

**TÉCNICAS
BÁSICAS
DE
BIOLOGIA MOLECULAR II**





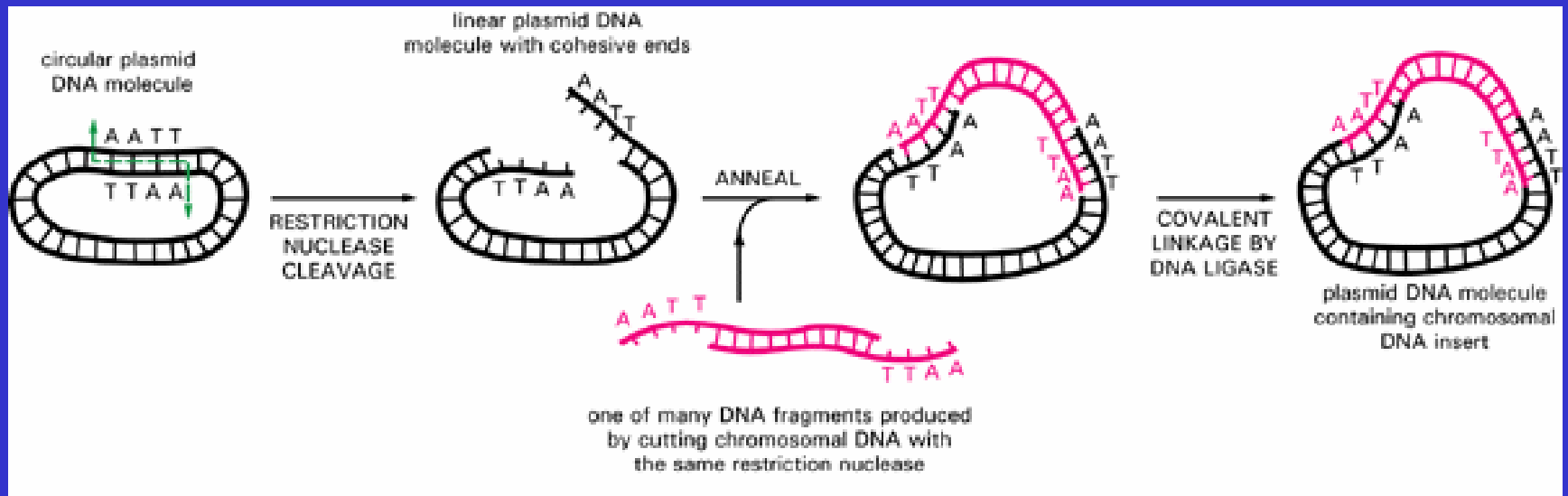
**BIBLIOTECAS: O QUE SÃO,
PARA QUE SERVEM?**

VÁRIOS OBJETIVOS

- BUSCA DE UM GENE ESPECÍFICO
- PROJETOS GENOMA

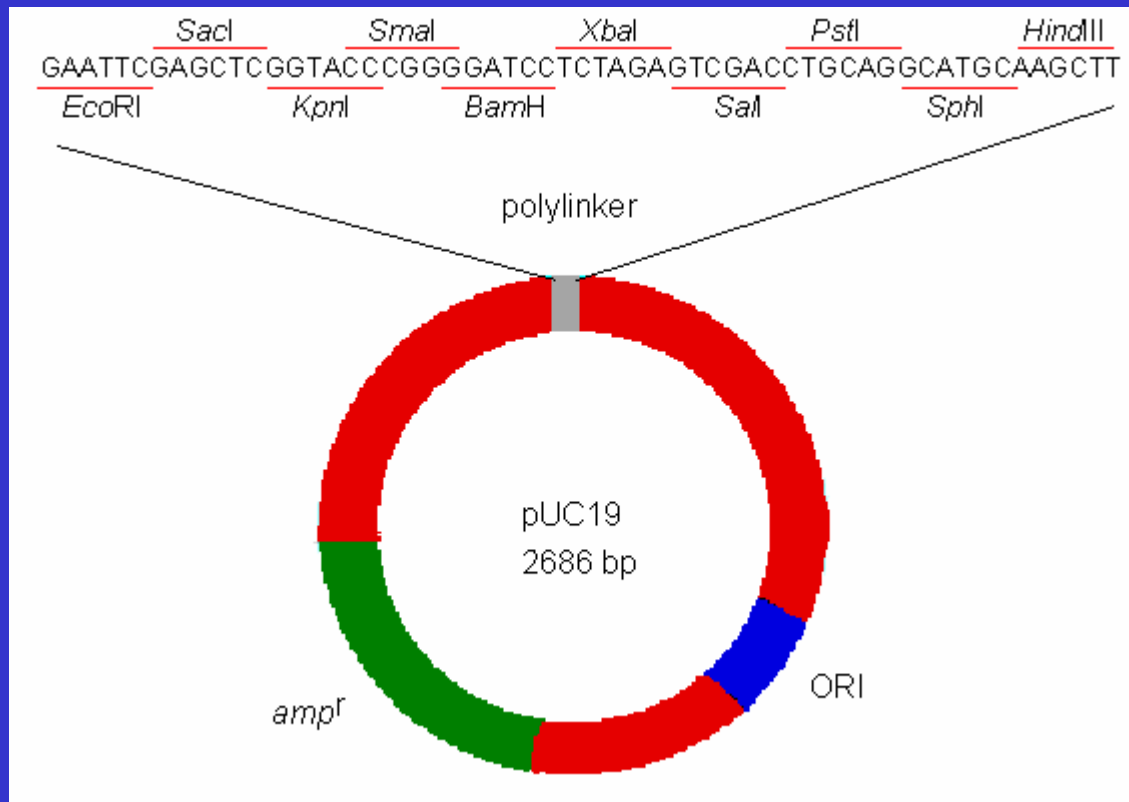
VÁRIOS VETÔRES

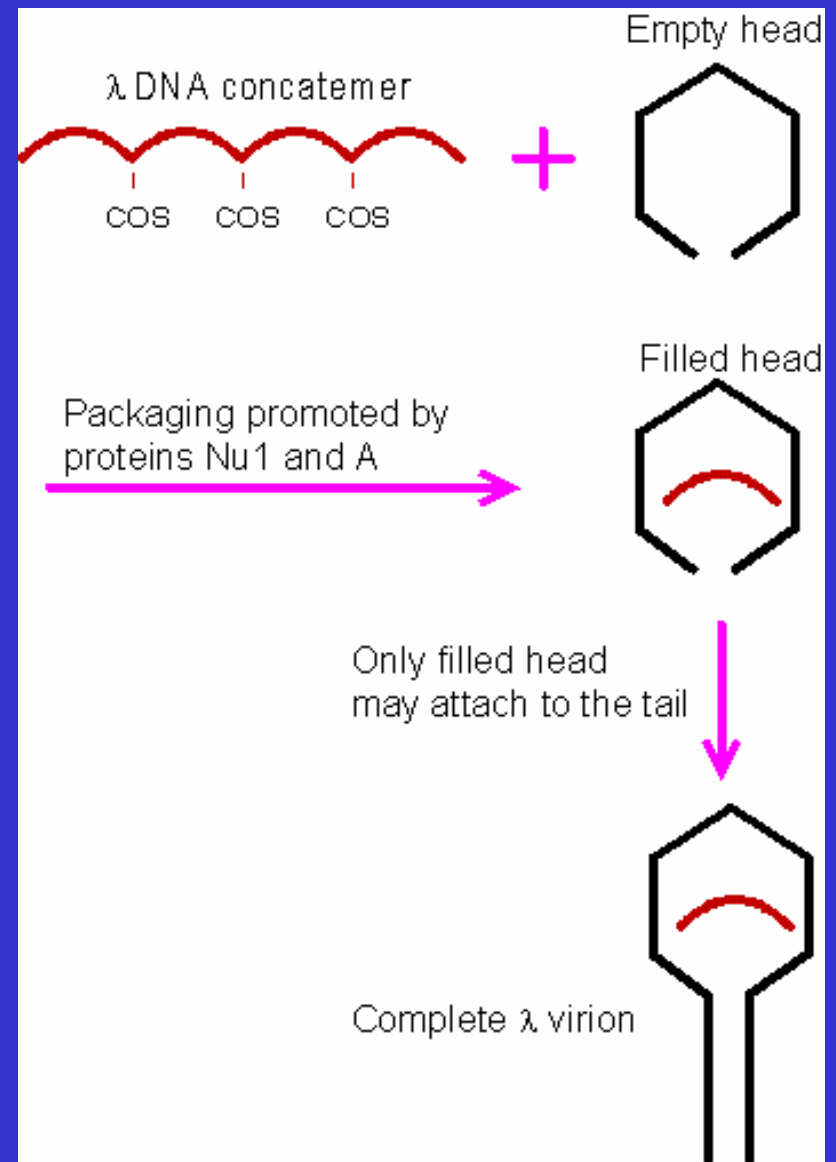
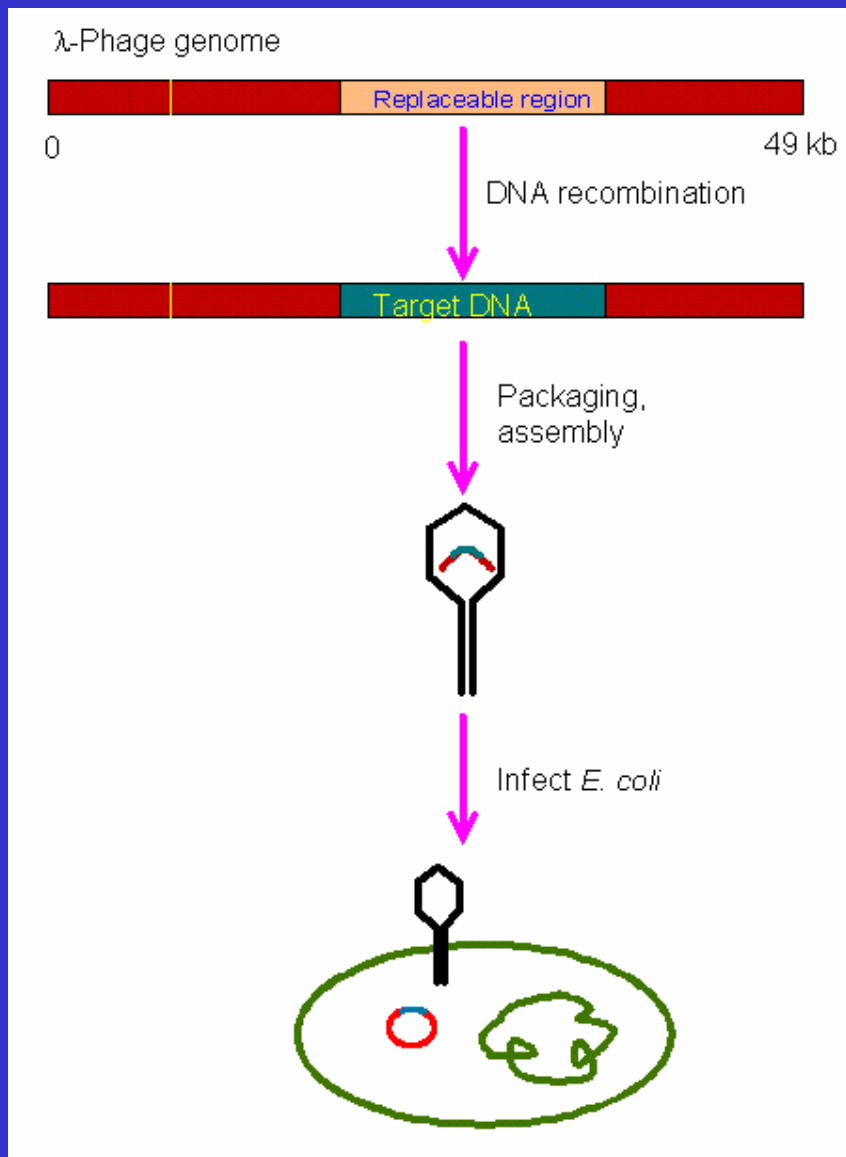
- PLASMÍDIO
- FAGO
- COSMÍDIO
- CROMOSOMAS ARTIFICIAIS DE BACTÉRIA OU LEVEDURA.



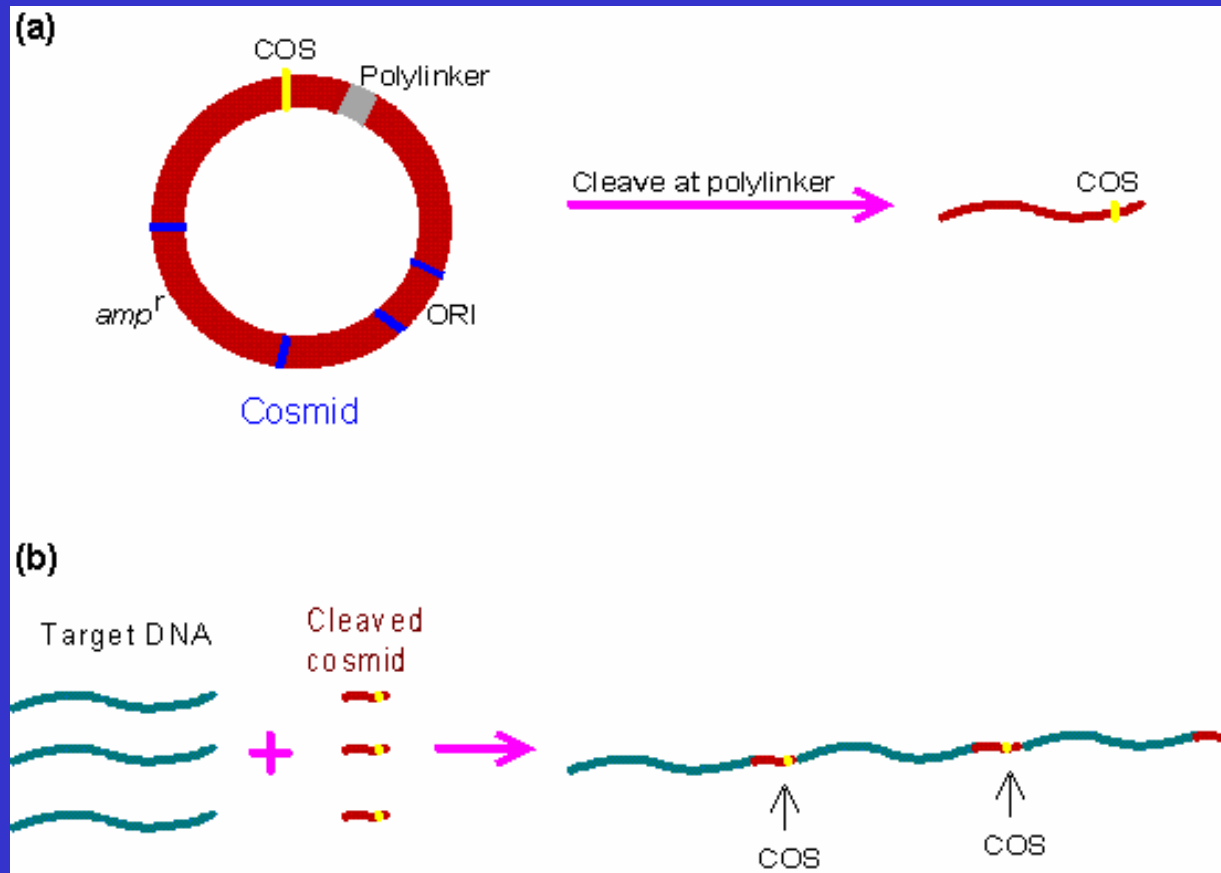
A CONSTRUÇÃO DE UMA BIBLIOTECA DE QUALQUER TIPO VAI SEMPRE COMEÇAR COM UMA CLONAGEM MOLECULAR.

