

SUPPLEMENTARY INFORMATION

Supplementary Figure Legends

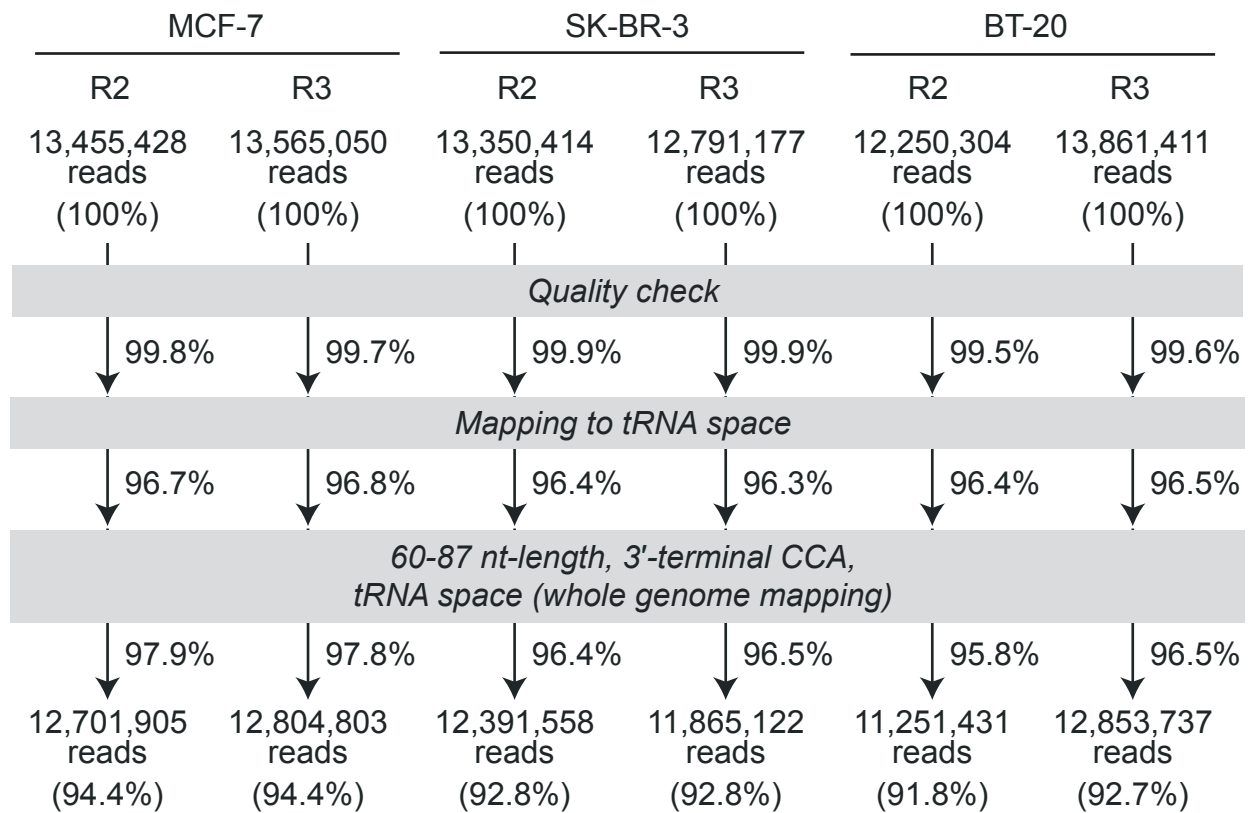
Fig. S1. Flow diagram of the YAMAT-seq read numbers of two (R2 and R3) of the triplicate filtered by the indicated sequence analyses.

Fig. S2. Scatter plots of tRNA reads per million between two libraries out of triplicates of YAMAT-seq.

