

Deciphering tRNA Repertoires and Translation Coordination during Mouse Early Embryogenesis by ORACLE-tRNAseq

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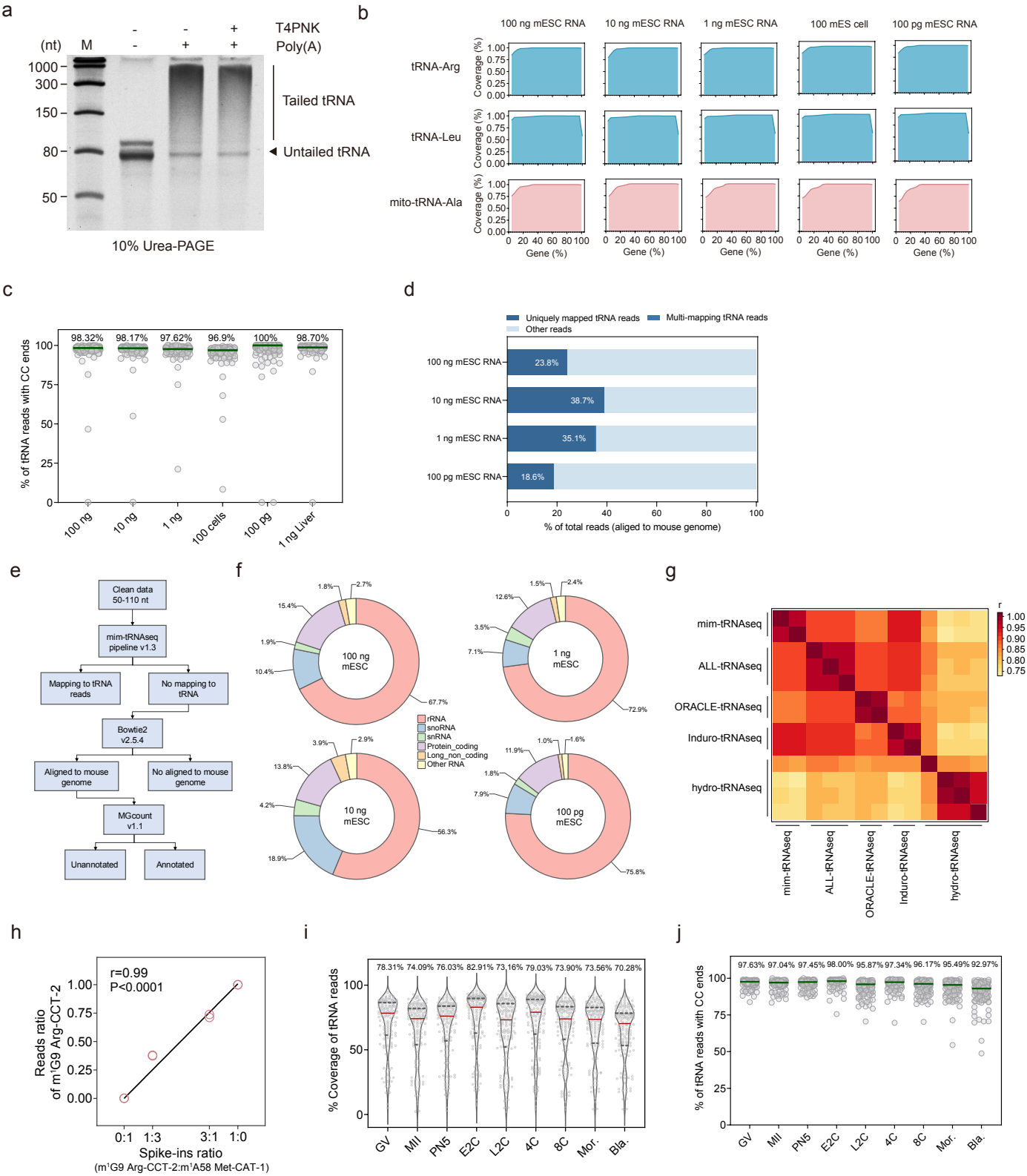
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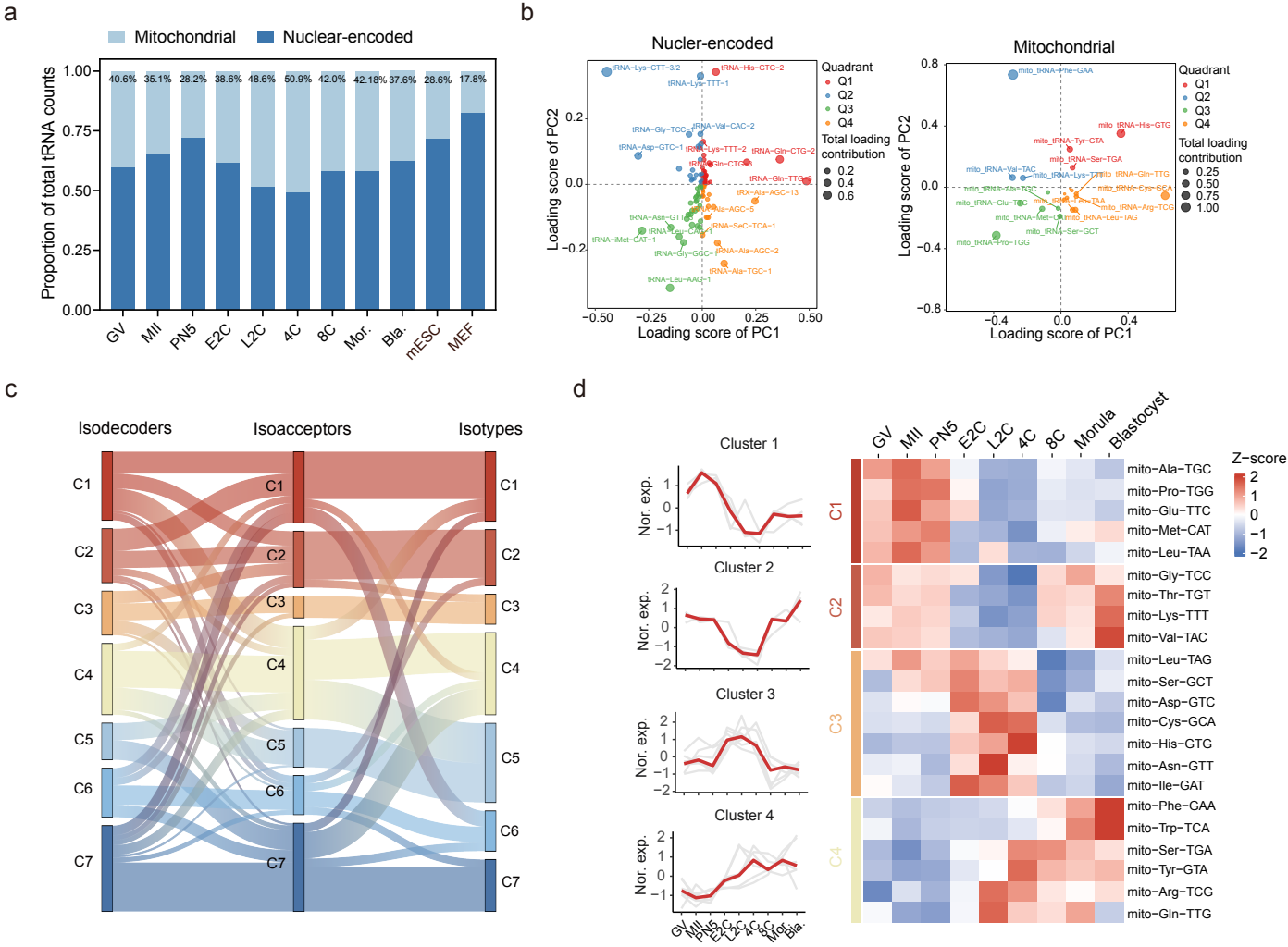
Supplementary Fig 1