VETOR PLASMÍDIO

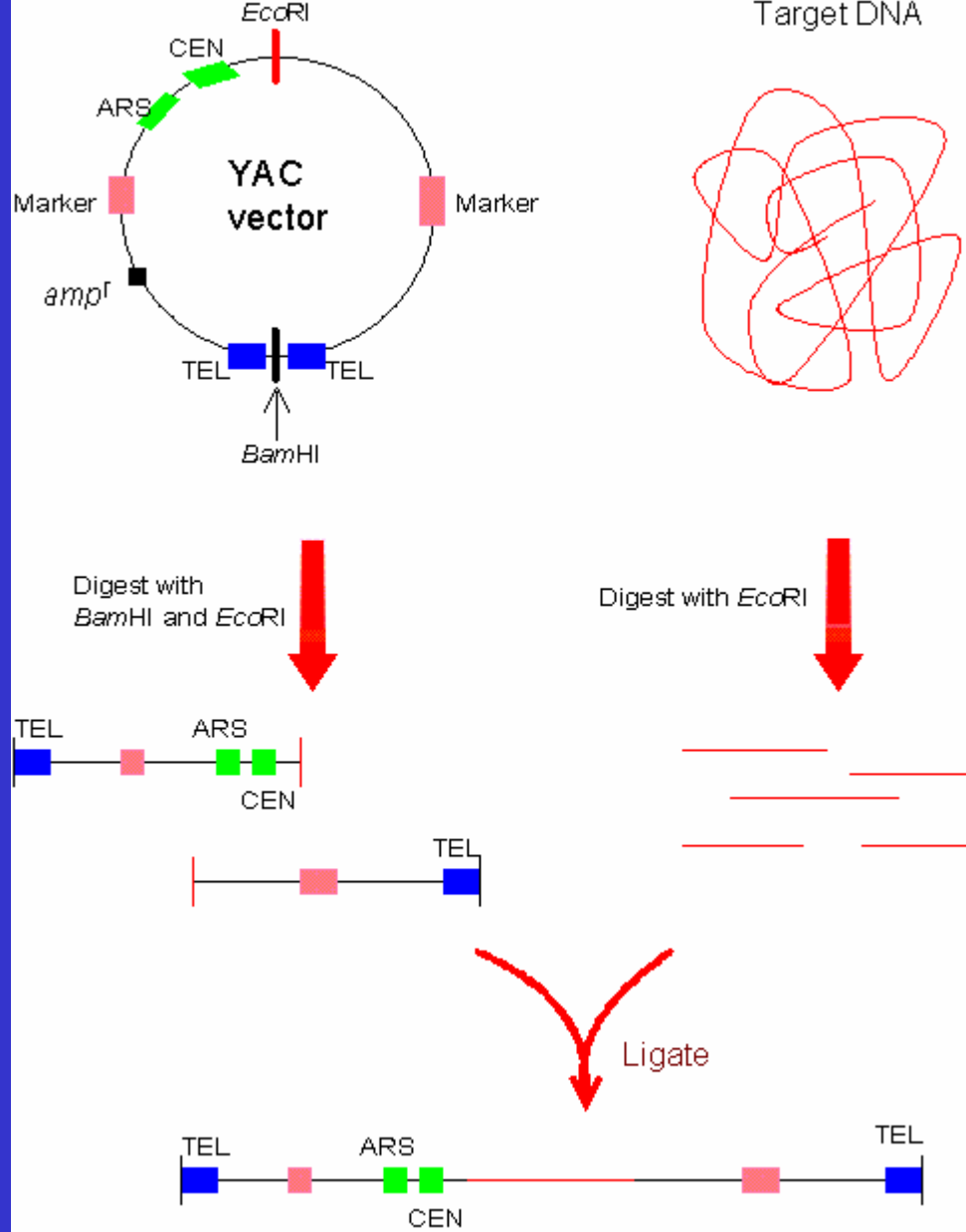




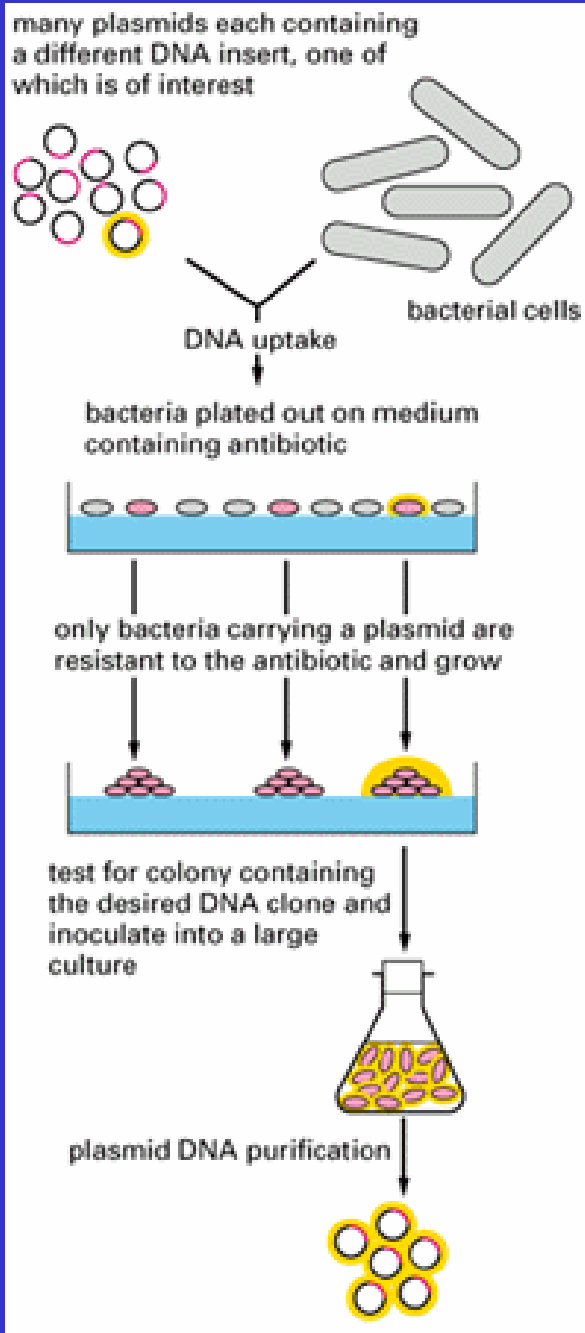
VETOR LAMBDA



VETOR COSMÍDIO

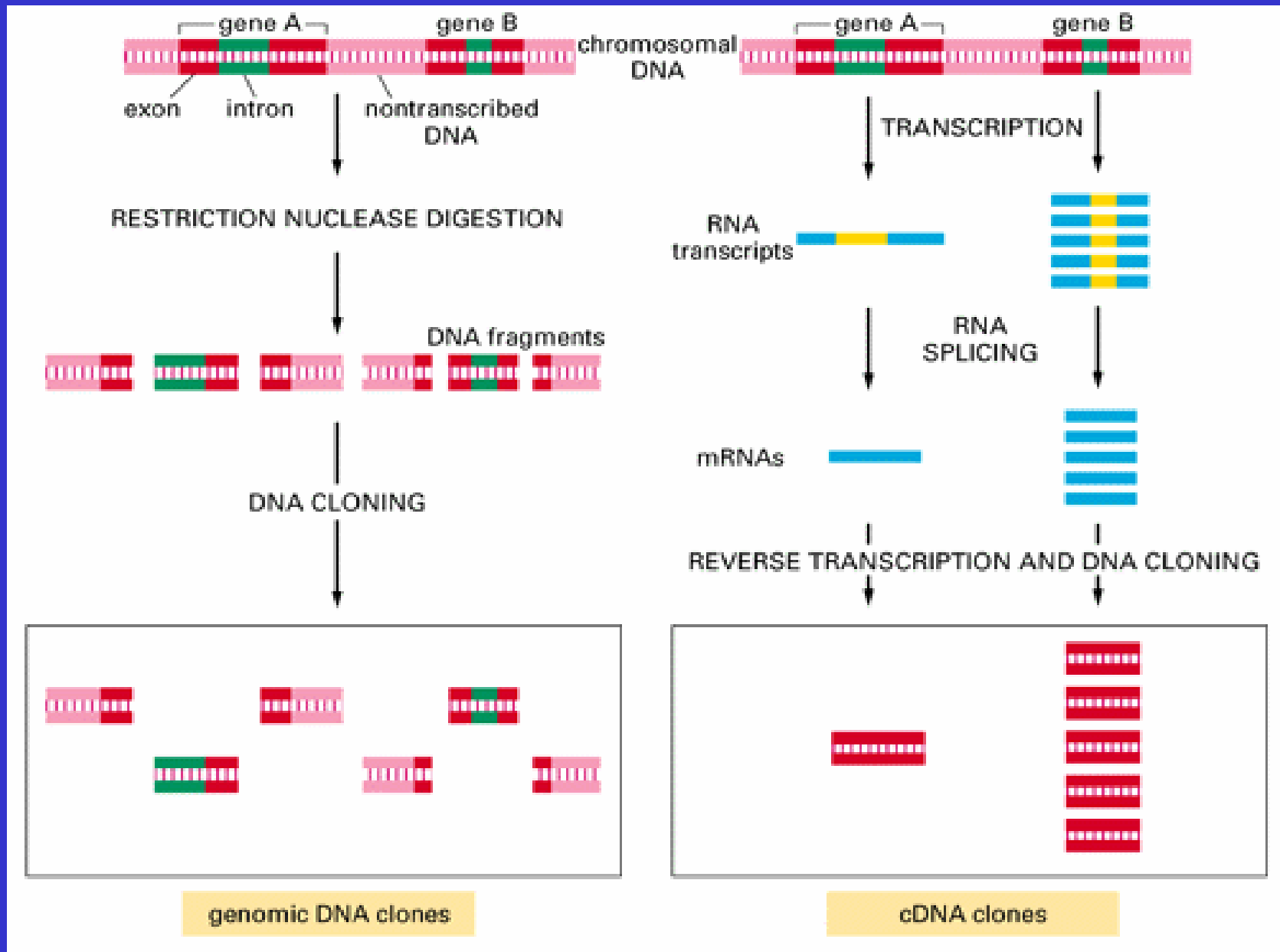


CROMOSOMA ARTIFICIAL DE LEVEDURA



**O DNA CLONADO É
AMPLIFICADO POR UMA
BACTÉRIA**

QUANDO GENÔMICA E QUANDO DE EXPRESSÃO?