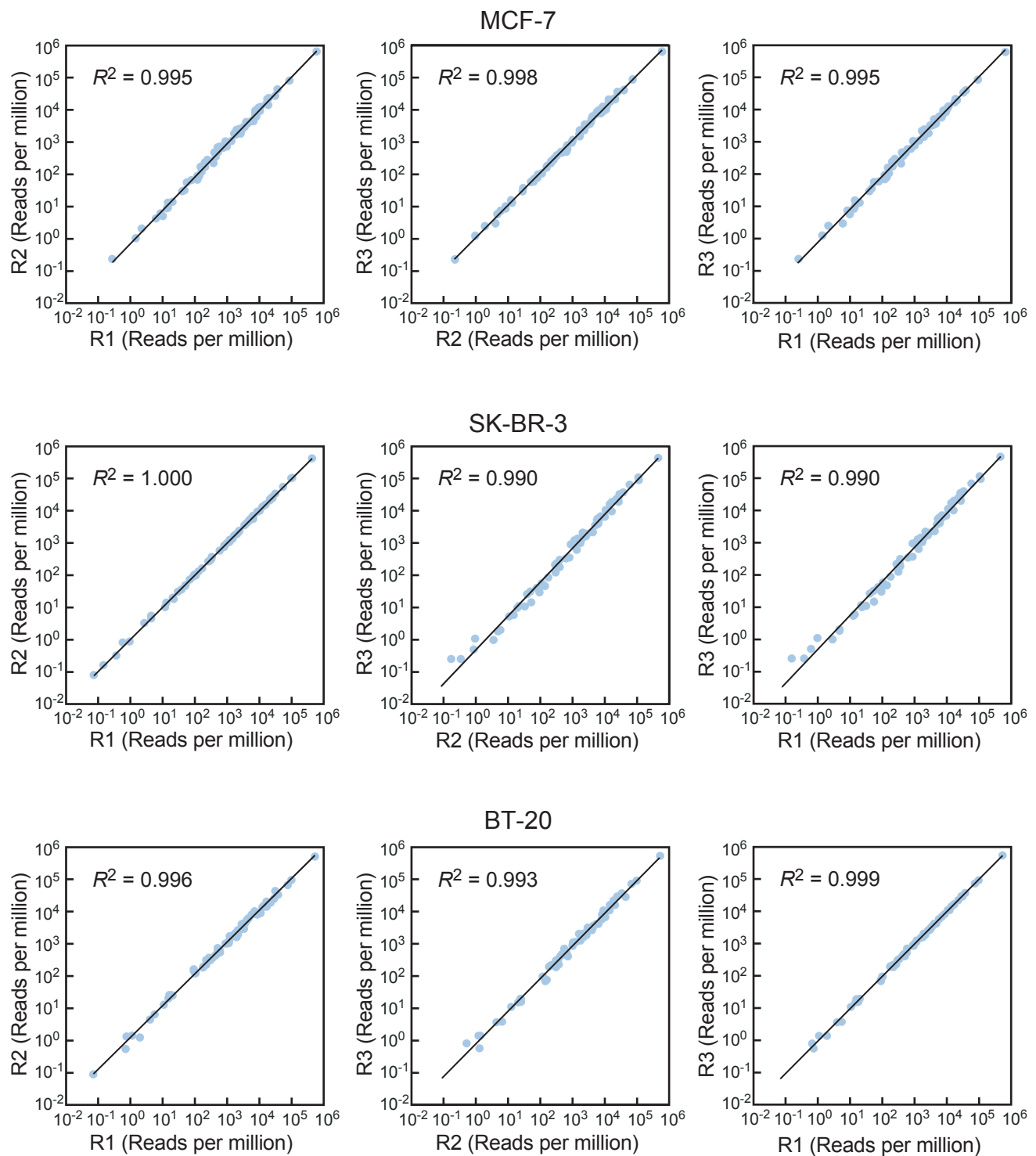
Fig. S3. Comparison of tRNA levels estimated by Northern blot and YAMAT-seq.

(A) Synthetic human cyto tRNA^{LysCUU}, cyto tRNA^{AspGUC}, mt tRNA^{AlaUGC}, and mt tRNA^{GluUUC} were synthesized by Integrated DNA Technologies and the indicated amounts of the tRNAs and 500 ng of MCF-7 total RNA were subjected to Northern blots using the following 5'-end labeled antisense probes: cyto tRNA^{LysCUU}, 5'-GTCTCATGCTCTACCGACT-3'; cyto tRNA^{AspGUC}, 5'-GAACCCCGGTCTCCCGCGTGA-3'; mt tRNA^{AlaUGC}, 5'-GGACTGCAAACCCCACT-3'; and mt tRNA^{GluUUC}, 5'-GGACTACAACCACGACCAAT-3'.

