ESALQ
Escola Superior de Agricultura Luiz de Queiroz
Universidade de São Paulo

Biotecnologia Animal

Luiz Lehmann Coutinho

llcoutinho@usp.br

Formação Acadêmica

- Engenheiro Agrônomo – ESALQ
- Oportunidades
- Estágios

Formação Acadêmica

- Graduação: ESALQ
- Mestrado e Doutorado Michigan State University, EUA

- Experiência Internacional
  - Outro idioma
  - Diferentes cursos
  - Mudança de foco
  - Desafio intelectual

Professor Titular

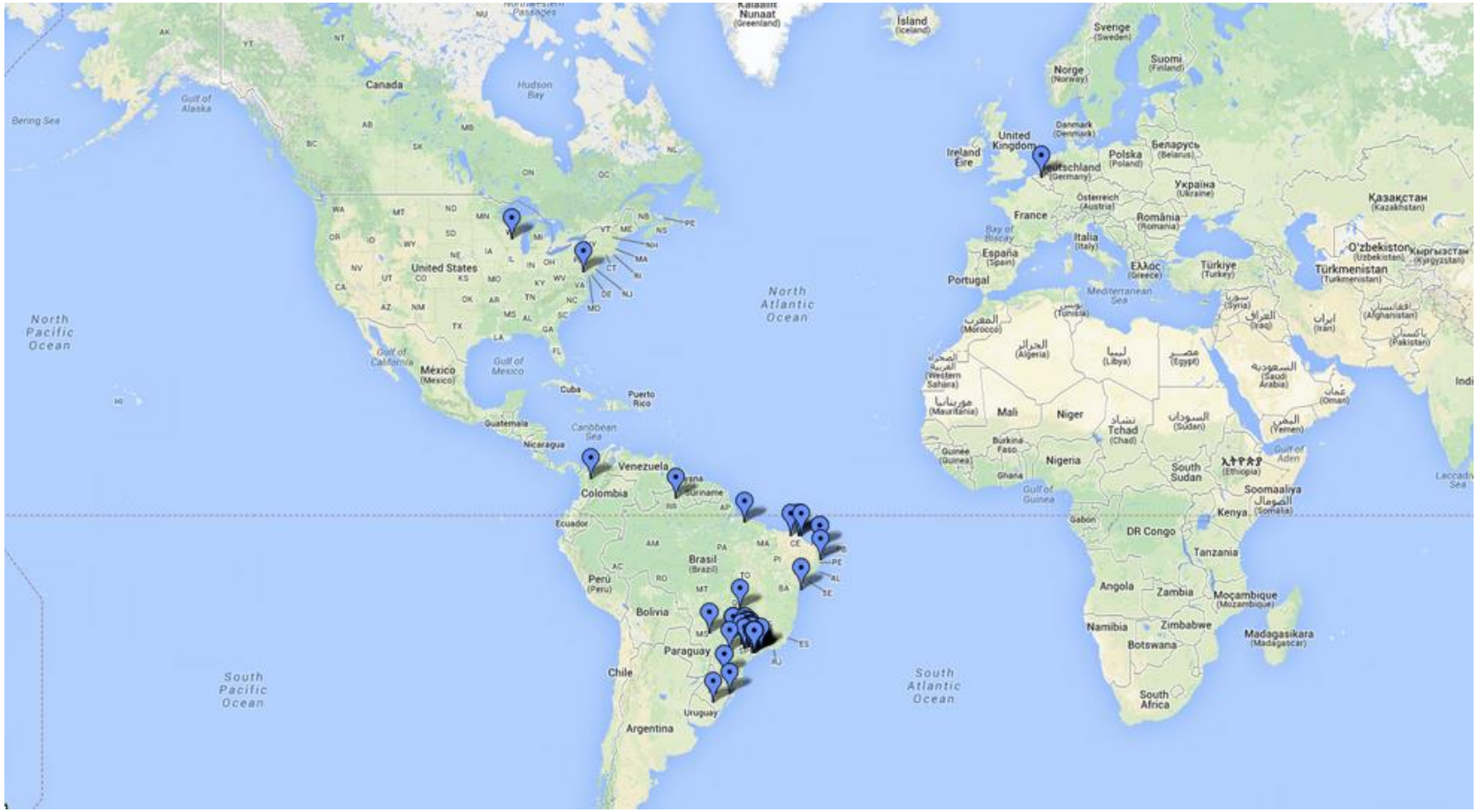
- Ensino de graduação e pós-graduação
- Coordenador do Centro de Genômica Funcional
- Formação de recursos humanos
  - IC, MS, DR, PD
- Avanço do conhecimento
  - Publicação
- Extensão
  - Palestras, cursos, testes genéticos, prestação de serviços em genômica

Objetivos do Centro de Genômica

- Dar suporte científico e tecnológico para pesquisadores atuando na área de genômica
- Gerar resultados com qualidade e agilidade
- Treinamento de recursos humanos na área de genômica

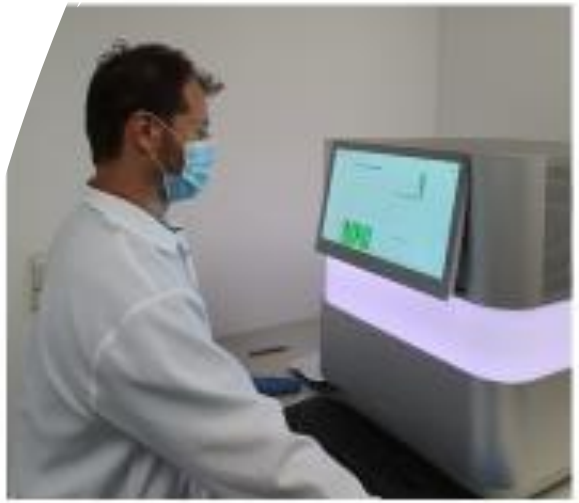
Usuários do Centro

- Mais de 300 pesquisadores realizaram projetos e em vários casos múltiplos projetos.
- A maioria dos usuários receberam treinamento



Tecnologias Disponíveis

- Sequenciamento
- Genotipagem
- Bioinformática
- Cultura de Tecidos



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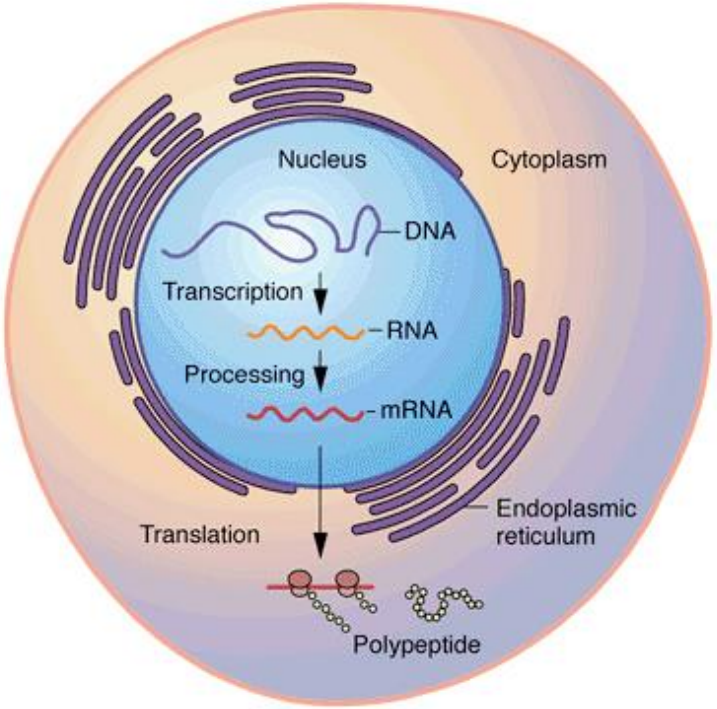
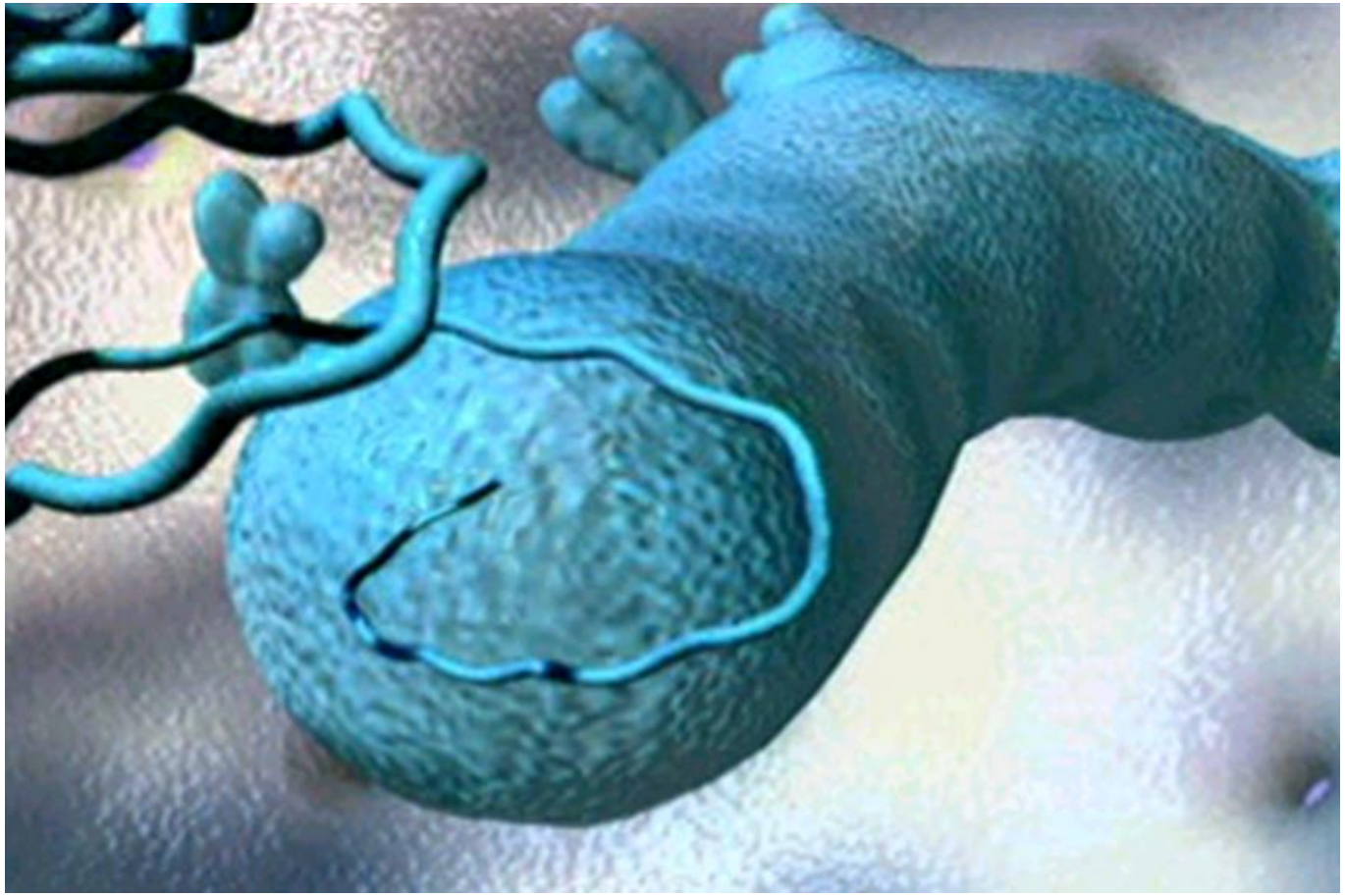
Por que estudar o genoma?

