Biotecnologia

ACH5545 Engenharia Genética

Atividades de Laboratório

2º Semestre 2024

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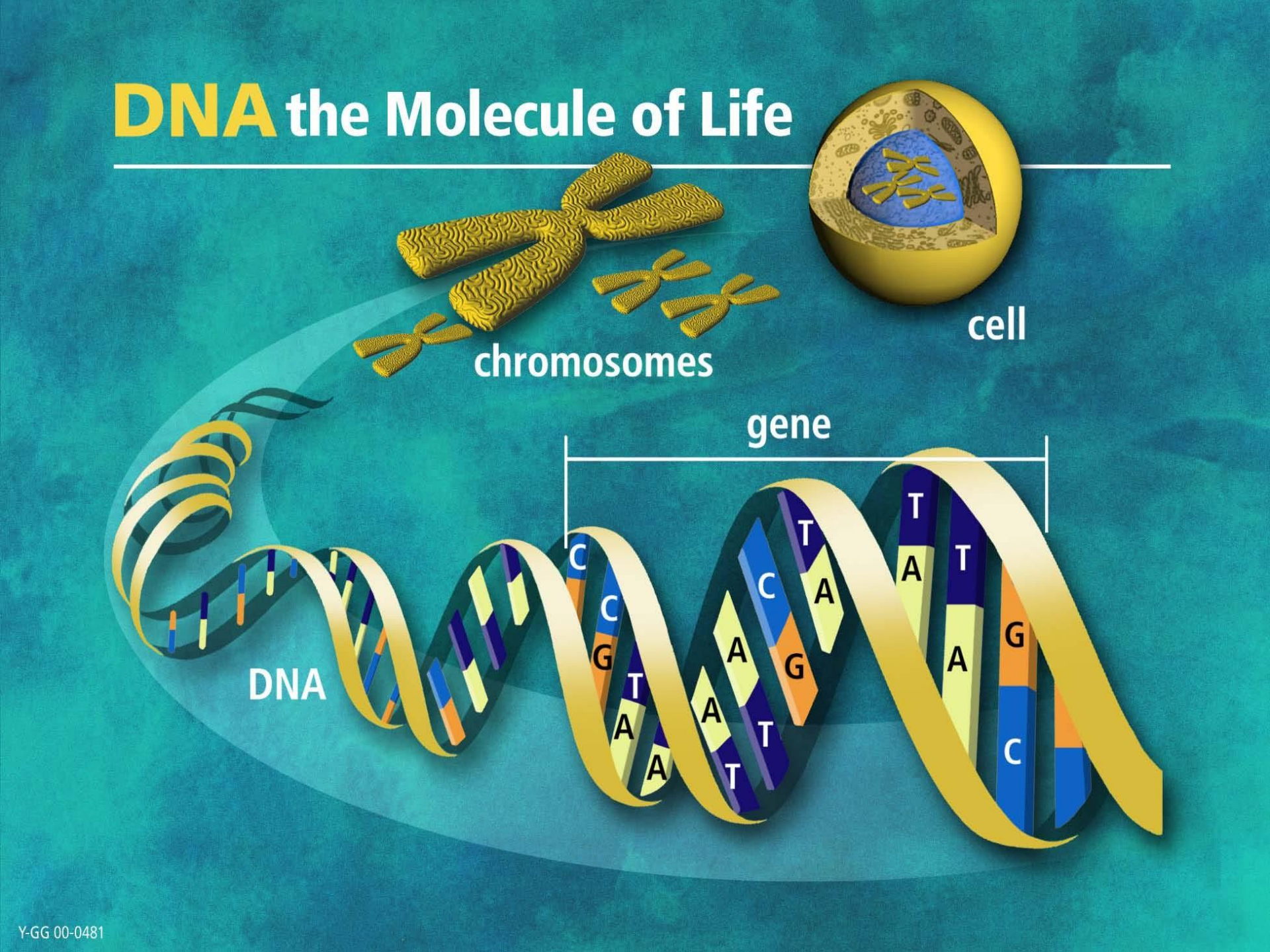
Tec. Pedro Manoel dos Santos - pedroms@usp.br

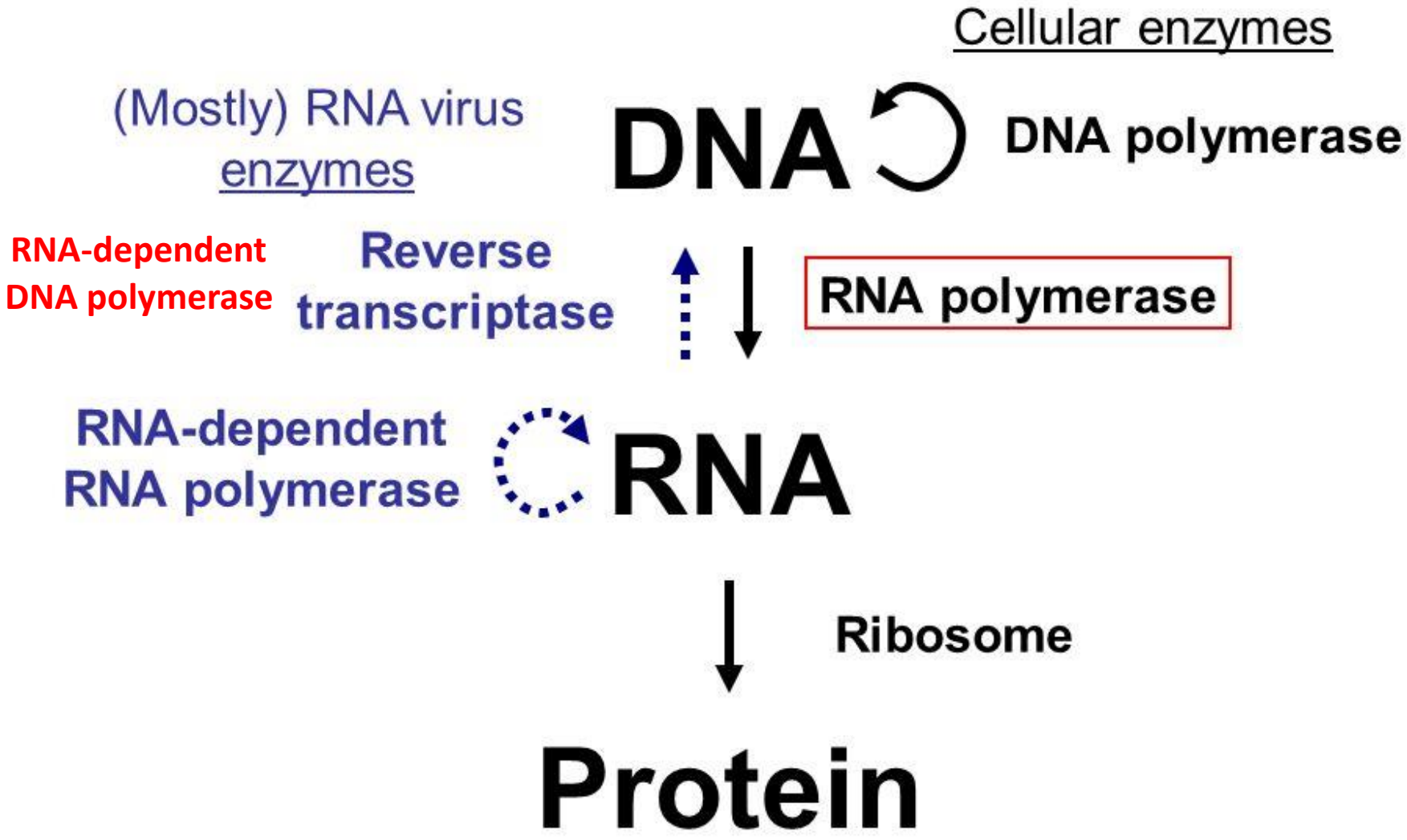
Créditos: 4
Período: Quinta-feira (14h00 -18h00), Laboratório de Biotecnologia – Edifício A2, 1º andar

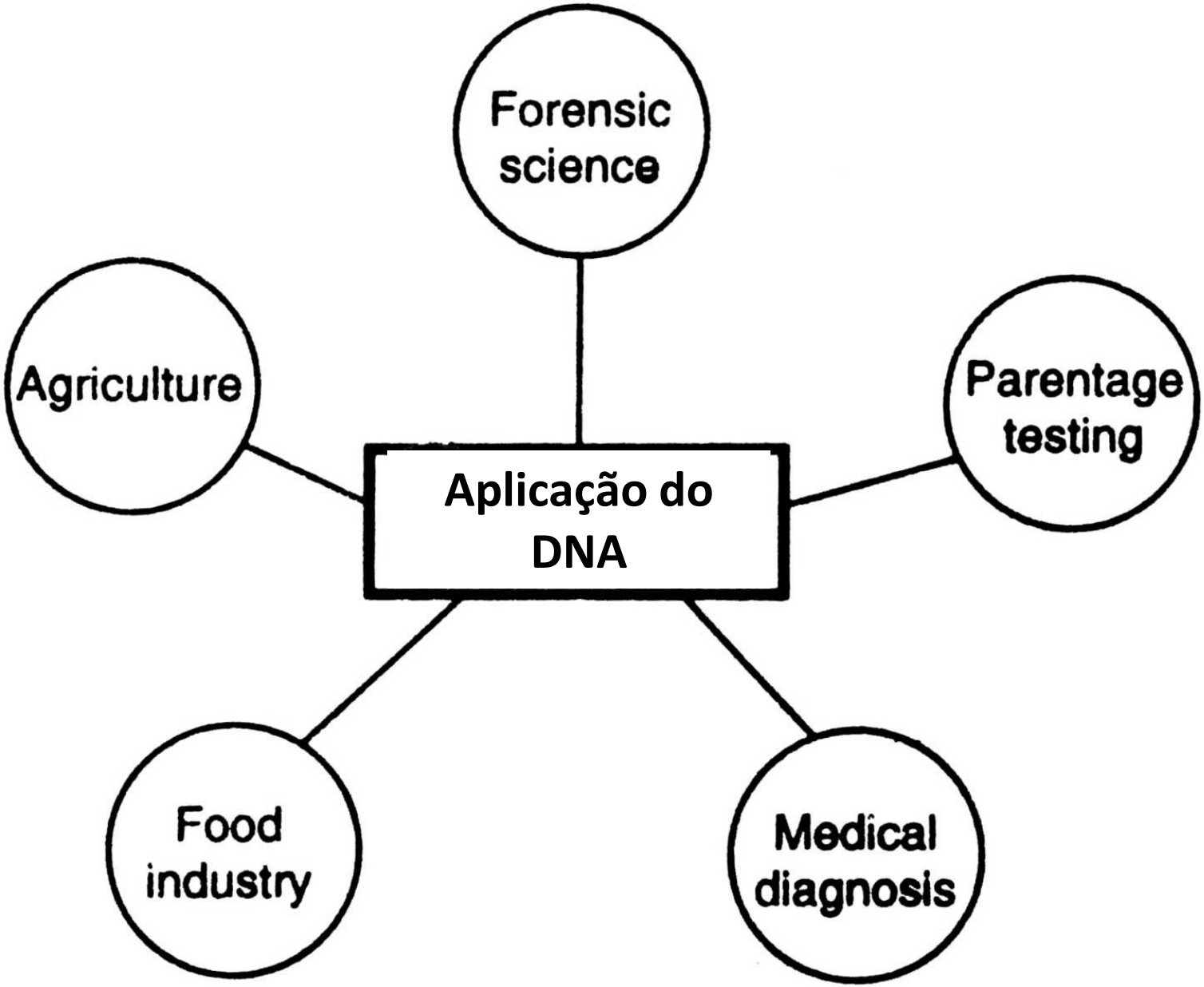
USP - 2024

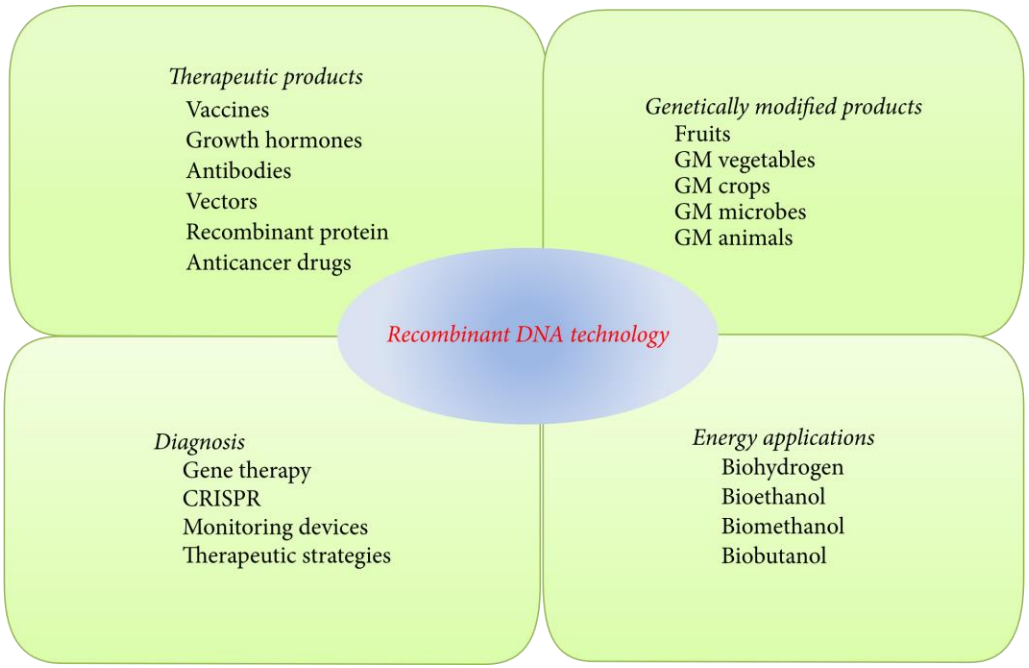
Extração de DNA genômico (células procarióticas) plasmidial e proteínas