Supplementary Fig 1. Effectiveness of ORACLE-tRNAseq for capturing tRNA molecules.

- a. Effect of T4PNK treatment on the tailing of enriched tRNA fragments. M, marker; Poly(A), Poly(A) polymerase.
 - b. Coverage of full-length tRNA-Arg, tRNA-Leu, and mito-tRNA-Ala at libraries generated from different inputs.
 - c. Proportion of tRNA reads containing the CC end at libraries generated from different inputs. Each dot represents a tRNA isodecoder.
 - d. Proportion of tRNA reads among all genome-mapped reads
 - e. Bioinformatic analysis pipeline of ORACLE-tRNAseq and the analysis workflow for non-tRNA reads
 - f. Proportion of various classes of annotated non-tRNA reads.
 - g. Heatmap shows Spearman correlation comparison of HEK-293T datasets generated by five tRNA-seq methods (at the tRNA isodecoder level).
 - h. Pearson Correlation between the input ratio of m¹A58 Met-CAT-1 and A58 Met-CAT-1 oligos and the misincorporation ratio detected by ORACLE-tRNAseq.
 - i. Sequencing coverage of tRNA reads in embryo samples. Each dot represents a tRNA isodecoder.
 - j. Proportion of tRNA reads containing the CC end at libraries generated from embryo samples. Each dot represents a tRNA isodecoder.
- a, c, h-j. Source data are provided as a Source Data file.

