

# Single Cell Genomics

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# Single Cell Concept

- Single-cell analysis allows the study of cell-to-cell variation within a cell population
- higher resolution of cellular differences and a better understanding of the function of an individual cell in the context of its microenvironment
- Single-cell genomics is **the study of the individuality of cells using omics approaches**
- **Single Cell Genomics**
- **Single Cell Transcriptomics**
- **Single Cell Proteomics**
- **Single Cell Epigenomics-scMethylome (bisulfite sequencing)**

# Bisulfite method

- Treatment of DNA with bisulfite converts cytosine residues to uracil, but leaves 5-methylcytosine residues unaffected. Therefore, DNA that has been treated with bisulfite retains only methylated cytosines. To obtain the methylome readout, the bisulfite-treated sequence is aligned to an unmodified genome. Whole genome bisulfite sequencing was achieved in single cells in 2014.

# Single cell Genome Seq

## TYPES

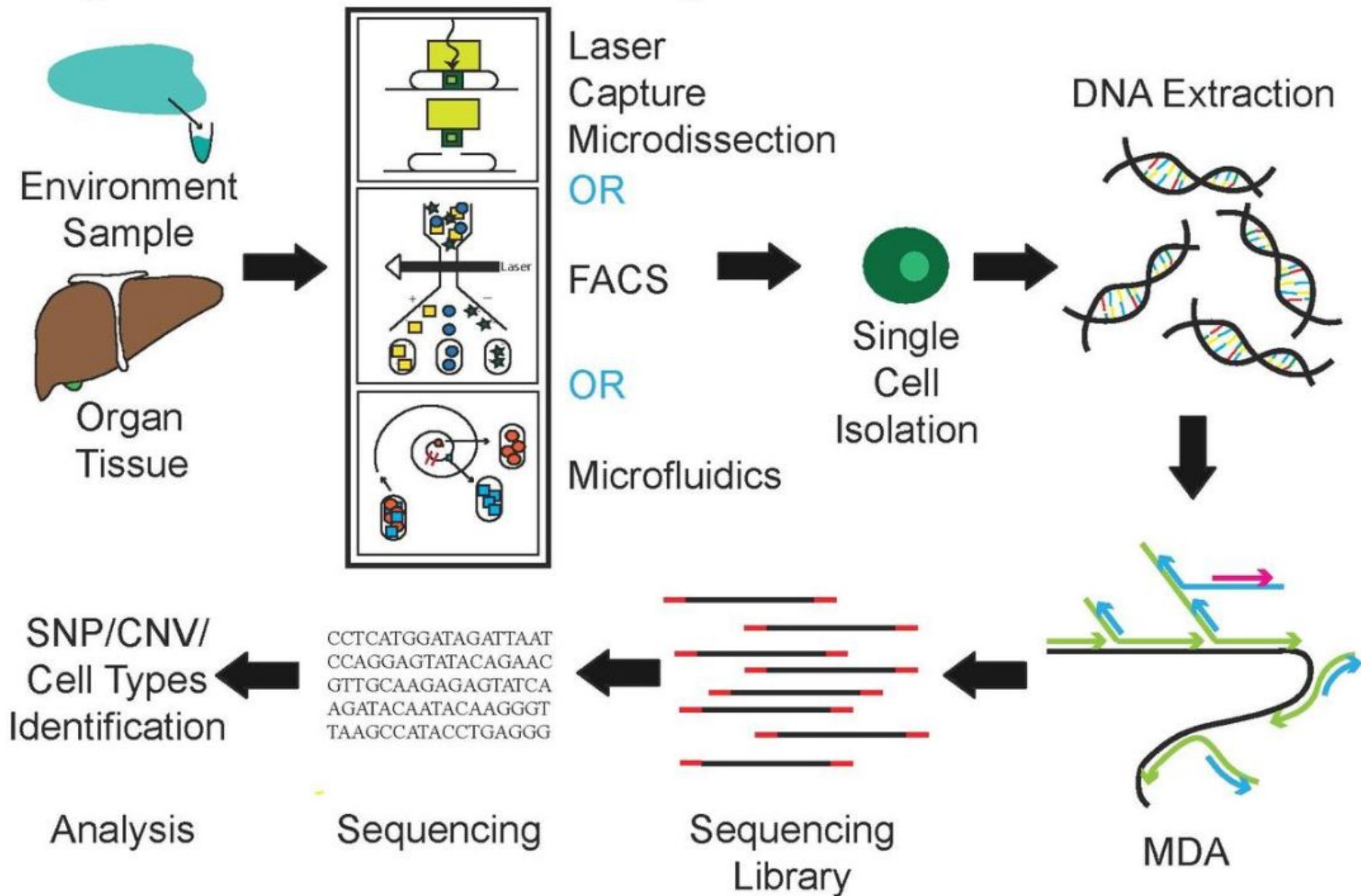
### A. Single-cell Genome Sequencing (scDNA-seq)