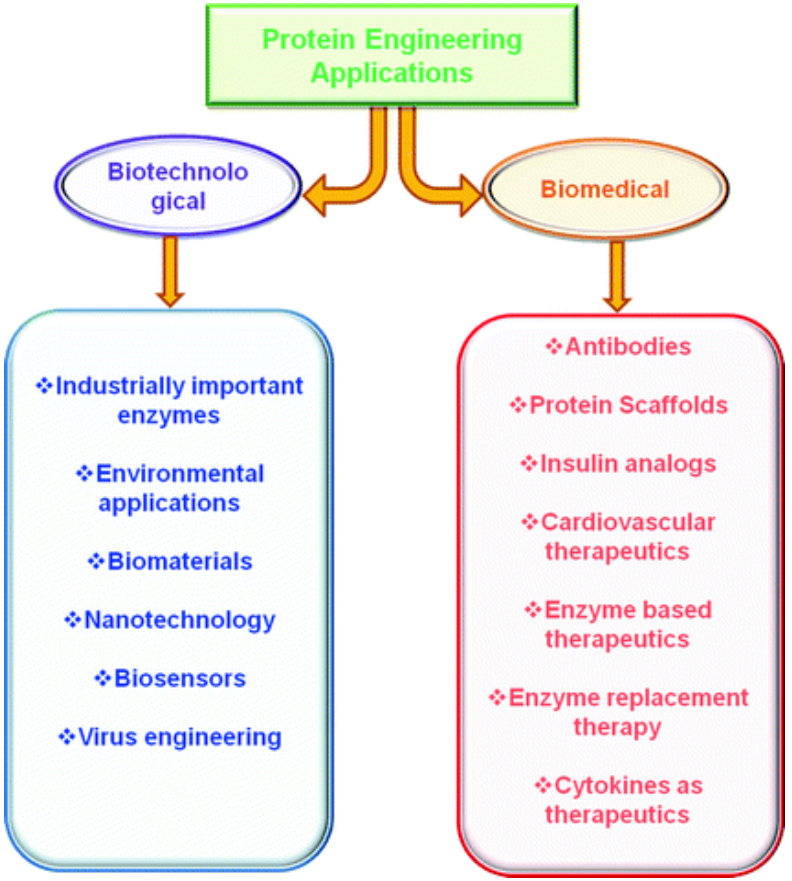

DNA the Molecule of Life
chromosomes
cell
gene
DNA
C
C
G
T
A
A
A
T
A
G
C
T
T
A
T
A
G
C
Y-GG 00-0481

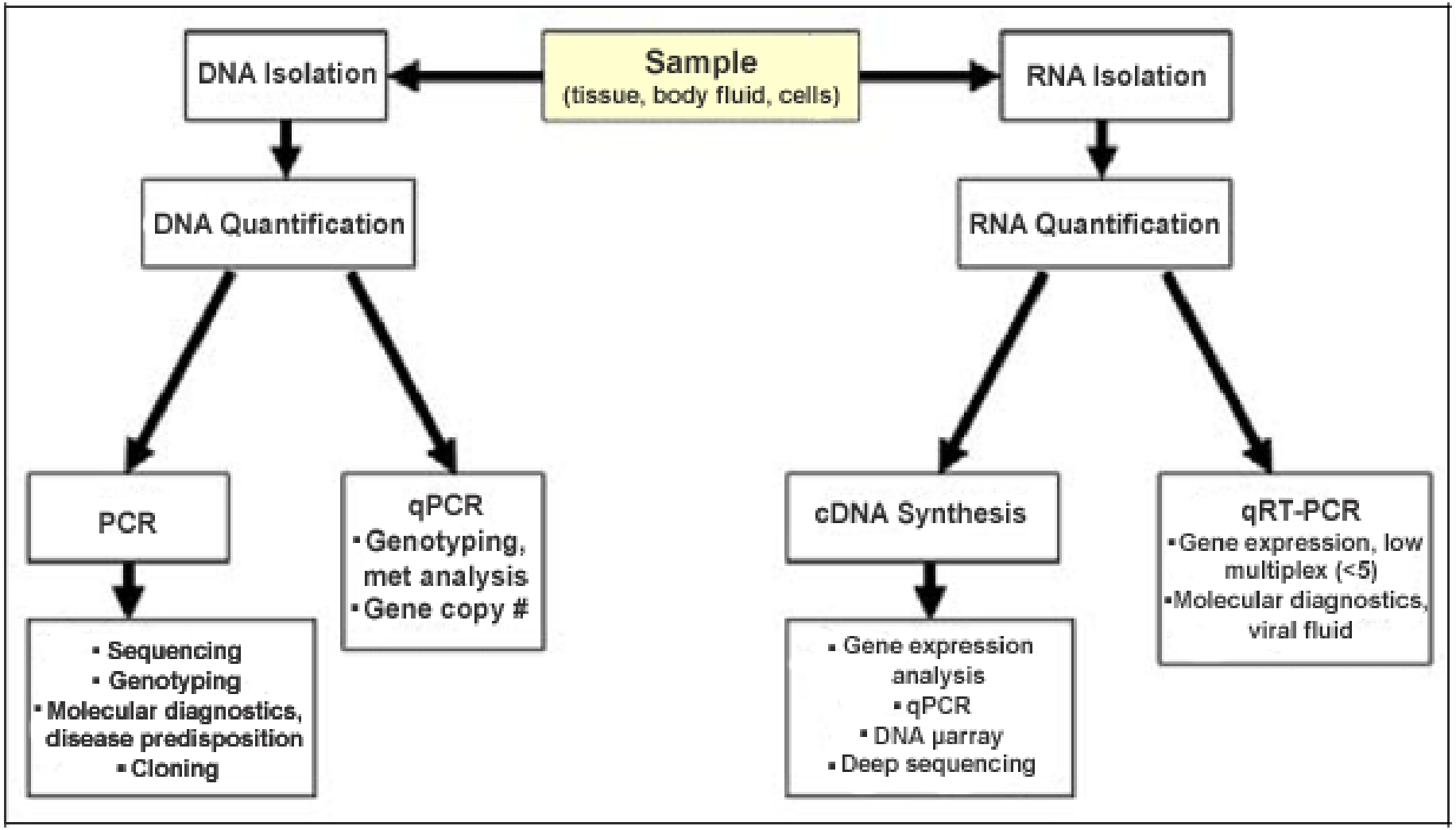
Enzymes in the central dogma



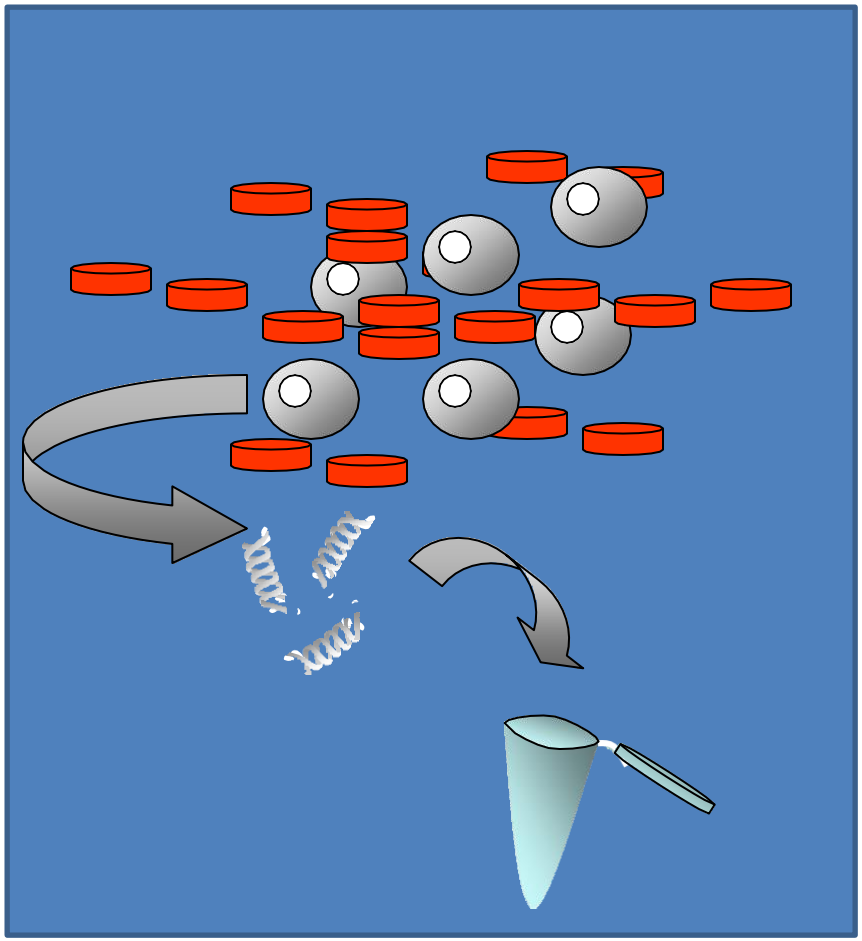

Forensic science
Agriculture
Parentage testing
Aplicação do DNA
Food industry
Medical diagnosis


Therapeutic products
Vaccines
Growth hormones
Antibodies
Vectors
Recombinant protein
Anticancer drugs
Genetically modified products
Fruits
GM vegetables
GM crops
GM microbes
GM animals
Recombinant DNA technology
Diagnosis
Gene therapy
CRISPR
Monitoring devices
Therapeutic strategies
Energy applications
Biohydrogen
Bioethanol
Biomethanol
Biobutanol


Protein Engineering Applications
Biotechnological
Biomedical
❖Industrially important enzymes
❖Environmental applications
❖Biomaterials
❖Nanotechnology
❖Biosensors
❖Virus engineering
❖Antibodies
❖Protein Scaffolds
❖Insulin analogs
❖Cardiovascular therapeutics
❖Enzyme based therapeutics
❖Enzyme replacement therapy
❖Cytokines as therapeutics


Sample
(tissue, body fluid, cells)
DNA Isolation
RNA Isolation
DNA Quantification
RNA Quantification
PCR
qPCR
▪ Genotyping, met analysis
▪ Gene copy #
cDNA Synthesis
qRT-PCR
▪ Gene expression, low multiplex (<5)
▪ Molecular diagnostics, viral fluid
▪ Sequencing
▪ Genotyping
▪ Molecular diagnostics, disease predisposition
▪ Cloning
▪ Gene expression analysis
▪ qPCR
▪ DNA µarray
▪ Deep sequencing

EXTRAÇÃO DE DNA/RNA



Possíveis fontes de DNA/RNA:

- sangue periférico
- células da mucosa oral
- células do bulbo capilar
- células descamadas da pele
- etc

Metodologia Geral:

- rompimento das células nucleadas
- precipitação das proteínas (solventes orgânicos, “salting-out”)
- precipitação do DNA/RNA (etanol e sal - NaCl, NaAc)
- em muitos casos o isolamento do DNA é desnecessário.

