Genetic engineering and biotechnology institute for postgraduates studies
University of Baghdad

Affordable facilities promote functional genomic studies:

PCR cloning, Transcriptomics/Microarray and *in silico* analysis

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Motivations to present this lecture!

1- Highlight some of available facilities.
(Free and Commercial)

2- Design a research project with clear target.

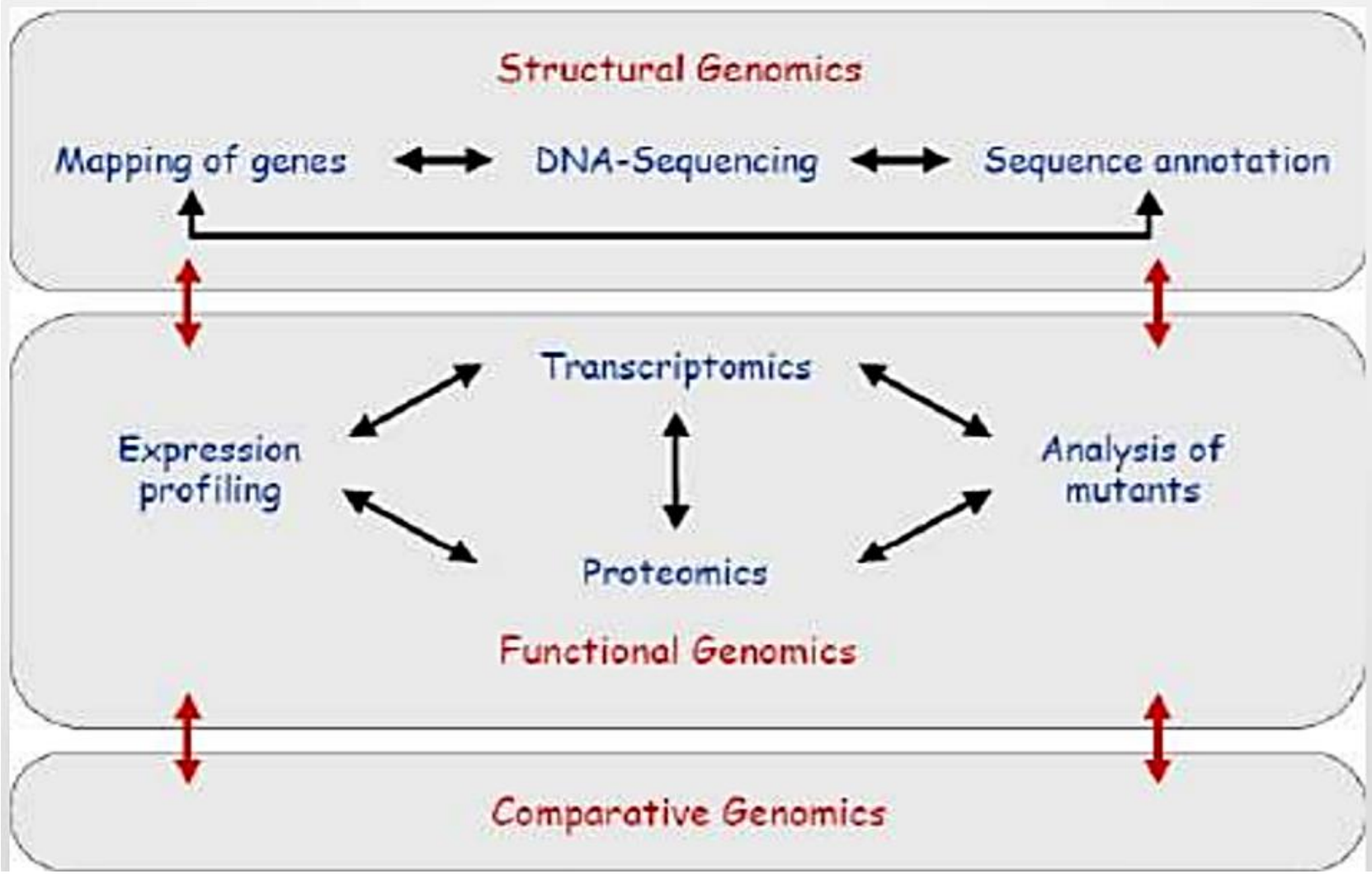
3- Techniques are means, not goals.

4- Encouraging collaborative work.

The goal is

Manuscript will be accepted in high rank journal

Levels of genome studies



Importance of structural and functional genomic studies:

- Discovery of whole new classes of genes.
- Functions and expression
- Determining the functions of proteins and of individual domains within proteins
- Allowing the regulatory regions of genes to understanding complex regulatory network.
- Show the way to efficient mass production of proteins (hormones and vaccines).

Microbial related studies

1- Genomic characterisation of viruses: DNA and RNA genomes, single-stranded, double stranded.

2- Genomic comparisons of microbial strains in the monitoring of outbreaks and infectious disease. epidemic control and drug research.

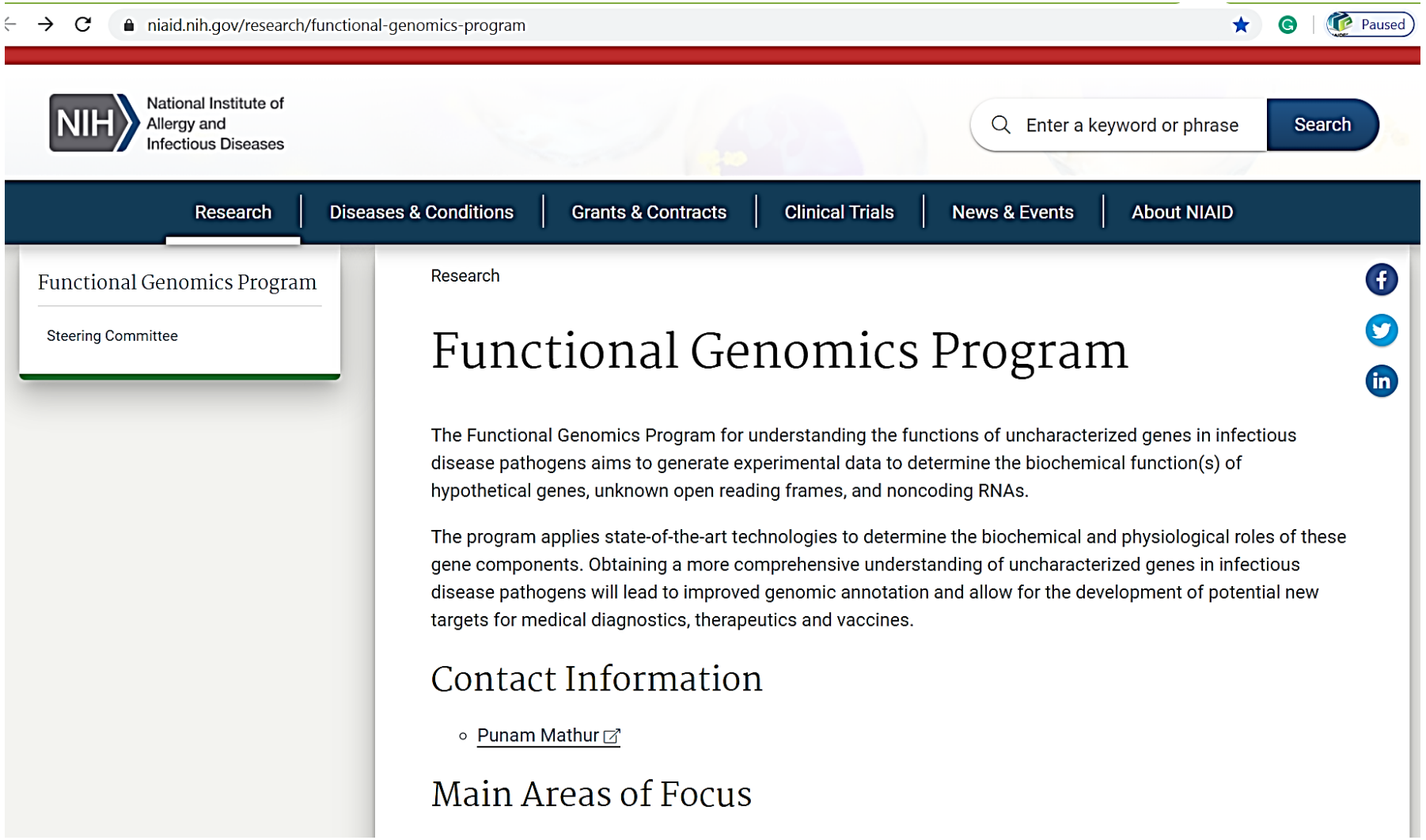
3- Revealing a host pathogen relationships.

4- Laboratory diagnosis.

5- Determine: A- Virulence genes
B- Mutation rate
C- Antibiotics resistance.

6- Metagenomics: Analyse DNA acquired from community of microorganisms present, without the necessity of pure cultures.

International web



Program examples

niaid.nih.gov/research/functional-genomics-program

- Dave Wentworth (Vice-Chair) – CDC

Award Project Descriptions

The research activities were carried out by Harvard University School of Public Health, University of Chicago, University of North Carolina at Chapel Hill and University of Washington, see center websites for more detailed information projects and accomplishments.

- Functionalizing Lists of Unknown TB Entitites (FLUTE)
- The Chicago Center for Functional Annotation (CCFA)
- Genes Unknown in Acinetobacter baumannii (GUNK)

1- Gene expression studies

- **Gene expression** is a highly regulated mechanism that controls the function and adaptability of all living cells.