Informação genética + Ambiente ===➔ Fenótipo

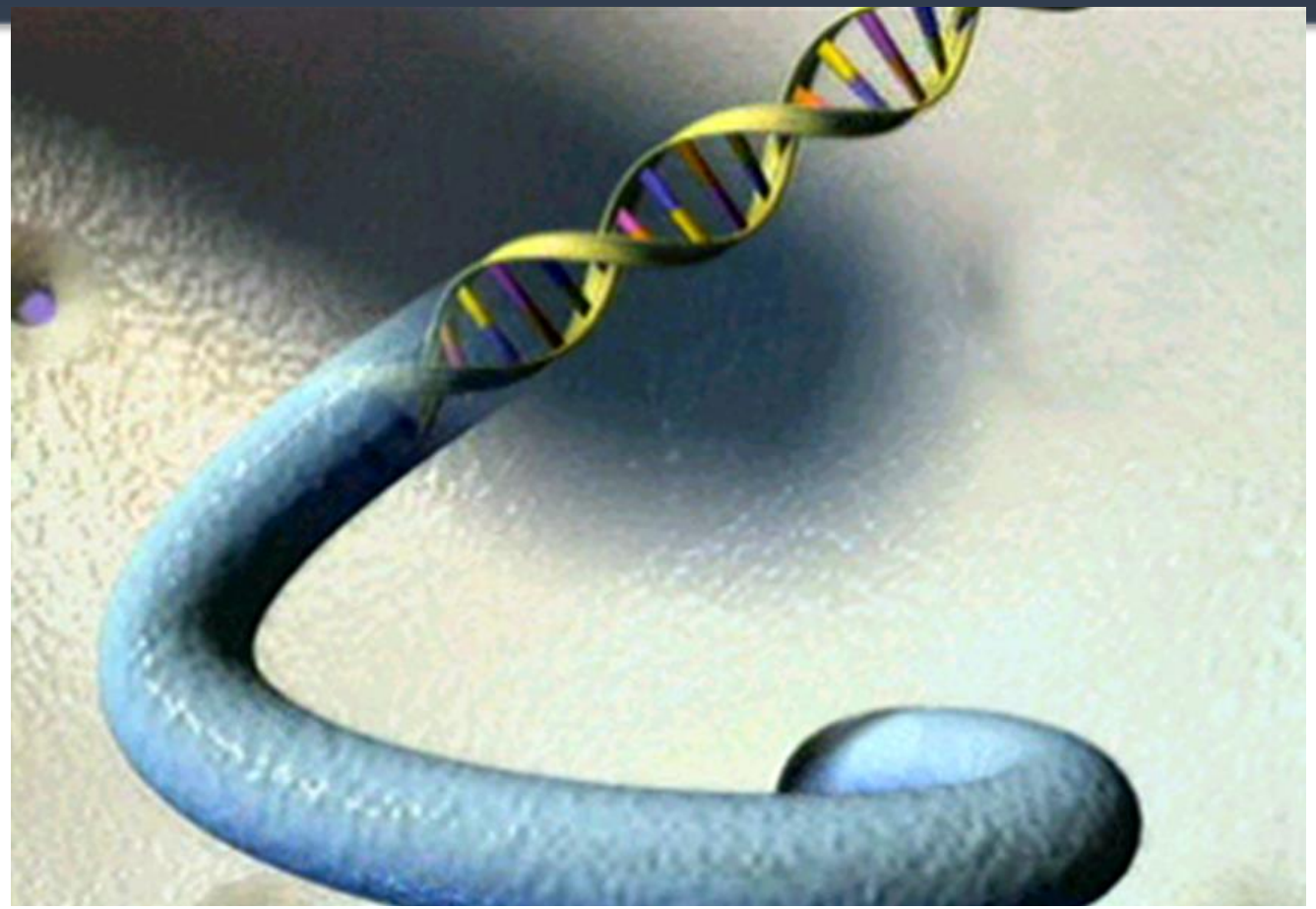


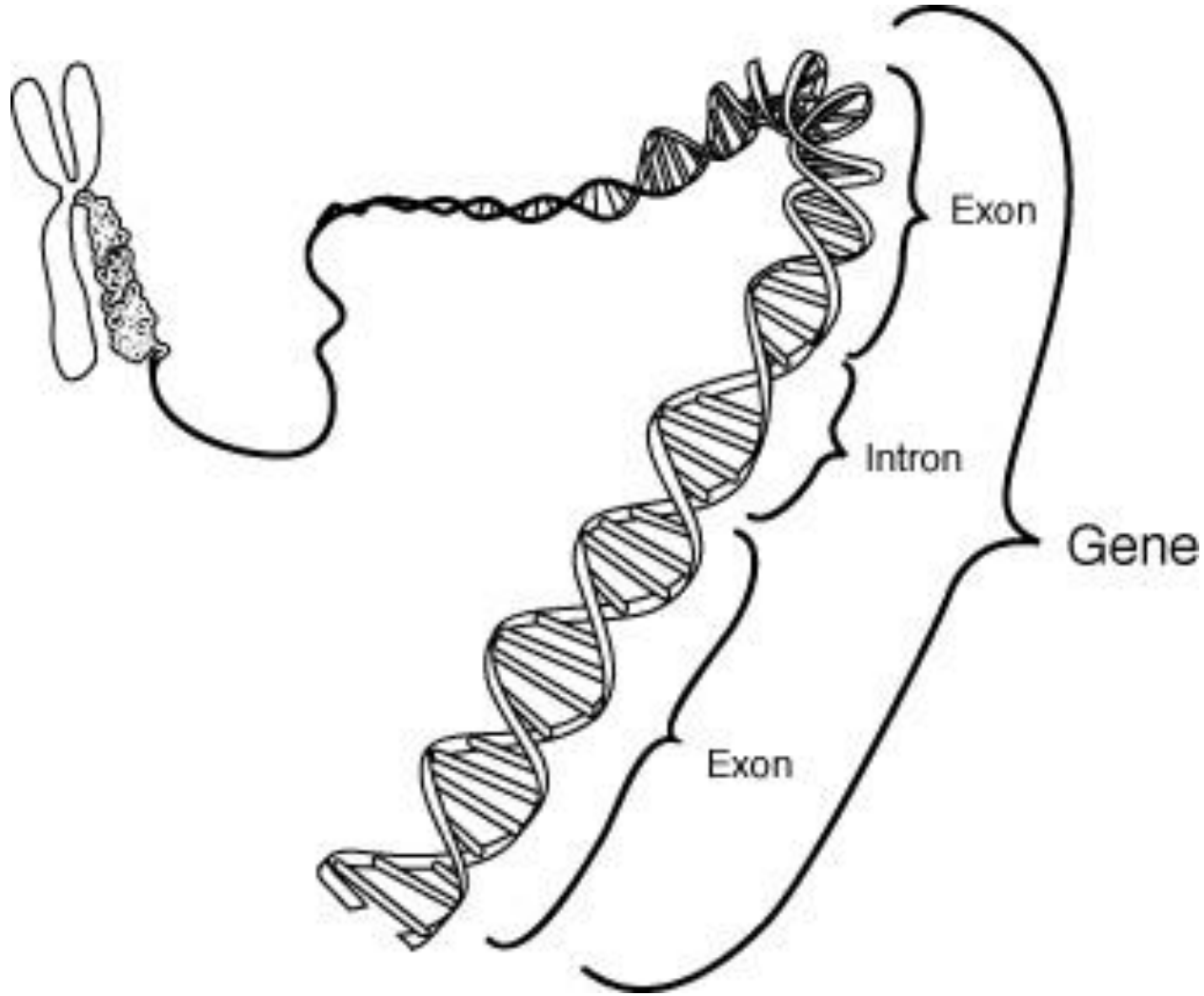
Célula

Cromossomo

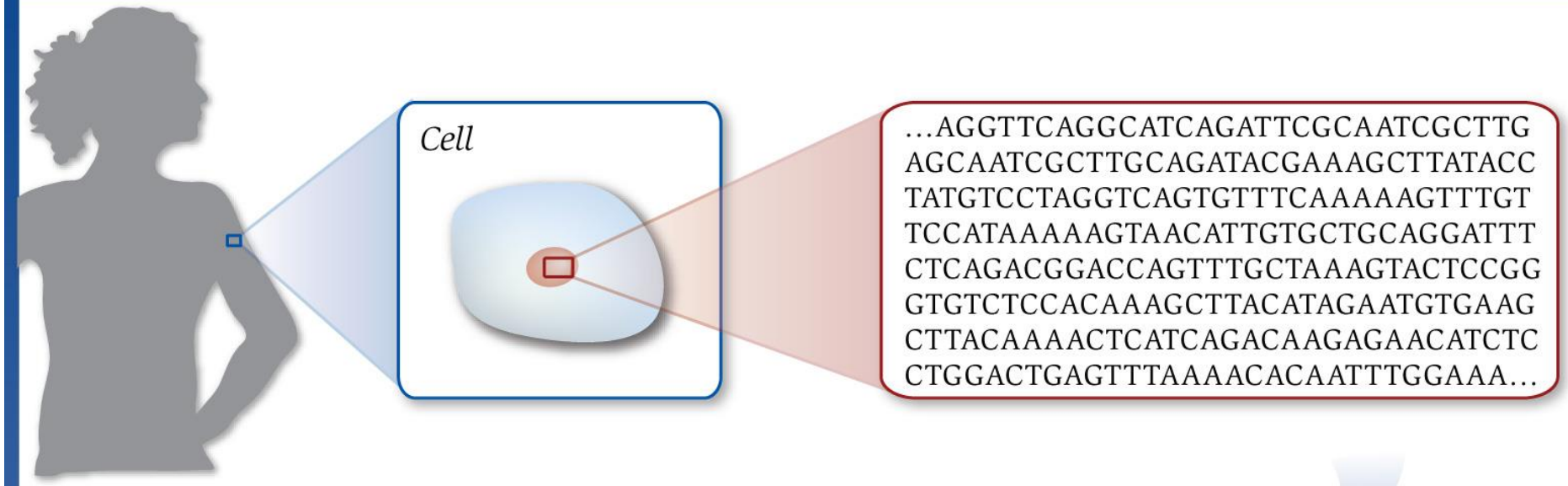


Dupla Hélice DNA

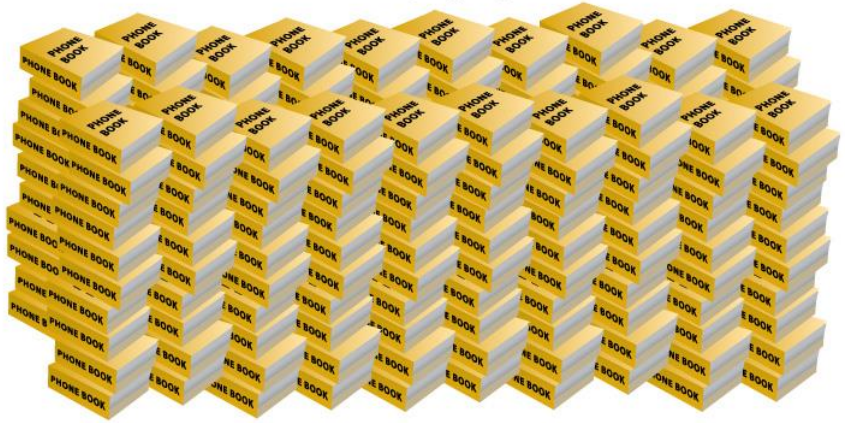



Exon
Intron
Gene
Exon

The human genome contains about 3 billion nucleotides

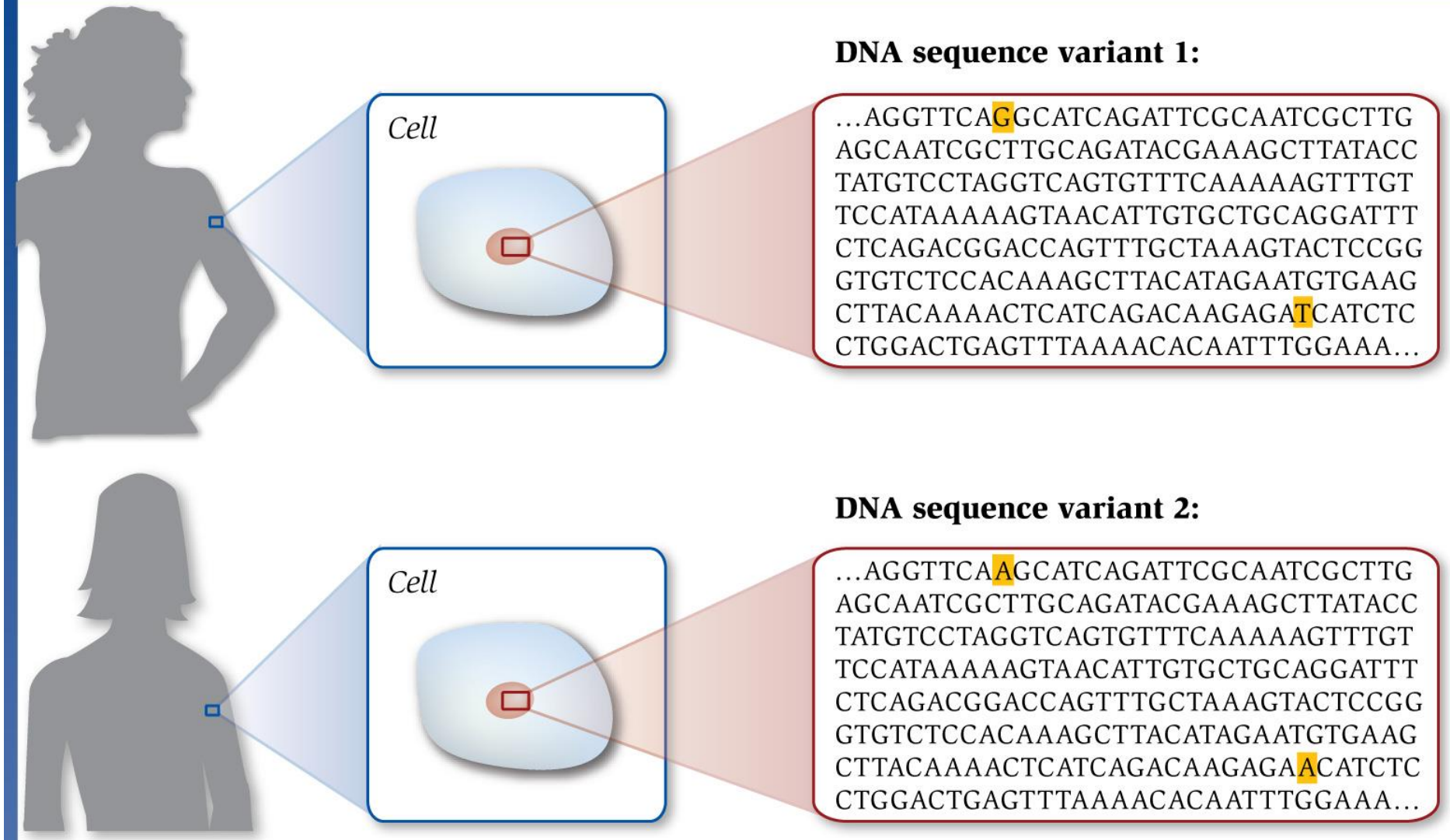


3 billion nucleotides would fill about 200 1,000-page phone books



National Human Genome Research Institute

Each individual has a unique DNA sequence



Diferenças vs. Semelhanças

- Entre espécies próximas?
- Entre indivíduos de uma mesma espécie?

Observações após o sequenciamento de vários genomas

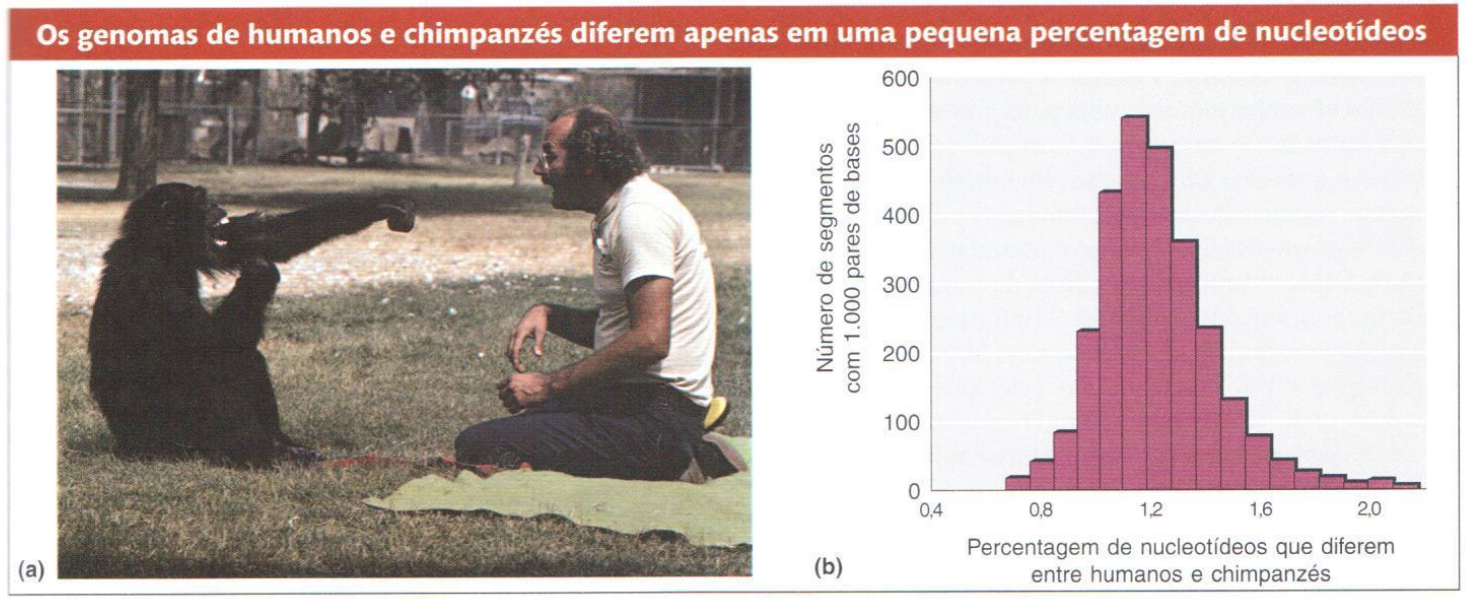


FIG. 1.11 (a) Esses dois primatas diferem um do outro apenas em 1% do DNA. (b) A comparação das seqüências de nucleotídeos de humanos e chimpanzés. Um gráfico de barras mostra o número de segmentos do genoma, de 24.000 segmentos diferentes examinados (eixo *y*), que diferem entre o chimpanzé e o humano por uma percentagem de todos os nucleotídeos em um segmento (eixo *x*). Cada segmento examinado tinha 1.000 nucleotídeos de tamanho. A diferença média era de cerca de 1,2% por segmento. [(a) Vic Cox/Peter Arnold.]

Each individual has a unique DNA sequence

Our uniqueness lies in just 0.1% of our DNA sequence

- The DNA sequences of any two people are 99.9% identical
- 1 difference in every 1,000 nucleotides
- 3 million total nucleotide differences

DNA sequence variant 1:

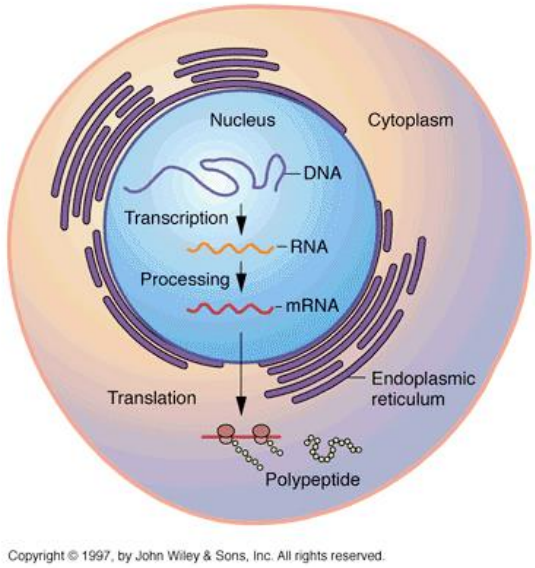
…AGGTTCAGGCATCAGATTCGCAATCGCTTG
AGCAATCGCTTGCAGATACGAAAGCTTATACC
TATGTCCTAGGTCAGTGTTTCAAAAAGTTTGT
TCCATAAAAAGTAACATTGTGCTGCAGGATTT
CTCAGACGGACCAGTTTGCTAAAGTACTCCGG
GTGTCTCCACAAAGCTTACATAGAATGTGAAG
CTTACAAAACTCATCAGACAAGAGATCATCTC
CTGGACTGAGTTTAAAACACAATTTGGAAA…

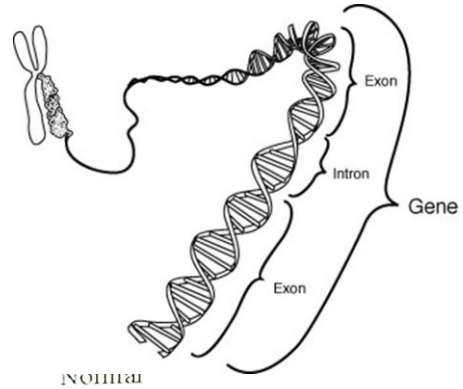
DNA sequence variant 2:

…AGGTTCAAGCATCAGATTCGCAATCGCTTG
AGCAATCGCTTGCAGATACGAAAGCTTATACC
TATGTCCTAGGTCAGTGTTTCAAAAAGTTTGT
TCCATAAAAAGTAACATTGTGCTGCAGGATTT
CTCAGACGGACCAGTTTGCTAAAGTACTCCGG
GTGTCTCCACAAAGCTTACATAGAATGTGAAG
CTTACAAAACTCATCAGACAAGAGAACATCTC
CTGGACTGAGTTTAAAACACAATTTGGAAA…

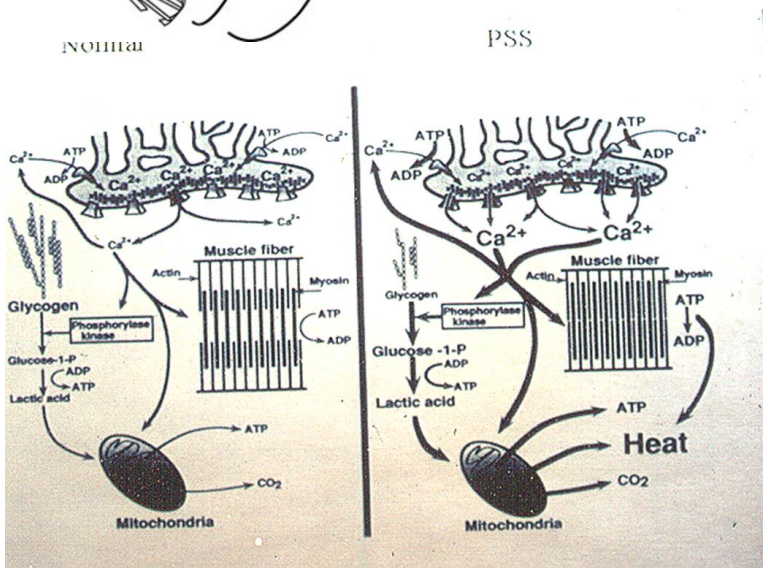
National Human Genome Research Institute

