# INTRODUCTION TO RECOMBINANT DNA TECHNOLOGY

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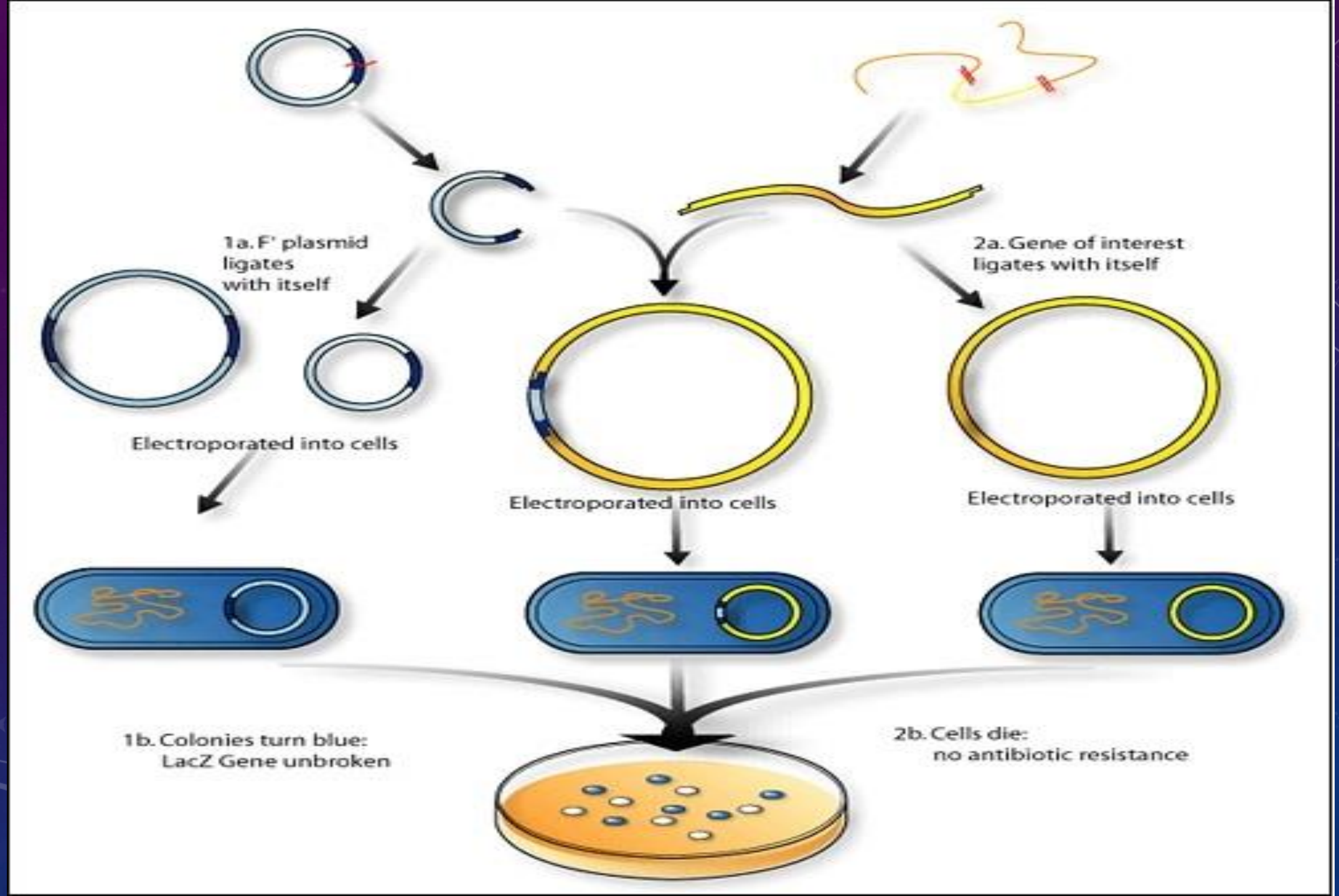
# INTRODUCTION

- Recombinant DNA Technology is defined by the Encyclopedia Britannica as “the joining together of DNA molecules from different organisms and inserting it into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture and industry.”
- Recombinant DNA (rDNA) is a technology that uses enzymes to cut and paste together DNA sequences of interest. The recombined DNA sequences can be placed into vehicles called vectors that ferry the DNA into a suitable host cell where it can be copied or expressed.

# BASIC PRINCIPALS OF RDT

- Generation of DNA fragment & selection of the desired piece of DNA.
- Insertion of the selected DNA into a cloning vector to create a rDNA or chimeric DNA.
- Introduction of the recombinant vectors into host cells.
- Multiplication & selection of clones containing the recombinant molecules.
- Expression of the gene to produce the desired product.


