Tutorial for SRA-BLAST:

Search for SRA datasets based on specific topics/conditions
https://www.ncbi.nlm.nih.gov/sra

For example, searching for meRIP gives 2295 entries
Limiting by Human, gives 1215 entries
Limiting by RNA-seq, gives 521 entries, etc

Task: Search for presence of TERT_R4 in specific SRA sets: the position of m6A site is highlighted in grey

>TERT_R4_42bp
GGACACCCGGGGACCGCGCCTCACTCACCCTGCACGTGACAG

Selected SRA sets: Hela meRIP-seq

- Filters activated: RNASeq. Clear all

Select item 96383361.
GSM4216728: **HeLa** hypoxia 24h **meRIP**-seq; **Homo sapiens**; RIP-Seq
1 ILLUMINA (HiSeq X Ten) run: 12.3M spots, 3.7G bases, 1.3Gb downloads

Accession:

SRX7354763

Select item 96383342.
GSM4216726: **HeLa** hypoxia 12h **meRIP**-seq; **Homo sapiens**; RIP-Seq
1 ILLUMINA (HiSeq X Ten) run: 11.7M spots, 3.5G bases, 1.3Gb downloads

Accession:

SRX7354761

Select item 96383323.
GSM4216724: **HeLa** hypoxia 6h **meRIP**-seq; **Homo sapiens**; RIP-Seq
1 ILLUMINA (HiSeq X Ten) run: 11.8M spots, 3.5G bases, 1.3Gb downloads

Accession:

SRX7354759

Select item 96383304.
GSM4216722: **HeLa** normoxia **meRIP**-seq; **Homo sapiens**; RIP-Seq
1 ILLUMINA (HiSeq X Ten) run: 15.8M spots, 4.7G bases, 1.7Gb downloads

Accession:

