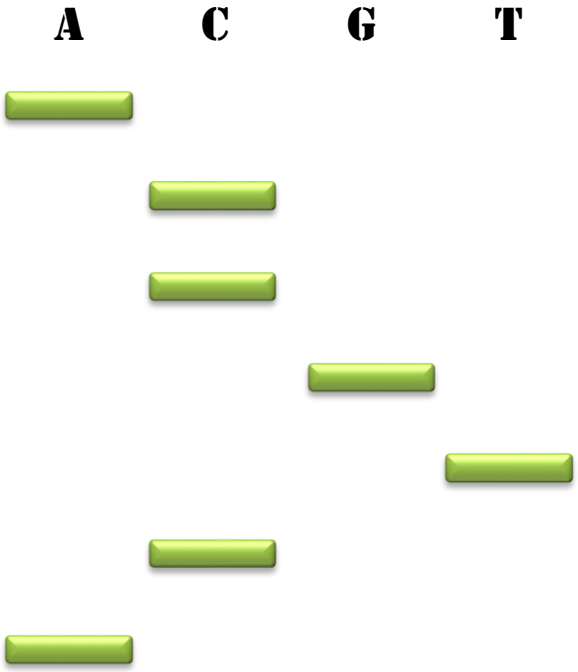
Bioinformatics

Sanger Sequencing



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2.11.2016

Sanger sequencing needs the following steps:

1. Polymerase chain reaction
2. Sequencing reactions
3. Gel loading
4. Fragment separation

1. Polymerase chain reaction PCR

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

1. Polymerase chain reaction PCR

Separate the two strands (95° C)

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

1. Polymerase chain reaction PCR

Anneal two primers (55° C)

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

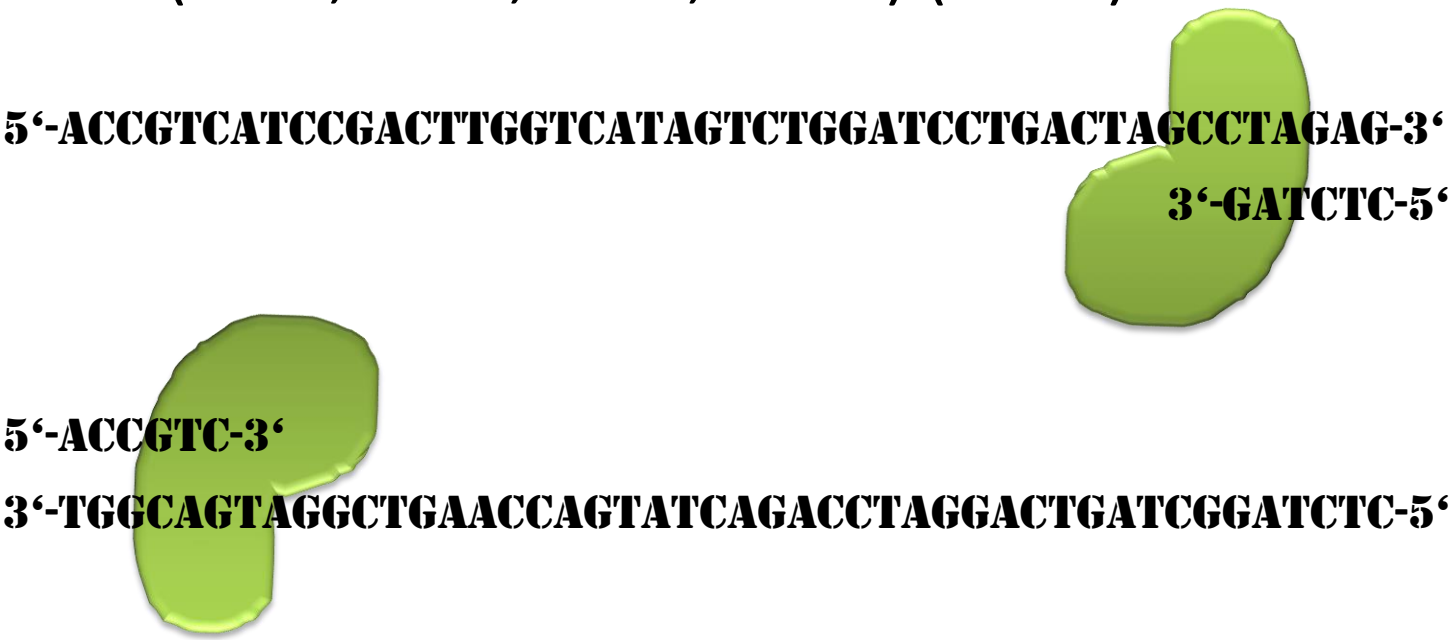
3‘-GATCTC-5‘

5‘-ACCGTC-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

1. Polymerase chain reaction PCR

Add DNA polymerase and nucleotides (dTTP, dCTP, dATP, dGTP) (72° C)



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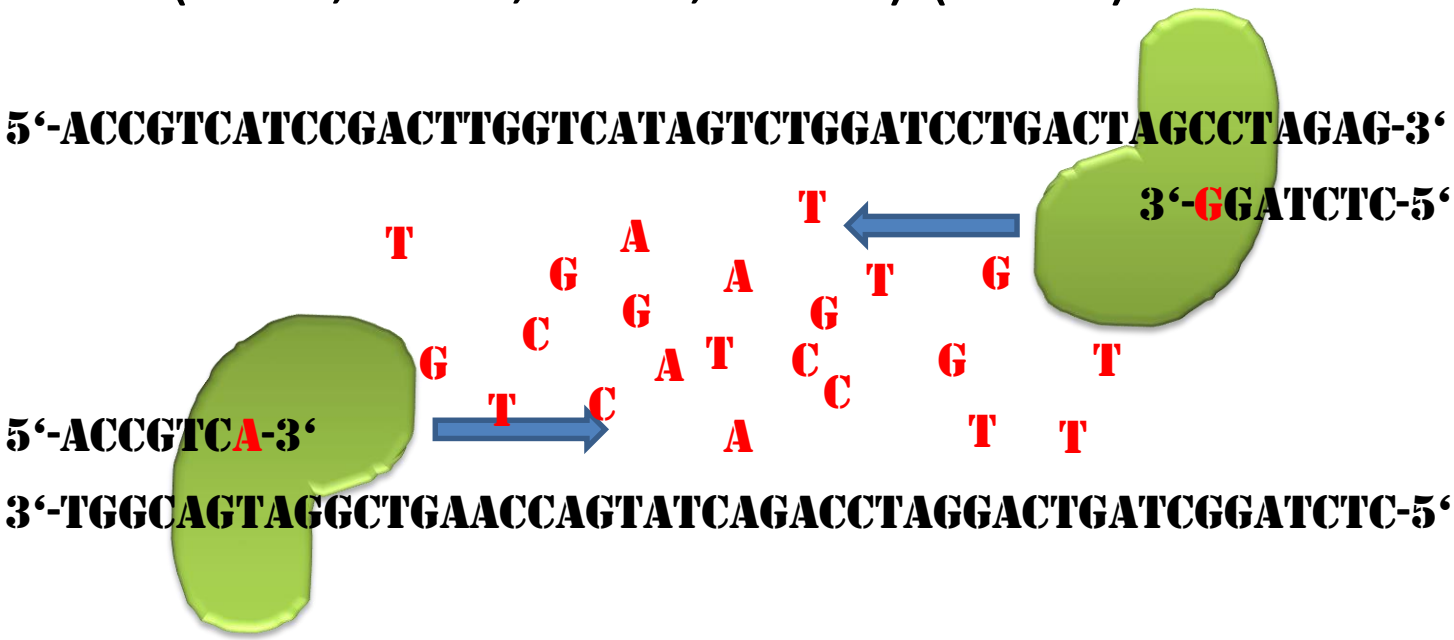

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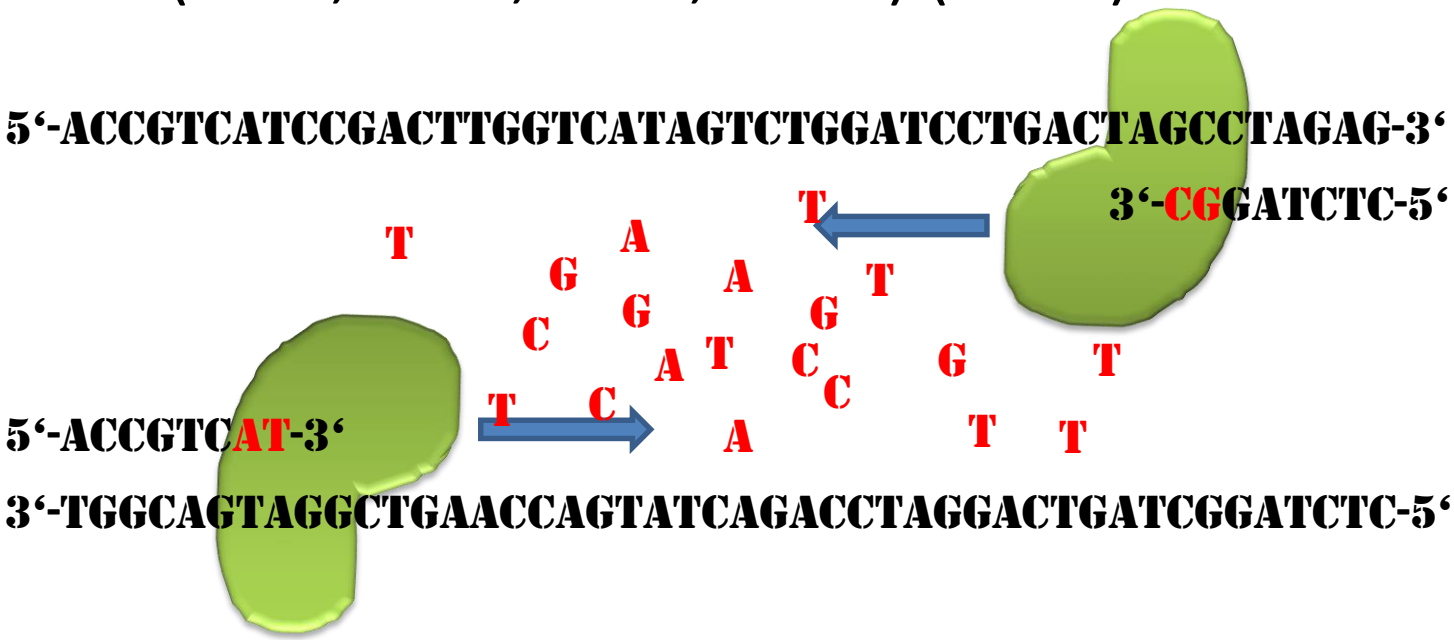

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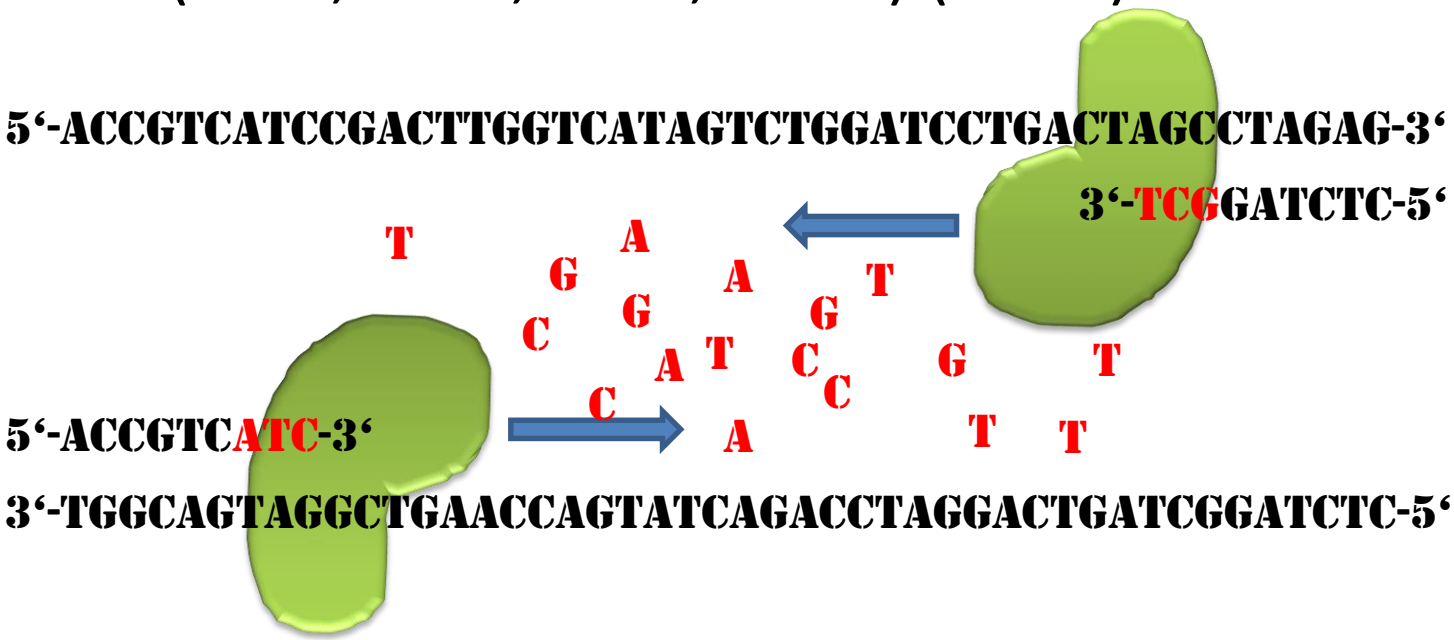

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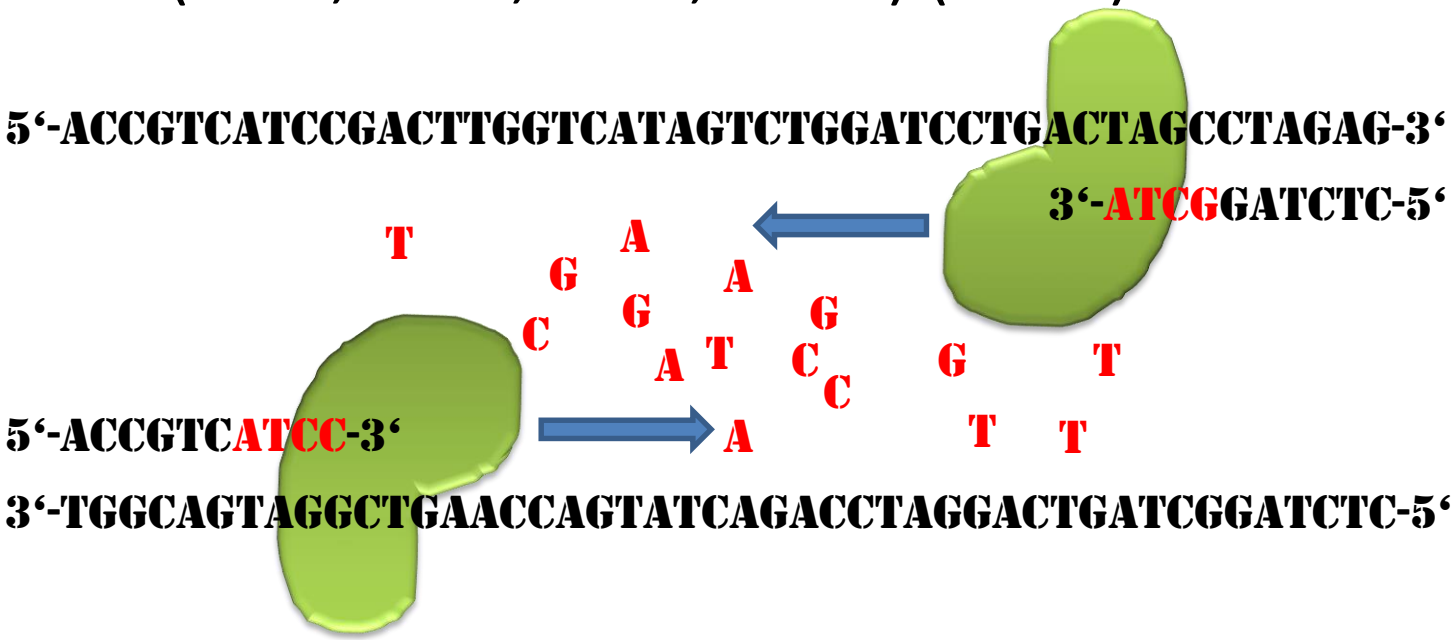