Extração de DNA genômico (gDNA) de bactérias

1 Cultura de Bactéria

2 Crescimento

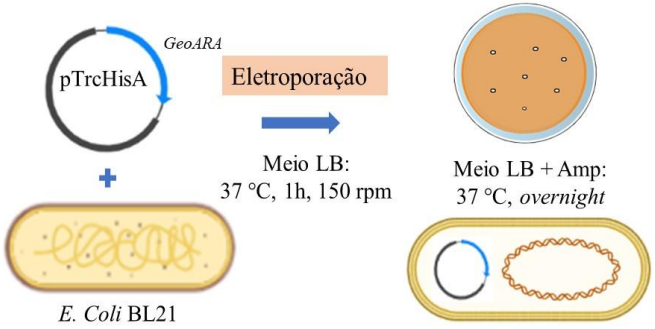
50 mL de meio LB 37 °C/150 rpm/18 h

3 Extração de gDNA

Lise celular

Extração de DNA plasmidial de bactérias

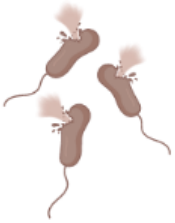
1 Transformação e Seleção



2 Crescimento

50 mL de meio LB + Amp 37 °C/150 rpm/18 h

3 Extração DNA plasmidial



Lise celular

Extração de Proteínas de Levedura

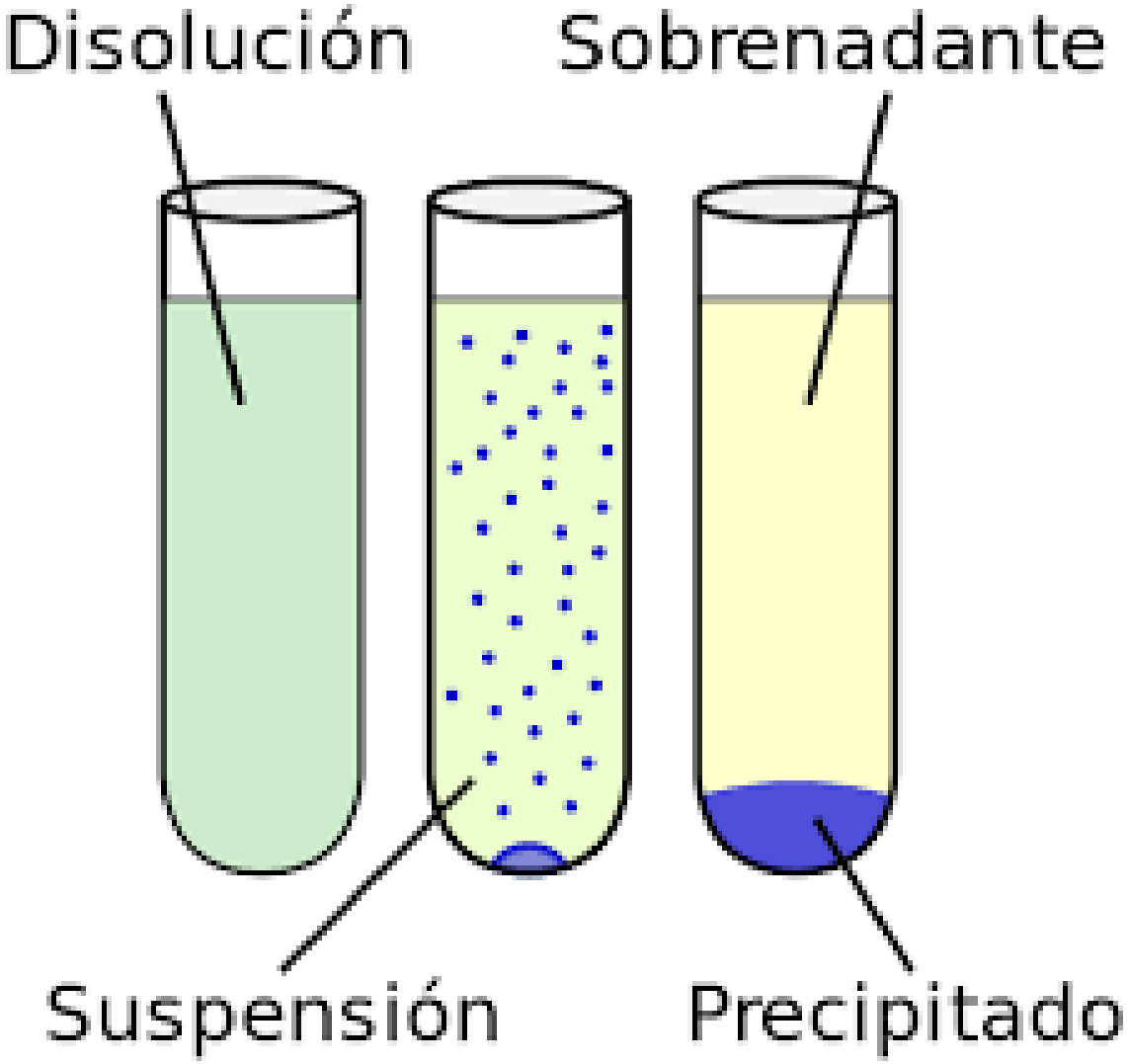
1 Cultura de Levedura

2 Crescimento

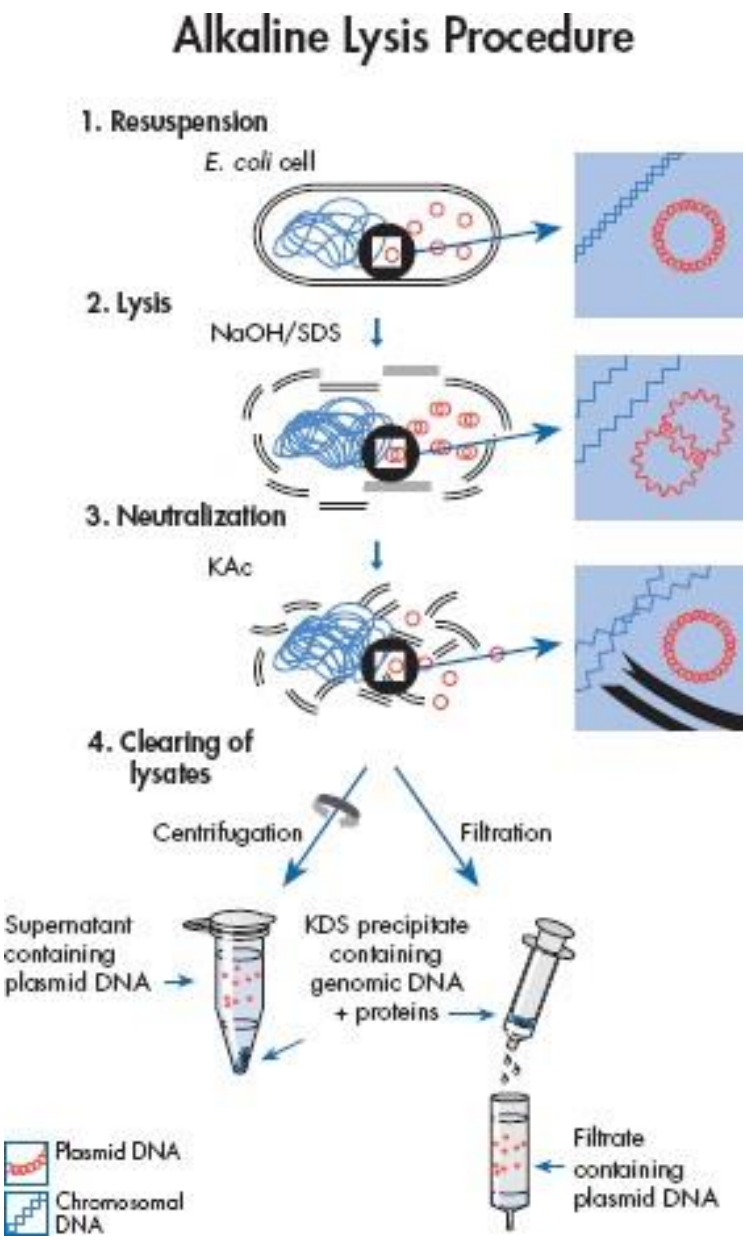
50 mL de meio YPD 30 °C/150 rpm/24 h

3 Extração de Proteína

Lise celular


Disolución
Sobrenadante
Suspensión
Precipitado

DNA cromossômico x DNA plasmidial



Ressuspende as células em Buffer + RNAse

Lise com NaOH/SDS

Neutralização com alta concentração de sais.
Sais-SDS, precipita proteínas, DNA cromossômico e restos celulares.
DNA plasmidial renatura e permanece em solução.

Restos celulares são removidos por centrifugação ou filtração

1-Precipita com álcool e recupera ácido nucleico 2-DNA liga a sílica na presença de sais.

Atividade 1: Extração de DNA genômico de bactéria

1- Extração de DNA genômico de Bactéria - (EasyPure Genomic DNA kit, Trans LifeSciences)

O procedimento é utilizado para extração de DNA genômico (gDNA) de diversas fontes biológicas.

Procedimento

Preparar banho maria ou banho seco a 55ºC

1. Realizar uma cultura de bactéria, por exemplo *Escherichia coli* (*E. coli*) em 10 mL de meio LB liquido (10 g Peptona/ 5 g extrato levedura /5 g NaCl, por litro), incubar 18 h sob agitação 37°C.
2. Centrifugar a cultura (3-5mL; 10.000 rpm/8 min, temperatura ambiente) e obtenha uma quantidade adequada de massa celular ~100 µl.
3. Suspenda a massa de bactéria em 100 µl de tampão LB2 + 20 uL de Proteinase K, misture por pipetagem até dissolver a massa de bactéria.
4. Incubar a 55ºC/15 min.
5. Adicionar 500 uL de tampão BB2, misturar por inversão 5 segs, incubar a temperatura ambiente (TA) por 10 min.
6. Transferir o lisado a coluna, com seu correspondente tubo reservatório e centrifugar à 10.000 rpm, TA por 1 minuto. Descartar o líquido do tubo reservatório.
7. Adicionar 500 uL de tampão CB2, centrifugar à 10.000 rpm, TA por 1 minuto. Descartar o líquido do tubo reservatório.
8. Repetir a etapa anterior
9. Adicionar 500 uL de tampão WB2, centrifugar à 10.000 rpm, TA por 1 minuto. Descartar o líquido do tubo reservatório.
10. Repetir a etapa anterior
11. Centrifugar a coluna, 10.000 rpm, TA por 1 minuto. Descartar o tubo reservatório e transferir a coluna a um novo tubo de microcentrífuga de 1,5 mL.
12. Adicionar no centro da coluna 50 uL de tampão de eluição ou água estéril (aquecidos a 65ºC). Incubar a TA por 2 minutos. Centrifugar à 10.000 rpm, temperatura ambiente, por 1 minuto, para eluição do DNA genômico, coletado no microtubo de centrifuga.
13. Conservar o gDNA isolado ~20ºC.
14. Realizar a Eletroforeses e quantificar.

Atividade 2: Extração de DNA plasmidial de bactérias em pequena escala: "Miniprep" (Plasmid DNA Purification using the QIAprep Spin Miniprep Kit, www.qiagen.com)

Procedimento:

1. Realizar uma cultura de *E.coli,* contendo o plasmídeo, em 5 mL de meio LB com antibiótico, incubar por 12-18 h sob agitação à 37°C.

2. Posteriormente, colocar 1,5 mL da cultura em microtubo de 1,5 mL.

3. Centrifugar à 10.000 rpm em temperatura ambiente por 5 minutos e remover o sobrenadante.

4. Acrescentar 1,5 mL da cultura nos respectivos tubos e repetir os passos 2 e 3.

5. Suspender o sedimento bacteriano em 250 µL da Solução P1.

6. Acrescentar 250 µL da Solução P2. Misturar por inversão, **NÃO VORTEXAR OS TUBOS**. Uma solução viscosa deverá ser formada imediatamente devido à lise das células.

7. Acrescentar 350 µL da Solução N3. Misturar por inversão, **NÃO VORTEXAR OS TUBOS**. Um precipitado branco deverá ser formado imediatamente. Este precipitado contém DNA genômico e restos celulares. O DNA plasmidial permanecerá em solução.

8. Centrifugar os tubos à 12.000 rpm, temperatura ambiente, por 10 minutos.

9. Remover 750 µL do sobrenadante cuidadosamente e transferi-lo para coluna QIAprep Spin, com tubo reservatório.

10. Centrifugar os tubos à 10.000 rpm, temperatura ambiente, por 1 minuto, descartar o líquido do tubo reservatório.

11. Lavar a coluna QIAprep Spin, adicionando 0.75 mL de solução PE

12. Em seguida, centrifugar à 10.000 rpm, temperatura ambiente, por 1 minuto.

13. Descartar o líquido do tubo reservatório, centrifugar à 10.000 rpm, temperatura ambiente, por 1 minuto.

14. Transferir a coluna QIAprep Spin a tubo limpo, eluir o DNA por adição de 50 uL de solução EB (10 mM Tris·Cl, pH 8.5) ou água.

15. Centrifugar os tubos à 10.000 rpm, temperatura ambiente, por 1 minuto, descartar a coluna QIAprep Spin, o DNA está em solução.

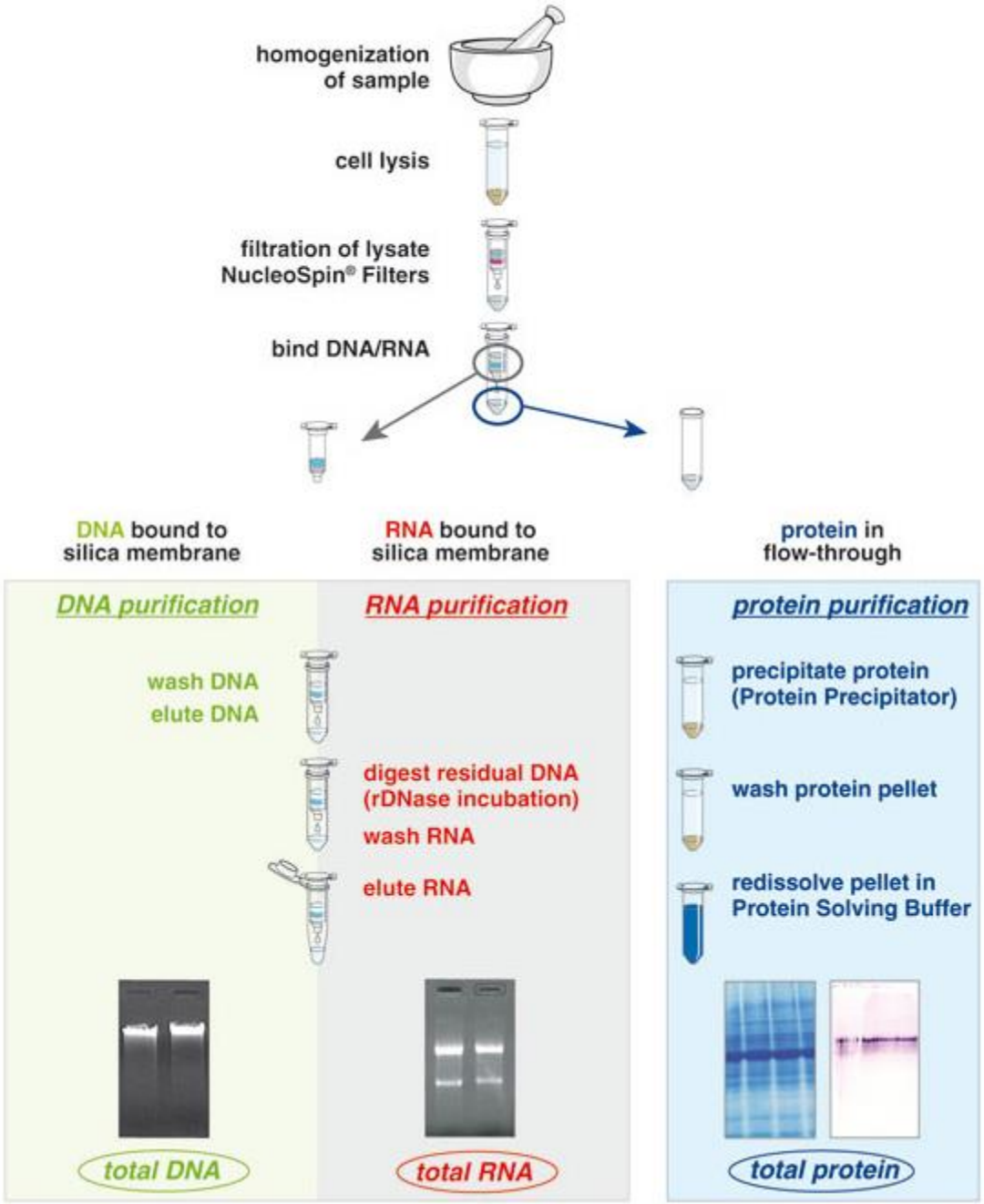
16. Realizar a Eletroforeses e quantificar.