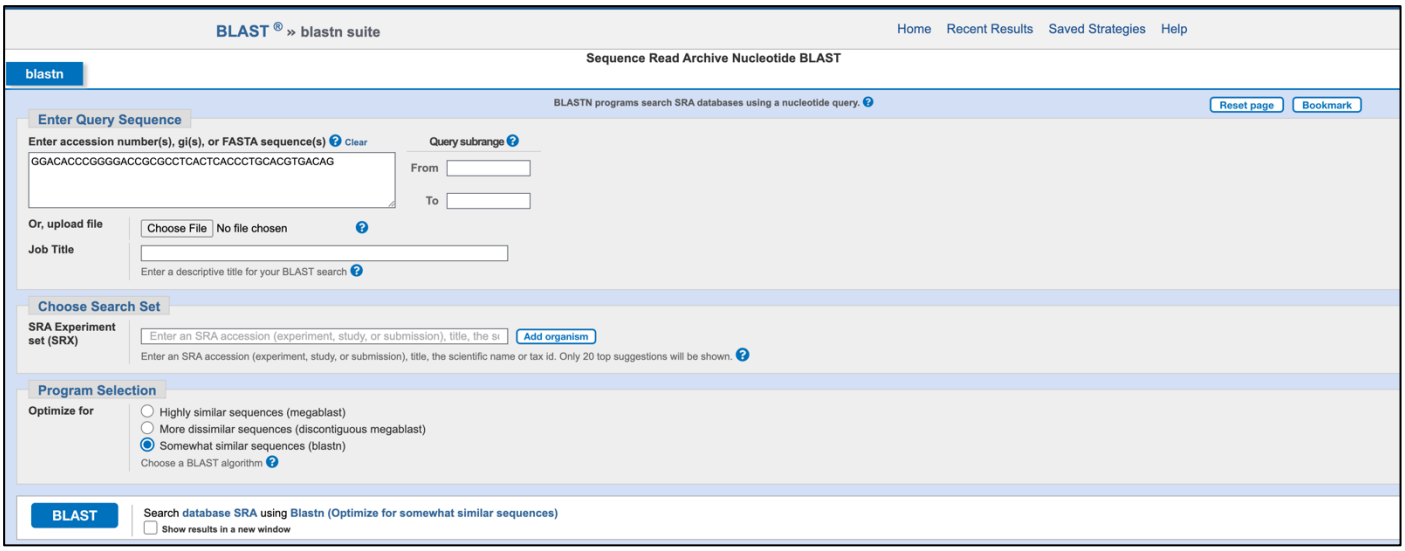
SRX7354757

TERT: NM_198253

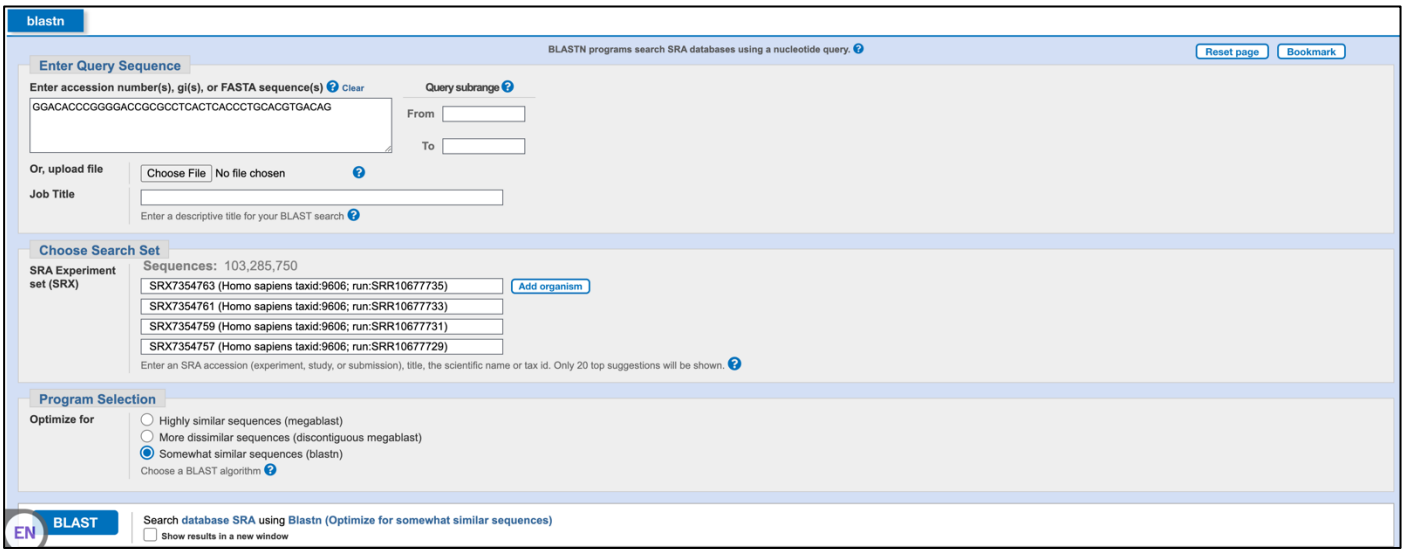
CLPTML1: NM_030782

Tool: SRA BLAST
https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&BLAST_SPEC=SRA&SHOW_DEFAULTS=on

