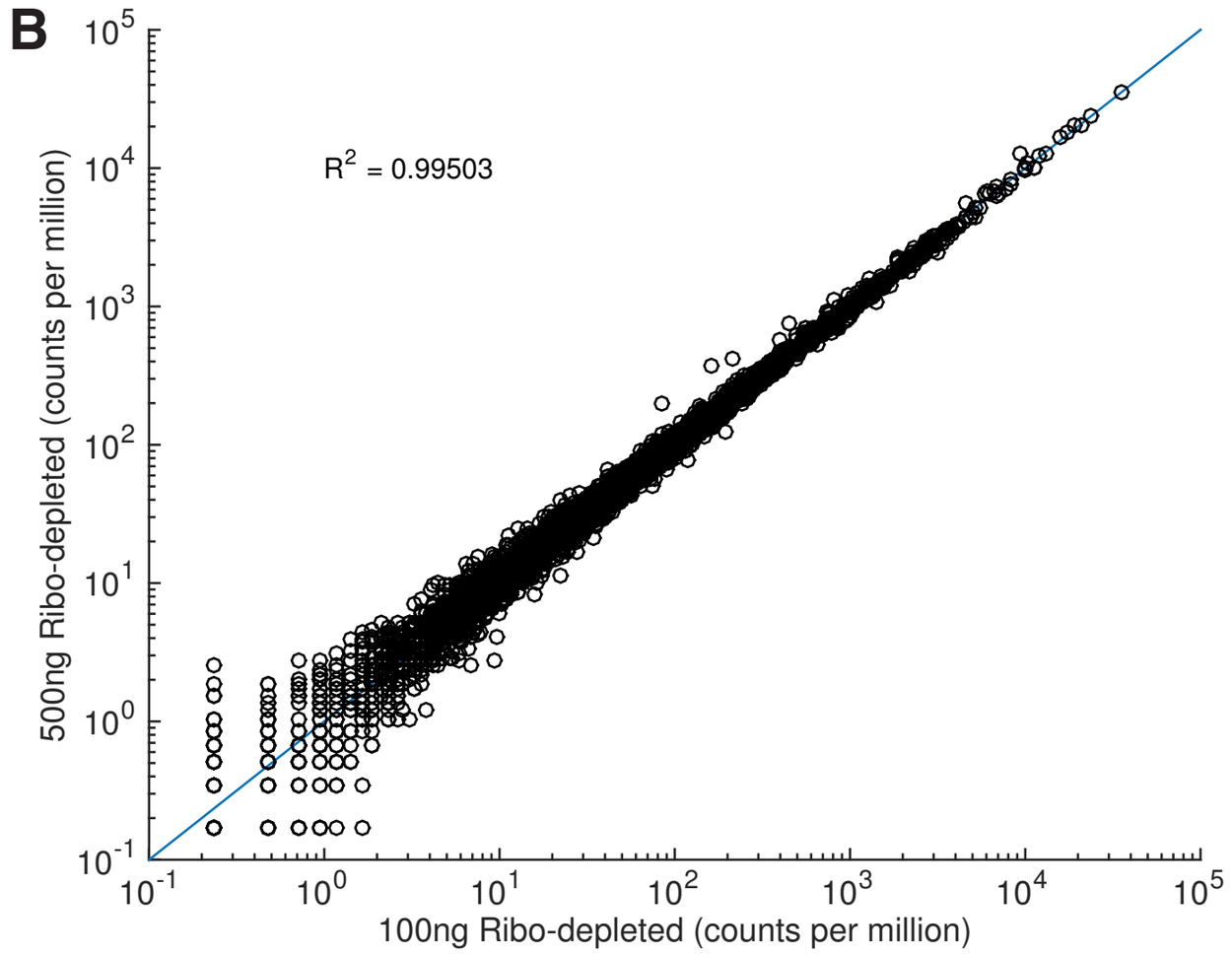
100ng Ribo-depleted (counts per million)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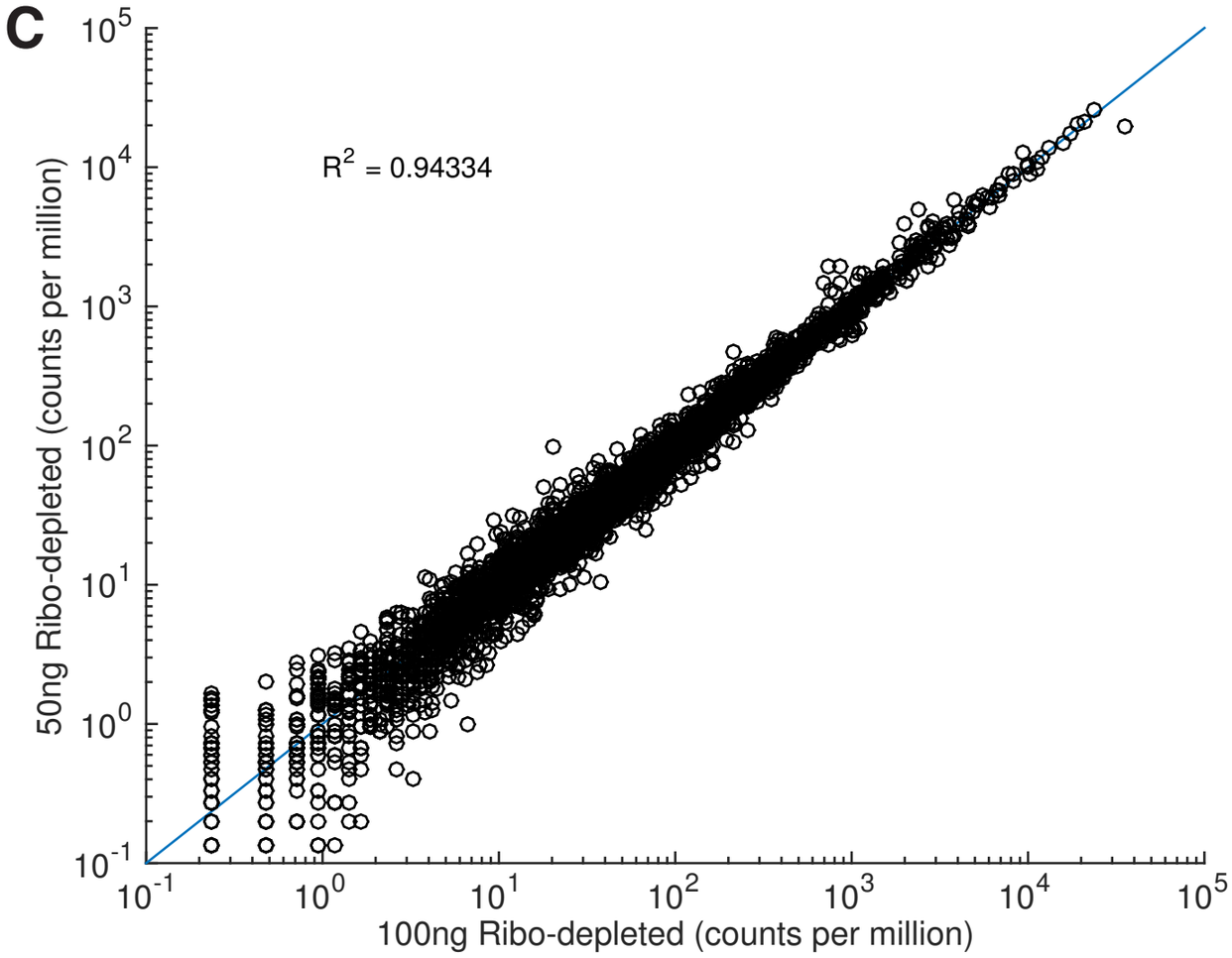