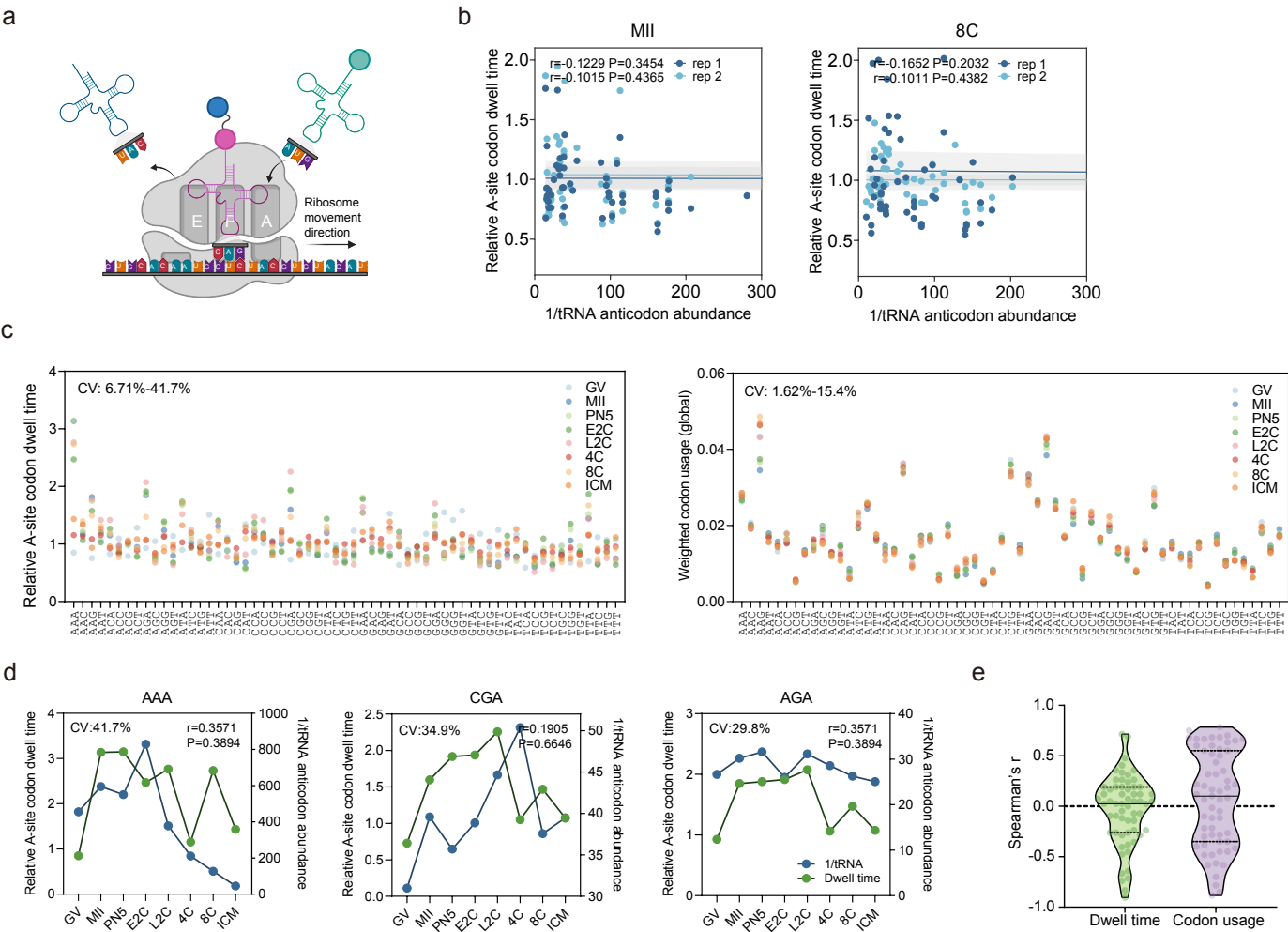
Supplementary Fig 2



Supplementary Fig 2. Abundance variation of embryonic tRNA pools.

- a. Ratio changes between nuclear-encoded tRNAs and mitochondrial tRNAs across different stages and cell types.
- b. The four-quadrant plot showing the combined contribution and direction of each tRNA isodecoder on PC1 and PC2.
- c. Sankey diagram showing the relationship between 7 clusters across three levels (isodecoders, isoacceptors and isotypes). Source data are provided as a Source Data file.
- d. K-means based clustering heatmap and line plots for each cluster of mitochondrial tRNAs, Z-score normalized by row.

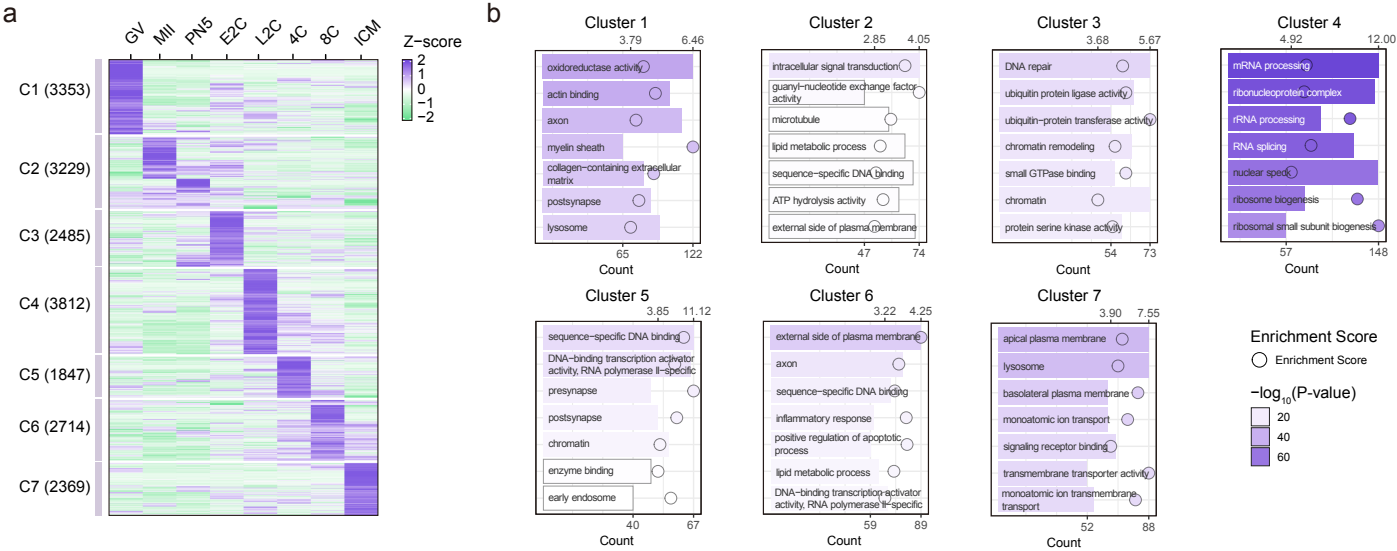
Supplementary Fig 3



Supplementary Fig 3. The relationship between changes in tRNA abundance and decoding time differences or codon usage shifts.

- a. Translation model illustrates the stepwise occupancy of tRNA at the three elongation sites on the ribosome, created in BioRender. Li, Y. (2026) <https://BioRender.com/plyrp5n>.
 - b. Spearman Correlation between relative A-site codon dwell time and $1/\text{tRNA}$ anticodon abundance. Fitted line, linear regression, shading, 95% confidence interval. P-values were calculated using a two-sided Spearman correlation test.
 - c. Differences in relative A-site codon dwell time and global weighted codon usage across developmental stages.
 - d. Line plots showing stage-wise changes in relative A-site codon dwell time for selected codons and $1/\text{tRNA}$ anticodon abundance, along with the Spearman correlation between the two.
 - e. Violin plots showing the Spearman correlation between relative A-site codon dwell time or global weighted codon usage and $1/\text{tRNA}$ anticodon abundance or tRNA anticodon abundance across developmental stages for all codons. Each dot represents a codon. Solid lines represent the median, and dashed lines indicate the quartiles.
- b-e. Source data are provided as a Source Data file.