Atividade 3: Extração de proteínas de Levedura

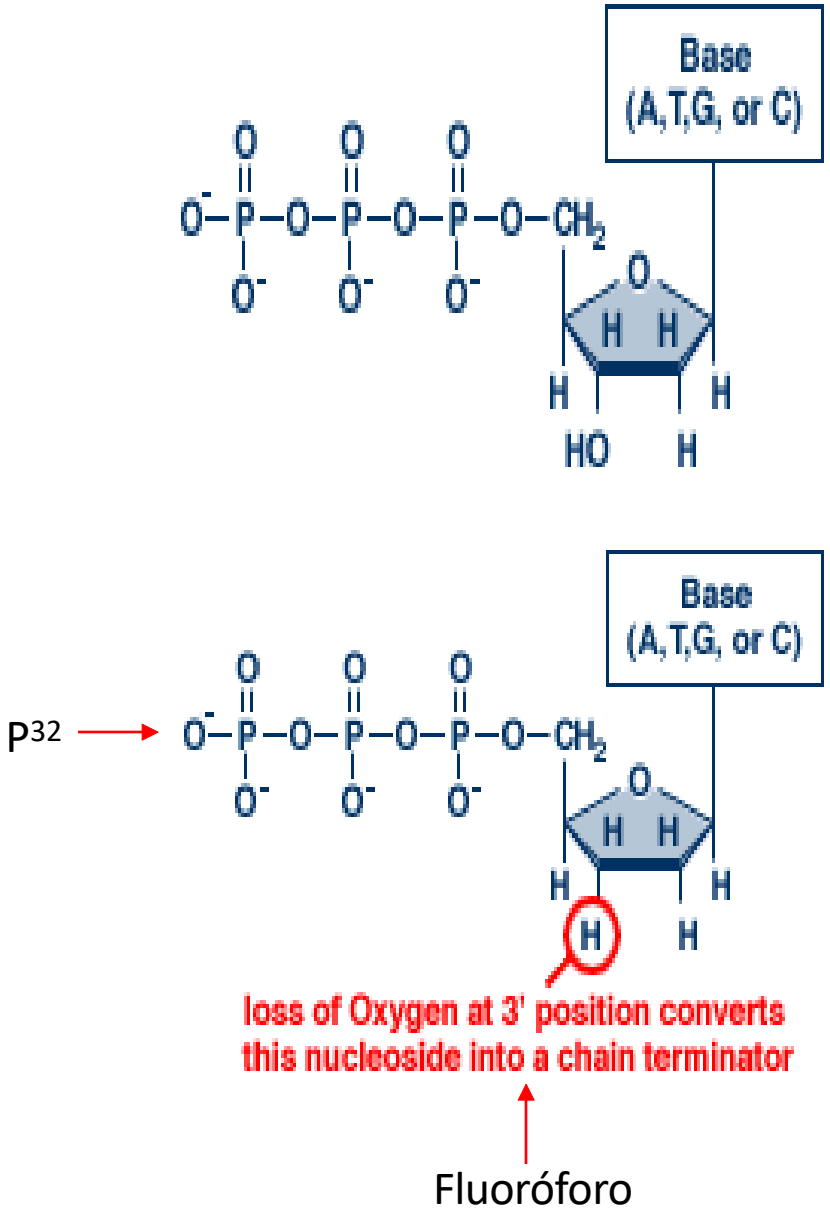
Procedimento:

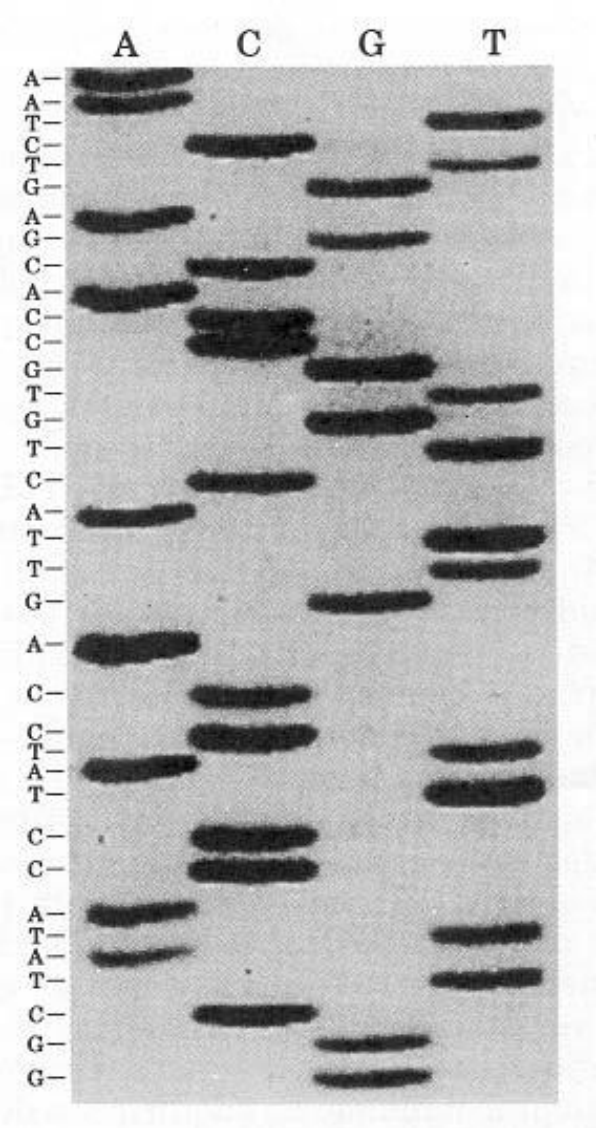
1. Realizar uma cultura de *Saccharomyces cerevisiae* em 5 mL de meio YPD por 12-18 h sob agitação à 37°C. Colocar 1,5 mL da cultura em microtubos de 1,5 mL;
2. Centrifugar a cultura (10000 rpm/8 min, temperatura ambiente) e obtenha uma quantidade adequada de massa celular ~200 µl;
3. Suspender o precipitado em 250 µl de tampão de extração;
4. Adicionar um volume equivalente a 100 µl de microesferas/perolas de vidro (tamanho de 450 a 600 µm);
5. Agitar em vortex por 20 min, em intervalos de 5 min, mantendo no gelo, velocidade máxima;
6. Centrifugar a 10.000 rpm por 10 min, TA;
7. Separar o sobrenadante do material sedimentado.
8. Realizar eletroforese em gel de SDS-PAGE, utilizando 20 µL do sobrenadante obtido.

