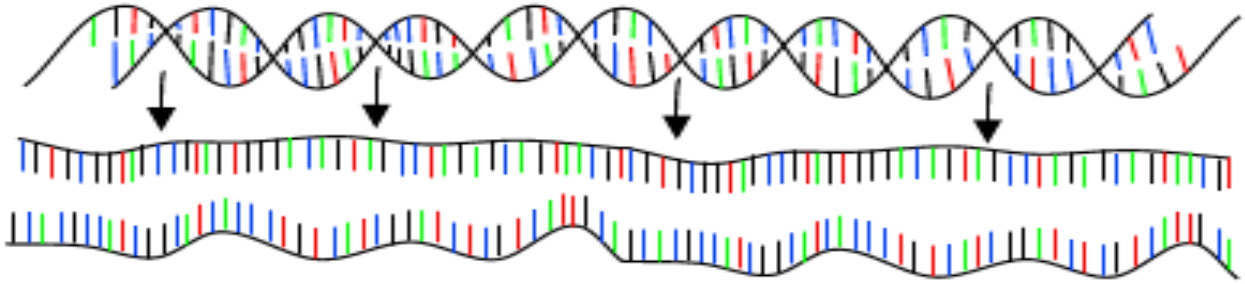
Lab 5

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

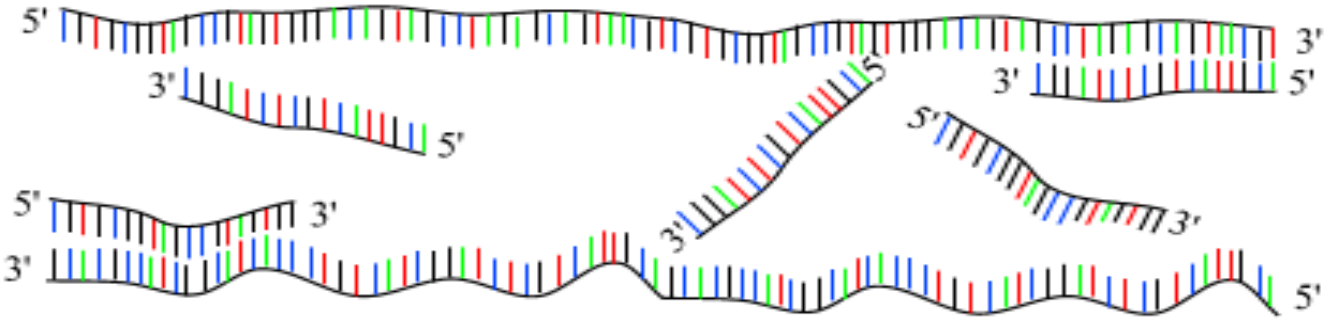
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

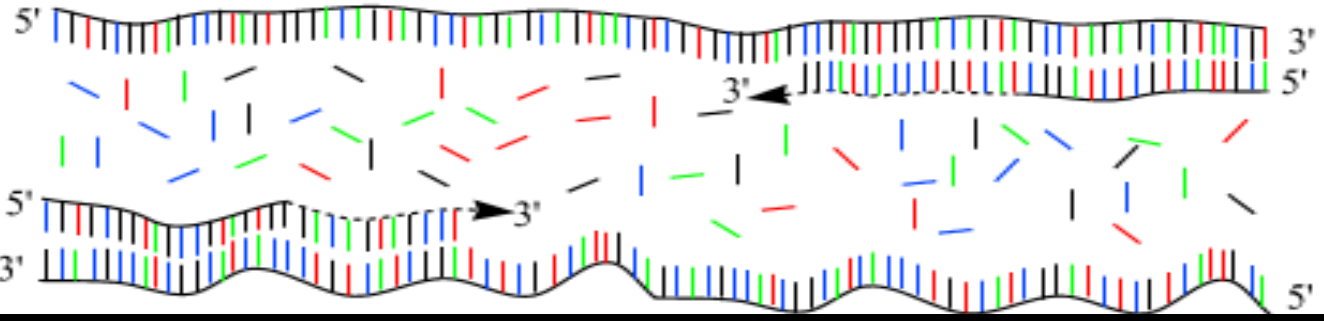
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

2 minutes 72 °C

only dNTP's

(Andy Vierstraete 1999)