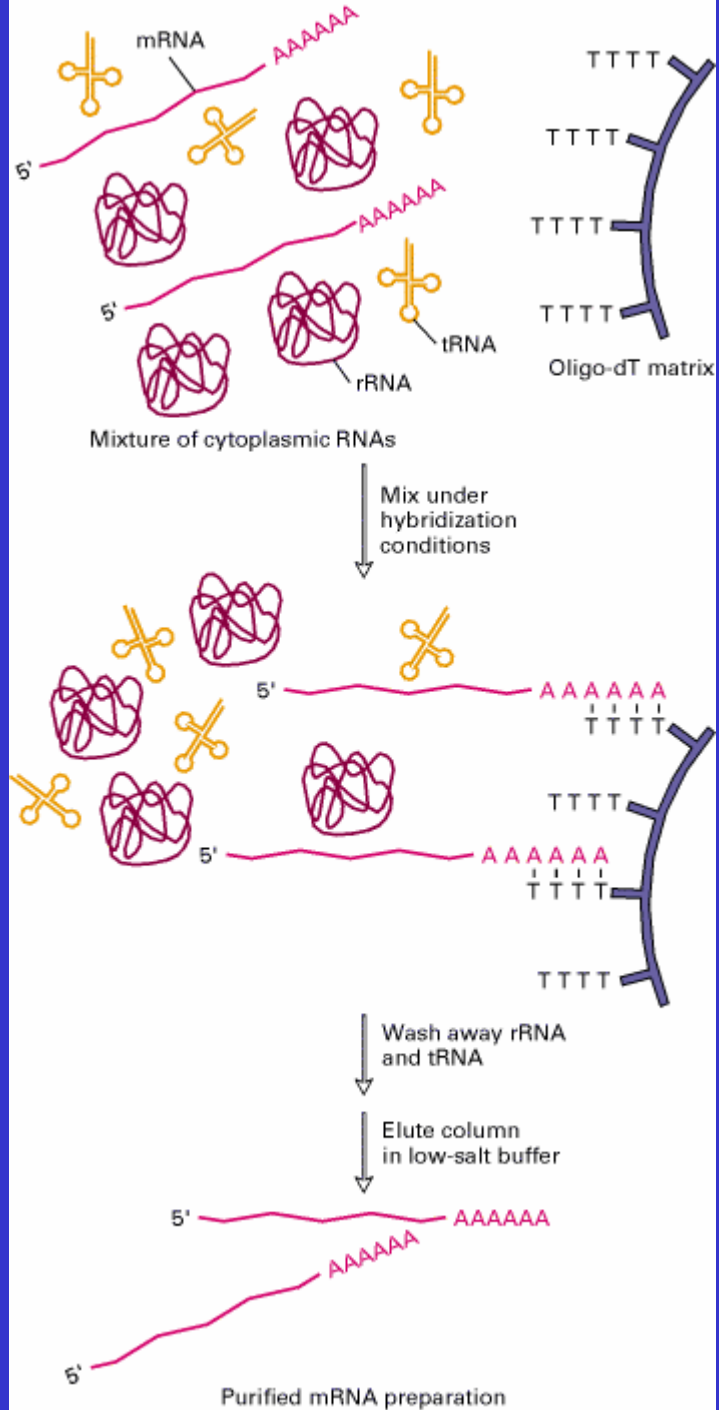
Tamanho máximo aproximado de DNA que pode ser clonado em vários tipos de vetôres

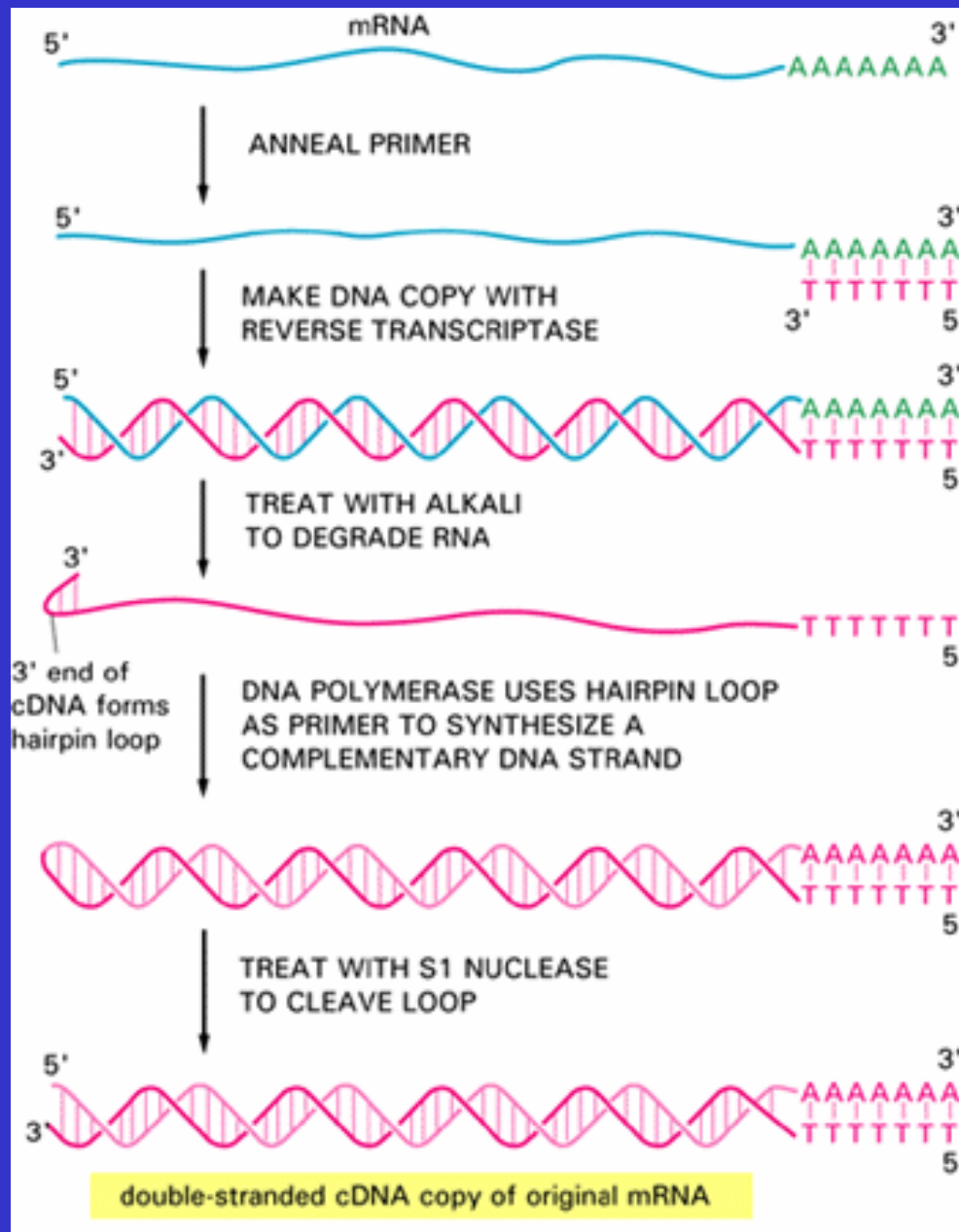
Vector Type	Cloned DNA (kb)
Plasmid	20
λ phage	25
Cosmid	45
BAC (bacterial artificial chromosome)	300
YAC (yeast artificial chromosome)	1000

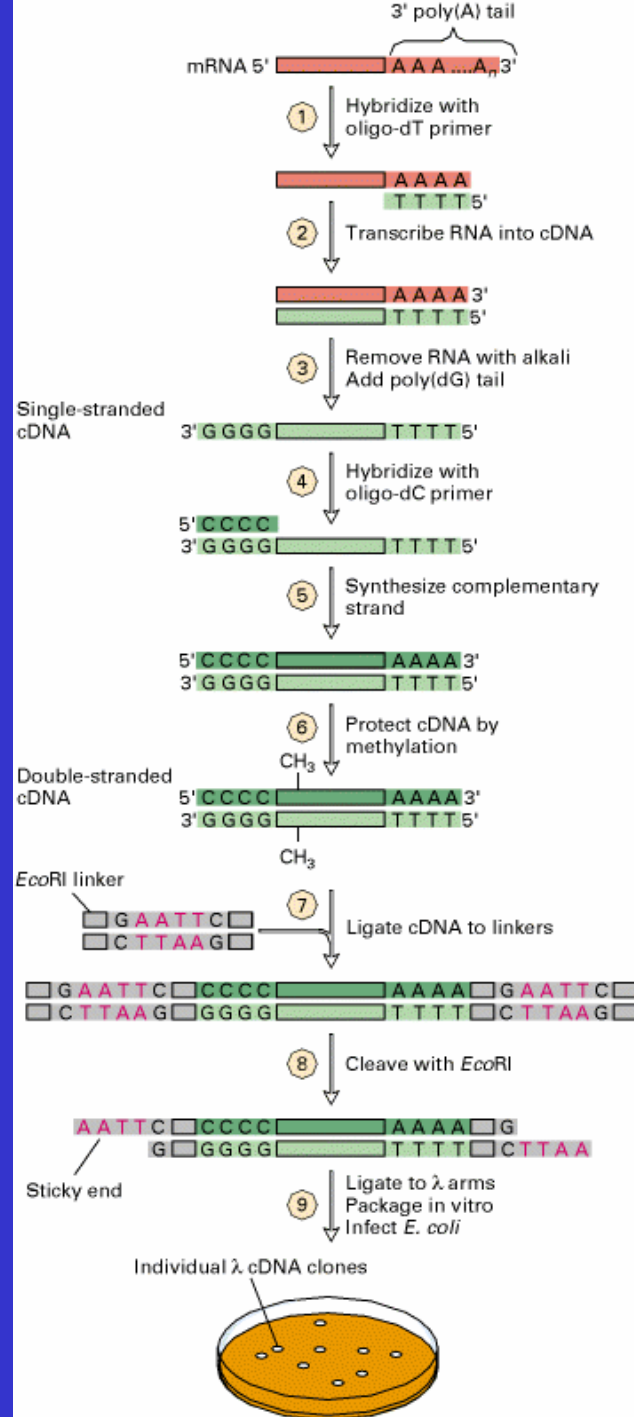
BIBLIOTECA DE EXPRESSÃO

A PARTIR DE mRNA, PORTANTO...

- só regiões transcritas
- especificidades, por exemplo, de tecidos
- células especializadas, mais fácil de isolar genes específicos:
eritrócitos->hemoglobina
- sequencias não interrompidas dos genes (sem introns)

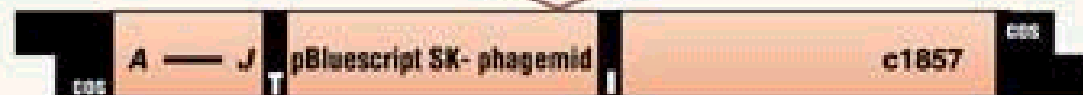




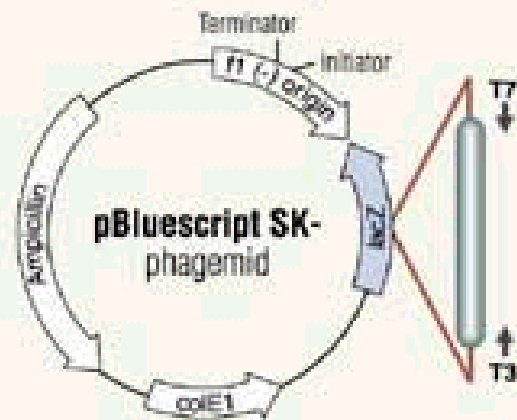




1. Construct DNA library
2. Isolate positive clone



3. Excise the pBluescript plasmid containing the cloned DNA insert by co-infection with helper phage



Vetor Lambda ZAP[®] II (Stratagene)

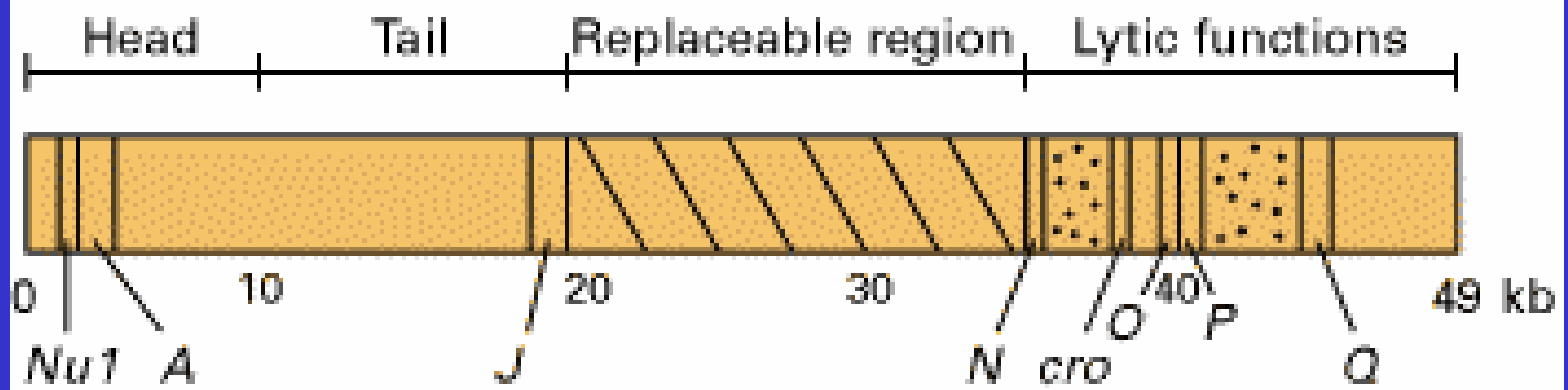
- Vetor lambda de alta eficiência com versatilidade de plasmídeo.
- Excisão *in vivo* de fragmentos clonados em vetor fagemídeo.
- Expressão de proteínas de fusão sob o controle de um promotor lac.
- Sondagem com anticorpos ou sondas de ácidos nucleicos.

Aplicações

- Construção de bibliotecas de cDNA em vetor lambda excisável.
- Bibliotecas genômicas de organismos com genomas pequenos.

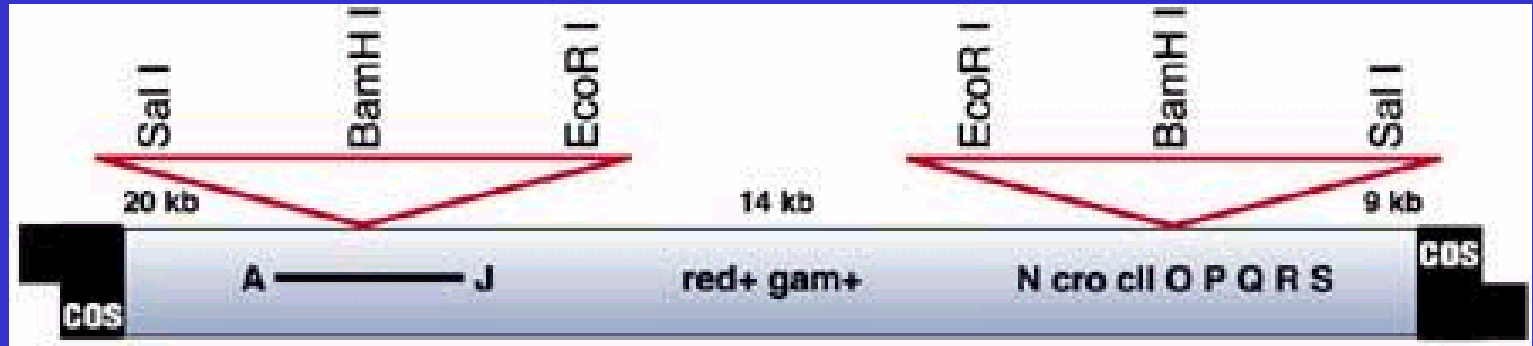
BIBLIOTECA GENÔMICA

(b) λ Phage genome



GENOMA SIMPLIFICADO DE UM FAGO LAMBDA.

