

Supplementary Material

Differential Expression Profiles and Bioinformatics Analysis of tRNA-derived Small RNAs in Muscle-Invasive Bladder Cancer in Chinese Population

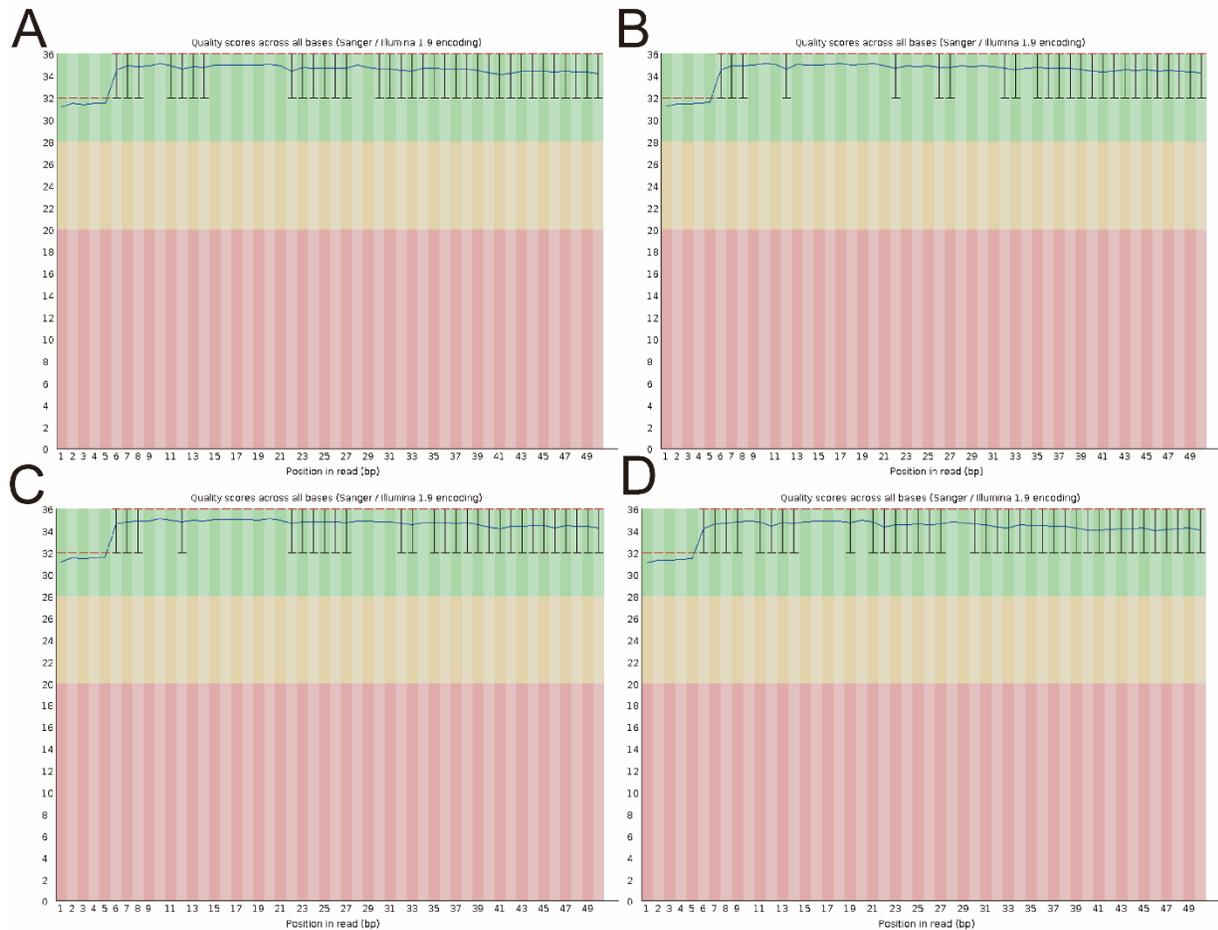