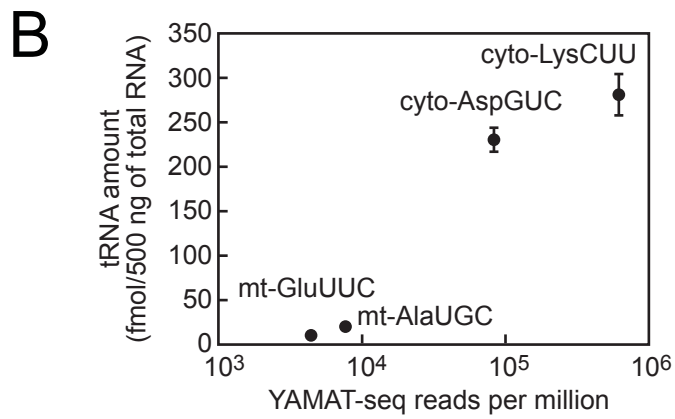
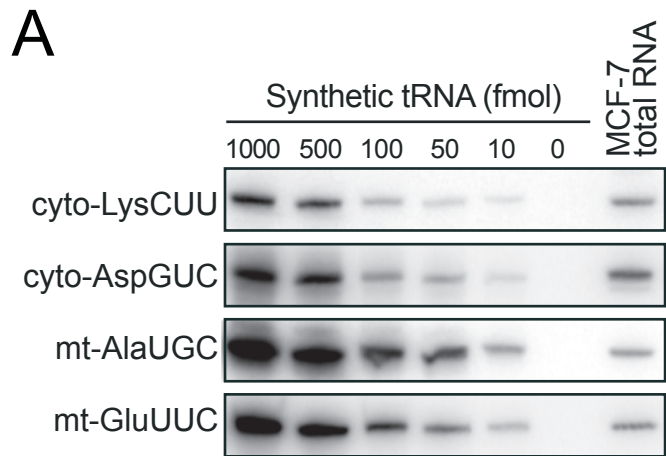
(B) Band intensities of Northern blots were measured using Typhoon 9400 and ImageQuant ver. 5.2 (GE Healthcare). Based on standard curves obtained from dilution series of synthetic RNAs, the amounts of respective tRNAs in MCF-7 total RNA were determined. YAMAT-seq reads per million (the average of triplicate) obtained from MCF-7 cells were plotted against the determined tRNA amounts (the average of three independent Northern blot analyses with bars showing the SD).



Shigematsu and Honda et al. Supplementary Figure S1



Shigematsu and Honda et al. Supplementary Figure S2



Shigematsu and Honda et al. Supplementary Figure S3

Supplementary Tables

Table S1. tRNA species cloned from Bands 1–3 in Fig. 2D.

Band 1 (total 22 clones)		Band 2 (total 12 clones)		Band 3 (total 9 clones)	
tRNA species	clones	tRNA species	clones	tRNA species	clones
cyto tRNA ^{LysCUU}	7	mt tRNA ^{ValUAC}	4	mt tRNA ^{SerGCU}	7
cyto tRNA ^{AspGUC}	6	mt tRNA ^{MetCAU}	3	mt tRNA ^{GlnUUG}	1
cyto tRNA ^{ValCAC}	2	mt tRNA ^{AspGUC}	2	mt tRNA ^{MetCAU}	1
cyto tRNA ^{AsnGUU}	1	mt tRNA ^{GlyUCC}	2		
cyto tRNA ^{GluCUC}	1	mt tRNA ^{LysUUU}	1		
cyto tRNA ^{LeuAAG}	1				
cyto tRNA ^{ValAAC}	1				
mt tRNA ^{GlyUCC}	1				
mt tRNA ^{ProUGG}	1				
mt tRNA ^{ValUAC}	1				

Table S2. YAMAT-seq sequence reads (reads per million) of three cell lines mapped to cyto tRNA isoacceptors