MAPA DE EMBL3



Applications

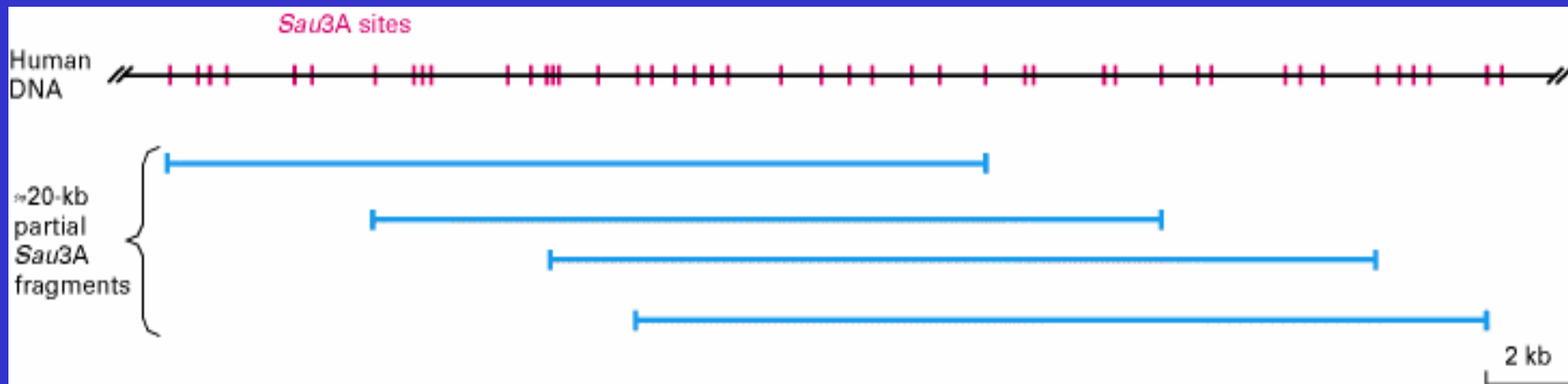
- High-quality genomic libraries
- High-resolution restriction mapping and rapid chromosomal walking

Cloning Capacity

- 9-23 kb

Host Strains

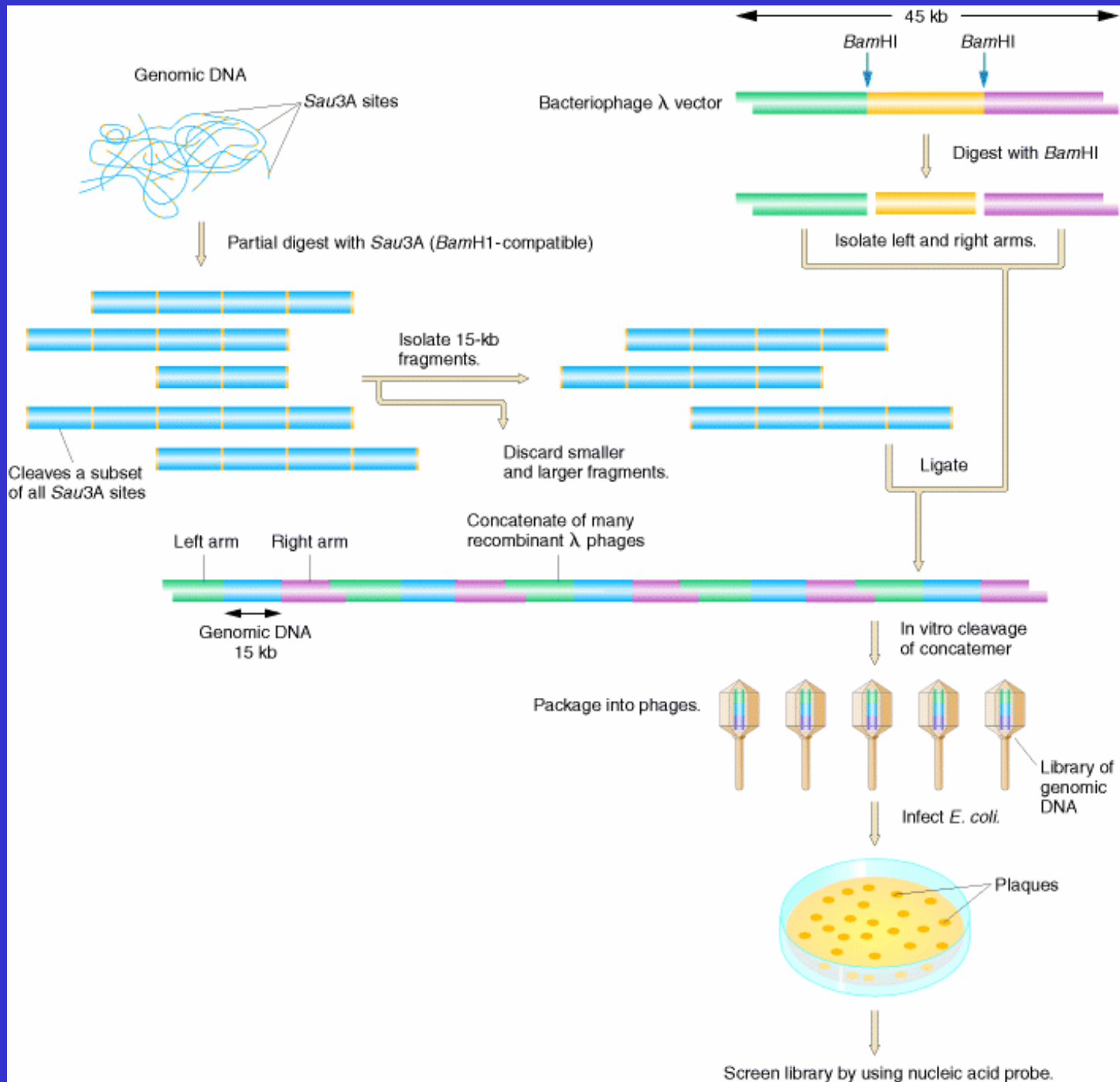
- XL1-Blue MRA and XL1-Blue MRA(P2)

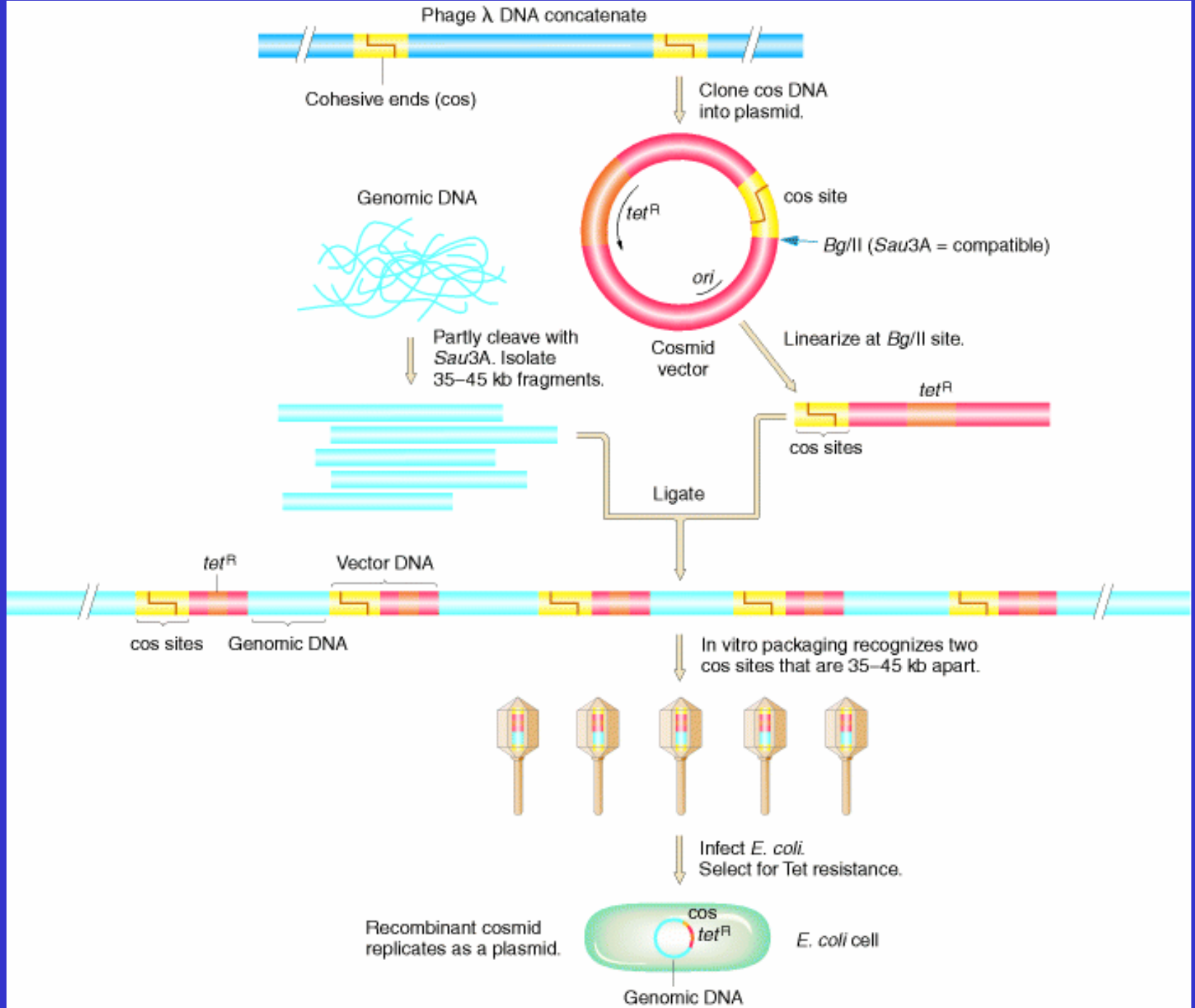


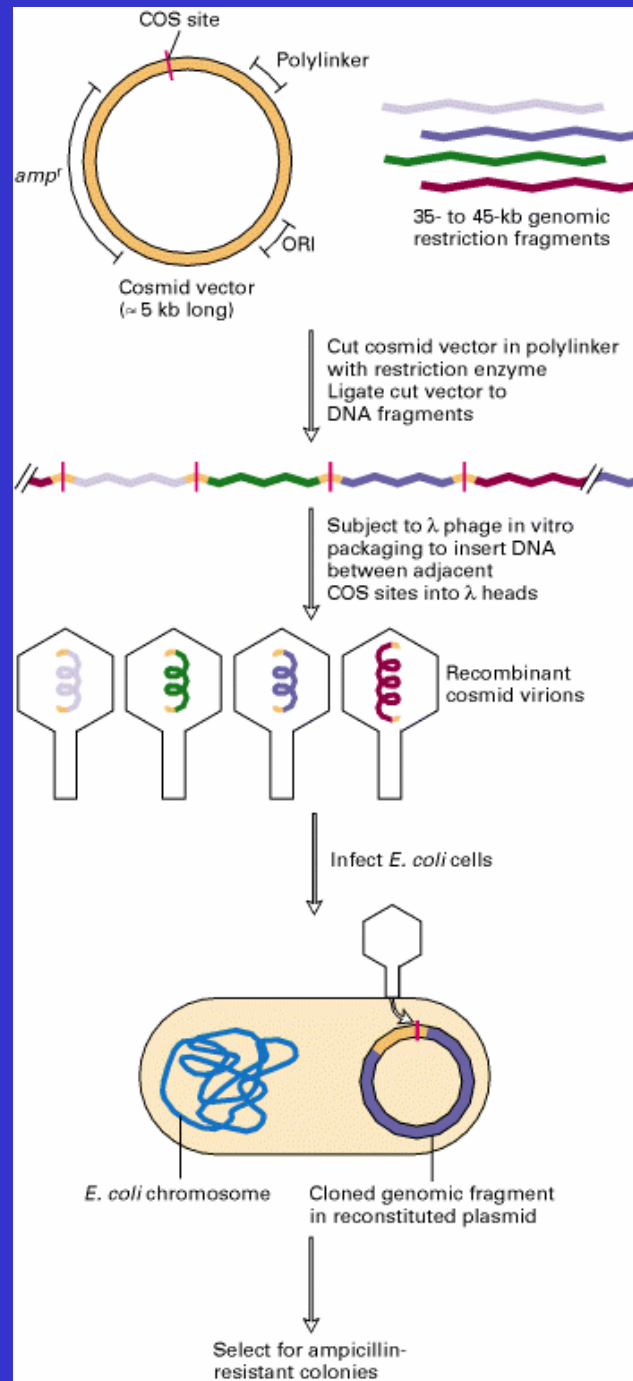
DIGESTÃO PARCIAL DE DNA HUMANO PARA CONSTRUÇÃO DE BIBLIOTECA GENÔMICA

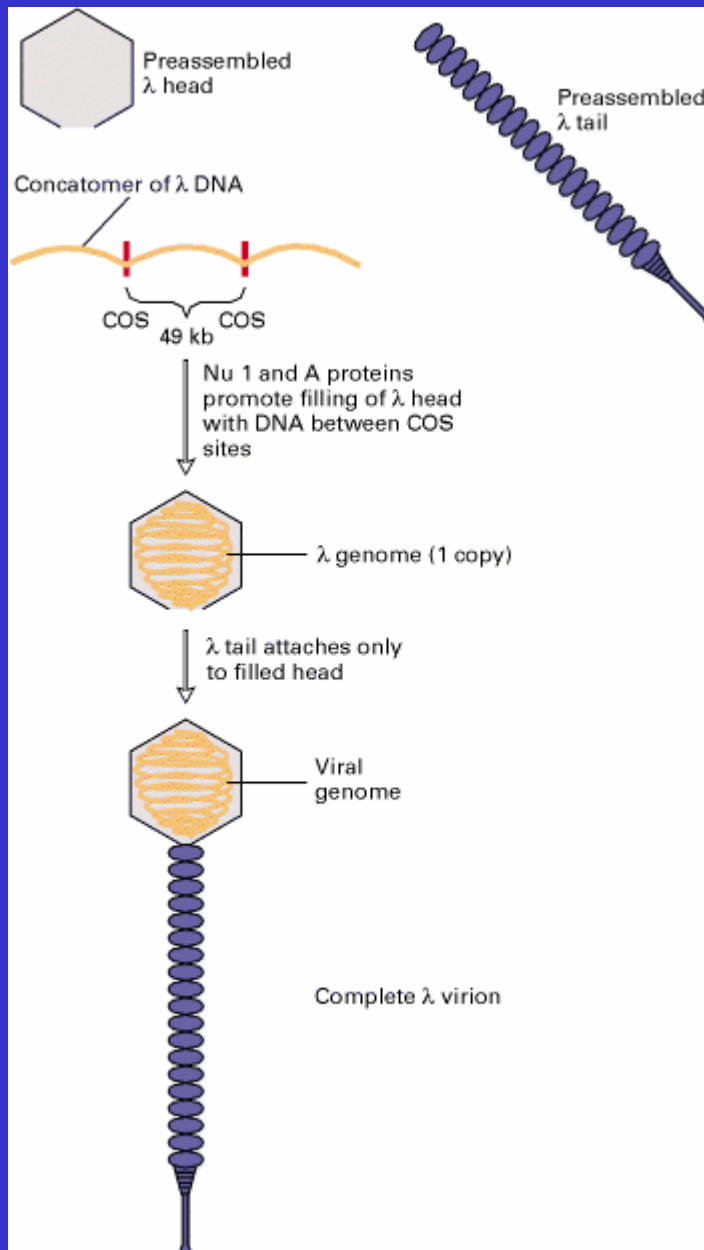
Enzyme	Recognition Sequence
BamH I	GGATCC CCTAGG
Not I	GCGGCCGC CGCCGGCG
Sau3A I	GATC CTAG
Sac I	GAGCTC CTCGAG
Sst I	GAGCTC CTCGAG
Hinf I	GANTC CTNAG
Xho II	PuGATCPy PyCTAGPu

