**First download putty(where u give commands) and winscp(where you find your files and folders) on your PC**

**Go to array express search for your disease, choose the experiment but it must be with raw data.  
know the names of files in raw data.**

**1- to download the files:**

./sratoolkit.2.9.2-unbuntu64/bin/fastq-dump –split-files (file name)

To download more than one file at a time do loop:

Create a txt file containing names of all the files u need  
ex. File name=list1.txt  
 SRR2422919  
 SRR2422920  
 SRR2422921  
Loop command for run in `cat list1.txt` ;do ./sratoolkit…………………same as above…. $run; done  
run= this is the file name

**2- quality control:**

Fastqc (name of the file)  
for a loop:

List2.txt with names of the files after download  
SRR2422919.1.fastq  
SRR2422919.2.fastq  
 SRR2422920.1.fastq  
 SRR2422920.2.fastq  
 SRR2422921.1.fastq  
SRR2422921.2.fastq  
  
command: for run in `cat list2.txt` ; do fastqc $run; done

**3-trimming(** find in pdf) this step to cut bases with low quality

**4-quality again**

**5-alignment: on human reference genome**

for run in `cat list2.txt` ; do tophat2 –output-dir /output2 /data1/ayaosama/bowtie38/grch38\_1kgmaj /data1/ayaosama/$run

tophat -o /data1/ayaosama/output2/19 -G /data1/ayaosama/gtf/Homo\_sapiens.GRCh38.96.gtf /data1/ayaosama/bowtie38/grch38\_1kgmaj /data1/ayaosama/qual\_trim\_SRR2422919\_1.fastq /data1/ayaosama/qual\_trim\_SRR2422919\_2.fastq; done

-wget (URL for the GRCh38.96)

**6-Normalization:**

**cufflinks -o /data1/ayaosama/32 -g /data1/ayaosama/gtf/Homo\_sapiens.GRCh38.96.gtf 32.accepted\_hits.bam**

**OR use the pseusoalignment**

**With kallisto**