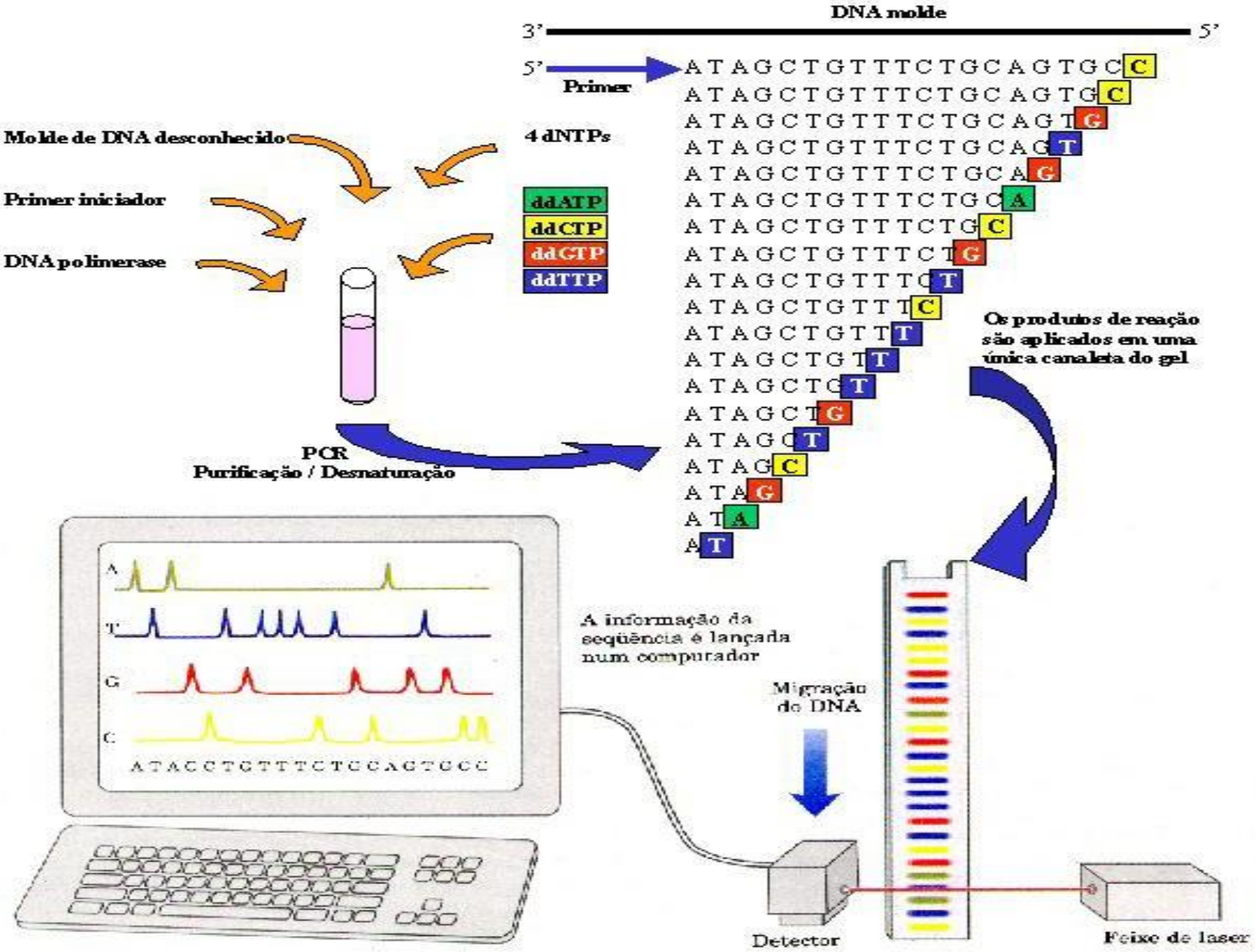
NucleoSpin® TriPrep procedure

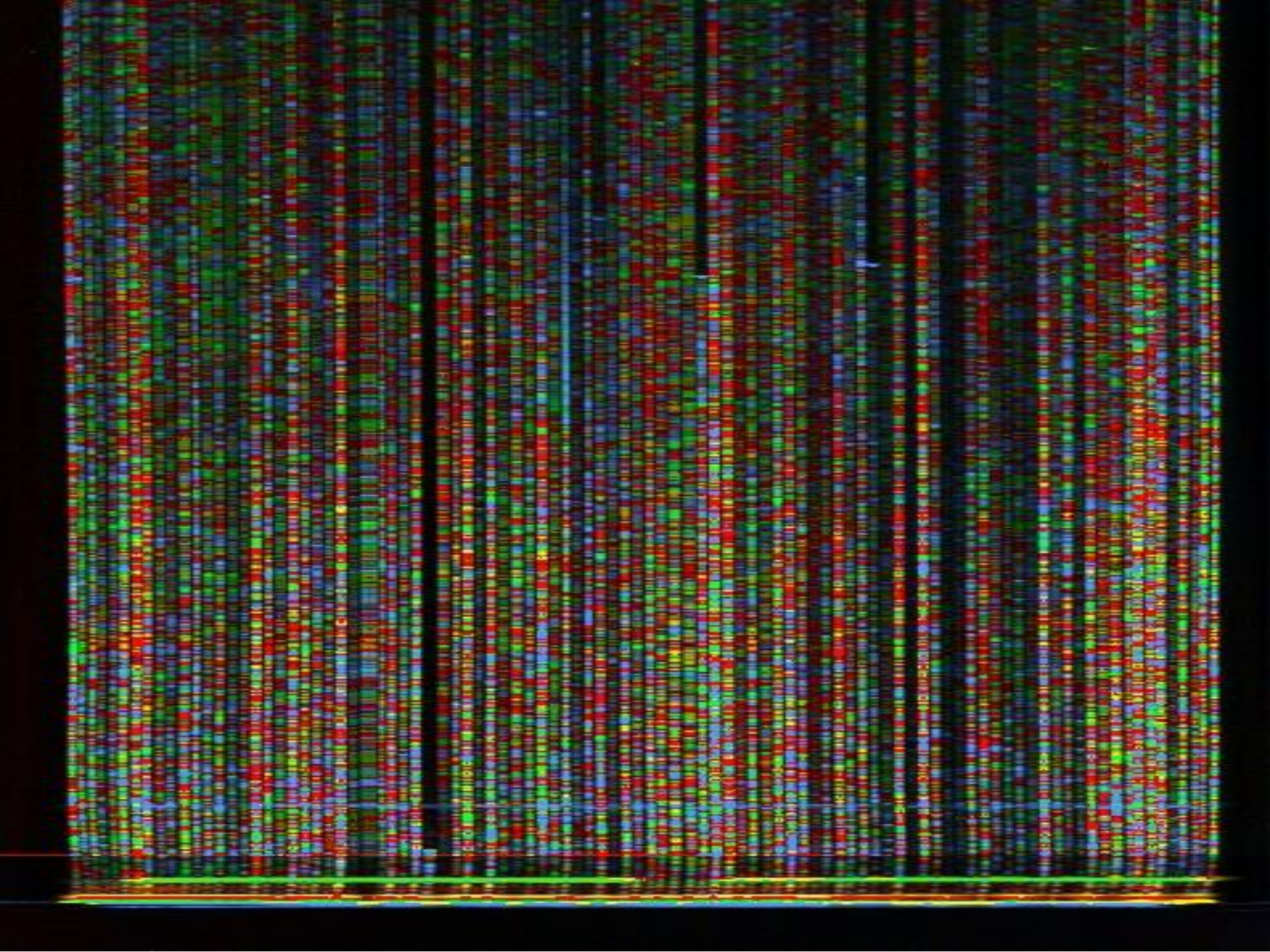


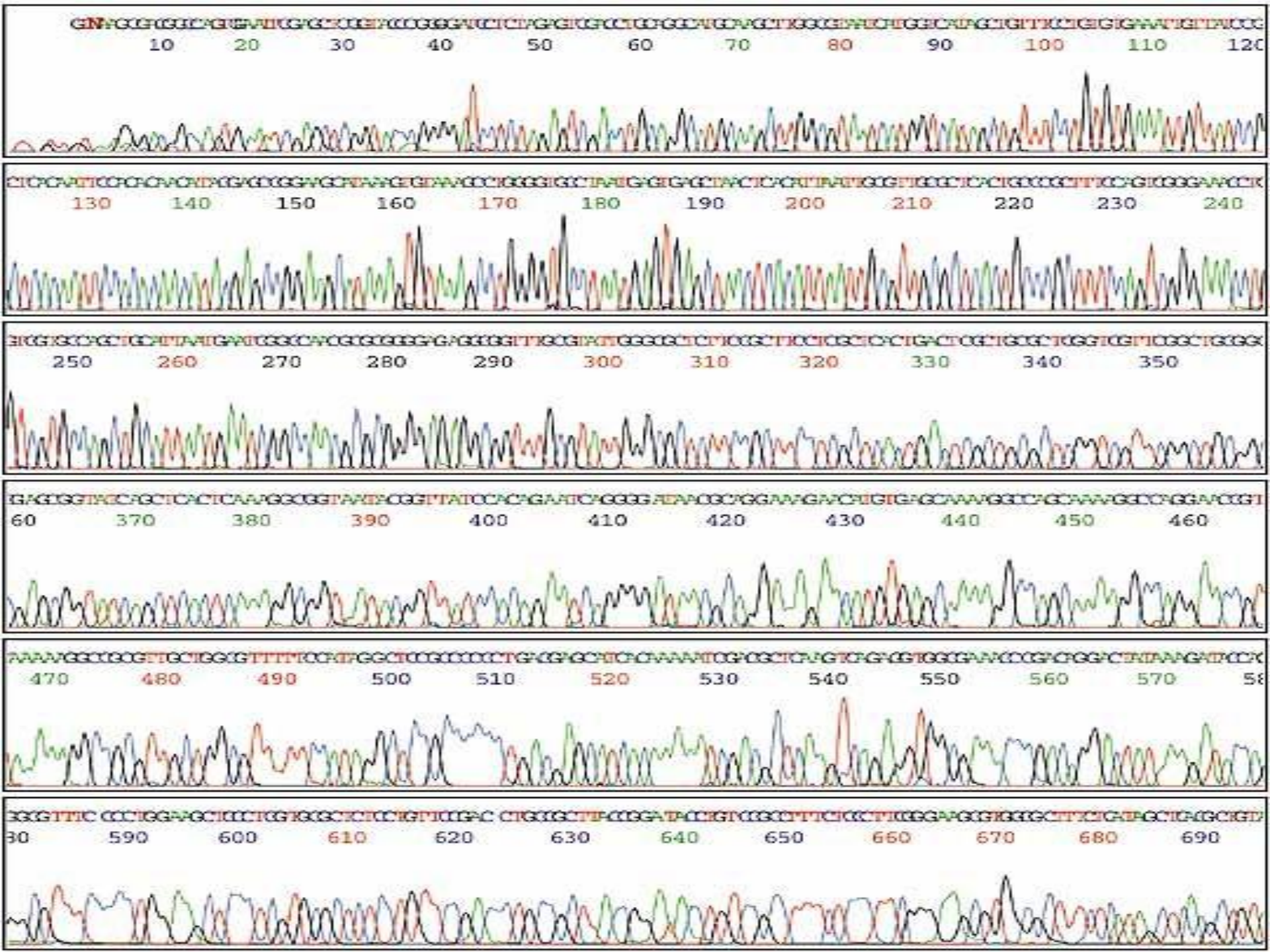
Aplicação: Seqüênciamento de DNA






DNA molde
3'
5'
5'
Primer
A T A G C T G T T T C T G C A G T G C C
A T A G C T G T T T C T G C A G T G C
A T A G C T G T T T C T G C A G T G
A T A G C T G T T T C T G C A G T
A T A G C T G T T T C T G C A G
A T A G C T G T T T C T G C A
A T A G C T G T T T C T G C
A T A G C T G T T T C T G
A T A G C T G T T T C T
A T A G C T G T T T C
A T A G C T G T T T
A T A G C T G T T
A T A G C T G T
A T A G C T G
A T A G C T
A T A G C
A T A G
A T A
A T
Molde de DNA desconhecido
Primer iniciador
DNA polimerase
4 dNTPs
ddATP
ddCTP
ddGTP
ddTTP
PCR
Purificação / Desnaturação
Os produtos de reação são aplicados em uma única canaleta do gel
A
T
G
C
A T A C C T C T T T C T C C A G T C C C
A informação da seqüência é lançada num computador
Migração do DNA
Detector
Feixe de laser




10 20 30 40 50 60 70 80 90 100 110
130 140 150 160 170 180 190 200 210 220 230 240
250 260 270 280 290 300 310 320 330 340 350
60 370 380 390 400 410 420 430 440 450 460
470 480 490 500 510 520 530 540 550 560 570
30 590 600 610 620 630 640 650 660 670 680 690

NCBI results of BLAST

BLASTX 2.1.3 [Apr-11-2001]

Reference:
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs", Nucleic Acids Res. 25:3389-3402.

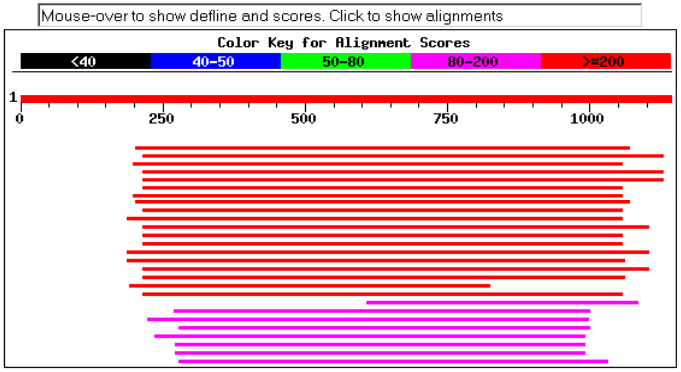
RID: 989884248-13757-21712

Query= TRESTO857.fasta.screen.Contig1
(1142 letters)
Database: nr
687,743 sequences; 216,528,054 total letters

If you have any problems or questions with the results of this search
please refer to the BLAST FAQs

Taxonomy reports

Distribution of 210 Blast Hits on the Query Sequence

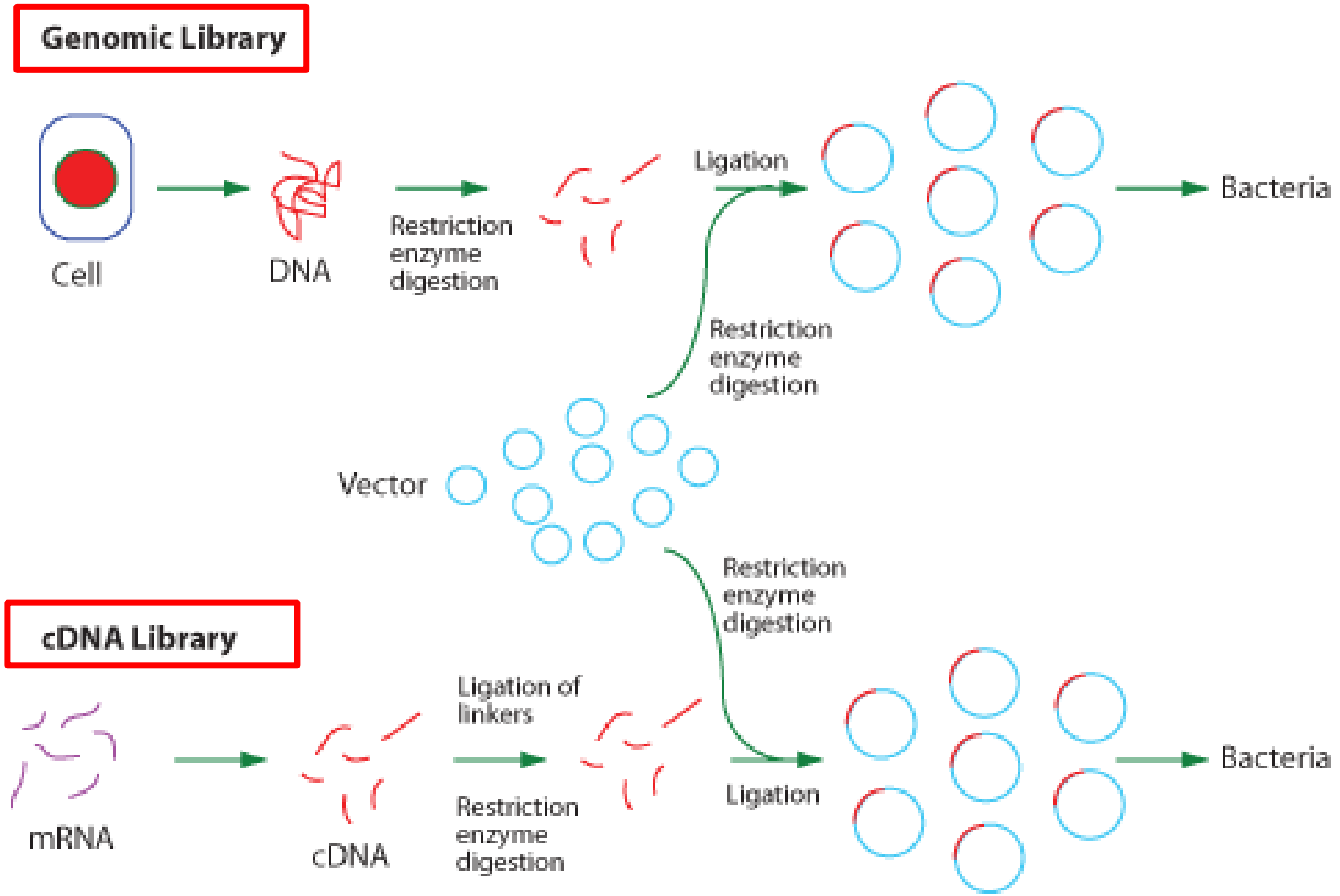


Sequences producing significant alignments:	Score (bits)	E Value
gi|5763968|emb|CAB53336.1| (AJ249117) methylcitrate synthas...	446	e-124
gi|10800928|emb|CAC12961.1| (AJ296102) mitochondrial citrat...	352	4e-96
gi|7267133|emb|CAB77625.1| (AJ243204) citrate synthase [Asp...	349	5e-95
gi|11268307|pir||T49379 citrate synthase, mitochondrial [im...	349	5e-95
gi|1705871|sp|P51044|CISY_ASPNG CITRATE SYNTHASE, MITOCHOND...	346	3e-94
gi|461744|sp|P34085|CISY_NEUCR CITRATE SYNTHASE, MITOCHONDR...	346	3e-94
gi|2493725|sp|O00098|CISY_EMENI CITRATE SYNTHASE, MITOCHOND...	346	4e-94

>TREST0554 (Contig)-Glycogen Synthase 5'3' Frame 1

```
gacgaggtcaaccagctgtgtcaattcatgtttgattactgtggcaagagccggcgtcaa
 D  E  V  N  Q  L  C  Q  F  M  F  D  Y  C  G  K  S  R  R  Q
cgtatcaaccagagaaaccgtactgagcgactcagcgacctgcttgactggaagcgcatg
 R  I  N  Q  R  N  R  T  E  R  L  S  D  L  L  D  W  K  R  M
ggtatggagtatatcaaggcccgccagctcgccctccgacgagcataccccaactcgttc
 G  M  E  Y  I  K  A  R  Q  L  A  L  R  R  A  Y  P  N  S  F
cacggagacgaggaagaagaggagctggaggactttatccgtgggccggagcagaagatc
 H  G  D  E  E  E  E  E  L  E  D  F  I  R  G  P  E  Q  K  I
tccaggccattctccgtccctggttcgcctcgcgatcggactggcatgatgacccctgga
 S  R  P  F  S  V  P  G  S  P  R  D  R  T  G  M  M  T  P  G
gactttgccagcttgcaggaagggcgagaaggcttgagcacggaggattacgttgcctgg
 D  F  A  S  L  Q  E  G  R  E  G  L  S  T  E  D  Y  V  A  W
aagctgcctgaggaggaggaccccgaggagtatcccttctcgttgacccttcggacaaag
 K  L  P  E  E  E  D  P  E  E  Y  P  F  S  L  T  L  R  T  K
cagcccggccctgtgagcccagagactcaggccactctcaacggtaactaaaagaactta
 Q  P  G  P  V  S  P  E  T  Q  A  T  L  N  G  N  -  K  N  L
gcaaatcaaggcgtttttttctcaaggtagcatccatttgcgtccacagccagcattgcg
 A  N  Q  G  V  F  F  S  R  -  H  P  F  A  S  T  A  S  I  A
tcggatcggggacgttgtacgtgtgatttctcgttcgtgtcgaatctatgatatatggtt
 S  D  R  G  R  C  T  C  D  F  S  F  V  S  N  L  -  Y  M  V
tttgtctcttccaggactgcgtccgaggtgccatgggagtagcaggttgggtaaaggtgg
 F  V  S  S  R  T  A  S  E  V  P  W  E  -  Q  V  G  -  R  W
gacaggagaaggaaaccaggcggaaaagaaaaggcgcgggttatt
 D  R  R  R  K  P  G  G  K  E  K  A  R  V  I
```