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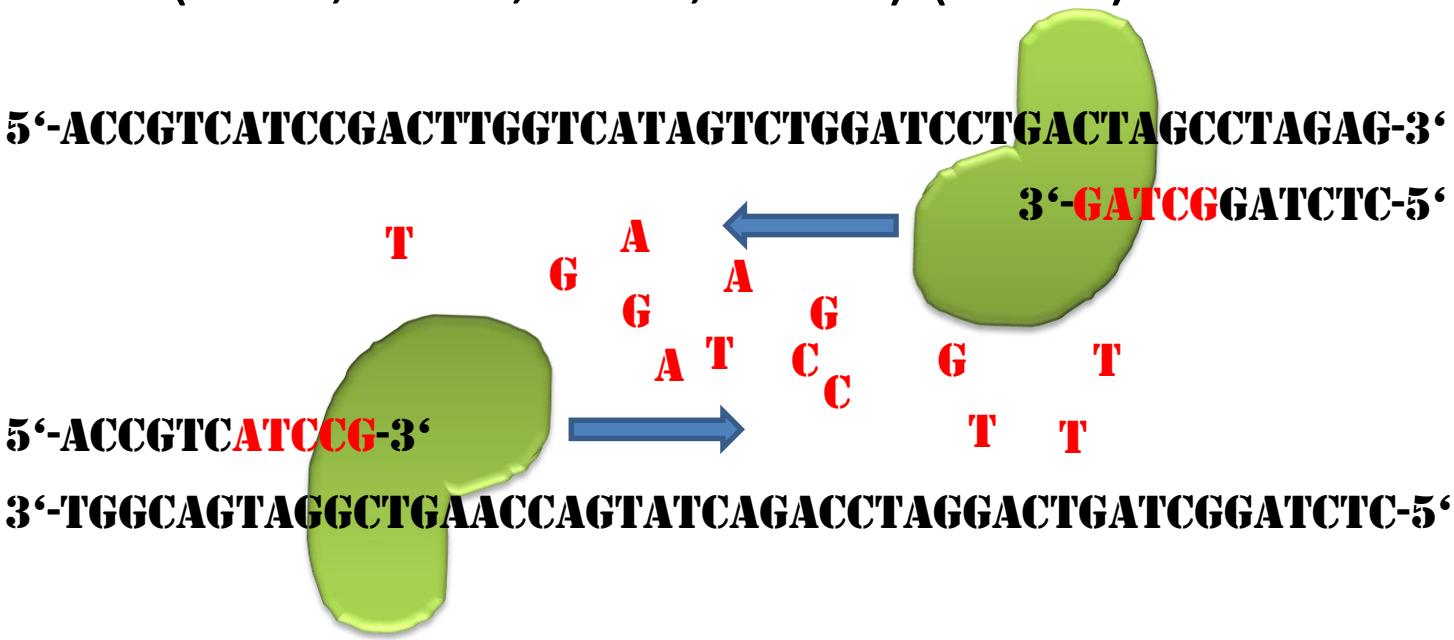

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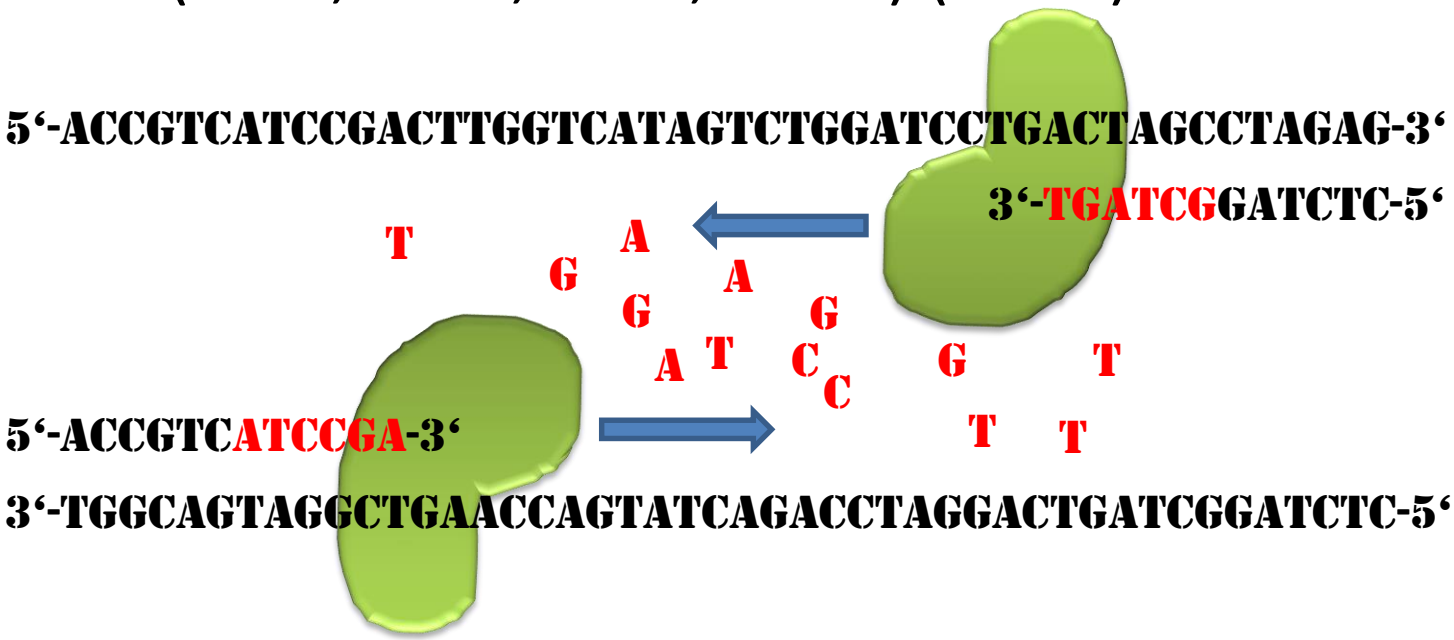

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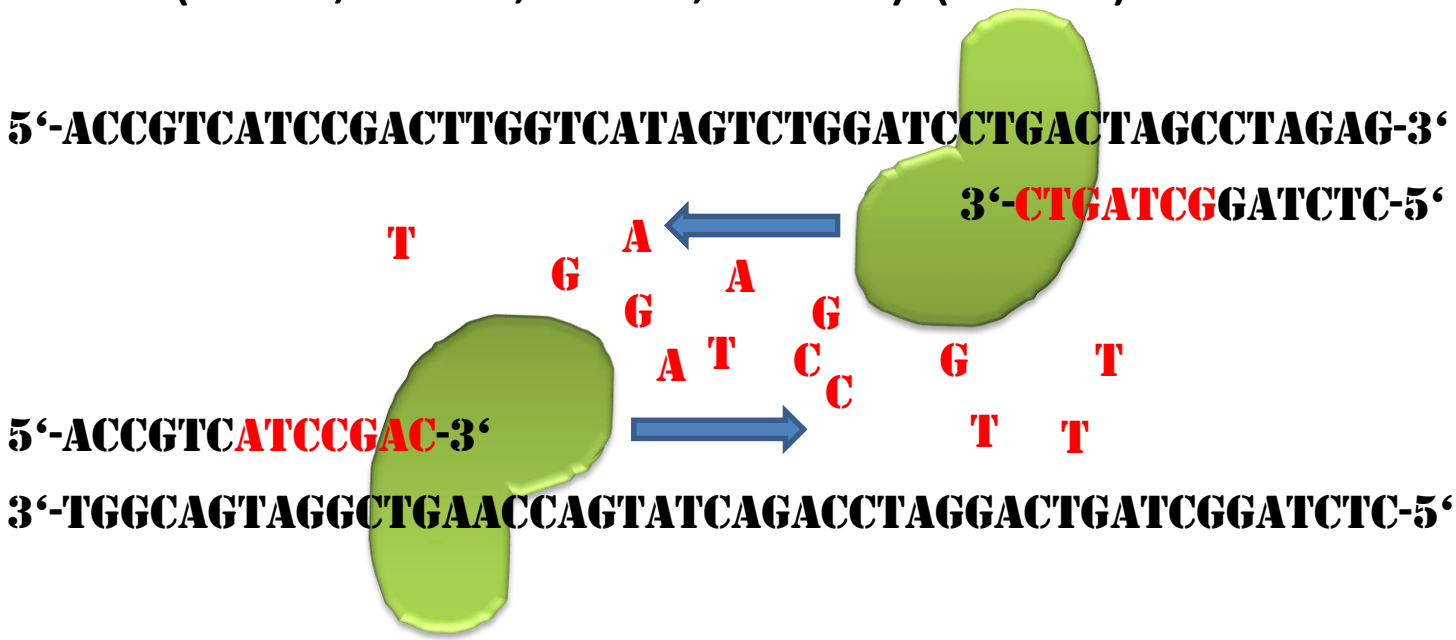

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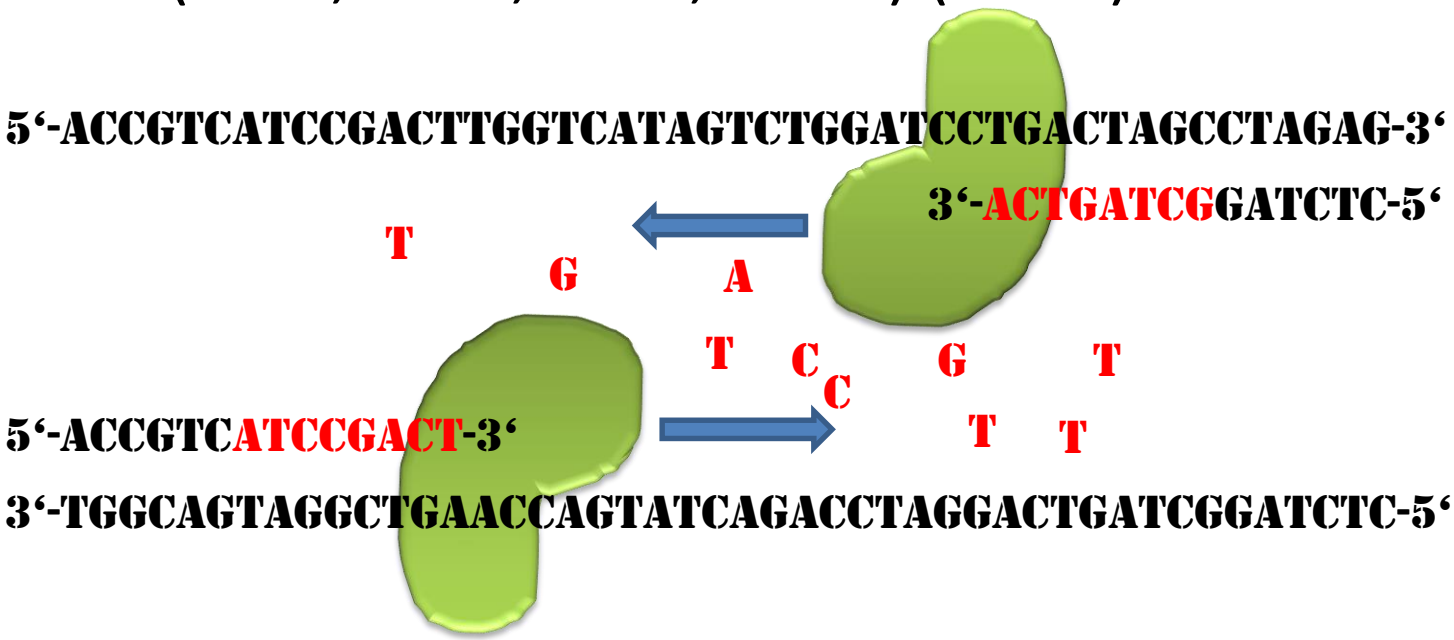

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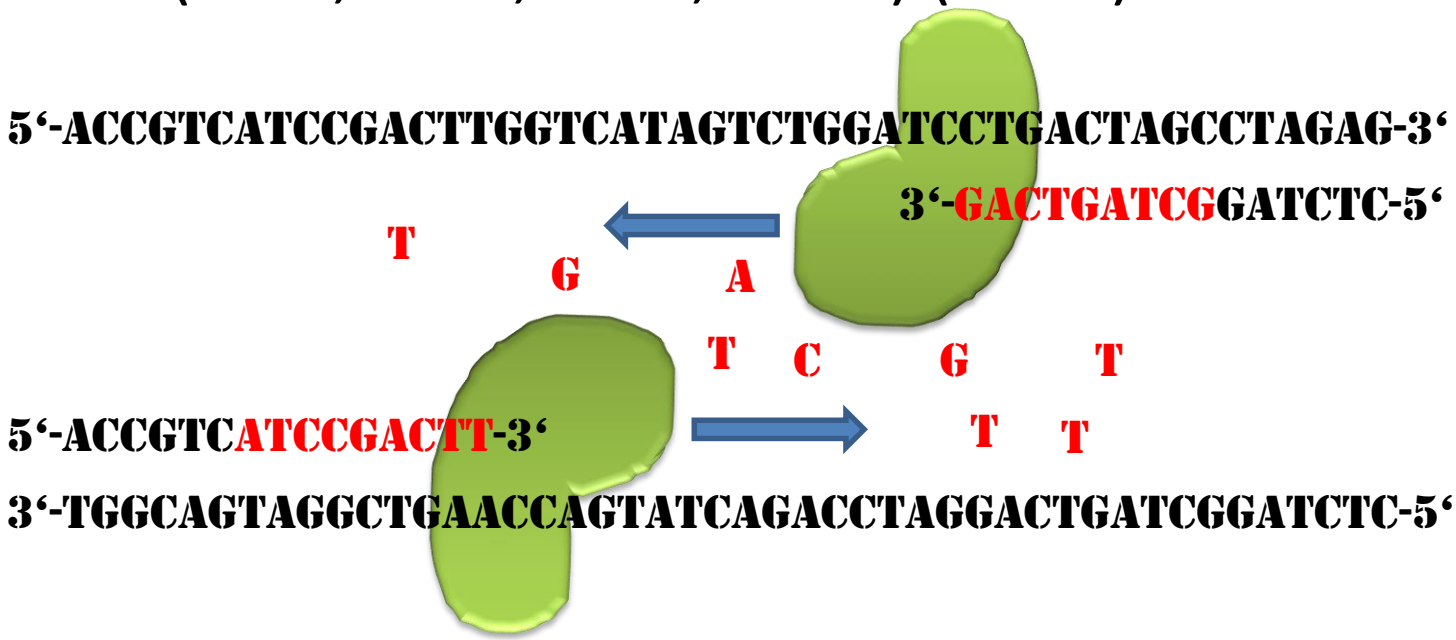