100ng Ribo-depleted (counts per million)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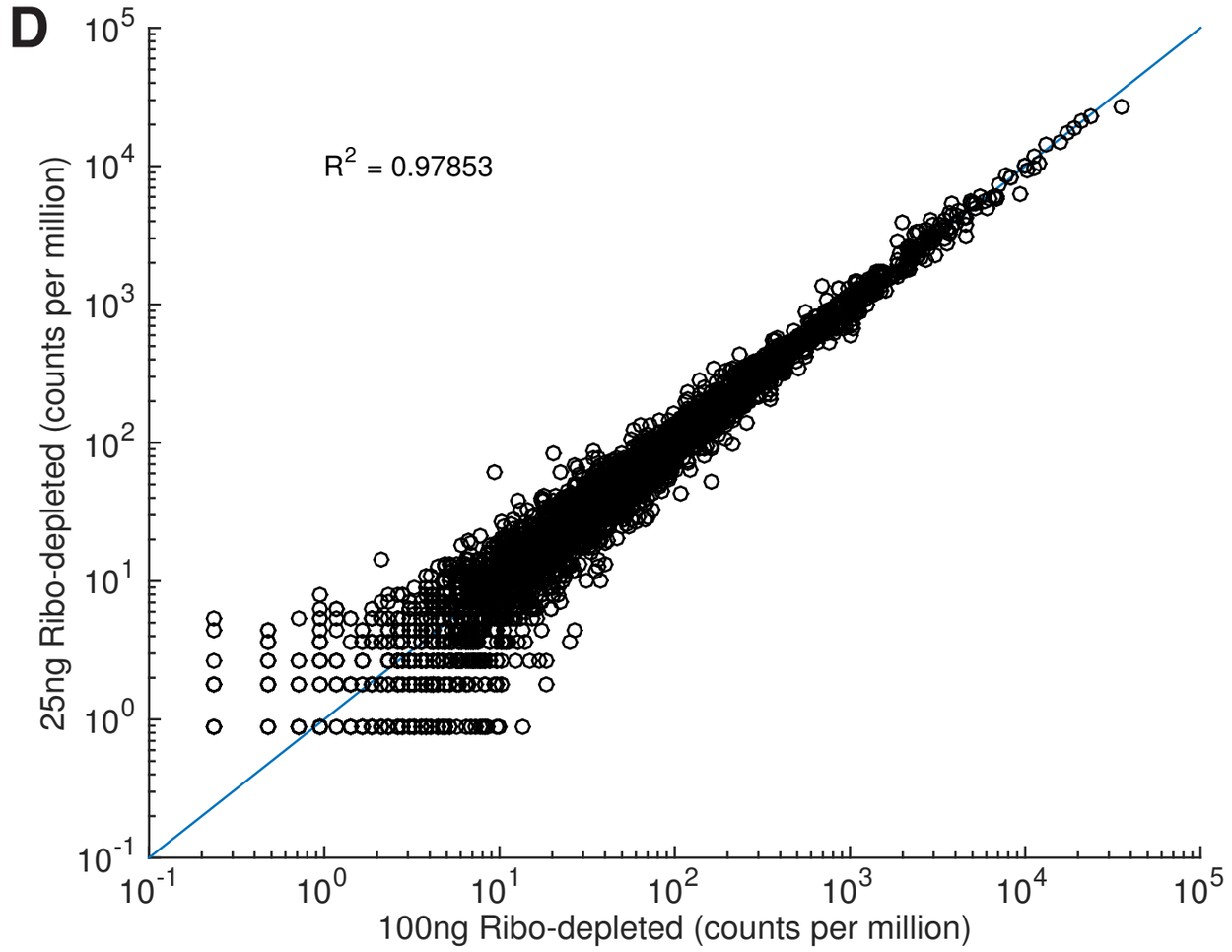
100ng Ribo-depleted (counts per million)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R^2 = 0.97853