1a. F' plasmid ligates with itself
2a. Gene of interest ligates with itself
Electroporated into cells
Electroporated into cells
Electroporated into cells
1b. Colonies turn blue:
LacZ Gene unbroken
2b. Cells die:
no antibiotic resistance

# ENZYMES FOR DNA MANIPULATION

- **RESTRICTION ENDONUCLEASES**

- Enzymes for the manipulation of DNA
- Are bacterial enzymes that can cut/split DNA at specific sites.
- These were first discovered in E. coli restricting the replication of bacteriophages by cutting the viral DNA.
- The host E. coli DNA is protected from cleavage by addition of methyl ($CH_3$) groups.
- Thus the enzymes that restrict the viral replication are known as restriction enzymes or restriction endonuclease.

- TEMPLATE DEPENDENT DNA POLYMERASE

- DNA polymerase enzymes that synthesize new polynucleotides complementary to an existing DNA or RNA template are included in this category. Ex. DNA polymerase (Kornberg enzyme), Reverse transcriptase, Taq DNA Polymerase.

- **NUCLEASES**
- Nucleases are enzymes that degrade nucleic acids by breaking the phosphodiester bond that link one nucleotide to the next. Ex. Mung bean nuclease, S1 nuclease, RNase A , RNase H.

# END MODIFICATION ENZYMES

- End modification enzymes make changes to the ends of DNA molecules.

- **Terminal deoxyncleotidyl transferase**
- It is a template –independent DNA polymerase, because it is able to synthesize a new DNA polynucleotide without base-pairing of the incoming nucleotides to an existing strand of DNA or RNA.
- This enzyme is used for the formation of a cohesive end by homopolymer tailing.

**Alkaline phosphatase**

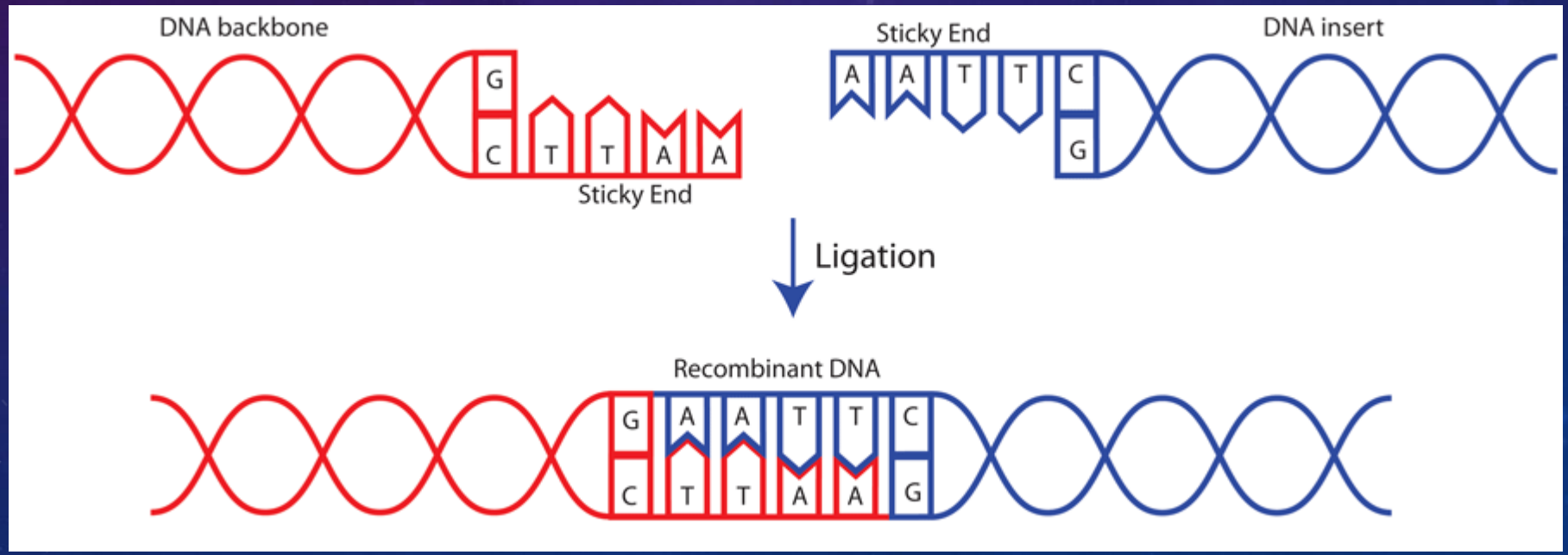
- Alkaline phosphatase, obtained from various sources, including E.coli and calf intestinal tissue, removes phosphate groups from the 5’ends of DNA molecules, which prevents these molecules from being ligated to one another.