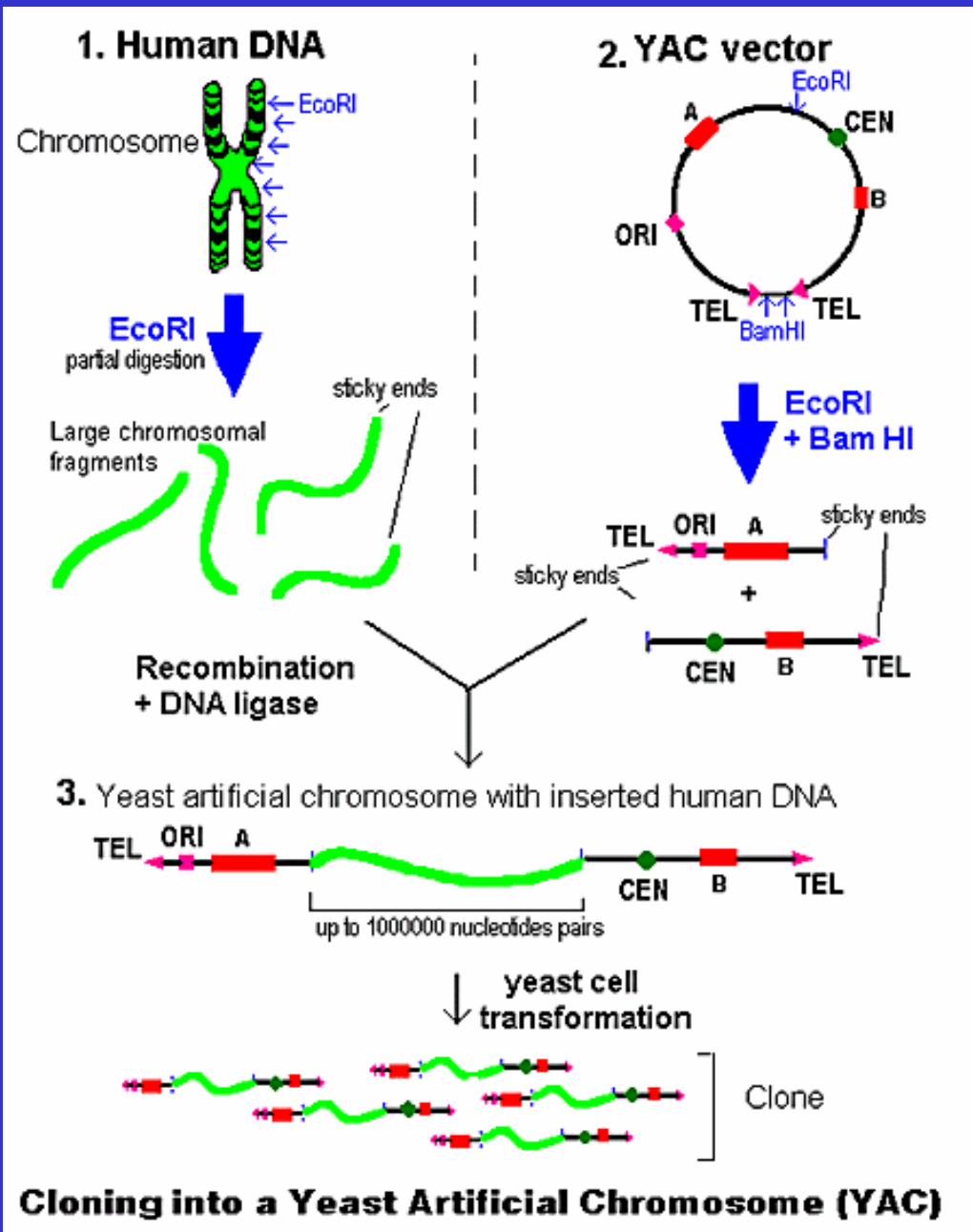












- **TEL: TELÔMERO, PROTEGE DE DEGRADAÇÃO**

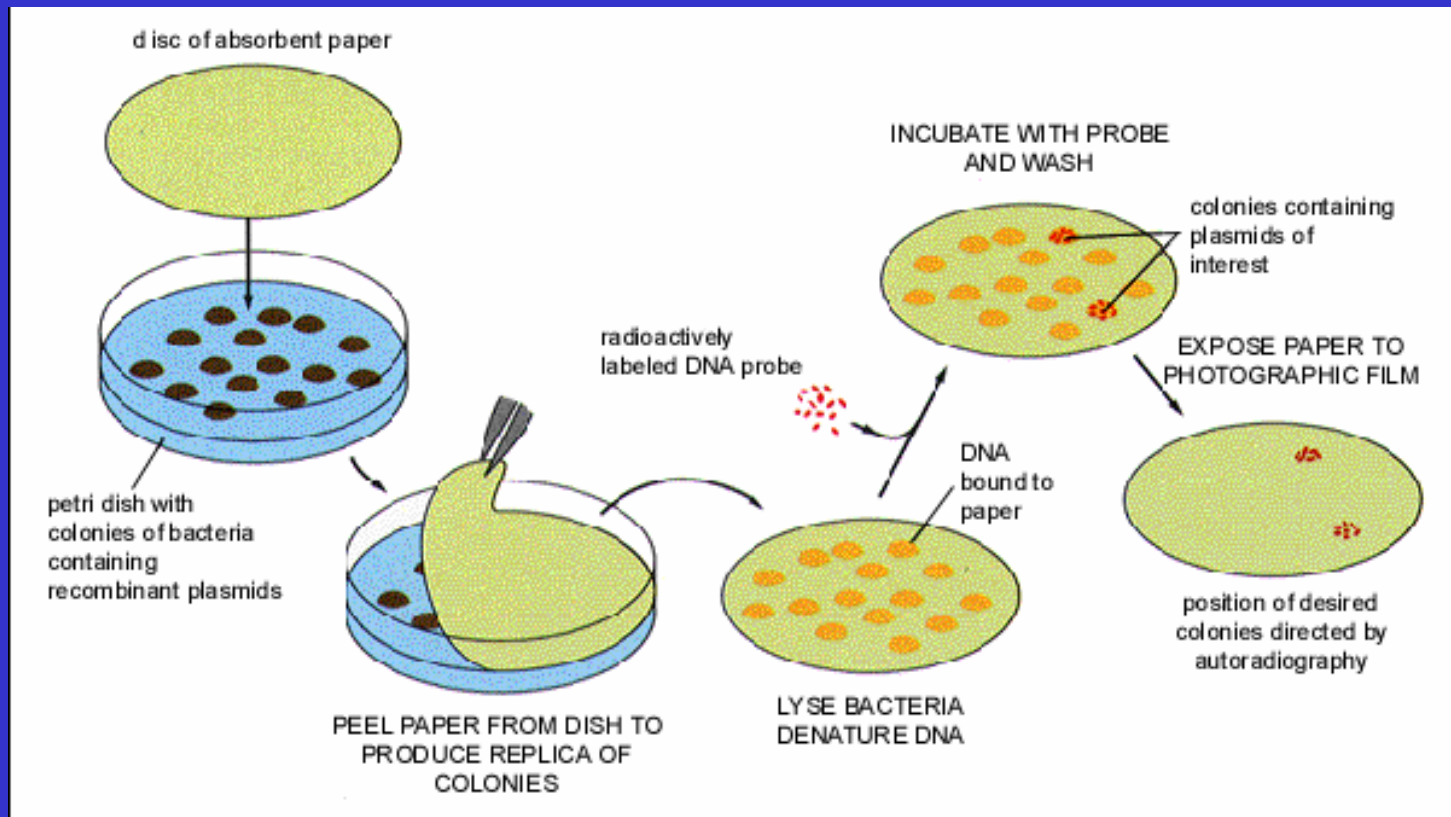
- **CEN: CENTRÔMER, NECESSÁRIO PARA A SEPARAÇÃO CORRETA DE CROMOSOMAS EM CÉLULAS EM DIVISÃO.**

