Core course
BMS361N
Genetic Engineering

In vitro mutagenesis

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In vitro mutagenesis

- Many methods are available
  - Random mutagenesis
  - Site directed mutagenesis
  - Deletion mutagenesis
  - Insertion mutagenesis

  - Random mutagenesis is used generally to screen particular genetic functional analysis

Mutagenesis

- One can clone gene to express it in a specific host organism to produce a large amount and very pure protein that can be used commercially.
- However physico chemical properties of these proteins (which are naturally occurring) are often not suited for particular task at extreme environment.
- Ex. Glucose oxidase, purified naturally occurring enzyme's optimal temperature is at 37oC but if one commercialize this, harsh handling, changing environment may inactivate. To over come this type of problem, molecular biologist came up with idea to create or modify or changing aa encodes an enzyme with desired properties so that which can be used at both extreme conditions or particular task.

By using a set of techniques what specifically change aa encoded by a cloned gene
Protein with properties that are better suited than naturally occurring enzymes.
Ex.

By altering both substrate binding and maximal rate of conversion of the substrate to product.

By changing the thermal tolerance or pH stability or both.

By changing an enzyme so that a cofactor is no longer required for large scale reaction.

By changing substrate binding site to increase its specificity, so that non-specific reactions and its products are reduced.

By increasing resistance to cellular protease, so that protease cannot act on it thereby increase the yield of proteins.

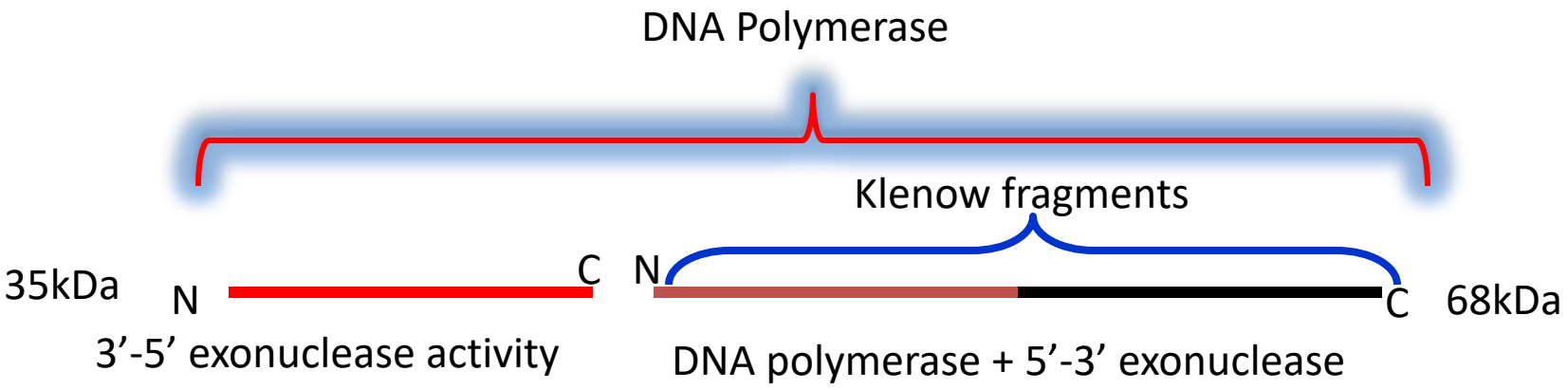
By altering the allosteric regulation to diminish the impact of feedback inhibition and increase the yield.

Mutants

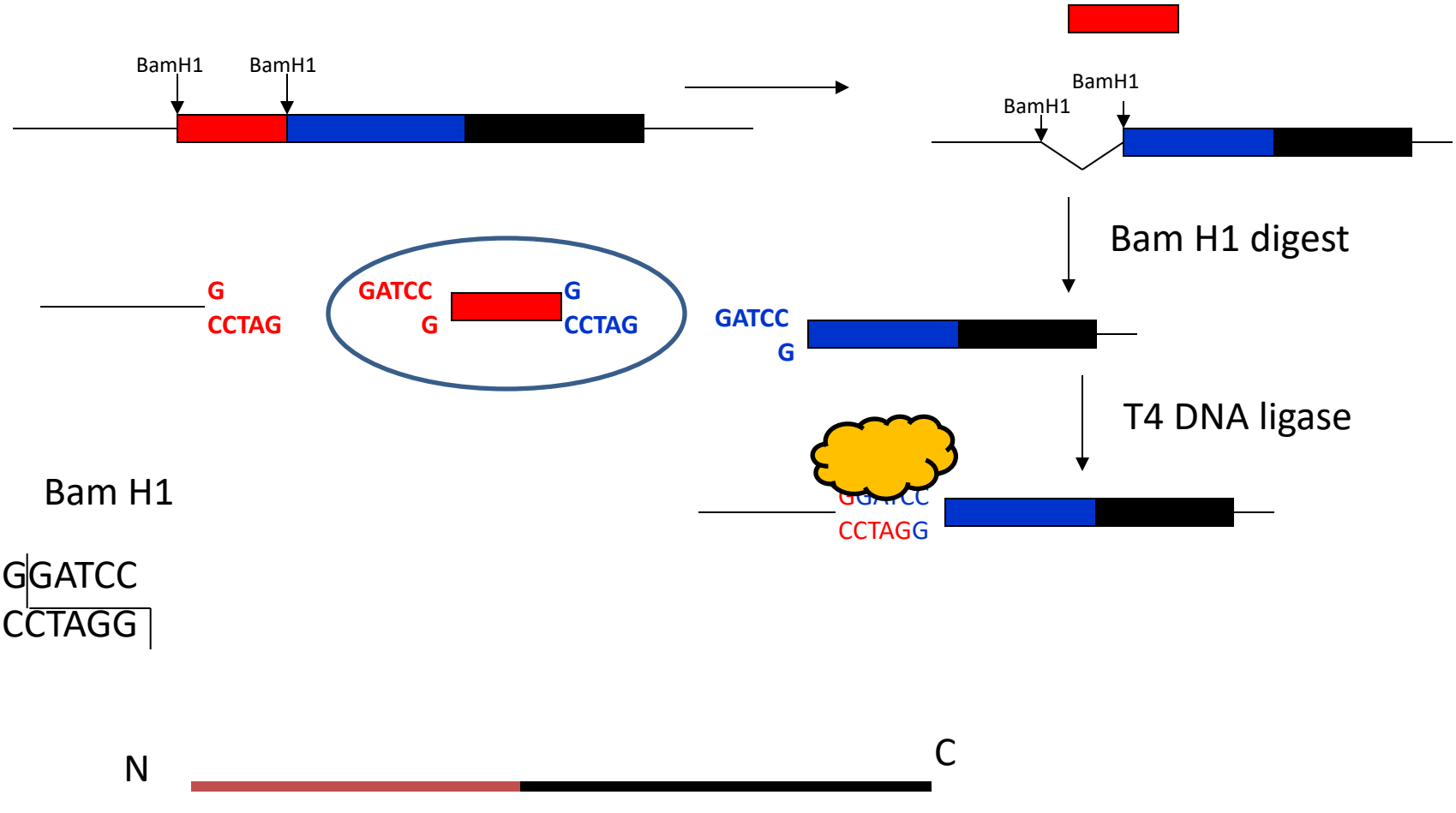
- Deletions
  - One can delete one nucleotide to few hundred nucleotides depending upon their experimental need.
- Insertions
  - One can insert one nucleotide to few hundred nucleotide depending upon their experimental need.

Deletion

- If RE site present in site where one wants to make deletion, they can use RE to digest and remove part of the DNA fragment from the cloned DNA fragments