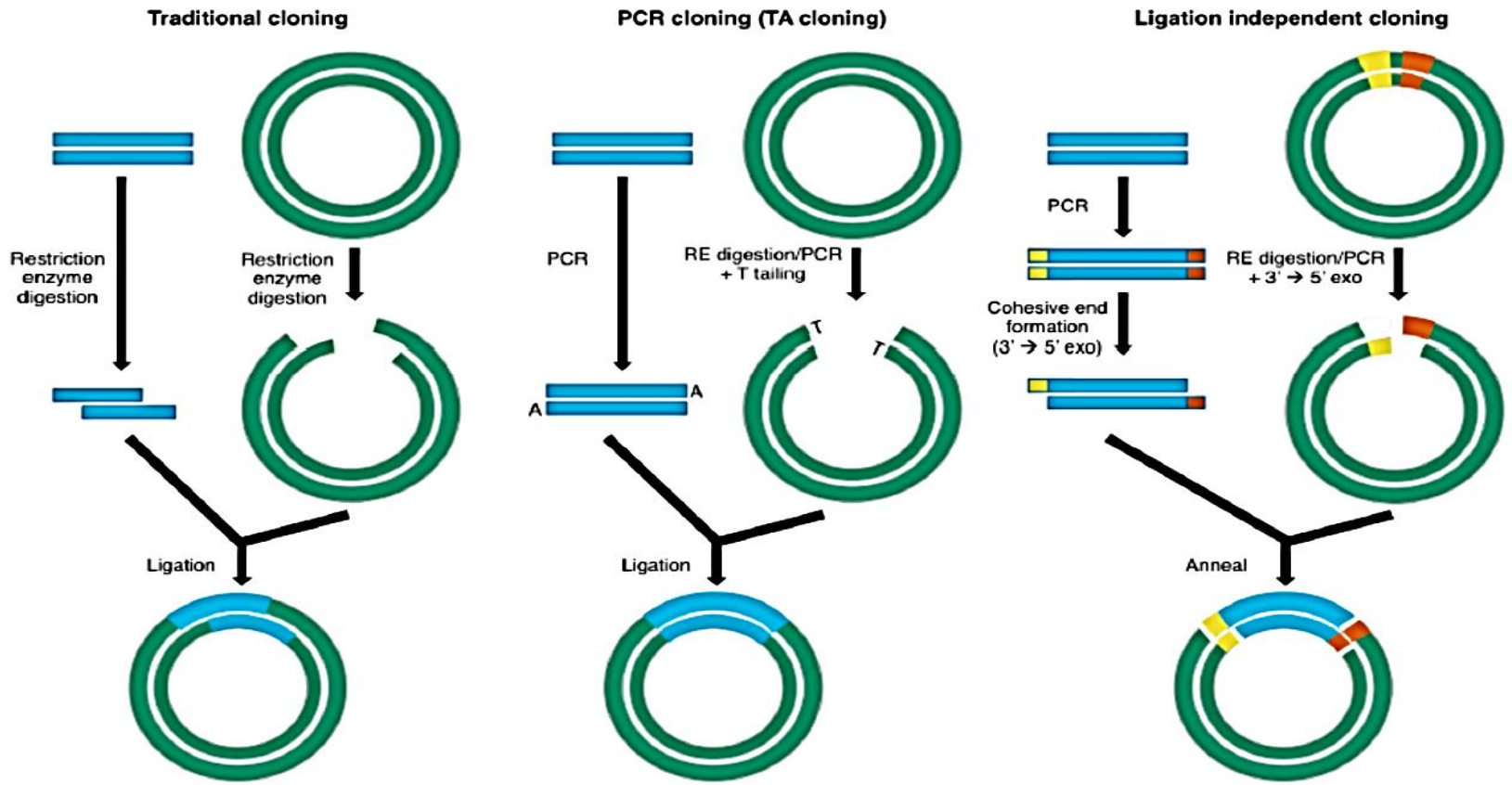
- **First step is get knowledge about gene structure**
- A gene contains two functional segments.
  1- is a coding DNA sequence, which contains the instructions for making a protein.
- 2- The other is a DNA sequence called a promoter.
  which regulates the gene's transcription, either by activating or suppressing its expression.

- Several techniques exist for studying and quantifying gene expression and its regulation.
  This techniques keep updated and developed (effort, time, cost, precise)

- **Cloning**
- protein characterization
- DNA sequencing
- Trancreptome analysis: RT real time PCR, **Microarray,** RNAseq, NGS

Cloning methods

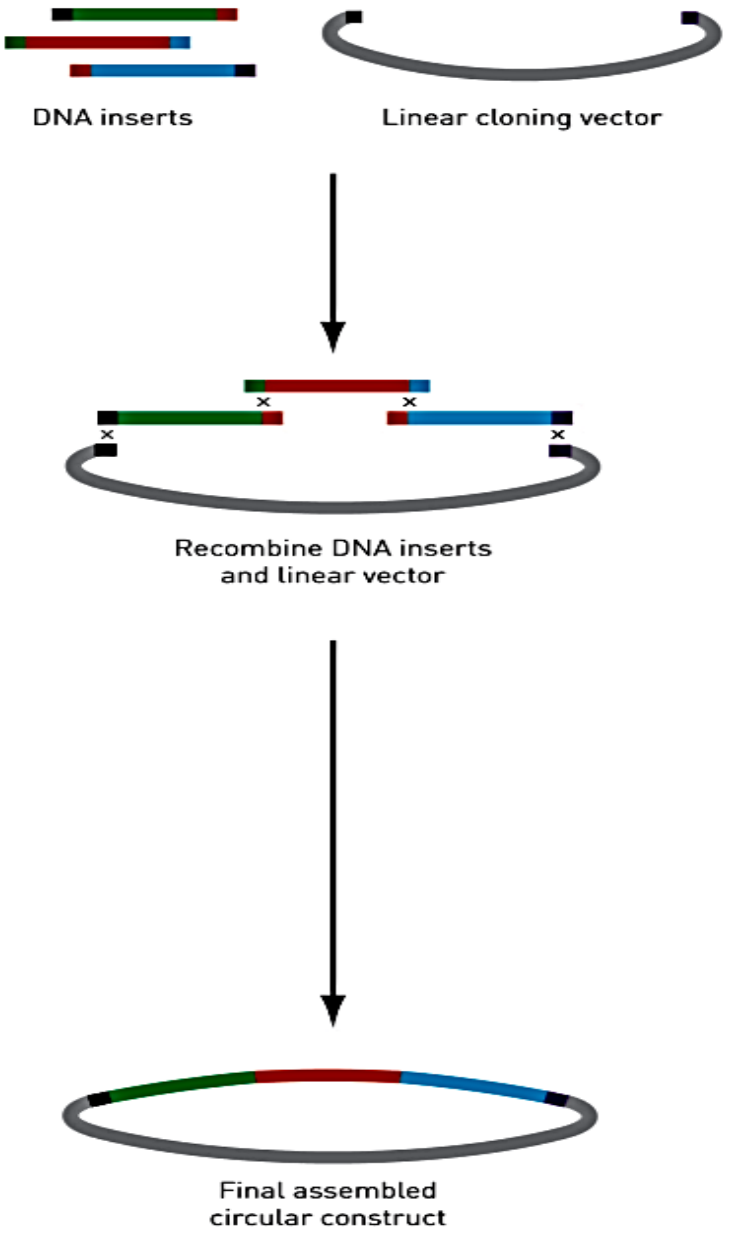
- Produce large numbers of identical combinations DNA molecules (clones)



Seamless cloning

- Seamless cloning technologies eliminate the requirement for restriction enzymes.
- Usful when an insert contains a number of restriction sites within its sequence.
- In general, the procedure consists of adding flanking sequences approximately 15 bp in length to both the insert and vector via PCR.
- The DNA is joined using recombinase enzymes or DNA ligase.
- For instance, GenScript's GenBuilder™ Kit can clone inserts up to 10 kb in 30 minutes.

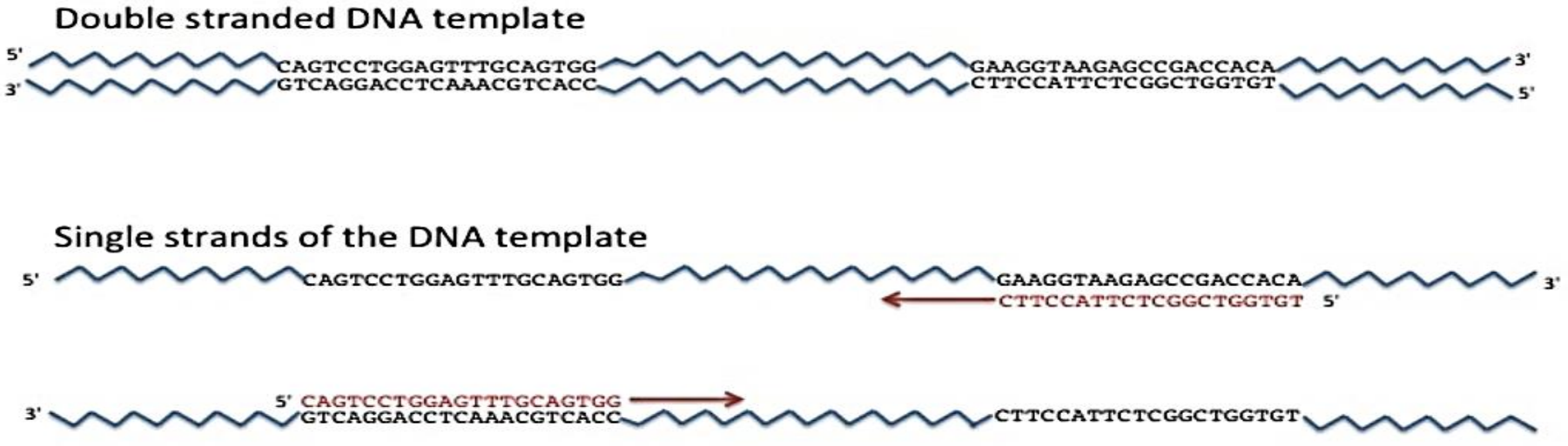
about $200



The PCR cloning strategy

- PCR cloning strategy consists of the following steps:
- The gene of interest is amplified by PCR.
- The PCR product is cloned into intermediate vector.
- The sequence of a positive clone is checked by sequence analysis.
- The gene of interest is sub-cloned into a variety of expression vectors.