- **ORI: ORIGEM DE REPLICAÇÃO.**

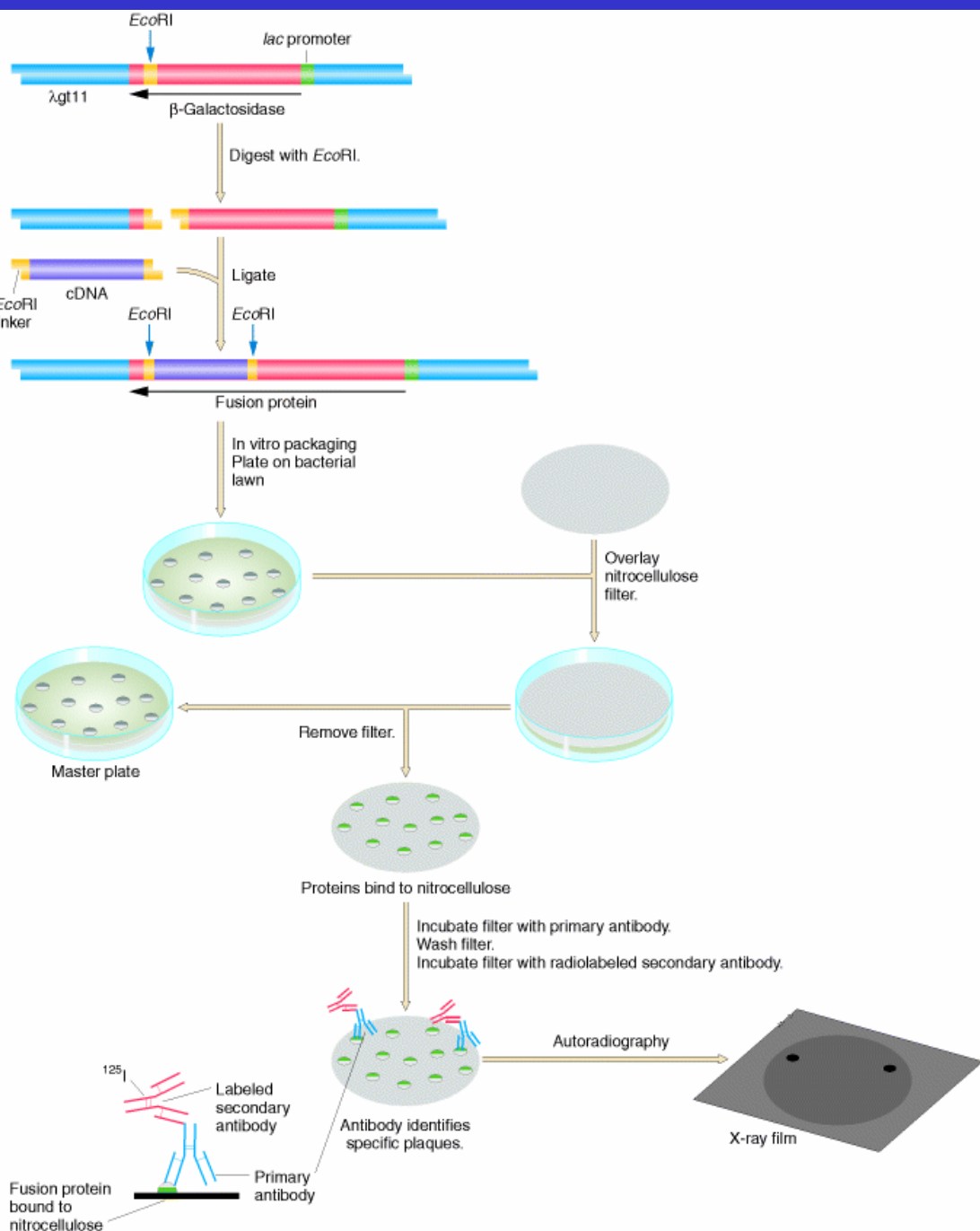
- **A and B: MARCADORES DE SELEÇÃO.**

- **SÍTIOS DE RESTRIÇÃO PARA EcoRI and BamHI.**

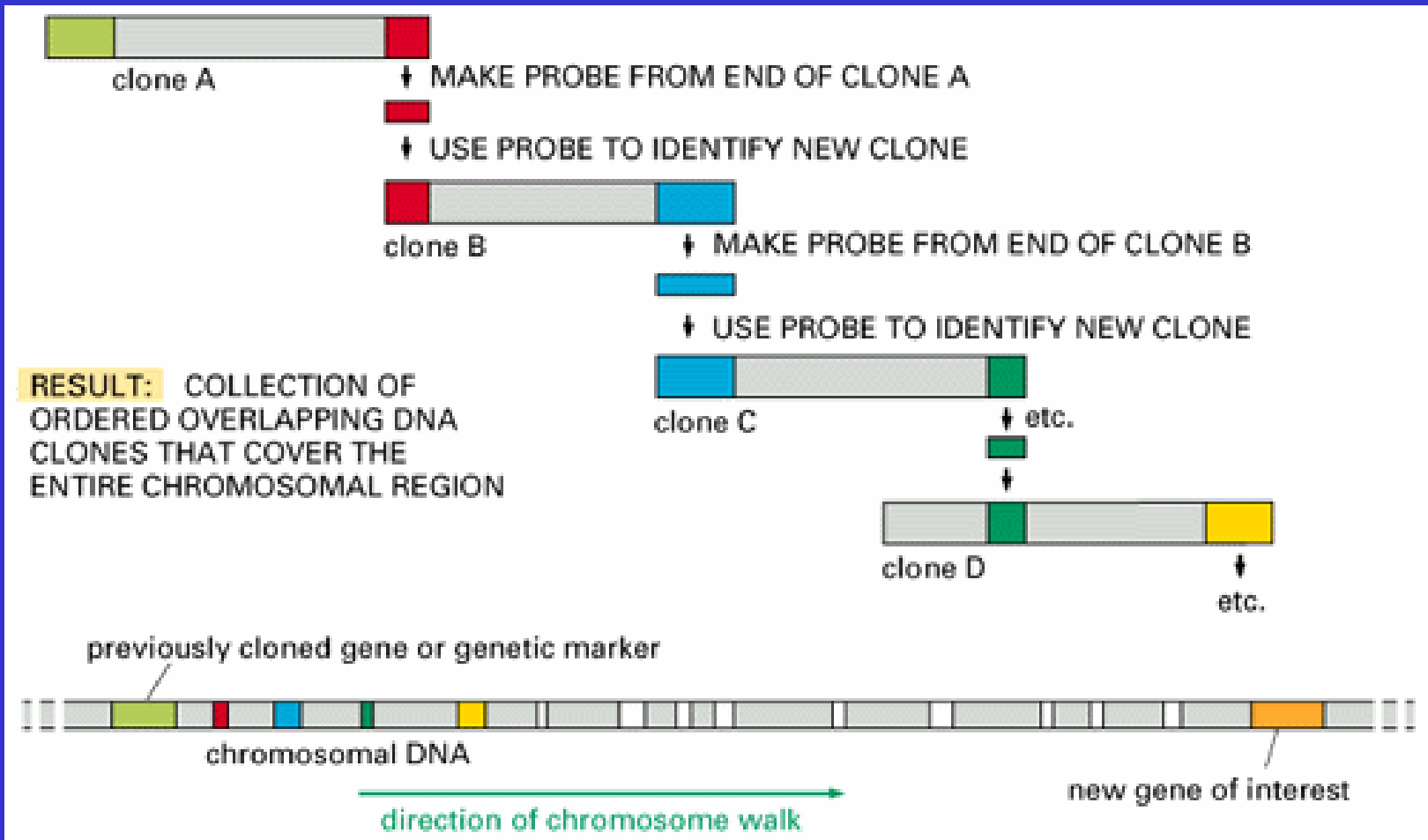
**E...O QUE FAZER COM AS
BIBLIOTECAS??**



TANTO BIBLIOTECAS EM PLASMÍDIOS COMO EM FAGOS PODEM SER SONDADAS PARA ACHAR GENES ESPECÍFICOS.



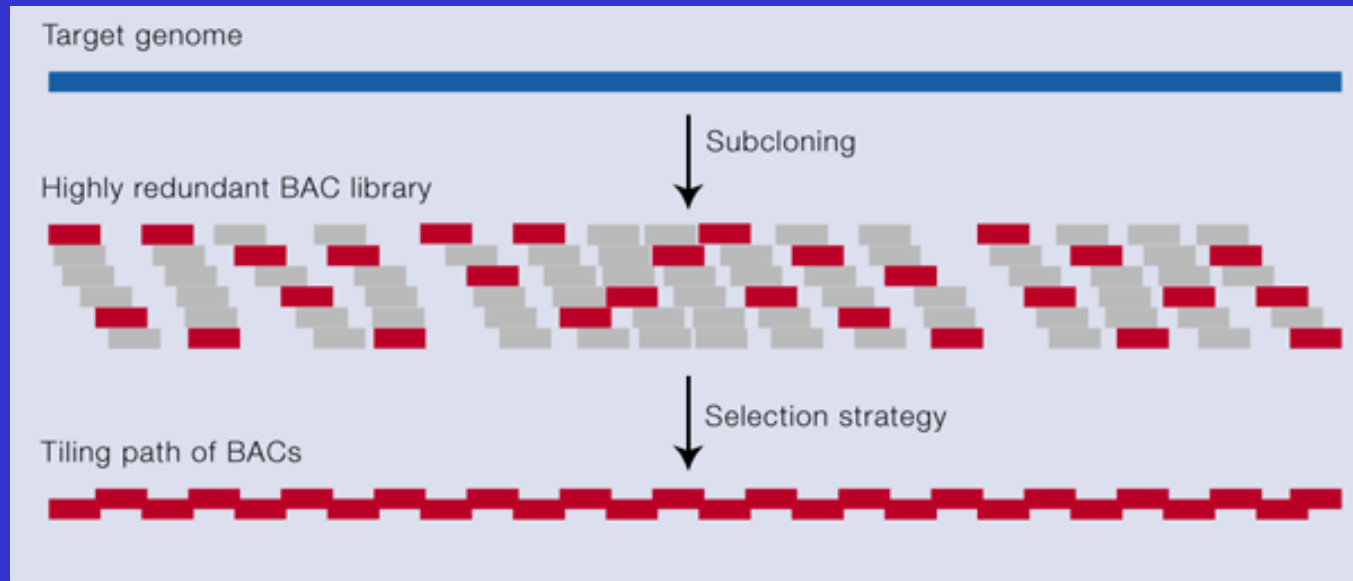
BIBLIOTECAS DE EXPRESSÃO PODEM SER SONDADAS COM ANTICORPOS ESPECÍFICOS



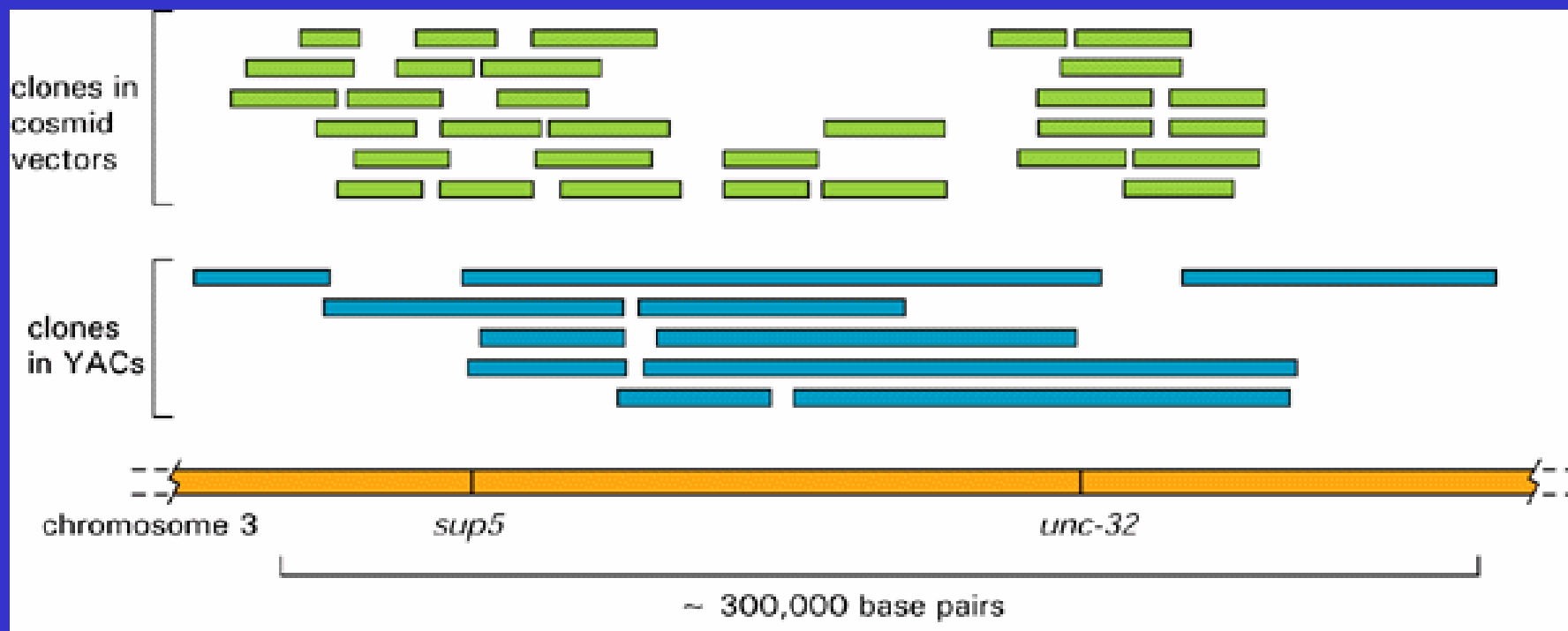
**”CHROMOSOME WALKING”:
CLONES GENÔMICOS DEVEM SER GRANDES**

**EXEMPLOS DE USOS DOS
VÁRIOS TIPOS DE
BIBLIOTECA**

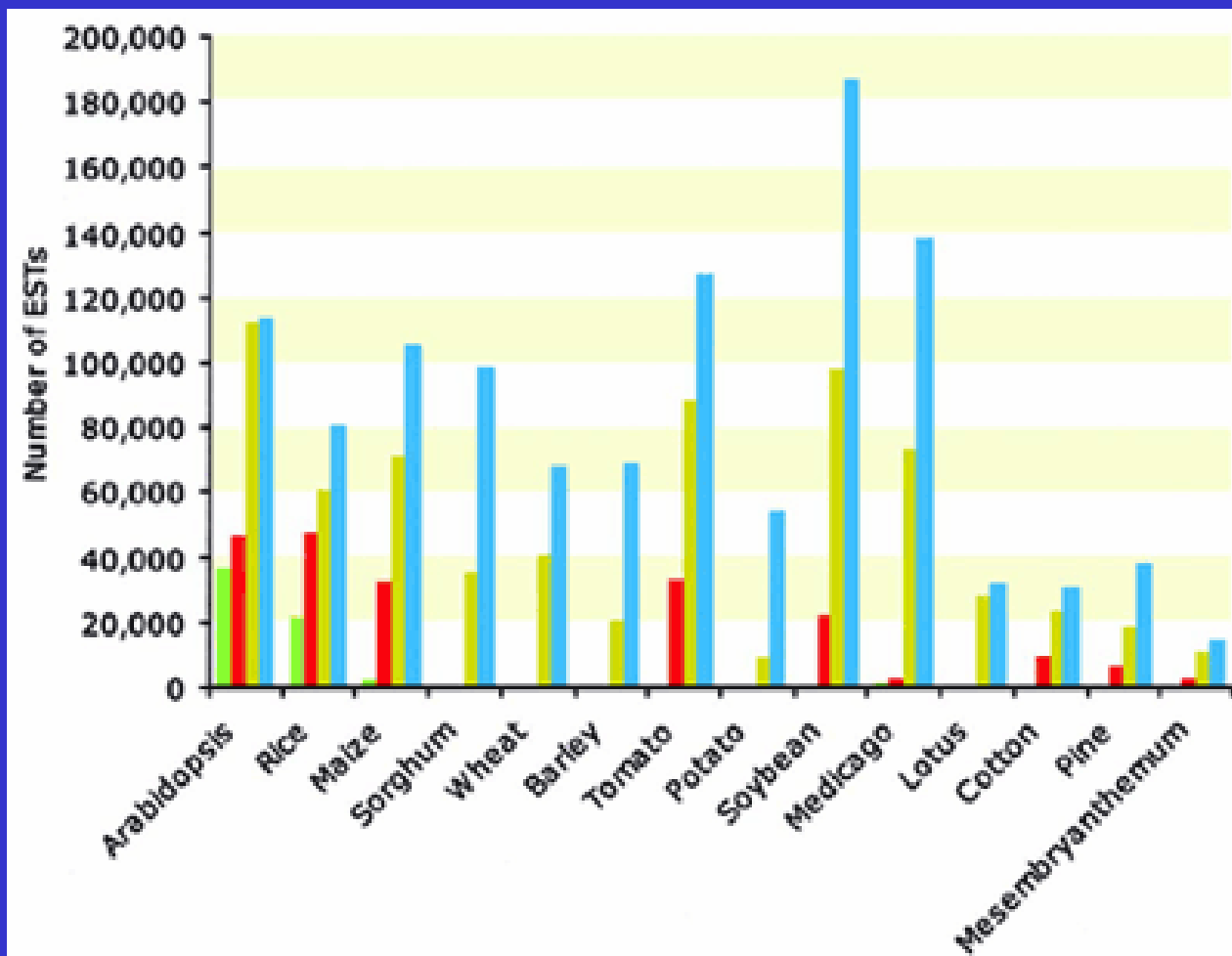
TODOS PROJETOS GENOMA DEPENDEM DA CONSTRUÇÃO DE BIBLIOTECAS



**ESTRATÉGIA DE SEQUENCIAMENTO
DO GENOMA HUMANO (BACs)**



Clones genômicos (YACs E COSMÍDIOS) sobrepostos, cobrindo uma pequena região de um cromosoma do nematódio *Caenorhabditis elegans* representando 0.3% do genoma total



SEQUENCIAMENTO DE ESTs (expressed sequence tags, ou etiquetas de seqüências expressas)

1998, 1999, 2000, 2001

PERGUNTAS PP

Towards the Physical Map of the *Trypanosoma cruzi* Nuclear Genome: Construction of YAC and BAC Libraries of the Reference Clone *T. cruzi* CL-Brener

Vol. 92(6): 843-852

I Ferrari, H Lorenzi, MR Santos*, S Brandariz, JM Requena, A Schijman, M Vázquez, JF da Silveira*, C Ben-Dov, C Medrano, S Ghío, P López Bergami, I Cano*, B Zingales***, TP Urmenyi****, E Rondinelli****, A González*****, A Cortes*****, MC Lopez*****, MC Thomas*****, C Alonso**, JL Ramírez*****, MA Chiurrillo*****, R Rangel Aldao*****, A Brandão*****, W Degrave*****, V Perrot, *****, M Saumier*****, A Billaut*****, D Cohen*****, D Le Paslier *****, MJ Levin/+**

pYAC + PREPARATION

linearized with Bam HI
dephosphorylated with BAP
EcoRI digested

DNA INSERT PREPARATION

T. cruzi DNA embedded
in agarose
partial digestion with EcoRI
preparative CHEF

LIGATION

↓
preparative CHEF II

↓
Agarose treatment

↓
ABI380 spheroplast transformation

↓
Selection on SD Ura^r plates

↓
Selection on SD Ura^r, Trp^r plates

↓
Analytical PFGE of randomly chosen YACs and
size distribution

↓
Storage in AHC, 20% glycerol at -80°C in 96
wells microtiter plates.