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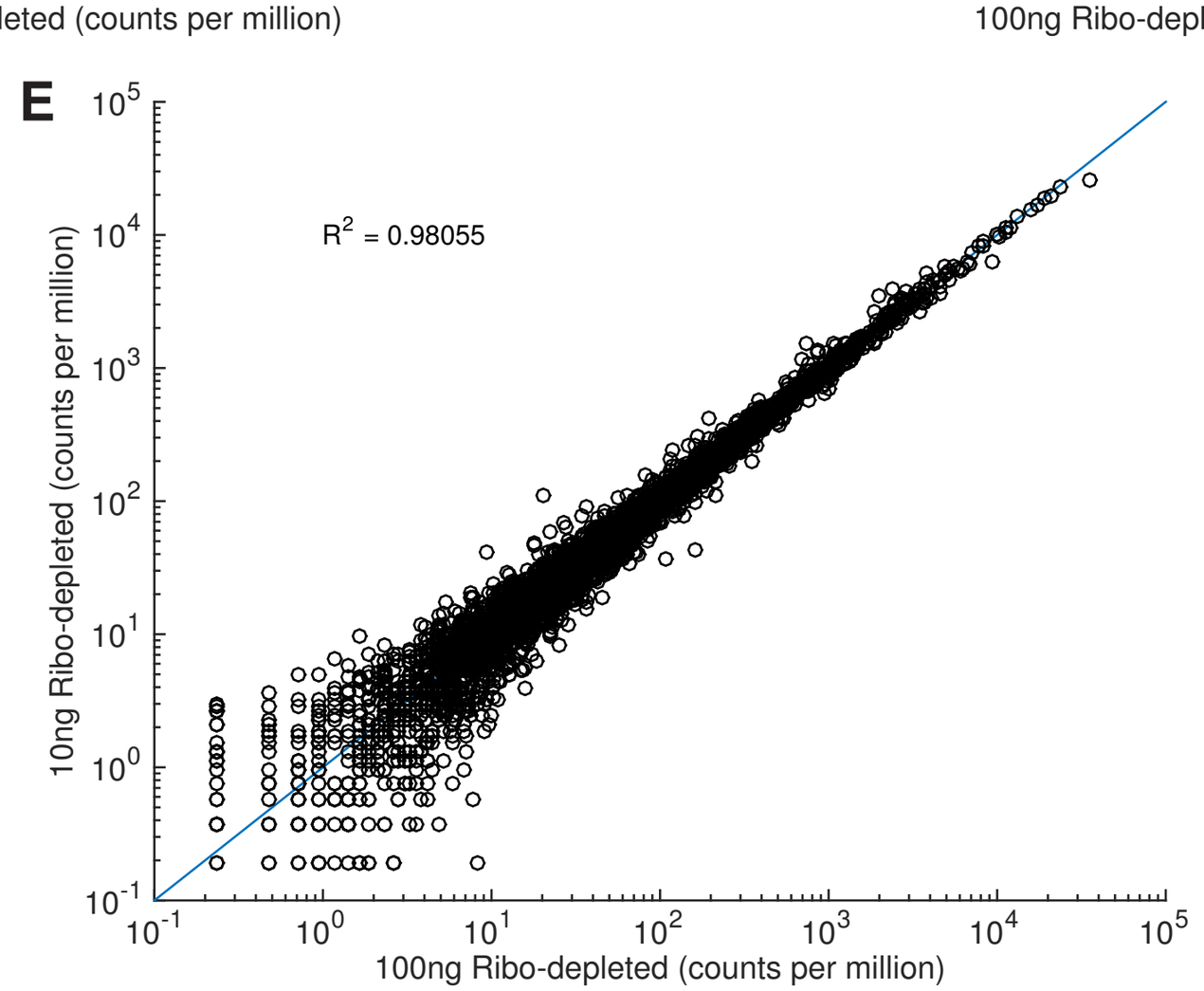
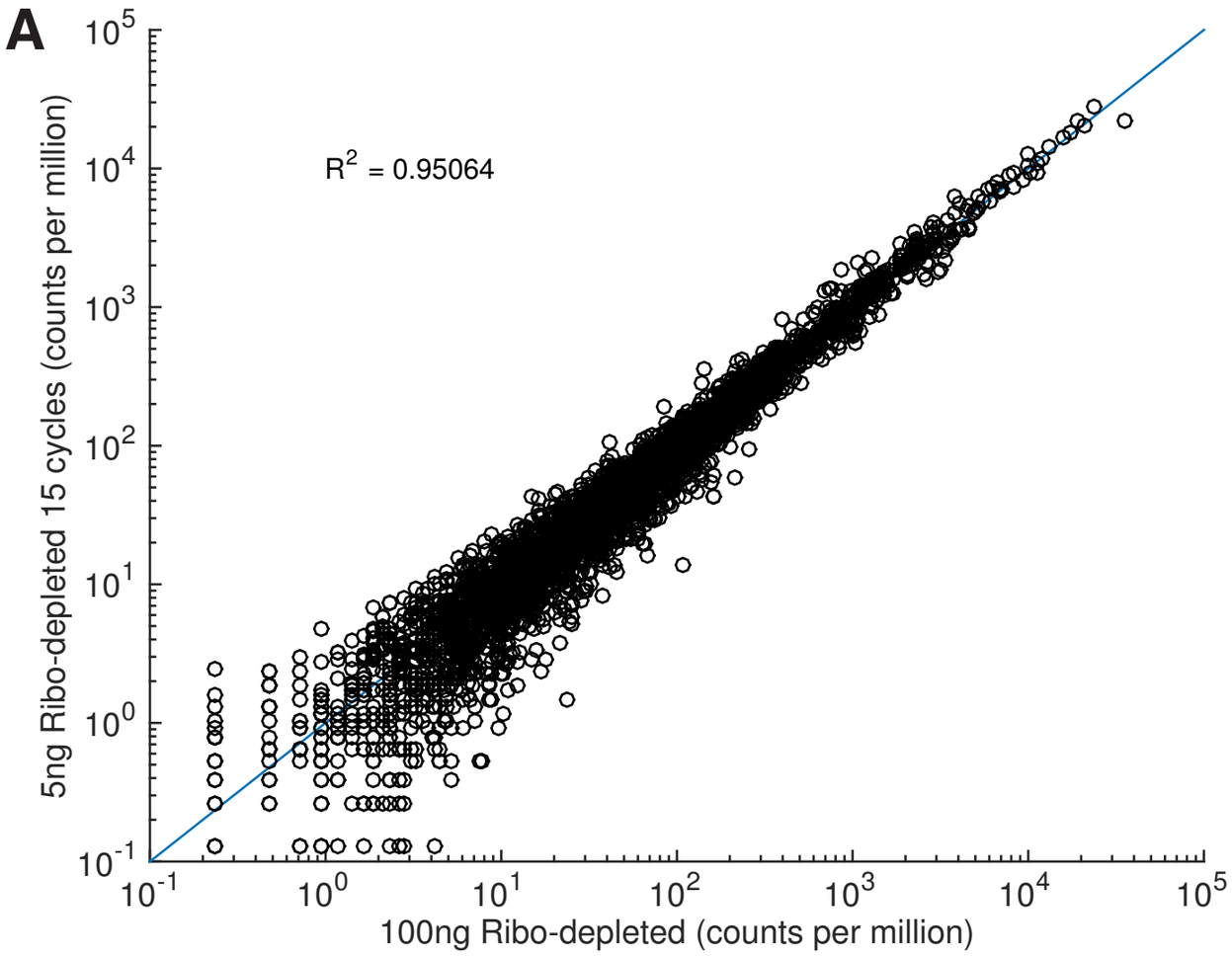
100ng Ribo-depleted (counts per million)