Isoacceptors	Gene copy number	MCF-7			SK-BR-3			BT-20		
		R1	R2	R3	R1	R2	R3	R1	R2	R3
cyto-AlaAGC	29	1410.34	1085.68	1114.99	294.82	277.64	124.91	2124.55	2673.78	1819.54
cyto-AlaCGC	5	122.00	87.73	94.82	49.99	46.52	32.29	103.79	124.78	91.57
cyto-AlaUGC	9	3318.15	2910.07	3268.92	1260.37	1196.72	1008.02	1799.23	2152.32	1543.28
cyto-ArgACG	7	122.27	67.34	69.62	52.29	49.52	14.45	99.68	119.23	92.50
cyto-ArgCCG	4	2665.45	1829.44	1853.69	1258.86	1258.96	626.35	894.06	1010.19	883.09
cyto-ArgCCU	5	993.38	721.55	759.81	605.34	595.00	340.94	1601.25	1889.71	1490.85
cyto-ArgUCG	6	769.53	661.40	607.38	331.70	323.19	215.01	464.60	480.14	463.23
cyto-ArgUCU	6	3668.10	3012.97	3297.33	2424.52	2416.29	1624.23	2748.34	3203.66	2631.14
cyto-AsnAUU	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cyto-AsnGUU	32	4347.12	3688.36	3626.45	108.69	99.27	46.06	580.27	659.75	517.21
cyto-AspGUC	19	87232.52	78763.43	83580.91	108711.92	107584.51	90864.84	96779.10	97206.19	92465.08
cyto-CysGCA	30	363.65	251.98	256.83	344.81	374.16	185.77	183.49	195.71	193.68
cyto-GlnCUG	20	4544.92	3878.93	3901.57	1642.35	1633.52	1016.30	2555.58	2802.85	2440.51
cyto-GlnUUG	11	532.85	484.67	452.12	317.72	302.77	237.07	428.90	434.17	405.96
cyto-GluCUC	13	19132.66	14032.38	19600.56	15299.31	15533.99	9755.75	16491.84	20809.59	16075.71
cyto-GluUUC	13	7452.09	5700.78	7434.86	3567.12	3614.63	2181.18	3322.71	4348.73	3190.70
cyto-GlyCCC	7	7779.28	6941.14	9139.41	978.23	972.32	909.64	1193.08	1755.45	1233.58
cyto-GlyGCC	15	5761.16	4377.43	6052.44	4815.83	4816.05	3534.79	5661.99	8121.71	5595.51
cyto-GlyUCC	9	32270.64	26478.61	34009.45	26060.10	25199.39	19386.92	30281.67	43764.87	28330.61
cyto-HisGUG	11	1959.91	1766.76	1454.32	834.82	749.46	360.12	1805.62	1884.43	1599.38
cyto-IleAAU	14	80.03	65.37	59.58	20.74	20.02	11.33	14.90	20.66	14.53
cyto-IleGAU	3	0.00	0.00	0.00	0.07	0.08	0.00	0.07	0.09	0.00
cyto-IleUAU	5	0.27	0.24	0.24	0.00	0.00	0.00	0.00	0.00	0.00
cyto-LeuAAG	12	449.12	344.61	369.23	171.57	168.08	87.56	502.22	732.56	397.75
cyto-LeuCAA	7	41.02	30.03	29.51	11.88	10.29	5.32	89.93	145.53	67.74
cyto-LeuCAG	10	48.81	31.30	36.42	30.40	30.80	10.99	90.82	162.52	73.60
cyto-LeuUAA	7	6.31	4.27	2.98	4.47	4.62	1.86	5.49	6.53	3.67
cyto-LeuUAG	3	210.76	170.41	175.51	97.89	104.70	56.96	222.62	312.16	181.81
cyto-LysCUU	17	612826.57	631303.95	606849.93	436810.62	432333.73	439956.52	529006.66	512375.31	542050.54
cyto-LysUUU	16	17214.64	15395.26	16919.72	9007.81	9364.01	6626.80	13595.77	15024.81	13933.73
cyto-MetCAU	20	6773.21	4362.96	5646.79	4020.69	4081.58	2207.98	4097.66	5500.43	4079.11
cyto-PheGAA	12	10.14	4.98	5.81	22.19	18.56	9.80	17.92	25.40	15.08
cyto-ProAGG	10	20.41	14.15	13.03	4.39	5.51	2.03	10.71	12.88	10.55
cyto-ProCGG	4	136.70	86.86	78.89	89.39	89.71	30.17	173.74	186.85	188.68
cyto-ProUGG	7	380.90	223.60	215.54	130.95	131.37	47.16	297.31	313.24	294.47
cyto-SeCUCA	3	153.56	167.48	158.24	340.99	374.73	303.33	333.69	360.02	327.67
cyto-SerAGA	11	56.53	54.77	57.46	38.54	36.06	26.45	20.32	24.87	19.06
cyto-SerCGA	4	165.51	118.32	106.20	70.30	72.85	40.65	266.69	375.22	219.00
cyto-SerGCU	8	14.64	13.04	15.46	2.74	3.32	1.01	3.98	4.47	3.52
cyto-SerUGA	5	13.83	8.77	8.63	13.40	14.10	5.92	15.86	25.22	18.59
cyto-ThrAGU	10	8.59	6.24	7.46	0.36	0.32	0.25	0.69	0.54	0.78
cyto-ThrCGU	6	2.22	2.06	2.51	0.94	0.89	1.10	1.92	1.25	1.33
cyto-ThrUGU	6	1947.96	1714.52	1699.92	260.46	271.40	217.04	218.71	221.73	217.20
cyto-TrpCCA	9	1.48	1.03	1.26	0.14	0.16	0.25	0.76	1.34	0.55
cyto-TyrAUA	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cyto-TyrGUA	14	14.91	9.01	10.13	0.58	0.81	0.51	1.10	1.43	1.33
cyto-ValAAC	11	3033.06	2534.15	3298.59	1848.50	1846.50	1380.57	2741.55	4030.40	2560.91
cyto-ValCAC	16	8107.88	6175.81	8257.78	5780.59	5798.66	3969.96	6978.90	10430.82	6567.43
cyto-ValUAC	5	10855.18	9036.16	11711.16	5789.67	6061.63	4227.91	4147.64	5636.21	4026.13