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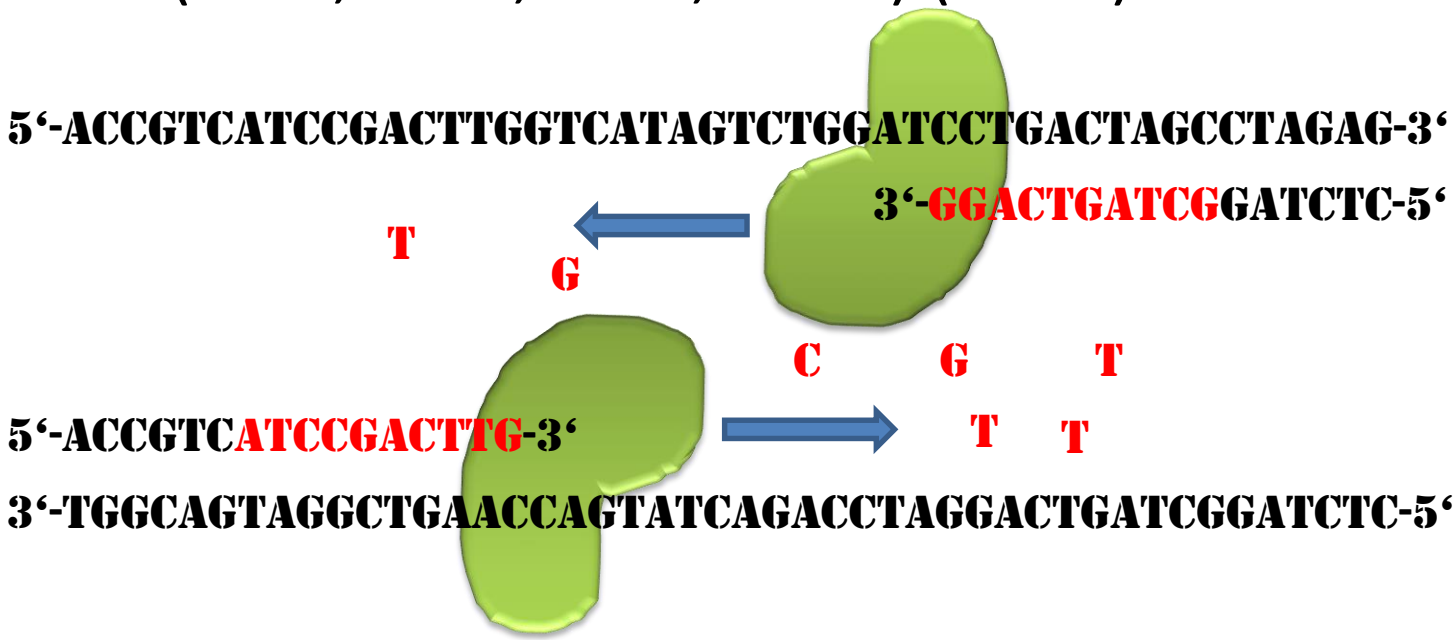

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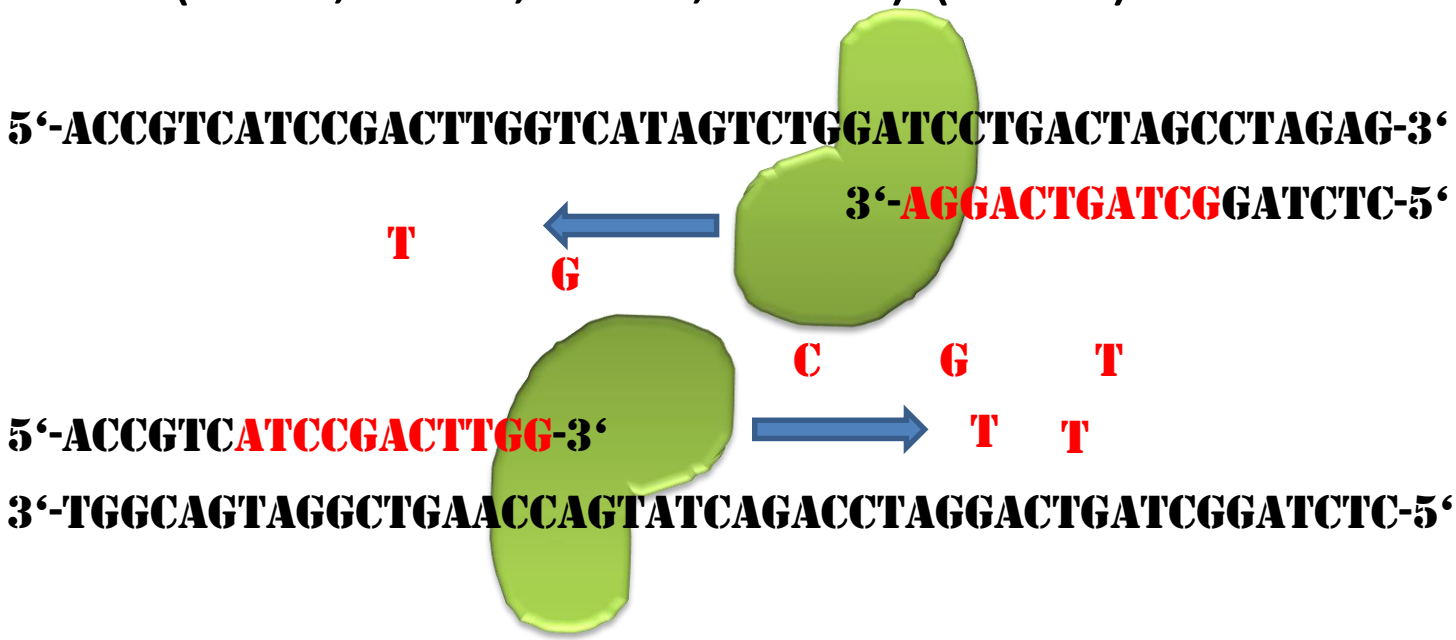

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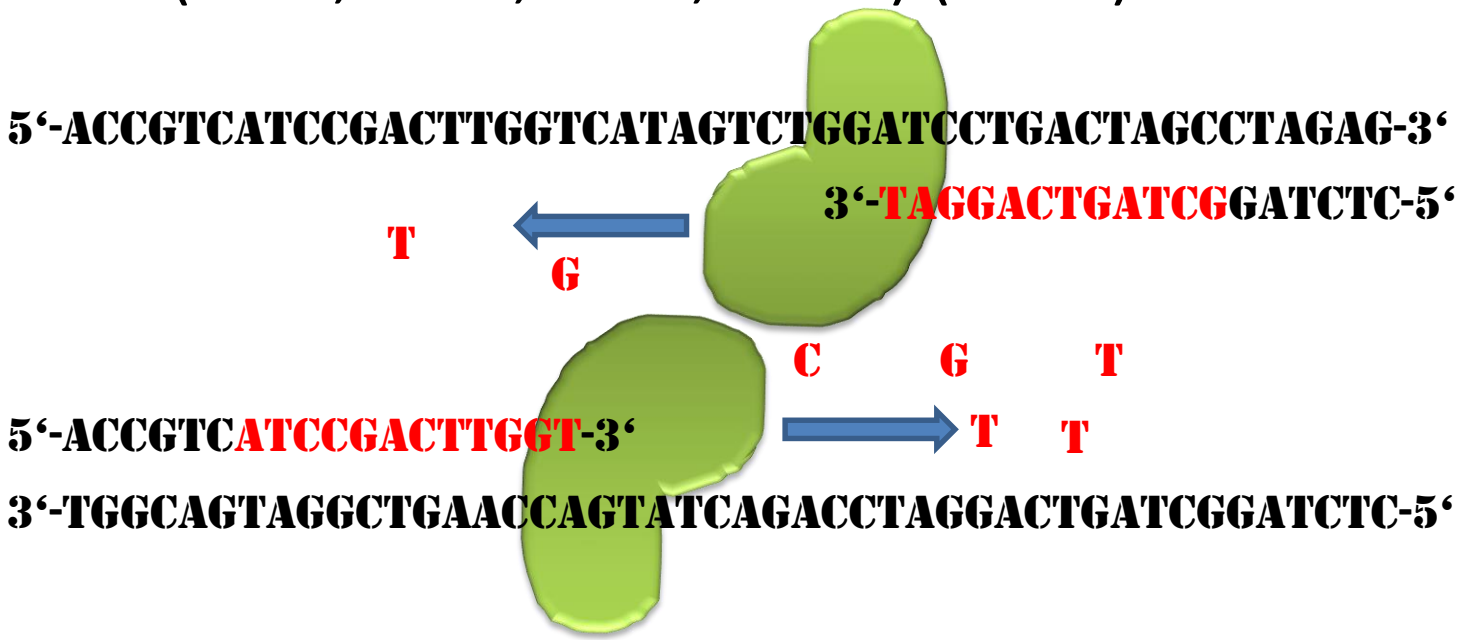

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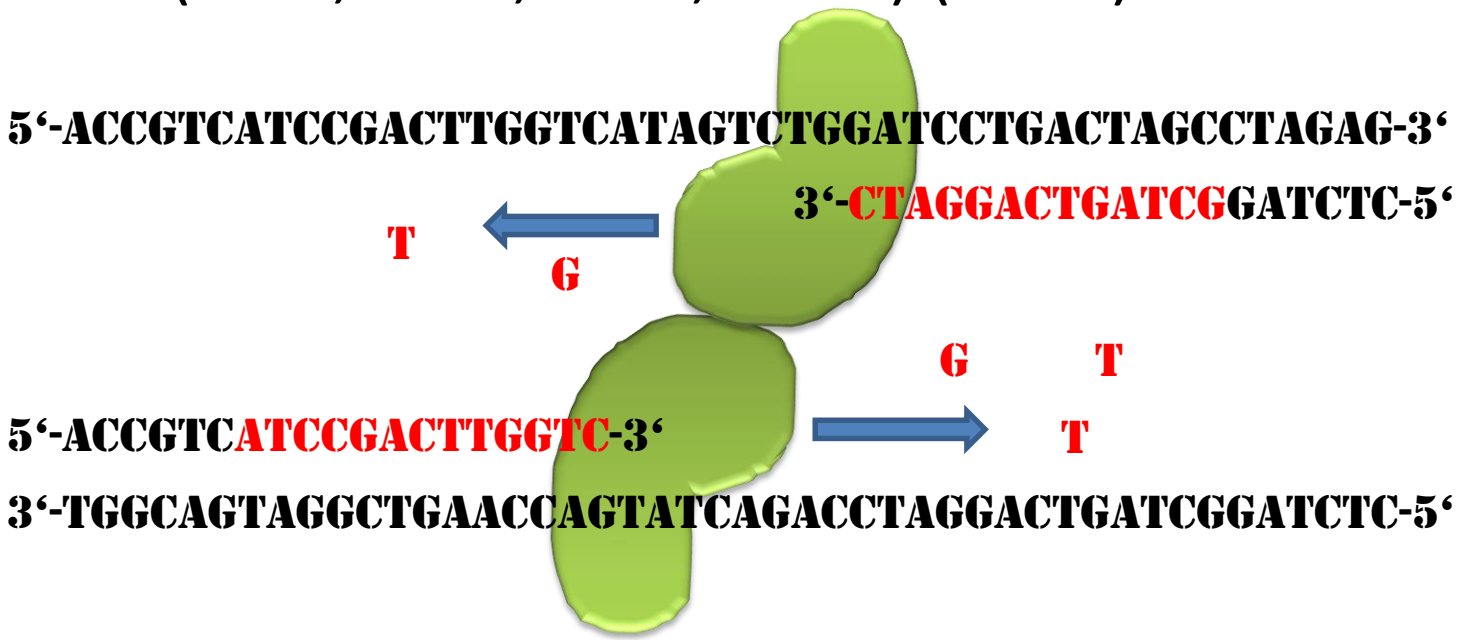

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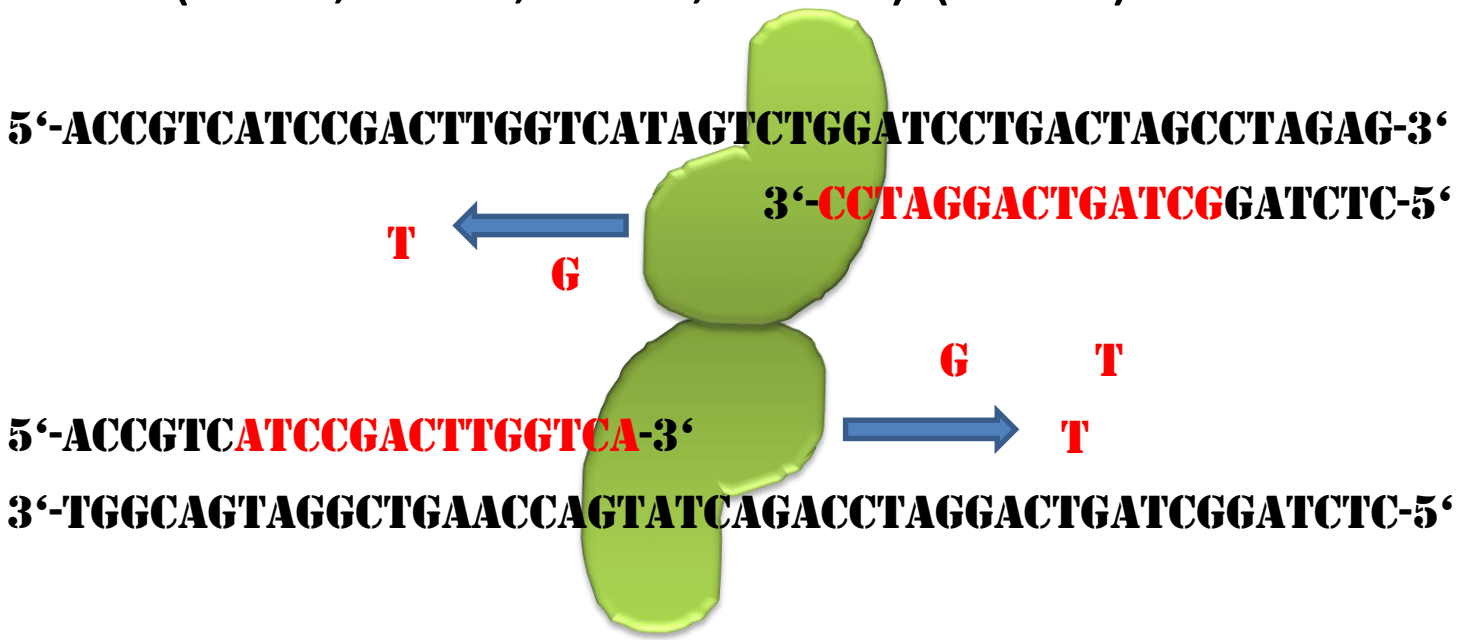

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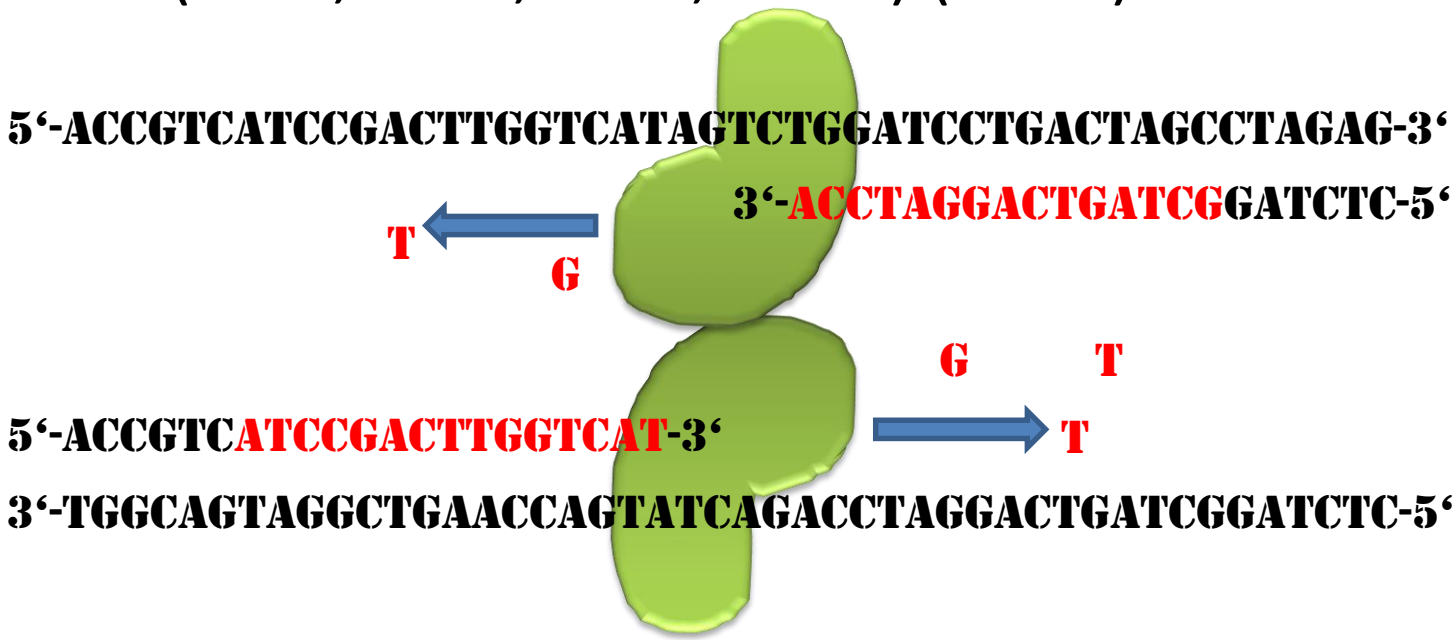