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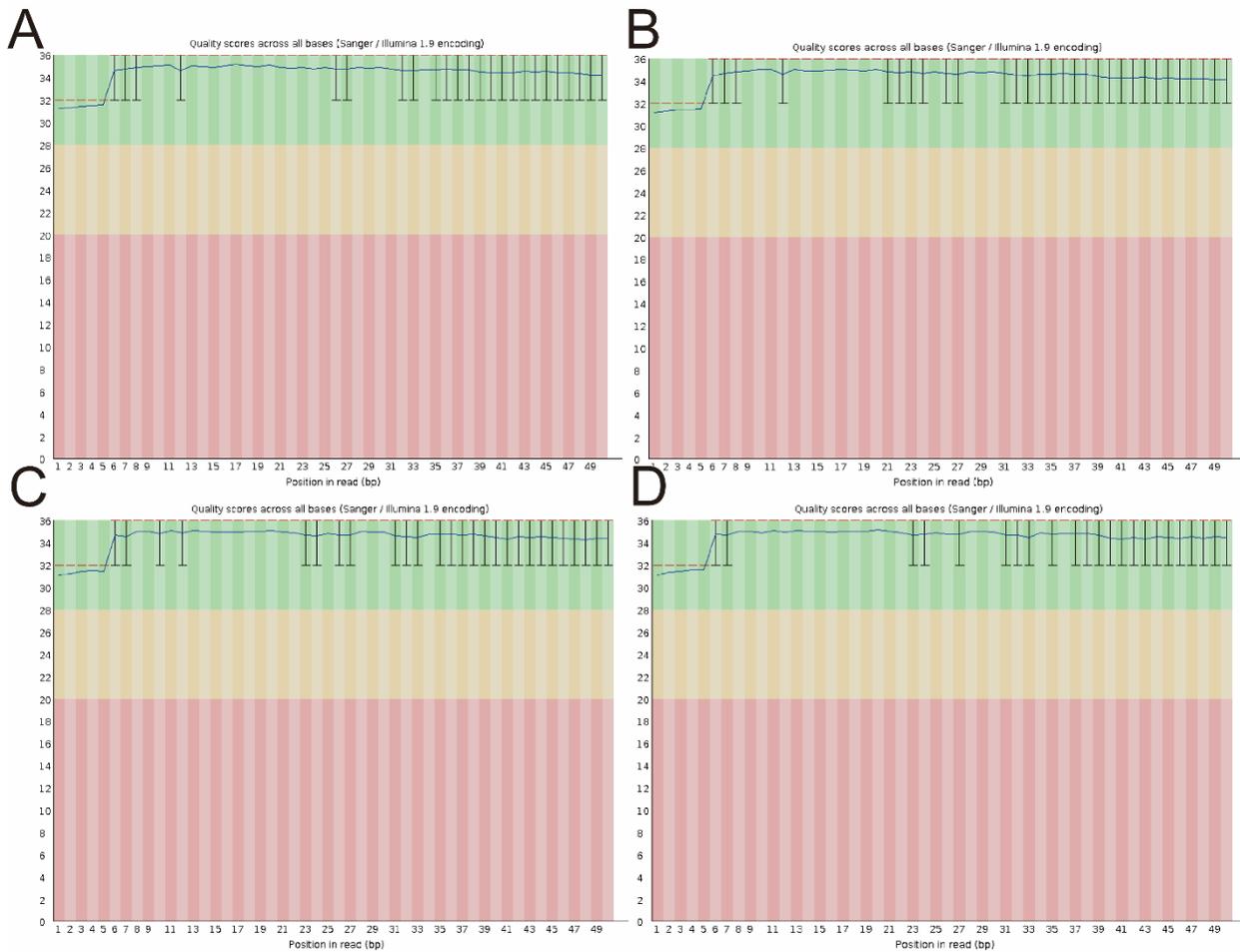
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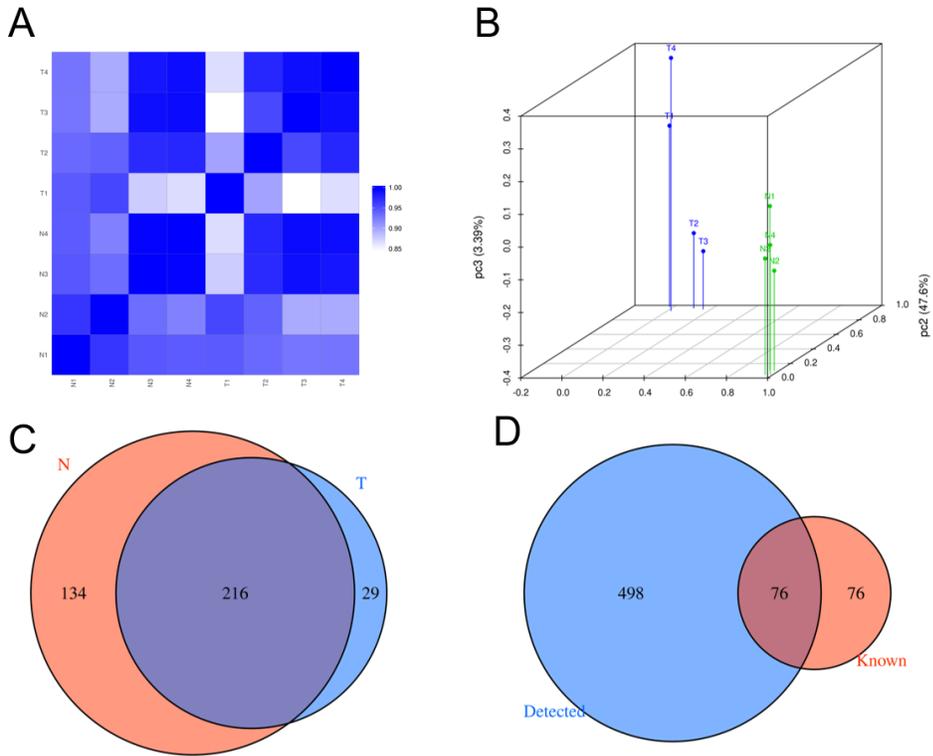