Supplementary Fig 4

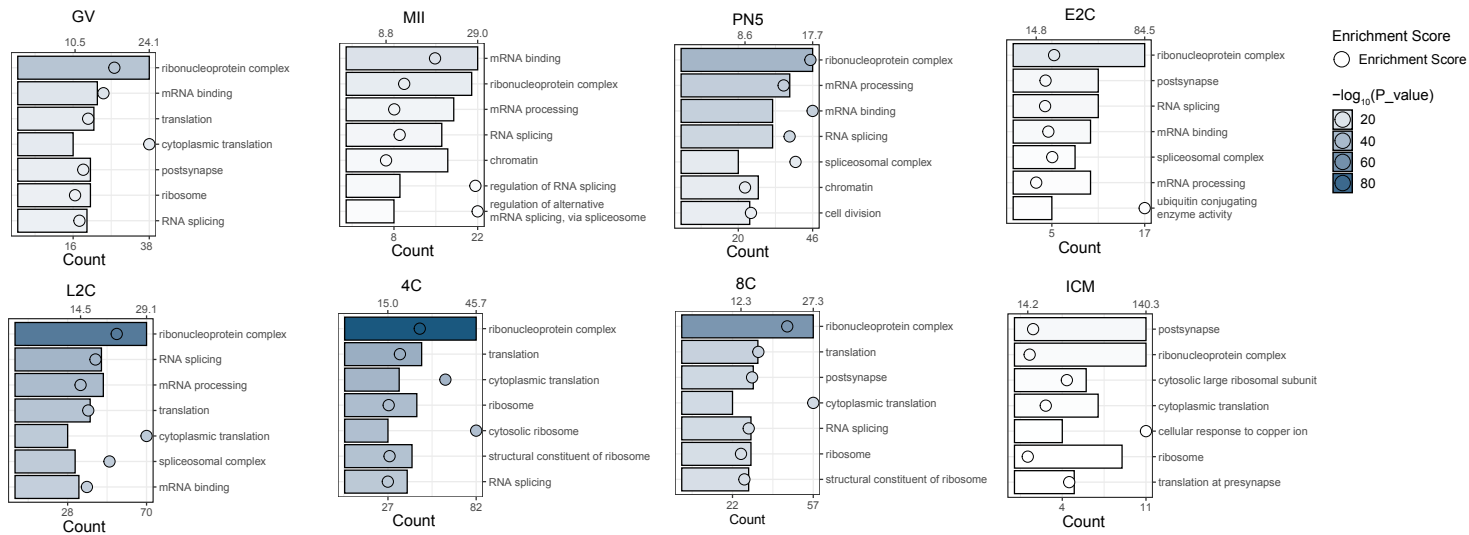


Supplementary Fig 4. Identifitaion of stage-specific high-TE gene pools and their functions.

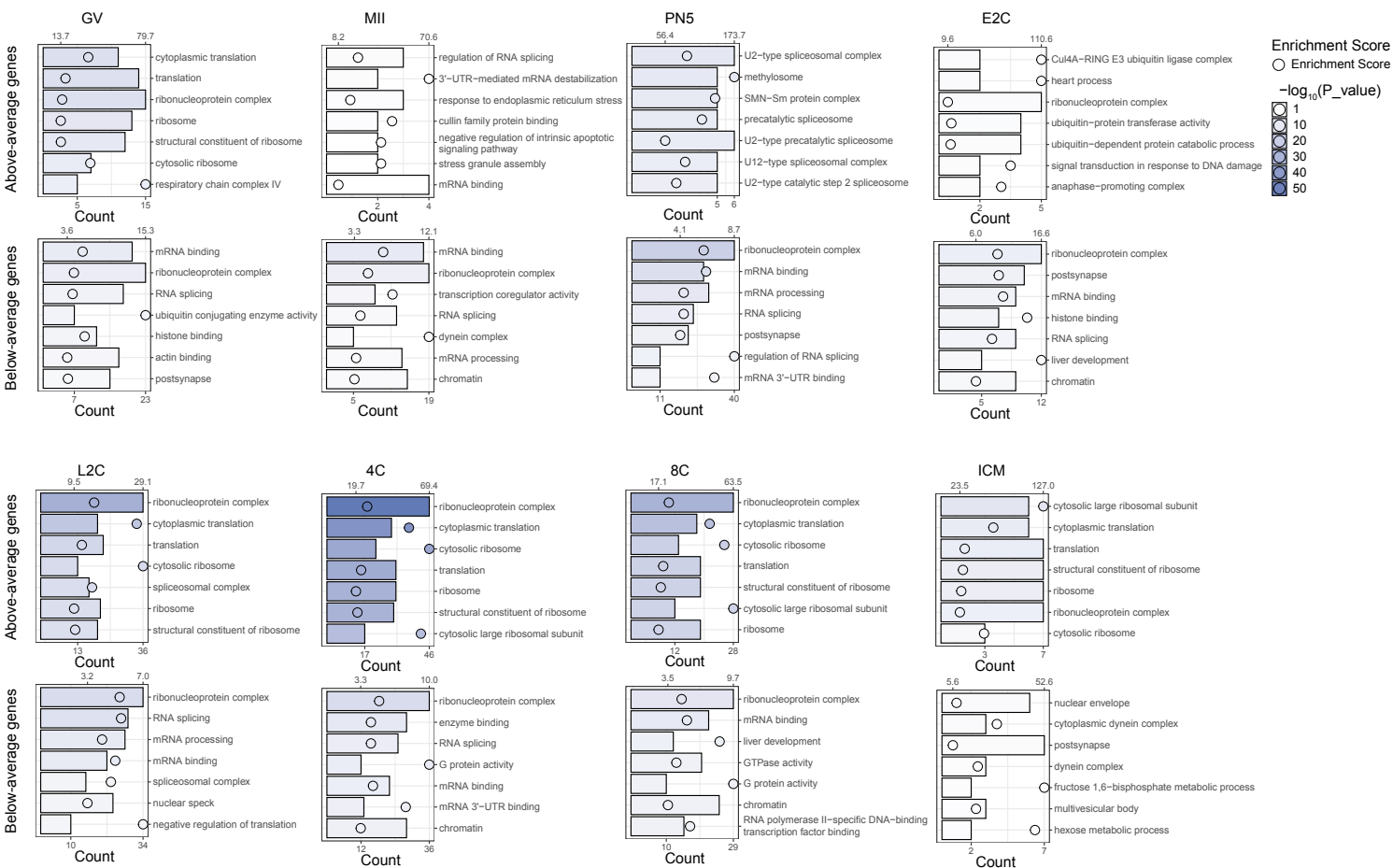
- a. K-means-based clustering heatmap displays stage-specific high-TE genes, Z-score normalized by row.
- b. GO enrichment analysis results for each cluster, showing the top 7 terms with the largest $-\log_{10}(P\text{-value})$.