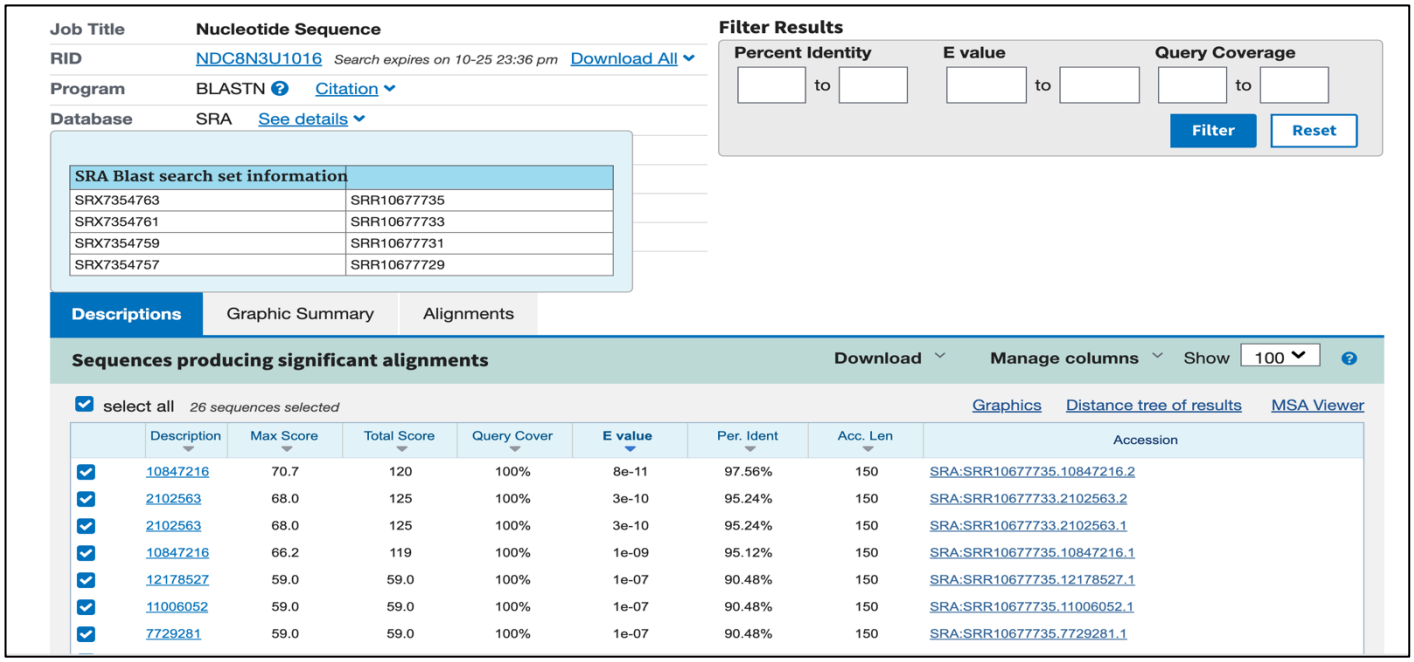
1. Open SRA BLAST link, enter target fasta sequence in **"Enter Query Sequence"** box.



2. enter SRX number set on "Choose Search Set" section, can add multiple SRA by click "Add organism"



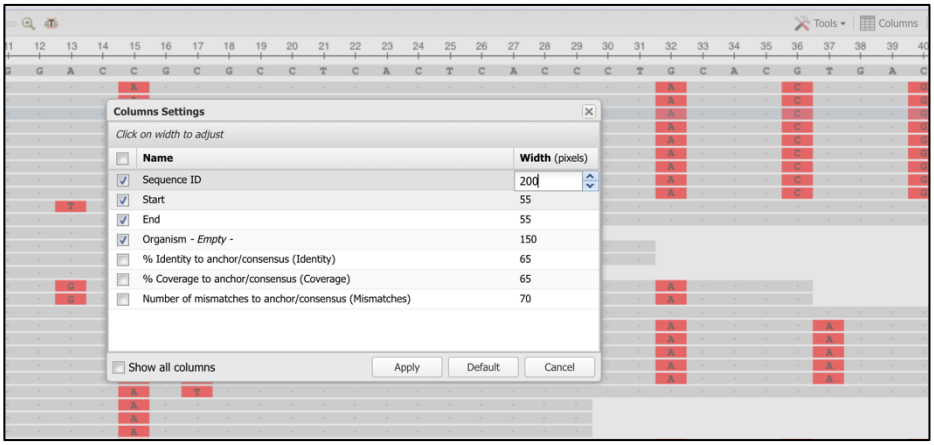
3. Select “somewhat similar sequences (blastn)” and hit **BLAST**
4. The result will show on next page with multiple tables, select “See details” can show relation between SRX number and SRR number