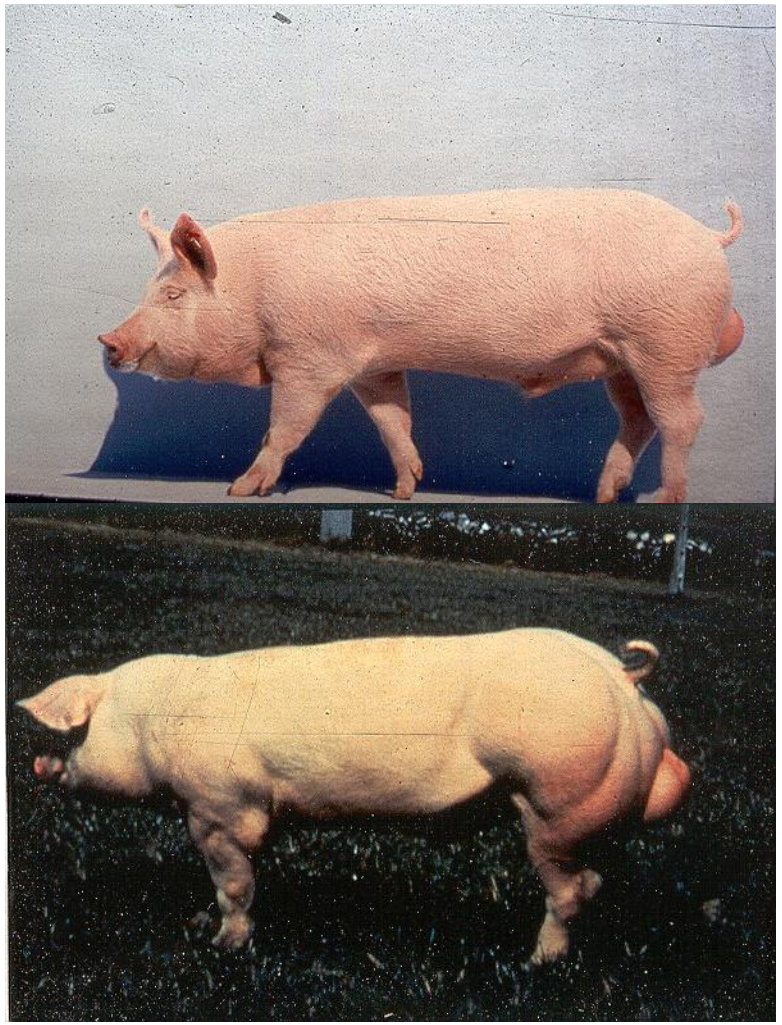
Mutação pode mudar o fenótipo





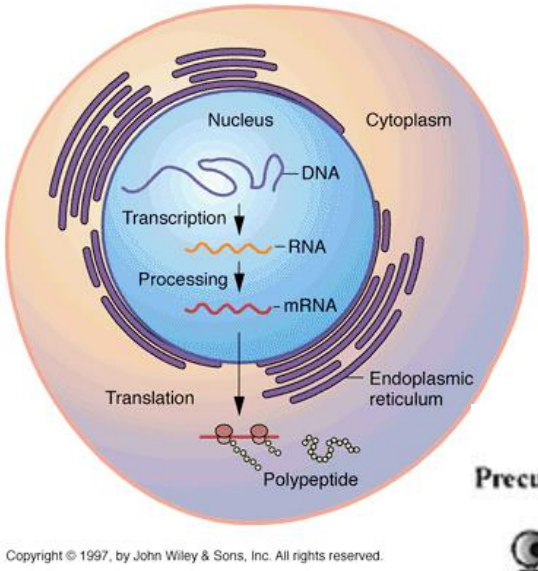
Receptor de Rianodina

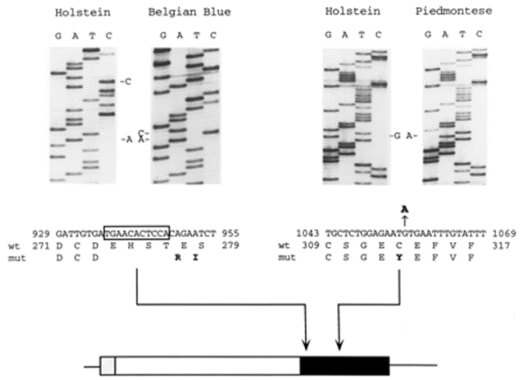


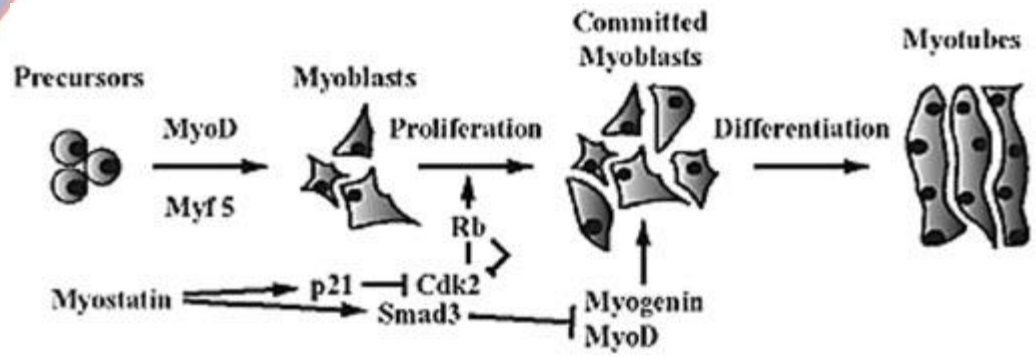


Mutação de grande efeito

Dentro de uma mesma espécie, mutação em região codificante pode mudar o fenótipo

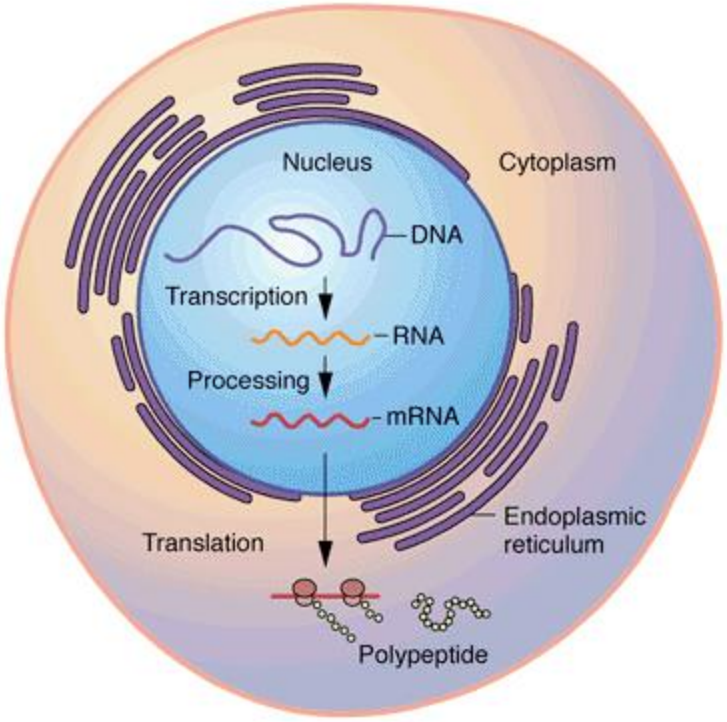


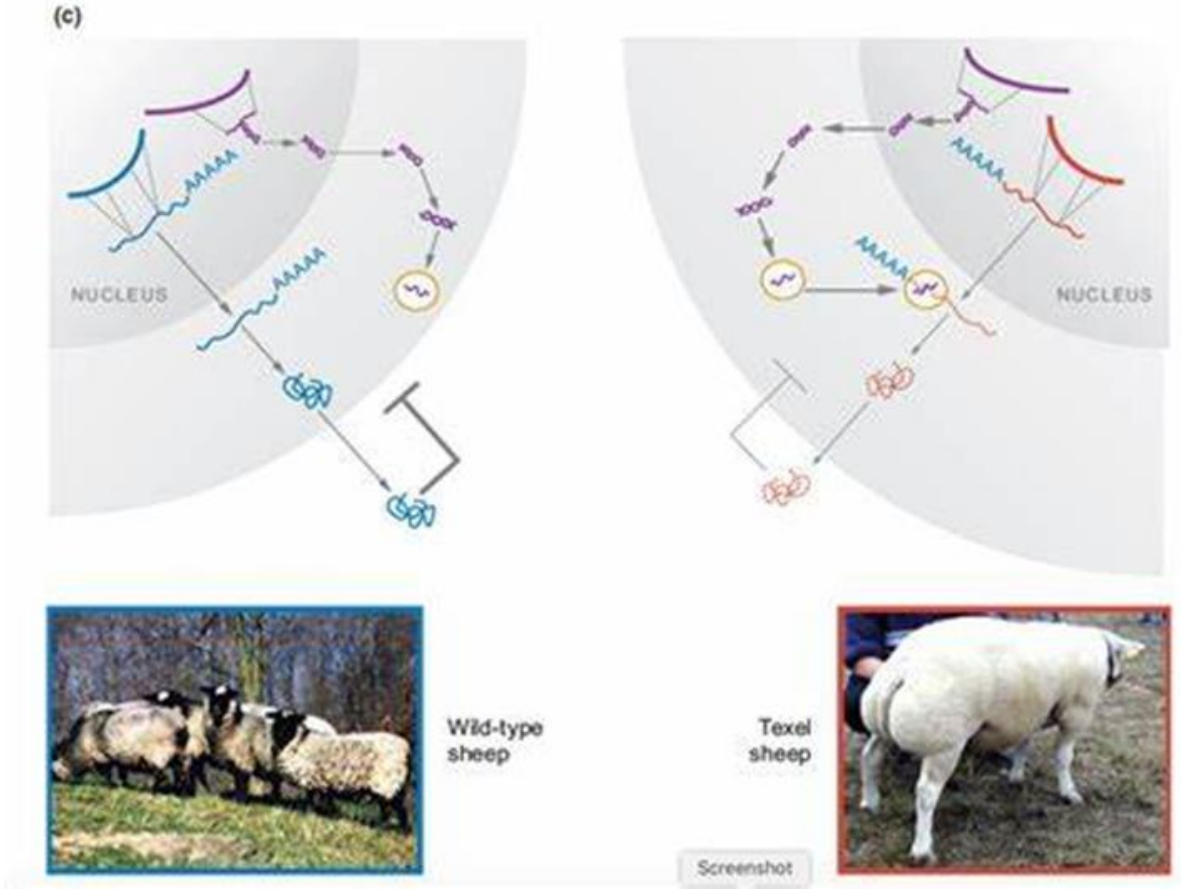




Mutação de grande efeito

Mutação em região não codificante pode alterar a expressão gênica e mudar o fenótipo





Atividades do Laboratório

- Diagnóstico e identificação de variantes de SARS-CoV-2
- Sequenciamento de Genomas
- Sequenciamento de RNA
- Estudo do Microbioma
- Cultura de células musculares
- Edição Gênica

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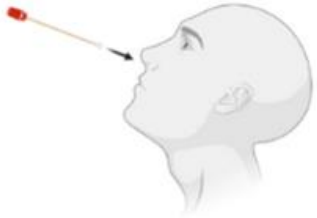
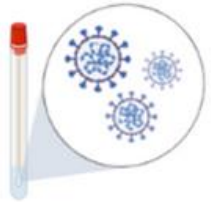
Diganóstico de SARS-CoV-2

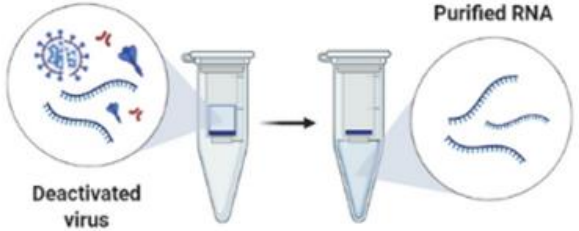
Coleta de amostra

Armazenamento

Extração de RNA



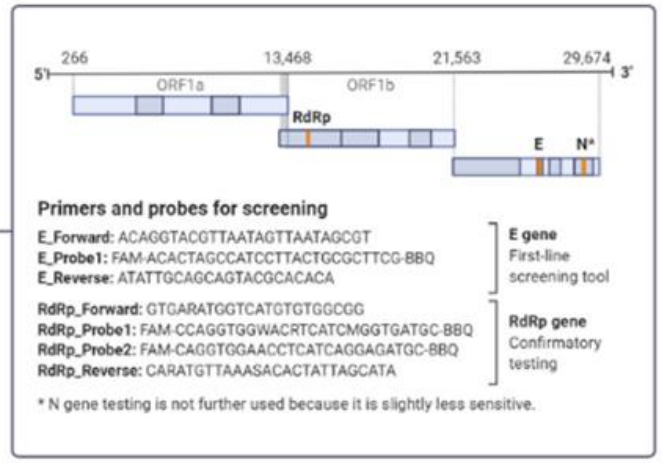




4 **RT-PCR** ~1 h per primer set

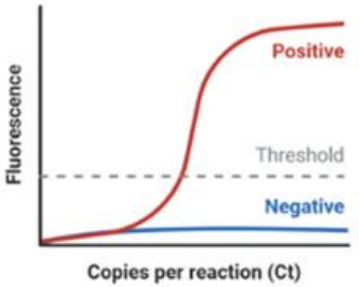
Purified RNA is reverse transcribed to DNA and amplified by PCR.

Retro transcription

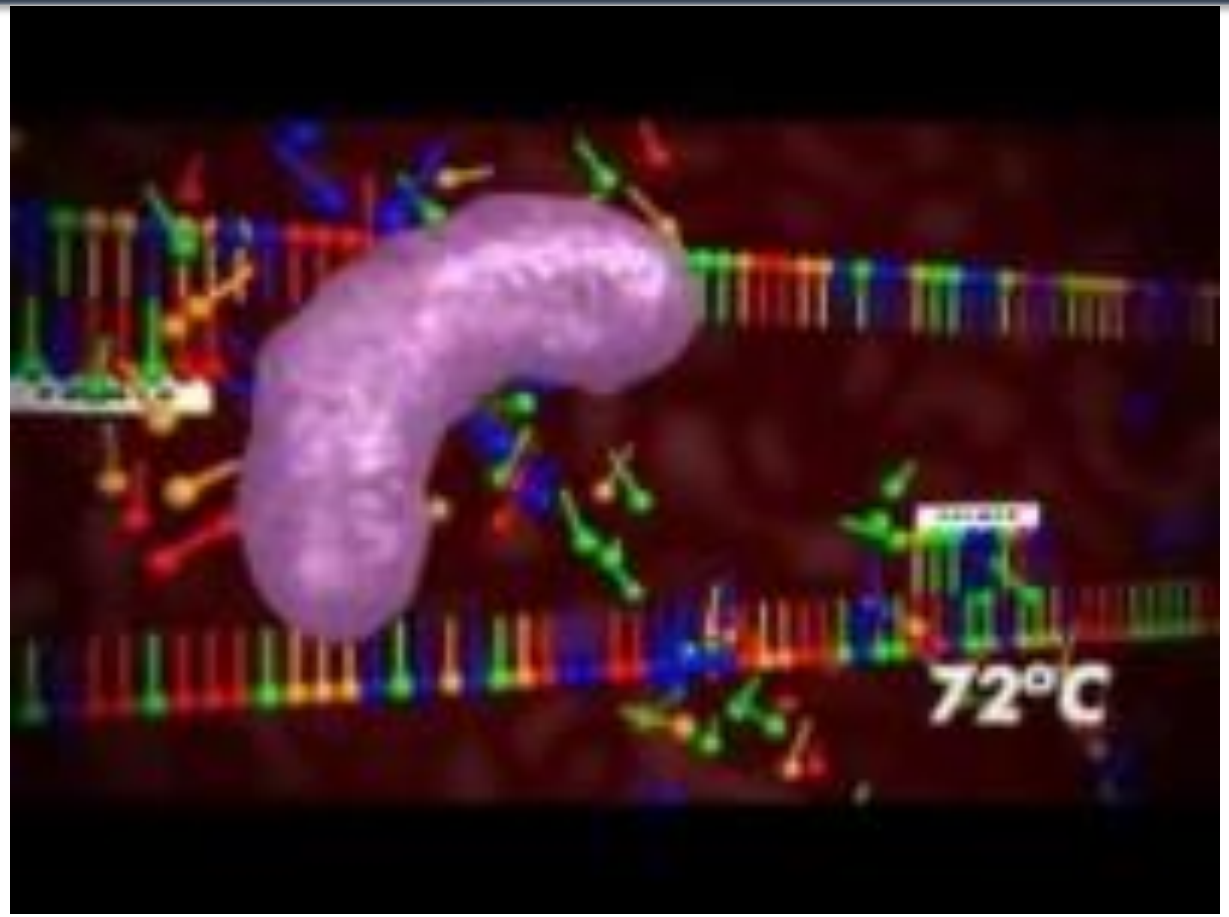


5 **Test results** real-time

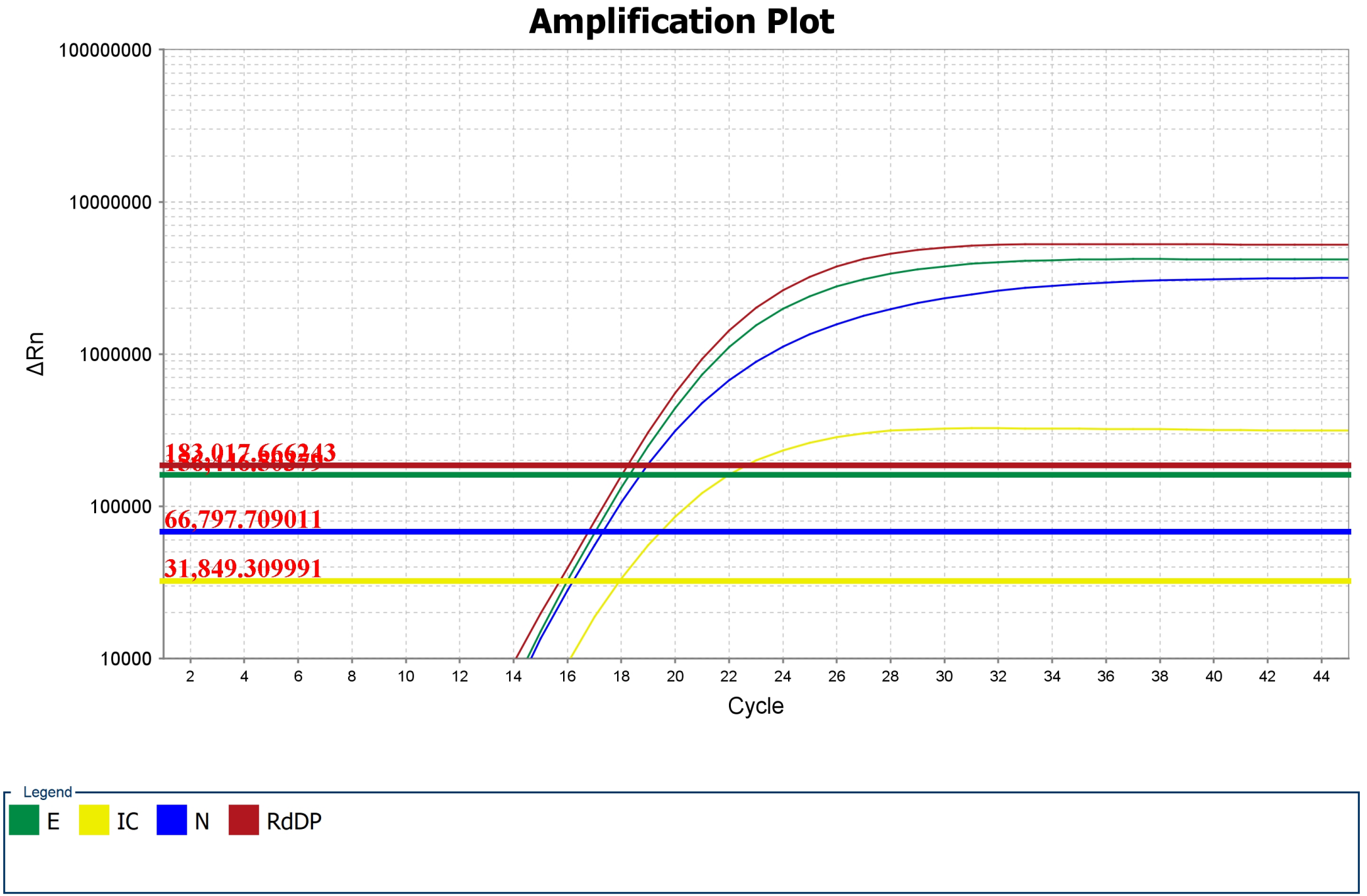
Positive SARS-CoV2 patients cross the threshold line within 40.00 cycles (< 40.00 Ct).