Primer design/Restriction site adding in PCR cloning



- For insertion of double stranded DNA into a cloning vector, addition of sticky ends on both termini is necessary.
- Create sticky ends

a) Linkers are ligated to blunt end by T4-DNA ligase.

b) Using terminal transferase the synthesis of homopolymer tails.

c) Adding restriction site to primers.

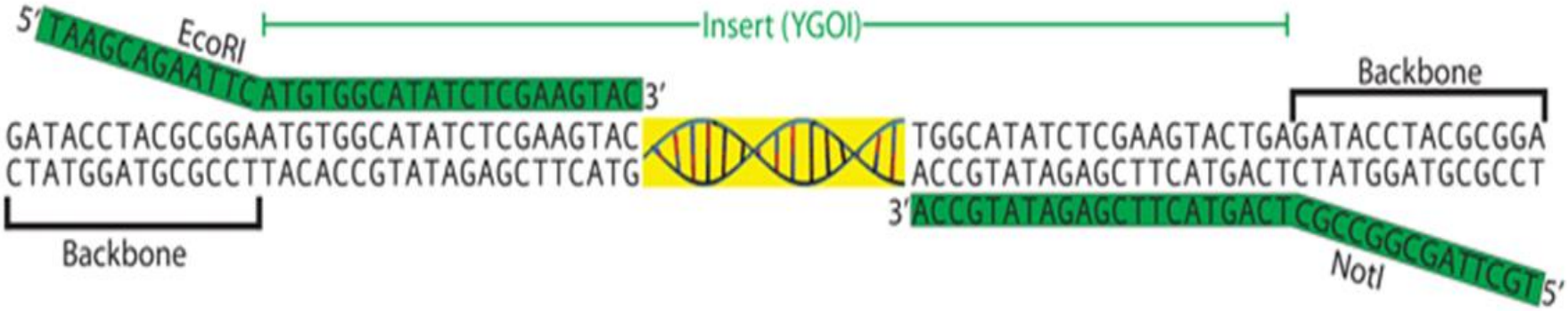
R.E. adding 2-3$ extra

- Restriction sites are in green

(GGATCC for *Bam*HI and GAATTC for *Eco*RI)

Sequence 5' - 3'

- **For:** CACGAATTC**TAAGCCAGAGGAGGTGATGGCGATT**
- **Rev:** GAGGGATCC**AGGCGTGTAACGCCTGCTTCTGATT**



Polymerase selection

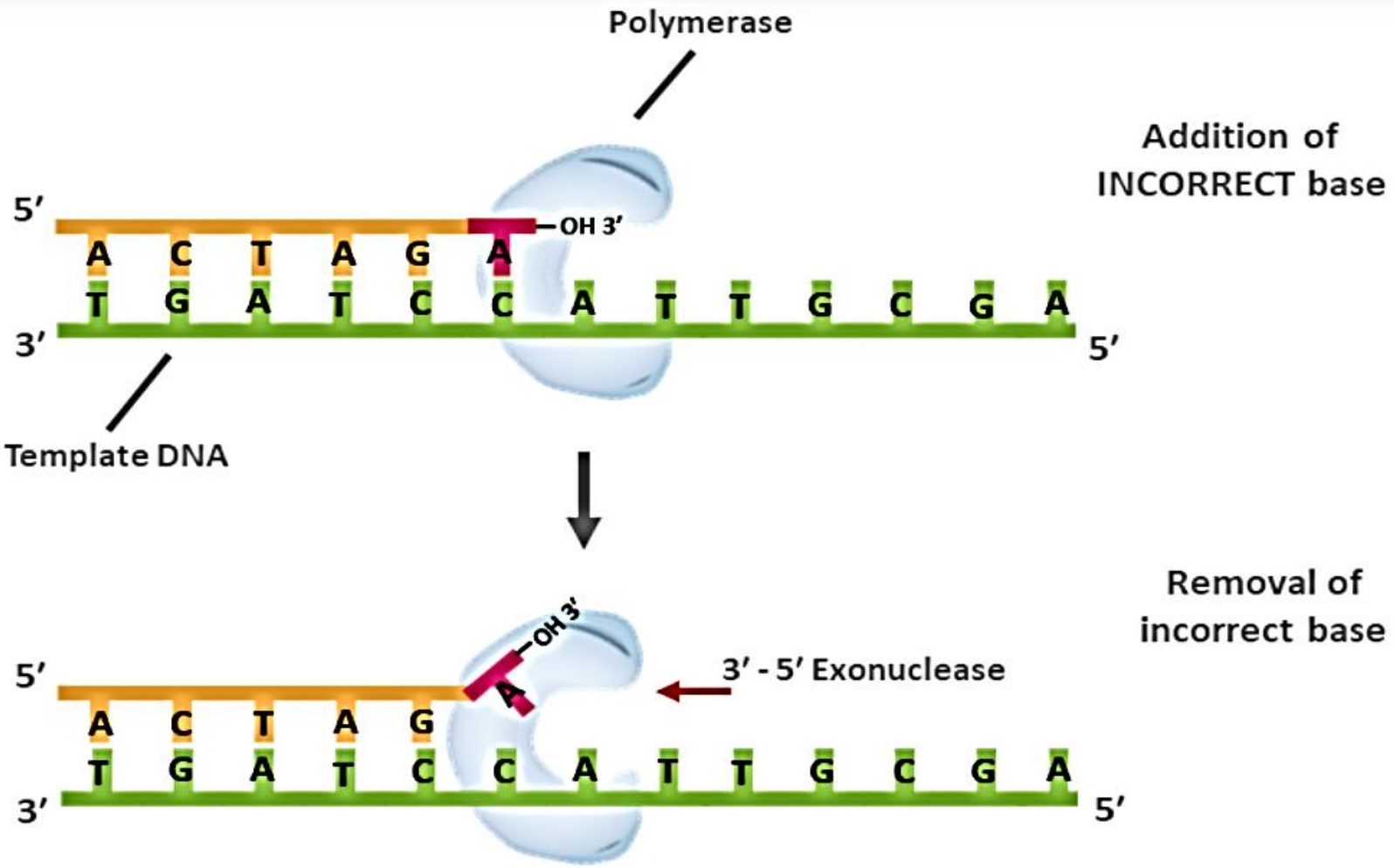
DNA Polymerases

GoldBio Polymerases

GOLDBIO

Polymerase	Mol. Weight	5'→3' Exonuc.	Proof-reading	Thermostability	Fidelity[a]	Processivity	DNA Ends	Applications
DNA Polymerase I	109 kDa	✓	✓	----	1 x 10^{-5} - 10^{-7}	Low	Blunt	• Excision repair • Removal of RNA primer • Nick translation • 3' DNA end removal or filling
Klenow Fragment	68 kDa	✗	✓	----	1 x 10^{-5} - 10^{-7}	Low	Blunt	• Double-strand synthesis • Filling of 3' ends • Primer labeling • DNA sequencing
T4 DNA Polymerase Cat # T-412	104 kDa	✗	✓	----	1 x 10^{-6}	Low	Blunt	• Filling in 5' ends • 3' end labeling/removal • Nick translation • Mutagenesis
Taq Polymerase Cat # T-514	94 kDa	✓	✗	✓	1 – 20 x 10^{-5}	High	3' A overhang	• Routine PCR • TA cloning • Sequencing
Pfu Polymerase Cat # P-665	92 kDa	✗	✓	✓	1 – 2 x 10^{-6}	Low	Blunt	• Mutation analysis • Cloning • Sequencing • Gene expression analysis
Hot Start Taq DNA Polymerase Cat # T-510	94 kDa	✓	✗	✓	1 – 20 x 10^{-5}	High	3' A overhang	• Routine PCR • Suitable for low amount of template or complex template
Hot Start Pfu DNA Polymerase Cat # P-650	90 kDa	✗	✓	✓	1 – 2 x 10^{-6}	High	Blunt	• Routine PCR • Suitable for low amount of template or complex template

3-5 exonuclease activity/proofreading



Intermediate cloning Vector (148$ for 20 reactions)