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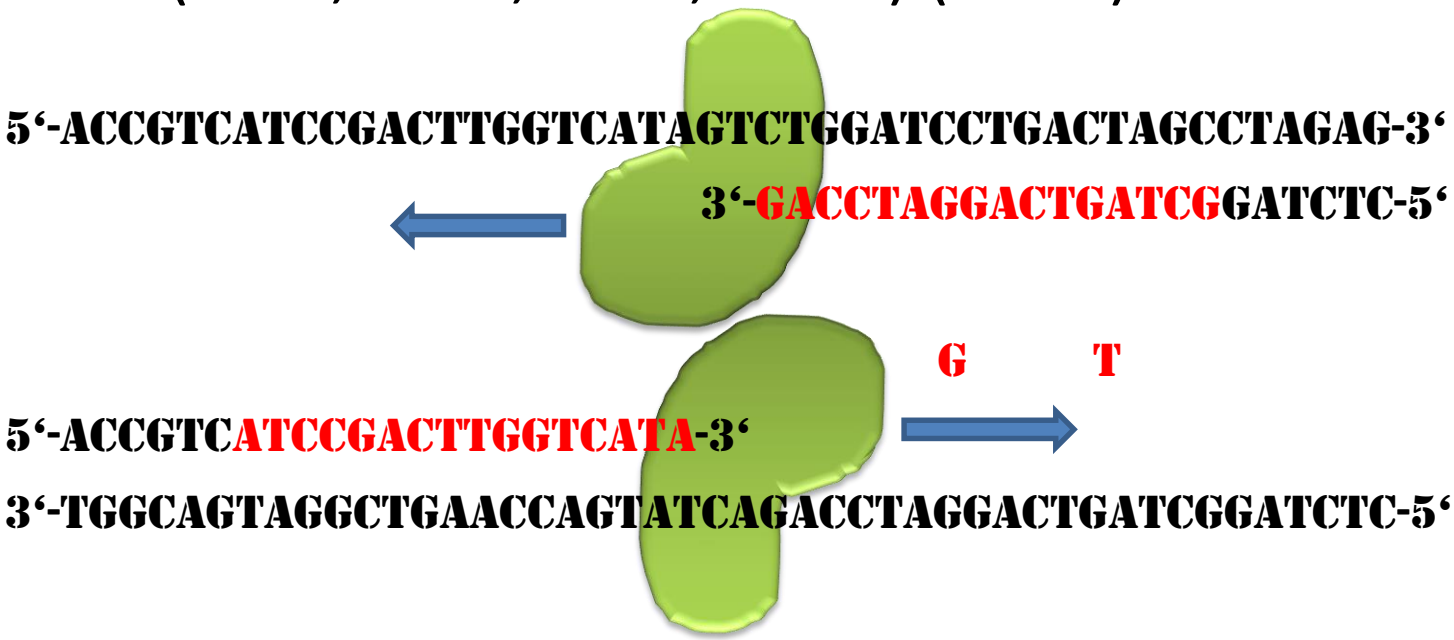

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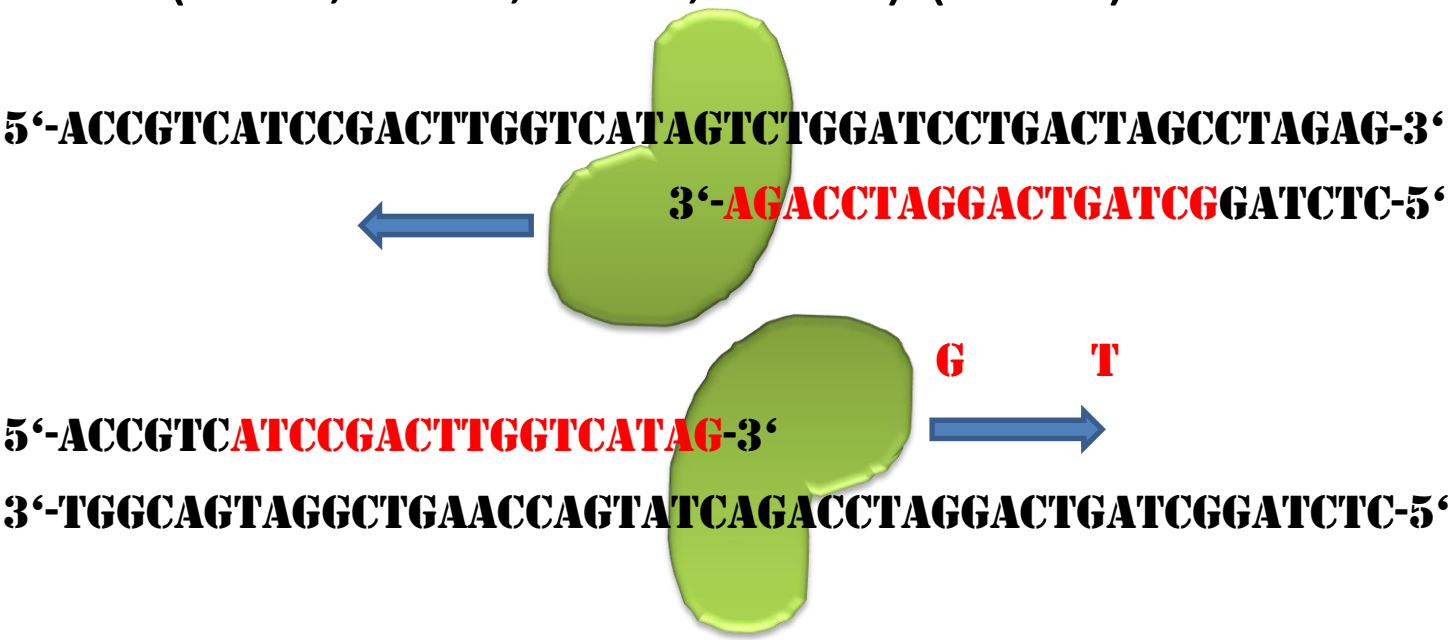

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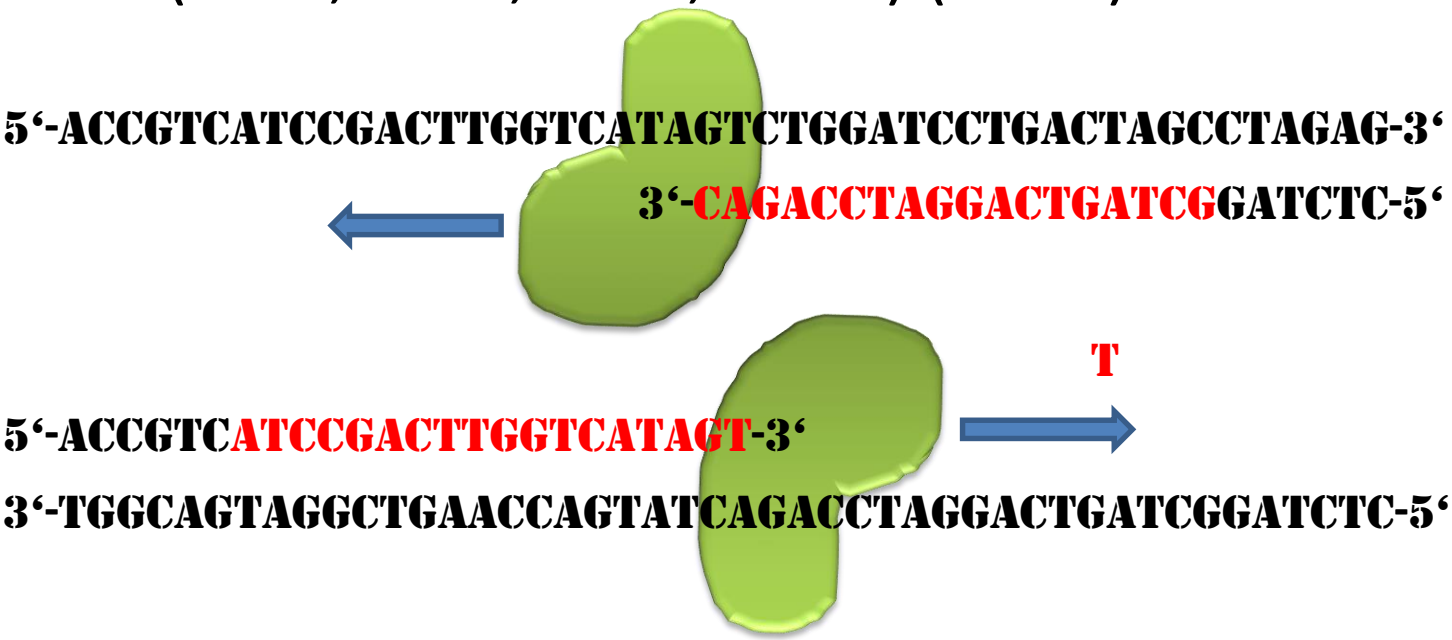

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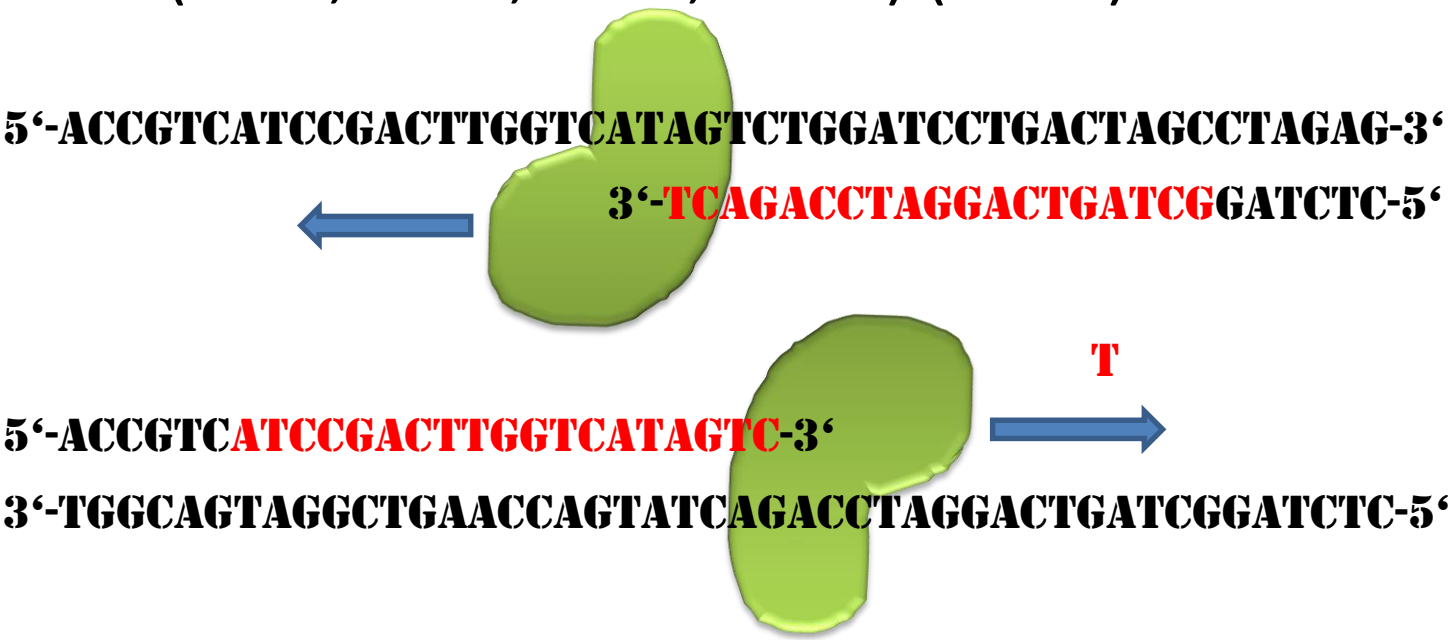

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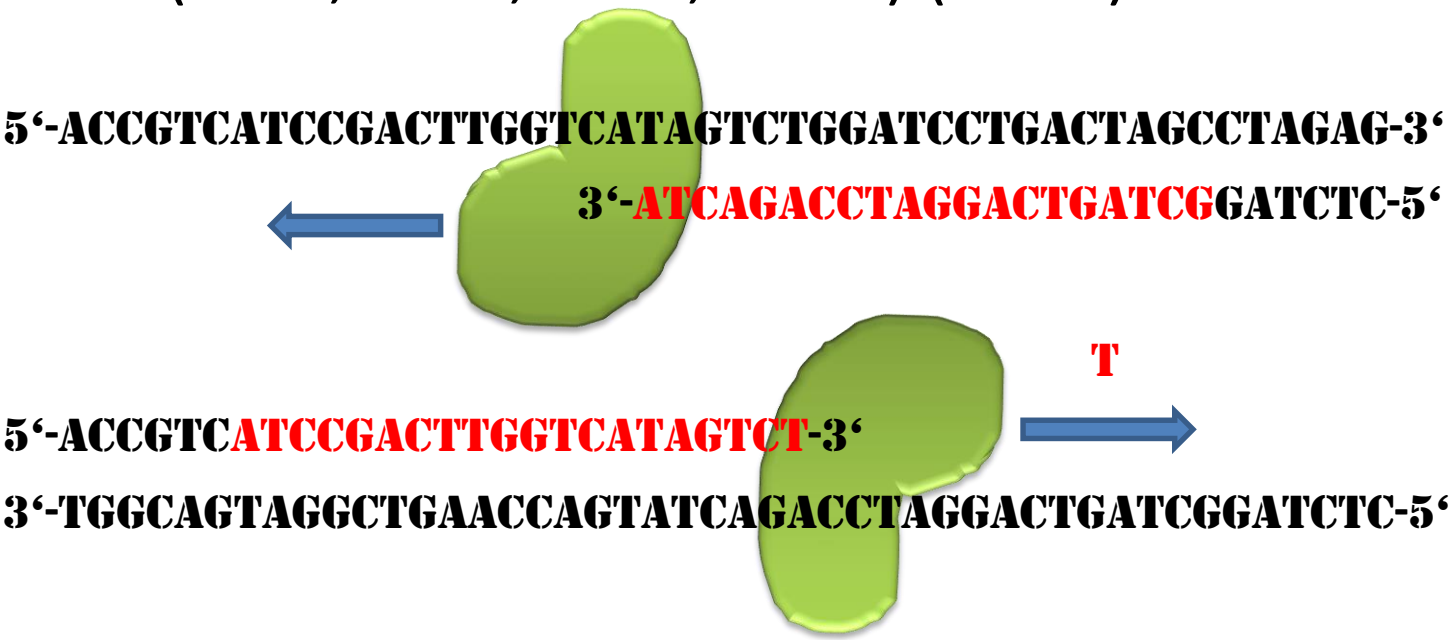