Supplementary Fig 5

a



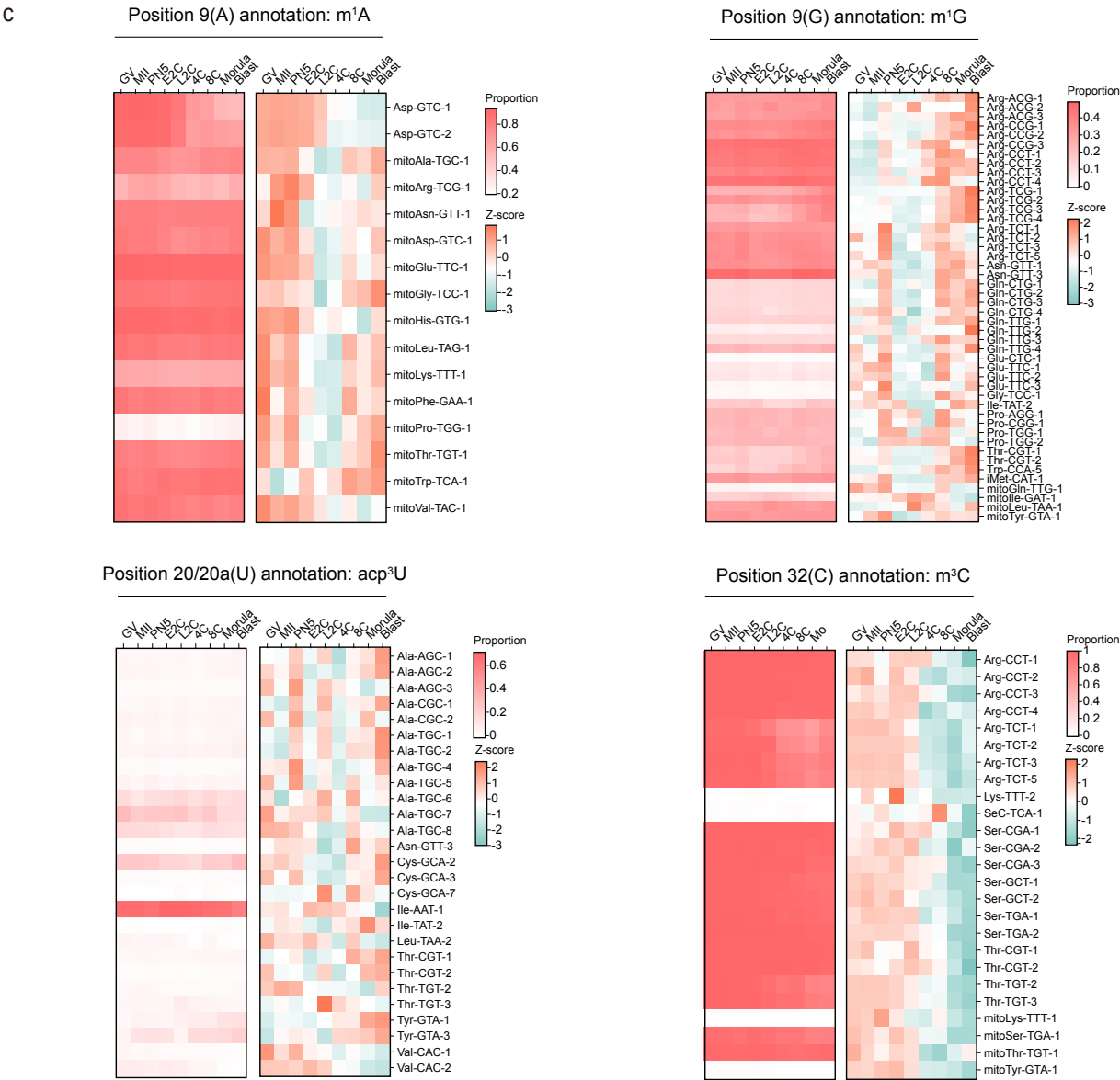
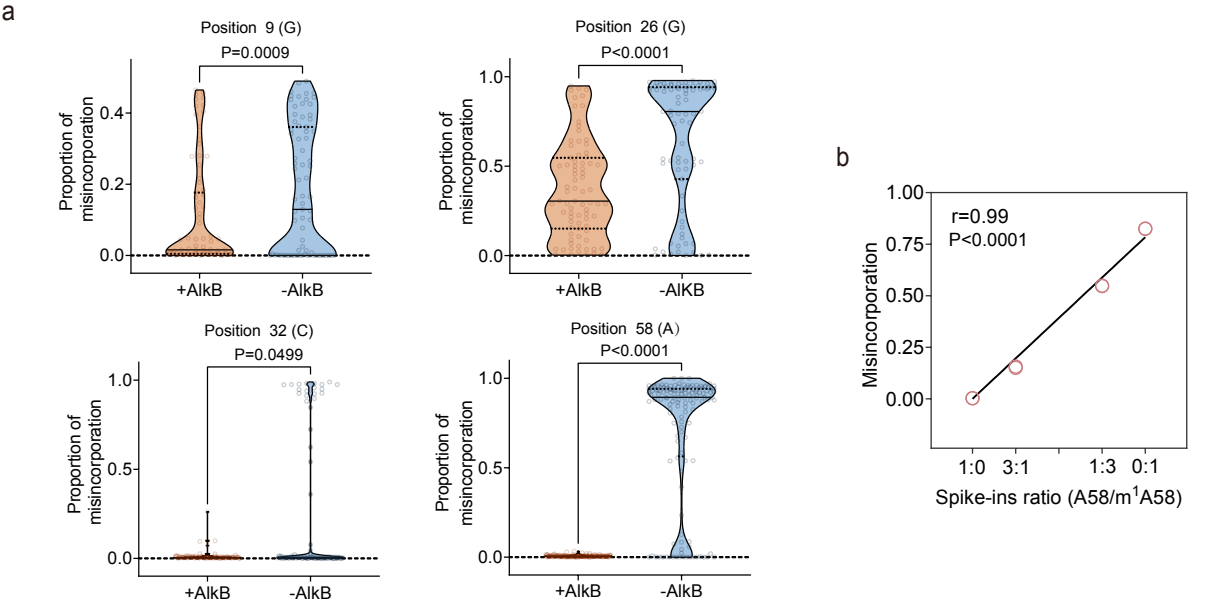
b



Supplementary Fig 5. Functional analysis of the high TE gene pool most adapted to the tRNA pool.