### B. Single-cell RNA Sequencing (scRNA-seq)

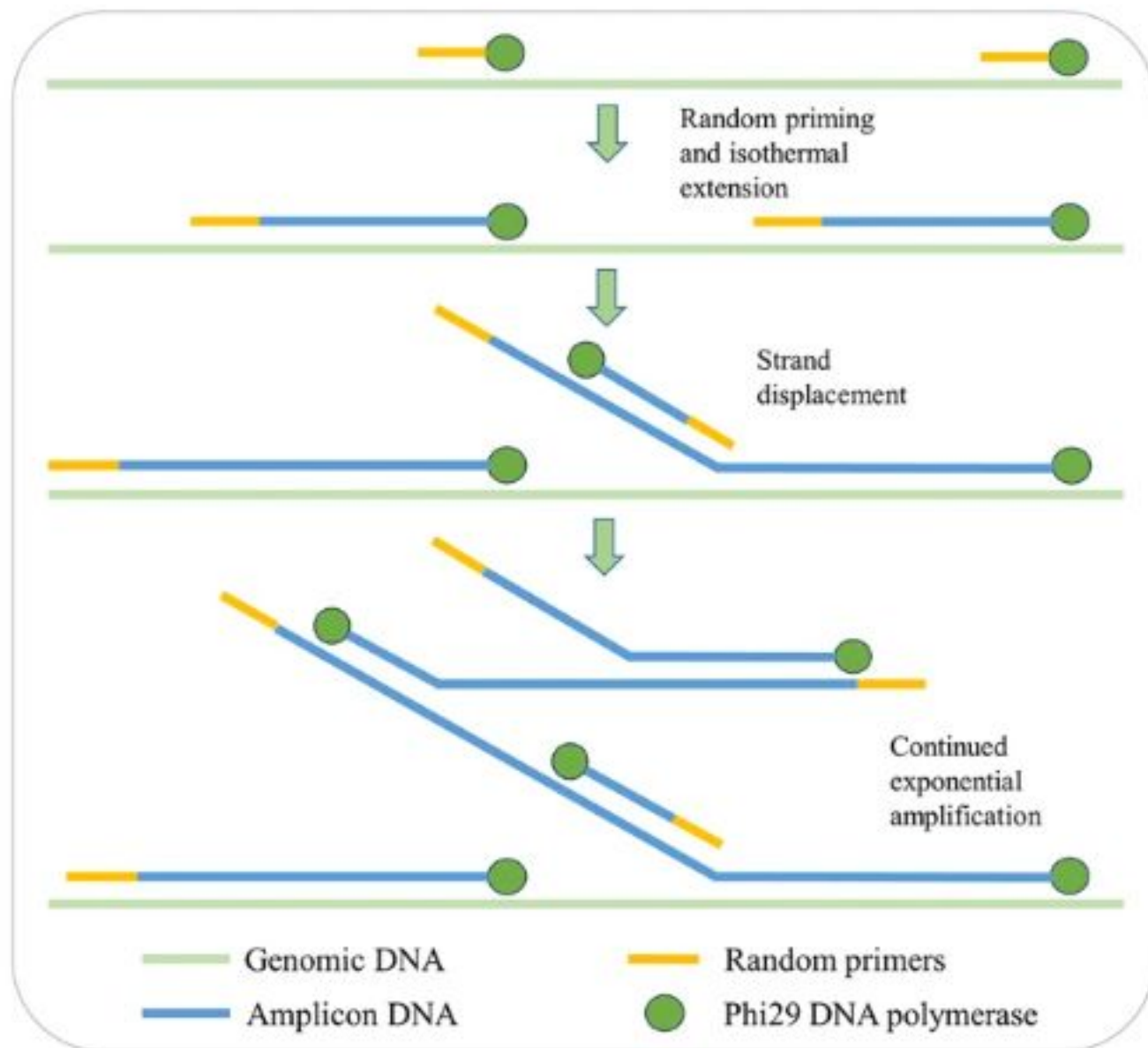


# Single Cell Genome Sequencing Workflow



# Multiple Displacement Amplification

- Multiple displacement amplification (MDA) is a widely used technique, enabling amplifying femtograms of DNA from bacterium to micrograms for sequencing.
- Reagents required for MDA reactions include: random primers and DNA polymerase from **bacteriophage phi29**.
- In 30 degree isothermal reaction, DNA is amplified with included reagents. As the polymerases manufacture new strands, a strand displacement reaction takes place, synthesizing multiple copies from each template DNA. At the same time, the strands that were extended antecedently will be displaced. MDA products result in a length of about 12 kb and ranges up to around 100 kb, enabling its use in DNA sequencing.
- In 2017, a major improvement to this technique, called WGA-X, was introduced by taking advantage of a thermostable mutant of the phi29 polymerase, leading to better genome recovery from individual cells, in particular those with high G+C content.
- MDA has also been implemented in a microfluidic droplet-based system to achieve a highly parallelized single-cell whole genome amplification. By encapsulating single-cells in droplets for DNA capture and amplification, this method offers reduced bias and enhanced throughput compared to conventional MDA