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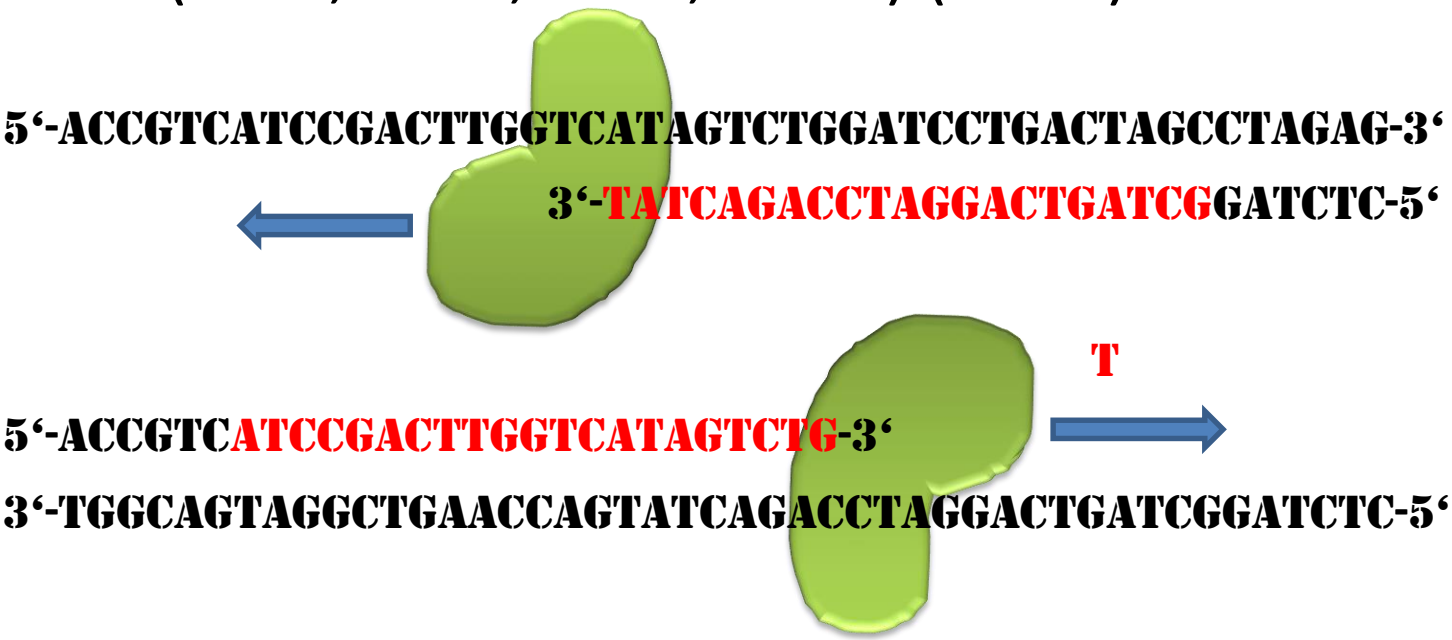

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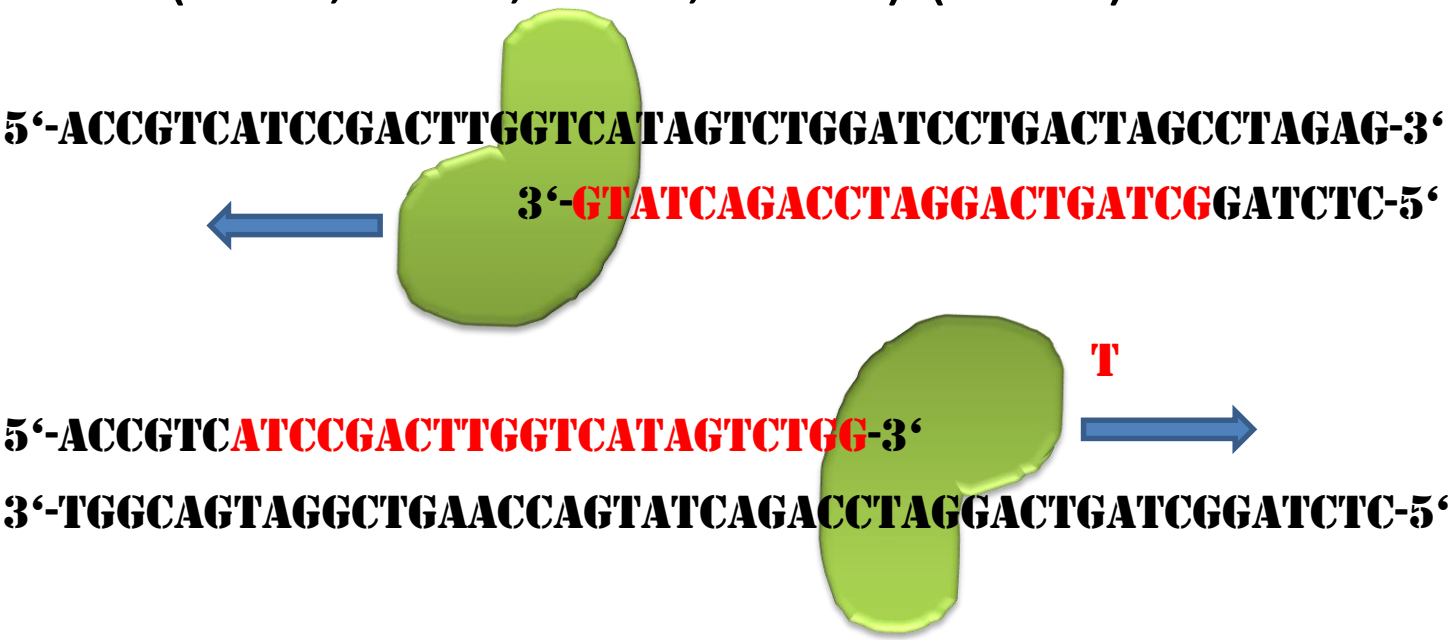

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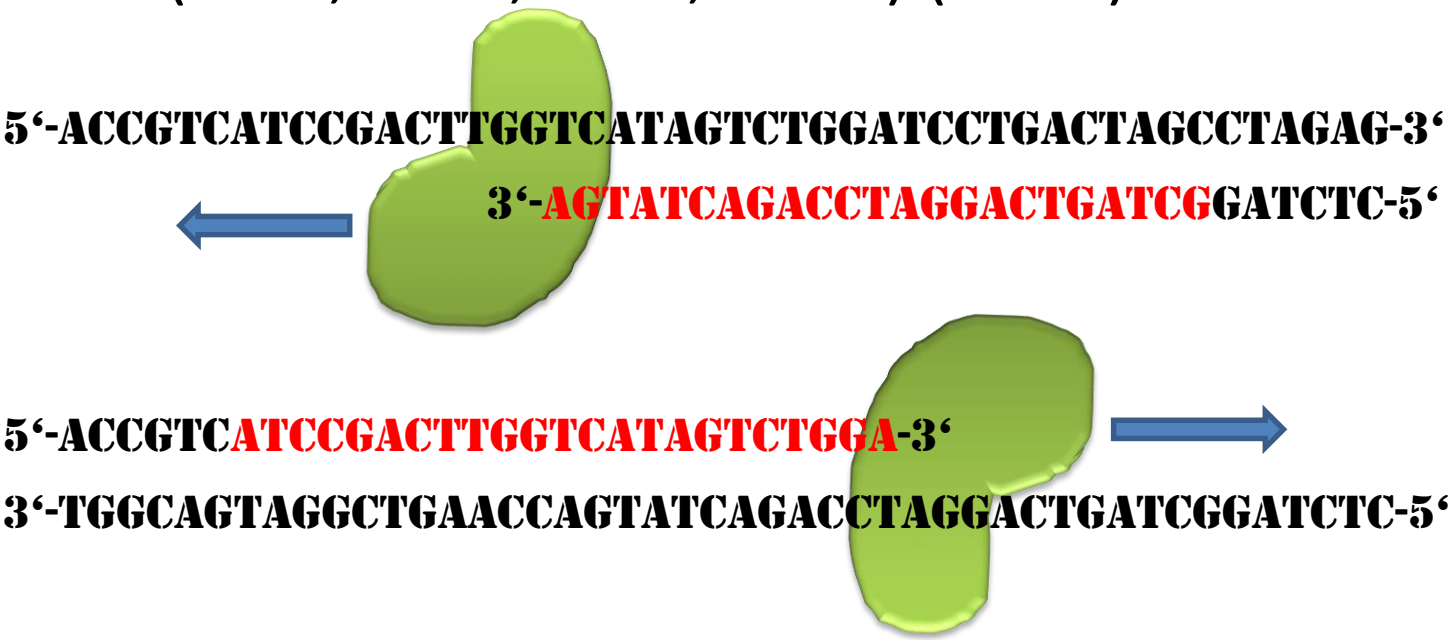

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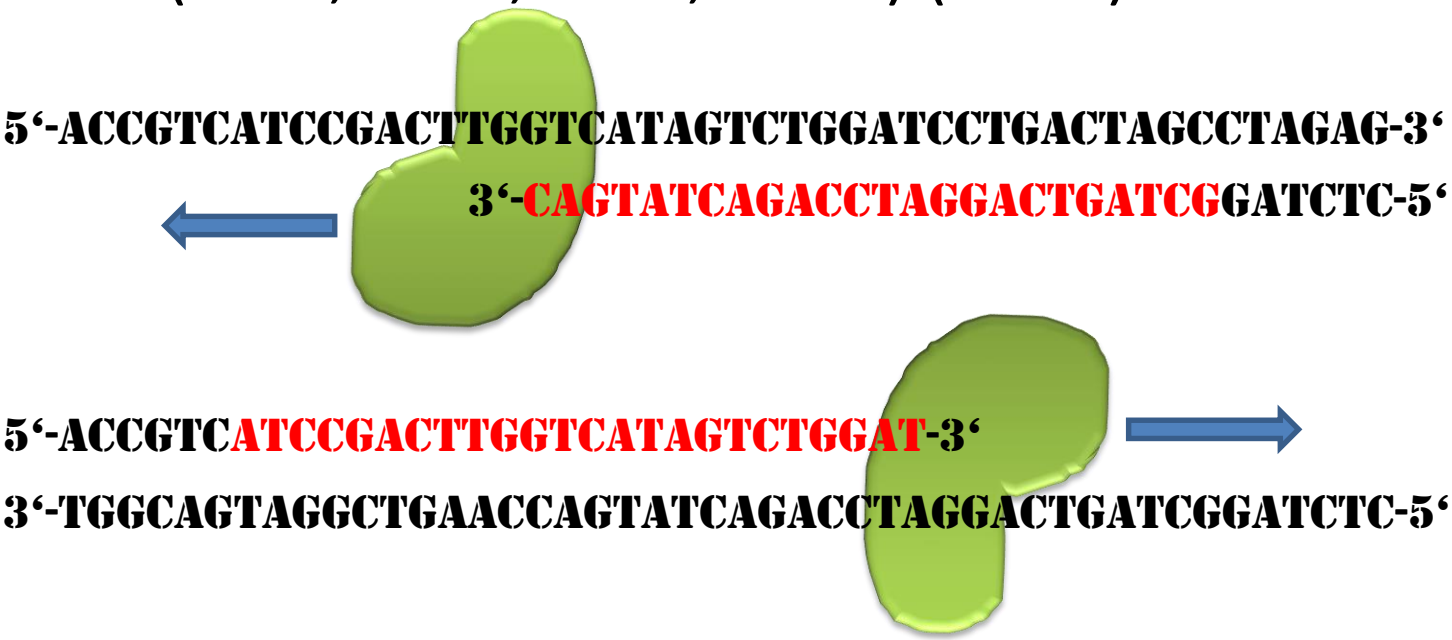

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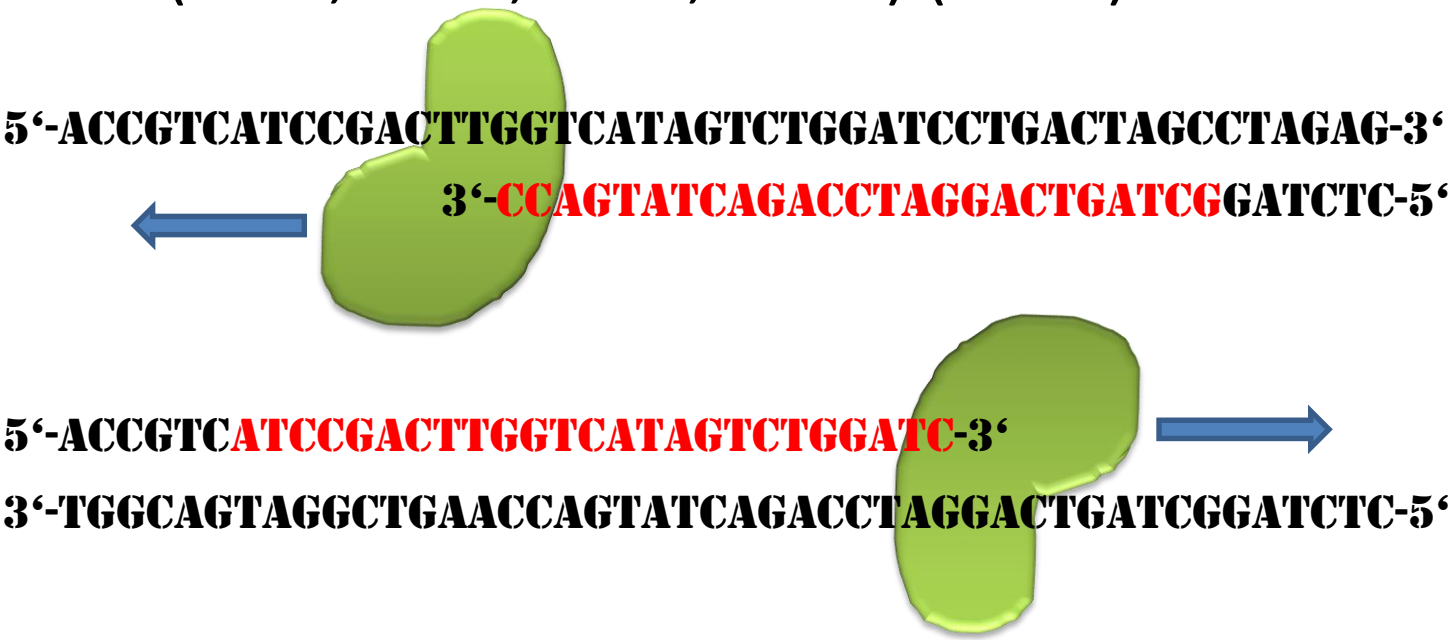

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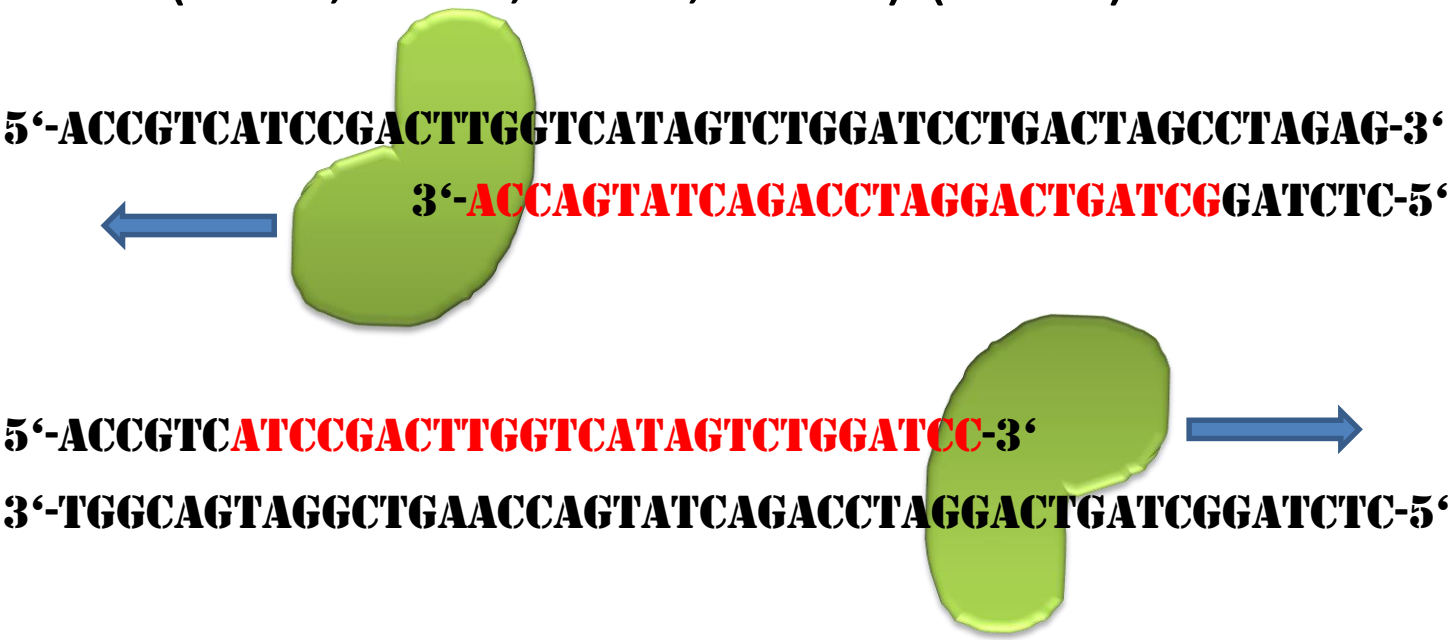