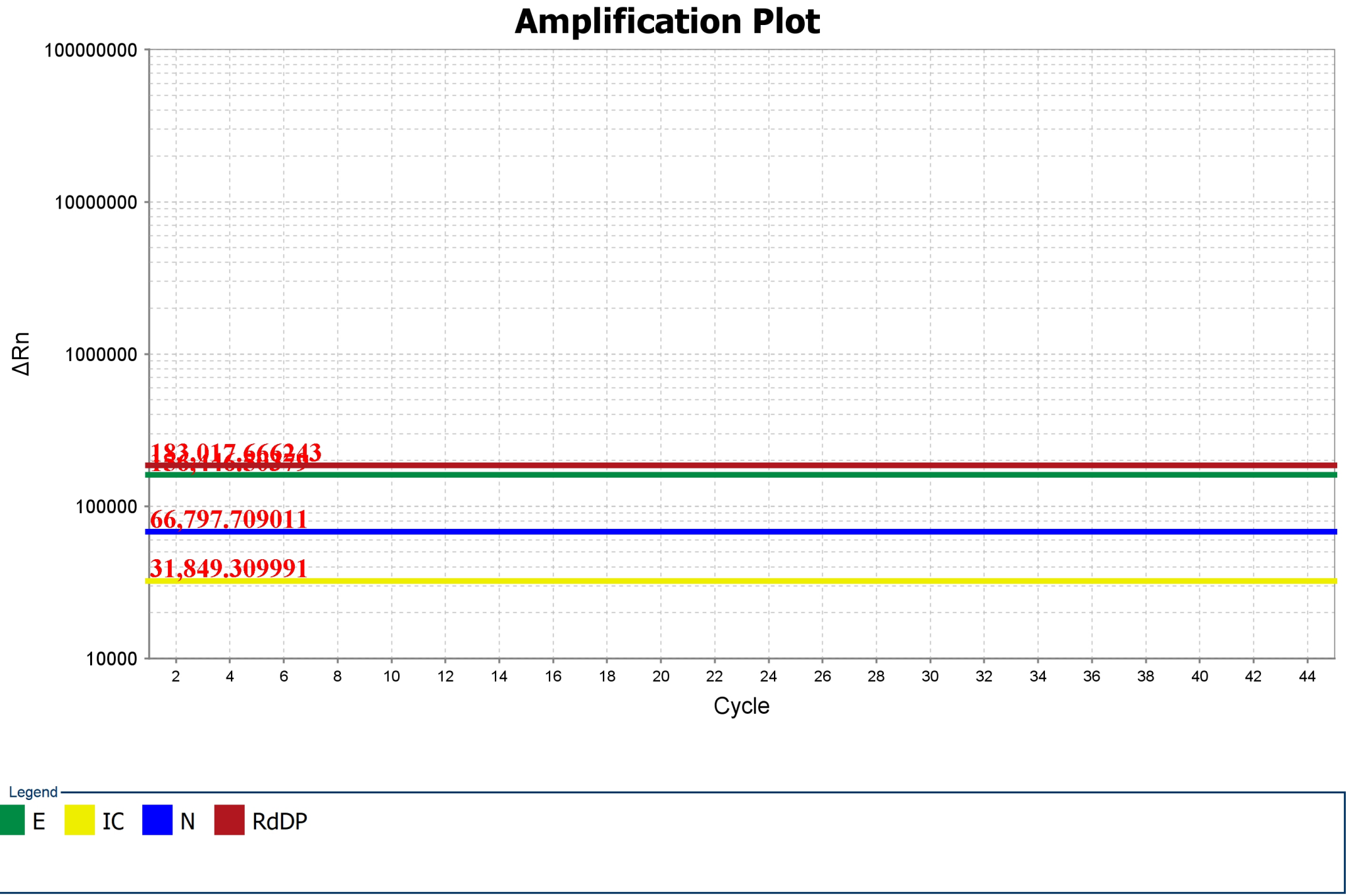
PCR: Reação em cadeia da Polimerase



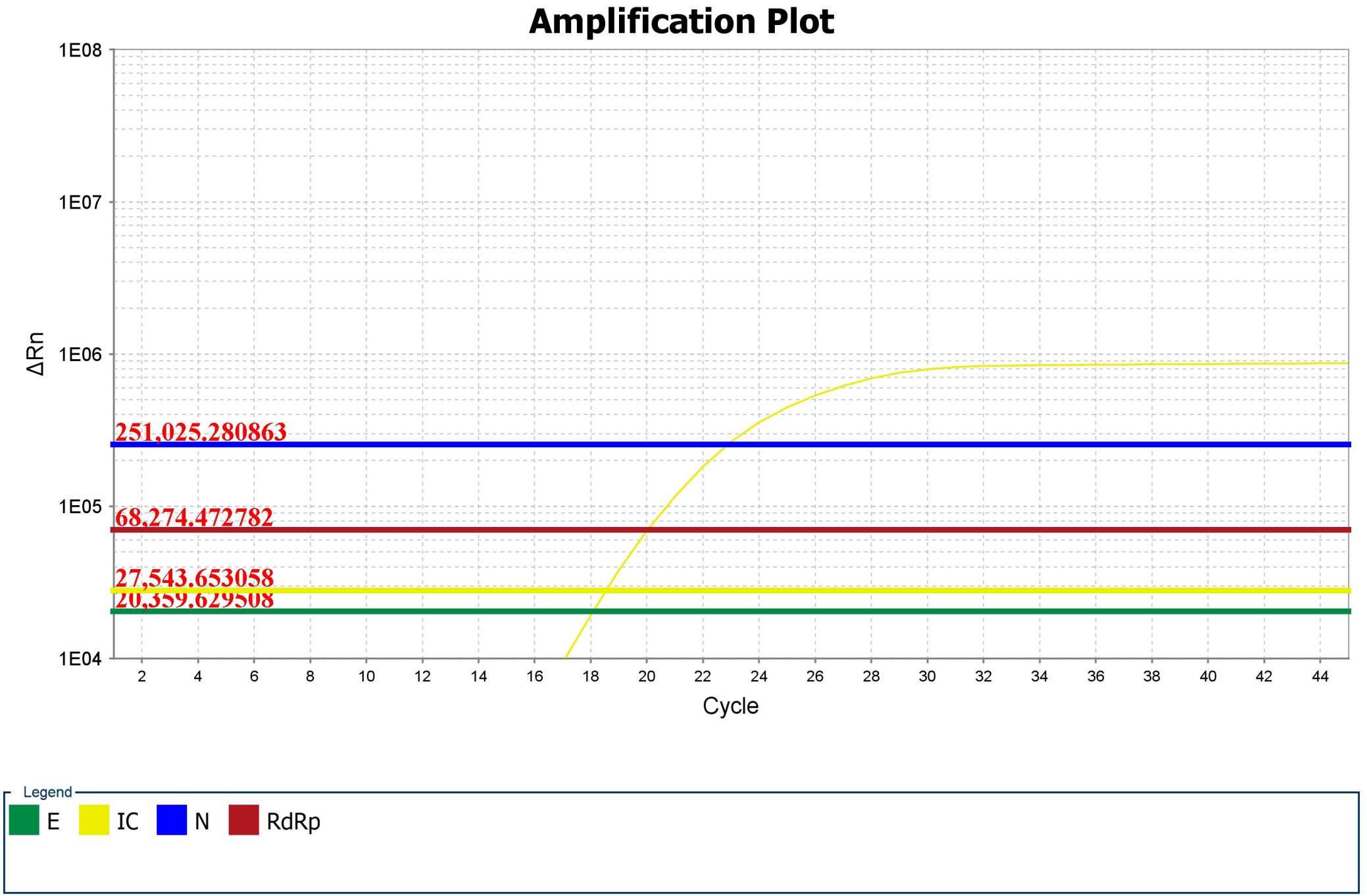
Controle positivo



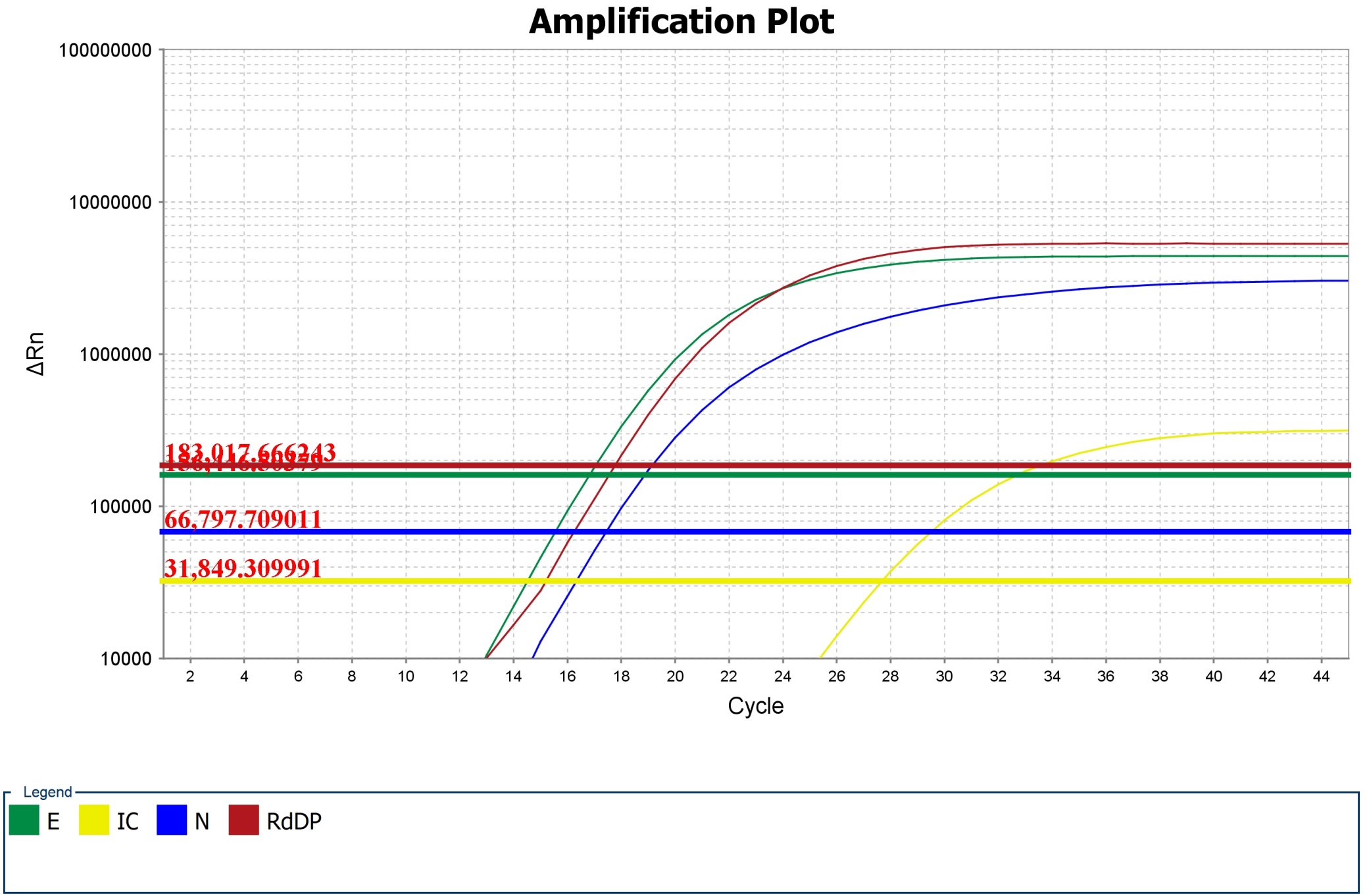
Contole negativo



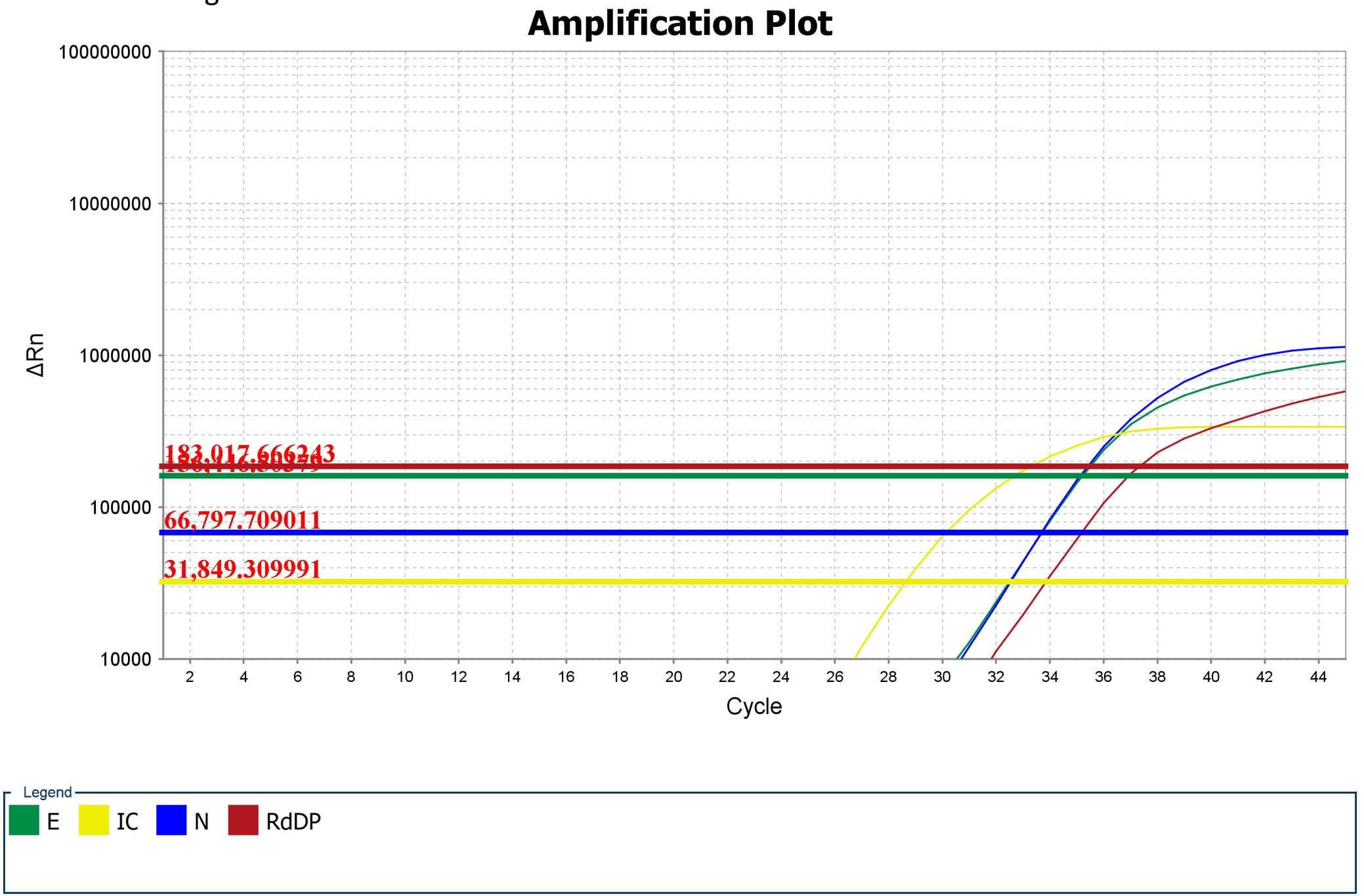
Célula


Amplification Plot
ΔRn
1E08
1E07
1E06
1E05
1E04
251,025.280863
68,274.472782
27,543.653058
20,359.629508
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
Cycle
Legend
E
IC
N
RdRp

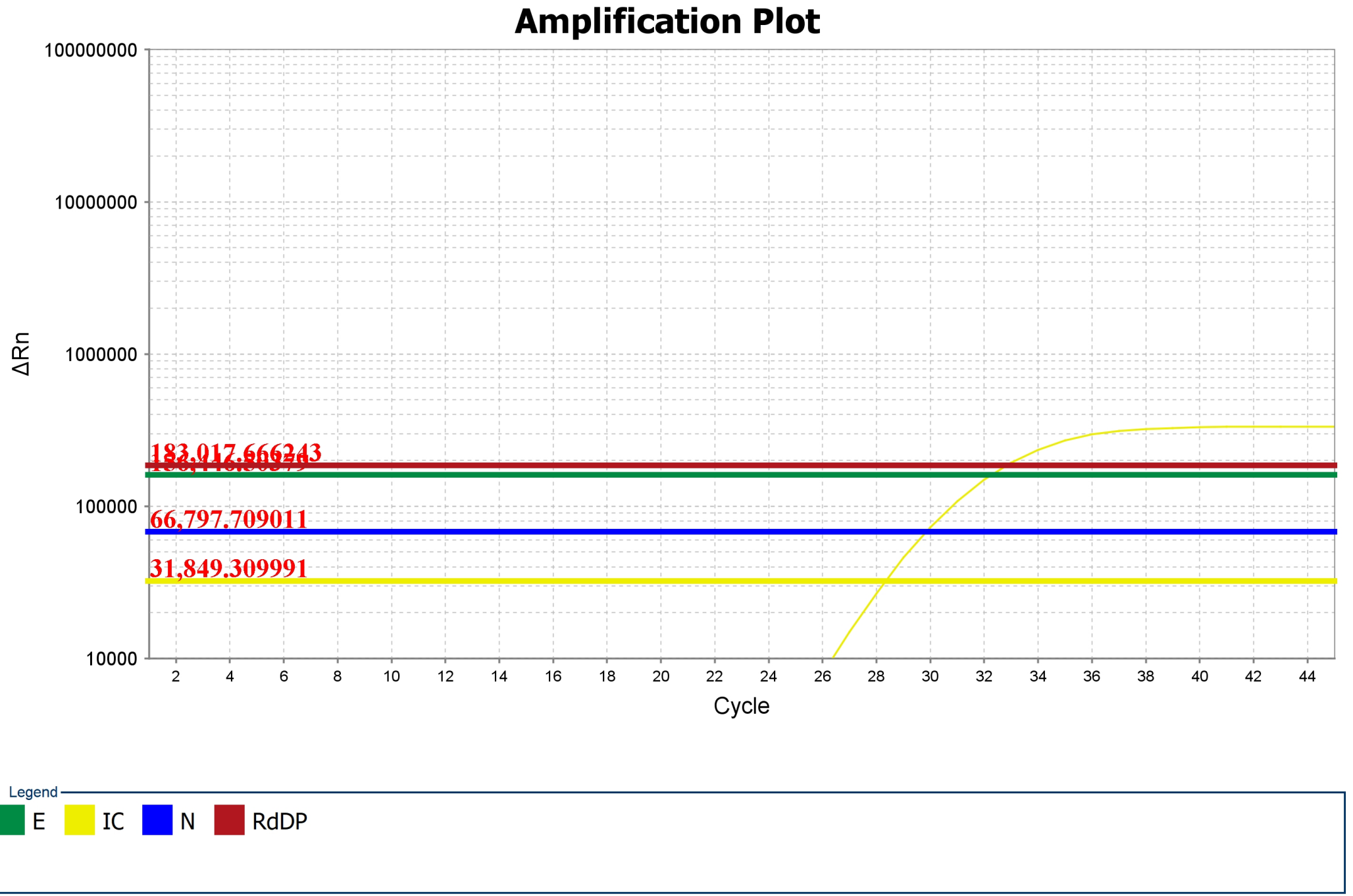
Amostra positiva



Amostra positiva menor carga



Amostra negativa



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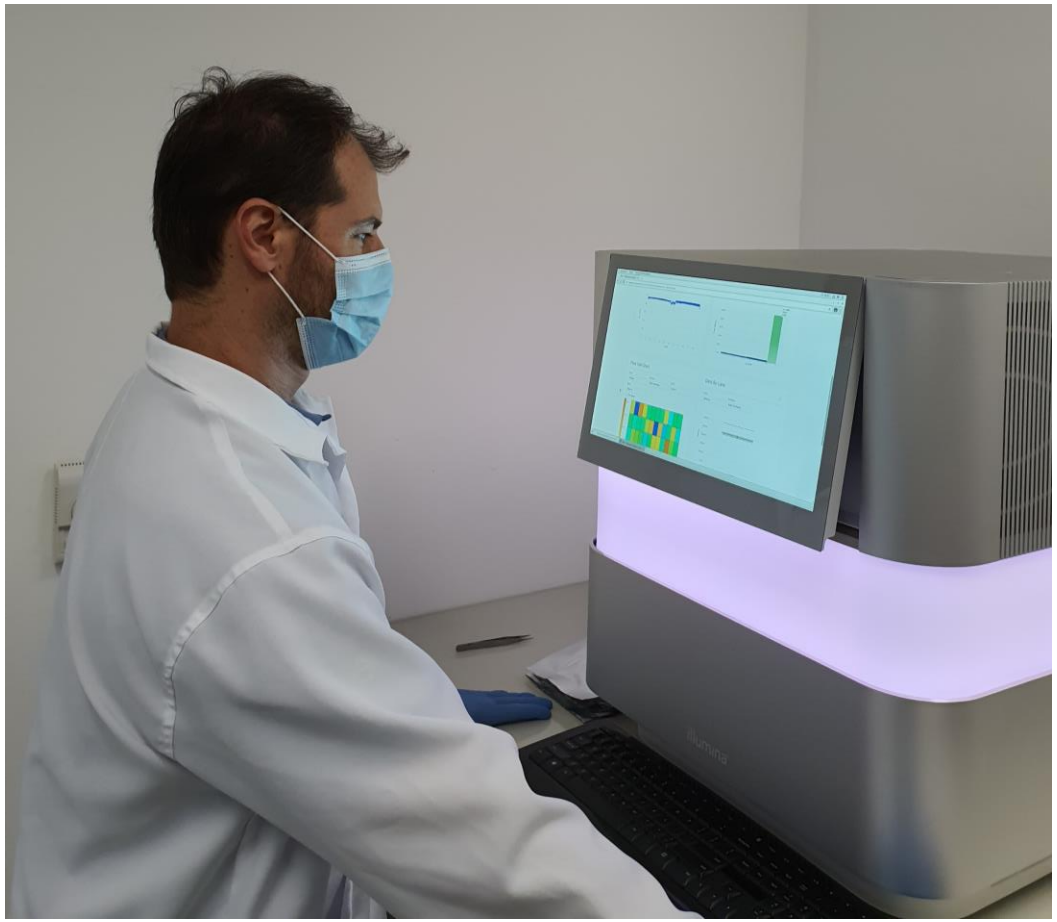
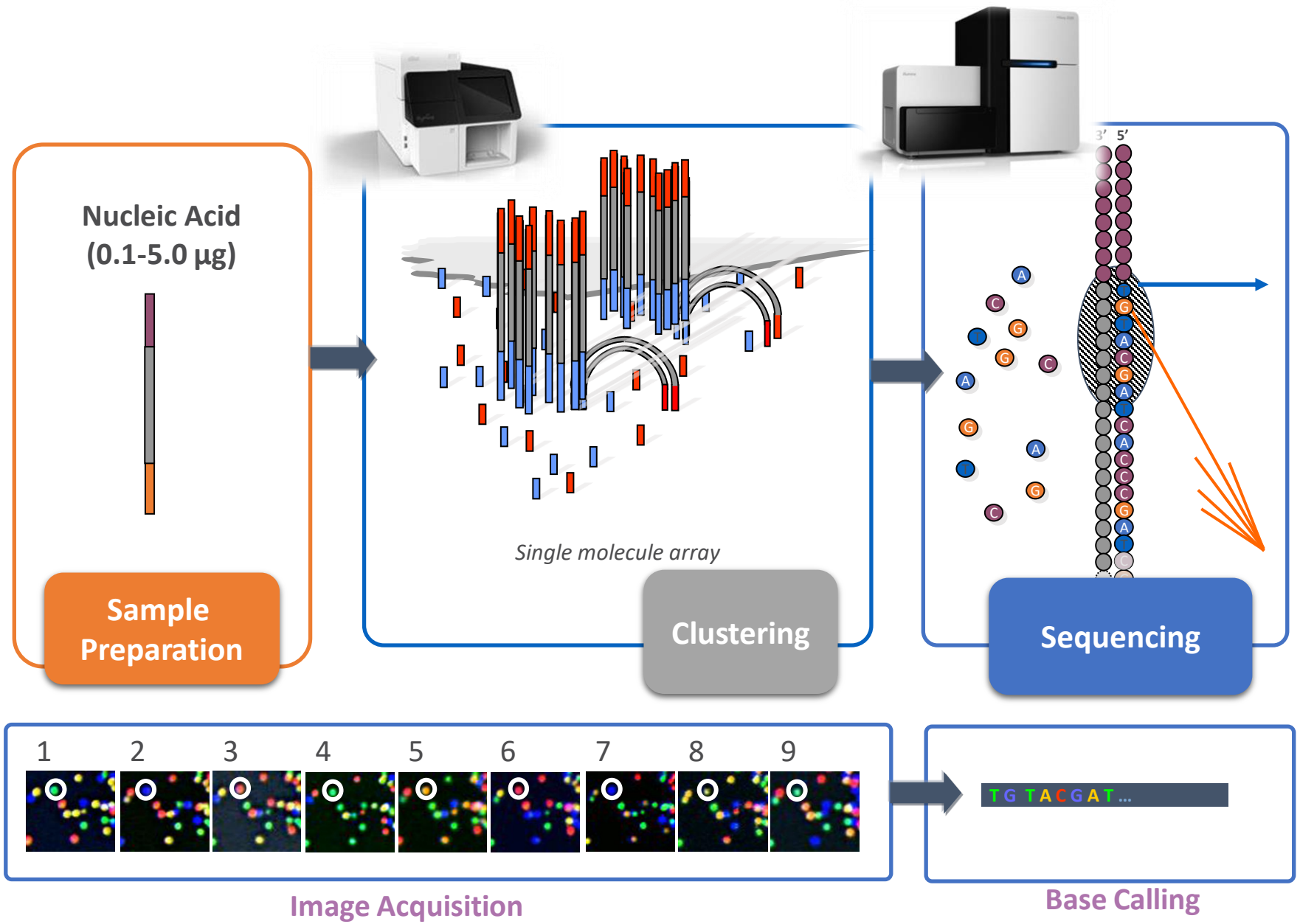
Sequenciamento de Genomas



