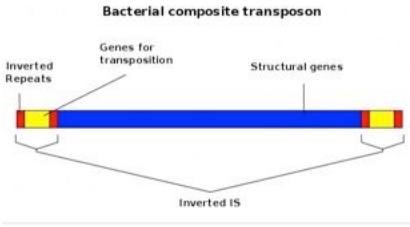
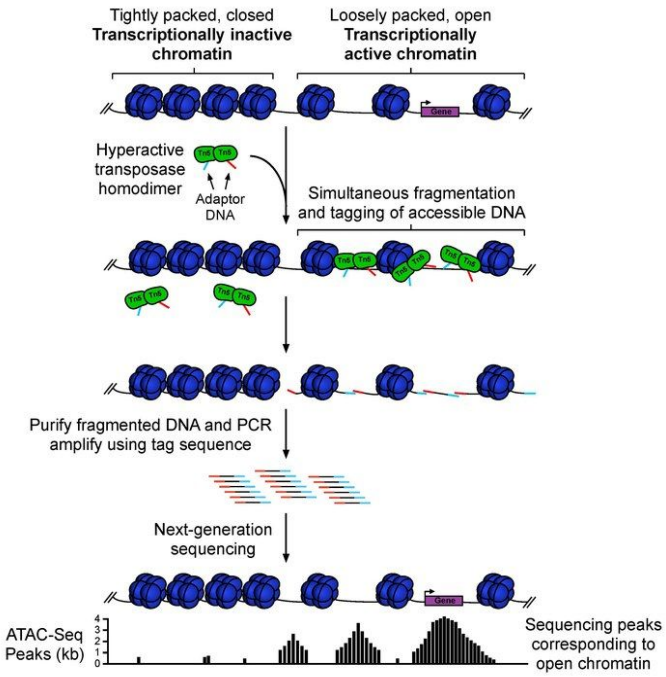
1. Laboratory Methods
    a. Illumina Sequencing (created by ISB Founder)
        i. Has been used to sequence many genomes and has enabled the comparison of DNA sequences to improve understanding of health and disease
        ii. generates many millions of highly accurate reads making it much faster and cheaper than other available sequencing methods.
        iii. This sequencing method is based on reversible dye-terminators that enable the identification of single bases as they are introduced into DNA strands. It can also be used for whole-genome and region sequencing transcriptome analysis, sRNA discovery, methylation profiling, and genome-wide protein–nucleic acid interaction analysis.
        iv. How does it work?
            1. DNA is broken into fragments, then DNA adaptors (an adaptor is a short, chemically-synthesised, double-stranded DNA molecule which is used to link together two other DNA molecules) are attached to the other fragments. The fragments attached to the adaptors are then made into single strands.
            2. Complementary DNA binds to primers on the surface of the flow-cell and DNA that doesn't attach is washed away. The DNA on the flow-cell is replicated to form small clusters of DNA with the same sequence. When sequenced, each cluster of DNA molecules will emit a signal.
            3. Unlabelled nucleotide bases and DNA polymerase are then added to lengthen/join the strands of DNA on the flow-cell. This creates "bridges" of double-stranded DNA between the primers on the flow-cell. The double-stranded DNA is then broken down into single-stranded DNA using heat, leaving several dense clusters of identical DNA sequences.
            4. Primers and terminators (ddNTPs) are added to the flowcell. The primer attaches to the DNA being sequenced. The DNA polymerase then binds to the primer and adds the first terminator to the new DNA strand. Once a base has been added, no more bases can be added to the strand of DNA until the terminator base is cut from the DNA.
            5. Lasers are passed over the flowcell to activate the fluorescent label on the nucleotide base. The ddNTPs are removed from one base and added alongside another as it continues this process. The sequence generated can then

be aligned to a reference sequence; this looks for matches or changes in the sequenced DNA.