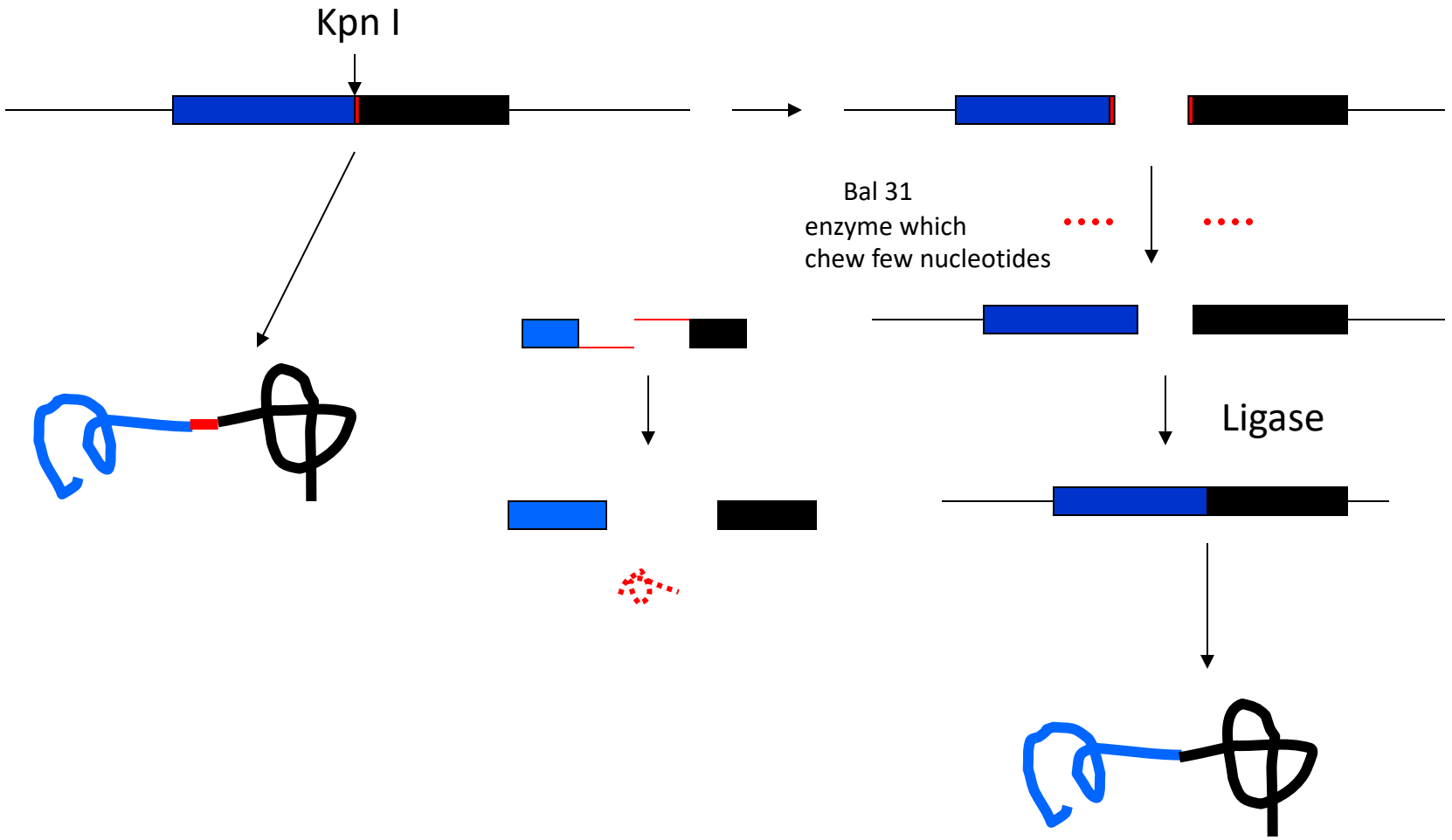


Few nucleotides Deletion

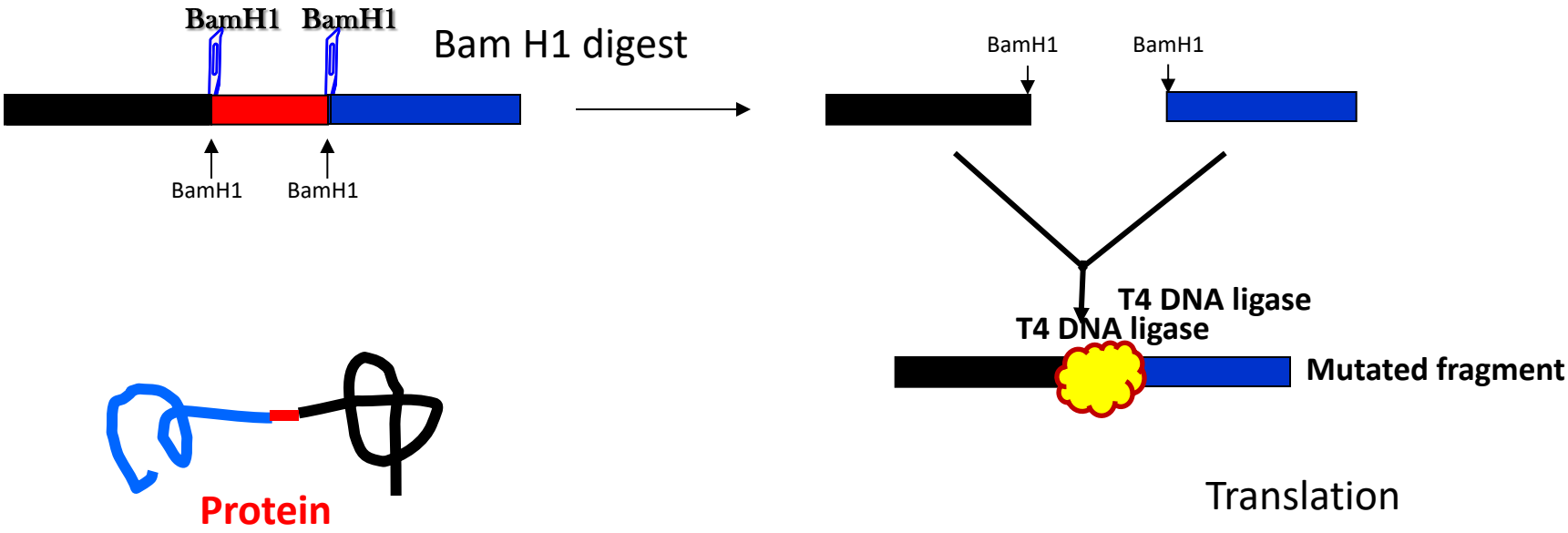


Few nucleotides Deletion

Removing few nucleotide



Deletion

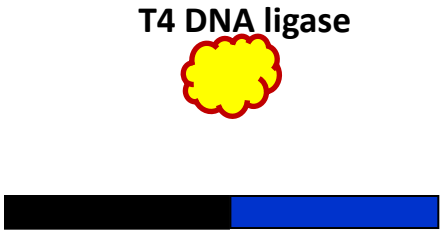


Bam H1

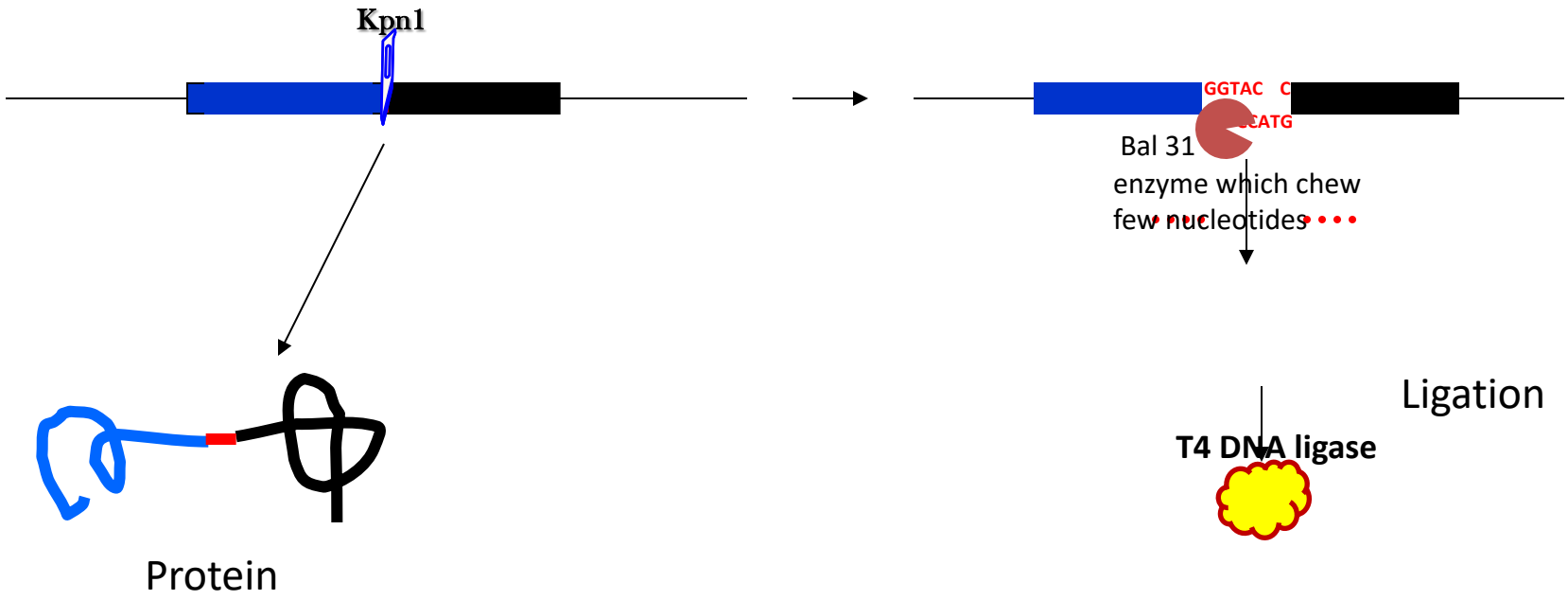
GGATCC
CCTAGG

Mutated Protein

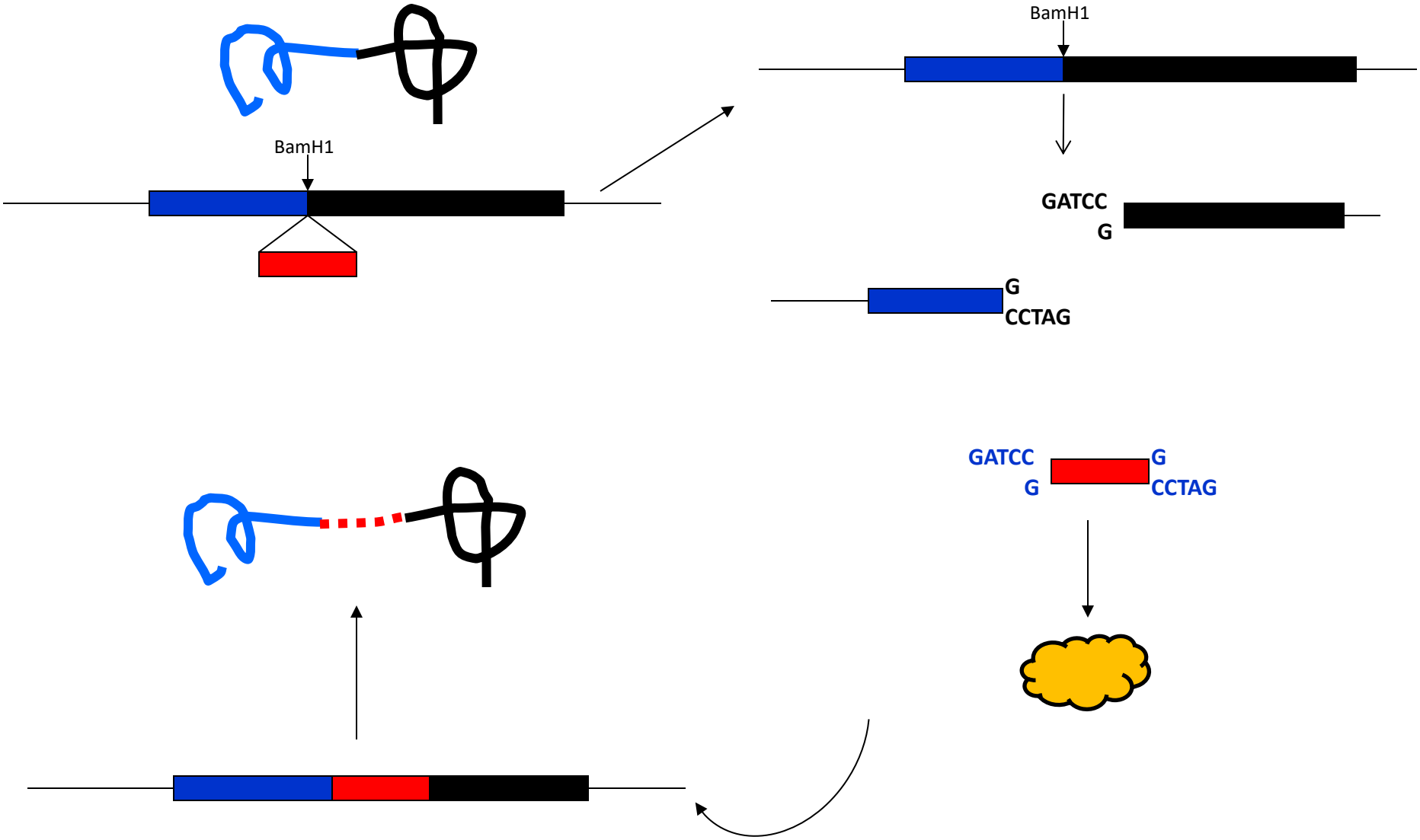
Few nucleotides Deletion



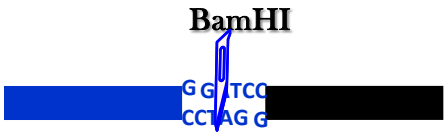
Removing few nucleotide



Insertion



Insertion



Site directed mutagenesis

- By many method one can achieve this mutagenesis.
- Main mechanism is Oligonucleotides can be synthesized and used to make mutant DNA. This mutant DNA used to express mutant protein and then analyzed the mutant induced effect by comparing wild type protein.

Site Directed mutagenesis

- Site directed mutagenesis:
- A single nucleotide (point mutation) change can make various functions
- Ex. Single nucleotide can make
  - » non-sense mutant
  - » Sense mutant
  - » Frame shift

The Good, the Bad, and the Silent

- Mutations can serve the organism in three ways:

- The Good : A mutation can cause a trait that enhances the organism's function:

  Mutation in the sickle cell gene provides resistance to malaria.

- The Bad : A mutation can cause a trait that is harmful, sometimes fatal to the organism:

  Huntington's disease, a symptom of a gene mutation, is a degenerative disease of the nervous system.

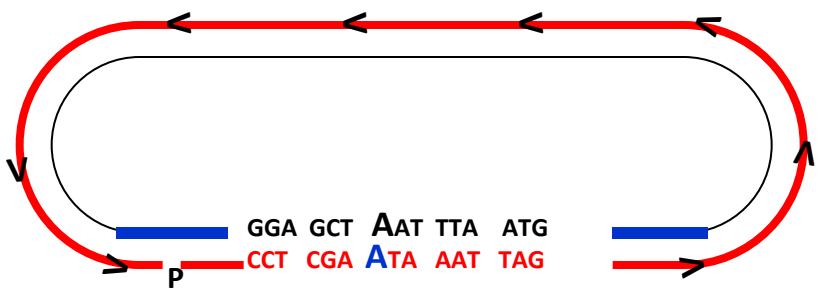
- The Silent: A mutation can simply cause no difference in the function of the organism.

Campbell, Biology, 5th edition, p. 255

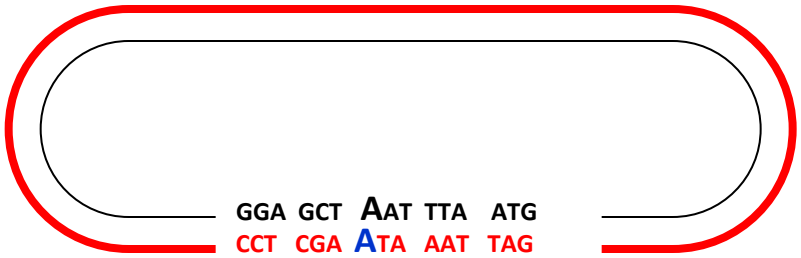
Oligonucleotide-directed mutagenesis

Oligonucleotide-directed mutagenesis

Plasmid based



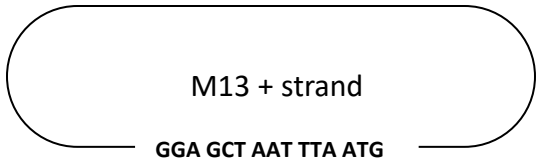
T4 DNA ligase

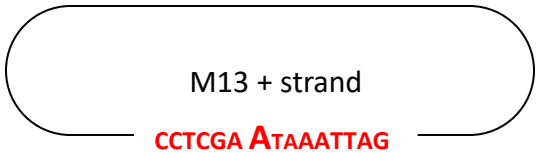


Transform to E.coli

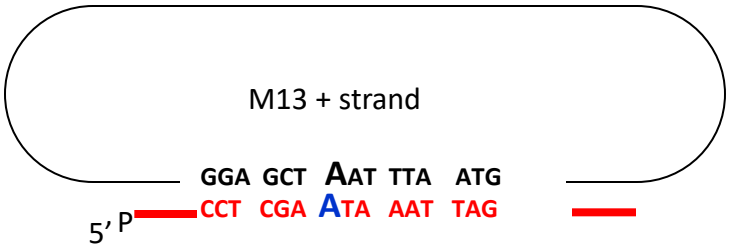
50%

50%





Klenow enzyme

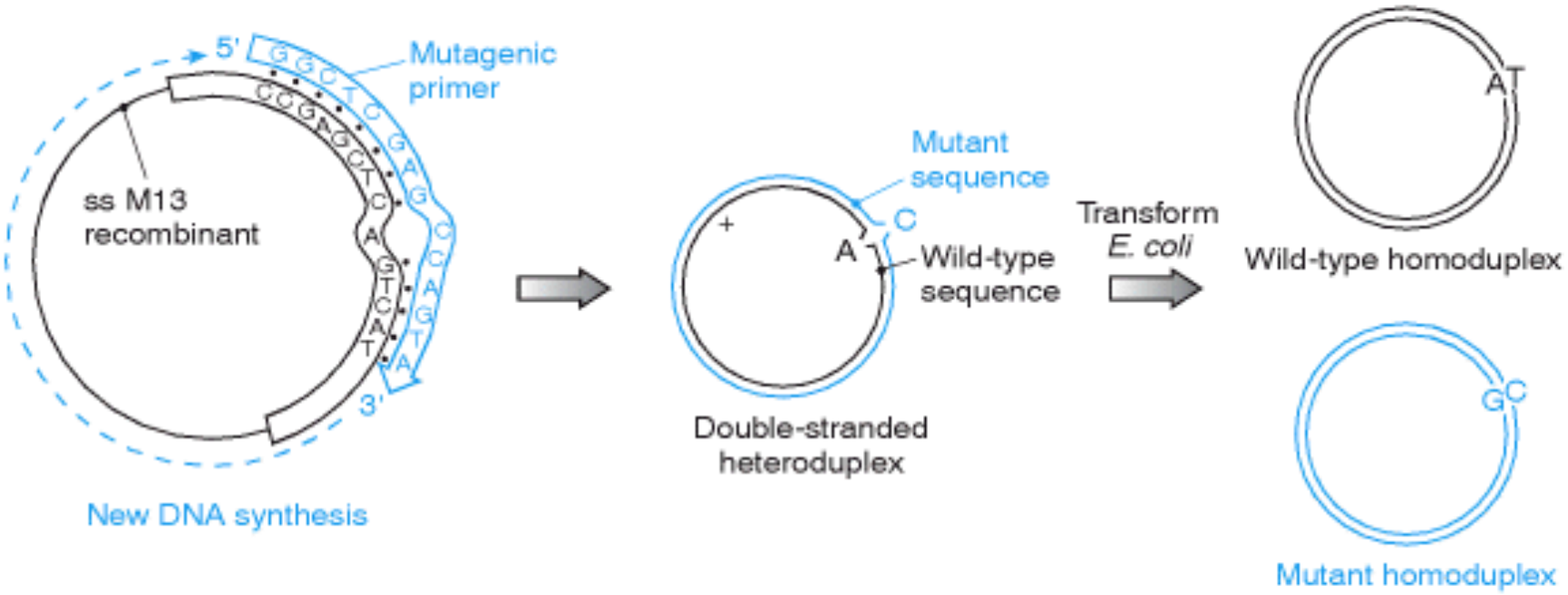


Gly	ala	asn	leu	met
GGA	GCT	AAT	TTA	ATG
CCT	CGA	TTA	AAT	TAG
CCT	CGA	ATA	AAT	TAG
Gly	ala	tyr	leu	met

Select the mutant by high stringency hybridization using mutant Oligo as probe.

