 # The CRISPR Guide Capture analysis provided by 10X CellRanger was used to generate UMI count matrices for both mRNA and gRNA. Detailed methods and procedures are described in the 10x Genomics official documentation.

```
cellranger count --id=sample345 \
                 --libraries=library.csv \
                 --transcriptome=/opt/refdata-gex-GRCh38-2020-A \
                 --feature-ref=feature_ref.csv
```

 # Generate the UMI count matrix for aptamers.

Step 1: Using the aptamer primer sequence GCAGCTCGGCCCATATAAGAAA, the random aptamer sequence located 45–47 bp upstream of the primer in Read2 was extracted. The corresponding Read1 sequence was retained.

```
perl step1_reads_extract.pl    --R1 adaptmer_data/$i/${i}_R1.fastq.gz
     --R2 adaptmer_data/$i/${i}_R2.fastq.gz --o1 step1_reads_extract/$i/${i}_R1.fastq.gz
     --o2 step1_reads_extract/$i/${i}_R2.fastq.gz
```

Step 2: Cell barcodes were extracted based on the results of CRISPR Guide Capture analysis.

```
perl step2_barcode_correct.pl possorted_genome_bam.bam barcodes.tsv
     step2_barcode_correct/${i}_result.txt
```

##**Step 3**: Read1 was filtered based on the cell barcodes extracted in Step 2, and the corresponding Read2 was retained. Additionally, aptamer sequences obtained in Step 1 were subjected to a secondary length-based filtering to remove non-compliant sequences. The final output includes aptamer sequences along with their associated cell barcodes and UMI information.

```
perl step3_cell_barcode_extract.pl --barcode step2_barcode_correct/${i}_result.txt
     --R1 step1_reads_extract/$i/${i}_R1.fastq.gz
     --R2 step1_reads_extract/$i/${i}_R2.fastq.gz
     -o step3_barcode_extract/$i.result.reads.txt
```

Step 4: The aptamer data were consolidated into standard file formats compatible with Seurat analysis.

```
perl step4_cell_adapter_umi_stat_add_matrix.pl
     step3_barcode_extract/$i.result.reads.uniq.txt step4_result_test/$i.result.txt
     step4_result_test/$i
```