

## BioIT guidelines

Bioinformatics guide for using DeMux nanoT to demultiplex nano-tRNAseq sequencing datasets.  
(NTRSQ-12)

FOR RESEARCH USE ONLY

## Supplemental product information and tips for success

### Required hardware:

- Linux operating system
- CPU with 4 cores
- 16 GB RAM memory
- Nvidia GPU with at least 8 GB vRAM
- 500 GB SSD storage

### Required software:

- cuda (version 11.3 or newer)
- docker (version 23 or newer)
- nvidia-container-toolkit
- dorado (v0.7.2 or newer)
- minimap2 (version 2.24 or newer)
- samtools (version 1.15 or newer)

### Steps to perform before running the DeMux nanoT application:

#### Step 1. Basecalling:

```
dorado basecaller -r -x cuda:all rna004_130bps_hac@v5.0.0 --emit-moves pod5_directory > bam_file.bam
```

Parameter	Definition
pod5_directory	Pod5 output directory from MinKNOW
bam_file.bam	Output bam file

#### Step 2. Mapping:

```
samtools fastq -T ts,mv bam_file.bam | minimap2 -y -t8 -ax splice -k7 -w3 -n1 -m13 -s30 -A2 -B1 -O1,32 -E1,0 fasta_tRNA_database.fa - | samtools sort -@8 --write-index -o mapped.bam
```

Parameter	Definition
bam_file.bam	Output bam file from dorado
fasta_tRNA_database.fa	FASTA database file containing tRNA sequences. It is recommended to retain only tRNA sequences with sequence homology below 87-90% to reduce multimapping reads.
mapped.bam	Output BAM file containing aligned reads.

### Step 3. Demultiplexing bam file with DeMuxnanoT app:

To activate the DeMuxnanoT application, please send an email to [bioinformatics@immaginabiotech.com](mailto:bioinformatics@immaginabiotech.com).

After receiving a confirmation email from the bioinformatics group, the application will be activated and *will remain active for 45 days*.

Before launching DeMuxnanoT, create a folder containing the following:

- the pod5 folder generated by MinKNOW
- the BAM file generated in step 2.

To launch the application, follow the command provided below:

```
./DeMuxnanoT --inputDir input_directory --bamName bam_name --outputDir output_directory --outputName exp_name
```

Parameter	Definition
DeMuxnanoT	DeMuxnanoT is the binary file located on the USB drive sent by Immagina Biotechnology.
input_directory	Path to input folder containing pod5 folder and bam file obtained in Step 2
bam_name	Name of the bam file present in input_directory (Example: test.bam)
output_directory	Path to output folder
exp_name	Name of the experiment (Example: test)

#### Step 4. Counts tRNA:

For the analysis of tRNA abundances, basecalling errors, and the generation of plots for visualizing these results, you can use the scripts available on GitHub: <https://github.com/novoalab/Nano-tRNAseq/tree/main/src>

4.1 The expression of tRNAs can be estimated as follows:

```
get_counts.py -o counts.tsv -i /path/*bam_file.bam > stats.tsv
```

Parameter	Definition
/path/*bam_file.bam	Path to bam files obtained in Step 3
counts.tsv	output file with counts for each sample and each tRNA
stats.tsv	output file with statistics on bam files

4.2 Scatterplot of tRNA count for all samples using:

```
plot_scatter.py -i counts.tsv -o scatter.pdf
```

Parameter	Definition
counts.tsv	Input file with counts for each sample and each tRNA
scatter.pdf	Output file name

4.3 Generate differences in sum of basecalling errors between WT and some other sample(s)/condition(s) as follows:

```
get_sum_err.py -o heatmap/sample1 -f fasta_tRNA_database.fa -i /path/*bam_file.bam
```

Parameter	Definition
fasta_tRNA_database.fa	FASTA database file containing tRNA sequences.

/path/*bam_file.bam	Path to bam files obtained in Step 3. The first BAM file will be used as WT.
heatmap/sample1	Output name

#### 4.4 Plots heatmaps of basecalling errors:

```
plot_heatmap.py -i heatmap/sample1.err_diff.tsv.gz -f fasta_tRNA_database.fa -a  
fasta_tRNA_database.aln.fa
```

Parameter	Definition
heatmap/sample1.err_diff.tsv.gz	File gz obtained in Step 4.3
fasta_tRNA_database.fa	FASTA database file containing tRNA sequences.
fasta_tRNA_database.aln.fa	FASTA file containing alignments of tRNA sequences.

## Contacts



### Info

[info@immaginabiotech.com](mailto:info@immaginabiotech.com)

**Sale support (quoting, ordering, and order status update)**

[orders@immaginabiotech.com](mailto:orders@immaginabiotech.com)

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