Because of some reason only 1-5% of mutated phage can be recovered.

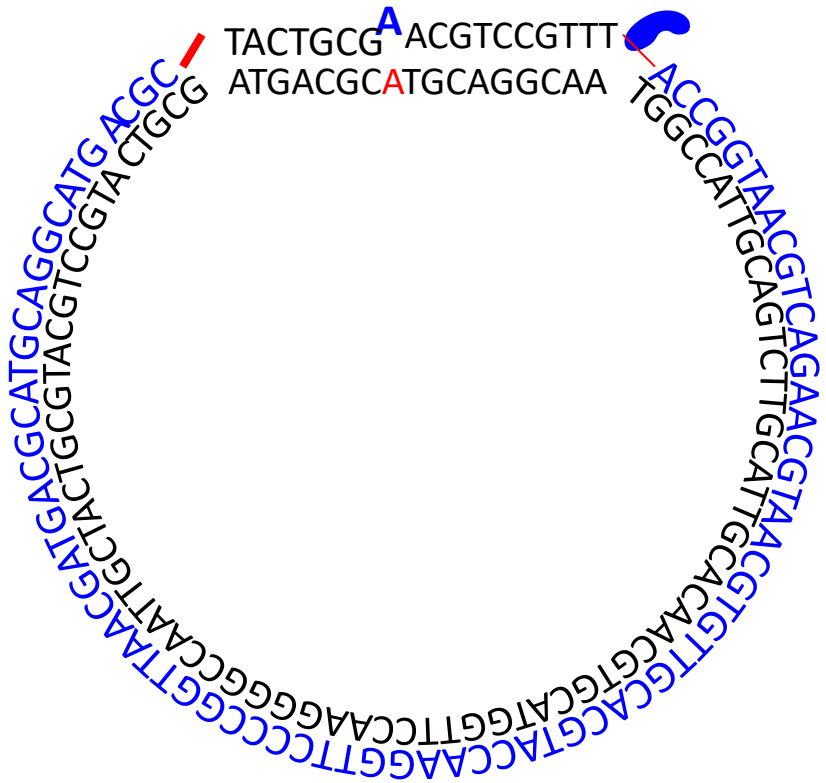
Oligonucleotide-directed mutagenesis

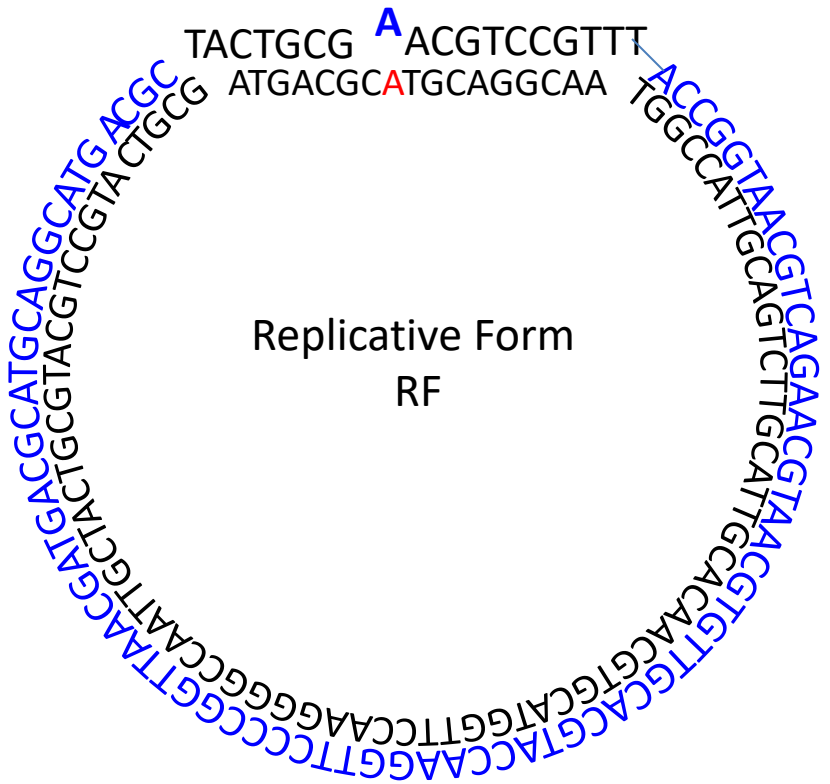


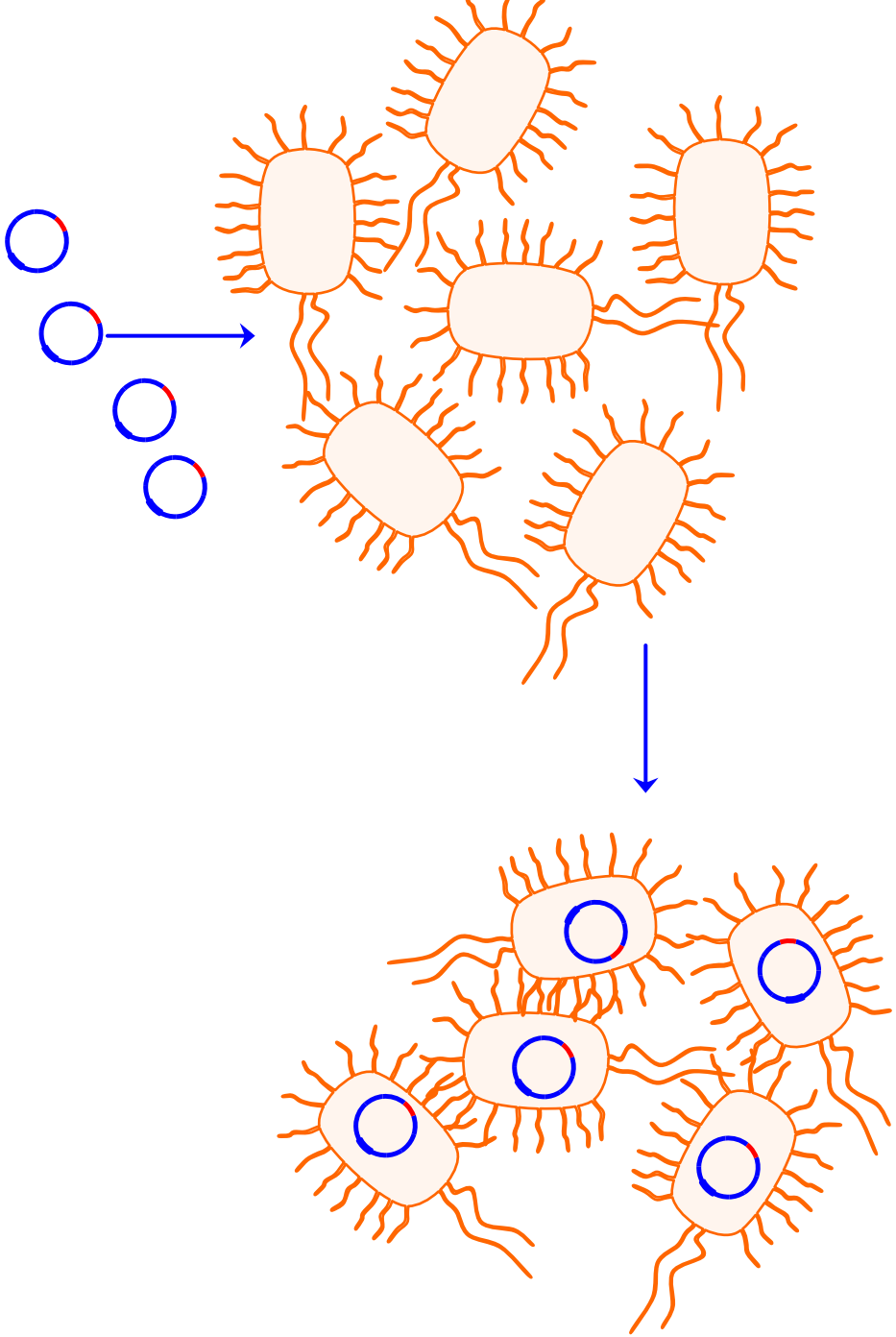
Oligonucleotide-directed mutagenesis

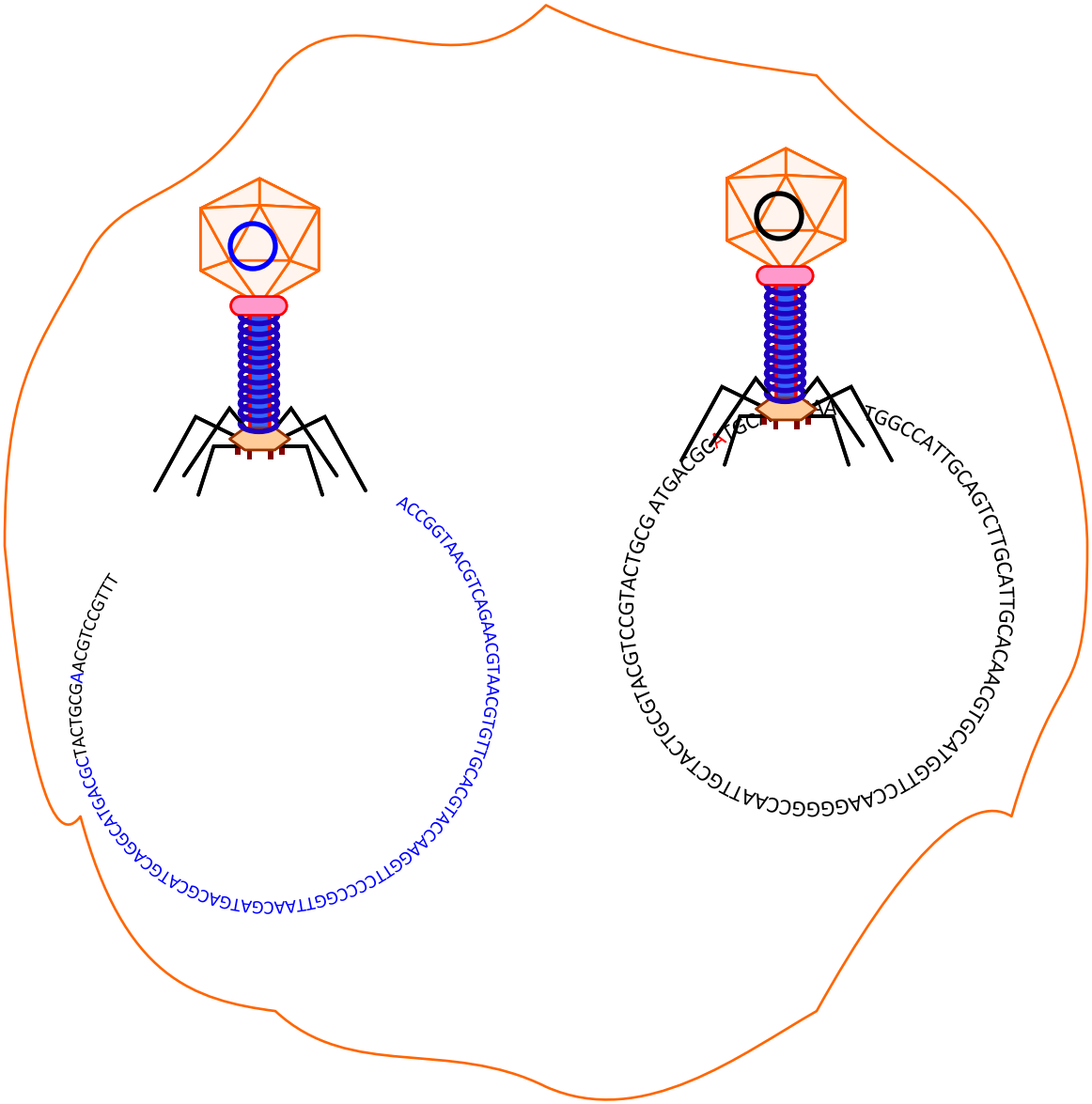
Site Directed mutagenesis

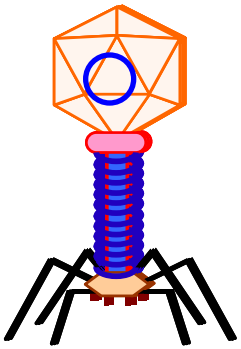
T4 DNA ligase

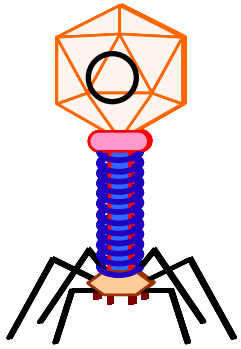


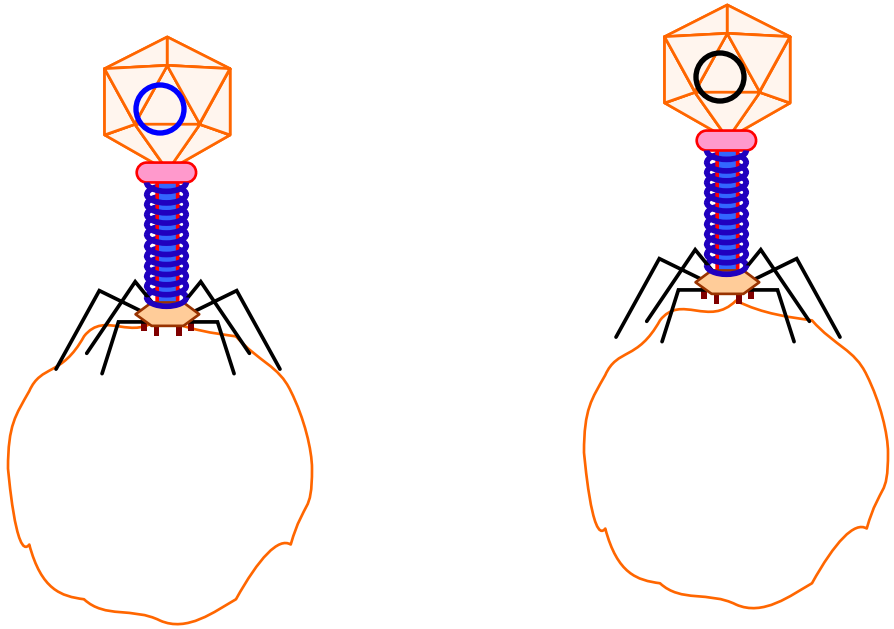