Supplementary Figure S1. tsRNA-seq quality score plot for T group (tumor tissue). The position in the read is plotted on the X-axis and the Q value is plotted on the Y-axis. The red line is the median Q score, and the blue line is the mean Q score. The boxplot represents the inter-quartile range, while the whiskers represent the 10% and 90% points. A Q score above 30 ($>99.9\%$ correct) is considered high quality of data. **A-D** represents tumor sample 1-4 (T1-T4).

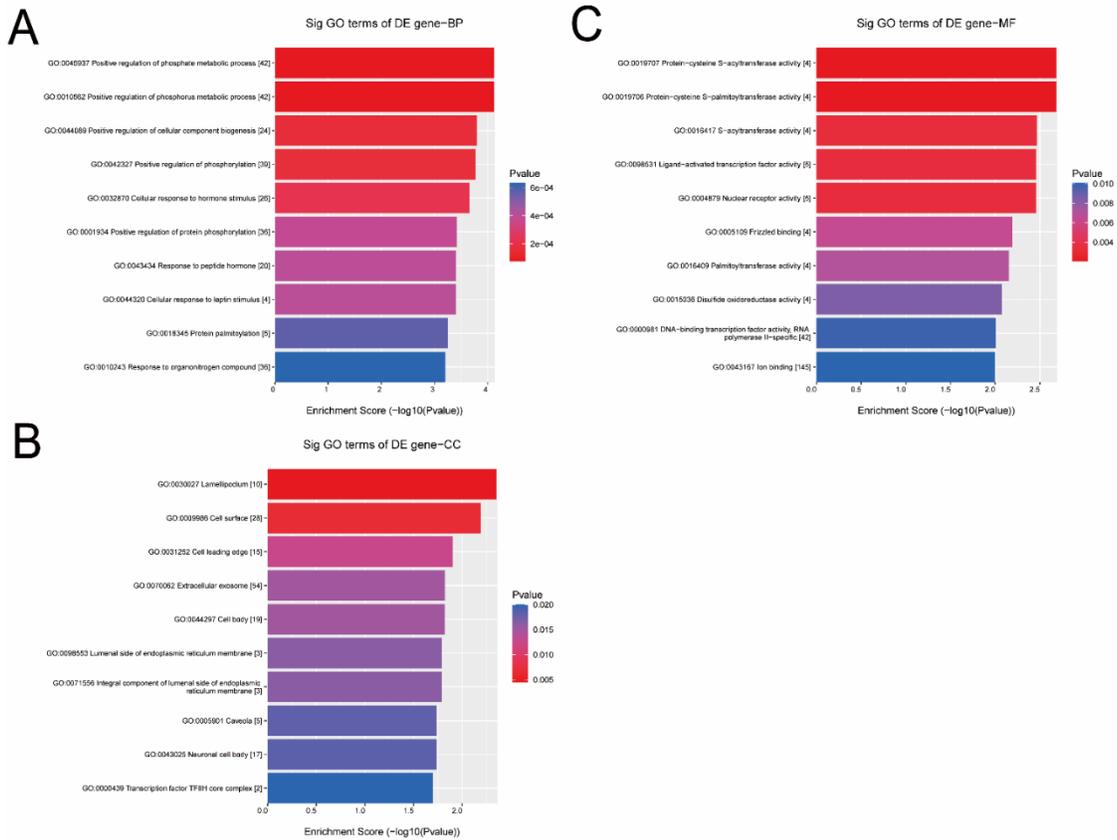