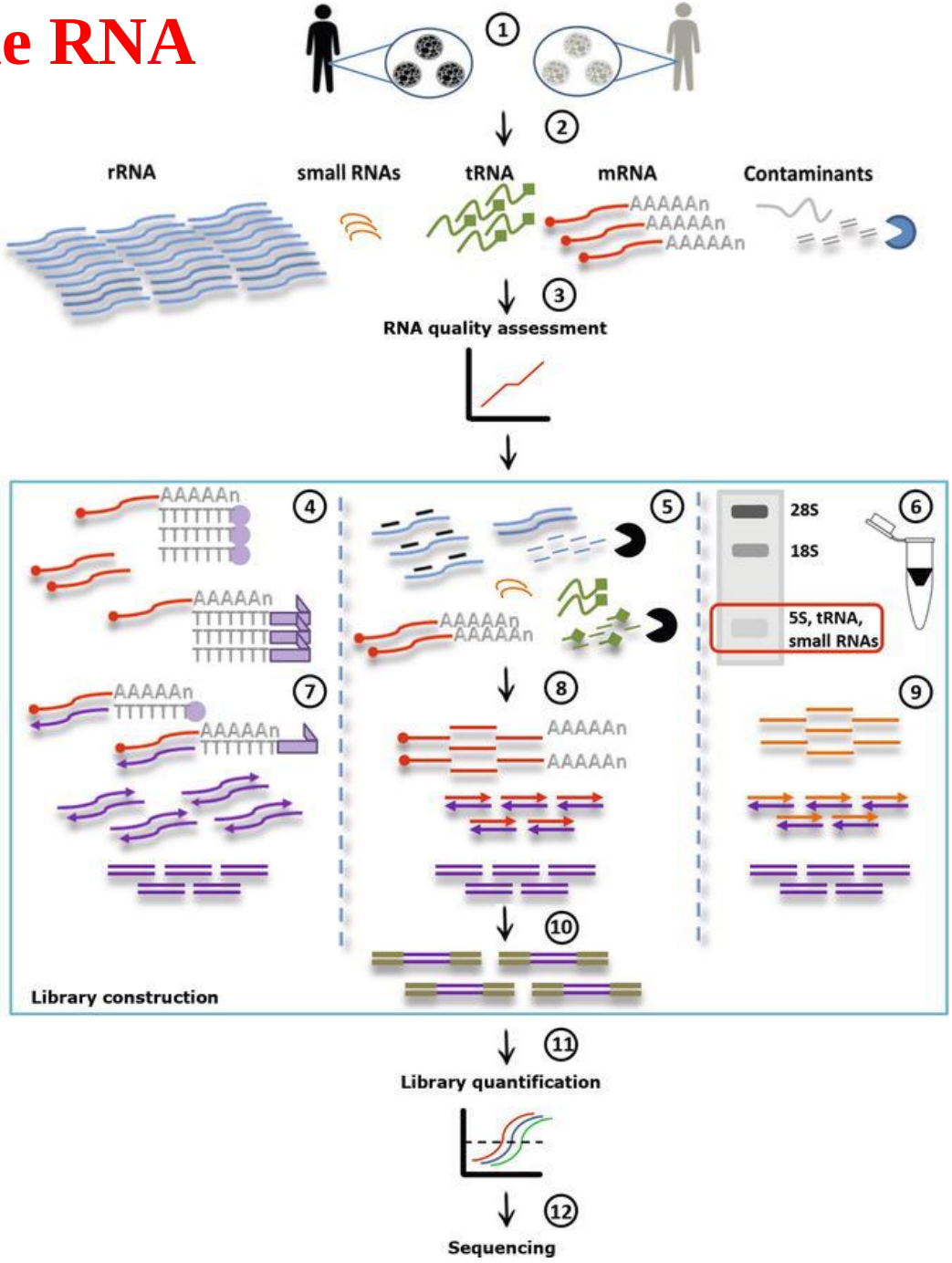
Aplicação: Bibliotecas de DNA



Aplicação: Bibliotecas de RNA

A typical RNA-seq workflow.

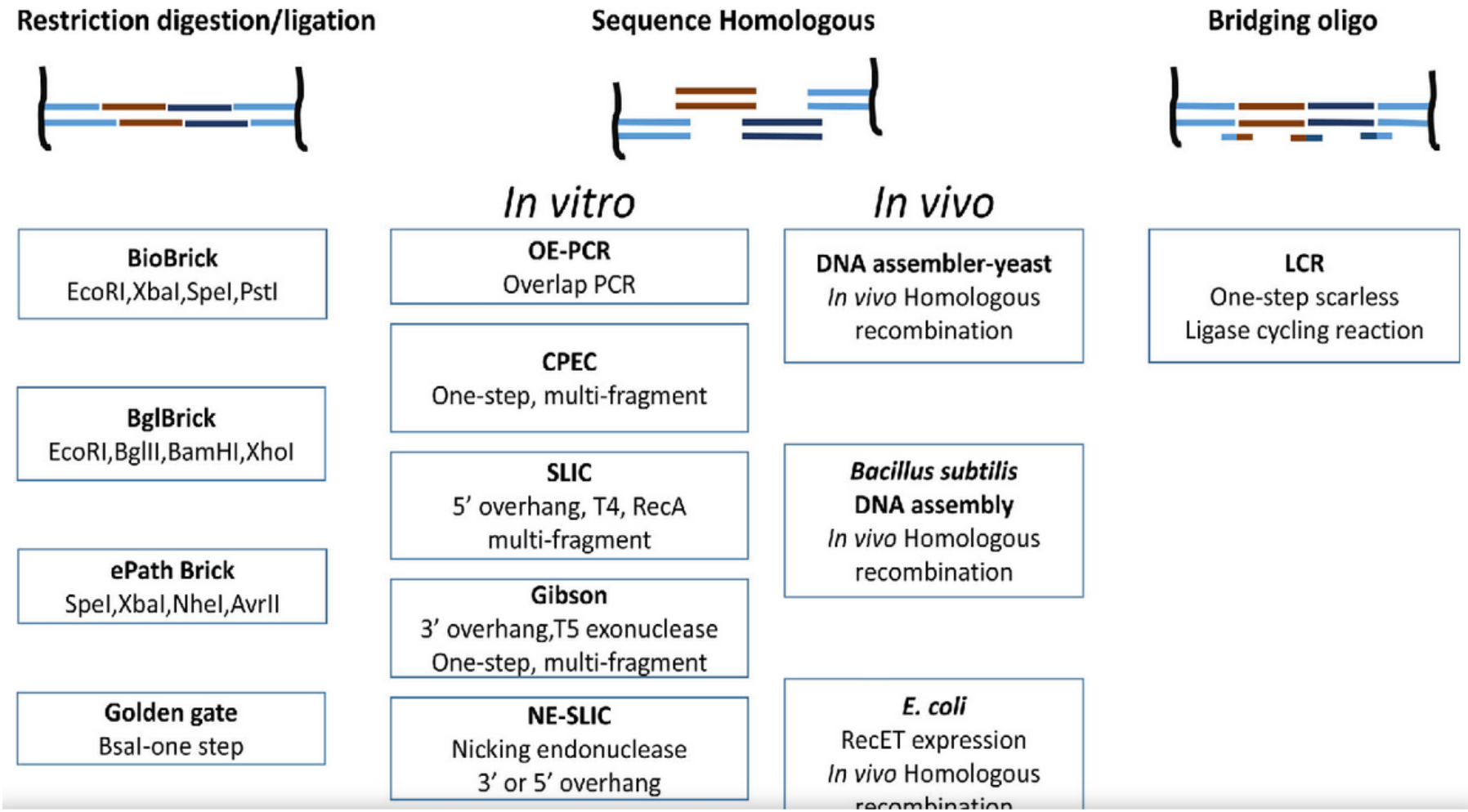
1) Experimental design definition of qualitative and quantitative goals. Differential gene expression among different conditions is exemplified;
2) Sample selection, RNA extraction and elimination of contaminants such as genomic DNA;
3) Assessment of RNA integrity; (4-6) RNA enrichment.
4) mRNA enrichment using magnetic or cellulose beads coated with oligo(dT) molecules or oligo(dT) priming;
5) mRNA enrichment through rRNA depletion with conserved probes or Selective Depletion of abundant RNA (SDRNA);
6) Small RNA size-selection through electrophoresis or based on solid phase extraction; (7-9) cDNA single/double strand synthesis.
7) cDNA synthesis followed by fragmentation;
8) mRNA fragmentation followed by cDNA synthesis;
9) cDNA synthesis for small RNA without fragmentation;
10) Adapters ligation;
11) Library quantification and
12) Library sequencing with NGS technology.



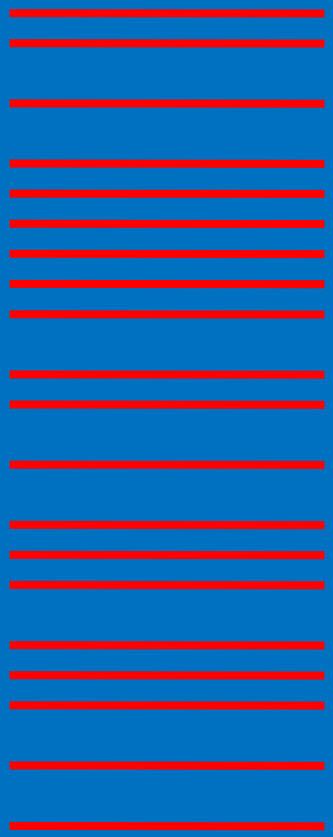
https://www.intechopen.com/chapters/55603

Aplicação: Recent advances in DNA assembly technologies.

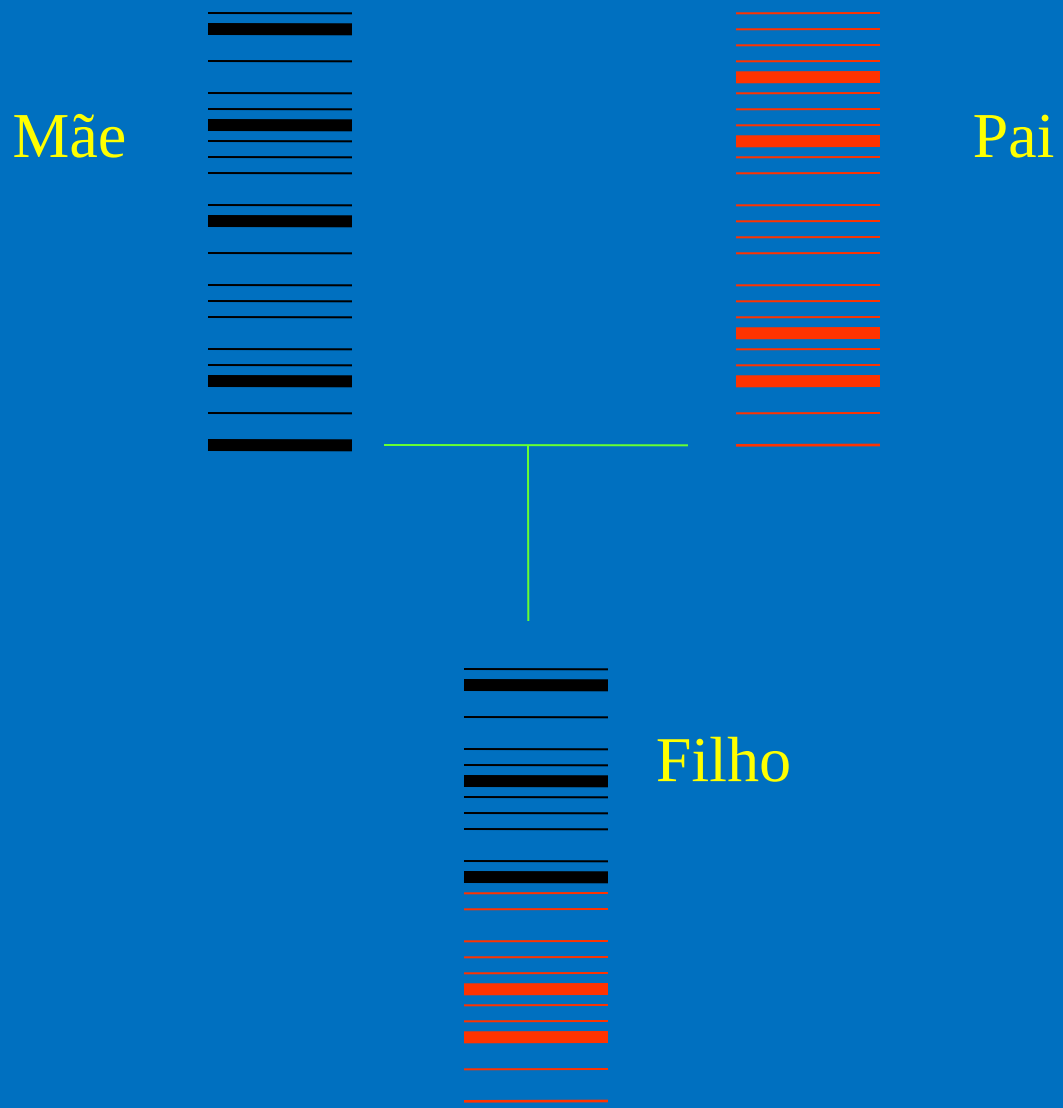
Ran Chao, Yongbo Yuan, H. Zhao. 2015. Biology, Medicine. FEMS yeast research
DOI:10.1111/1567-1364.12171