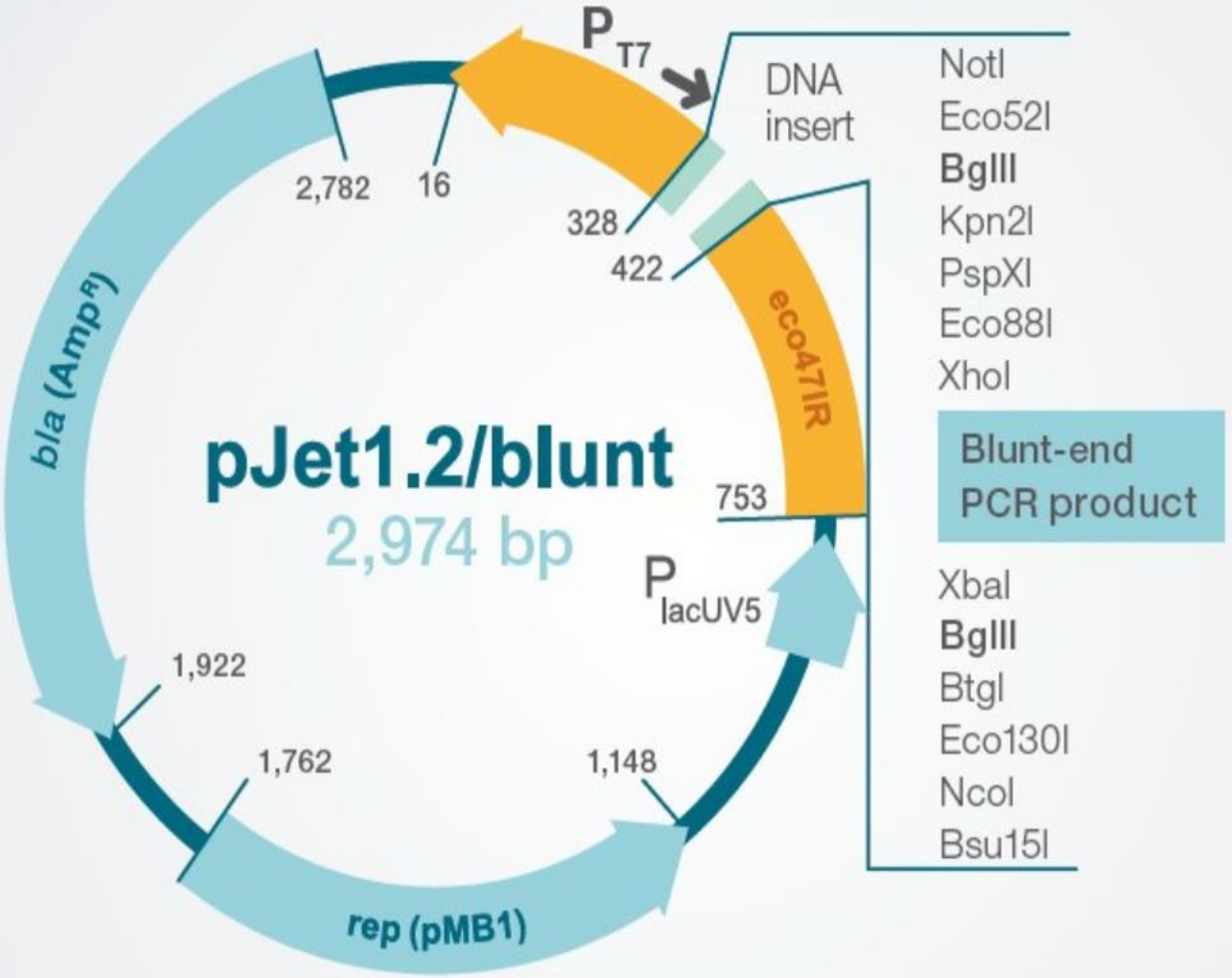
- Before going to destination vector, Intermediate is required!

- Thermo Scientific CloneJET PCR Cloning Kit, is an advanced positive selection system (lethal gene that is disrupted by ligation)

- Products generated with any thermostable DNA polymerase.

- Fast—PCR cloning in only 5 minutes
- Highest efficiency—>99% of positive clones
- Economical—no expensive blue/white screening
- Sequencing of cloned DNA (available primer).

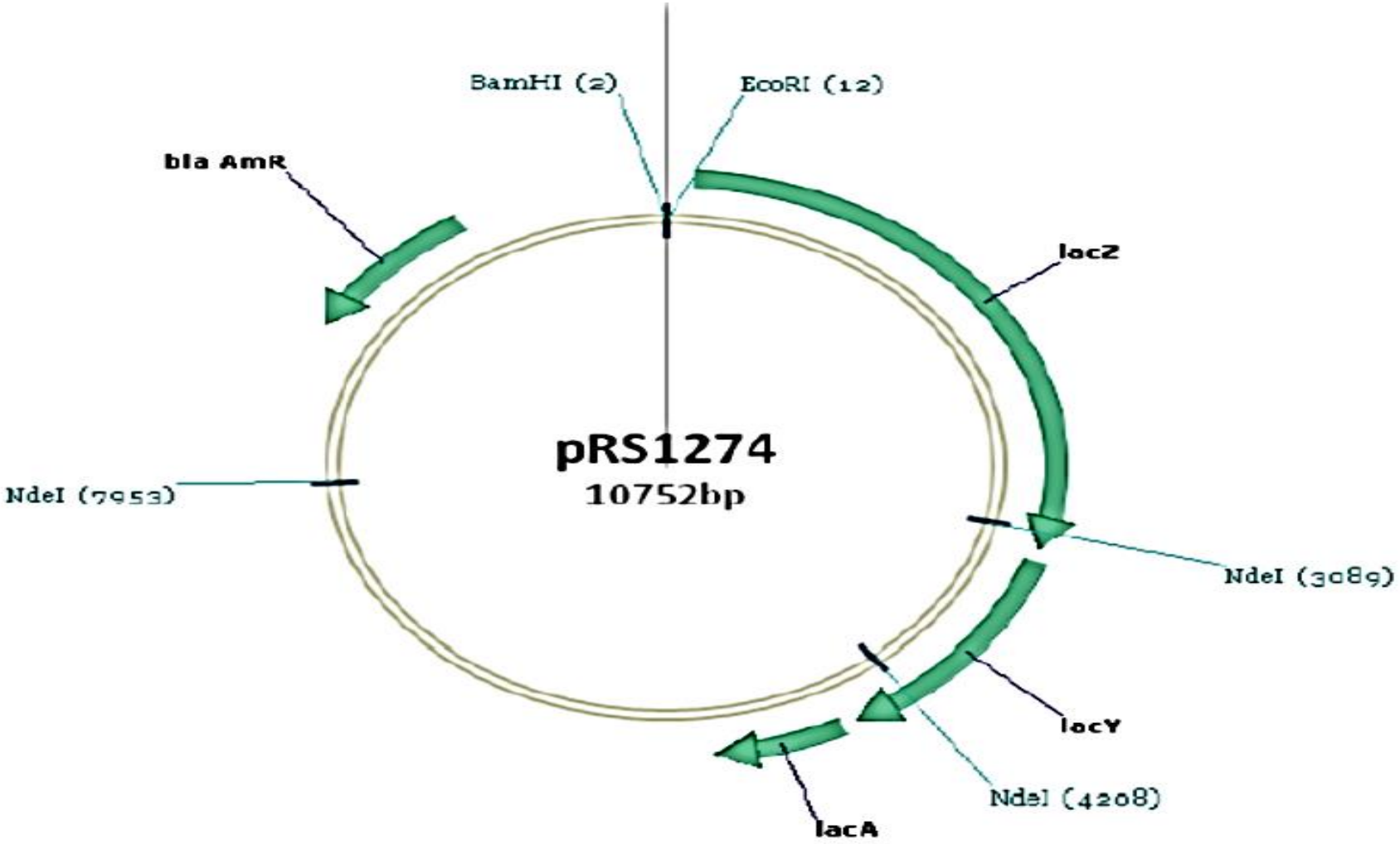
Thermo Scientific™ CloneJET™ vector map



thermofisher.com/tstoppicks

Destination vector 60-200$

pRS1274 *lacZ* transcriptional fusion vector containing *Bam*HI-*Sma*I-*Eco*RI-*lacZ* cloning site , *lacZ lacY lacA*