Supplementary Figure S2. tsRNA-seq quality score plot for N group (paracancerous tissue).

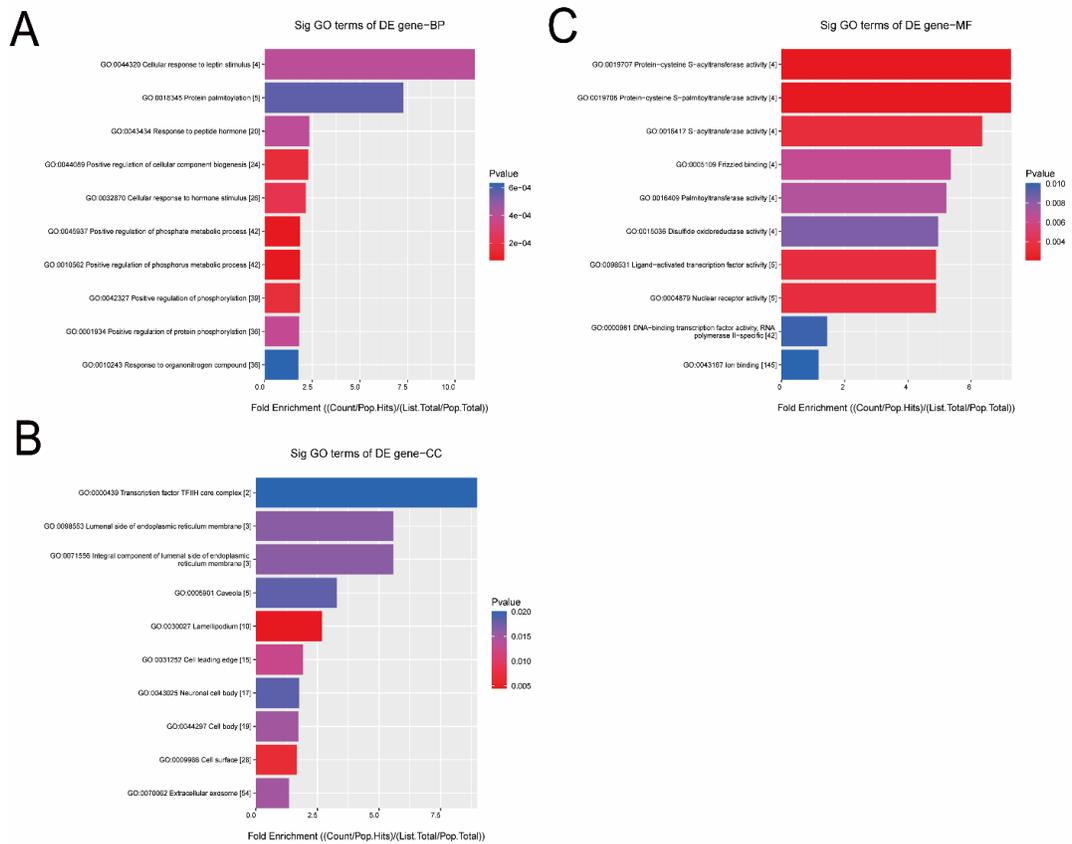
The position in the read is plotted on the X-axis and the Q value is plotted on the Y-axis. The red line is the median Q score, and the blue line is the mean Q score. The boxplot represents the inter-quartile range, while the whiskers represent the 10% and 90% points. A Q score above 30 (>99.9% correct) is considered high quality of data. **A-D** represents paracancerous tissue 1-4 (N1-N4).