- a. GO enrichment analysis results for the high-TE gene sets identified at each stage, showing the top 7 terms with the largest $-\log_{10}(P\text{-value})$.
- b. GO enrichment analysis results for the high-TE gene sets identified at each stage, grouped by RNA expression level (average expression), showing the top 7 terms with the largest $-\log_{10}(P\text{-value})$.

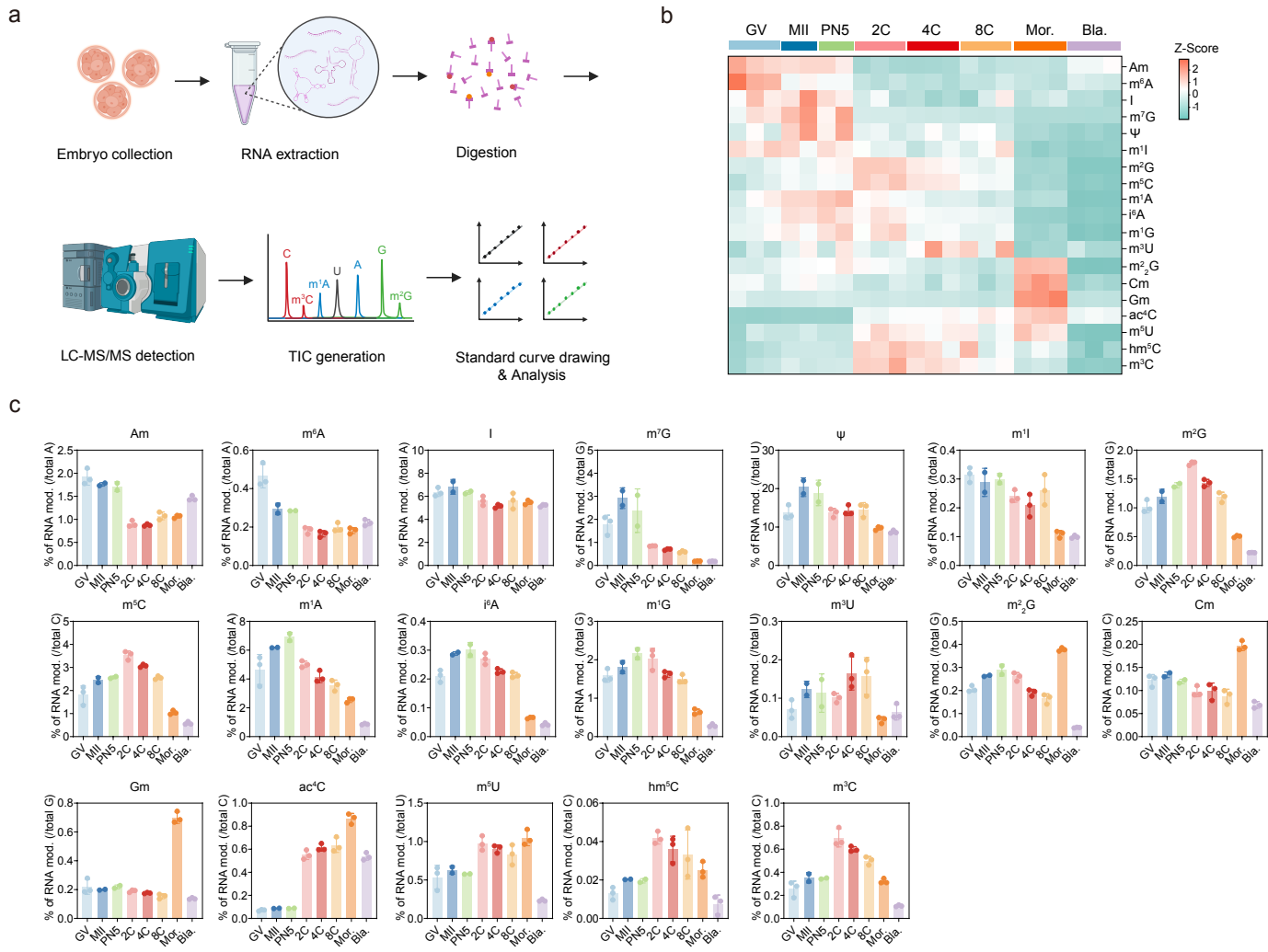
Supplementary Fig 6



Supplementary Fig 6. ORACLE-tRNAseq for quantitative analysis of tRNA modifications.

- a. Comparison of misincorporation proportion at specific sites on tRNA transcripts before and after AlkB treatment. Each dot represents a tRNA isodecoder. Solid lines represent the median, and dashed lines indicate the quartiles. Wilcoxon paired signed-rank test.
 - b. Pearson Correlation between the input ratio of m¹G9 Arg-CCT-2 and m¹A58 Met-CAT-1 oligos and the misincorporation ratio detected by ORACLE-tRNAseq.
 - c. Heatmaps display the misincorporation proportion of each tRNA isodecoder and their row-Z-score-normalized values across stages at positions 9, 20 (a), and 32.
- a, b. Source data are provided as a Source Data file.