E
R^2 = 0.98055
10ng Ribo-depleted (counts per million)
100ng Ribo-depleted (counts per million)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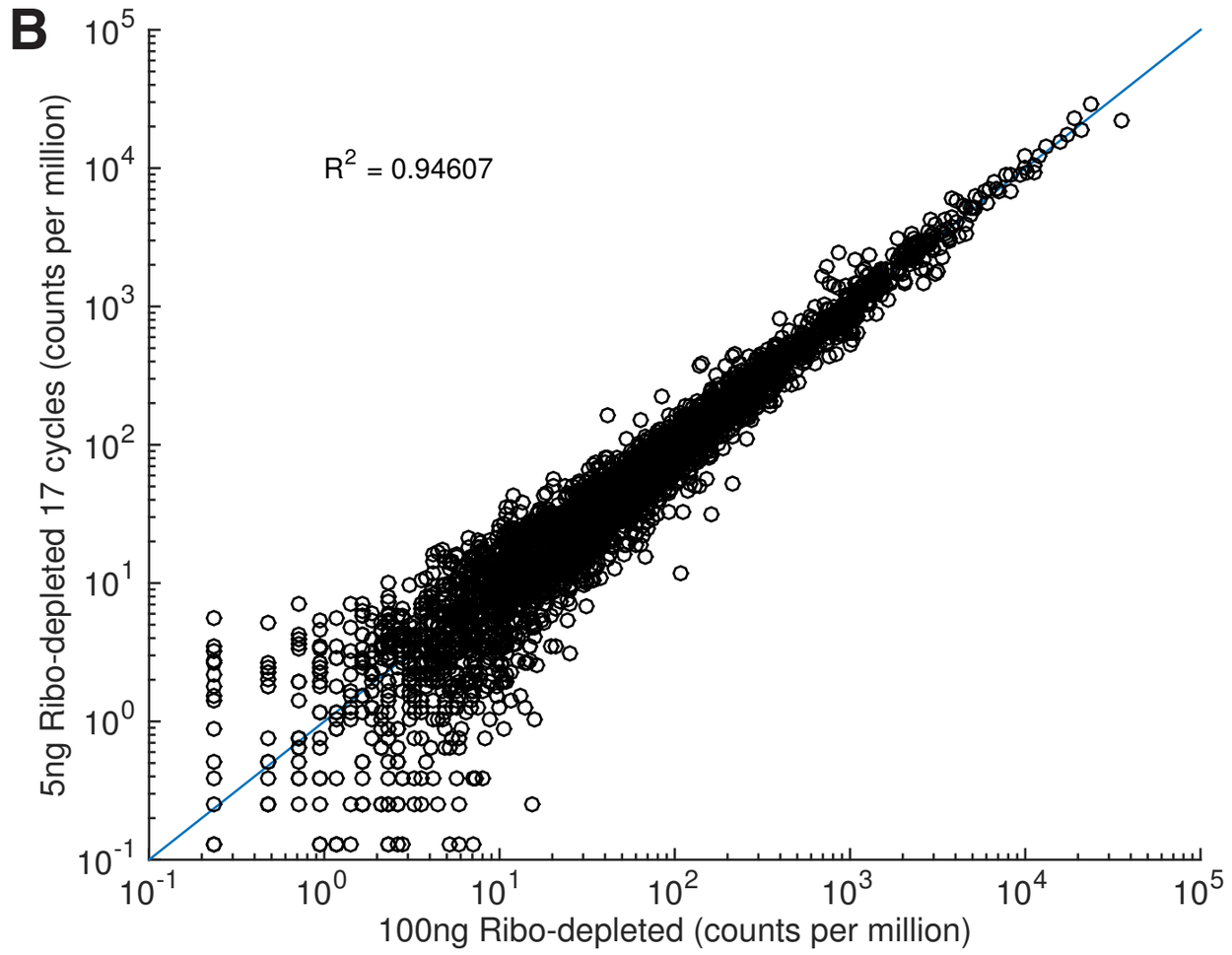
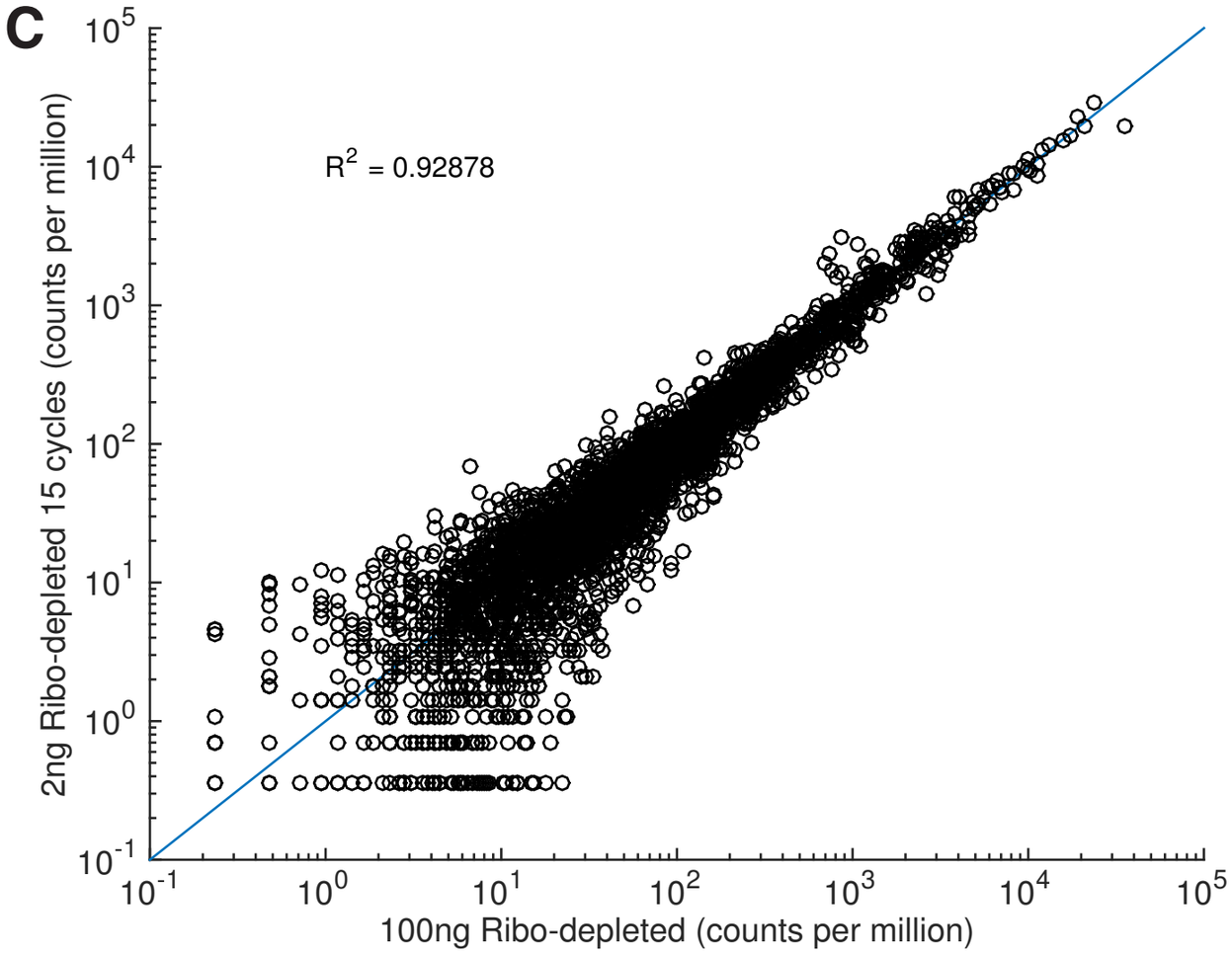
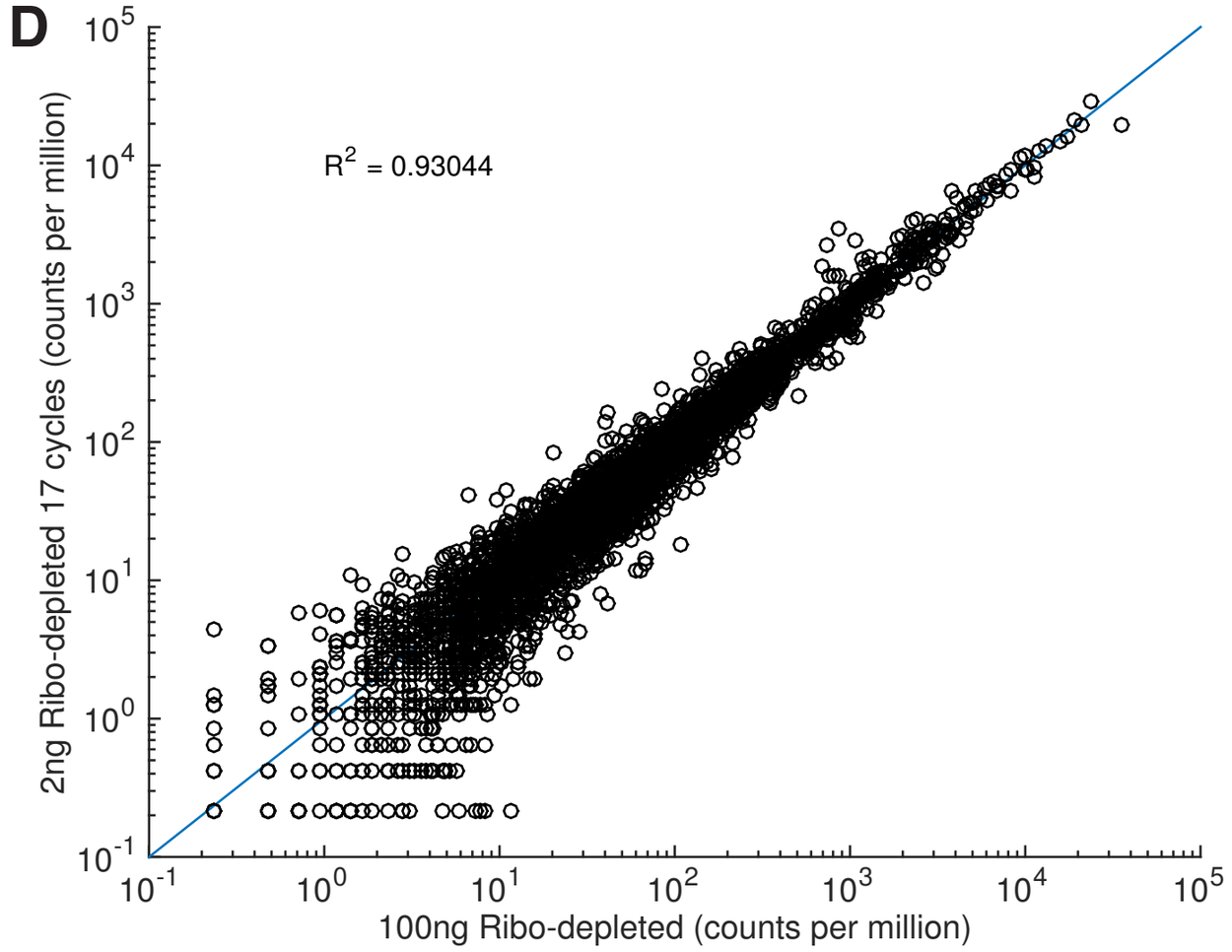
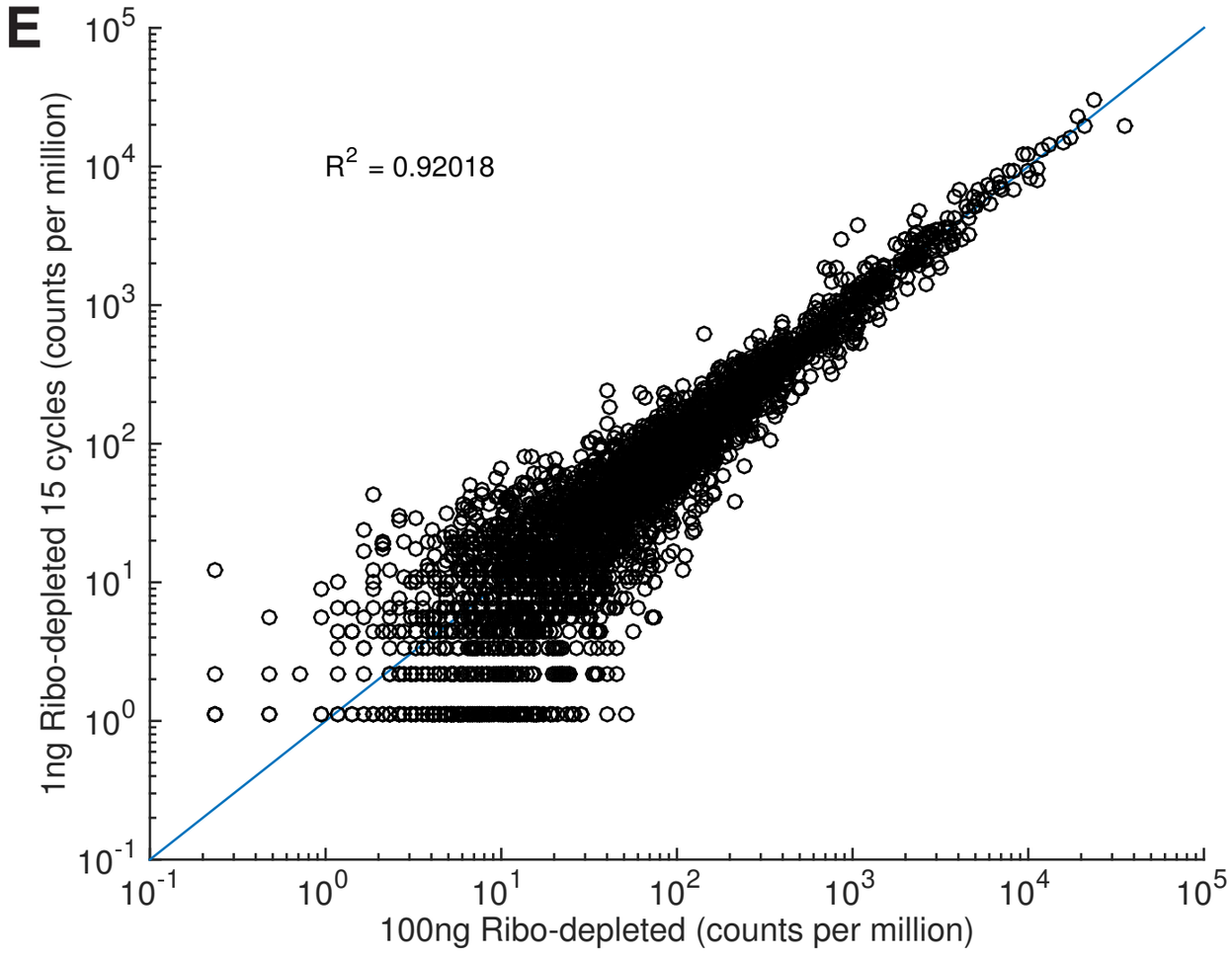