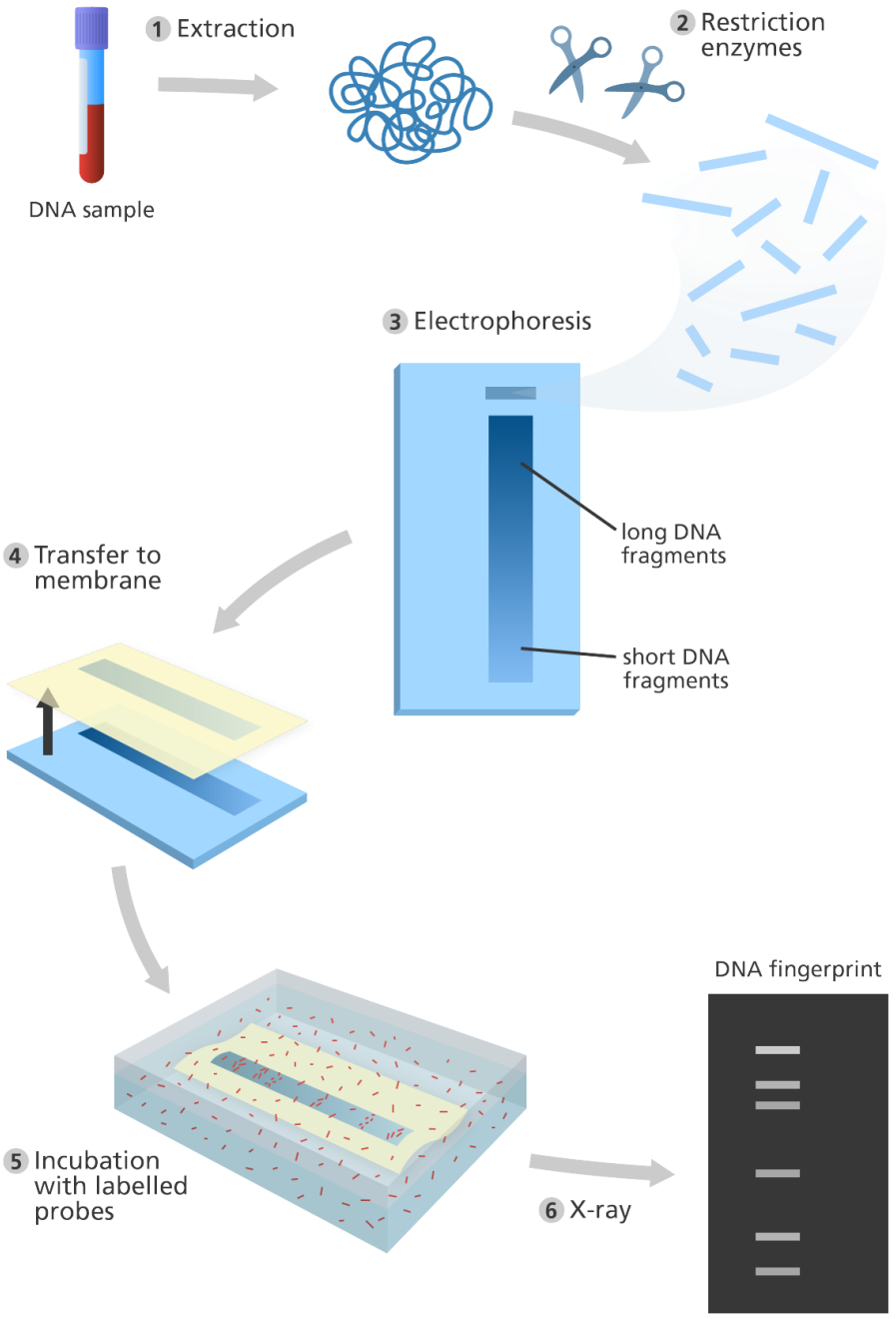
Código de Barras



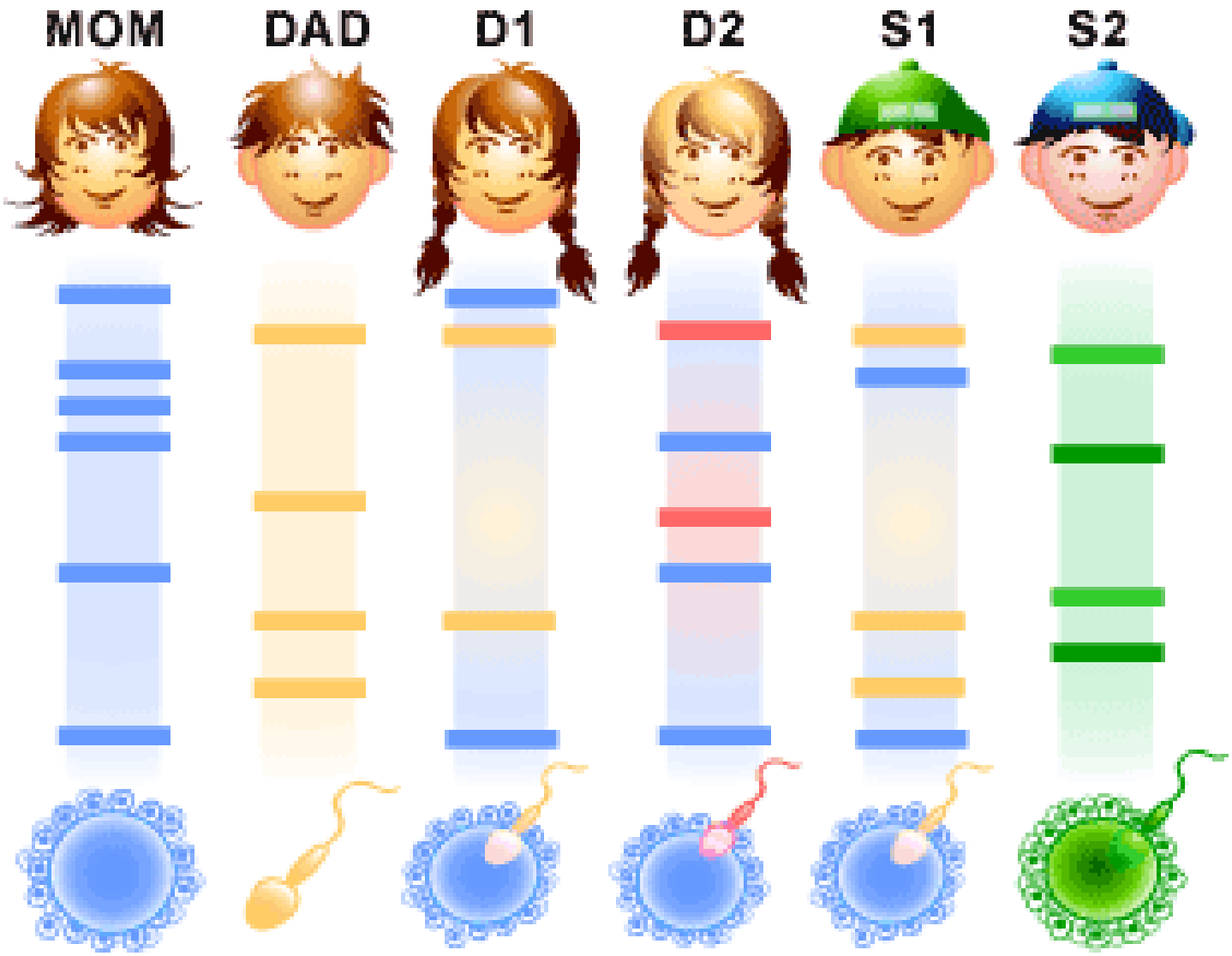
Investigação de Paternidade Através do DNA



Aplicação: Análise de DNA por Restriction fragment length polymorphism (RFLP)



Determine o grau de parentesco na família, após análise RFLP



Aplicação: Montagem de genomas

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Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome

DANIEL G. GIBSON, GWYNEDD A. BENDERS, CYNTHIA ANDREWS-PFANNKOCH, EVGENIYA A. DENISOVA, HOLLY BADEN-TILLSON, JAYSHREE ZAVERI, TIMOTHY B. STOCKWELL, ANUSHKA BROWNLEY, DAVID W. THOMAS, [...] HAMILTON O. SMITH +8 authors Authors Info & Affiliations

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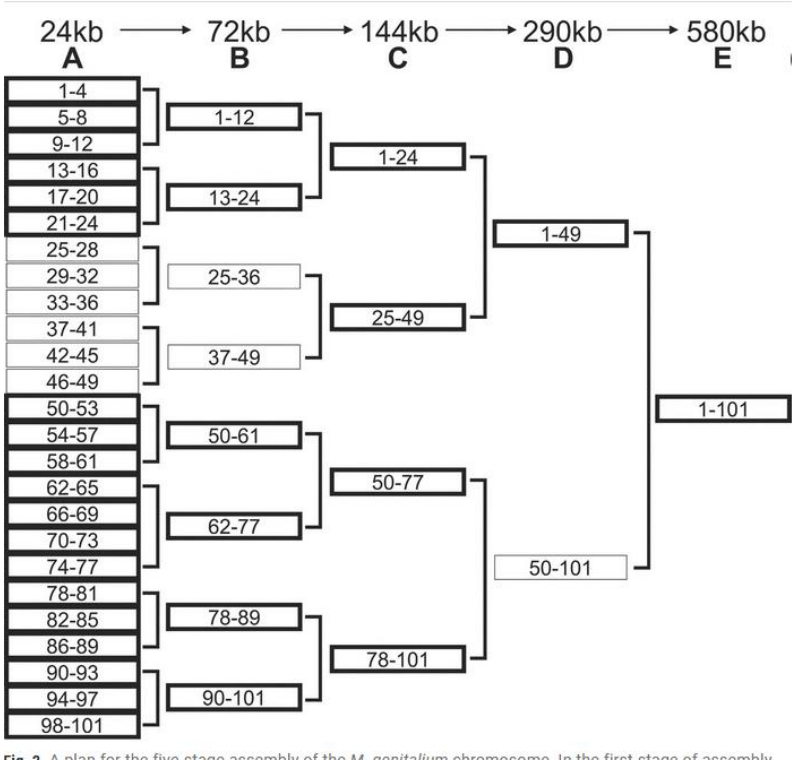
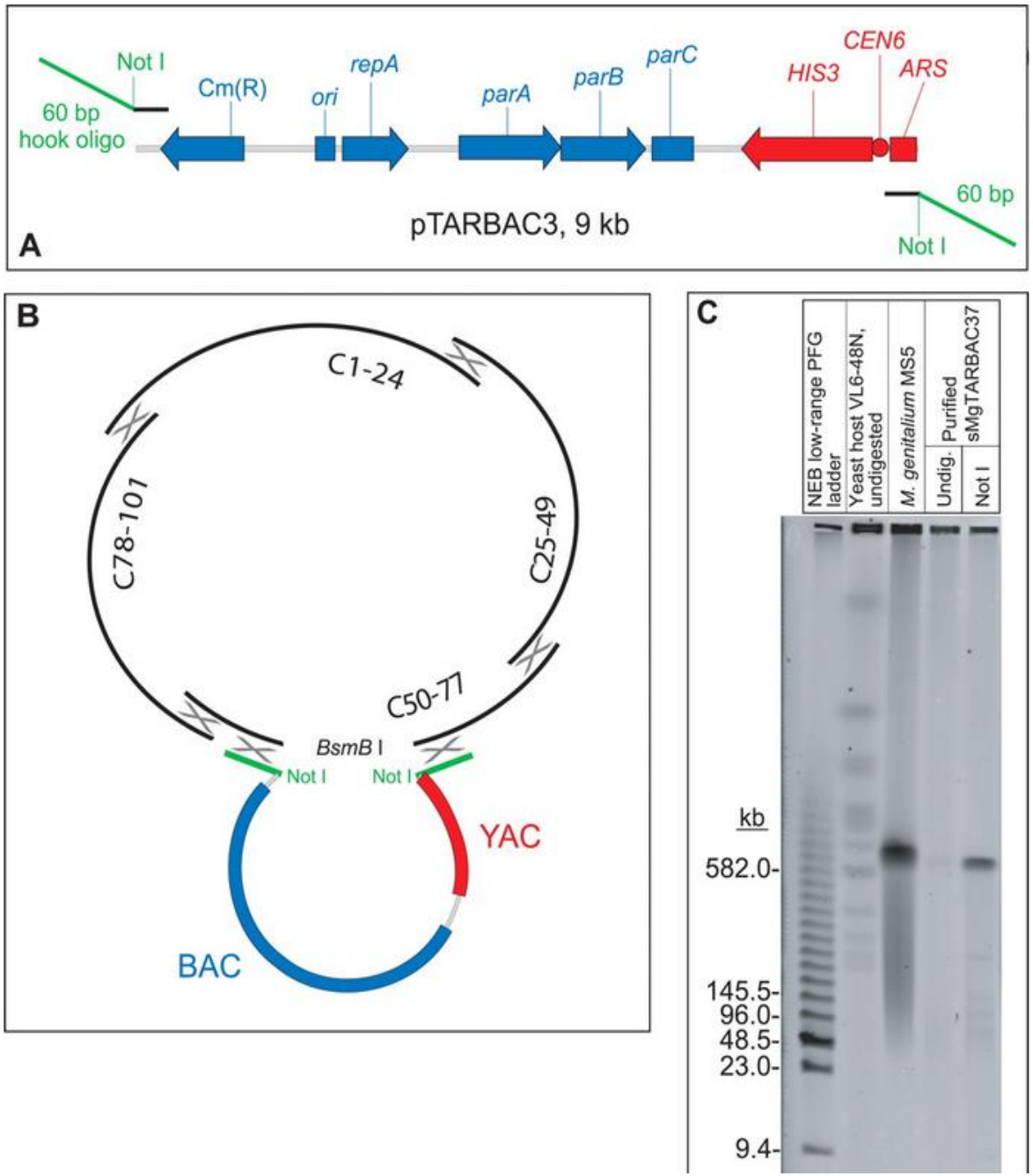
2 810