↓
Screening

↓
Preparation of YAC DNA pools and
hybridization filters.

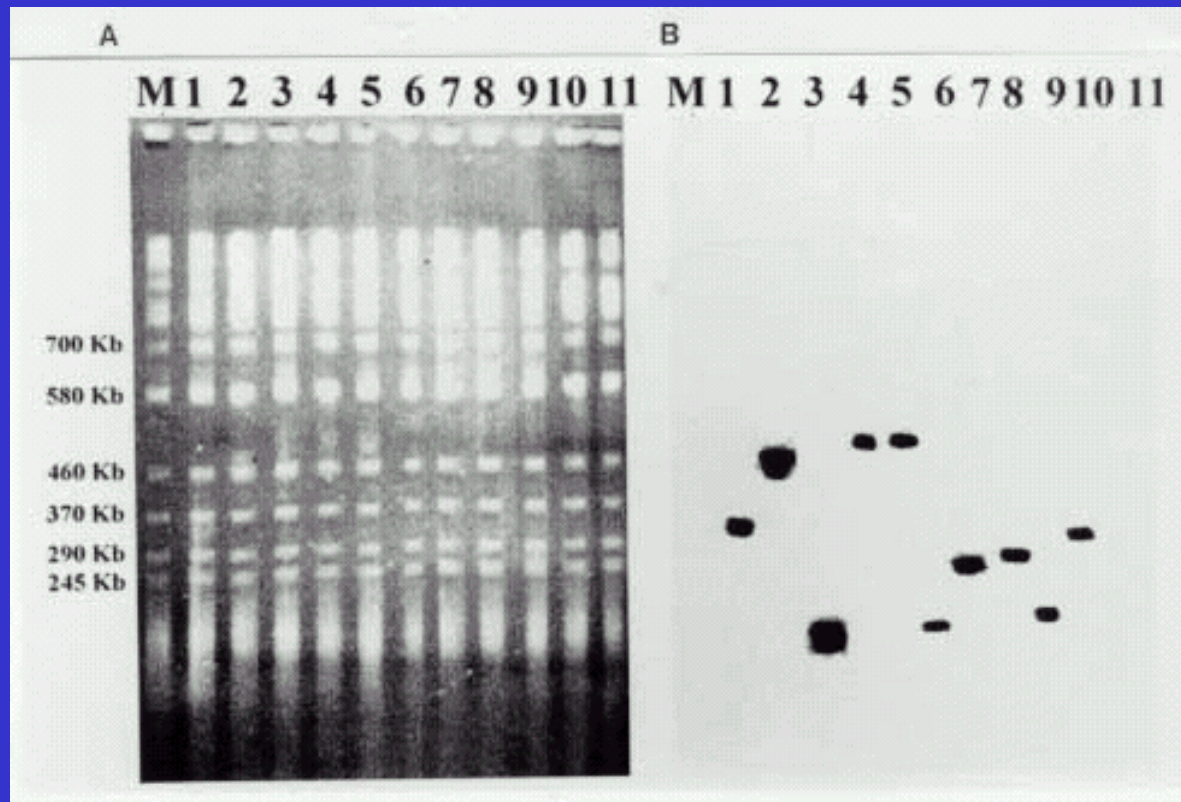
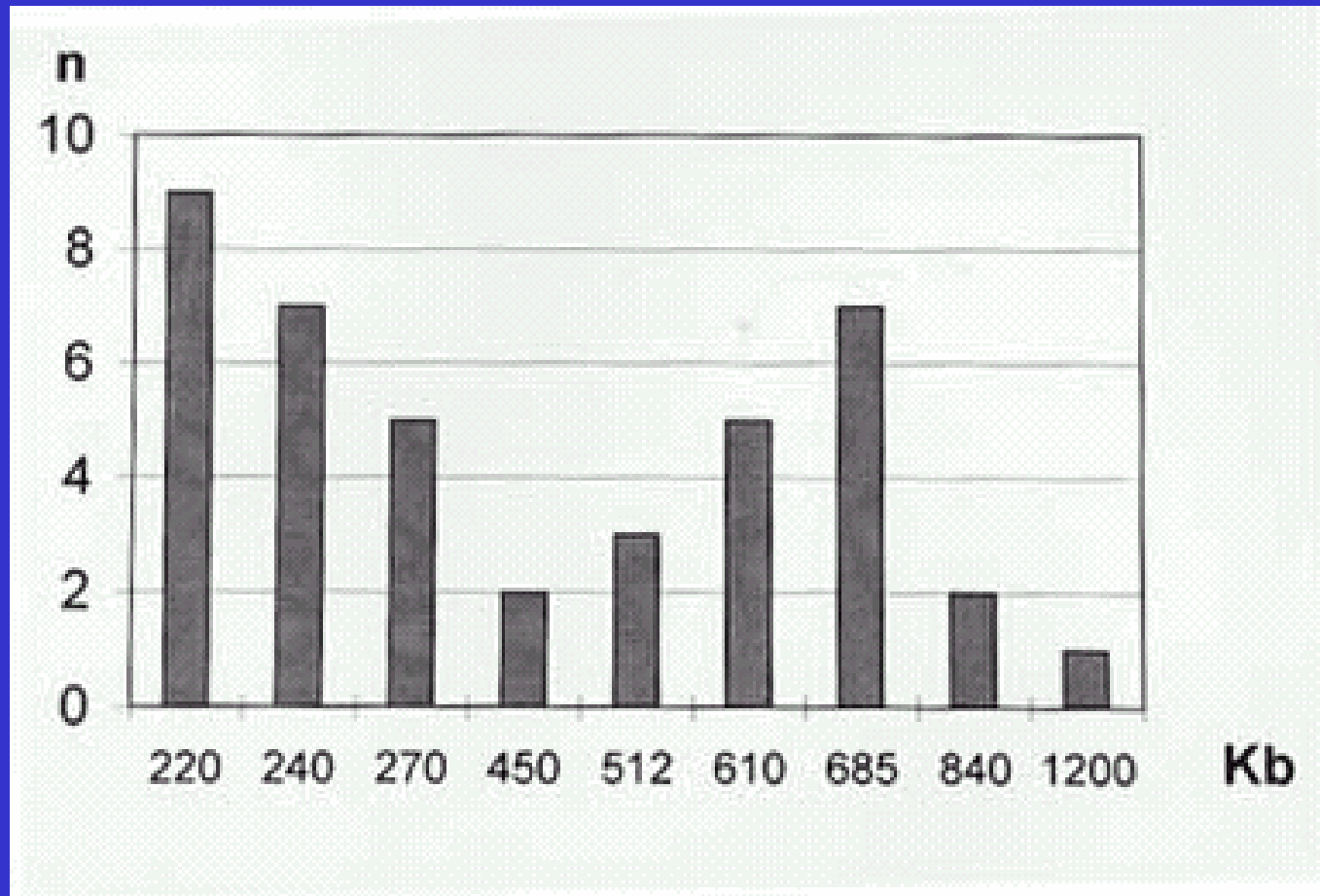


Fig. 2: analysis of sizes of YACs. Eleven randomly selected YAC clones were analyzed by (A) PFGE (conditions of the run are described in Materials and Methods); and (B) Southern blot. The Southern blots were probed with labeled *Trypanosoma cruzi* total DNA .



Distribuição por tamanho de uma biblioteca de *Trypanosoma cruzi* em YAC. 58 clones foram escolhidos ao acaso e o tamanho determinado por PFGE. O tamanho médio foi de 365 kb.

pBAC PREPARATION

linearized with BamHI
desphosphorylated with BAP

DNA INSERT PREPARATION

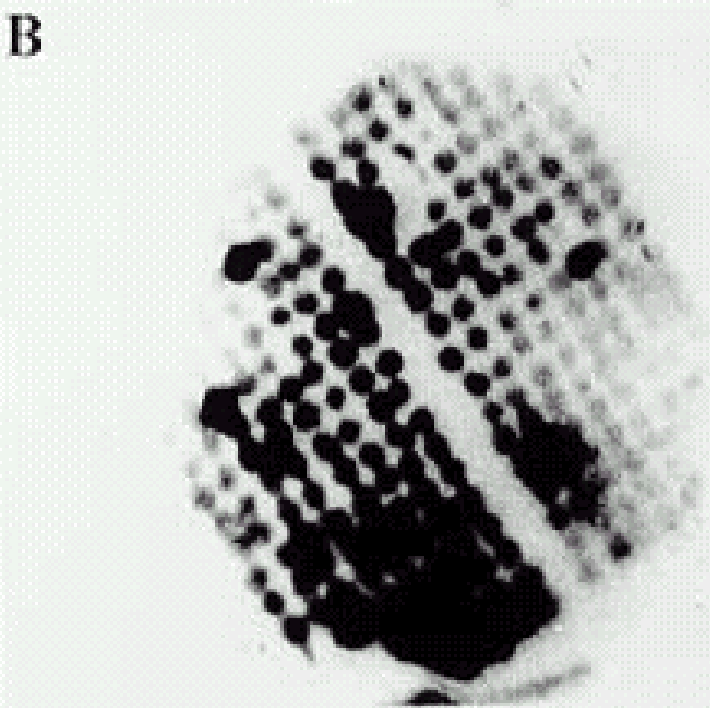
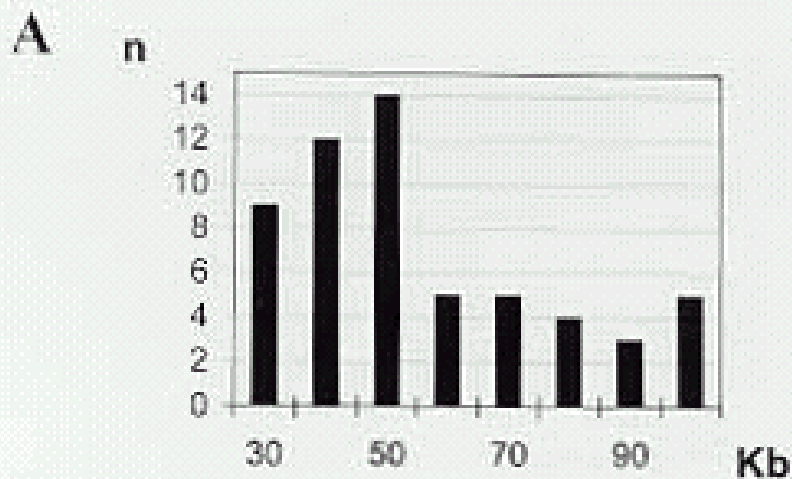
T. cruzi DNA embedded
in agarose
partial digestion with NdeII
(BAC I Library)
partial digestion with BamHI
(BAC II Library)
preparative CHEF
Excision from gel

LIGATION

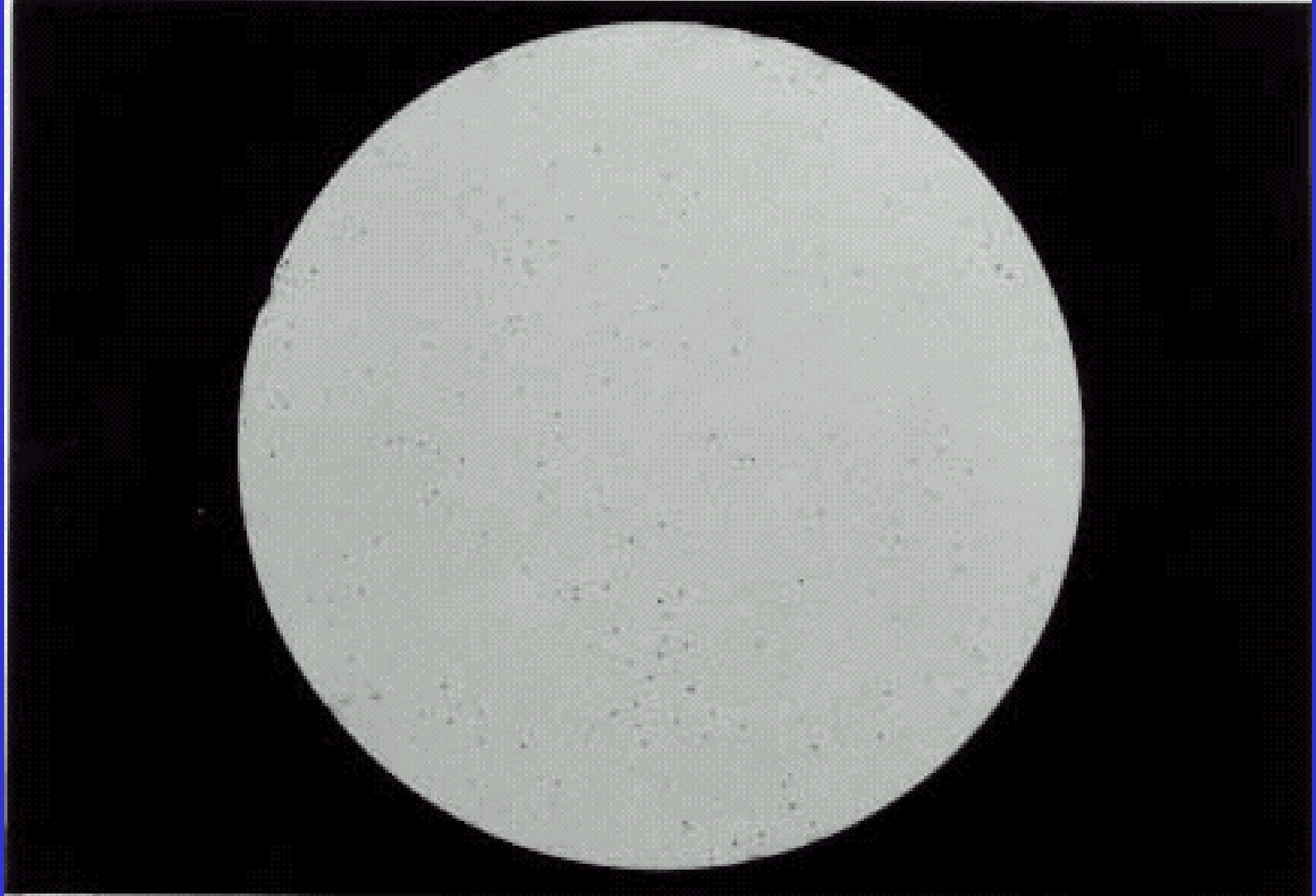
↓
Electroporation and
transformation of
Escherichia coli DH10B cells

PLATING

LB chloramphenicol (selection for
maintenance of BAC)
X-Gal / IPTG (color selector for insert
presence, only pBeloBAC)
↓
Analytical PFGE for size determination
↓
Storage in LB chloramphenicol, 20%
glycerol at -80°C in 96 wells microtiter
plates



A: Distribuição por tamanho de clones de uma biblioteca em BACs. Um total de 59 BACs foram analisados. B: sondagem do filtro contendo 236 BACs com uma sonda E13, uma sequência repetitiva. (Requena 1993).