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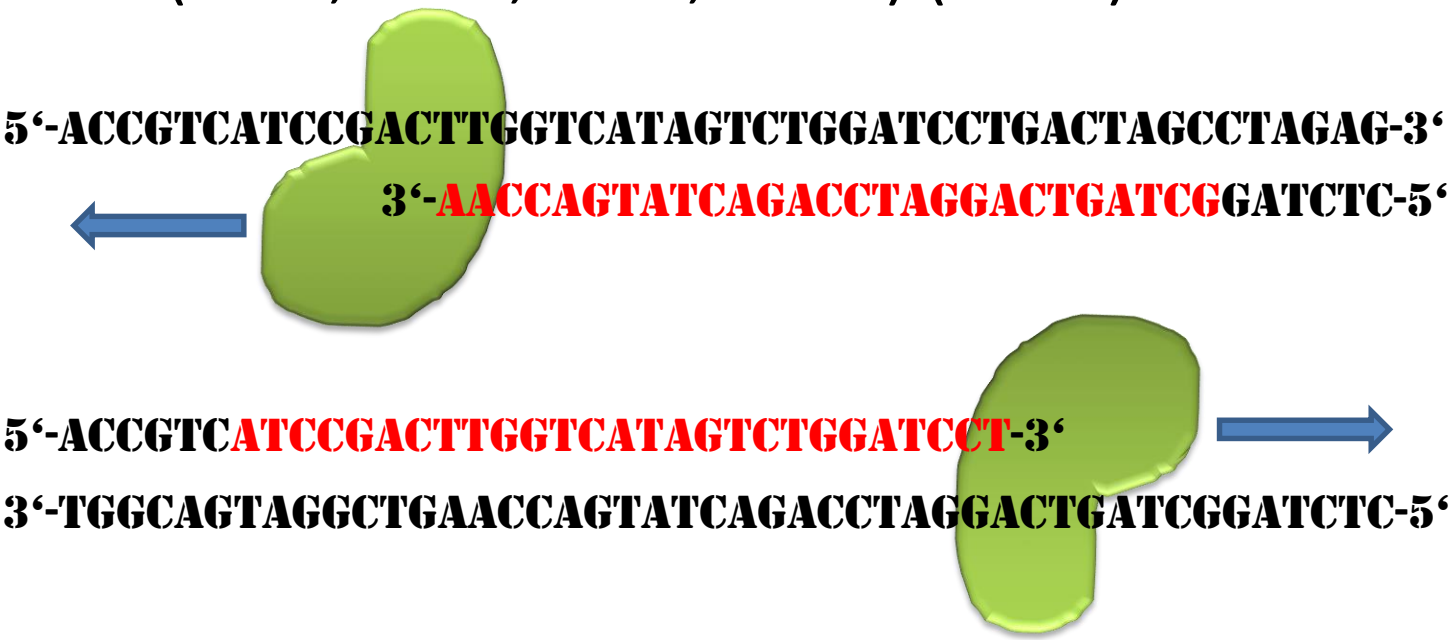

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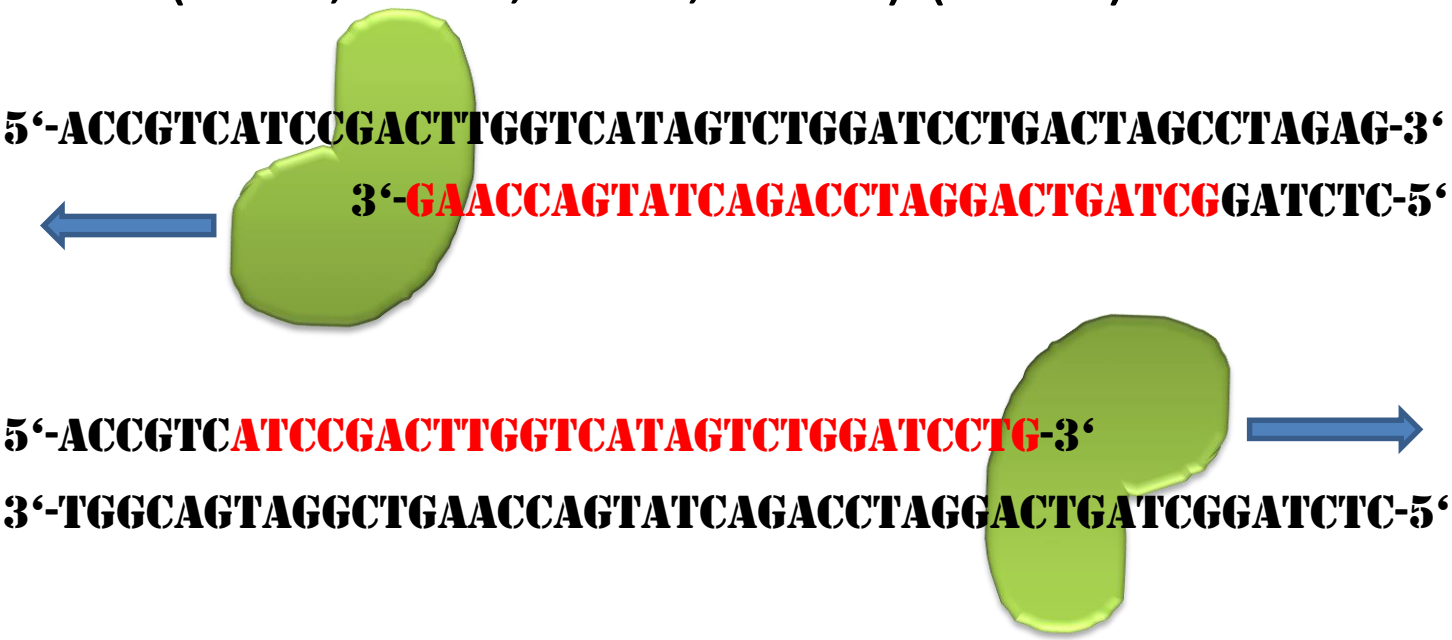

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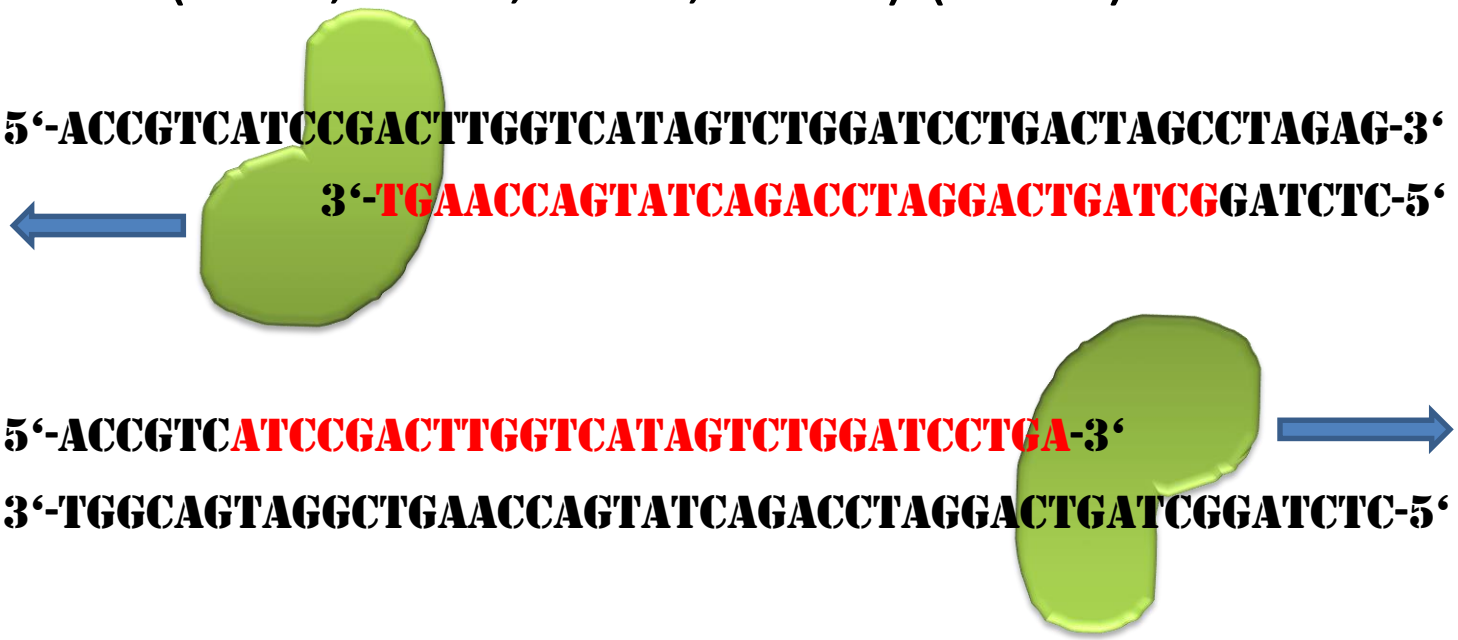

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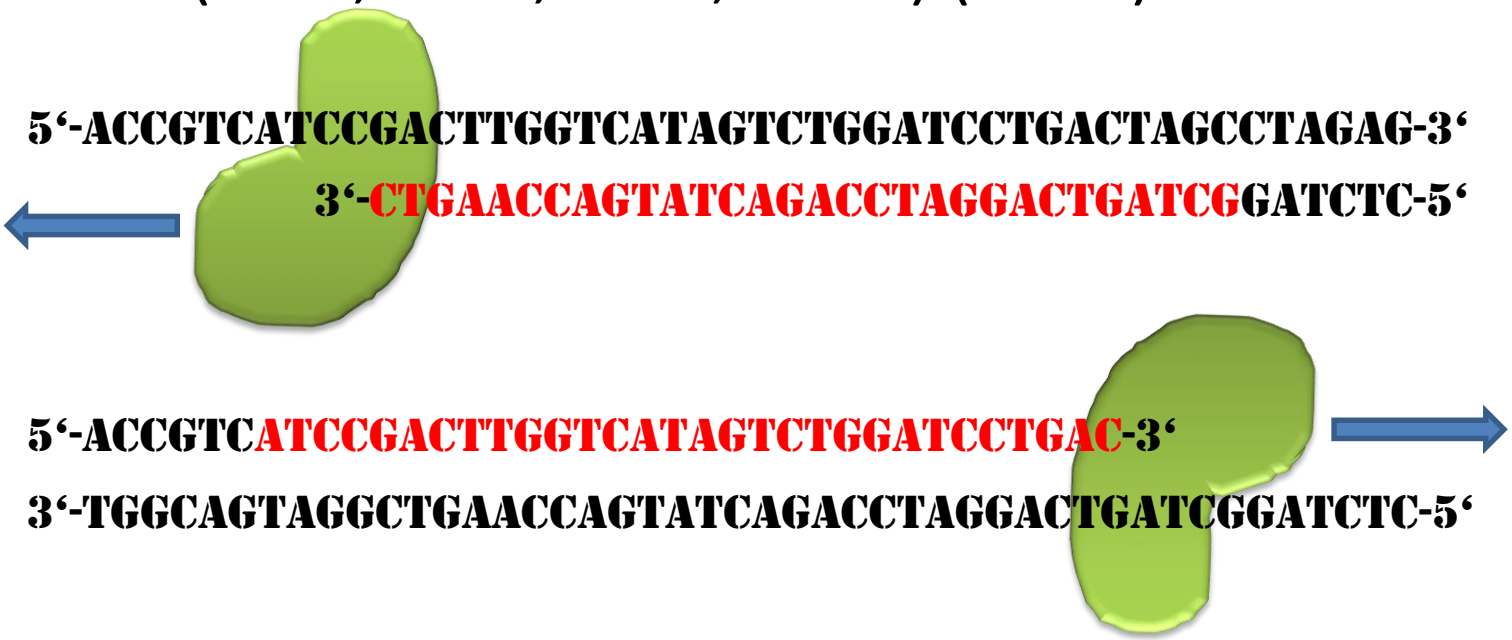

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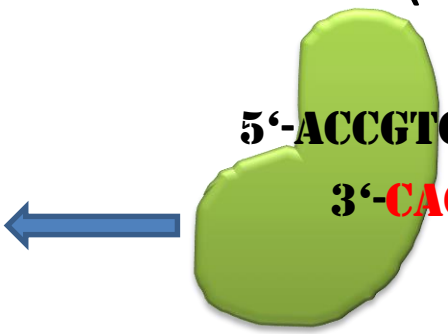

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3‘-CAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

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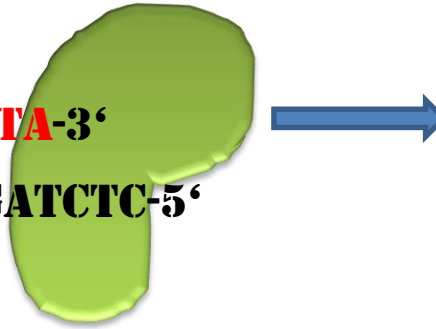

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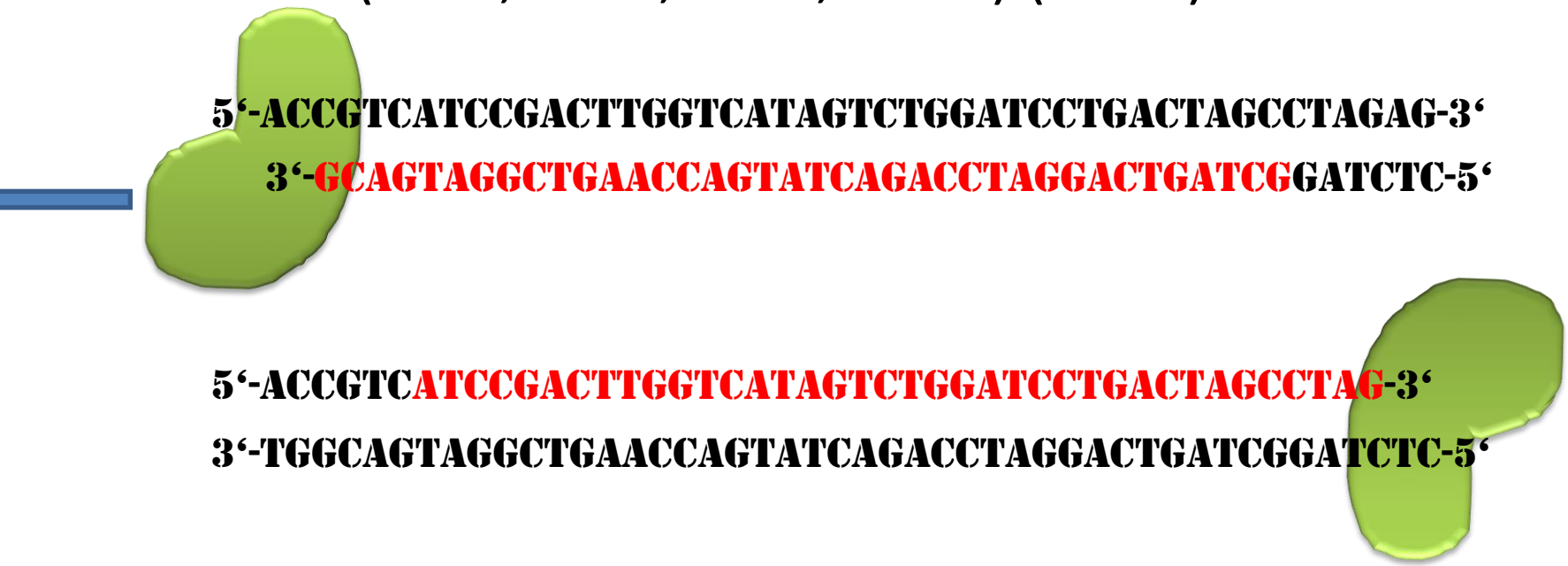

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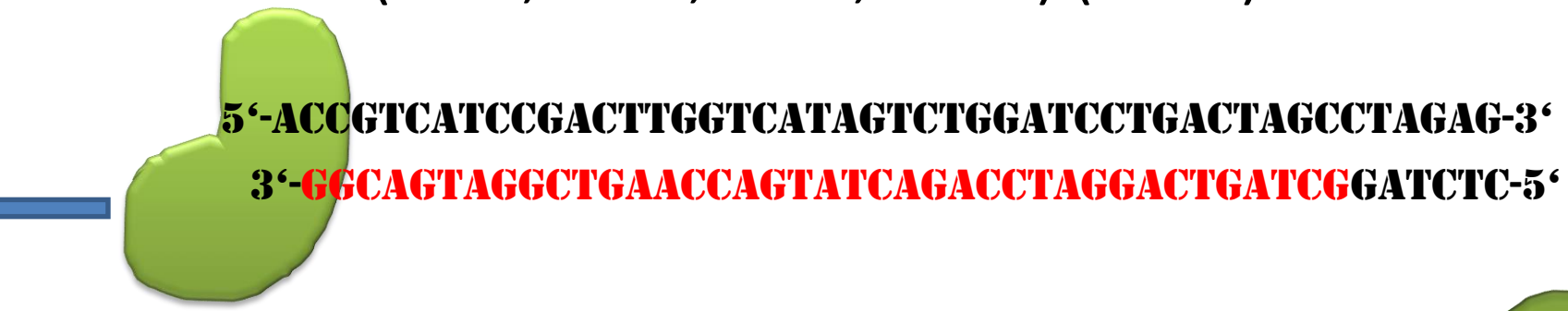

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5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

1. Polymerase chain reaction PCR

Separate the strands again

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

1. Polymerase chain reaction PCR

Add primers again

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘
3‘-GATCTC-5‘

5‘-ACCGTC-3‘
3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘
3‘-GATCTC-5‘

5‘-ACCGTC-3‘
3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

1. Polymerase chain reaction PCR

Resynthesize the complementary strands again.

