Supplemental Table 4. Divergence from expected non-labeled vs. ^{15}N-labeled nucleoside isoform distribution in cross-dilution series

Isoform divergence (non-labeled:^{15}N-labeled) and standard deviation (n=3) determined by manual Xcalibur™ quantification

Nucleoside	100:0	99:1	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90	1:99	0:100	Average
A	0.71 ±0.04	0.14 ±0.04	0.74 ±0.53	0.72 ±1.86	0.06 ±1.53	0.00 ±2.45	1.32 ±0.14	0.63 ±1.70	2.71 ±0.94	0.26 ±0.97	1.60 ±1.41	0.31 ±0.01	0.11 ±0.02	0.72 ±0.89
C	0.46 ±0.03	0.08 ±0.01	0.57 ±0.66	0.49 ±0.08	0.80 ±1.05	0.16 ±0.57	1.19 ±0.25	0.05 ±1.35	0.67 ±0.32	0.57 ±1.20	0.04 ±0.31	0.00 ±0.03	0.13 ±0.11	0.40 ±0.46
G	1.99 ±0.07	0.42 ±0.03	0.44 ±0.35	1.50 ±0.81	0.13 ±2.02	0.74 ±0.77	0.40 ±0.47	0.57 ±0.78	1.89 ±0.85	0.57 ±0.98	1.09 ±0.12	0.25 ±0.13	0.20 ±0.07	0.78 ±0.57
U	0.32 ±0.02	0.03 ±0.08	0.84 ±0.90	0.51 ±0.61	2.18 ±0.38	3.46 ±0.72	1.46 ±1.78	2.28 ±3.72	0.97 ±0.48	0.73 ±0.86	1.09 ±0.58	0.03 ±0.01	0.06 ±0.01	1.07 ±0.78
m^1A	0.92 ±0.12	0.47 ±0.21	0.27 ±0.09	1.42 ±0.84	1.64 ±0.46	2.97 ±0.80	2.43 ±0.45	3.11 ±0.54	3.83 ±0.62	0.22 ±0.77	2.36 ±0.22	0.39 ±0.12	0.24 ±0.26	1.56 ±0.42
Am	1.33 ±0.09	0.76 ±0.06	2.89 ±0.39	2.61 ±0.71	5.05 ±0.67	4.46 ±1.12	4.87 ±0.71	3.22 ±0.70	2.76 ±0.21	3.04 ±0.43	0.57 ±0.51	0.09 ±0.05	0.12 ±0.02	2.44 ±0.44
m^6A	0.55 ±0.08	0.15 ±0.07	0.82 ±1.25	0.37 ±2.05	2.15 ±0.20	0.32 ±5.38	2.27 ±0.67	0.81 ±0.64	0.99 ±2.17	0.48 ±0.83	0.36 ±0.34	0.02 ±0.09	0.09 ±0.03	0.72 ±1.06
Cm	0.23 ±0.02	0.18 ±0.02	2.63 ±1.19	5.53 ±1.11	5.71 ±0.19	8.07 ±1.84	6.78 ±1.09	8.17 ±2.13	7.15 ±0.60	5.49 ±1.12	2.85 ±1.20	0.33 ±0.11	0.13 ±0.10	4.10 ±0.83
m^3C	0.31 ±0.07	0.46 ±0.32	0.71 ±0.77	1.10 ±0.92	2.56 ±1.17	3.17 ±0.46	1.70 ±2.62	0.54 ±0.63	0.66 ±0.17	0.53 ±1.55	0.11 ±0.42	0.12 ±0.15	0.50 ±0.70	0.96 ±0.77
m^5C	0.18 ±0.03	0.27 ±0.05	1.94 ±0.39	4.50 ±0.60	5.22 ±1.20	7.44 ±1.04	7.15 ±1.22	9.99 ±0.66	7.49 ±1.08	3.94 ±1.26	3.66 ±0.52	0.18 ±0.07	0.03 ±0.01	4.00 ±0.62
m^1G	4.59 ±1.35	2.39 ±0.78	4.36 ±3.89	5.13 ±1.42	5.00 ±1.65	12.08 ±8.25	5.32 ±2.12	3.95 ±5.21	3.93 ±2.92	3.26 ±0.98	0.57 ±0.41	0.02 ±0.10	0.21 ±0.04	3.91 ±2.24
m^2G	1.35 ±0.15	0.45 ±0.05	0.47 ±0.45	1.87 ±0.38	0.55 ±0.61	2.90 ±1.98	2.74 ±2.21	3.07 ±0.47	3.89 ±1.22	0.27 ±0.57	2.10 ±0.28	0.37 ±0.17	0.28 ±0.07	1.56 ±0.66