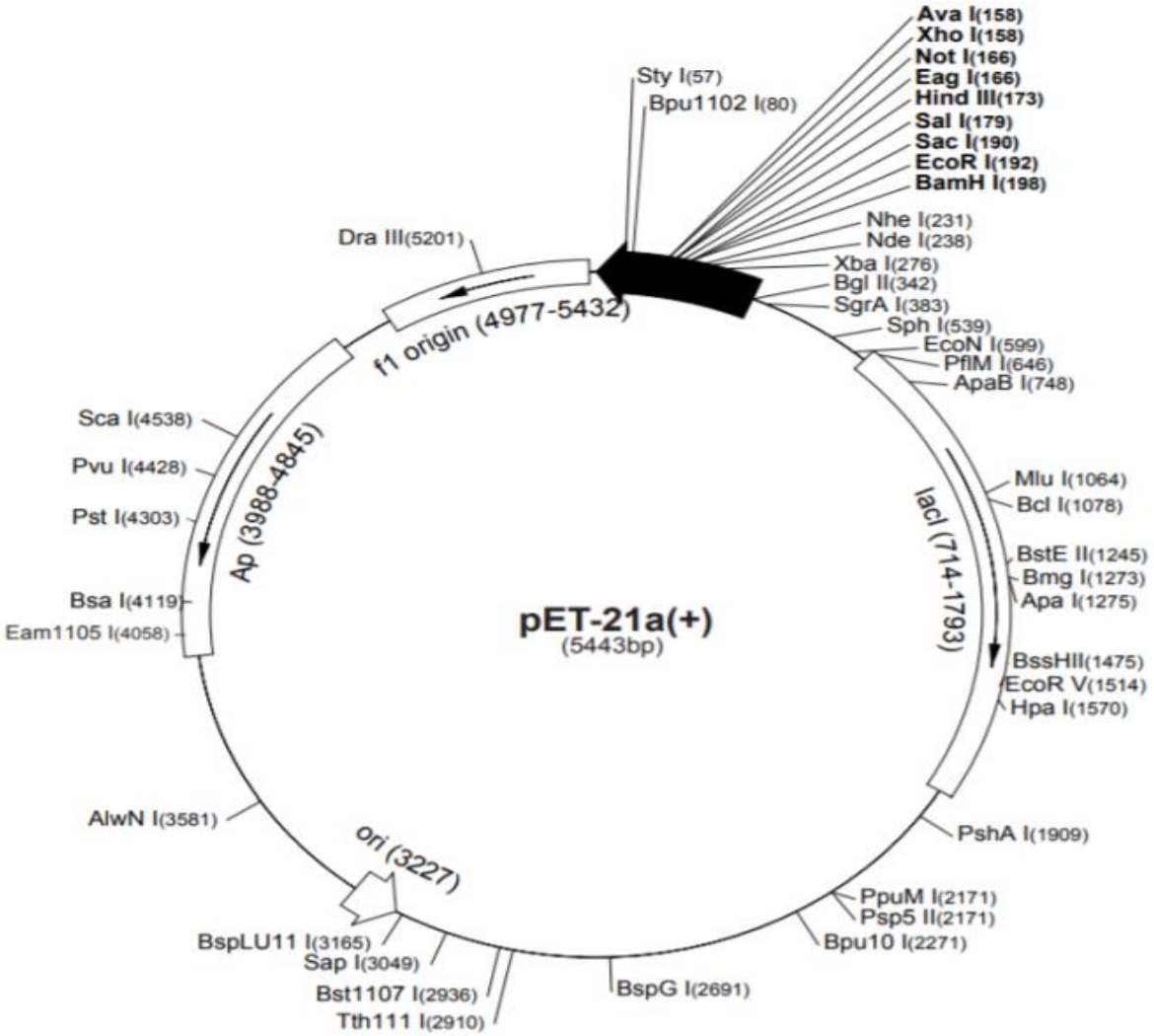


pET21a(+) Overexpression cloning vector

Novagen/225$

pET-21a(+) sequence landmarks	
T7 promoter	311-327
T7 transcription start	310
T7•Tag coding sequence	207-239
Multiple cloning sites (*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	714-1793
pBR322 origin	3227
bla coding sequence	3988-4845
f1 origin	4977-5432

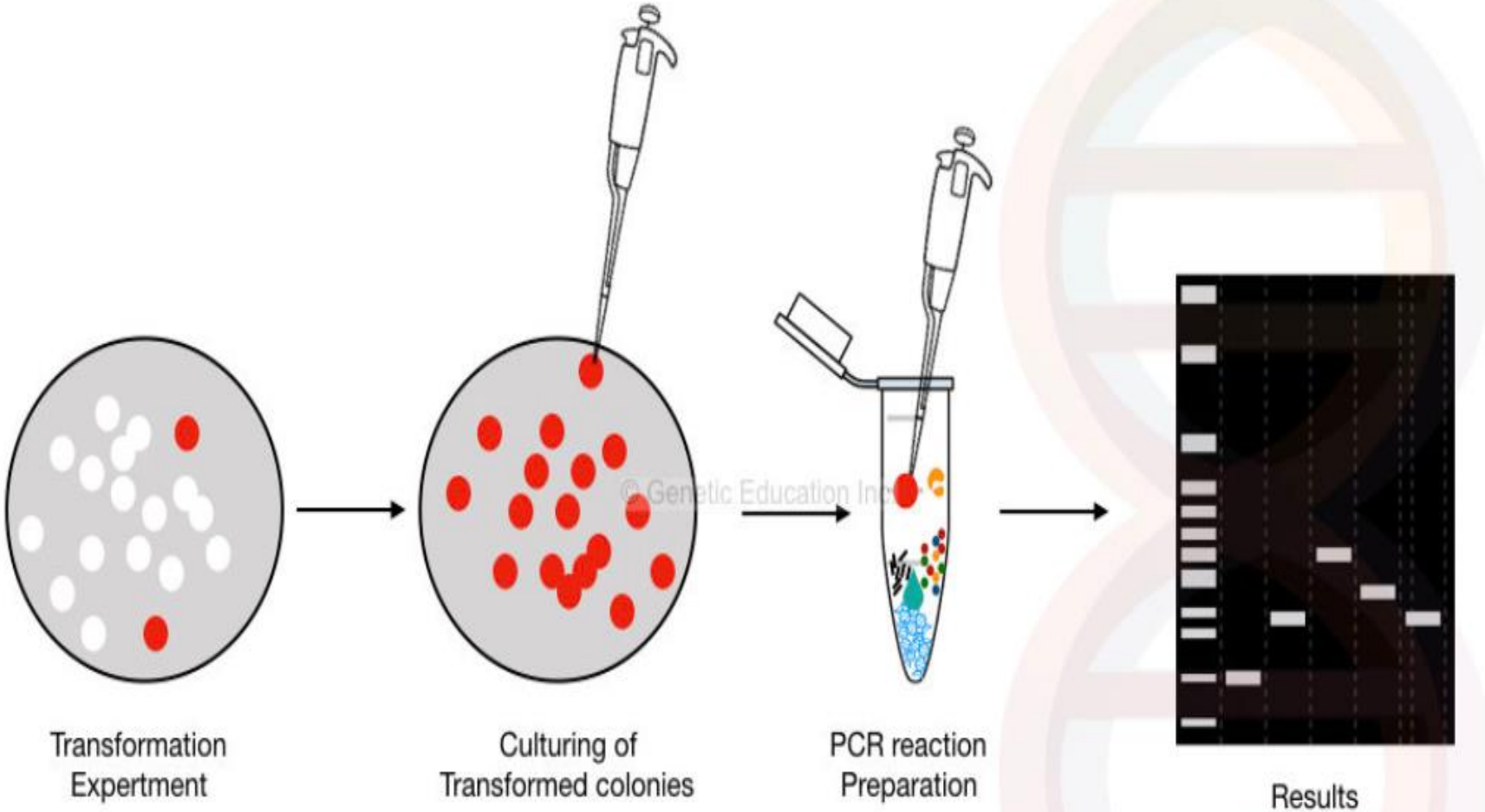
The maps for pET-21b(+), pET-21c(+) and pET-21d(+) are the same as pET-21a(+) (shown) with the following exceptions: pET-21b(+) is a 5442bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-21c(+) is a 5441bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198. pET-21d(+) is a 5440bp plasmid; the *Bam*H I site is in the same reading frame as in pET-21c(+). An *Nco* I site is substituted for the *Nde* I site with a net 1bp deletion at position 238 of pET-21c(+). As a result, *Nco* I cuts pET21d(+) at 234, and *Nhe* I cuts at 229. For the rest of the sites, subtract 3bp from each site beyond position 239 in pET-21a(+). *Nde* I does not cut pET-21d(+). Note also that *Sty* I is not unique in pET-21d(+).



Detection of Recombinant Clone

- **A classic way:** Blue-white screening using bacterial lactose metabolism
  Colourless substrate (X-gal) on cleavage by β-galactosidase
- **A powerful way:** Positive selection vectors conditionally express a lethal gene clones. OR insertional inactivation suitable genetic system (Abr).
- **A precise way:** Using restriction enzymes.
- **The most accurate way:** Sequencing using appropriate primers.
- **A quick way:** Colony screening with PCR is the most rapid initial screen.

Clones selection/Colony PCR



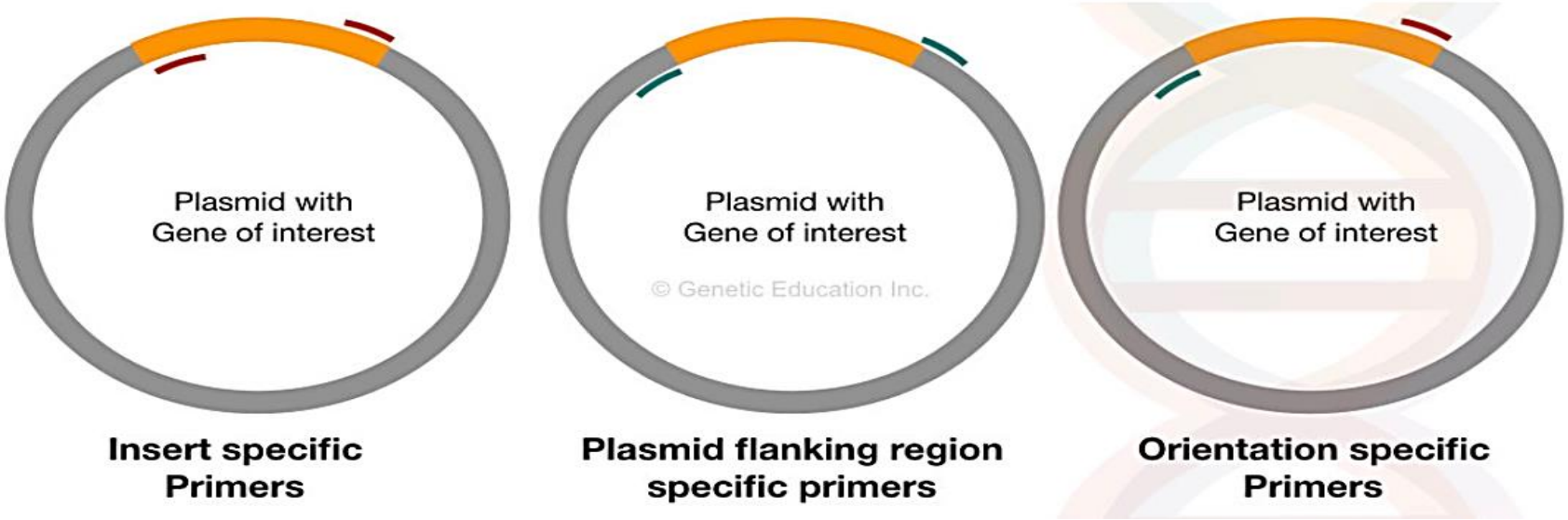
The general overview of the colony PCR method.

Colony PCR Info.

What type of information do we want from our colony PCR experiment?

1. Information about the presence or absence of the insert only.
2. Information about the size of the insert.
3. Information about the orientation of the insert.