# Sc methylome

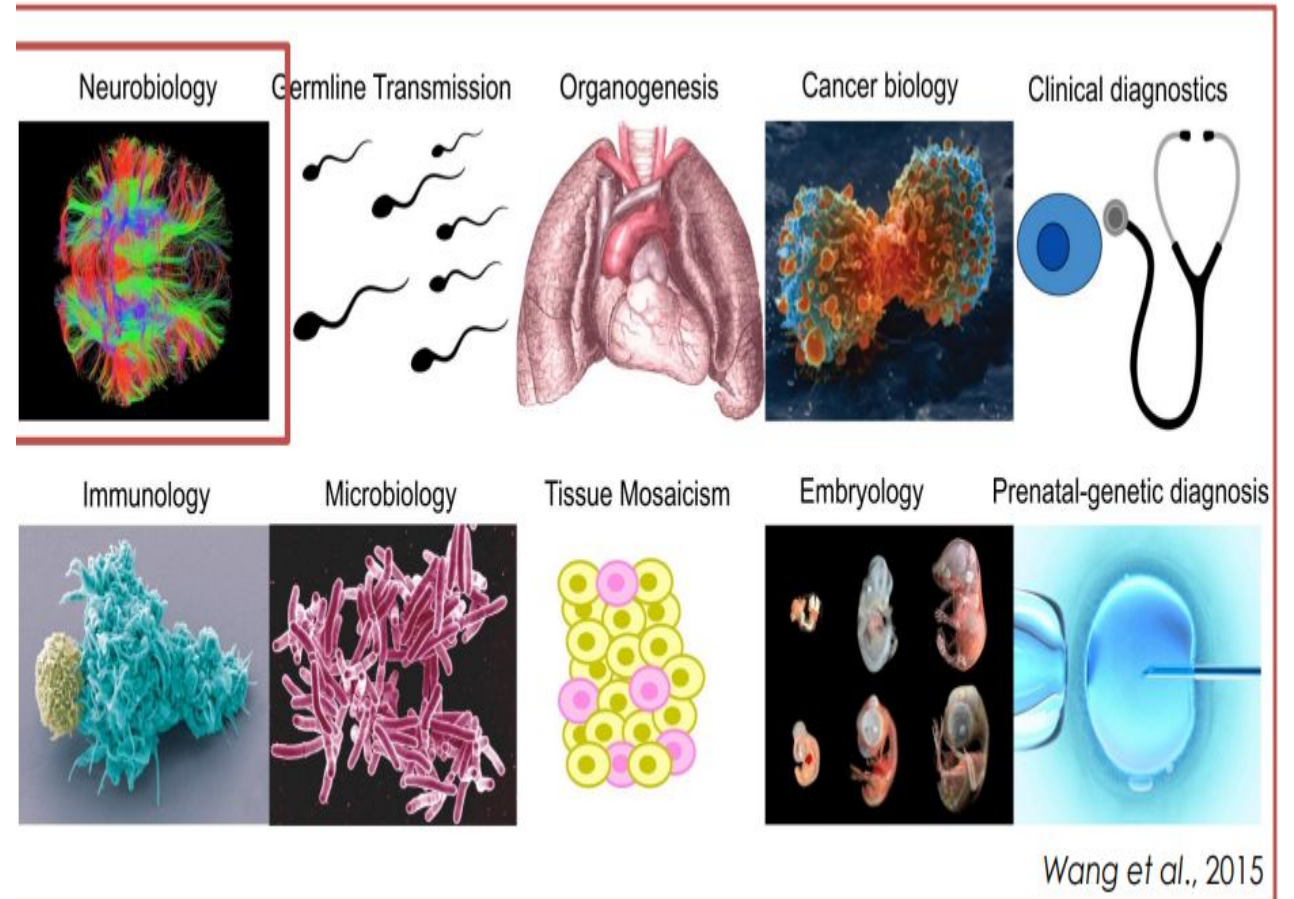
- Single-cell DNA methylome sequencing quantifies DNA methylation.
- There are several known types of methylation that occur in nature, including 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), 6-methyladenine (6mA), and 4mC 4-methylcytosine (4mC).
- In eukaryotes, especially animals, 5mC is widespread along the genome and plays an important role in regulating gene expression by repressing transposable elements.
- Sequencing 5mC in individual cells can reveal how epigenetic changes across genetically identical cells from a single tissue or population give rise to cells with different phenotypes.





# Applications

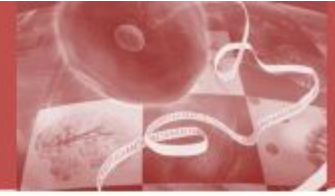
- Microbiomes: uncultured cells
- Cancer biology- study of heterogenous tissue