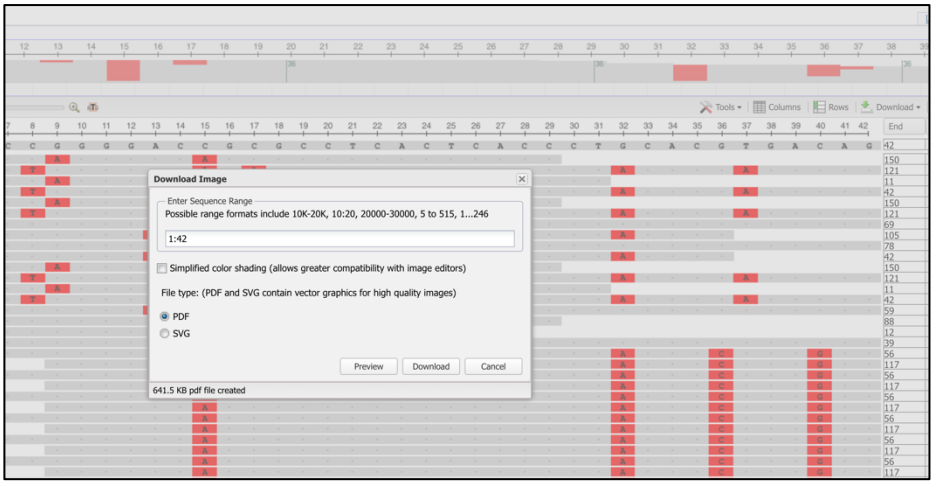
Also check the Graphic Summary and Alignment Tabs

5. To view all the alignment, click “MSA Viewer” on the right side of page. For example (https://www.ncbi.nlm.nih.gov/projects/msaviewer/?anchor=0&coloring=diff&key=NCID_1_20641650_130.14.18.128_9147_1666626263_588037759_0MetA0__S_NC_PhyloTree&columns=d:200,b:55,x:17,aln,e:55,o:150) This link is copied from select “Link to View” on the top right.

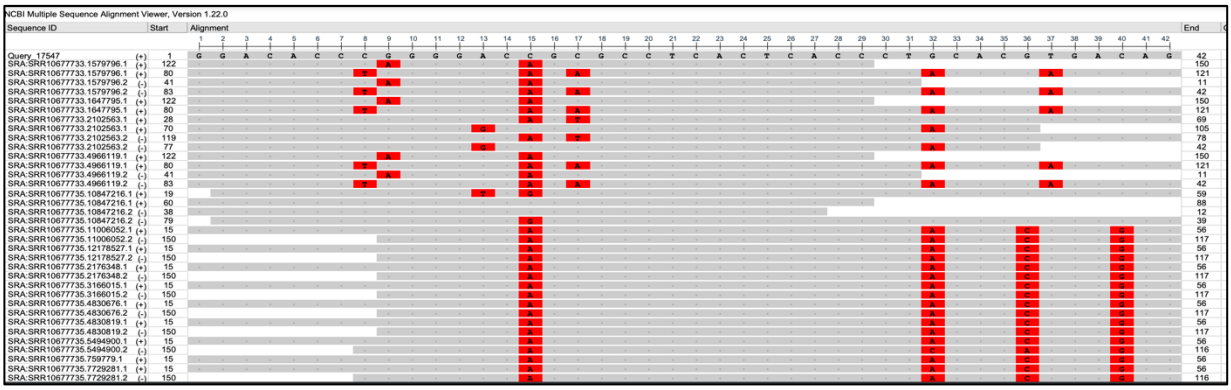
6. It can change the size of the column text to see all the information by selecting “Columns” and type desired Width (Pixels) in the box and hit Apply.



7. To output pdf format of alignment, select “Download” button and select range and type of output format (PDF or SVG). Click “preview” to save the file.



8. The output will look like:



9. Conclusions:
   - TERT-R4 sequences are detectable in PolyA sequences (read the protocol for sample preps):

     For meRIP-seq, the libraries were constructed by Truseq Stranded mRNA Sample Prep Kit (Illumina) according to the manufacturer's instructions and quantified by BioAnalyzer High Sensitivity DNA chip, and then deeply sequenced on the Illumina HiSeq X10 to generate 150-bp paired-end reads. For RNA-seq library, mRNA enrichment, cDNA synthesis, adaptor addition, circularization, PCR amplification and library examination were performed on the BGIseq 500 at Beijing Genome Institute (BGI; Shenzhen, China)

   - Read the reporting paper:
     Wang YJ *et al.*, "Reprogramming of m^{6}A epitranscriptome is crucial for shaping of transcriptome and proteome in response to hypoxia.", *RNA Biol*, 2021 Jan;18(1):131-143 https://pubmed.ncbi.nlm.nih.gov/32746693/

   - TERT_R4 expression is increased on hypoxia compared to normoxia conditions in HeLa cells