Supplementary Fig 7

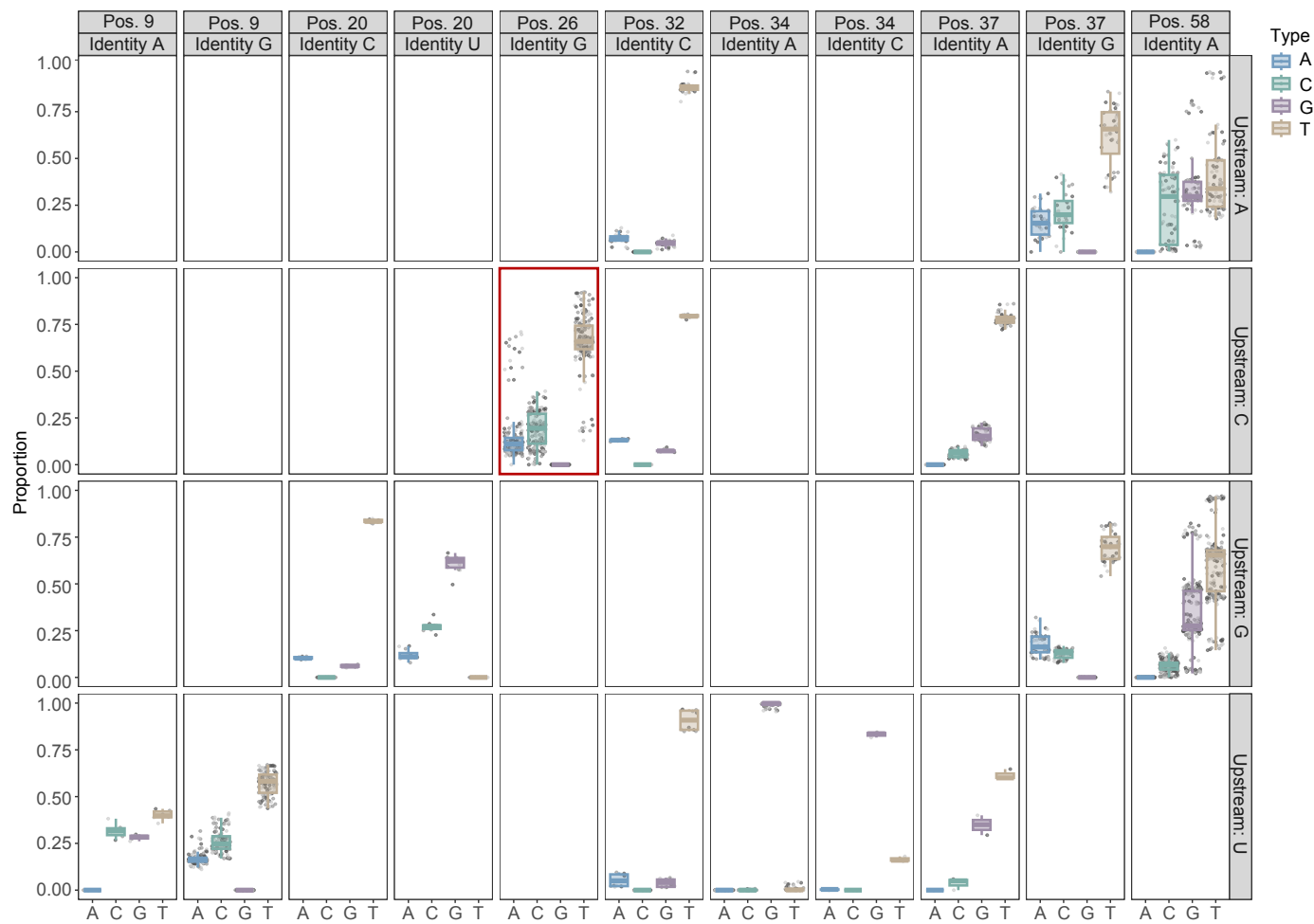


Supplementary Fig 7. Analysis of RNA modifications in early-stage embryos using LC-MS/MS.

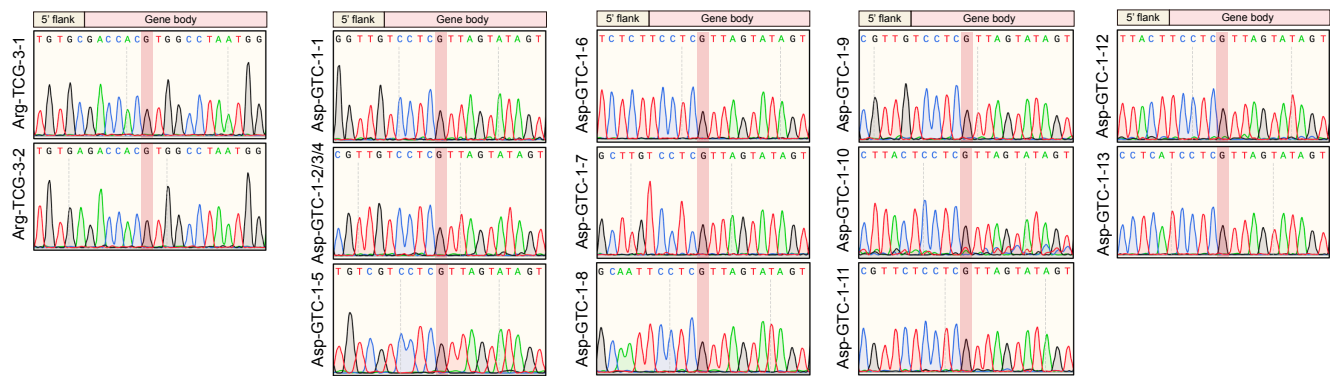
- a. Workflow of LC-MS/MS detection of total RNA modifications in embryos, created in BioRender. Li, Y. (2026) <https://BioRender.com/hecsibj>.
- b. Heatmap of relative abundance changes of 19 RNA modifications, Z-Score normalized by row.
- c. Relative abundance changes of 19 RNA modifications. Scatter plots represent biological replicates (n=3), bars represent the mean, and error bars represent standard deviation. Source data are provided as a Source Data file.

Supplementary Fig 8

a



b



Supplementary Fig 8. Identification of modifications at novel tRNA sites.

- a. Boxplots showing misincorporation signatures at canonical modified sites. The center line indicates the median, box limits represent the lower and upper quartiles, and whiskers extend to $1.5 \times$ the interquartile range. Signatures are stratified by upstream sequence context (rows) and modification type (columns), with the proportion at each nucleotide scaled to the total misincorporation at that site.
- b. Sanger sequencing chromatograms of the specified regions for all gene copies of Arg-TCG-3 and Asp-GTC-1.