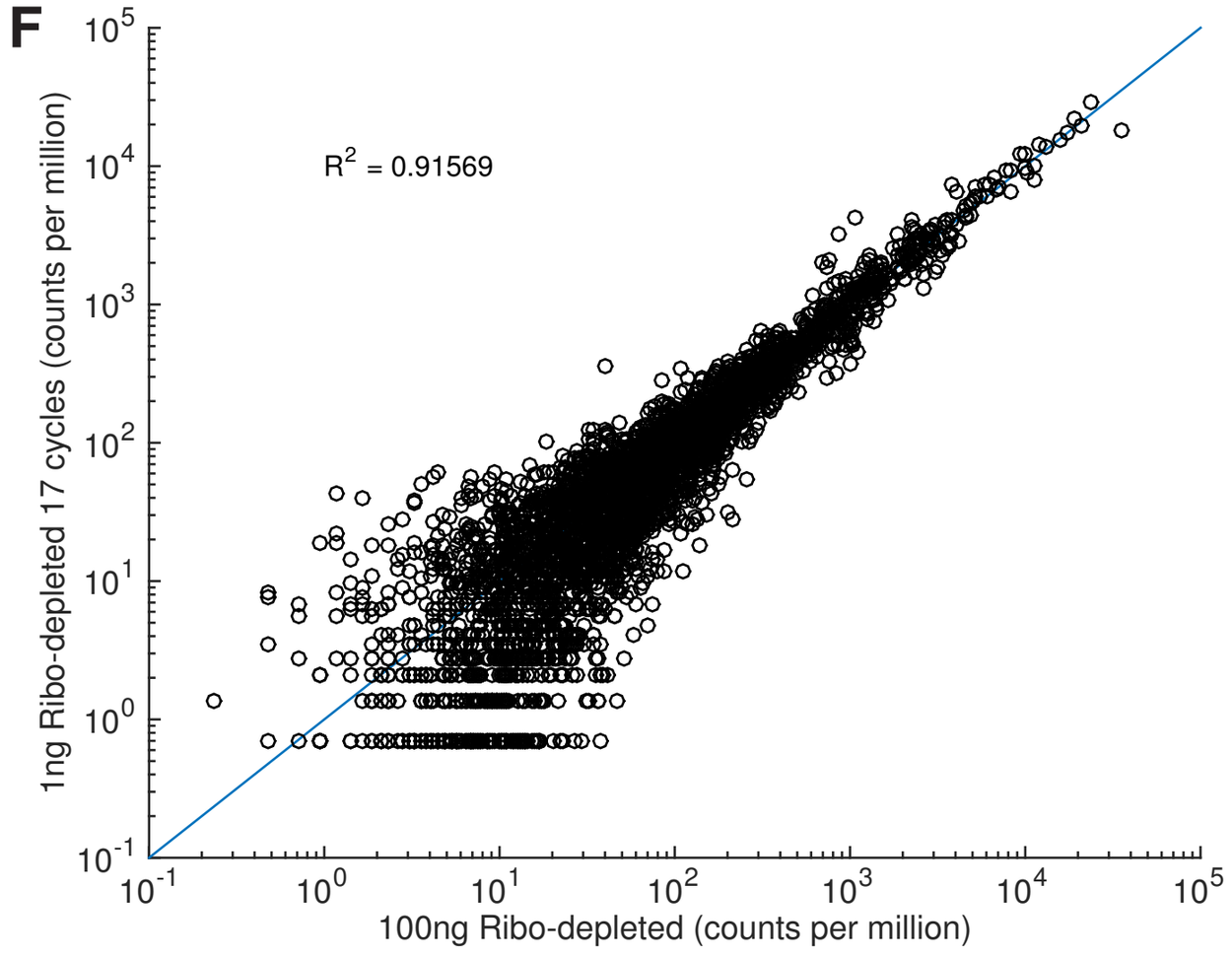
R^2 = 0.95064
5ng Ribo-depleted 15 cycles (counts per million)
100ng Ribo-depleted (counts per million)