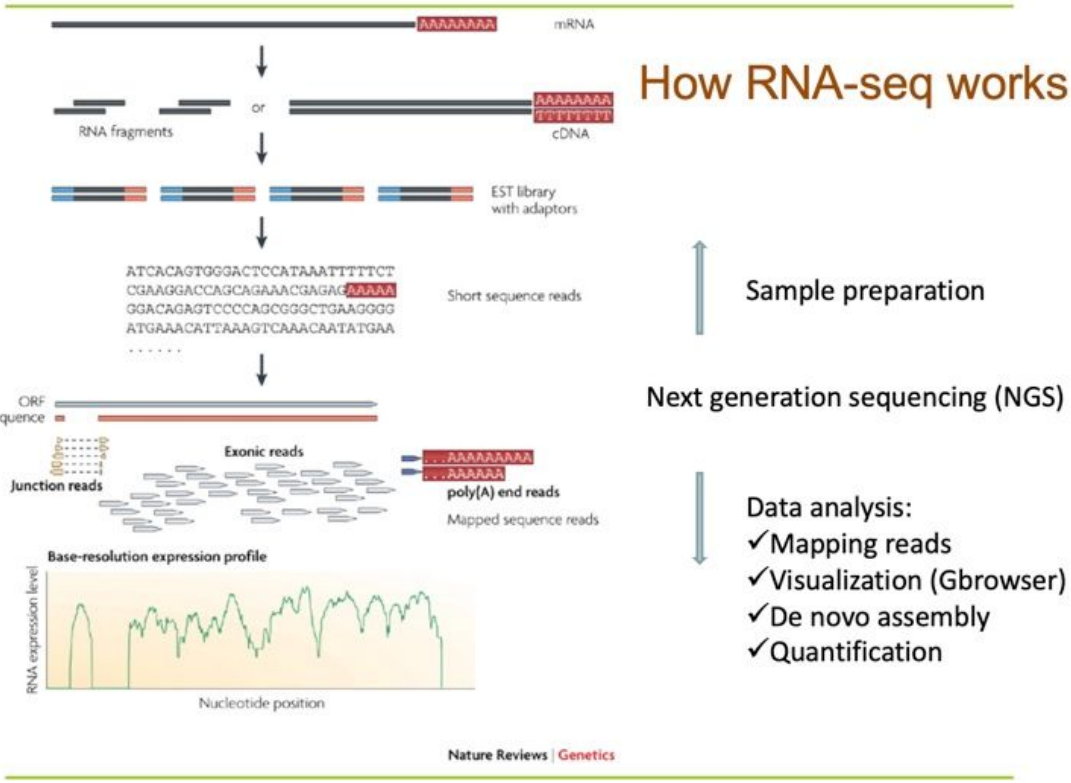
b. ATAC-seq
   i. Assay for Transposase Accessible Chromatin with high-throughput sequencing
   ii. What happens in ATAC-seq is that chromatin splitting/cleavage is performed by Tn5 transposase and during the process, Tn5 introduces a DNA adaptor to the cleaved site of accessible regions of chromatin. ATAC-seq protocol is faster and requires fewer steps in comparison to DHS-seq (used for assaying chromatin accessibility genome-wide).
      1. A transposase is an enzyme that binds to the end of a transposon (shown in the 1st figure on the right) and moves around the transposon to another part of the genome by a cut&paste mechanism.
      2. Tn5 is a member of the RNase superfamily of proteins which includes retroviral integrases ("enzymes produced by a retrovirus that enables its genetic material to be integrated into the DNA of the infected cell"). The Tn5 transposon codes for antibiotic resistance to kanamycin and other aminoglycoside antibiotics.
   iii. Sequencing reads can be used to infer regions of increased accessibility and to map regions of transcription factor binding and nucleosome-position.



A **transposable element** is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genetic identity and genome size. Transposition often results in duplication of the same genetic material.



c. RNA-seq
    i. Ribonucleic-Acid high-throughput sequencing
    ii. Involves the direct sequencing of mRNAs using next generation sequencing techniques (NGS) aka DNA sequencing. It can be used to characterize transcripts (and splicing variants) as well asdentify/give expression levels of known transcripts.



d. INTACT
    i. Isolation of Nuclei Tagged in Specific Cell Types

2. Molecules
    a. H3K4me3
        i.
    b. H3K27me3
        i.