TACTGCG A ACGTCCGTTT
ATGACGCATGCAGGCAA
Replicative Form
RF




ACCGGTAACGTCAGAACGTAACGTGTTGCAACGTACCAAGGTTCCCGGTTAACGATGACGCATGCAGGCATGACGCTACTGCGAACGTCCGTTT
AGGCCATTGCAGTCTTGCATTGCACAACGTGCATGGTTCCAAGGGGCCAATTGCTACTGCGTACGTCCGTACTGCG ATGACGCATGC







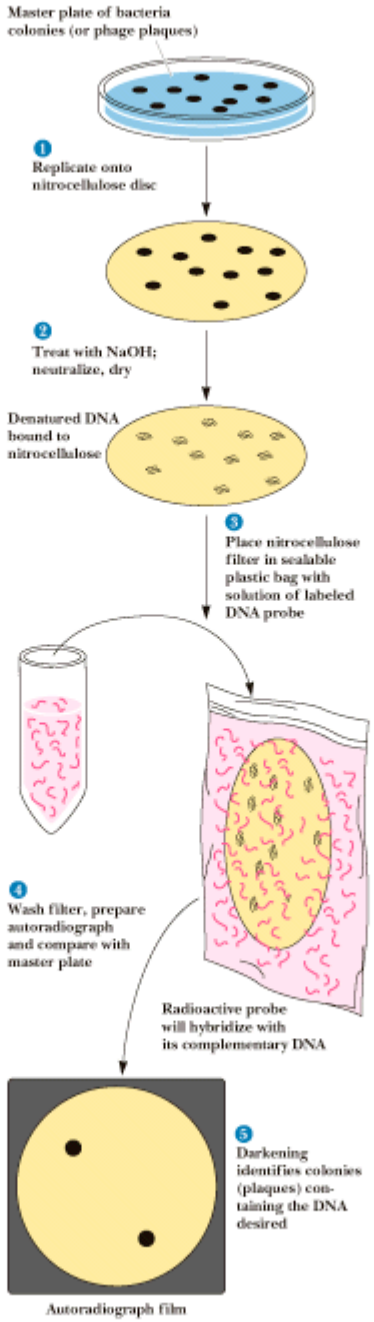
By hybridization method one can isolate mutated Plasmid.

Colony Hybridization

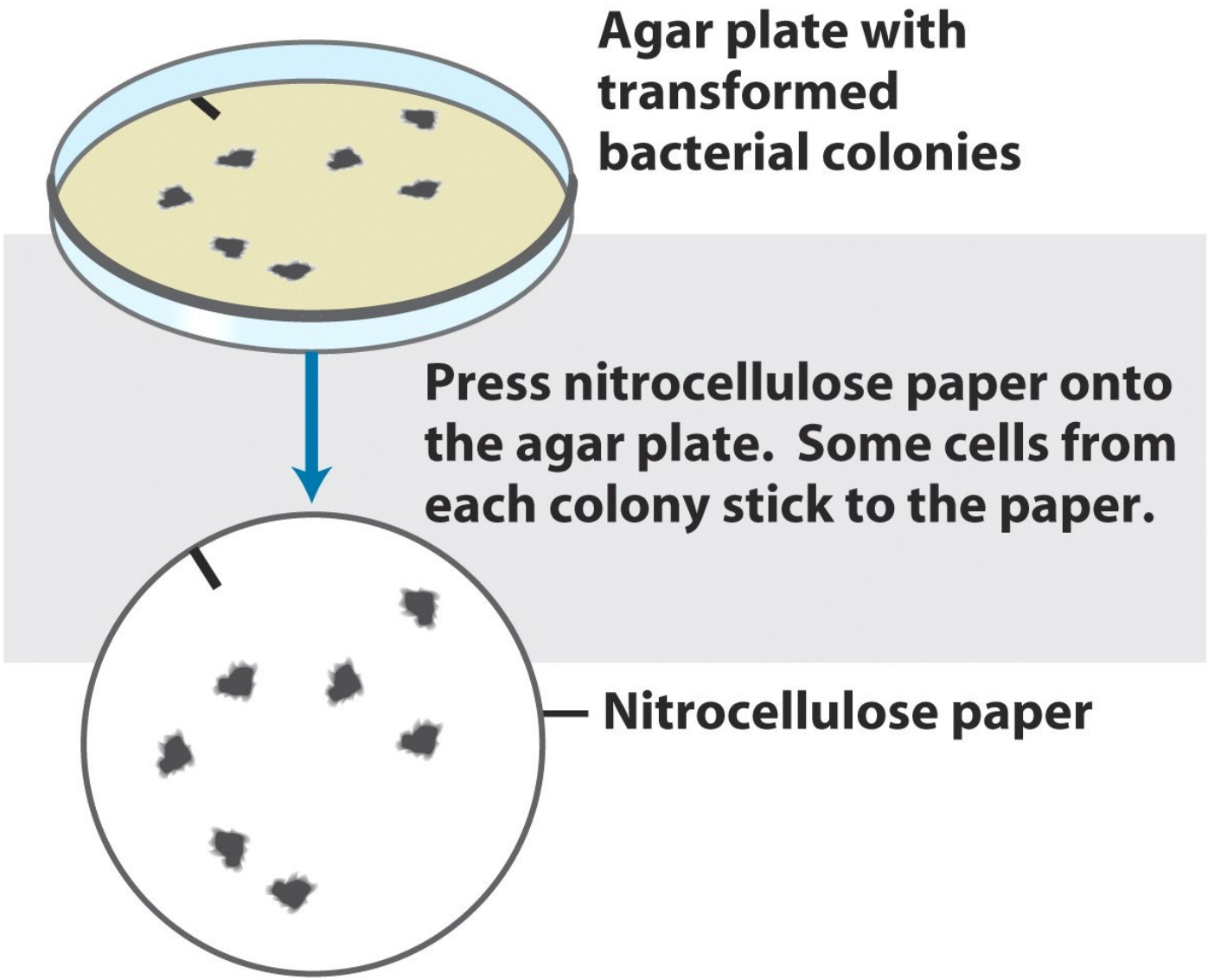
A way to screen plasmid-based genome libraries for a DNA fragment of interest