- **T4 polynucleotide kinase**
- T4 polynucleotide kinase, obtained from E.coli cells infected with T4 phage, performs the reverse reaction to alkaline phosphatase, adding phosphate to 5’ends.

# LIGASES

- DNA ligases join DNA molecules together by synthesizing phosphodiester bond between nucleotides at the end of two different molecules, or at the two end of a single molecule.

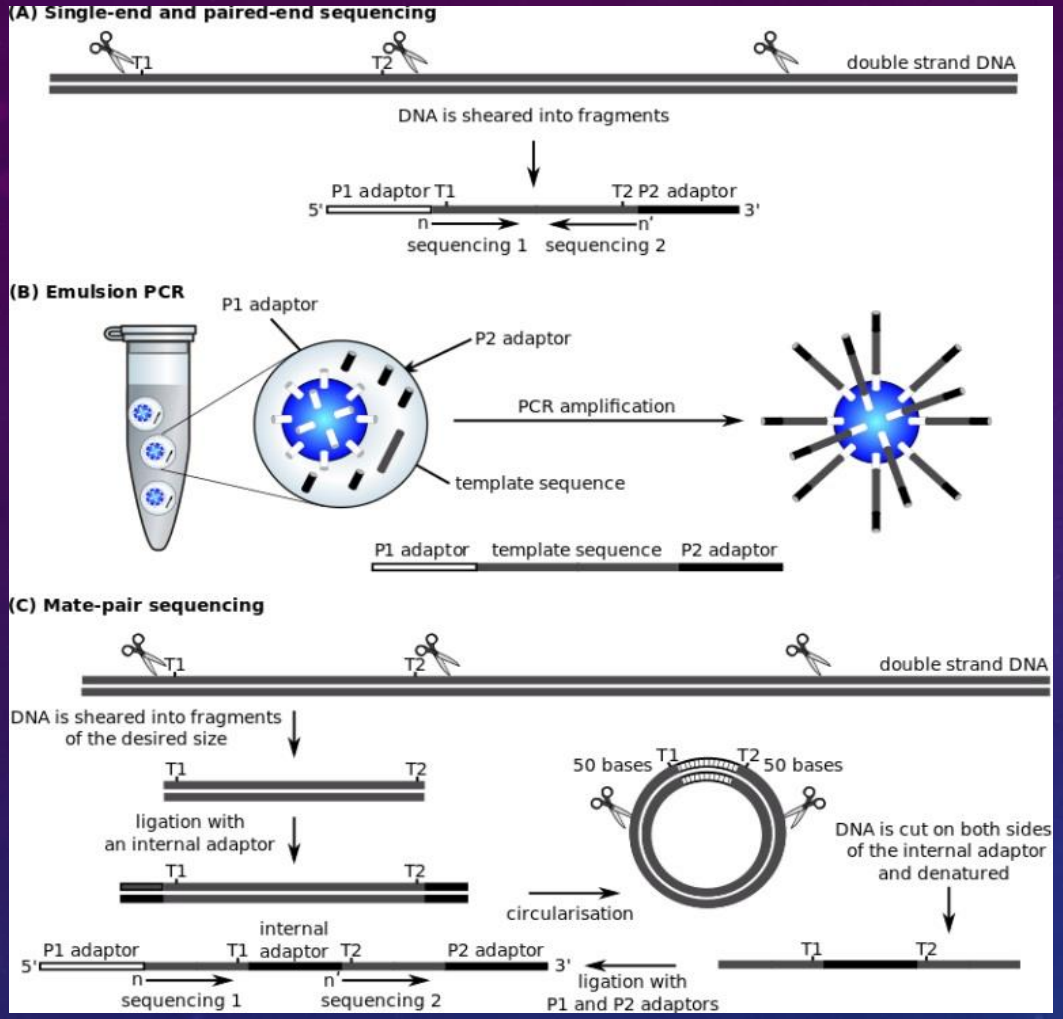
- DNA ligases commonly used in cloning experiments are those obtained from E.coli or from the bacteriophage T4