Gm	1.21 ±0.15	0.45 ±0.09	1.12 ±0.61	1.62 ±0.43	3.38 ±0.19	2.78 ±0.20	2.76 ±0.83	1.92 ±0.44	2.25 ±1.72	1.85 ±0.94	0.18 ±0.19	0.06 ±0.06	0.10 ±0.01	1.51 ±0.45
m^7G	0.29 ±0.01	0.16 ±0.12	1.80 ±0.59	3.73 ±1.45	3.98 ±1.18	4.17 ±2.13	6.62 ±1.57	6.32 ±1.82	3.73 ±1.04	4.67 ±1.68	3.79 ±1.37	0.41 ±0.02	0.09 ±0.04	3.06 ±1.00
m^5U	0.11 ±0.03	0.40 ±0.32	0.66 ±0.70	1.37 ±1.88	1.82 ±1.77	3.93 ±1.36	3.17 ±1.10	3.84 ±0.81	3.91 ±1.96	1.05 ±0.31	1.97 ±0.88	0.02 ±0.05	0.05 ±0.02	1.72 ±0.86
Ψ	0.52 ±0.26	0.00 ±0.02	0.35 ±0.19	1.13 ±1.23	1.58 ±0.63	1.61 ±1.69	2.23 ±1.10	3.47 ±0.68	2.50 ±0.42	1.18 ±0.40	1.28 ±0.49	0.00 ±0.10	0.11 ±0.03	1.23 ±0.56
D	0.07 ±0.00	0.15 ±0.03	0.83 ±0.23	2.34 ±0.36	1.87 ±0.12	2.53 ±0.12	2.85 ±0.17	3.62 ±0.35	3.33 ±0.41	1.68 ±0.46	1.67 ±0.36	0.07 ±0.05	0.02 ±0.00	1.62 ±0.20
I	0.10 ±0.09	0.44 ±0.02	4.13 ±0.40	7.89 ±0.18	9.69 ±1.07	13.02 ±0.22	13.34 ±0.28	14.57 ±0.98	13.39 ±0.55	8.87 ±0.93	5.67 ±0.66	0.87 ±0.03	0.30 ±0.07	7.10 ±0.42
Im	0.26 ±0.06	0.04 ±0.06	0.28 ±1.03	2.59 ±0.75	1.61 ±0.83	3.40 ±1.38	2.36 ±0.51	1.86 ±1.64	3.19 ±0.14	1.72 ±1.43	2.19 ±0.75	0.00 ±0.09	0.01 ±0.01	1.50 ±0.67
Average	0.87 ±0.17	0.41 ±0.14	1.38 ±0.96	2.46 ±0.95	2.79 ±0.98	4.01 ±1.79	3.73 ±1.28	3.74 ±1.49	3.56 ±1.00	2.16 ±1.11	1.85 ±0.62	0.20 ±0.07	0.15 ±0.08	2.10 ±0.73

Isoform divergence (non-labeled:^{15}N-labeled) and standard deviation (n=3) determined by automated pyQms quantification

Nucleoside	100:0	99:1	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90	1:99	0:100	Average
A	0.73 ±0.04	0.18 ±0.04	1.05 ±0.58	0.23 ±1.90	0.74 ±1.52	0.67 ±2.31	1.43 ±1.68	0.01 ±1.71	2.08 ±1.04	0.14 ±0.91	1.36 ±1.34	0.27 ±0.01	0.04 ±0.07	0.69 ±1.01
C	1.41 ±0.13	0.09 ±0.01	0.69 ±0.59	0.71 ±0.07	1.07 ±1.04	0.55 ±0.63	1.71 ±0.38	0.43 ±1.34	0.28 ±0.31	0.85 ±1.21	0.11 ±0.25	0.02 ±0.04	0.08 ±0.14	0.62 ±0.47
G	2.04 ±0.07	0.46 ±0.02	0.67 ±0.36	1.14 ±0.81	0.68 ±2.05	1.07 ±0.61	1.12 ±0.45	1.08 ±2.21	1.02 ±0.99	0.57 ±0.74	1.09 ±0.09	0.25 ±0.14	0.20 ±0.16	0.88 ±0.67
U	0.33 ±0.02	0.05 ±0.08	0.93 ±0.90	0.65 ±0.54	2.33 ±0.49	3.73 ±0.88	1.74 ±1.72	1.93 ±3.82	0.69 ±0.50	0.99 ±0.86	0.95 ±0.55	0.04 ±0.02	0.00 ±0.00	1.11 ±0.80
m^1A	2.75 ±0.16	0.51 ±0.21	0.27 ±0.04	1.42 ±0.92	1.64 ±0.43	2.97 ±0.85	2.43 ±0.40	3.11 ±0.61	3.83 ±0.57	0.22 ±0.79	2.36 ±0.19	0.39 ±0.12	0.24 ±0.19	1.70 ±0.42
