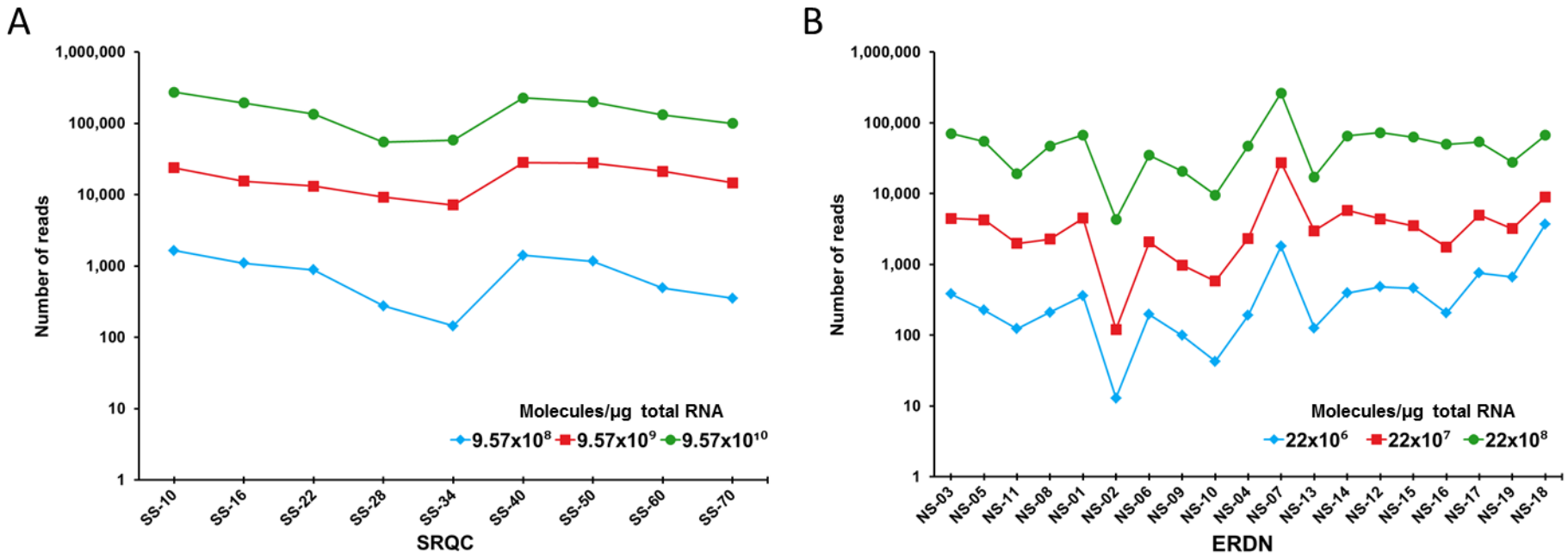

Supplementary Figure 1.1. *Sequencing efficiency of the individual spike-in oligonucleotides.* NGS results of three increasing concentrations of the size (A) and normalization (B) spike-in control sets. All spike-in controls were used at an equal concentration (blue, red, and green) in each test experiment that contained an identical miRNA background. Spike-in controls SS-19 and SS-25 were not ready at the time of these experiments. The NGS-library preparation was done without any size selection.

Name	molec/μl stock	DF	CF	Final DF	molec/μl final
SS-10	6.022E+13	1.61E+04	1.39	2.24E+04	2.69E+09
SS-16	6.022E+13	1.61E+04	0.30	4.84E+03	1.24E+10
SS-19	6.022E+13	1.61E+04	1.00	1.61E+04	3.75E+09
SS-22	6.022E+13	1.61E+04	0.33	5.36E+03	1.12E+10
SS-25	6.022E+13	1.61E+04	0.38	6.15E+03	9.79E+09
SS-28	6.022E+13	1.61E+04	0.13	2.04E+03	2.95E+10
SS-34	6.022E+13	1.61E+04	0.44	7.13E+03	8.45E+09
SS-40	6.022E+13	1.61E+04	0.71	1.15E+04	5.24E+09
SS-50	6.022E+13	1.61E+04	1.34	2.16E+04	2.79E+09
SS-60	6.022E+13	1.61E+04	1.13	1.82E+04	3.30E+09
SS-70	6.022E+13	1.61E+04	0.76	1.22E+04	4.94E+09

Supplementary Table 1.2. *Concentration-correction table for the size spike-in controls*
Name: short name for each size spike-in control; molec/μl stock: the number of molecules per μl at which each oligonucleotide was supplied (100 μmol/l); DF: the dilution factor that was needed to obtain an adequate final number of reads; CF: correction factor. An additional dilution factor that is needed to obtain a similar number of reads for all size spike-ins. Spike-in SS-19 is used as a reference (CF=1) to which the other spike-ins are scaled; Final DF: the final dilution factor, equaling DF × CF; molec/μl final: the number of molecules in the final spike-in mix, of which 1 μl is to be added per 5 μg of total RNA.

Name	molec/µl	DF	CF	Final DF	molec/µl
NS-18	6.02E+13	1.24E+03	1.15	1.43E+03	4.23E+10
NS-19	6.02E+13	2.48E+03	0.49	1.21E+03	4.96E+10
NS-17	6.02E+13	4.95E+03	0.97	4.82E+03	1.25E+10
NS-16	6.02E+13	9.90E+03	0.95	9.44E+03	6.38E+09
NS-15	6.02E+13	1.98E+04	1.18	2.34E+04	2.58E+09
NS-12	6.02E+13	3.96E+04	1.35	5.36E+04	1.12E+09
NS-14	6.02E+13	7.92E+04	1.18	9.35E+04	6.44E+08
NS-13	6.02E+13	1.58E+05	0.28	4.48E+04	1.34E+09
NS-7	6.02E+13	3.17E+05	4.70	1.49E+06	4.04E+07
NS-4	6.02E+13	6.34E+05	0.89	5.64E+05	1.07E+08
NS-10	6.02E+13	1.27E+06	0.18	2.26E+05	2.67E+08
NS-8	6.02E+13	1.79E+06	0.89	1.59E+06	3.78E+07
NS-9	6.02E+13	2.54E+06	0.39	9.92E+05	6.07E+07
NS-11	6.02E+13	3.59E+06	0.34	1.22E+06	4.94E+07
NS-6	6.02E+13	5.07E+06	0.65	3.29E+06	1.83E+07
NS-5	6.02E+13	7.17E+06	1.00	7.16E+06	8.40E+06
NS-2	6.02E+13	1.01E+07	0.08	8.45E+05	7.13E+07
NS-3	6.02E+13	1.43E+07	1.31	1.88E+07	3.20E+06
NS-1	6.02E+13	2.03E+07	1.25	2.54E+07	2.37E+06

Supplementary Table 1.3. *Concentration-correction table for the normalization spike-in controls*
Name: short name for each normalization spike-in control; molec/µl stock: the number of molecules per µl at which each oligonucleotide was supplied (100 µmol/l); DF: the dilution factor that is needed to obtain 2-fold decreasing read counts from top to bottom of the list; CF: correction factor. An additional dilution factor that is needed to correct for differences in sequencing efficiency. The average of all ERDN read counts is used as a reference (CF=1) to which the other spike-ins are scaled; Final DF: the final dilution factor, equaling DF × CF; molec/µl final: the number of molecules in the final spike-in mix, of which 1 µl is to be added per 5 µg of total RNA.