Table S3. YAMAT-seq sequence reads (reads per million) of three cell lines mapped to mt tRNA isoacceptors

Isoacceptors	MCF-7			SK-BR-3			BT-20		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
mt-AlaUGC	7582.41	7927.63	7489.57	6500.31	6450.38	5574.50	8504.30	8166.70	8105.79
mt-ArgUCG	2025.31	2364.77	2404.08	6691.55	7025.20	6939.93	2117.41	1987.11	2017.29
mt-AsnGUU	9692.73	11408.76	10055.58	12923.99	13573.23	16209.89	10670.18	9041.38	10947.02
mt-AspGUC	913.01	1030.67	1060.05	2369.63	2304.70	1886.39	2593.68	2868.51	2446.37
mt-CysGCA	11084.67	12359.04	12612.57	15958.38	15870.63	19318.38	16554.92	16563.63	16442.52
mt-GlnUUG	19874.86	22513.60	20123.88	21250.90	21556.64	23065.73	28320.70	24623.35	29186.51
mt-GluUUC	4022.15	4149.95	5039.64	6599.43	5697.03	5897.26	3680.29	4461.43	3616.90
mt-GlyUCC	9430.06	10781.90	10291.30	32347.78	34894.78	38158.73	22309.72	18952.53	22263.28
mt-HisGUG	250.65	281.85	293.49	817.97	839.98	921.22	1091.41	1055.72	1072.16
mt-IleGAU	414.61	464.99	478.02	1245.03	1278.89	1106.23	928.72	1022.27	840.82
mt-LeuUAA	1700.33	1796.96	2201.02	5549.23	5717.62	5815.28	4603.52	6317.87	4026.45
mt-LeuUAG	7676.82	9288.61	8727.09	25095.91	25655.16	26487.18	16893.83	15565.15	16508.54
mt-LysUUU	9071.65	9537.58	8595.14	22411.73	23306.70	22750.90	16284.04	14502.81	16115.01
mt-MetCAU	18410.73	21149.94	20994.76	56180.65	55653.18	66434.95	36910.82	33949.17	36562.03
mt-PheGAA	243.73	270.63	273.63	1511.18	1356.61	1385.13	500.98	588.64	453.62
mt-ProUGG	2431.46	2499.54	2259.97	5135.71	5354.39	5318.24	3262.03	2944.53	3106.09
mt-SerGCU	7779.55	8911.43	9392.86	25666.17	27031.95	33982.72	12082.86	13988.23	10857.88
mt-SerUGA	513.71	672.15	487.13	2089.07	1947.15	2125.57	595.79	553.67	689.10
mt-ThrUGU	1002.72	995.50	956.75	2354.94	2382.90	2004.46	3025.67	3468.06	2704.66
mt-TrpUCA	618.05	721.79	588.70	10415.55	10216.88	10645.02	1921.70	1605.63	2031.82
mt-TyrGUA	205.46	231.58	246.23	1183.23	1068.35	1251.93	1149.63	1254.38	1118.73
mt-ValUAC	38035.08	42021.11	39115.10	102172.64	102929.25	111026.27	74020.43	66949.50	72549.57