- Sars-CoV-2
- Mico Leão
- Onça
- Tambaqui
- Galinha
- Bovinos
- Soja


Nucleic Acid
(0.1-5.0 µg)
Sample Preparation
Single molecule array
Clustering
3′
5′
Sequencing
1
2
3
4
5
6
7
8
9
Image Acquisition
TG TACGAT…
Base Calling

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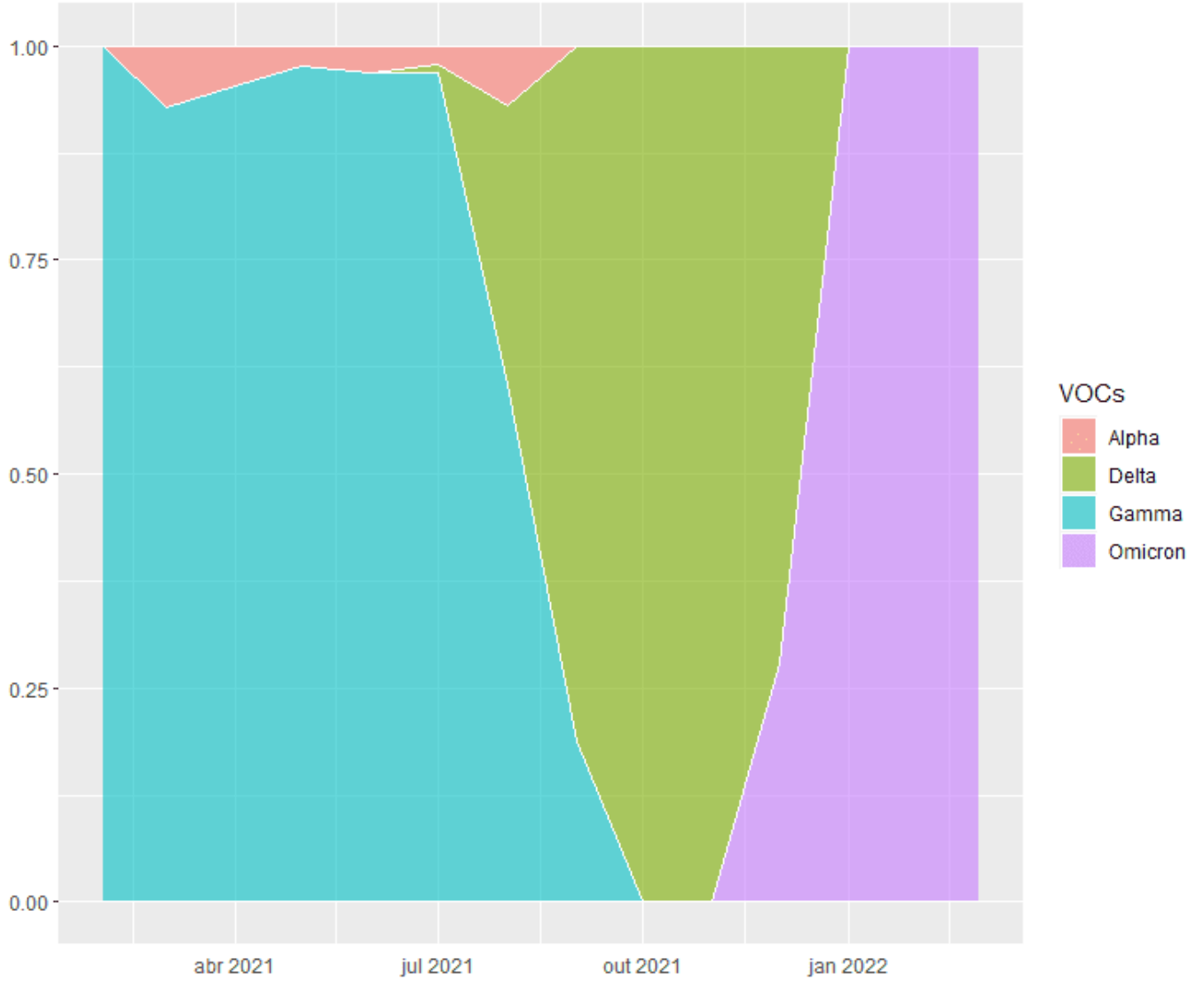
Sequenciamento de Variantes SARS-CoV-2



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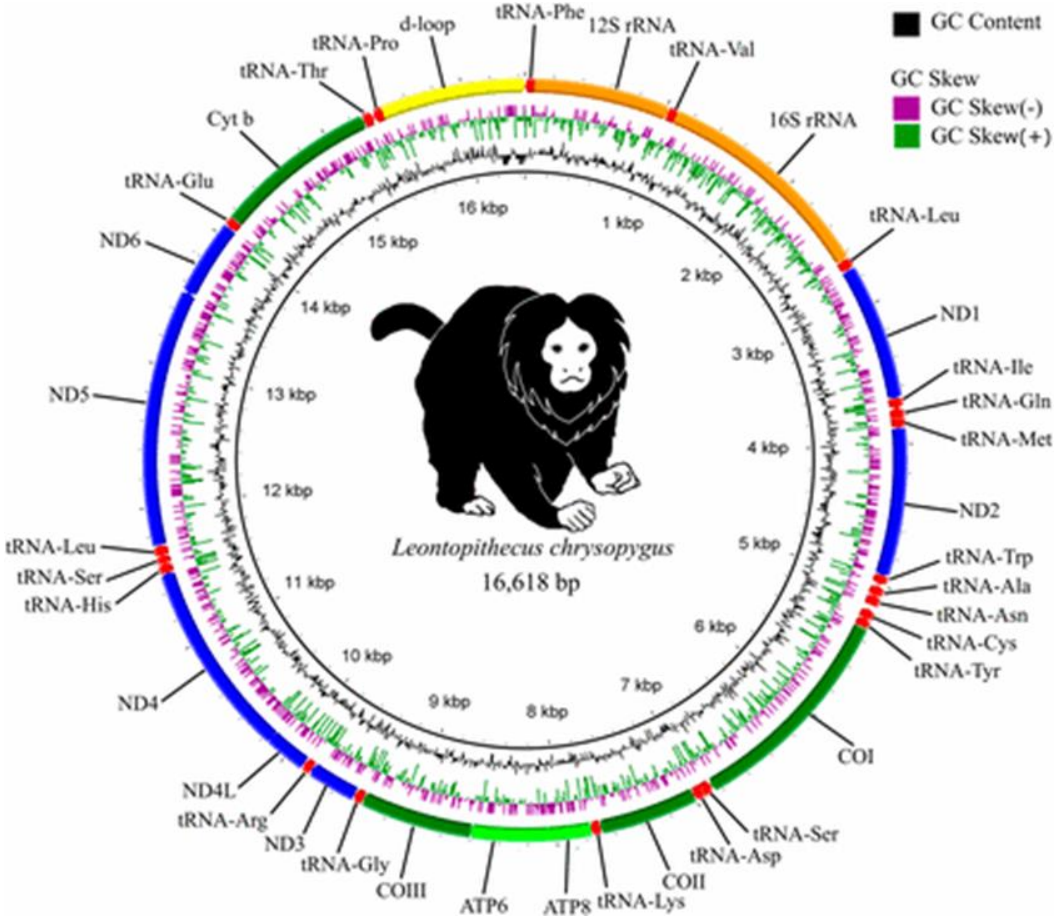
Next-Generation Sequencing of the Complete Mitochondrial Genome of the Endangered Species Black Lion Tamarin *Leontopithecus chrysopygus* (Primates) and Mitogenomic Phylogeny Focusing on the Callitrichidae Family

Patrícia Domingues de Freitas, Fernando Luis Mendez, Karla Chávez-Congrains, Pedro Manoel Galetti, Jr., Luiz Lehmann Coutinho, Alcides Pissinatti, Carlos Daniel Bustamante *G3 Genes|Genomes|Genetics*, Volume 8, Issue 6, 1 June 2018, Pages 1985–1991, https://doi.org/10.1534/g3.118.200153



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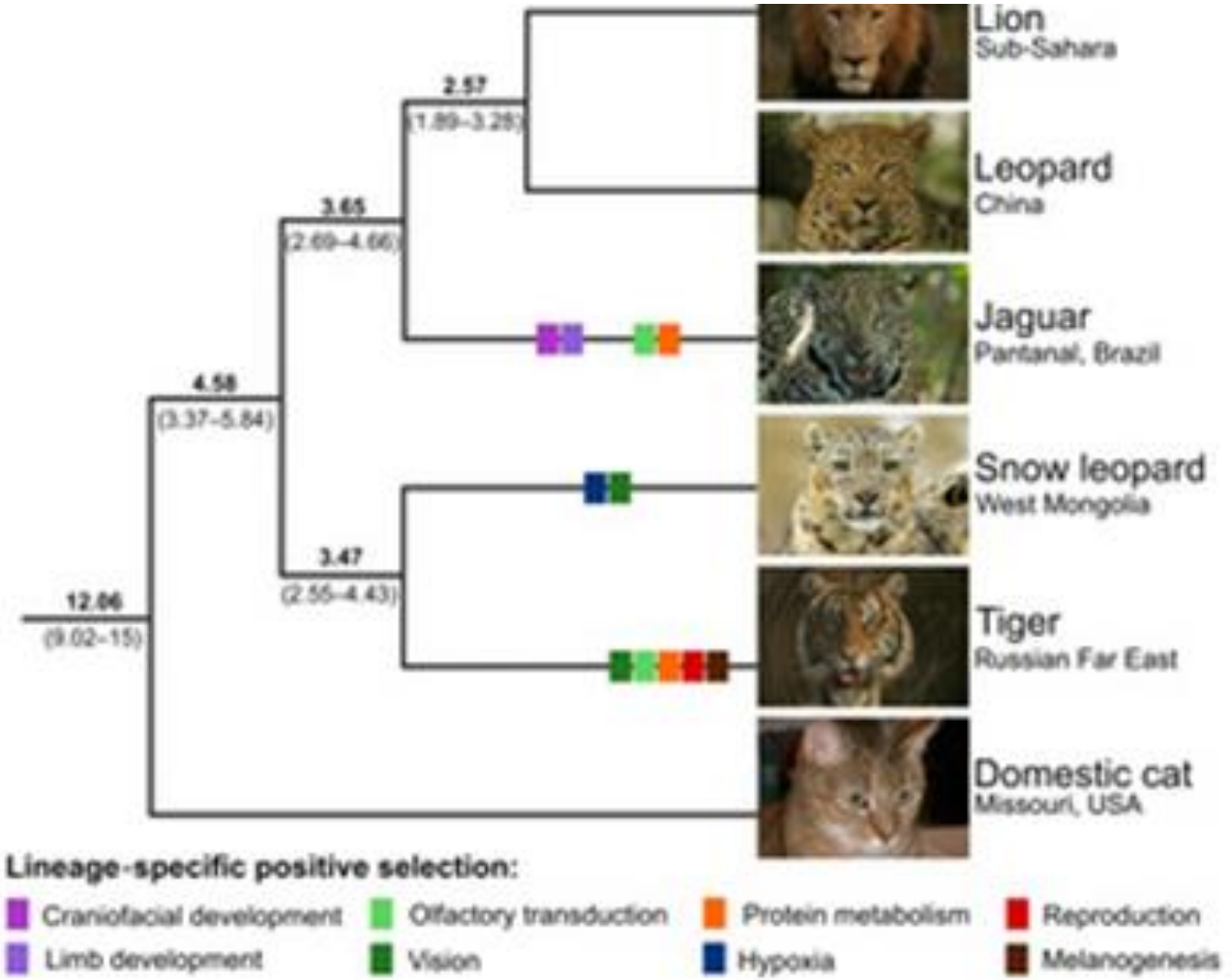
Genome-wide signatures of complex introgression and adaptive evolution in the big cats

HENRIQUE V. FIGUEIRÓGANG LIFERNANDA J. TRINDADEJULIANA ASSIS HTTPS://ORCID.ORG/0000-0001-5995-8684FABIANO PAISGABRIEL FERNANDESSARAH H. D. SANTOSGRAHAM M. HUGHESALEKSEY KOMISSAROV[...]EDUARDO EIZIRIK

SCIENCE ADVANCES • 19 Jul 2017 • Vol 3, Issue 7



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Genoma do Tambaqui

(GIGA)^n DB
Revolutionizing data dissemination, organization, and use

Home About Help Terms of use

Supporting data for "Genome assembly and annotation of the tambaqui (*Colossoma macropomum*): an emblematic fish of the Amazon River basin"

Dataset type: Genomic

Data released on September 21, 2021

Hilsdorf AWS; Uliano-Silva M; Coutinho LL; Montenegro H; Almeida-Val VMF; Pinhal D (2021): Supporting data for "Genome assembly and annotation of the tambaqui (*Colossoma macropomum*): an emblematic fish of the Amazon River basin" GigaScience Database. http://dx.doi.org/10.5524/100933

DOI 10.5524/100933

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Genoma da Galinha: Identificação de seleção de regiões associadas com desempenho

Almeida *et al. BMC Genomics* (2019) 20:449
https://doi.org/10.1186/s12864-019-5811-1

BMC Genomics

RESEARCH ARTICLE Open Access

Identification of selection signatures involved in performance traits in a paternal broiler line

Octávio Augusto Costa Almeida[1], Gabriel Costa Monteiro Moreira[1], Fernanda Marcondes Rezende[2], Clarissa Boschiero[3], Jane de Oliveira Peixoto[4], Adriana Mercia Guaratini Ibelli[4], Mônica Corrêa Ledur[4], Francisco José de Novais[1] and Luiz Lehmann Coutinho[1*]

Abstract

Background: Natural and artificial selection leads to changes in certain regions of the genome resulting in selection signatures that can reveal genes associated with the selected traits. Selection signatures may be identified using different methodologies, of which some are based on detecting contiguous sequences of homozygous identical-by-descent haplotypes, called runs of homozygosity (ROH), or estimating fixation index (F_{ST}) of genomic windows that indicates genetic differentiation. This study aimed to identify selection signatures in a paternal broiler TT line at generations 7th and 16th of selection and to investigate the genes annotated in these regions as well as the biological pathways involved. For such purpose, ROH and F_{ST}-based analysis were performed using whole genome sequence of twenty-eight chickens from two different generations.