Supplementary Table 1. Ion pair parameters for LC-MS/MS detection of 4 standard nucleosides and 19 modified nucleosides

Symbol	Chemical formula	Precursor ion	Product ion
G	C10H13N5O5	284.1	152.1
Gm	C11H15N5O5	298.1	152.1
m1G	C11H15N5O5	298.1	166.1
m2G	C11H15N5O5	298.1	166.1
m7G	C11H15N5O5	298.1	166.1
m2,2G	C12H17N5O5	312.1	180.1
C	C9H13N3O5	244.1	112.1
Cm	C10H15N3O5	258.1	112.1
hm5C	C10H15N3O6	274.1	142.1
m5C	C10H15N3O5	258.1	126.1
ac4C	C11H15N3O6	286.1	154.1
m3C	C11H15N3O6	258.1	126.1
A	C10H13N5O4	268.1	136.1
m1A	C11H15N5O4	282.1	150.1
m6A	C11H15N5O4	282.1	150.1
Am	C11H15N5O4	282.1	136.1
i6A	C15H21N5O4	336.1	204.1
U	C9H12N2O6	245.1	113.1
m5U	C10H14N2O6	259.1	127.1
m3U	C10H14N2O6	259.1	127.1
Psi	C9H12N2O6	245.1	209.1
I	C10H12N4O5	269.1	137.1
m1I	C11H14N4O5	283.1	151.1

Supplementary Table 2. Gradient dilution scheme of 4 standard nucleosides and 19 modified nucleosides standards

Component	Std#1 (ng/mL)	Std#2 (ng/mL)	Std#3 (ng/mL)	Std#4 (ng/mL)	Std#5 (ng/mL)	Std#6 (ng/mL)	Std#7 (ng/mL)	Std#8 (ng/mL)	Std#9 (ng/mL)
A	1000	500	250	100	40	20	10	5	2.5
U	1000	500	250	100	40	20	10	5	2.5
C	1000	500	250	100	40	20	10	5	2.5
G	1000	500	250	100	40	20	10	5	2.5
m5C	200	100	50	25	10	5	2.5	1	0.5
Cm	100	50	25	10	4	2	1	0.5	0.25
m1A	100	50	25	10	4	2	1	0.5	0.25
Gm	100	50	25	10	4	2	1	0.5	0.25
m5U	50	25	12.5	5	2	1	0.5	0.25	0.125
m7G	50	25	12.5	5	2	1	0.5	0.25	0.125
I	50	25	12.5	5	2	1	0.5	0.25	0.125
Am	50	25	12.5	5	2	1	0.5	0.25	0.125
m2G	50	25	12.5	5	2	1	0.5	0.25	0.125
m2,2G	50	25	12.5	5	2	1	0.5	0.25	0.125
Psi	50	25	12.5	5	2	1	0.5	0.25	0.125
m3C	50	25	12.5	5	2	1	0.5	0.25	0.125
m3U	20	10	5	2.5	1	0.5	0.25	0.1	0.05
m1G	20	10	5	2.5	1	0.5	0.25	0.1	0.05
ac4C	20	10	5	2.5	1	0.5	0.25	0.1	0.05
m6A	20	10	5	2.5	1	0.5	0.25	0.1	0.05
hm5C	20	10	5	2.5	1	0.5	0.25	0.1	0.05
i6A	20	10	5	2.5	1	0.5	0.25	0.1	0.05
m1I	20	10	5	2.5	1	0.5	0.25	0.1	0.05

Supplementary Table 3. Primer design of specific tRNA genes

Primer Name	Sequence 5' to 3'
Arg-TCG-3-1-loci-F	CGGCGTGCTGGGATTAAATG
Arg-TCG-3-1-loci-R	GGCACTGCAACACCAACATT
Arg-TCG-3-2-loci-F	CCTTGTGGAGACAGTGCAT
Arg-TCG-3-2-loci-R	GCCTTTTAGCCTTGCTTCCG
Asp-GTC-1-1-loci-F	ACTTTCCTATGGTGC GGCAA
Asp-GTC-1-1-loci-R	GGAGCCCTCTTGCAATTCCT
Asp-GTC-1-2/3/4-loci-F	AAGGGTTTGAGAGCTTCGGG
Asp-GTC-1-2/3/4-loci-R	TCGACGTGGAGACTCTGCTA
Asp-GTC-1-5-loci-F	GATAAGACCGAAGCTGGCGA
Asp-GTC-1-5-loci-R	GAATCGAACCCGGGTCAACT
Asp-GTC-1-6-loci-F	GCGATCTGCTACCTAGGCTG
Asp-GTC-1-6-loci-R	CTGGAGCTTCGTACCCTGTC
Asp-GTC-1-7-loci-F	CGGTTTTTGGAGTTCGCGTT
Asp-GTC-1-7-loci-R	CTGAAGCCTCGGGAGAAAGG
Asp-GTC-1-8-loci-F	GCAAGCGGTAGAAGAGGTGT
Asp-GTC-1-8-loci-R	GATGGCAGCACGAAAAGCAA
Asp-GTC-1-9-loci-F	TCCTTGATGGCATGGACCAC
Asp-GTC-1-9-loci-R	CTGCAGGCAAGCTCTCTTCT
Asp-GTC-1-10-loci-F	CCGTCTGTGCCCTTCAATCA
Asp-GTC-1-10-loci-R	TCTGCATGCGTTGGTGGTAT
Asp-GTC-1-11-loci-F	TGTCTCTGTCTCTCTCGGCA
Asp-GTC-1-11-loci-R	GGAATCCTGGGGAGGGTACT
Asp-GTC-1-12-loci-F	CAAGGCAGACCCACATCTGT
Asp-GTC-1-12-loci-R	CAAACCTCAGCTTTTCGCCCA
Asp-GTC-1-13-loci-F	CAAACCTGTGGCATTGGGCTC
Asp-GTC-1-13-loci-R	ATACTCTCAGAGCCAGCCGA