Depending upon that different PCR primers are designed for the colony PCR.



Insert specific Primers

Plasmid flanking region specific primers

Orientation specific Primers

Transcriptome profiling

- Global transcriptional profiling is a full range of messenger RNA expressed by an organism.
- This techniqiues has been widely used to understand the genetic regulation of a particular cell type.
- Transcriptomics provides guidance to select the genes for functional studies.
- **Methods have been used in profiling**
- **Microarray technology**
- Serial Analysis of Gene Expression (SAGE),
- RNA sequencing (RNA-Seq)

DNA microarrays: analyzing genome-wide expression

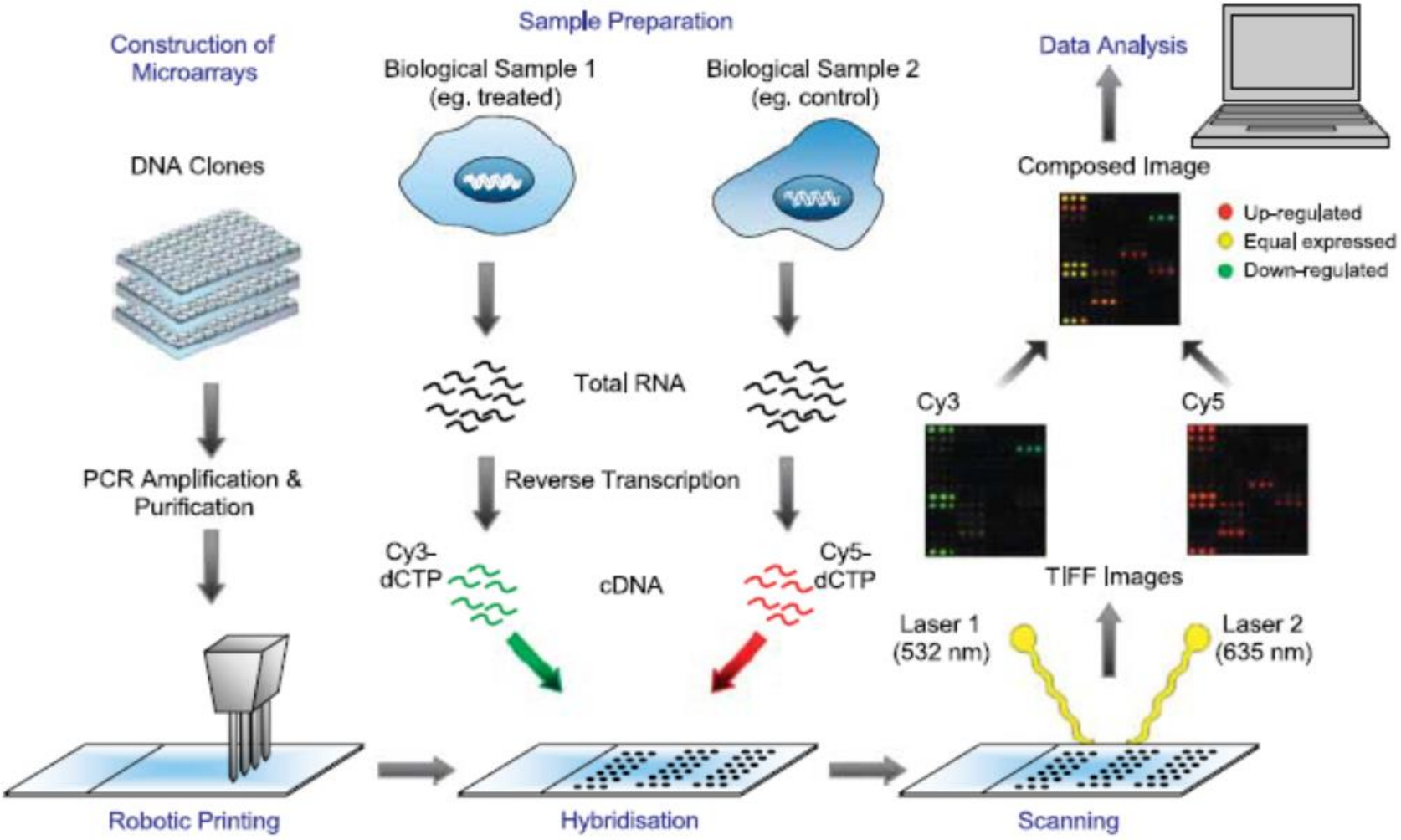
- DNA microarrays consist of thousands of individual gene sequences bound to closely spaced regions on the surface of a glass microscope slide or synthesized

  sequences on a chip surface.

- a DNA microarrays allow the simultaneous analysis of the expression of thousands of genes.

- The combination of DNA microarray technology with genome sequencing projects enables scientists to analyze the complete transcriptional program of an organism during specific physiological response or developmental processes

Microarray outline

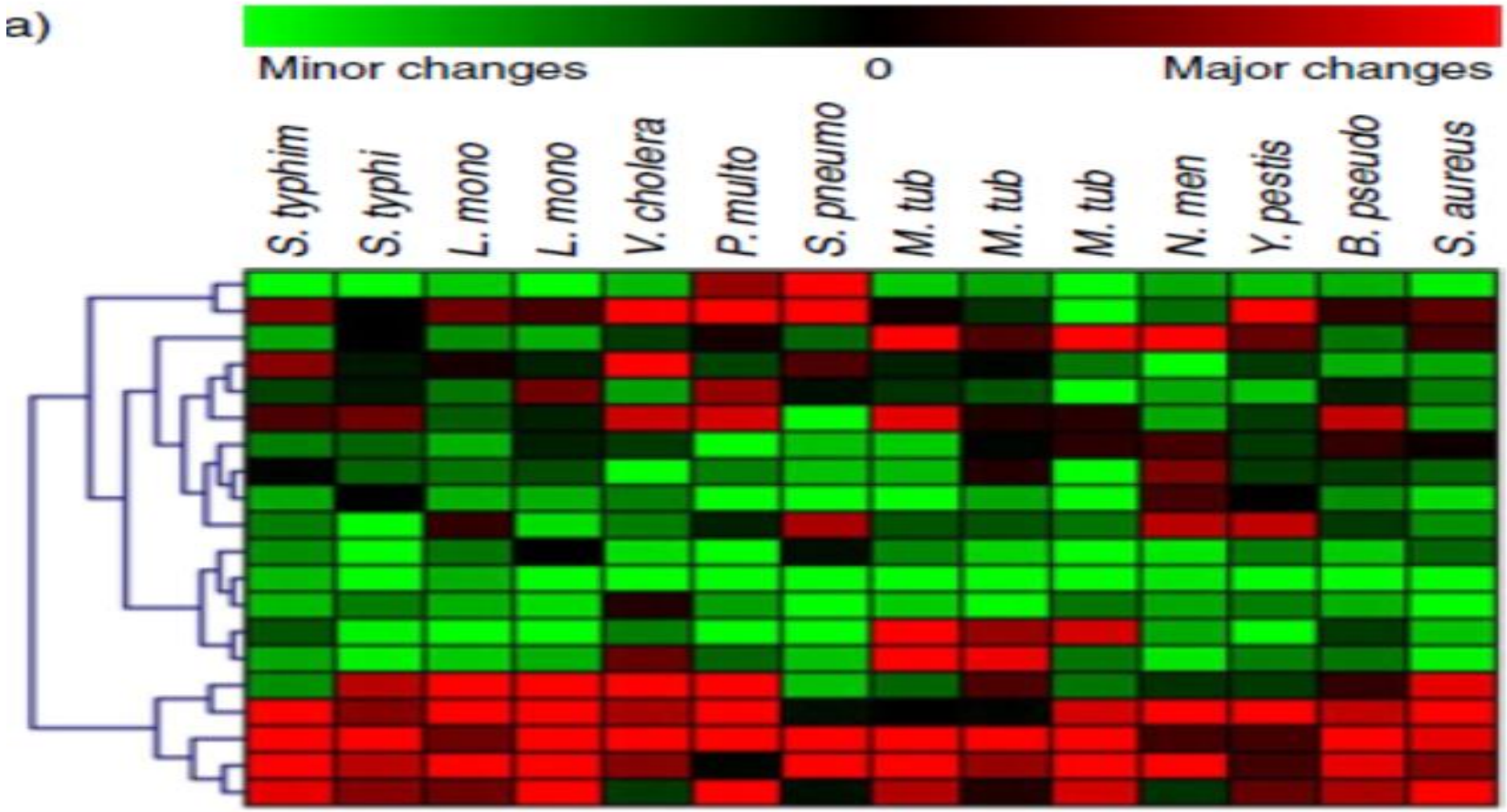


Microarray studies

Table 1. Microarray-based transcriptional profiles of bacterial pathogens during infection of host cells or tissues

Pathogen	Infected experimental model
Facultative intracellular pathogens	
Helicobacter pylori B128	Gastric bisopsy specimens from humans and gerbils vs. growth *in vitro* at 37 °C
Salmonella enterica	
serovar Typhi ISP1820	Human monocytes (THP-1) at different time-points after infection vs. extracellular growth
serovar Typhimurium SL1344	Murine macrophages J774-A.1 vs. complement-opsonized bacteria grown at 37 °C in complete culture medium
Shigella flexneri Sf301	Human macrophages-like (U-937) or human epithelial (HeLa) cells vs. growth in LB at 37 °C
Listeria monocytogenes EGD	Epithelials cells (Caco-2) vs. growth in BHI medium at 30, 37 or 42 °C Murine macrophage cells (P388D1) vs. growth in BHI broth at 37 °C