Results: ROH analysis identified homozygous regions of short and moderate size. Analysis of ROH patterns revealed regions commonly shared among animals and changes in ROH abundance and size between the two generations. Results also suggest that whole genome sequencing (WGS) outperforms SNPchip data avoiding overestimation of ROH size and underestimation of ROH number; however, sequencing costs can limited the number of animals analyzed. F_{ST}-based analysis revealed genetic differentiation in several genomic windows. Annotation of the consensus regions of

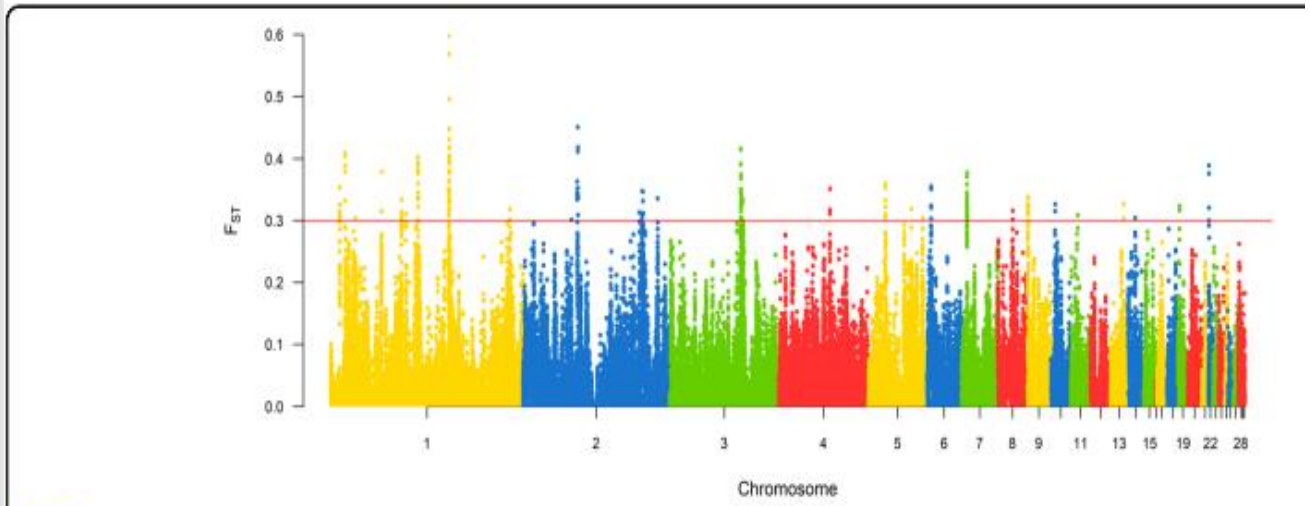
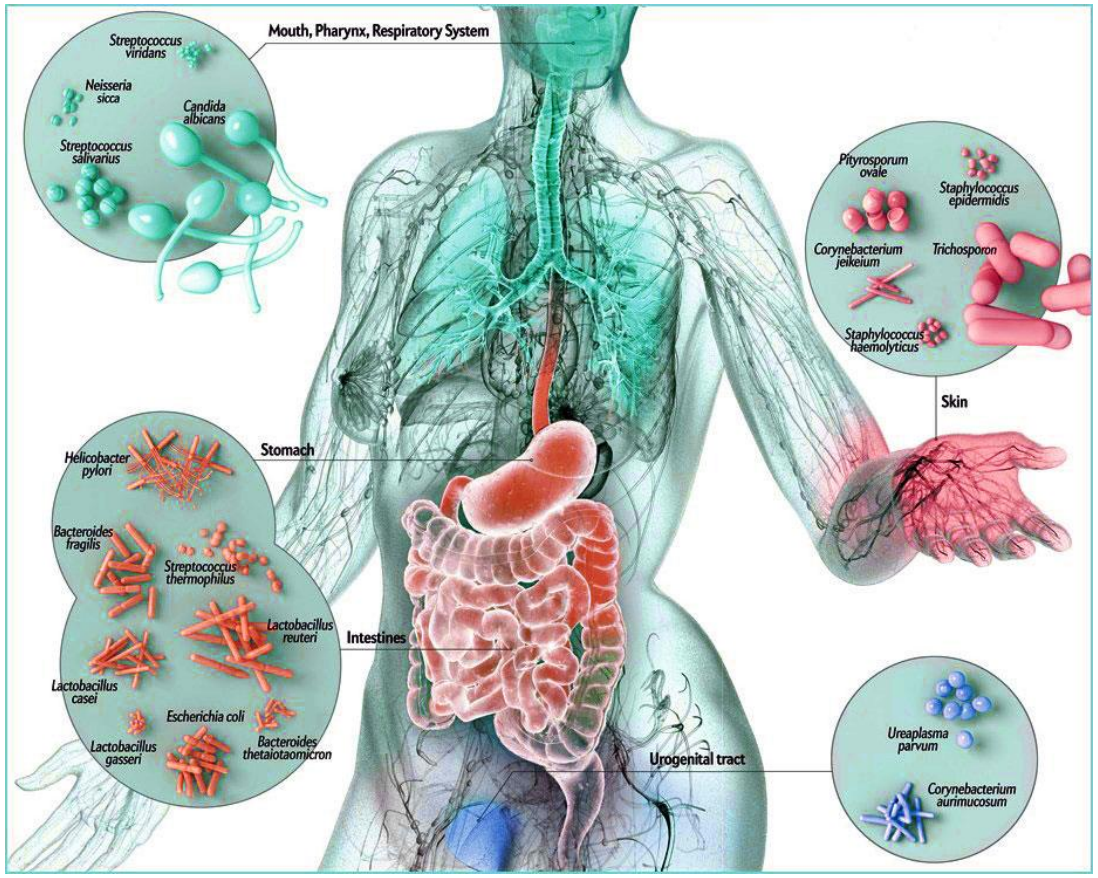


Fig. 5 Manhattan plot of genome wide distribution of F_{ST} windows for SNP dataset. Red line represents threshold of 0.3, windows value were considered candidate selection signature

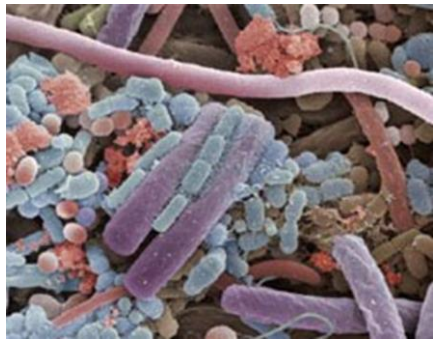
Microbioma: todos os microrganismos que vivem em um ambiente

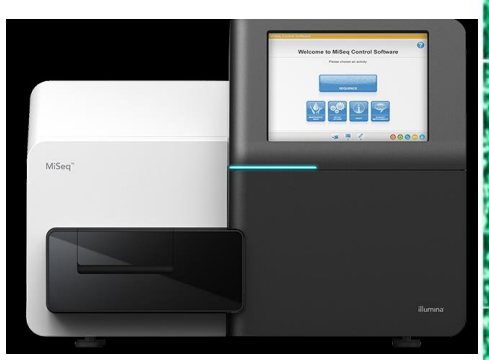


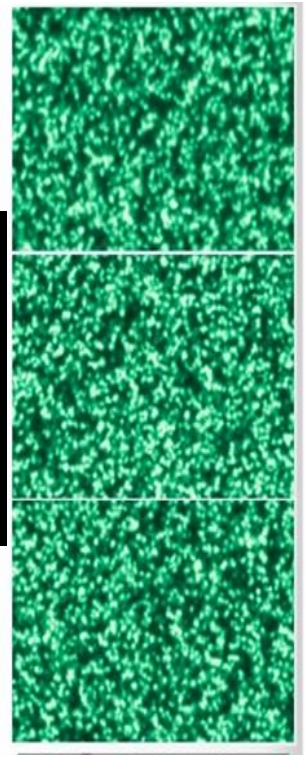
- O corpo humano tem entre 10 a 100 trilhões de células de mo
- Existe 10 vezes mais células de mo do que células humanas
- 100 vezes mais genes de mo do que genes humanos
- Mais de 10 mil espécies de mo vivendo no corpo humano

Metodologia independente de cultura

Sequenciamento de DNA







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+
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+
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+
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+
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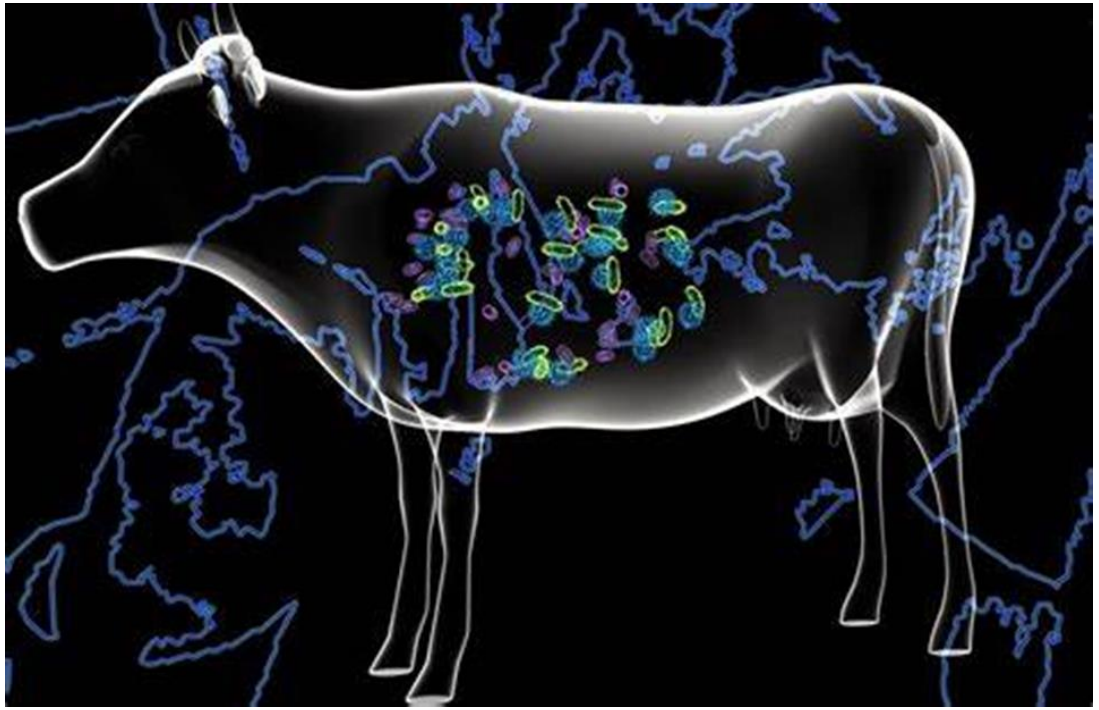
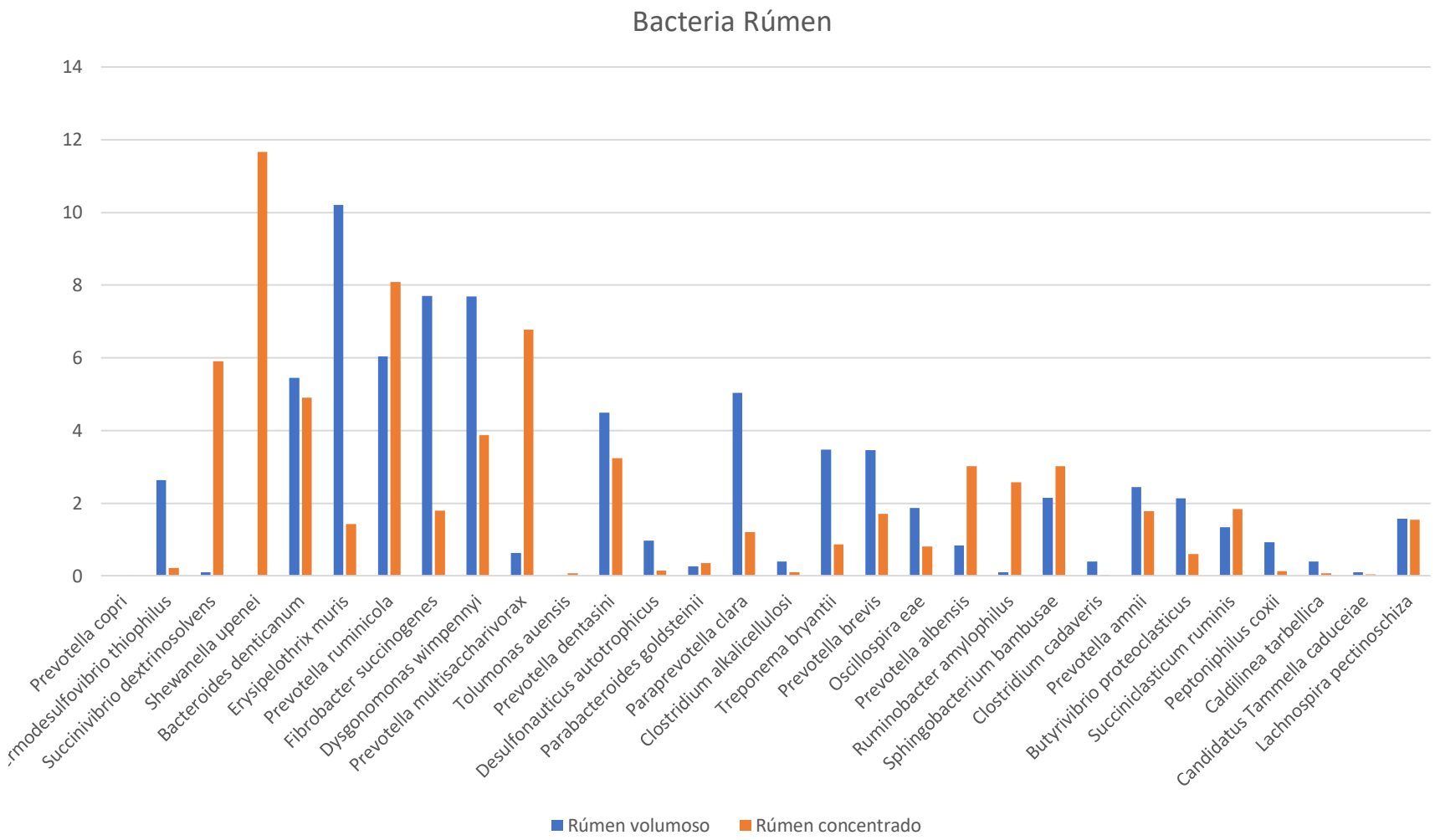
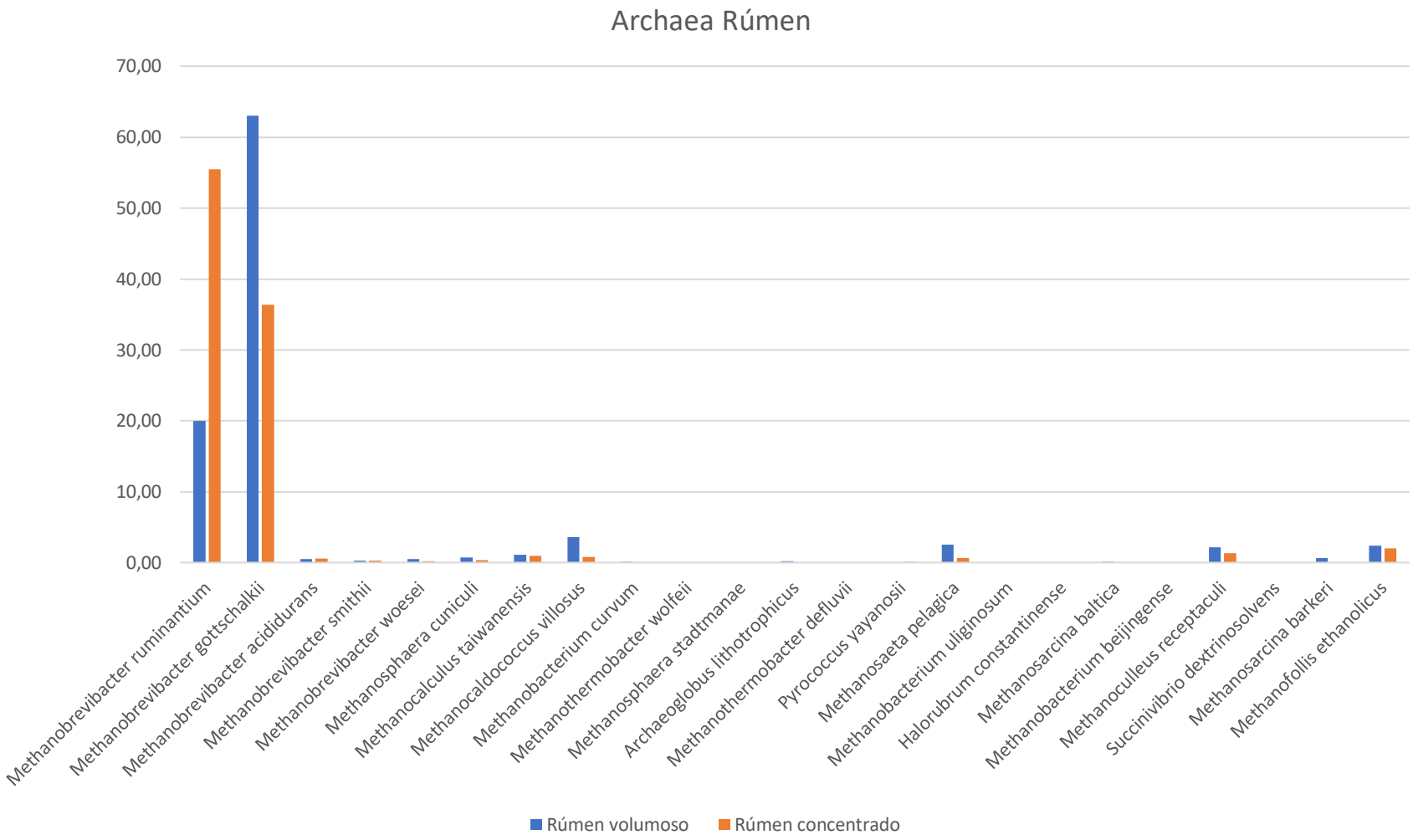
Microbioma do Rumen



- Estudo comparativo de população de microrganismos das fezes e rúmen de animais recebendo dietas de forragem ou concentrado


Bacteria Rúmen
14
12
10
8
6
4
2
0
Prevotella copri
-rmodesulfovibrio thiophilus
Succinivibrio dextrinosolvens
Shewanella upenei
Bacteroides denticanum
Erysipelothrix muris
Prevotella ruminicola
Fibrobacter succinogenes
Dysgonomonas wimpennyi
Prevotella multisaccharivorax
Tolumonas auensis
Prevotella dentasini
Desulfonauticus autotrophicus
Parabacteroides goldsteinii
Paraprevotella clara
Clostridium alkalicellulosi
Treponema bryantii
Prevotella brevis
Oscillospira eae
Prevotella albensis
Ruminobacter amylophilus
Sphingobacterium bambusae
Clostridium cadaveris
Prevotella amnii
Butyrivibrio proteoclasticus
Succiniclasticum ruminis
Peptoniphilus coxii
Caldilinea tarbellica
Candidatus Tammella caduceiae
Lachnospira pectinoschiza
Rúmen volumoso
Rúmen concentrado


Archaea Rúmen
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
Methanobrevibacter ruminantium
Methanobrevibacter gottschalkii
Methanobrevibacter acididurans
Methanobrevibacter smithii
Methanobrevibacter woesei
Methanosphaera cuniculi
Methanocalculus taiwanensis
Methanocaldococcus villosus
Methanobacterium curvum
Methanothermobacter wolfeii
Methanosphaera stadtmanae
Archaeoglobus lithotrophicus
Methanothermobacter defluvii
Pyrococcus yayanosii
Methanosaeta pelagica
Methanobacterium uliginosum
Halorubrum constantinense
Methanosarcina baltica
Methanobacterium beijingense
Methanoculleus receptaculi
Succinivibrio dextrinosolvens
Methanosarcina barkeri
Methanofollis ethanolicus
Rúmen volumoso
Rúmen concentrado

Microbioma do leite

- Qualidade nutricional do leite
- Estabilidade e tempo de prateleira
- Infecção da glândula mamária (mastite)
- Rastreamento de mo

Dados preliminares: Identificação de patógenos e prevalência em tanques de leite

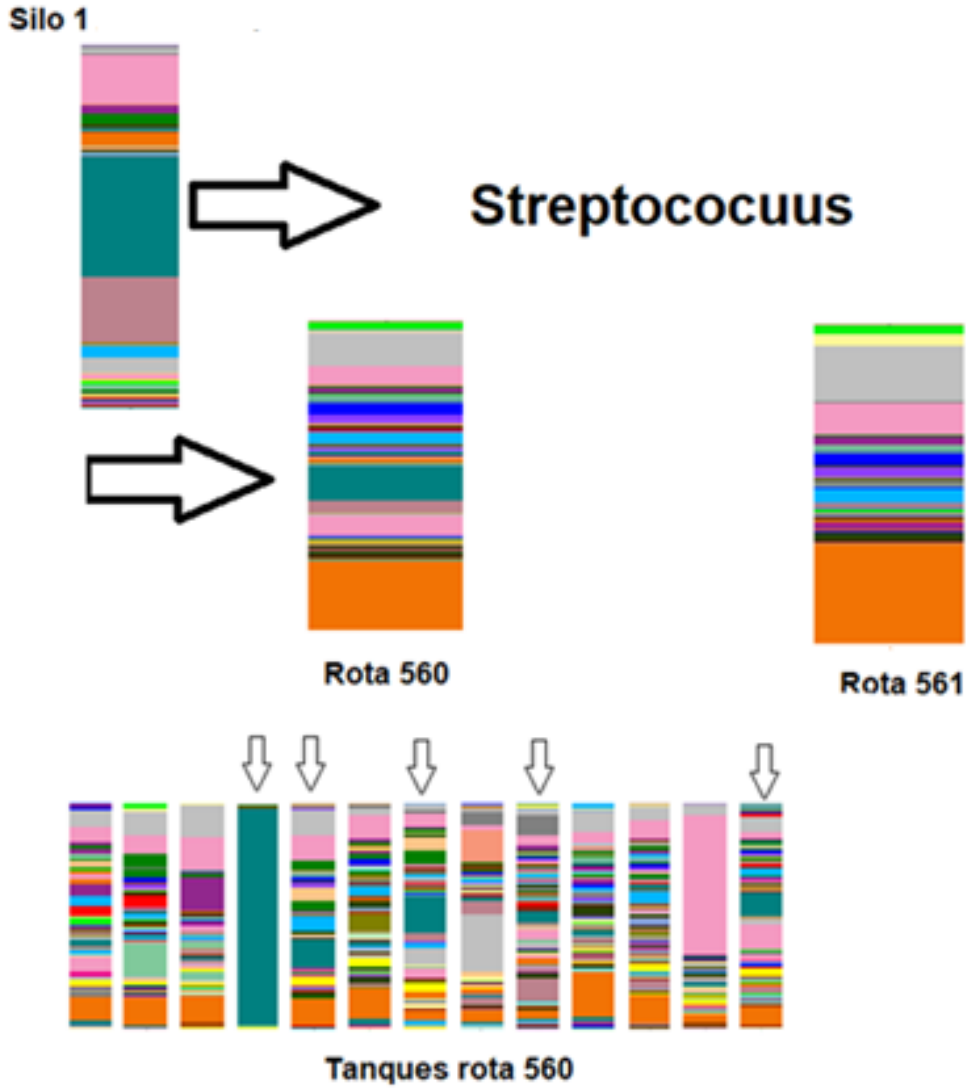
Tabela 1 – Prevalência de agentes causadores de mastite nas 36 fazendas analisadas.