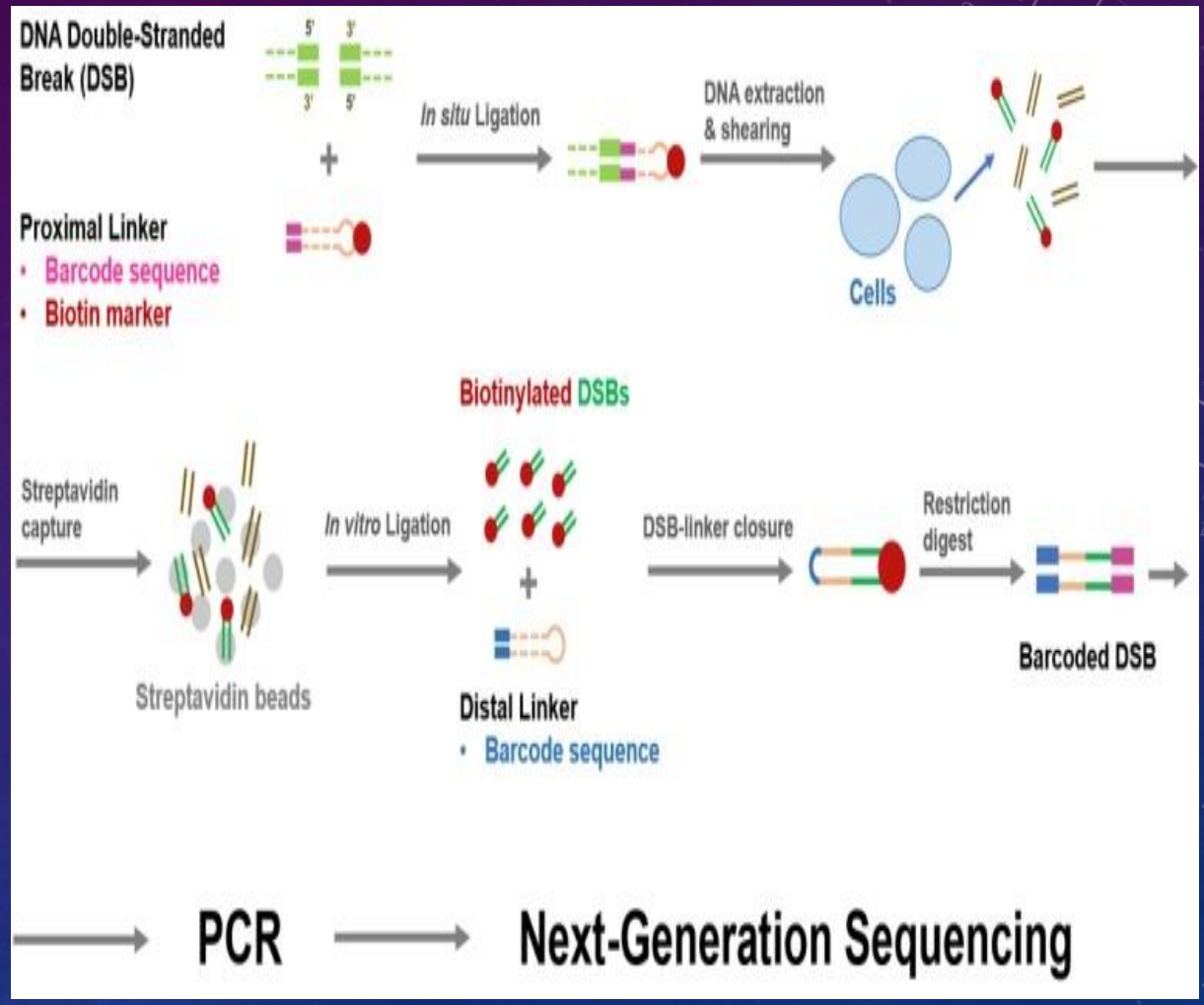


# LINKERS AND ADAPTORS

- **Linker** is a chemically synthesized oligonucleotide sequence that is double-stranded. Linker has two blunt ends. Linker is used to ligate DNA molecules that have blunt ends to vectors. It contains one or more internal restriction sites. These restriction sites work as recognition sites for restriction enzymes.

- An Adaptor is a double-stranded oligonucleotide sequence used to link two DNA molecules together. It is a short sequence with one blunt end and one sticky or cohesive end. Therefore, it consists of a single-stranded tail at one end, which enhances the efficiency of DNA ligation.


(A) Single-end and paired-end sequencing
T1
T2
double strand DNA
DNA is sheared into fragments
P1 adaptor T1
T2 P2 adaptor
5'
3'
n
n'
sequencing 1
sequencing 2
(B) Emulsion PCR
P1 adaptor
P2 adaptor
PCR amplification
template sequence
P1 adaptor
template sequence
P2 adaptor
(C) Mate-pair sequencing
T1
T2
double strand DNA
DNA is sheared into fragments of the desired size
ligation with an internal adaptor
50 bases
50 bases
DNA is cut on both sides of the internal adaptor and denatured
circularisation
P1 adaptor
internal adaptor
P2 adaptor
sequencing 1
sequencing 2
ligation with P1 and P2 adaptors


DNA Double-Stranded Break (DSB)
In situ Ligation
DNA extraction & shearing
Cells
Proximal Linker
Barcode sequence
Biotin marker
Biotinylated DSBs
Streptavidin capture
Streptavidin beads
In vitro Ligation
DSB-linker closure
Restriction digest
Barcoded DSB
Distal Linker
Barcode sequence
PCR
Next-Generation Sequencing

# THANK YOU