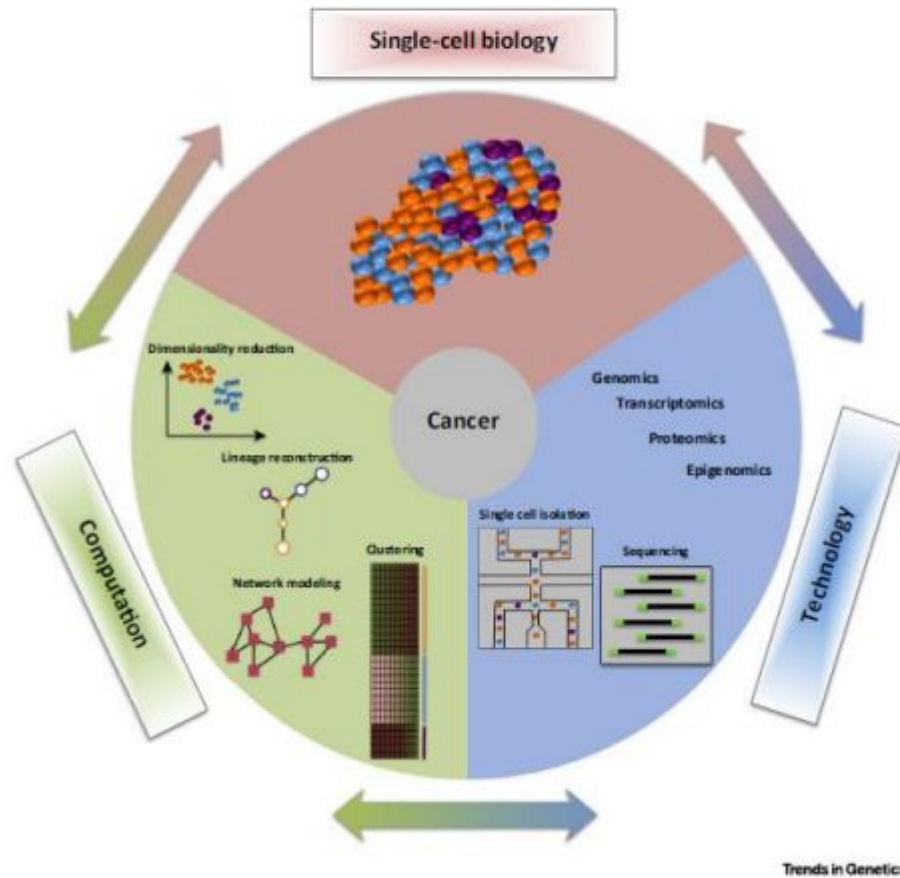
# Isolation methods of single cell

- Manual micro pipetting
- Single Cell Flow activated Cell sorting
- Microfluidics
- Laser capture microdissection