Espécies	Nº fazendas/espécie
Corynebacterium_bovis	32
Staphylococcus_aureus	30
Enterococcus_faecalis	29
Streptococcus_dysgalactiae	25
Streptococcus_agalactiae	21
Enterococcus_faecium	20
Streptococcus_uberis	17
Peptoniphilus_indolicus	9
Trueperella_pyogenes	7
Serratia_marcescens	3
Mycoplasma_bovis	2

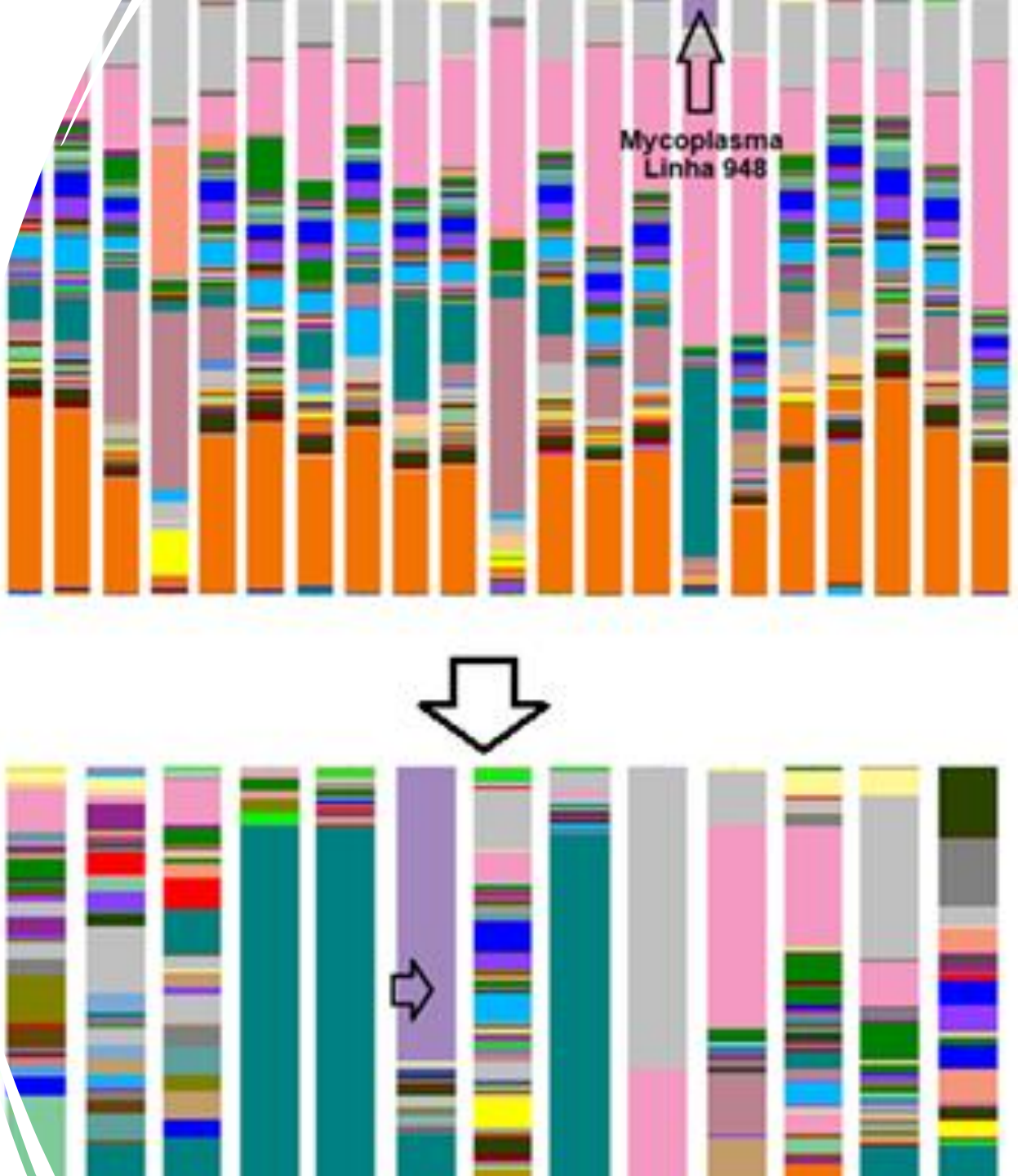
Trabalho de TCC, Giovanna Sartori

Rastreamento de contaminação

Rastreamento da bactéria *Streptococcus sp.* encontrada no Silo 1.



Rastreamento da bactéria *Mycoplasma sp*. Essa bactéria foi encontrada na linha 948, em uma proporção de 7,06% e foi rastreada sua origem em um único Tanque.



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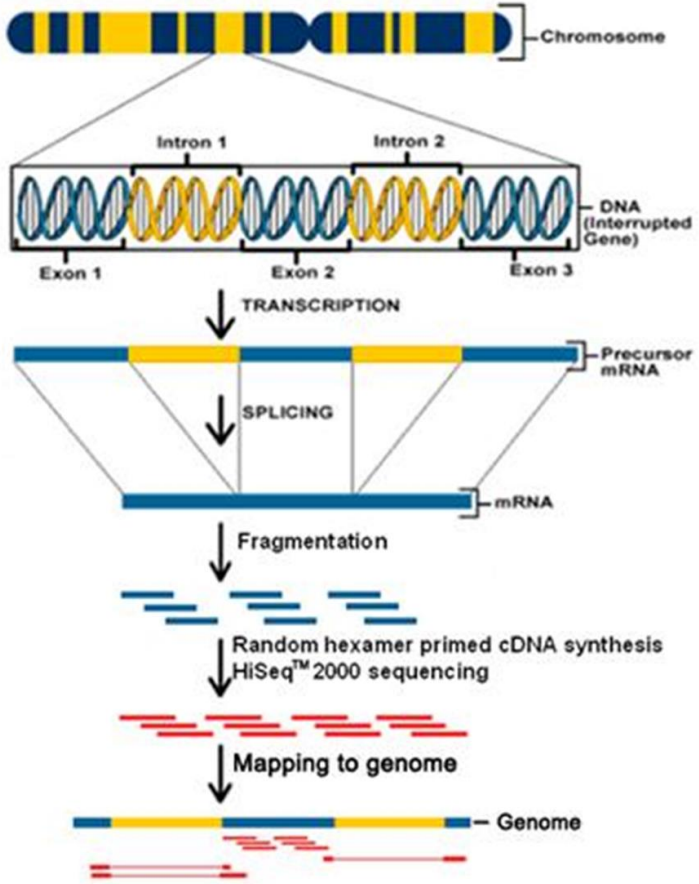
Sequenciamento de RNA: Qualidade de carne

Genes associados com gordura intramuscular



RNA Seq: Contagem de leituras para estimar a expressão gênica (exemplo ilustrativo)

FAS

IGF-2

Associação da expressão gênica com deposição de gordura intramuscular

PLOS ONE

RESEARCH ARTICLE

Putative Regulatory Factors Associated with Intramuscular Fat Content

Aline S. M. Cesar[1], Luciana C. A. Regitano[2], James E. Koltes[3], Eric R. Fritz-Waters[3], Dante P. D. Lanna[1], Gustavo Gasparin[1], Gerson B. Mourão[1], Priscila S. N. Oliveira[4], James M. Reecy[3], Luiz L. Coutinho[1]*

1 Department of Animal Science, University of São Paulo, Piracicaba, SP, 13418–900, Brazil, **2** Embrapa Southeast-Cattle Research Center, São Carlos, SP, 13560–970, Brazil, **3** Department of Animal Science, Iowa State University, Ames, IA, 50011, United States of America, **4** Department of Genetics and Evolution, Federal University of São Carlos, São Carlos, SP, 13565–905, Brazil

* llcoutinho@usp.br

OPEN ACCESS

Citation: Cesar ASM, Regitano LCA, Koltes JE, Fritz-Waters ER, Lanna DPD, Gasparin G, et al. (2015) Putative Regulatory Factors Associated with Intramuscular Fat Content. PLoS ONE 10(6): e0128350. doi:10.1371/journal.pone.0128350

Academic Editor: Roberta Davoli, University of Bologna, ITALY

Received: August 22, 2014

Accepted: April 26, 2015

Abstract

Intramuscular fat (IMF) content is related to insulin resistance, which is an important prediction factor for disorders, such as cardiovascular disease, obesity and type 2 diabetes in human. At the same time, it is an economically important trait, which influences the sensorial and nutritional value of meat. The deposition of IMF is influenced by many factors such as sex, age, nutrition, and genetics. In this study Nellore steers (*Bos taurus indicus* subspecies) were used to better understand the molecular mechanisms involved in IMF content. This was accomplished by identifying differentially expressed genes (DEG), biological pathways and putative regulatory factors. Animals included in this study had extreme genomic estimated breeding value (GEBV) for IMF. RNA-seq analysis, gene set enrichment analysis (GSEA) and co-expression network methods, such as partial correlation coefficient with information theory (PCIT), regulatory impact factor (RIF) and phenotypic impact factor (PIF) were utilized to better understand intramuscular adipogenesis. A total of 16,101 genes were

Cesar *et al. BMC Genomics* (2018) 19:499
https://doi.org/10.1186/s12864-018-4871-y

BMC Genomics

RESEARCH ARTICLE Open Access

Identification of putative regulatory regions and transcription factors associated with intramuscular fat content traits

Aline S. M. Cesar[1,2], Luciana C. A. Regitano[3], James M. Reecy[2], Mirele D. Poleti[1], Priscila S. N. Oliveira[3], Gabriella B. de Oliveira[1], Gabriel C. M. Moreira[1], Maurício A. Mudadu[4], Polyana C. Tizioto[1], James E. Koltes[2], Elyn Fritz-Waters[2], Luke Kramer[2], Dorian Garrick[5], Hamid Beiki[2], Ludwig Geistlinger[3], Gerson B. Mourão[1], Adhemar Zerlotini[4] and Luiz L. Coutinho[1*]

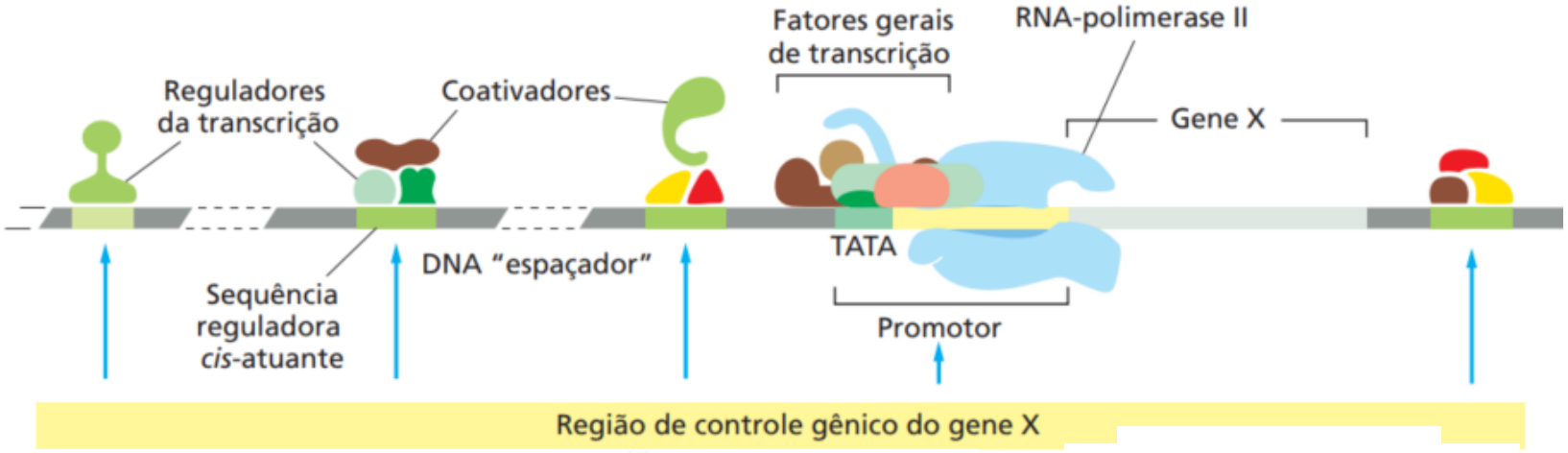
Abstract

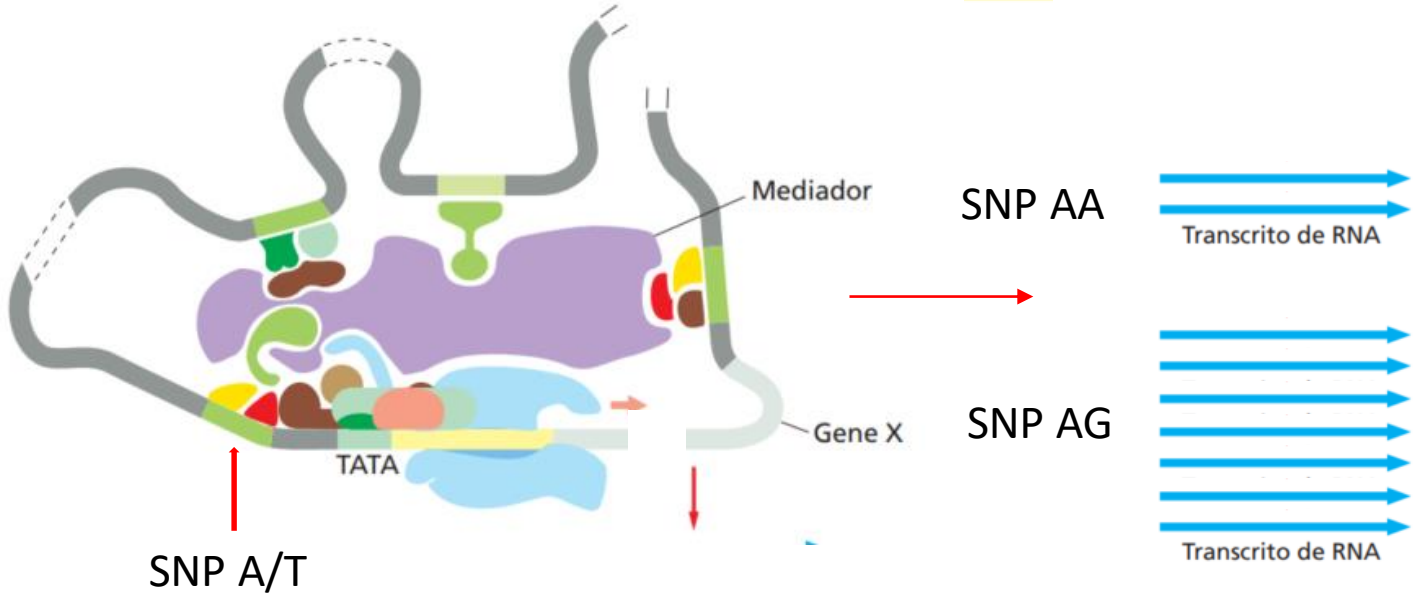
Background: Integration of high throughput DNA genotyping and RNA-sequencing data allows for the identification of genomic regions that control gene expression, known as expression quantitative trait loci (eQTL), on a whole genome scale. Intramuscular fat (IMF) content and carcass composition play important roles in metabolic and physiological processes in mammals because they influence insulin sensitivity and consequently prevalence of metabolic diseases such as obesity and type 2 diabetes. However, limited information is available on the genetic variants and mechanisms associated with IMF deposition in mammals. Thus, our hypothesis was that eQTL analyses could identify putative regulatory regions and transcription factors (TFs) associated with intramuscular fat (IMF) content traits.

Results: We performed an integrative eQTL study in skeletal muscle to identify putative regulatory regions and factors associated with intramuscular fat content traits. Data obtained from skeletal muscle samples of 192 animals was used for association analysis between 461,466 SNPs and the transcription level of 11,808 genes. This yielded 1268 *cis*- and 10,334 *trans*-eQTLs, among which we identified nine hotspot regions that each affected the expression of > 119 genes. These putative regulatory regions overlapped with previously identified QTLs for IMF content. Three of the hotspots respectively harbored the transcription factors *USF1*, *EGR4* and *RUNX1T1*, which are known to play important roles in lipid metabolism. From co-expression network analysis, we further identified modules significantly correlated with IMF content and associated with relevant processes such as fatty acid metabolism, carbohydrate metabolism and lipid metabolism.