B
R^2 = 0.94607
5ng Ribo-depleted 17 cycles (counts per million)
100ng Ribo-depleted (counts per million)

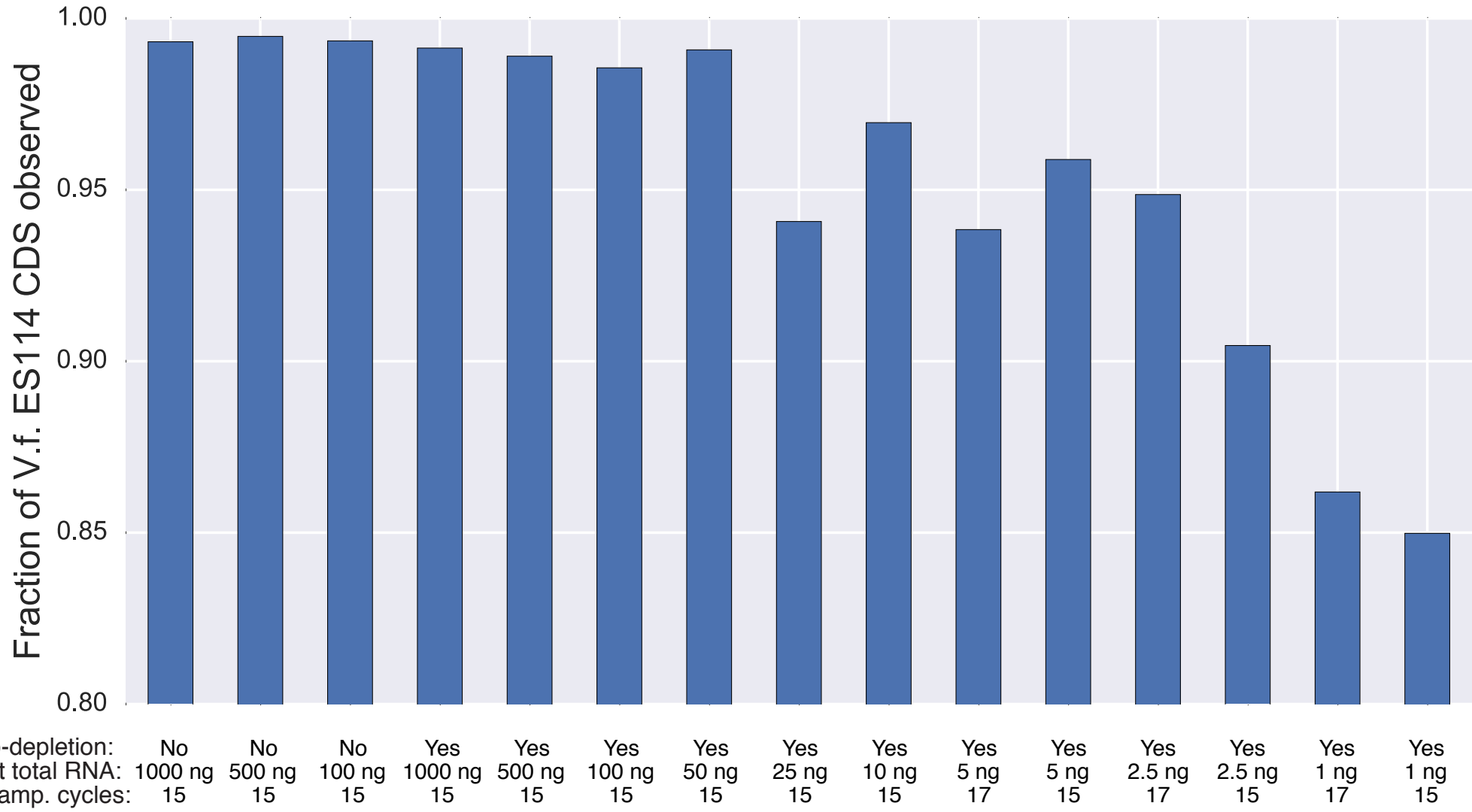

C
R^2 = 0.92878
2ng Ribo-depleted 15 cycles (counts per million)
100ng Ribo-depleted (counts per million)


D
R^2 = 0.93044
2ng Ribo-depleted 17 cycles (counts per million)
100ng Ribo-depleted (counts per million)


E
R^2 = 0.92018
1ng Ribo-depleted 15 cycles (counts per million)
100ng Ribo-depleted (counts per million)


F
R^2 = 0.91569
1ng Ribo-depleted 17 cycles (counts per million)
100ng Ribo-depleted (counts per million)

A


Fraction of V.f. ES114 CDS observed
1.00
0.95
0.90
0.85
0.80
Ribo-depletion: No No No Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes
Input total RNA: 1000 ng 500 ng 100 ng 1000 ng 500 ng 100 ng 50 ng 25 ng 10 ng 5 ng 5 ng 2.5 ng 2.5 ng 1 ng 1 ng
Lib. amp. cycles: 15 15 15 15 15 15 15 15 15 17 15 17 15 17 15

B

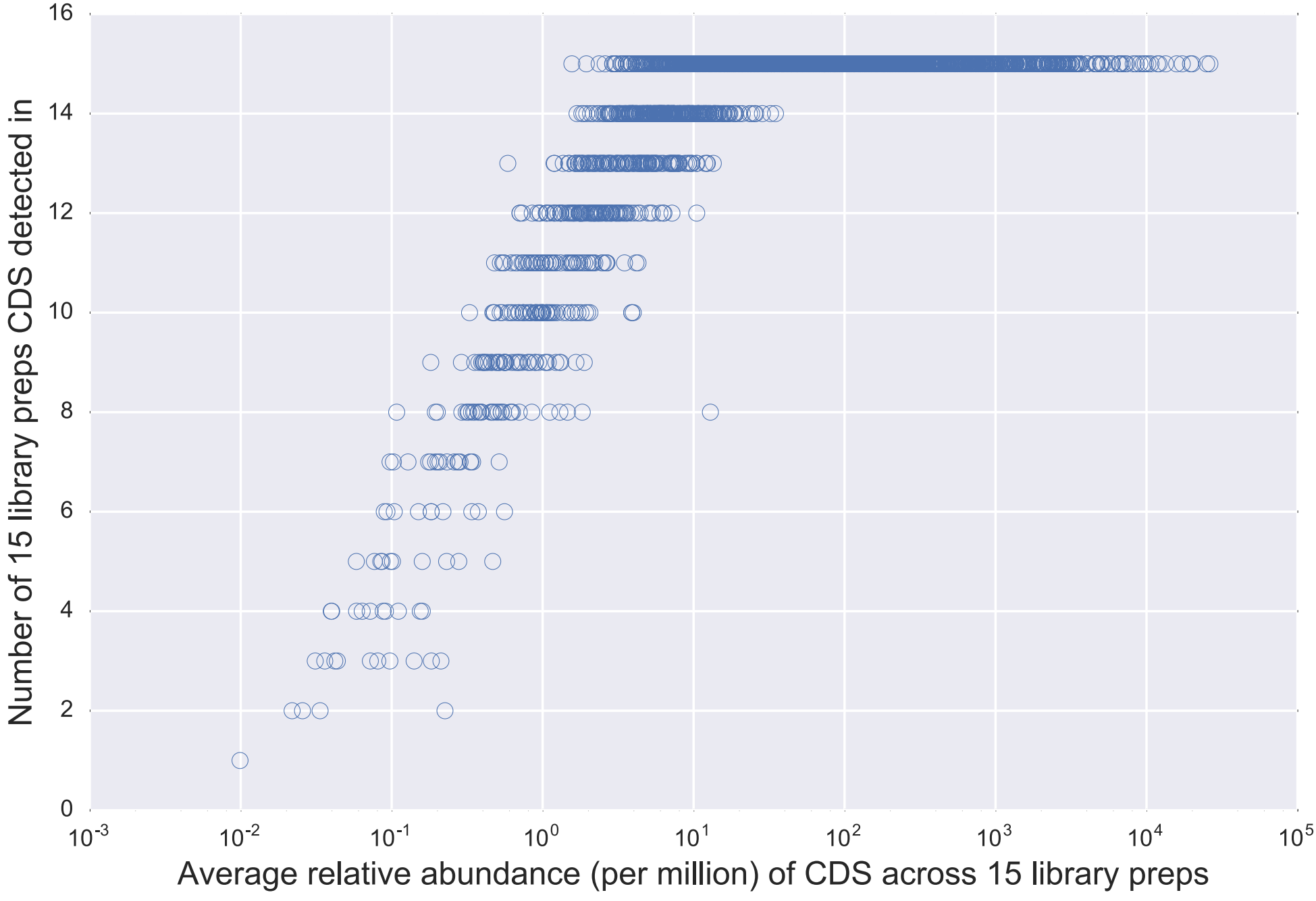

Number of 15 library preps CDS detected in
16
14
12
10
8
6
4
2
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}
Average relative abundance (per million) of CDS across 15 library preps

Table S1. Summary of *V. fischeri* samples analyzed by RNA-Seq.