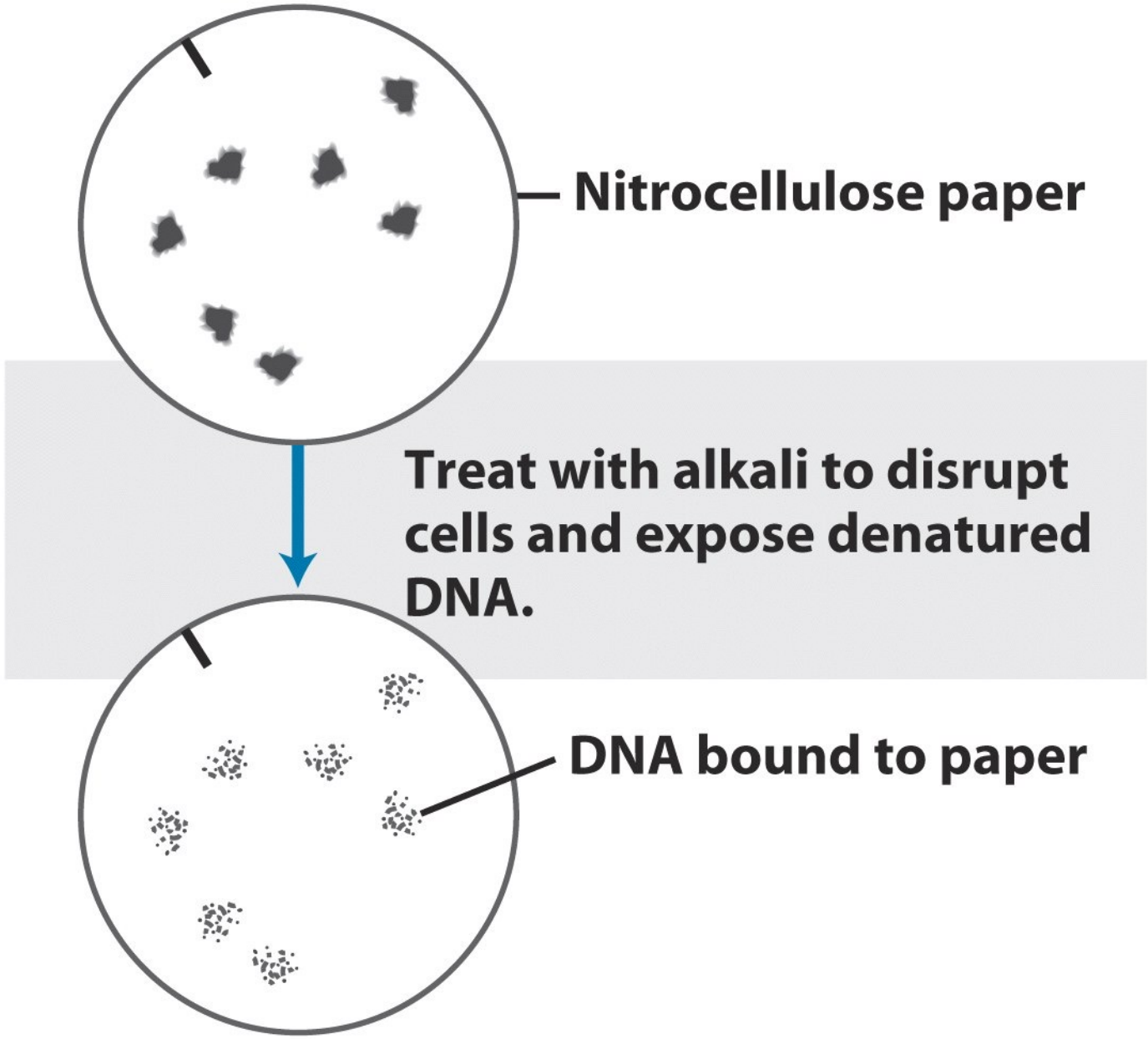
- Host bacteria containing a plasmid-based library of DNA fragments are plated on a petri dish and allowed to grow overnight to form colonies
- Replica of dish made with a nitrocellulose disk

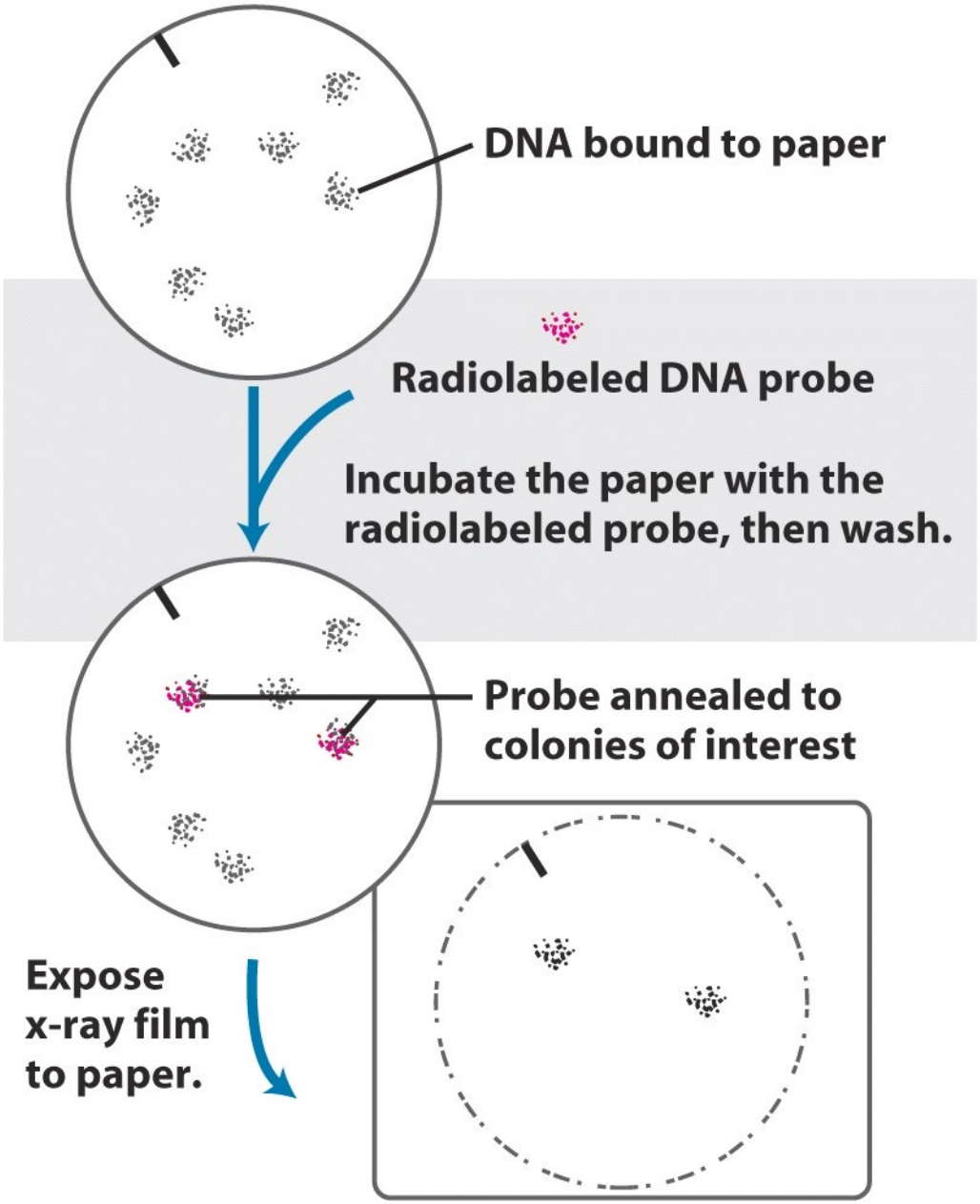
- Disk is treated with base or heated to convert dsDNA to ssDNA and incubated with probes
- Colonies that bind probe (with P-32) hold the fragment of interest