Conclusion: This study provides novel insights into the link between genotype and IMF content as evident from

Identificação de regiões que controlam a expressão gênica





SNP em regiões regulatórias podem modular a expressão

Adaptado de Alberts, 2017

Journal of Proteomics
Volume 179, 15 May 2018, Pages 30-41

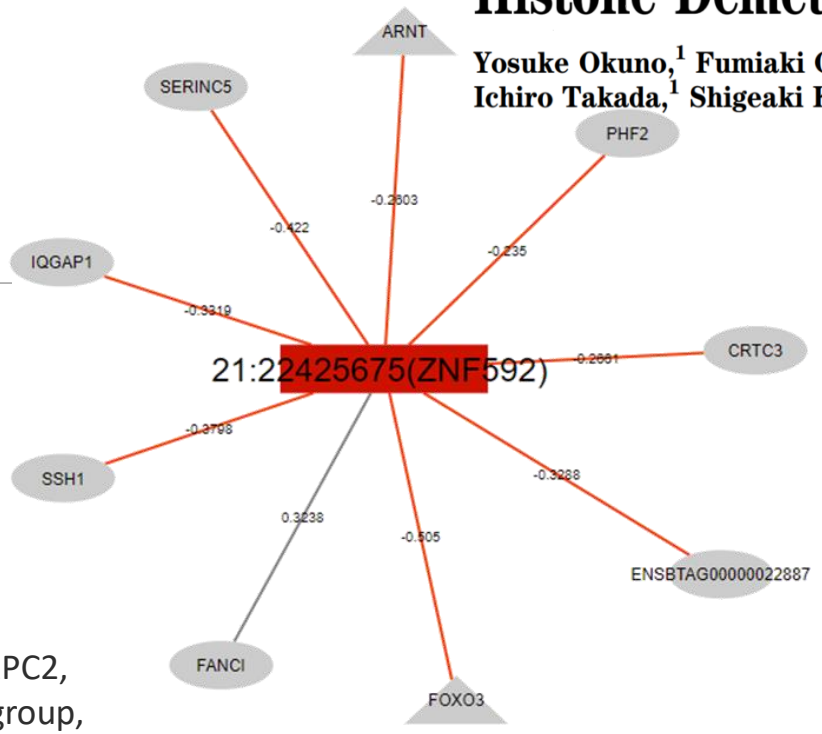
Longissimus dorsi muscle label-free quantitative proteomic reveals biological mechanisms associated with intramuscular fat deposition

Mirele D. Poleti [a], Luciana C.A. Regitano [b], Gustavo H.M.F. Souza [c], Aline S.M. Cesar [a], Rosineide C. Simas [a, 1], Bárbara Silva-Vignato [d], Gabriella B. Oliveira [a, 2], Sónia C.S. Andrade [a, 3], Luiz C. Cameron [e, f], Luiz L. Coutinho [a]

Most of the actin-binding proteins (ACTN1, ARPC2, SSH1, TTN) was down-regulated in the H IMF group, suggesting a cellular rearrangement to make space for the adipocyte

Epigenetic Regulation of Adipogenesis by PHF2 Histone Demethylase

Yosuke Okuno,[1] Fumiaki Ohtake,[1] Katsuhide Igarashi,[2] Jun Kanno,[2] Takahiro Matsumoto,[1] Ichiro Takada,[1] Shigeaki Kato,[3] and Yuuki Imai[1]



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC

Article

CRTC3 Regulates the Lipid Metabolism and Adipogenic Differentiation of Porcine Intramuscular and Subcutaneous Adipocytes by Activating the Calcium Pathway

Jiaqi Liu, Liyi Wang, Wentao Chen, Jie Li, and Tizhong Shan*

Inflammation Research (2021) 70:591–603
https://doi.org/10.1007/s00011-021-01463-0

Inflammat

ORIGINAL RESEARCH PAPER

FOXO3a regulates lipid accumulation and adipocyte inflammation in adipocytes through autophagy

Role of FOXO3a in obesity

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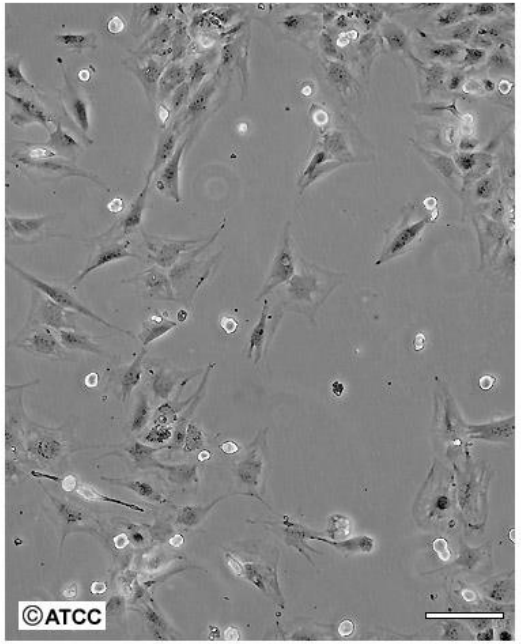
Universidade de São Paulo

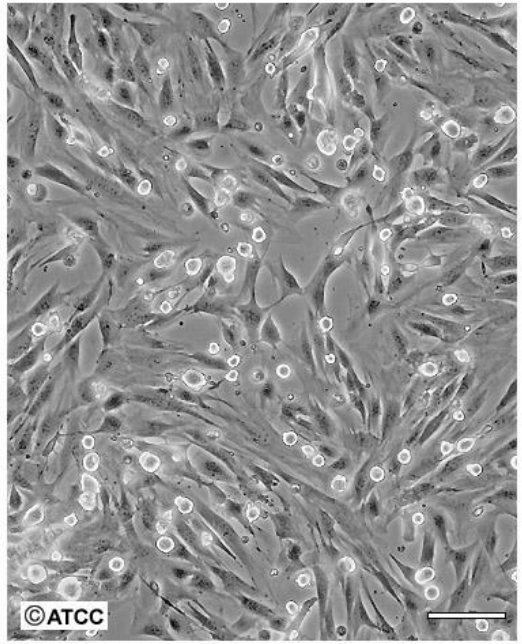
USP

Cultura de Células (células musculares)

ATCC Number: **CRL-1772**
Designation: **C2C12**



Low Density Scale Bar = 100µm



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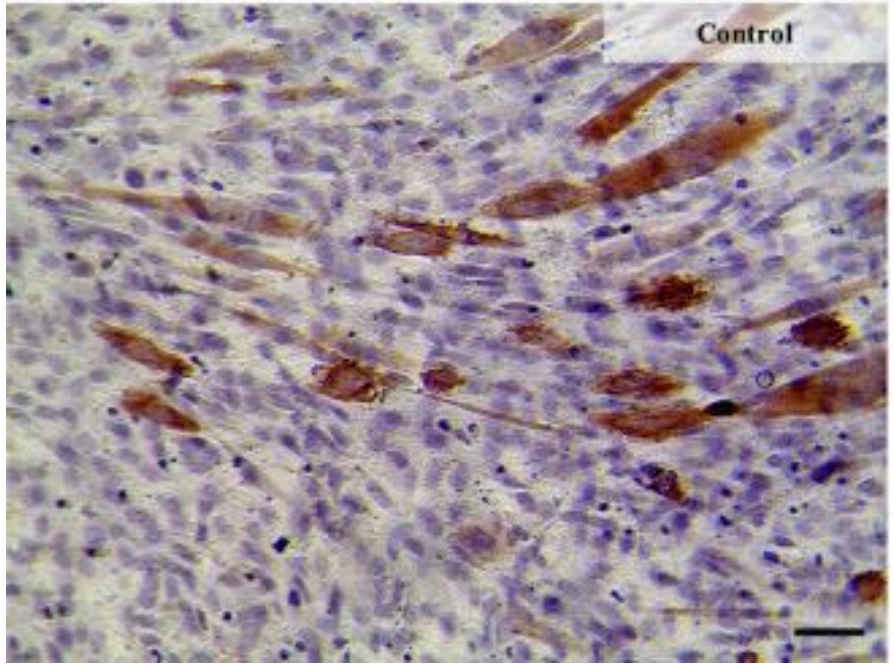
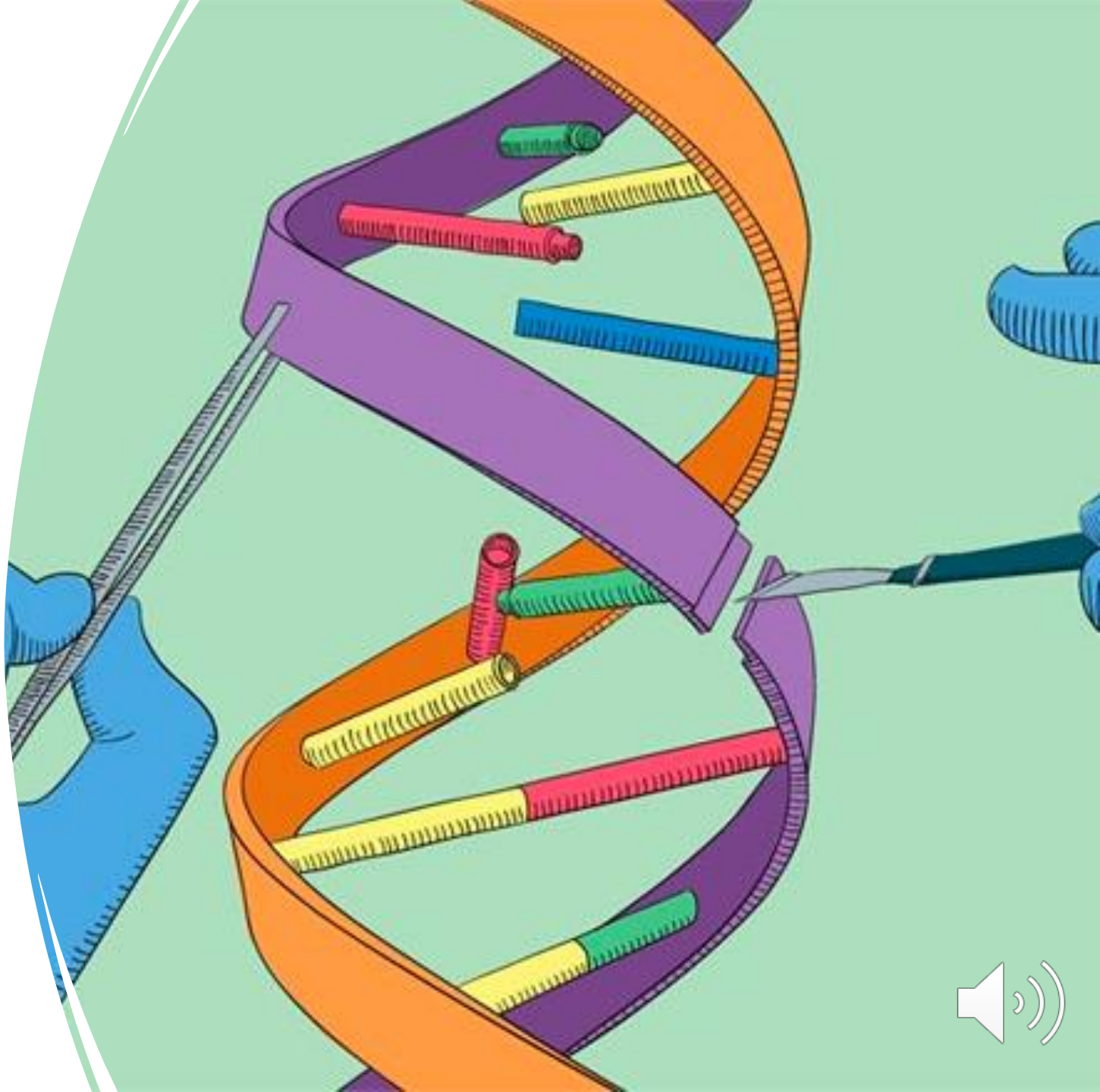


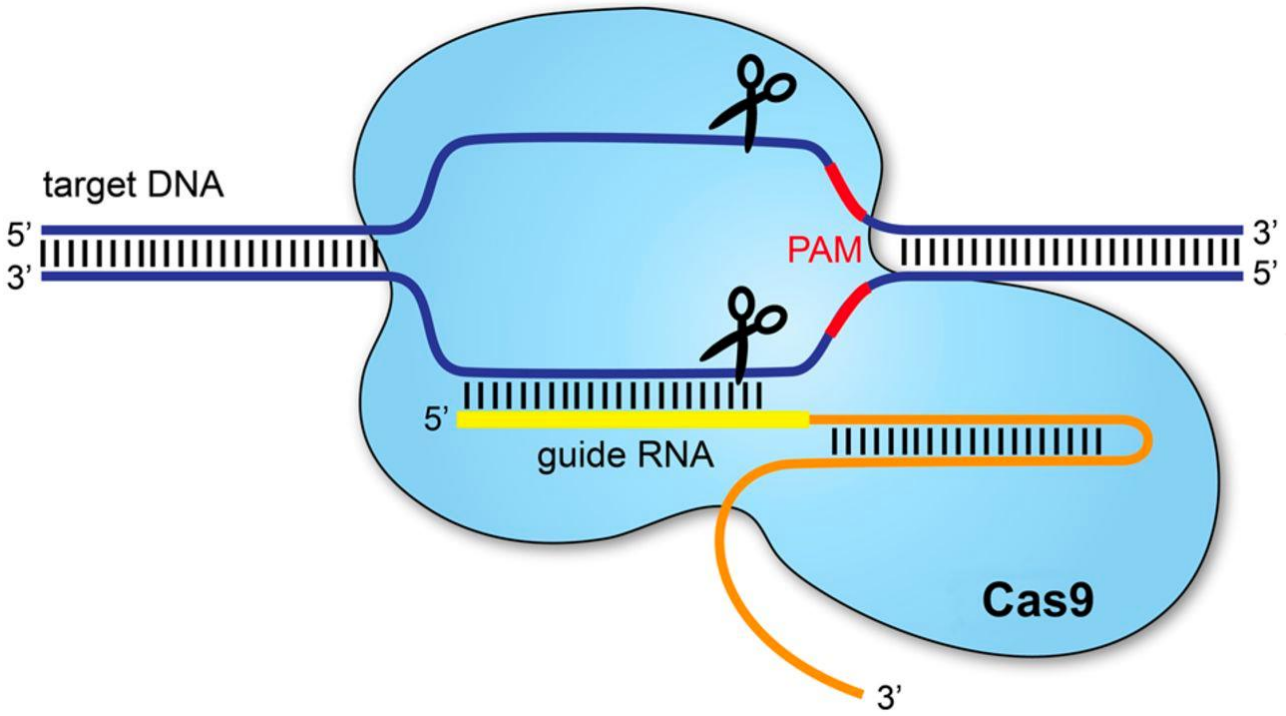
Edição Gênica

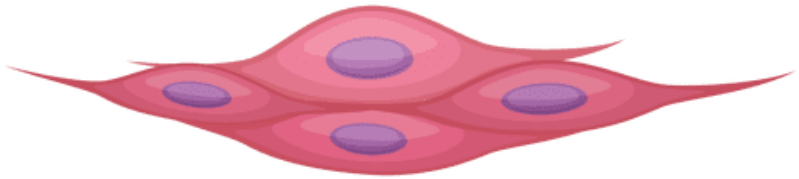
Validação de gene candidato para ao desenvolvimento muscular por meio de edição gênica (CRISPR/Cas9)

Objetivo: Identificar genes candidatos à regulação do desenvolvimento muscular em linhagem de frango de corte e testar o efeito da edição de um desses genes em culturas de células.



CRISPR/Cas9





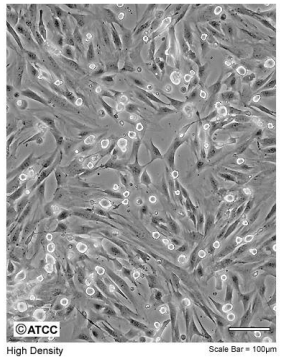
Ideia

Gene candidato: *SAP30*

Induzir a edição do gene em cultura celular (C2C12) e acompanhar as alterações no fenótipo das células, bem como a expressão diferencial de genes entre os grupos controle x tratado.

Cultura celular e imunocitoquímica

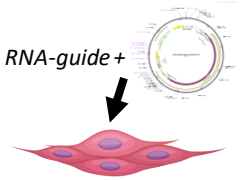
C2C12 cells
˜70% confluência



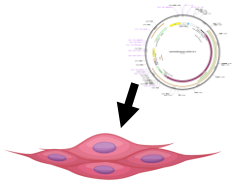
Meio de crescimento + transfecção

Tratado SAP30

RNA-guide +



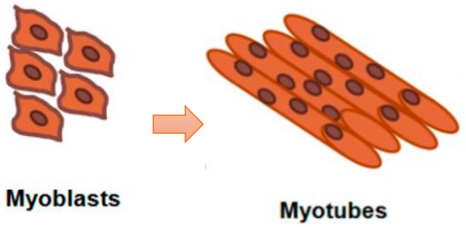
Controle SAP30



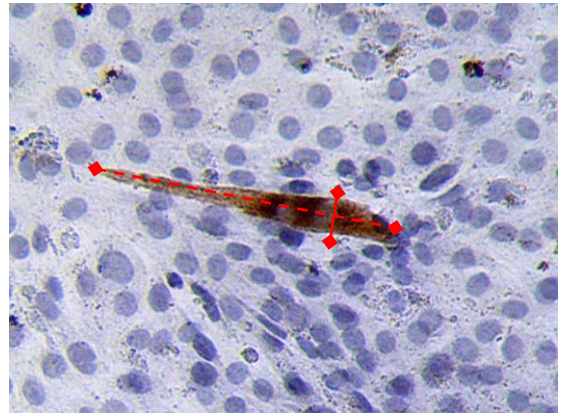
Isolamento dos clones

Mecanismo de reparo celular diferente em cada célula

˜70% confluência
Meio de diferenciação
3 dias



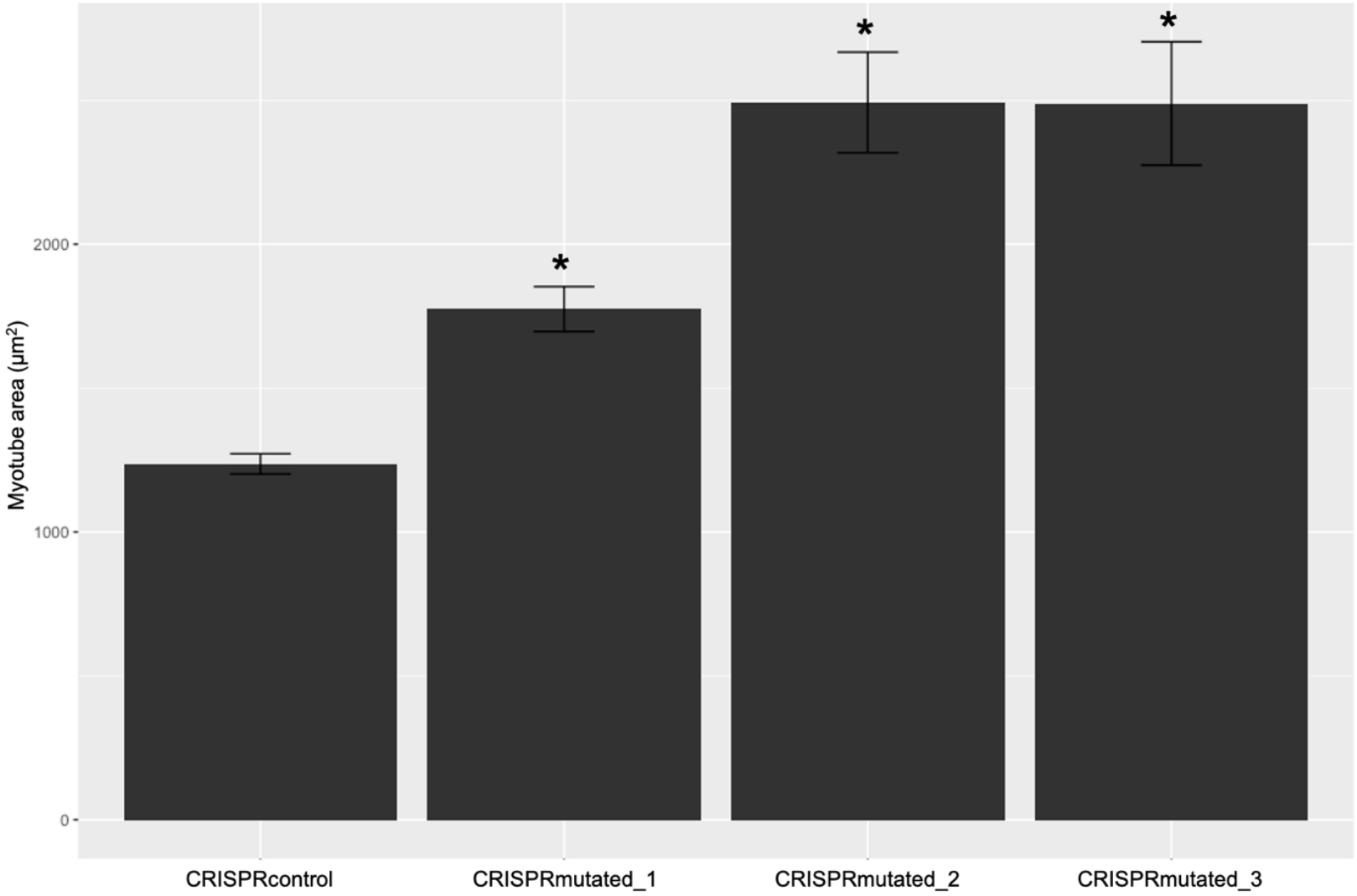
Imunocitoquímica e Análises morfométricas



- RNA-guia para gene SAP30
- Transfecção com Lipofectamina 3000

- Imagens das células *SAP30* CRISPR foram obtidas após ensaio de imunocitoquímica com marcador MF20.
- As células marcadas (positivas para MF20) são aquelas que se diferenciam em miotubos (200x).
- Análises morfométricas para caracterização de tamanho de área dos miotubos marcados com MF20 foram realizadas (comprimento x al[illegible]a).

Area dos miotubos



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Biotecnologia Animal

Luiz Lehmann Coutinho

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