Abstract

We have synthesized a 582,970–base pair *Mycoplasma genitalium* genome. This synthetic genome, named *M. genitalium* JCVI-1.0, contains all the genes of wild-type *M. genitalium* G37 except MG408, which was disrupted by an antibiotic marker to block pathogenicity and to allow for selection. To identify the genome as synthetic, we inserted "watermarks" at intergenic sites known to tolerate transposon insertions. Overlapping "cassettes" of 5 to 7 kilobases (kb), assembled from chemically synthesized oligonucleotides, were joined by in vitro recombination to produce intermediate assemblies of approximately 24 kb, 72 kb ("1/8 genome"), and 144 kb ("1/4 genome"), which were all cloned as bacterial artificial chromosomes in *Escherichia coli*. Most of these intermediate clones were sequenced, and clones of all four 1/4 genomes with the correct sequence were identified. The complete synthetic genome was assembled by transformation-associated recombination cloning in the yeast *Saccharomyces cerevisiae*, then isolated and sequenced. A clone with the correct sequence was identified. The methods described here will be generally useful for constructing large DNA molecules from chemically synthesized pieces and also from combinations of natural and synthetic DNA segments.




A
Not I
60 bp
hook oligo
Cm(R)
ori
repA
parA
parB
parC
HIS3
CEN6
ARS
60 bp
Not I
pTARBAC3, 9 kb
B
C1-24
C25-49
C50-77
C78-101
BsmB I
Not I
Not I
YAC
BAC
C
NEB low-range PFG ladder
Yeast host VL6-48N, undigested
M. genitalium MS5
Purified sMgTARBAC37
Undig.
Not I
kb
582.0-
145.5-
96.0-
48.5-
23.0-
9.4-

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

DANIEL G. GIBSON, JOHN I. GLASS, CAROLE LARTIGUE, VLADIMIR N. NOSKOV, RAY-YUAN CHUANG, MIKKEL A. ALGIRE, GWYNEDD A. BENDERS, MICHAEL G. MONTAGUE, LI MA, MONZIA M. MOODIE, CHUCK MERRYMAN, SANJAY VASHEE, RADHA KRISHNAKUMAR, NACYRA ASSAD-GARCIA, CYNTHIA ANDREWS-PFANNKOCH, EVGENIYA A. DENISOVA, LEI YOUNG, ZHI-QING QI, THOMAS H. SEGALL-SHAPIRO, CHRISTOPHER H. CALVEY, PRASHANTH P. PARMAR, CLYDE A. HUTCHISON, III, HAMILTON O. SMITH, AND J. CRAIG VENTER fewer Authors Info & Affiliations

SCIENCE · 2 Jul 2010 · Vol 329, Issue 5987 · pp. 52-56 · DOI: 10.1126/science.1190719

Let There Be Life

The DNA sequence information from thousands of genomes is stored digitally as ones and zeros in computer memory. Now, **Gibson *et al.*** (p. 52, published online 20 May; see the cover; see the Policy Forum by **Cho and Relman**) have brought together technologies from the past 15 years to start from digital information on the genome of *Mycoplasma mycoides* to chemically synthesize the genomic DNA as segments that could then be assembled in yeast and transplanted into the cytoplasm of another organism. A number of methods were also incorporated to facilitate testing and error correction of the synthetic genome segments. The transplanted genome became established in the recipient cell, replacing the recipient genome, which was lost from the cell. The reconstituted cells were able to replicate and form colonies, providing a proof-of-principle for future developments in synthetic biology.

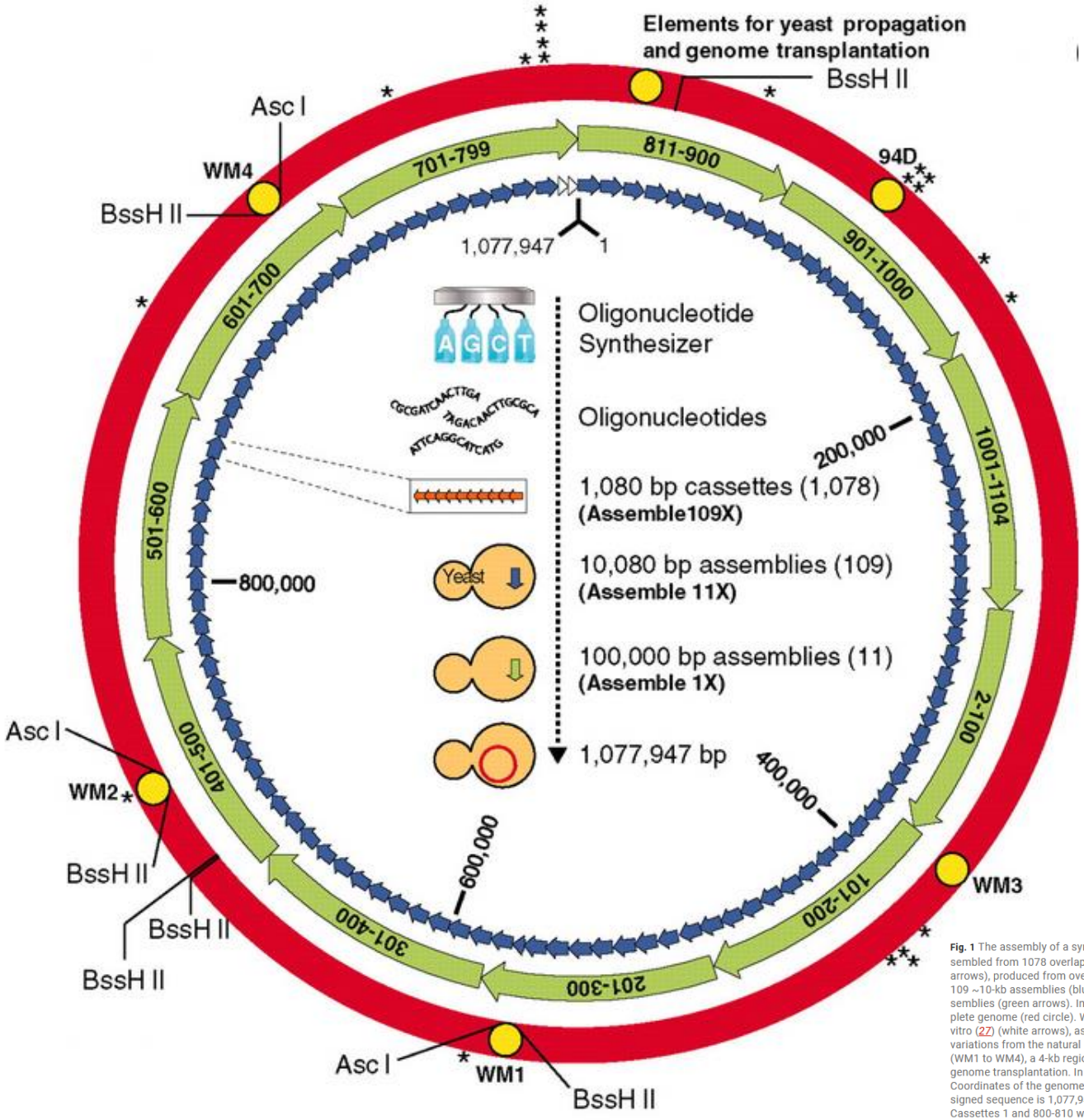
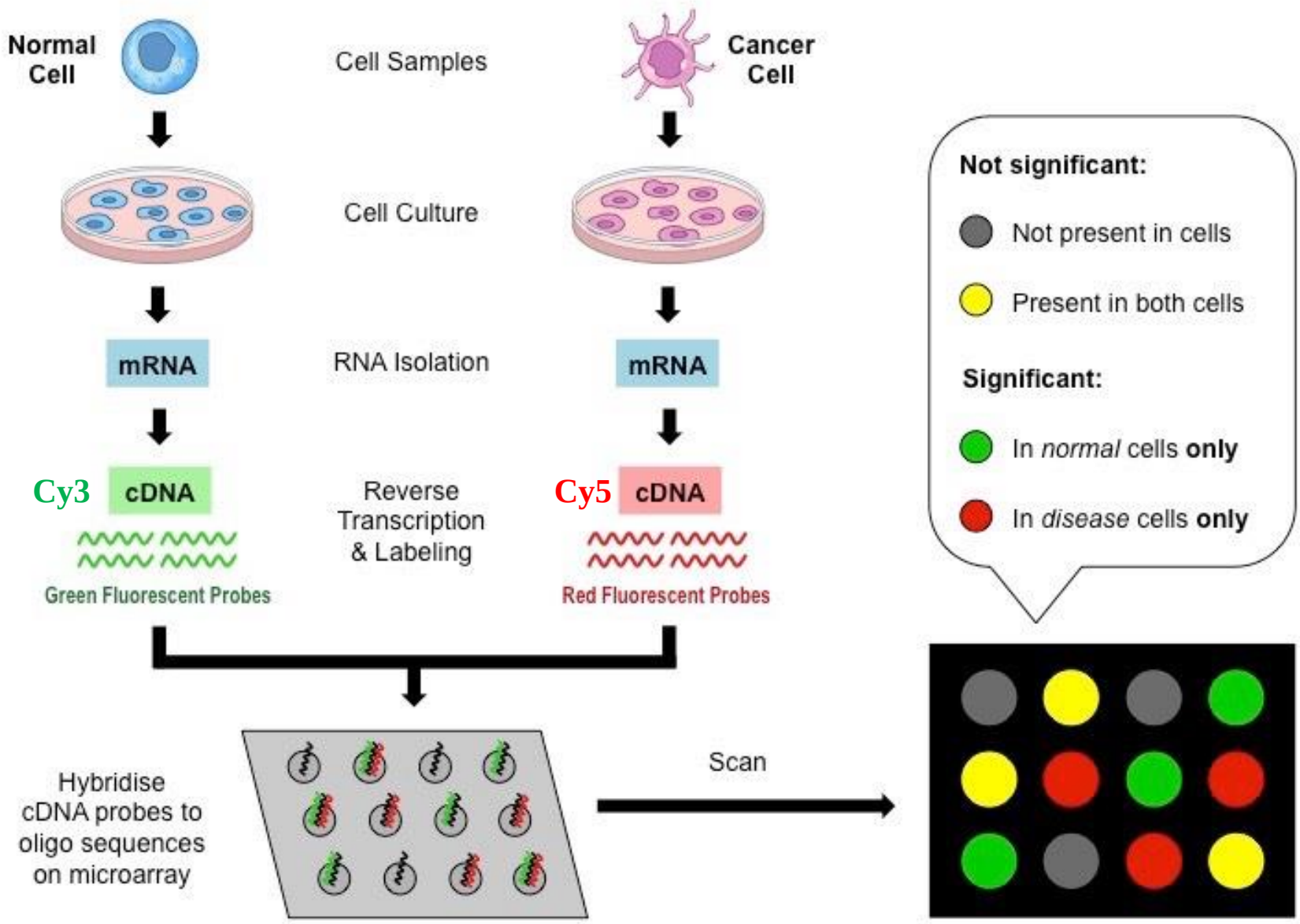


Fig. 1 The assembly of a synthetic *M. mycoides* genome in yeast. A synthetic *M. mycoides* genome was assembled from 1078 overlapping DNA cassettes in three steps. In the first step, 1080-bp cassettes (orange arrows), produced from overlapping synthetic oligonucleotides, were recombined in sets of 10 to produce 109 ~10-kb assemblies (blue arrows). These were then recombined in sets of 10 to produce 11 ~100-kb assemblies (green arrows). In the final stage of assembly, these 11 fragments were recombined into the complete genome (red circle). With the exception of two constructs that were enzymatically pieced together in vitro (27) (white arrows), assemblies were carried out by in vivo homologous recombination in yeast. Major variations from the natural genome are shown as yellow circles. These include four watermarked regions (WM1 to WM4), a 4-kb region that was intentionally deleted (94D), and elements for growth in yeast and genome transplantation. In addition, there are 20 locations with nucleotide polymorphisms (asterisks). Coordinates of the genome are relative to the first nucleotide of the natural *M. mycoides* sequence. The designed sequence is 1,077,947 bp. The locations of the Asc I and BssH II restriction sites are shown. Cassettes 1 and 800-810 were unnecessary and removed from the assembly strategy (11). Cassette 2 overlaps cassette 1104, and cassette 799 overlaps cassette 811.

Aplicação: Análise de expressão gênica: Microarranjos de DNA



Obrigado

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