Sample name	Accession no.	Description
1000ng_NoRibo	GSM2131511	Low-biomass test with 1000ng total RNA and no ribodepletion
500ng_NoRibo	GSM2131512	Low-biomass test with 500ng total RNA and no ribodepletion
100ng_NoRibo	GSM2131513	Low-biomass test with 100ng total RNA and no ribodepletion
1000ng_Ribodpl	GSM2131514	Low-biomass test with 1000ng total RNA and ribodepletion
500ng_Ribodpl	GSM2131515	Low-biomass test with 500ng total RNA and ribodepletion
50ng_Ribodpl	GSM2131516	Low-biomass test with 50ng total RNA and ribodepletion
25ng_Ribodpl	GSM2131517	Low-biomass test with 25ng total RNA and ribodepletion
10ng_Ribodpl	GSM2131518	Low-biomass test with 10ng total RNA and ribodepletion
5ng_Ribodpl_17cyc	GSM2131519	Low-biomass test with 5ng total RNA and ribodepletion (2 additional cycles)
5ng_Ribodpl_15cyc	GSM2131520	Low-biomass test with 5ng total RNA and ribodepletion
2ng_Ribodpl_17cyc	GSM2131521	Low-biomass test with 2.5ng total RNA and ribodepletion (2 additional cycles)
2ng_Ribodpl_15cyc	GSM2131522	Low-biomass test with 2.5ng total RNA and ribodepletion
1ng_Ribodpl_17cyc	GSM2131523	Low-biomass test with 1ng total RNA and ribodepletion (2 additional cycles)
1ng_Ribodpl_15cyc	GSM2131524	Low-biomass test with 1ng total RNA and ribodepletion
Plk1_Ribodpl	GSM2131525	Planktonic replicate 1 (100ng total RNA and ribodepletion)
Plk2_Ribodpl	GSM2131526	Planktonic replicate 2 (100ng total RNA and ribodepletion)
Plk3_Ribodpl	GSM2131527	Planktonic replicate 3 (100ng total RNA and ribodepletion)
Swt1_Ribodpl	GSM2131528	SWT replicate 1 (100ng total RNA and ribodepletion)
Swt2_Ribodpl	GSM2131529	SWT replicate 2 (100ng total RNA and ribodepletion)
Swt3_Ribodpl	GSM2131530	SWT replicate 3 (100ng total RNA and ribodepletion)
Vnt1_Ribodpl	GSM2131531	Vented replicate 1 (100ng total RNA and ribodepletion)
Vnt2_Ribodpl	GSM2131532	Vented replicate 2 (100ng total RNA and ribodepletion)
Vnt3_Ribodpl	GSM2131533	Vented replicate 3 (100ng total RNA and ribodepletion)

Table S2. Relative expression levels of bioluminescence and motility genes in *V. fischeri*

Gene*	Vnt / Plk log2(fc)†	FDR-adjusted *p*-value†	Description
Bioluminescence			
***luxR* (VF_A0925)**	2.3	1.7 E-06	LuxR family transcriptional regulator
***luxI* (VF_A0924)**	4.5	6.0 E-09	3-oxo-C6-HSL autoinducer synthesis protein
***luxC* (VF_A0923)**	4.8	1.8 E-12	acyl-CoA reductase
***luxD* (VF_A0922)**	5.0	9.5 E-14	acyl transferase
***luxA* (VF_A0921)**	5.4	1.5 E-14	luciferase alpha chain
***luxB* (VF_A0920)**	5.5	4.6 E-11	luciferase beta chain
***luxE* (VF_A0919)**	4.9	3.9 E-10	long-chain-fatty-acid ligase
***luxG* (VF_A0918)**	4.3	9.1 E-15	FMN reductase
Flagellar motility			
***motA* (VF_0714)**	-2.2	1.5 E-05	flagellar motor protein
***motB* (VF_0715)**	-2.8	9.6 E-07	flagellar motor protein
***flhA* (VF_1837)**	-2.2	6.0 E-03	flagellar biosynthesis protein
***flhB* (VF_1839)**	-3.0	9.0 E-05	flagellar biosynthesis protein
***fliR* (VF_1840)**	-2.6	2.0 E-06	flagellar biosynthesis protein
***fliQ* (VF_1841)**	-3.0	4.4 E-05	flagellar biosynthesis protein
***fliP* (VF_1842)**	-3.2	1.4 E-04	flagellar biosynthesis protein
***fliO* (VF_1843)**	-3.1	4.8 E-04	flagellar biosynthesis protein
***fliN* (VF_1844)**	-2.9	3.2 E-03	flagellar motor switch protein
***fliM* (VF_1845)**	-2.7	7.1 E-04	flagellar motor switch protein
***fliK* (VF_1847)**	-2.6	9.5 E-06	flagellar hook length control protein
***fliJ* (VF_1848)**	-2.5	1.2 E-03	flagellar biosynthesis chaperone
***fliI* (VF_1849)**	-1.7	2.4 E-03	flagellum-specific ATP synthase
***fliG* (VF_1851)**	-3.4	1.1 E-03	flagellar motor switch protein
***fliF* (VF_1852)**	-3.6	4.4 E-07	flagellar MS-ring protein
***fliE* (VF_1853)**	-2.6	4.0 E-05	flagellar hook-basal body protein
***flrC* (VF_1854)**	-3.5	9.0 E-04	two-component response regulator
***flrB* (VF_1855)**	-2.8	7.9 E-04	sensory histidine kinase
***fliD* (VF_1860)**	-3.0	7.5 E-09	flagellar capping protein
***flaG* (VF_1861)**	-3.9	9.8 E-10	flagellin
***flaE* (VF_1862)**	-2.4	3.8 E-03	flagellin
***flaD* (VF_1863)**	-3.1	3.9 E-05	flagellin
***flaA* (VF_1866)**	-2.2	7.7 E-03	flagellin
***flgK* (VF_1868)**	-1.3	7.0 E-03	flagellar hook-associated protein
***flgI* (VF_1870)**	-2.3	2.0 E-04	flagellar basal body P-ring biosynthesis protein
***flgG* (VF_1872)**	-2.7	3.0 E-05	flagellar basal body rod protein
***flgM* (VF_1881)**	-1.4	2.9 E-03	flagellar anti-sigma-28 factor
***flgN* (VF_1882)**	-2.0	3.1 E-04	chaperone
***flaF* (VF_2079)**	-3.4	2.7 E-04	flagellin
***motX* (VF_2317)**	-3.1	9.6 E-04	flagellar motor protein

* Orange: upregulated; Blue: down-regulated in squid relative to seawater.

† Genes listed showed differential expression between squid-associated (Vnt) and planktonic (Plk) *V. fischeri* of at least abs(log2(fc) > 1) and FDR-adjusted *p*-value < 0.01.