Results analysis: example



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Applied Biosystems™

GeneChip™ S. aureus Genome Array

Catalog number: 900514

Related applications: Microarray Analysis

	Catalog number
☆	900514

2700$

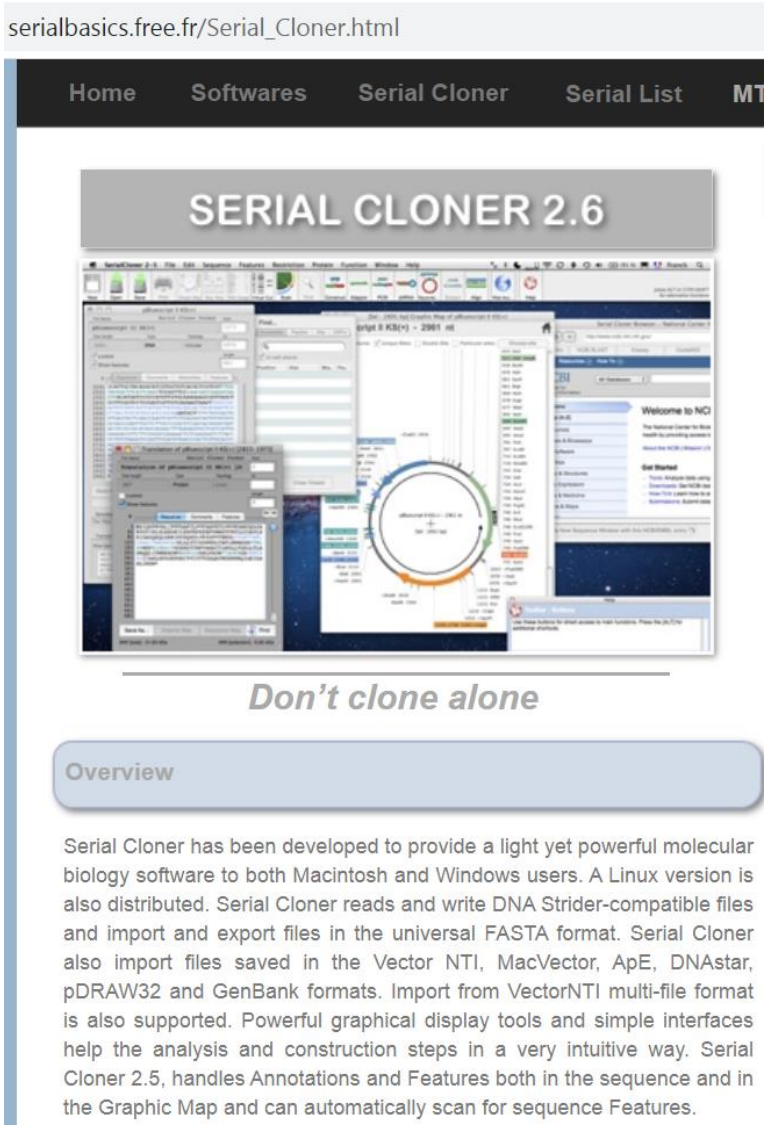
For comprehensive monitoring of the relative mRNA abundance of *S. aureus* sequences.

The GeneChip *S. aureus* Genome Array is useful for studying the expression of sequences in *Staphylococcus aureus*

The array contains probe sets to over 3,300 *S. aureus* open reading frames.

In silico services/Cloning webs

- Graphic Maps: Draws graphic ORF maps
- Quick annotation of sequence
- Virtual Digests
- Finds translationally silent restriction sites
- Translates sequences
- Finds potential primers with criteria (length, Tm, %GC, self/other complementarity)
- Aligns two DNA sequences with the alignment hyperlinked to the original sequence.
- Phylogenetic analysis



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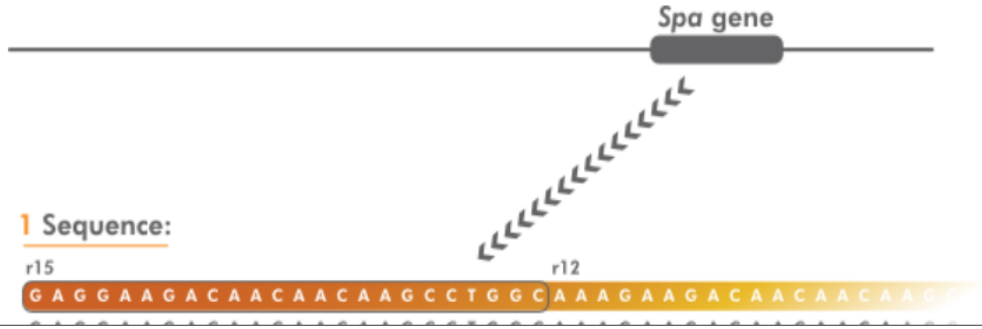
Staphylococcus aureus spa typing

The *spa* typing technique uses the sequence of a polymorphic VNTR in the 3' coding region of the *S. aureus*-specific staphylococcal protein A (*spa*). Each new base composition of the polymorphic repeat found in a strain is assigned a unique repeat code. The repeat succession for a given strain determines its *spa* type. The individual repeat length for the *spa* VNTR is usually 24 bp, but exceptions of 21 to 30 exist. Although *spa* typing is a single-locus typing technique, it offers a subtyping resolution comparable to more expensive and/or laborious techniques such as MLST and PFGE. The technique is widely used for sub-typing of *S. aureus* in hospital and outbreak settings.



Sequence: AF500262.1

Staphylococcus aureus IcaA (icaA) gene, partial cds.

Organism	Molecule type	Topology	Data class	Taxonomic Division
Staphylococcus aureus	genomic DNA	linear	STD	PRO

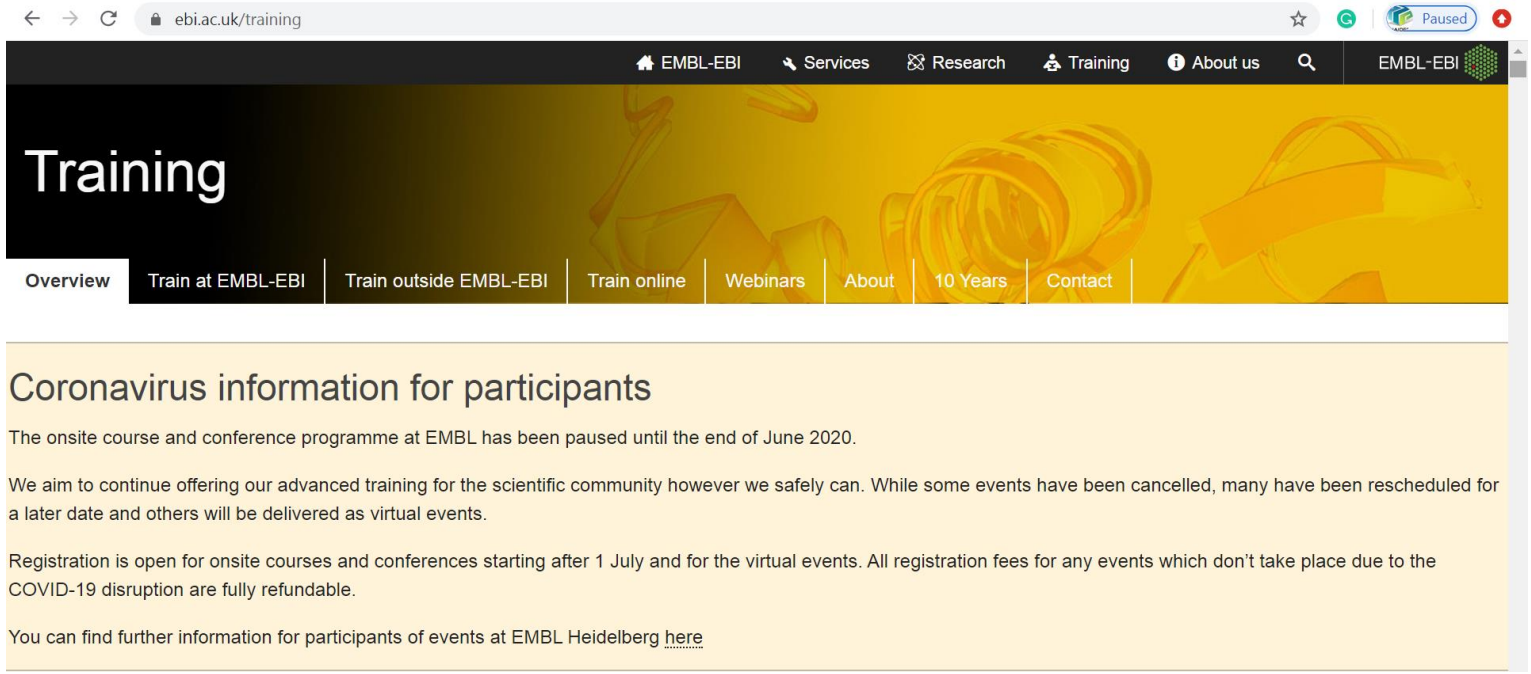
Sequence length	Sequence Version	First public	Last updated	Show Version History

1-866-757-2414 or Contact Us

Service Details

Core Services

SERVICE NAME	DESCRIPTION	UNIT	CAT. NO.	PRICE
Economy Gene Synthesis & Subcloning	• For non-complicated sequences • Synthesize up to 950 bp • Complimentary subcloning into pUC57, EcoRV site • Sequence verification to confirm full length sequence is generated • Sequence must be approved for economy synthesis by **abm**	1.0 µg	C078	**$375.00**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Coronavirus information for participants
The onsite course and conference programme at EMBL has been paused until the end of June 2020.
We aim to continue offering our advanced training for the scientific community however we safely can. While some events have been cancelled, many have been rescheduled for a later date and others will be delivered as virtual events.
Registration is open for onsite courses and conferences starting after 1 July and for the virtual events. All registration fees for any events which don't take place due to the COVID-19 disruption are fully refundable.
You can find further information for participants of events at EMBL Heidelberg here

models, which are provided by the InterPro's member databases.