You will need :

-RNA sample-------- mRNA------cDNA

- primers

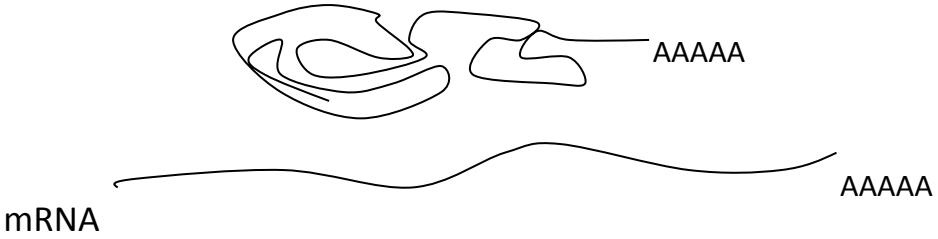
-dNTP

- buffers

- RNase and RNase inhibitor

-RT enzymes

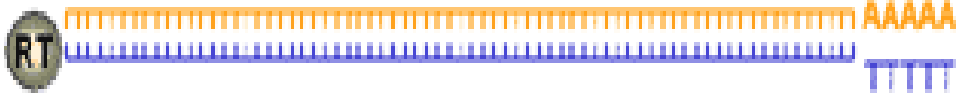
RT PCR: Reverse Transcription Polymerase Chain Reaction



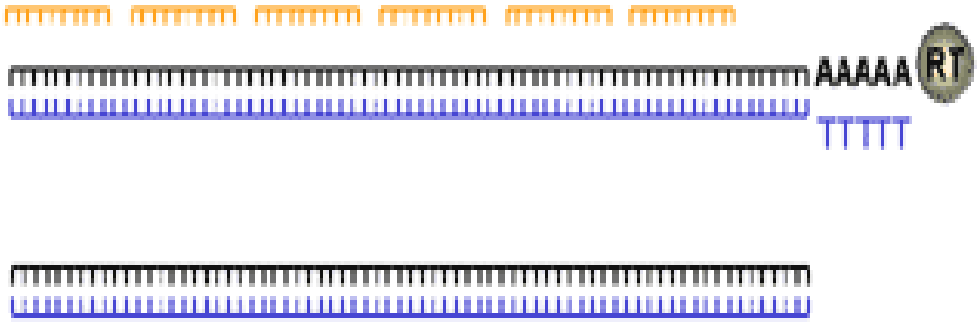
Step 1:Denaturation

Oligo dT primer is bound to mRNA

Step 2:Annealing



Reverse transcriptase (RT) copies first cDNA strand



Reverse transcriptase digests and displaces mRNA and copies second strand of cDNA

Step 3:Extention

One step and two step prosedures

-One step :RT and PCR performed consecutively
In a single tube

-Two step: RT and PCR performed in seperate tubes

Advantages of one step procedure:

-Minimize time requires

-Reduces risk of contamination

-Improve sensitive and specificity

Advantages of two step procedure:

-Allows optimal reaction conditions

-Provide maximum flexibility

-Amplifies long sequences

Choosing the RT-PCR enzymes:

-Maximum lenght of tamplet that can be transcribed into full-lenght cDNA

-Temperature optimum

-Snsetivity

-Specificity

-Rnase H activity

Choosing the primers for TR:

-Oligo (dt)n

-Anchored oligo (dt)n

-Random hexamers

-Sequence-specific

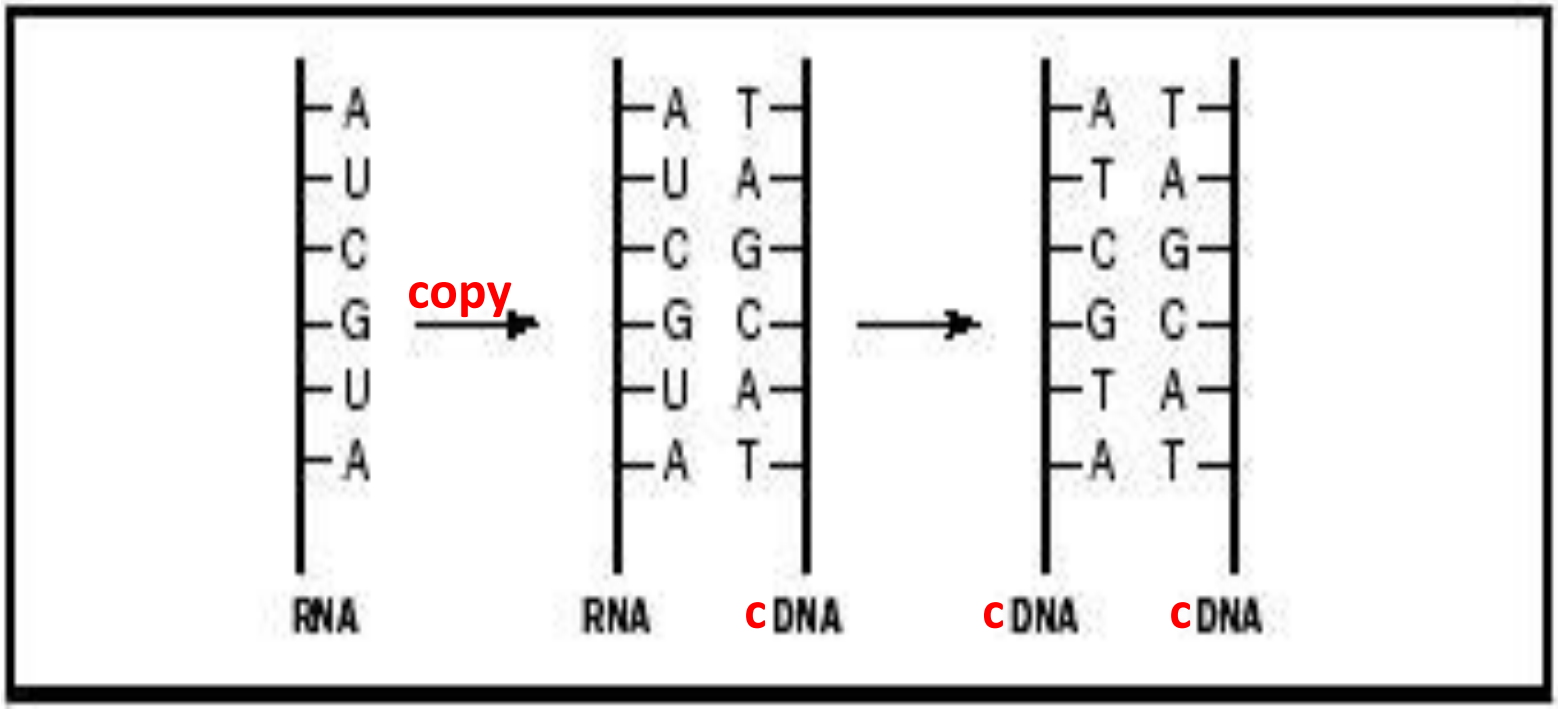
RT- PCR

Using Reverse Transcriptase to convert mRNA into complementary DNA (cDNA) which was then amplified by PCR and, again analyzed by agarose gel electrophoresis.

Reverse Transcriptase-PCR analysis of mRNA is often referred to "RT-PCR" which confused with "real time-PCR".

Complementary DNA

cDNA is synthesized from mature mRNA using the enzyme reverse transcriptase and the enzyme DNA polymerase. This enzyme operates on a single strand of mRNA, generating its complementary DNA based on the pairing of RNA base pairs (A, U, G and C) to their DNA complements (T, A, C and G respectively).



Reverse transcriptase uses a single-stranded RNA template to create a double-stranded cDNA.