Supplementary Figure S3. The expression level analysis. (A) Heat-map of the correlation coefficient from all samples. The color in the panel represents the correlation coefficient of the two samples. The blue represents the two samples with a high correlation coefficient, and the white represents the low similarity of the two samples. **(B)** Primary component analysis: the X, Y and Z axes represent the three main factors which affected the expression level of the sample. The colored point represents the corresponding sample, and its location shows the main character of the sample. The space distance represents the similarity of the data size. **(C)** Venn diagram based on the number of commonly expressed and specifically expressed tsRNAs. This diagram shows the number of tsRNAs which were expressed in both groups and also indicated the number of the specifically expressed tsRNAs. **(D)** Venn diagram based on the number of tsRNAs known and detected. This diagram shows the number of tsRNAs detected in this study and collected in tRFdb database.



Supplementary Figure S4. The top-ten significant and meaningful terms of enrichment score of target genes ranked by counts and *P*-values. (A) biological process. (B) cellular component. (C) molecular function.



Supplementary Figure S5. The top-ten significant and meaningful terms of fold enrichment of target genes ranked by counts and *P*-values. (A) biological process. (B) cellular component. (C) molecular function

Supplementary Table S1. Quality score for each sample

| Sample | TotalRead | TotalBase | BaseQ30 | BaseQ30 (%) |
|---------------|------------------|------------------|----------------|--------------------|
| T1 | 8274992 | 413749600 | 391179830 | 94.55 |
| T2 | 7617447 | 380872350 | 361491357 | 94.91 |
| T3 | 6159841 | 307992050 | 292418503 | 94.94 |
| T4 | 9513040 | 475652000 | 446833685 | 93.94 |
| N1 | 8946064 | 447303200 | 424246058 | 94.85 |
| N2 | 8172930 | 408646500 | 386027201 | 94.46 |
| N3 | 8346674 | 417333700 | 396028789 | 94.89 |
| N4 | 9856730 | 492836500 | 468729835 | 95.11 |

Sample: Sample name

TotalRead: Raw sequencing reads after quality filtering

TotalBase: Number of bases after quality filtering

BaseQ30: Number of bases of Q score more than 30 after quality filtering

BaseQ30 (%): The proportion of bases ($Q \geq 30$) number after quality filtering

Supplementary Table S2. Sequences of primers used for qRT-PCR

| Gene name | Primer sequence | Ta Opt (°C) | Product size(bp) |
|-----------------------------|--|-------------|------------------|
| <i>U6</i> | F:5'GCTTCGGCAGCACATATACTAAAAT 3' R:5'CGCTTCACGAATTTGCGTGTCAT 3' | 60 | 89 |
| <i>tiRNA-1:33-Gly-GCC-1</i> | F:5' TACAGTCCGACGATCGCATG 3' R:5' CGTGTGCTCTTCCGATCTCA 3' | 60 | 66 |
| <i>tiRNA-1:34-Val-CAC-2</i> | F:5' GATCGCTTCTGTAGTGTAGTGG 3' R:5' CGATCTGAGGCGAACGTGA 3' | 60 | 44 |
| <i>tRF-1:32-Gly-GCC-1</i> | F:5' TACAGTCCGACGATCGCATG 3' R:5' TCTAGGCGAGAATTCTACCACTGA 3' | 60 | 50 |
| <i>tRF-+1:T20-Ser-TGA-1</i> | F:5' TACAGTCCGACGATCGAAGC 3' R:5' TTCCGATCTAAAATAAGAGCACC 3' | 60 | 44 |