First come, first served

Protein families

July 2020

6

Cancer genomics (Virtual)

European Bioinformatics Institute (EMBL-EBI) - Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom

6th - 10th Jul

The delivery of this course has changed from face-to-face to virtual and that the date has moved back one week.

Open application with selection

Bioinformatics | Genomics | Oncology

6

Systems biology: From large datasets to biological insight (Cancelled)

associated bioactivities. Some basic understanding of...

Start now

ChEMBL: Quick tour

Author(s): Louisa Bellis

This quick tour provides a brief introduction to ChEMBL, the EBI's chemogenomics resource. For a more detailed walthrough of ChEMBL, have a look at our ChEMBL: Exploring bioactive drug-like molecules tutorial.

Start now

Complex Portal: Quick tour

Author(s): Birgit Meldal

This quick tour provides a brief introduction to EMBL-EBI's Complex Portal: a manually curated, encyclopedic resource of macromolecular complexes from a number of key model organisms.

Start now

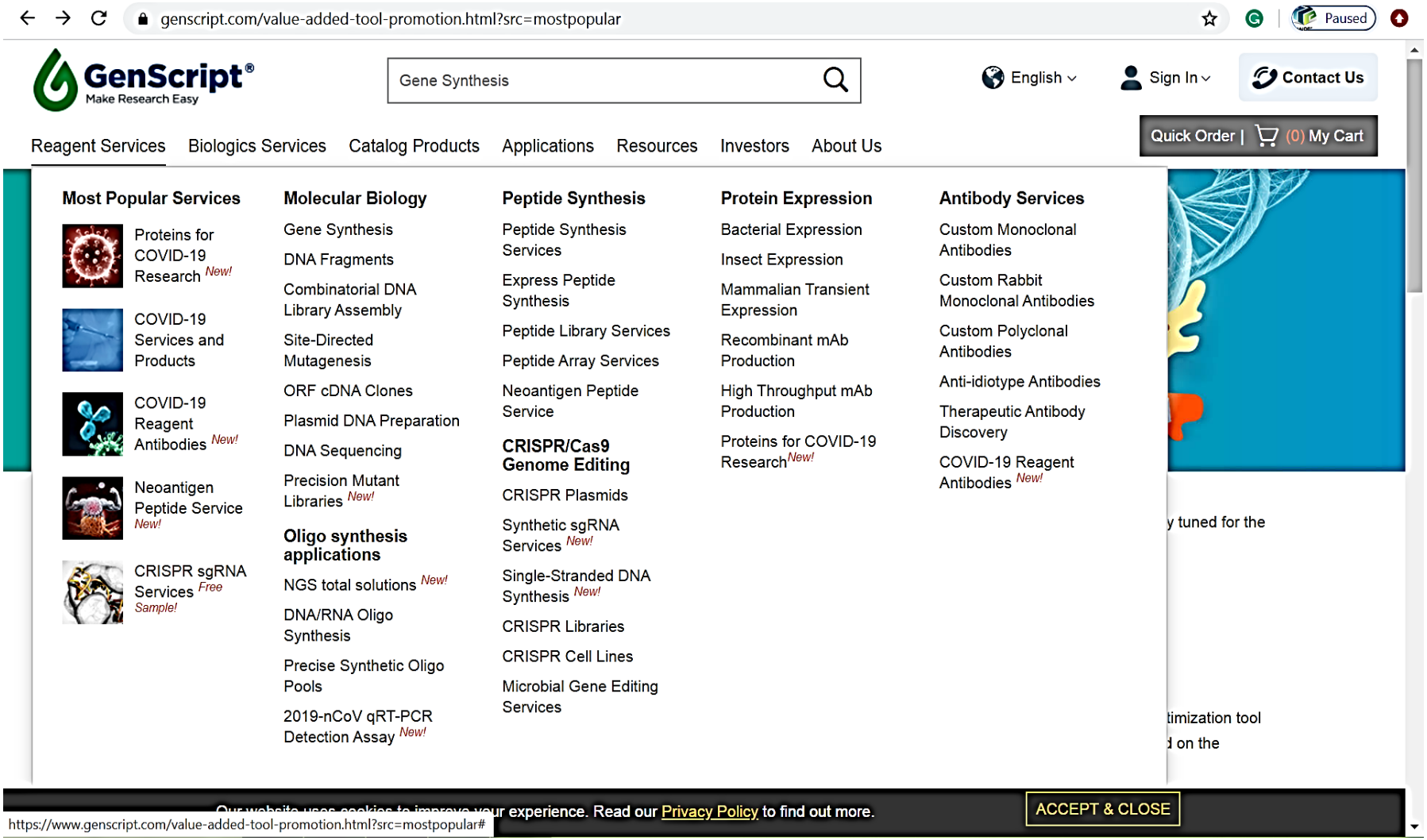
Complex Portal: webinar

Author(s): Birgit Meldal

Birgit Meldal gives an introduction to the Complex

	Start date
Imperial College London	*29th March 2021*
Queen Mary University of London / University College London	*2020*
St George's, University of London / King's College London	*07th December 2020*
University of Birmingham	*2020*
University of Cambridge	*05th April – 07th June 2021*
University of Exeter	*11th – 15th April 2021*
University of Manchester	*2020*

https://www.genscript.com/value-added-tool-promotion.html?src=mostpopular



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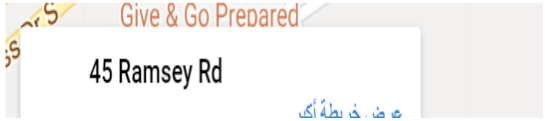

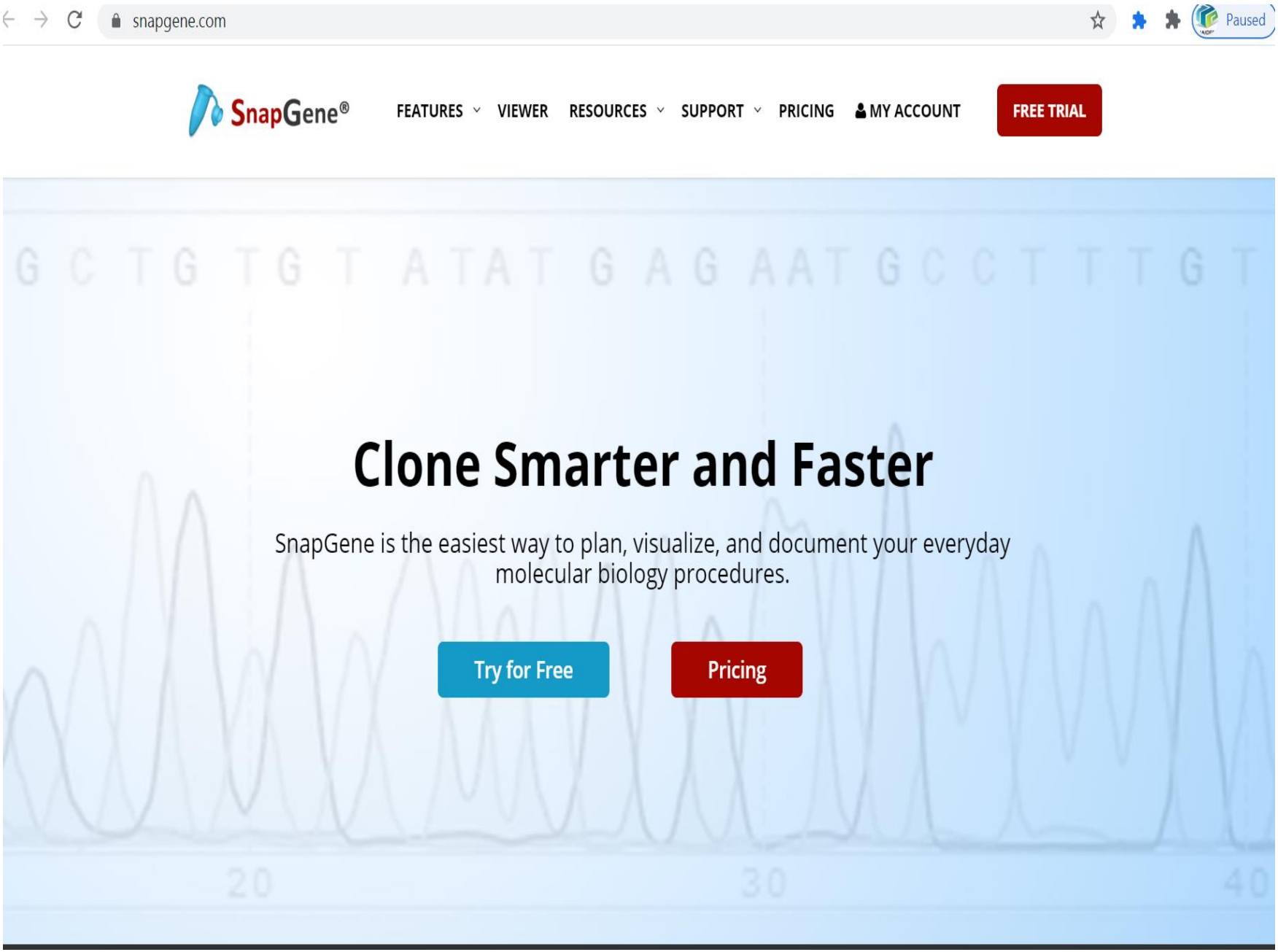
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