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘
3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘
3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘
3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘
3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

1. Polymerase chain reaction
PCR

Repeat this again and again...

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

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5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

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3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

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3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

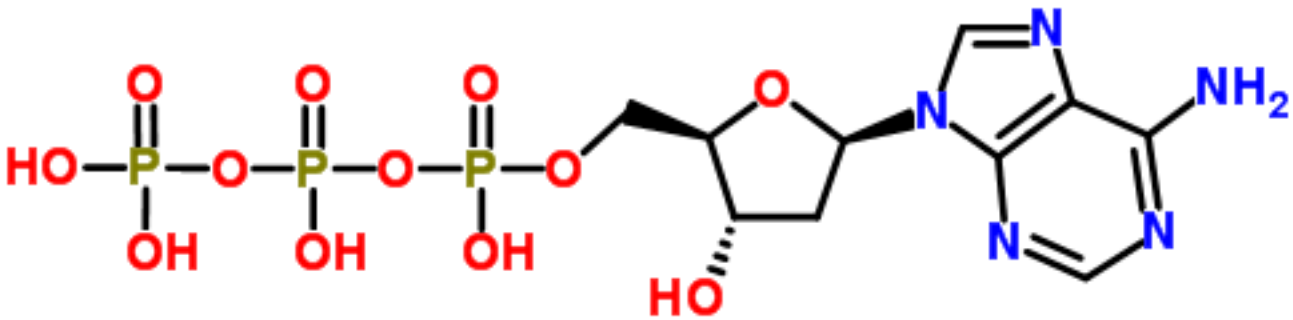
3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

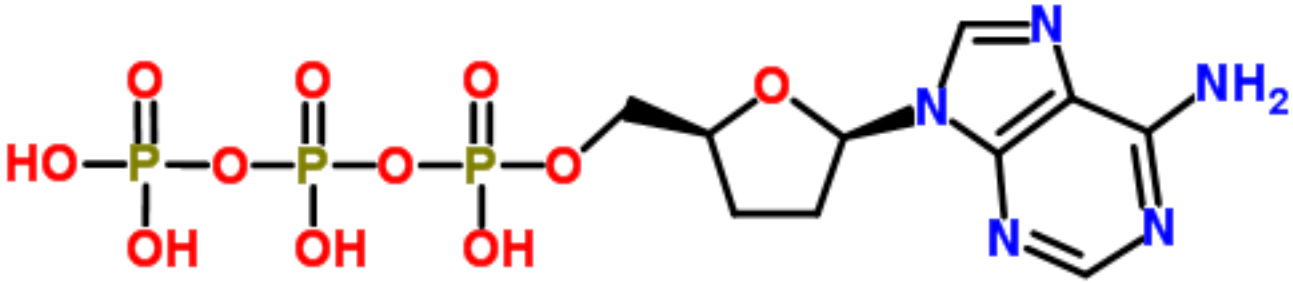
3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

2. Sequencing reactions

Dideoxy nucleotides will stop the DNA polymerisation.

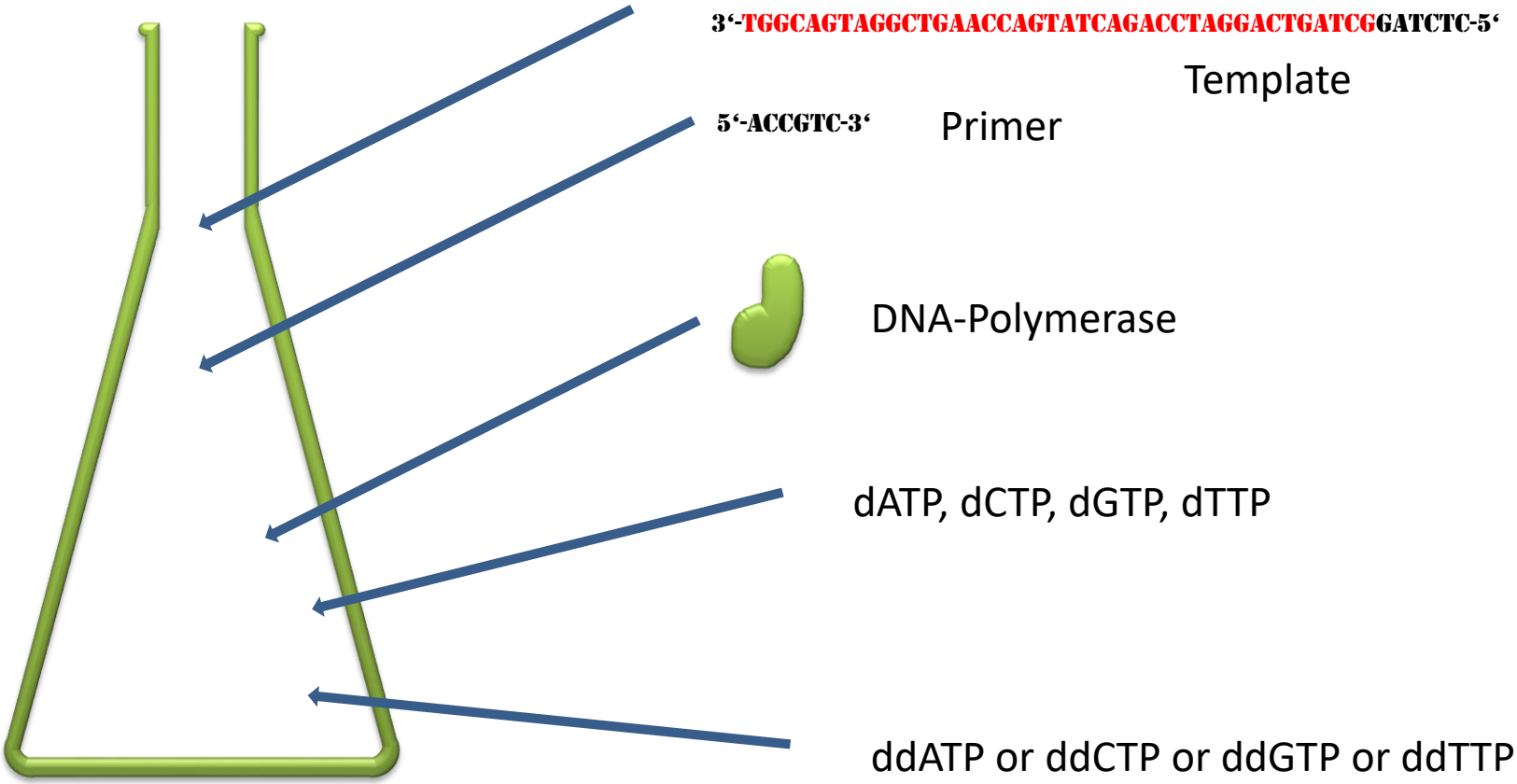


Deoxy-Adenosine-triphosphate, dATP

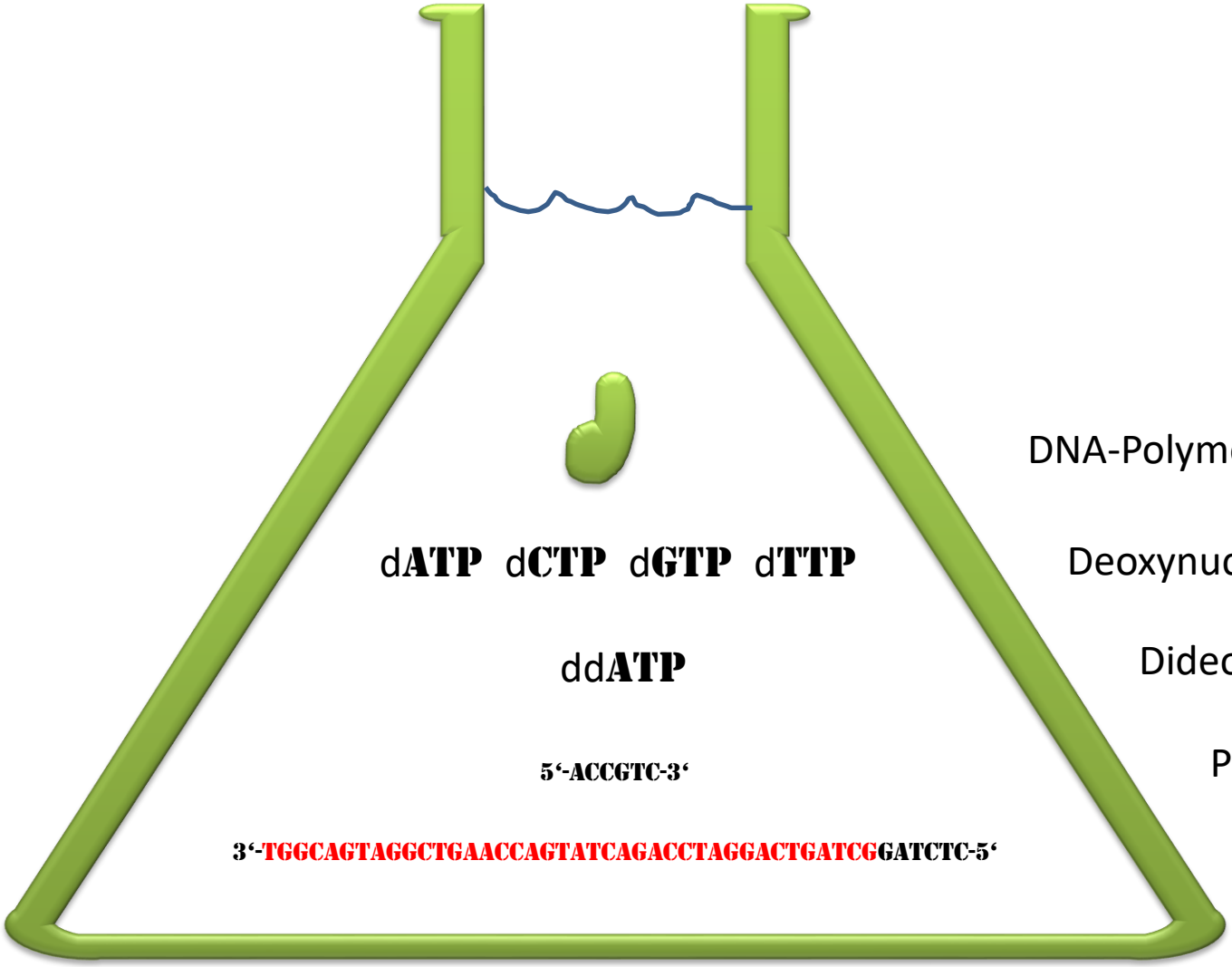


Dideoxy-Adenosine-triphosphate, ddATP

2. Sequencing reactions



2. Sequencing reactions



DNA-Polymerase

Deoxynucleotides

Dideoxynucleotide

Primer

Template

2. Sequencing reaction A

Add DNA polymerase and nucleotides (dTTP, dCTP, dATP, dGTP) (72° C)
Add a dideoxy nucleotide (e.g. ddATP):

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-AGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-AGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-AACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-ACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-AGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-ATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-AGACCTAGGACTGATCGGATCTC-5‘

3‘-ACCTAGGACTGATCGGATCTC-5‘

3‘-AGGACTGATCGGATCTC-5‘

3‘-ACTGATCGGATCTC-5‘

3‘-ATCGGATCTC-5‘

All fragments end in the same base: A

2. Sequencing reaction C

Add DNA polymerase and nucleotides (dTTP, dCTP, dATP, dGTP) (72° C)
Add a dideoxy nucleotide (e.g. ddCTP):

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-CAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-CTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-CCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-CAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-CAGACCTAGGACTGATCGGATCTC-5‘

3‘-CCTAGGACTGATCGGATCTC-5‘

3‘-CTAGGACTGATCGGATCTC-5‘

3‘-CTGATCGGATCTC-5‘

3‘-CGGATCTC-5‘

All fragments end in the same base: C

2. Sequencing reaction G

Add DNA polymerase and nucleotides (dTTP, dCTP, dATP, dGTP) (72° C)
Add a dideoxy nucleotide (e.g. ddGTP):

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-GGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-GCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-GTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-GGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-GCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-GAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-GTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-GACCTAGGACTGATCGGATCTC-5‘

3‘-GGACTGATCGGATCTC-5‘

3‘-GACTGATCGGATCTC-5‘

3‘-GATCGGATCTC-5‘

3‘-GGATCTC-5‘

All fragments end in the same base: G

2. Sequencing reaction T

Add DNA polymerase and nucleotides (dTTP, dCTP, dATP, dGTP) (72° C)
Add a dideoxy nucleotide (e.g. ddTTP):

5‘-ACCGTCATCCGACTTGGTCATAGTCTGGATCCTGACTAGCCTAGAG-3‘

3‘-TGGCAGTAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-TAGGCTGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-TGAACCAGTATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-TATCAGACCTAGGACTGATCGGATCTC-5‘