- Single nucleus suspension from fresh or cryopreserved tissue

# A. Cancer

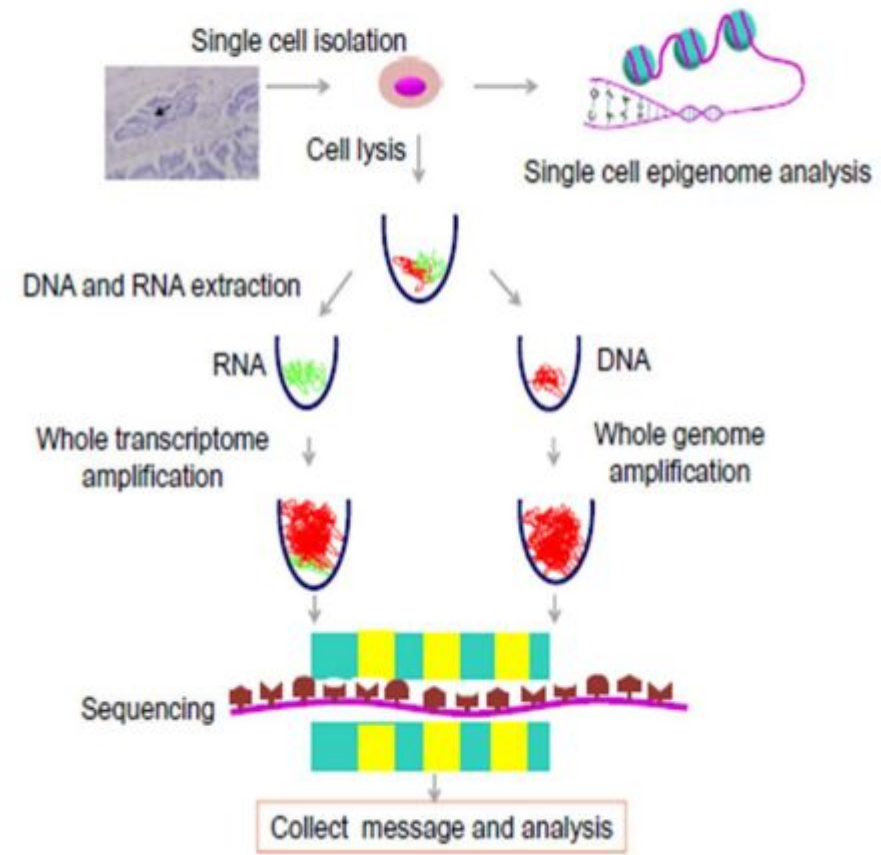


## An overview of Single-Cell Cancer Genomics



Saadatpour et al., 2015

## Single-cell sequencing of a tumor cells



Sun et al., 2015