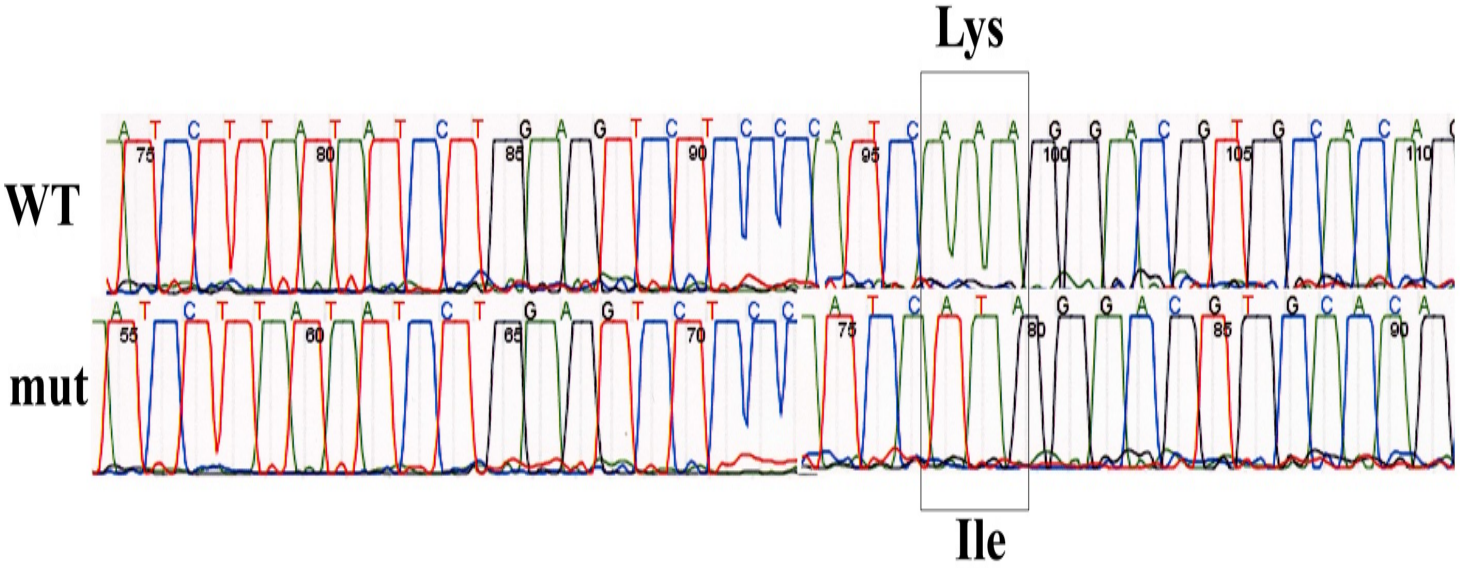
Specific sequences are detectable by hybridization




Nitrocellulose paper
Treat with alkali to disrupt cells and expose denatured DNA.
DNA bound to paper


DNA bound to paper
Radiolabeled DNA probe
Incubate the paper with the radiolabeled probe, then wash.
Probe annealed to colonies of interest
Expose x-ray film to paper.

Confirmation by DNA sequencing



AAA ⟶ ATA

Lys Ile

Modified Oligo-directed mutageneis

Oligo-directed mutagenesis is modified in a number of ways to enrich number of mutant phage.

One of the method is:

Use of different E.coli strains

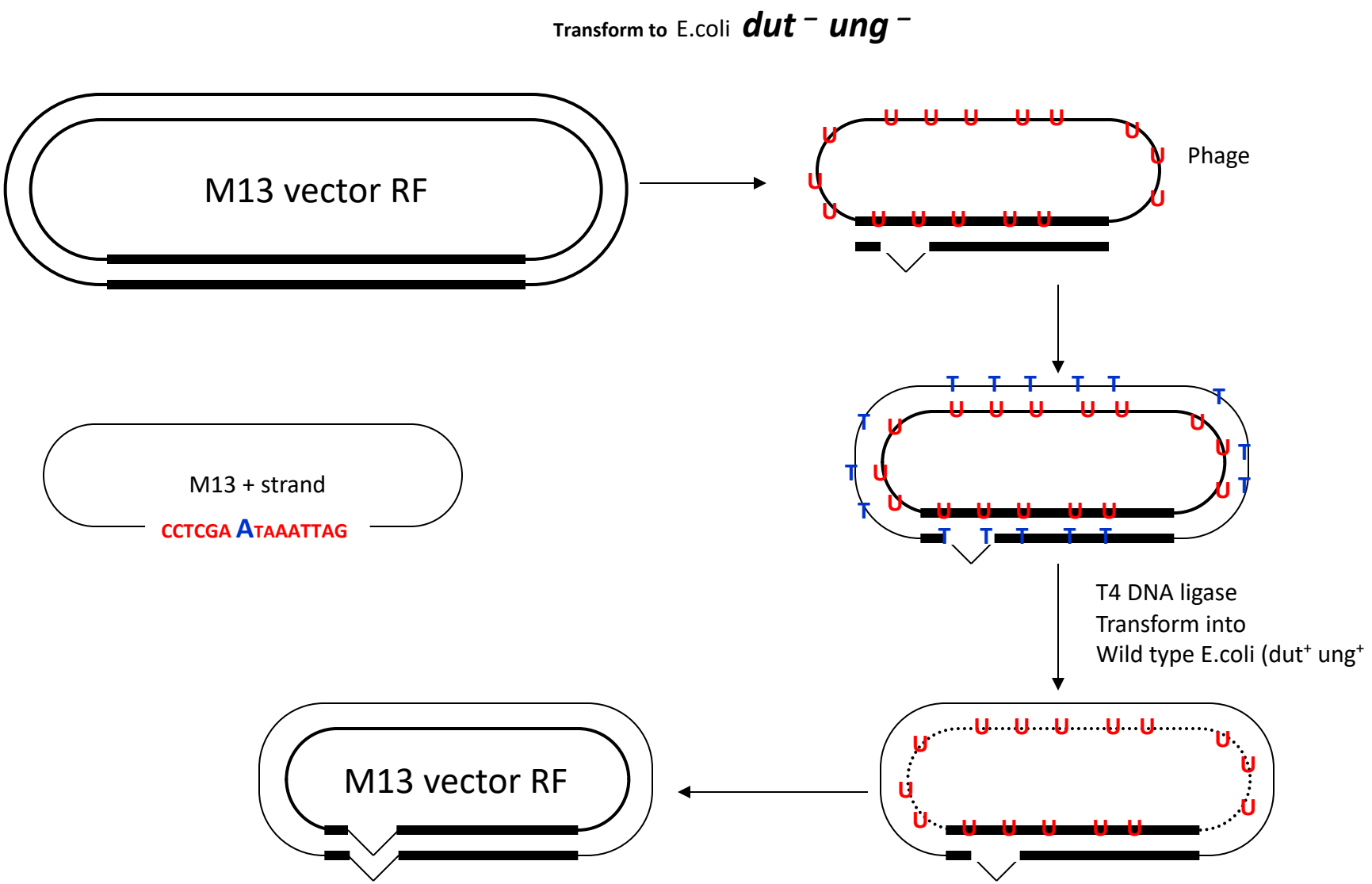
one of the E.coli strain has 2 defective DNA metabolism enzymes

(1) defective form of dUTPase (*dut*$^{-}$)

(2) defective form of Uracil N-glycosylase (*ung*$^{-}$)

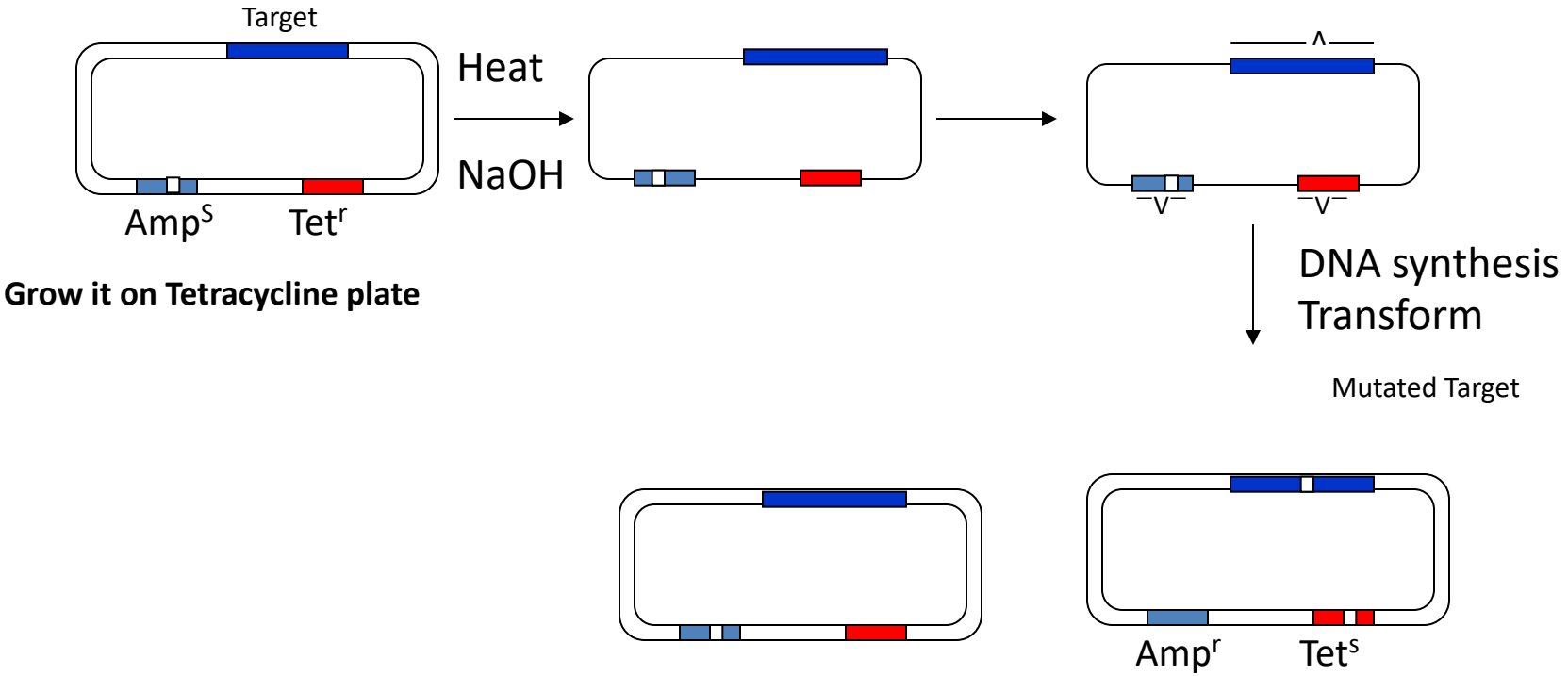
Because of these defective enzymes incorporation of UTP residue cannot be remove. So the genomic DNA and vectors transformed into this E.coli strains have UTPs in the place of TTPs. By make use of these properties one can enrich mutant vectors.