3‘-TCAGACCTAGGACTGATCGGATCTC-5‘

3‘-TAGGACTGATCGGATCTC-5‘

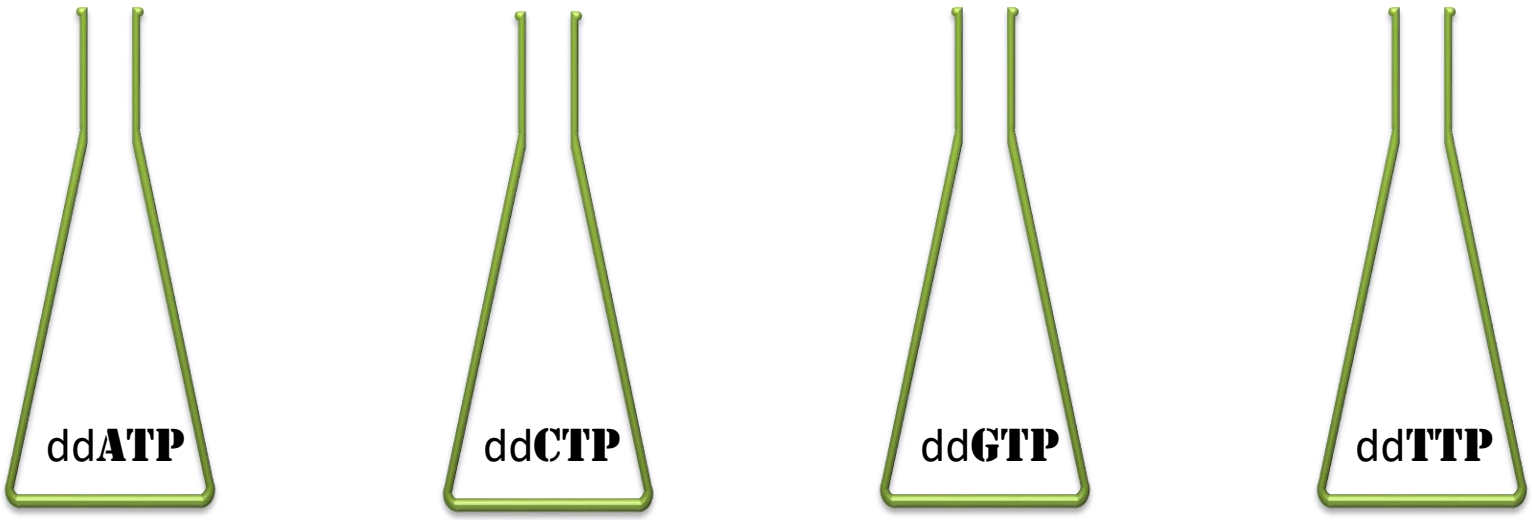
3‘-TGATCGGATCTC-5‘

3‘-TCGGATCTC-5‘

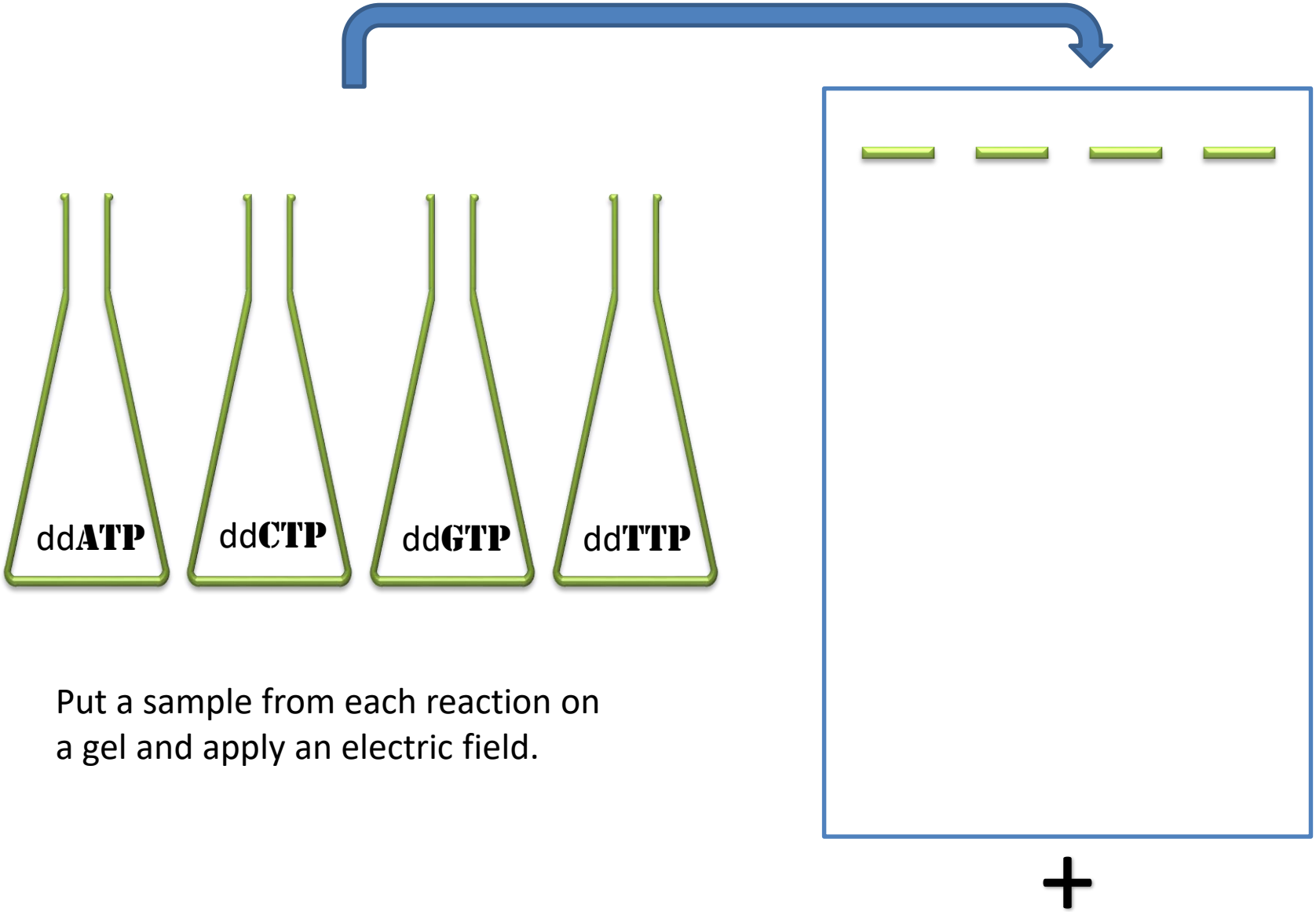
All fragments end in the same base: T

2. Sequencing reactions

4 reactions each with one of four different dideoxynucleotides added:



3. Gel loading



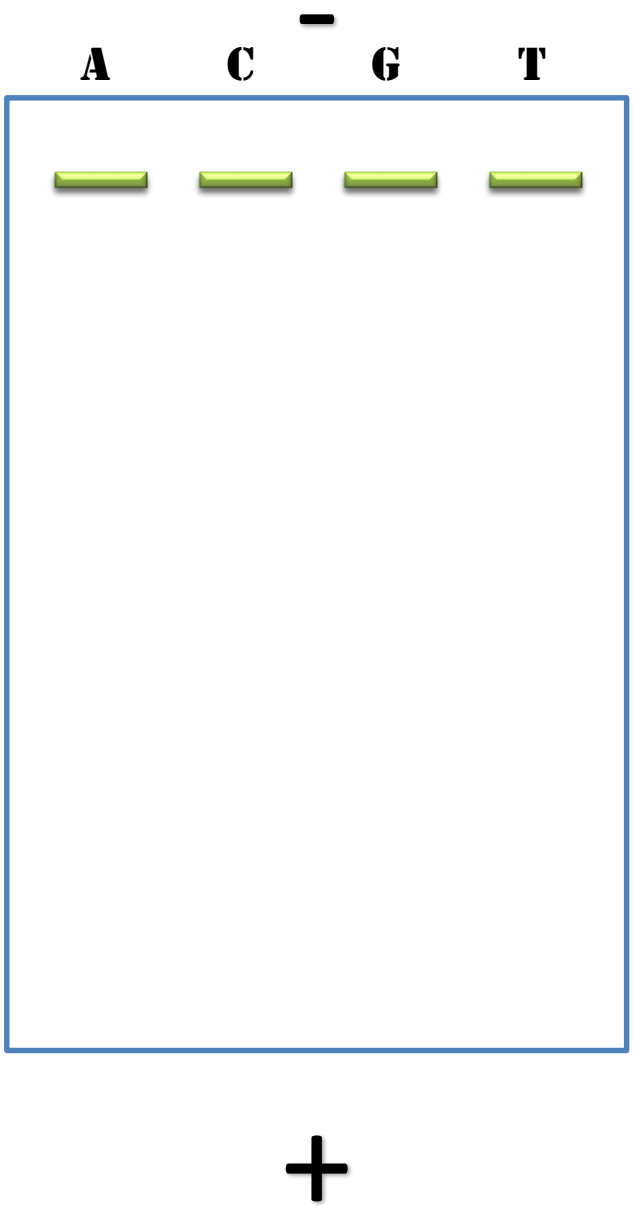
Put a sample from each reaction on a gel and apply an electric field.

4. Fragment separation

Put a sample from each reaction on a gel and apply an electric field.

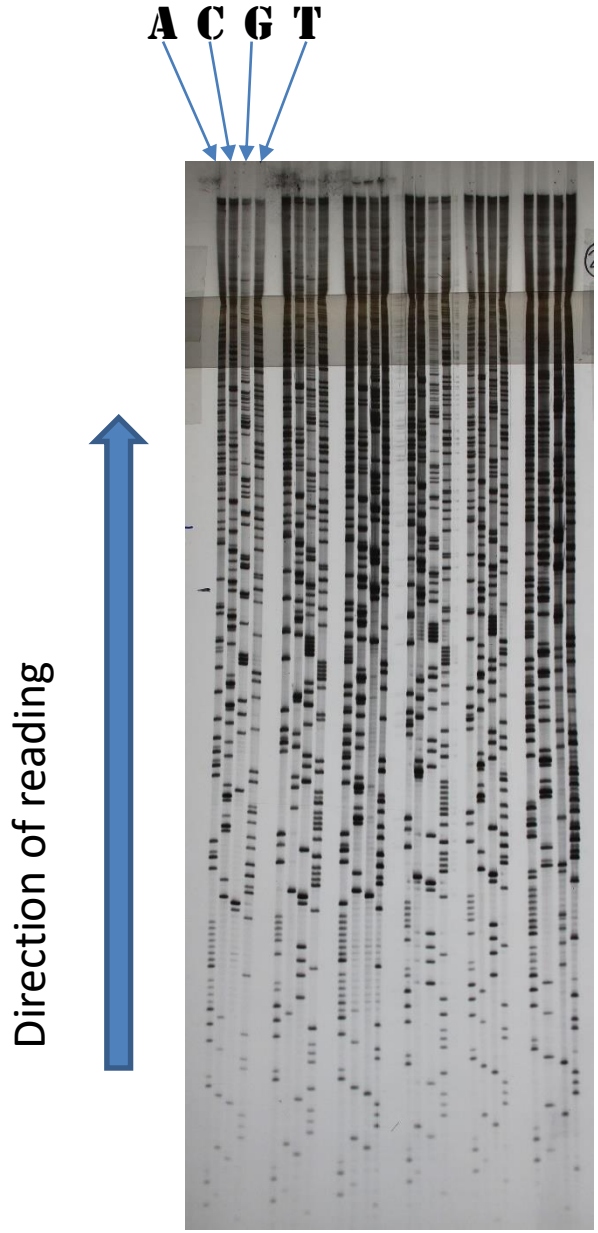
Short fragments travel faster.

Sequence read is:
AGTC



A real example

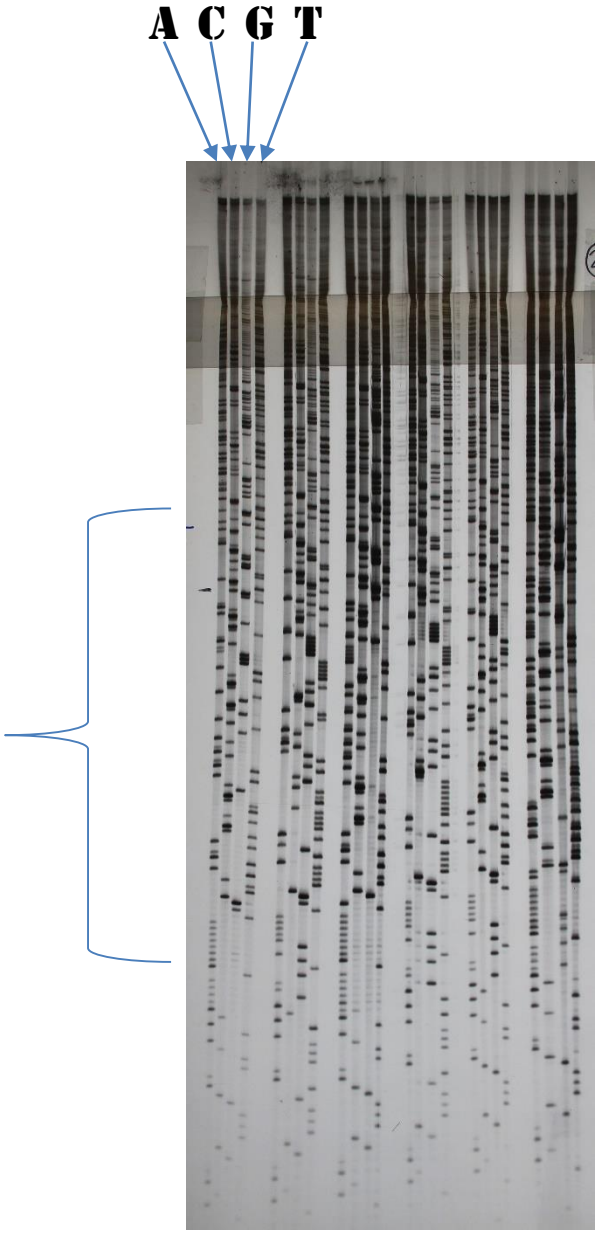
A real gel from a Sanger sequencing reaction. Fragments marked with radioactivity from phosphorus isotope P^{32}.



A real example

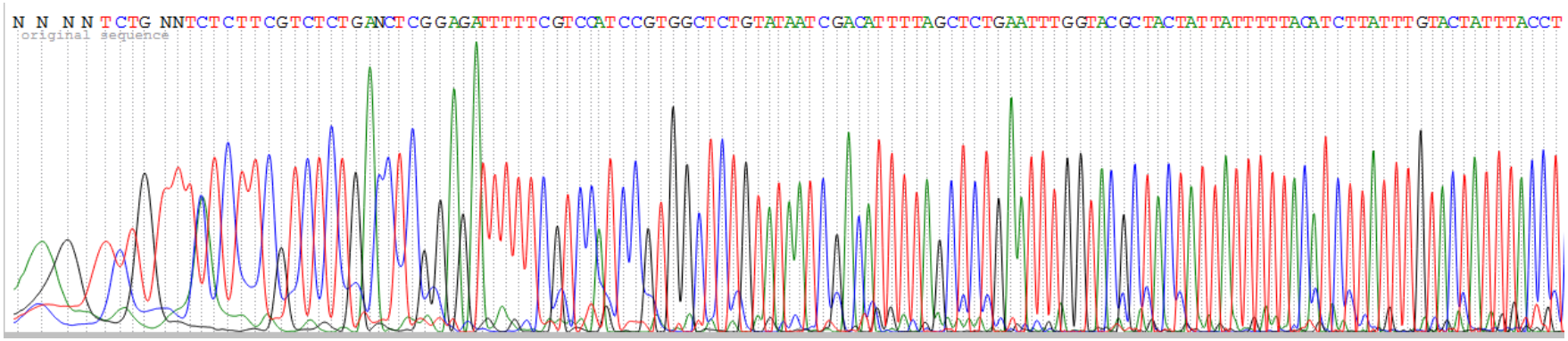
Gene: Ribulose bisphosphate carboxylase from pea chloroplast.

5‘-AAAAATGGCTTTTATGATATTTT
CTTTCGCTGTGACAACAGTTACC
GTGTTTTT-3‘



A real example

Fragments marked by nonradioactive colours and separated on a single gel. Sequence comes from tomato. The diagram below shows the measurements of the colour of the fragments as they emerge out of the separating gel.

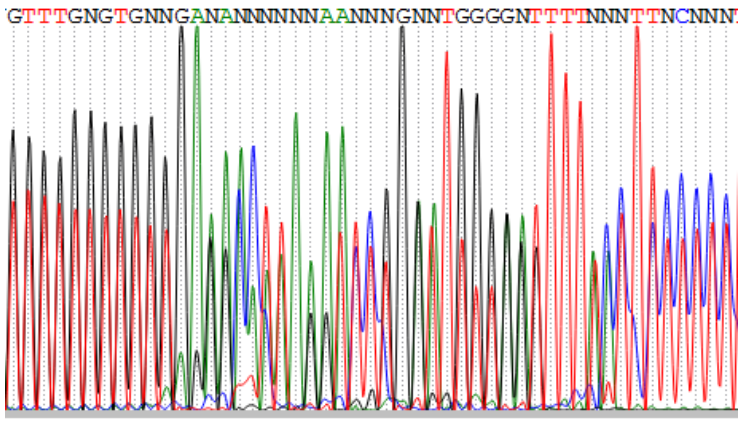


Direction of reading

The sequence starts with some non-readable peaks (marked with N bases) and then each peak stands for a fragment ending in a given base.
A = green, C = blue, G = black, T = red.
This is called a ‚chromatogram‘.

A real example

In some cases the sequence can not be read correctly, then the base N is inserted. This happens when the sequencing reaction fails or the sequencing contains a mixture of several different fragments.



Direction of reading