Am	0.91 ±0.79	0.80 ±0.07	3.14 ±0.39	3.04 ±0.70	5.53 ±0.66	5.05 ±1.15	5.74 ±0.64	3.91 ±0.69	3.25 ±0.14	3.53 ±0.41	0.82 ±0.44	0.12 ±0.05	0.00 ±0.00	2.76 ±0.47
m^6A	0.35 ±0.31	0.18 ±0.07	1.02 ±1.28	0.74 ±2.10	2.54 ±0.20	0.89 ±5.35	3.01 ±0.49	1.53 ±0.56	1.64 ±2.22	0.99 ±0.81	0.63 ±0.32	0.05 ±0.10	0.00 ±0.00	1.04 ±1.06
Cm	0.00 ±0.00	0.18 ±1.51	2.63 ±1.20	5.53 ±1.19	5.71 ±0.18	8.07 ±1.83	6.78 ±0.92	8.17 ±2.22	7.15 ±0.57	5.49 ±1.07	2.85 ±1.26	0.33 ±0.11	0.13 ±0.13	4.08 ±0.94
m^3C	1.14 ±1.02	0.48 ±0.31	0.89 ±0.72	1.47 ±0.89	3.02 ±1.27	3.64 ±0.30	2.10 ±2.51	0.15 ±0.68	1.02 ±0.02	0.71 ±1.59	0.05 ±0.42	0.11 ±0.15	0.59 ±0.60	1.18 ±0.81
m^5C	0.04 ±0.06	0.27 ±0.09	1.94 ±0.35	4.50 ±0.70	5.22 ±1.25	7.44 ±1.09	7.15 ±1.25	9.99 ±0.66	7.49 ±1.16	3.94 ±1.33	3.66 ±0.47	0.18 ±0.07	0.03 ±0.02	3.99 ±0.66
m^1G	1.05 ±1.81	0.45 ±1.31	1.73 ±7.17	5.63 ±1.34	5.67 ±1.73	12.82 ±8.34	6.04 ±2.05	4.47 ±5.17	4.50 ±2.86	3.66 ±0.88	0.85 ±0.35	0.07 ±0.14	0.00 ±0.00	3.61 ±2.55
m^2G	0.88 ±0.77	1.00 ±0.00	0.47 ±0.46	1.87 ±0.35	0.55 ±0.60	2.90 ±2.71	2.74 ±2.08	3.07 ±0.38	3.89 ±1.87	0.27 ±0.59	2.10 ±0.27	0.37 ±0.87	0.28 ±0.00	1.57 ±0.84
Gm	0.81 ±0.72	0.04 ±0.83	1.42 ±0.66	2.17 ±0.49	4.05 ±0.29	3.46 ±0.41	3.54 ±0.82	2.56 ±0.49	2.87 ±1.62	2.30 ±0.90	0.43 ±0.24	0.68 ±0.55	0.00 ±0.00	1.87 ±0.62
m^7G	0.20 ±0.18	0.16 ±0.46	1.80 ±0.63	3.73 ±1.56	3.98 ±1.18	4.17 ±2.15	6.62 ±1.53	6.32 ±1.84	3.73 ±2.11	4.67 ±1.64	3.79 ±1.37	0.41 ±0.80	0.09 ±0.00	3.05 ±1.19
m^5U	0.00 ±0.00	0.40 ±0.03	0.66 ±0.75	1.37 ±1.85	1.82 ±1.70	3.93 ±1.28	3.17 ±1.11	3.84 ±0.96	3.91 ±1.82	1.05 ±0.35	1.97 ±0.95	0.04 ±0.05	0.00 ±0.00	1.70 ±0.83
Ψ	0.53 ±0.26	0.02 ±0.02	0.35 ±0.26	1.13 ±1.27	1.58 ±0.65	1.61 ±1.68	2.23 ±1.25	3.47 ±0.82	2.50 ±0.45	1.18 ±0.35	1.28 ±0.49	0.00 ±0.10	0.11 ±0.00	1.23 ±0.58
D	0.07 ±0.00	0.15 ±0.03	0.83 ±0.22	2.34 ±0.35	1.87 ±0.18	2.53 ±0.05	2.85 ±0.32	3.62 ±0.24	3.33 ±0.50	1.68 ±0.41	1.67 ±0.40	0.40 ±0.52	0.00 ±0.00	1.64 ±0.25
I	0.00 ±0.00	0.44 ±0.34	4.13 ±0.41	7.89 ±0.19	9.69 ±1.07	13.02 ±0.21	13.34 ±0.34	14.57 ±0.94	13.39 ±0.60	8.87 ±0.93	5.67 ±0.67	0.87 ±0.02	0.30 ±0.07	7.09 ±0.45
Im	0.00 ±0.00	0.04 ±0.06	0.28 ±1.03	2.59 ±0.72	1.61 ±0.82	3.40 ±1.32	2.36 ±0.43	1.86 ±1.85	3.19 ±0.11	1.72 ±1.40	2.19 ±0.73	0.00 ±0.09	0.01 ±0.00	1.48 ±0.66
Average	0.70 ±0.30	0.31 ±0.29	1.31 ±0.95	2.54 ±0.94	3.12 ±0.91	4.31 ±1.75	4.01 ±1.07	3.90 ±1.43	3.67 ±1.02	2.26 ±0.90	1.78 ±0.57	0.24 ±0.21	0.11 ±0.07	2.17 ±0.80