Modified Oligo-directed mutageneis

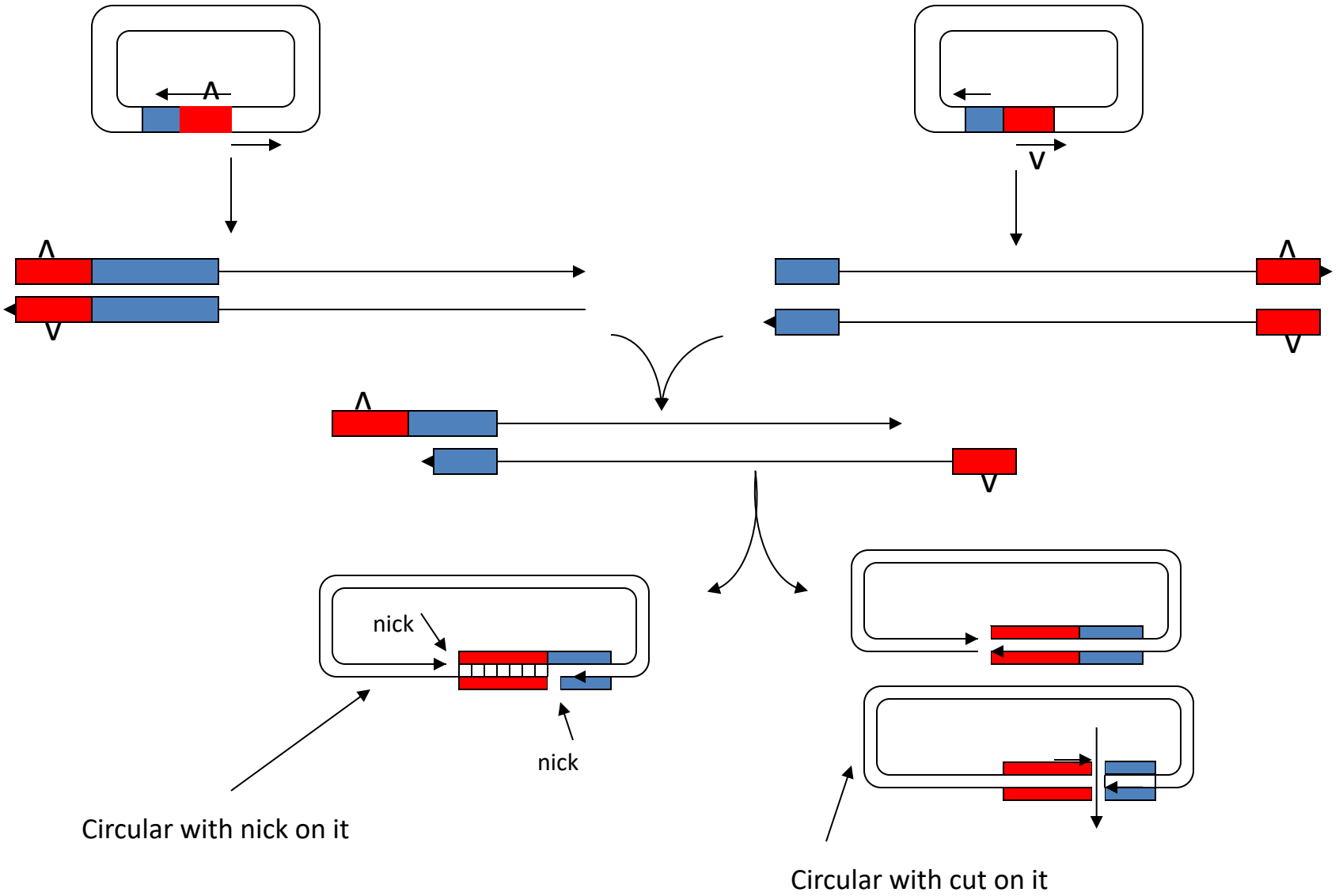


Modified Oligo-directed mutageneis

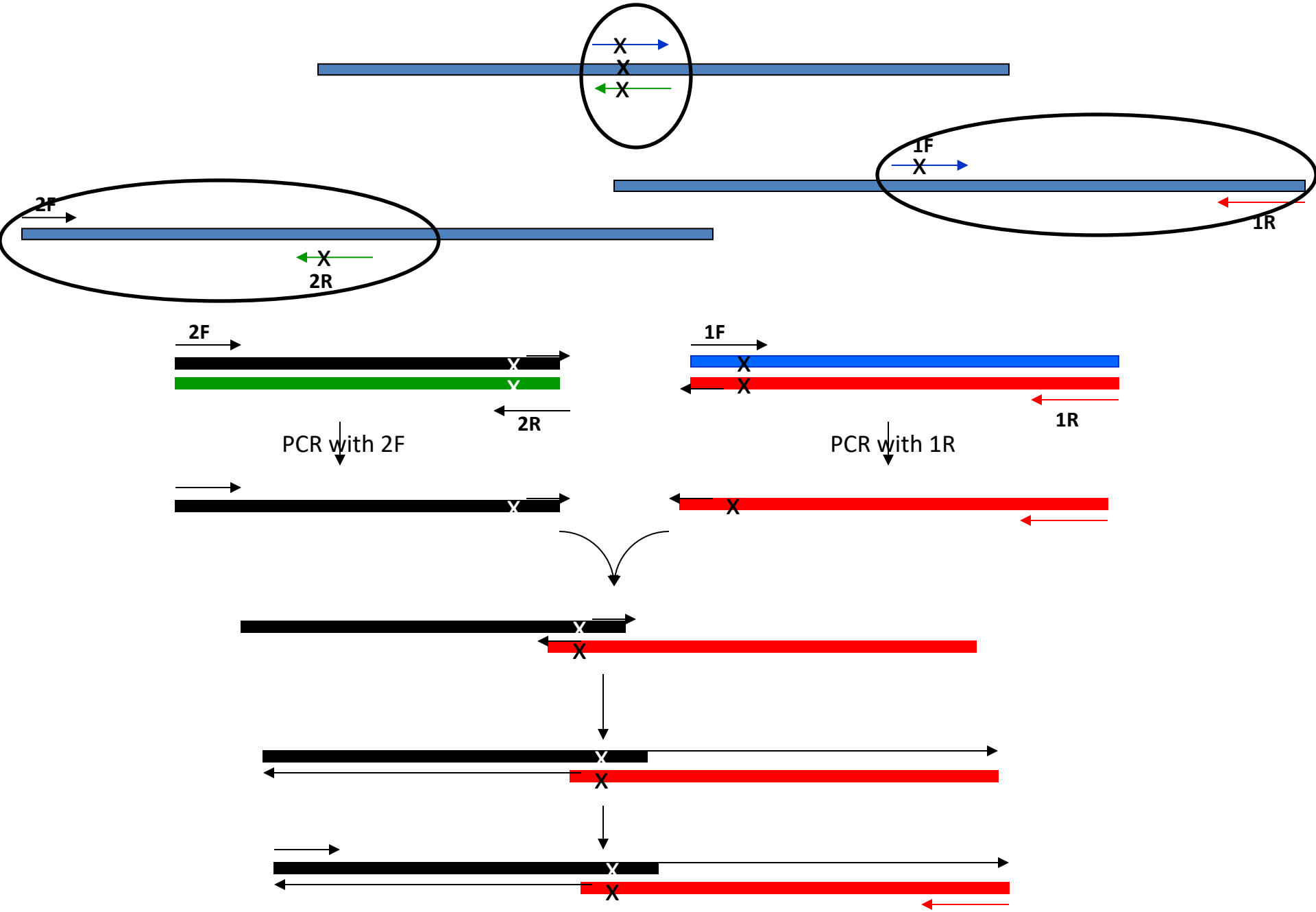
Using Ds Plasmid



PCR based Oligonucleotide directed mutagenesis



PCR based Mutation



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The End

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