DNA library

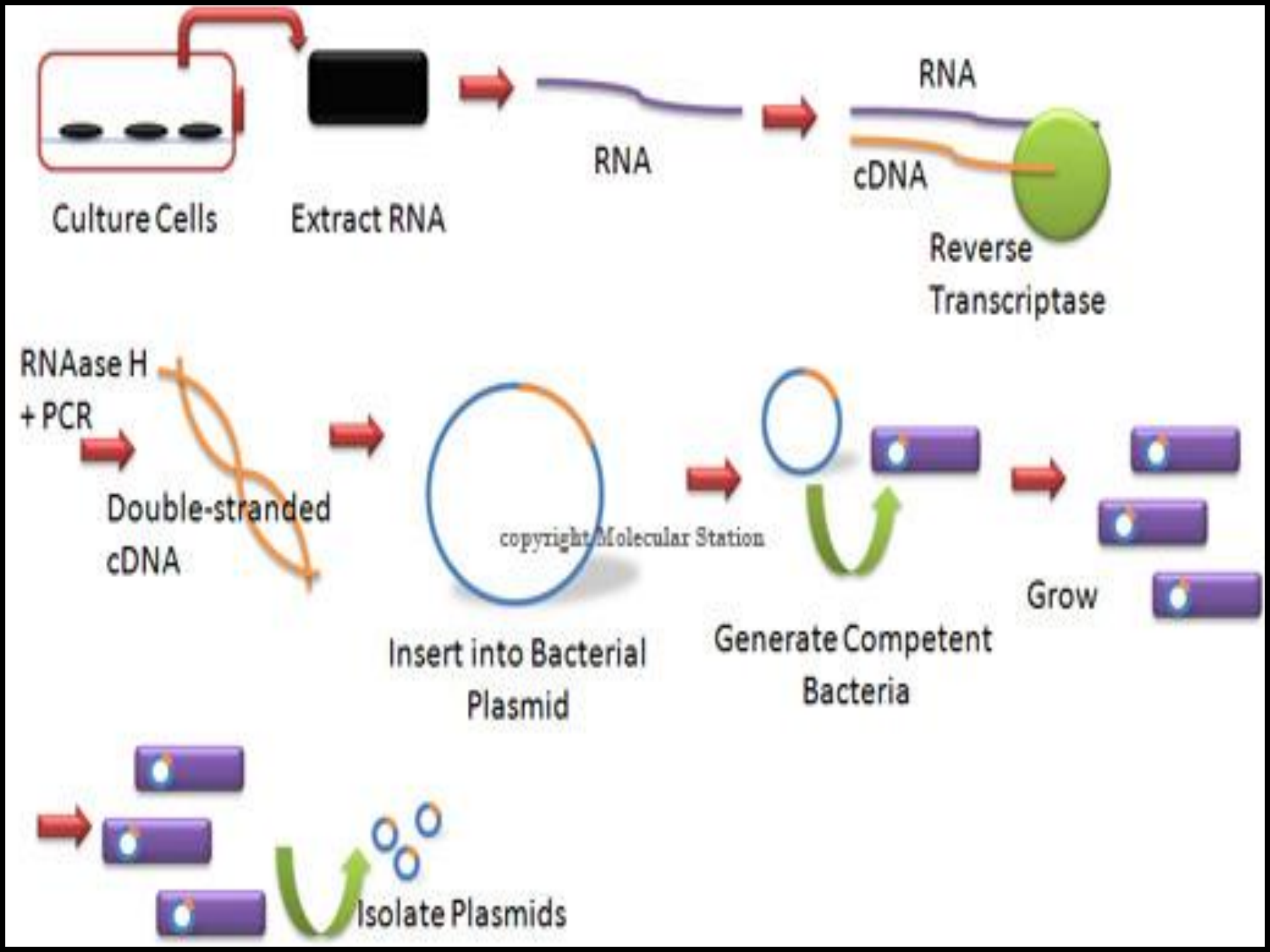
is a collection of cloned DNA fragments. There are two types of DNA library:

- **Genomic library** contains DNA fragments representing the entire genome of an organism.

- **cDNA library** contains only complementary DNA molecules synthesized from mRNA molecules in a cell. It contain all the coding information (the genetic code that produces proteins) from a given cell population.

cDNA Library Technique:

1- After the cDNA is synthesized, it is cloned into expression vectors or plasmids.
2- These plasmids each containing one cDNA are transformed into bacterial competent cells .
3- These plasmids are amplified in the growing bacteria.
4- The bacteria clones are then selected so that only bacteria containing the plasmid will survive.
5- This is commonly done through antibiotic resistance selection.
6- Once the bacteria are selected, stocks of the bacteria are created which can later be grown and sequenced to compile a cDNA library.


Culture Cells
Extract RNA
RNA
RNA
cDNA
Reverse Transcriptase
RNAase H + PCR
Double-stranded cDNA
copyright Molecular Station
Insert into Bacterial Plasmid
Generate Competent Bacteria
Grow
Isolate Plasmids