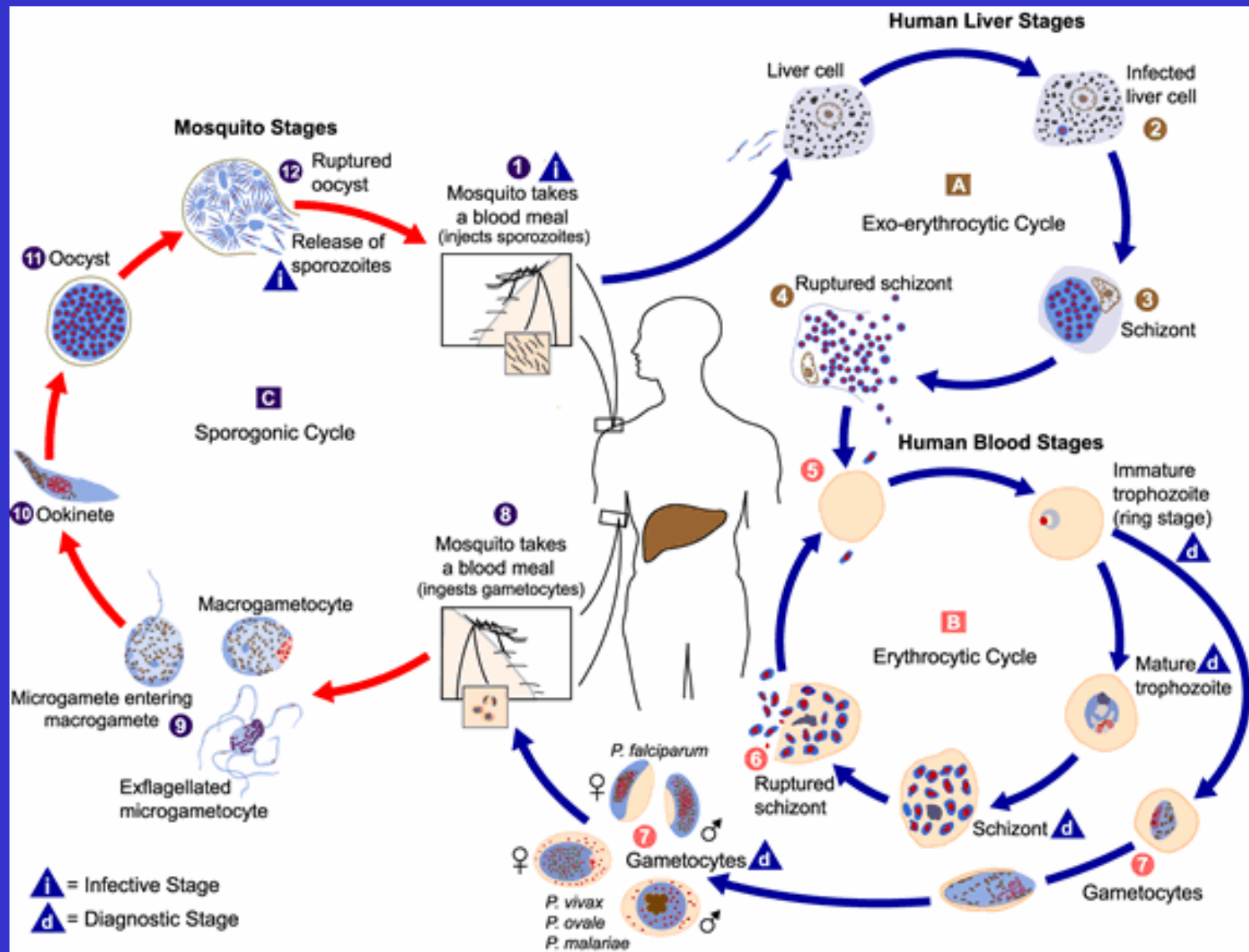


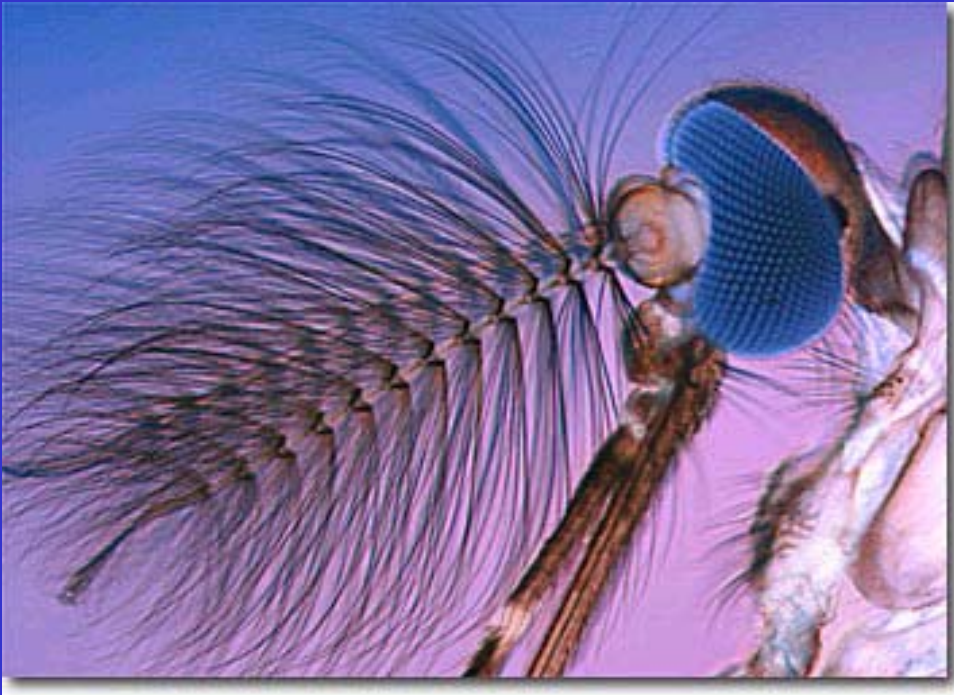
**Sondagem de uma biblioteca em λ gt 11 (Levy Yeyatti 1991)
com um pool de soros de pacientes com cardiopatia chagásica
grave. (Levin 1989).**

exemplo...



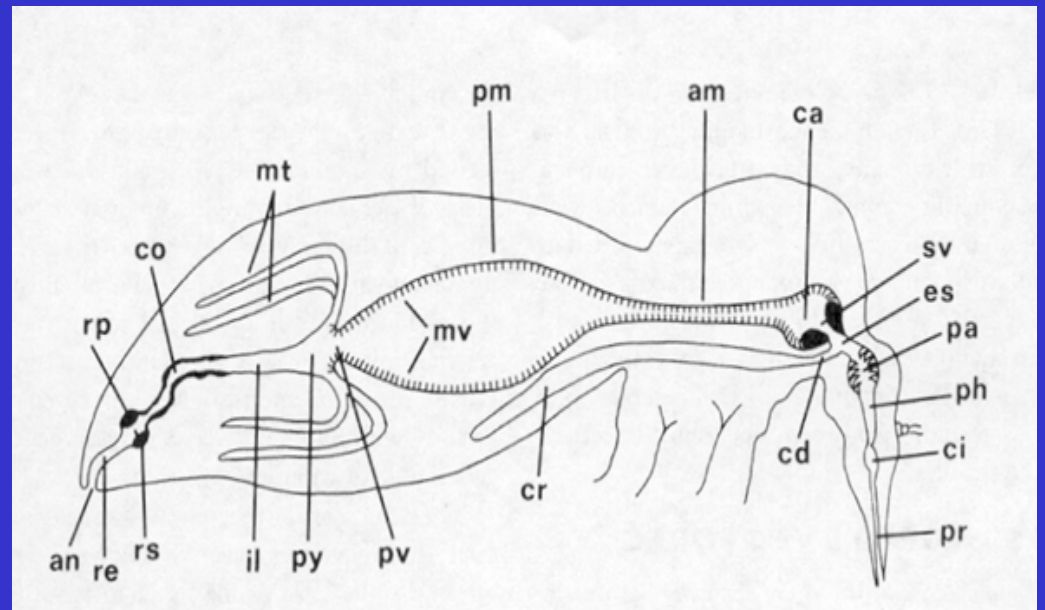
**CONHECIMENTO BÁSICO DA BIOLOGIA E
FISIOLOGIA DO MOSQUITO DA MALÁRIA,
COM O OBJETIVO, A MÉDIO E LONGO
PRAZO, DE INTERFERIR NA
TRANSMISSÃO DA DOENÇA.**





MOSQUITO

MATRIZ PERITRÓFICA



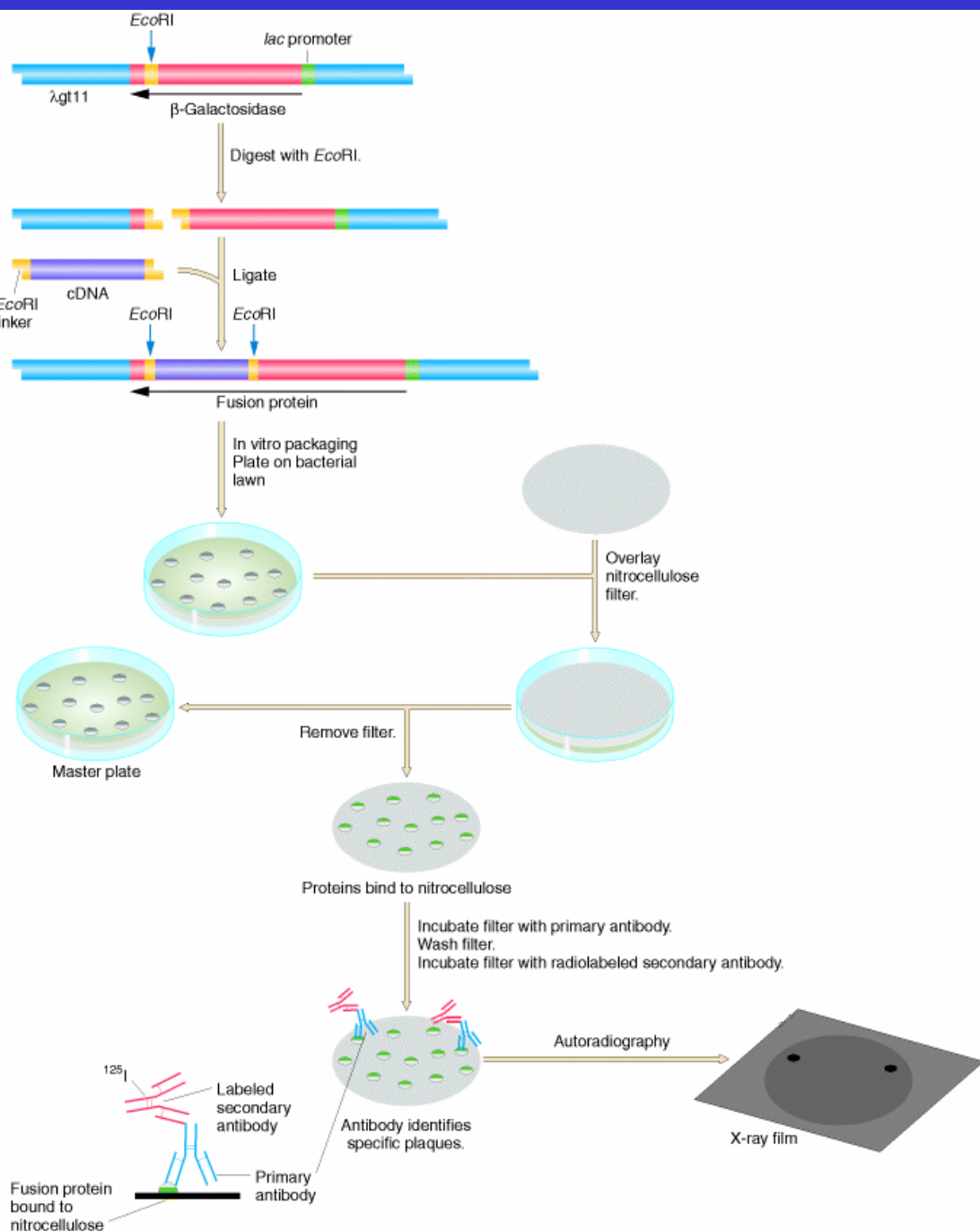
J Biol Chem 1998 Jul 10;273(28):17665-70

**A Type I Peritrophic Matrix Protein from the Malaria
Vector *Anopheles gambiae* Binds to Chitin CLONING,
EXPRESSION, AND CHARACTERIZATION***

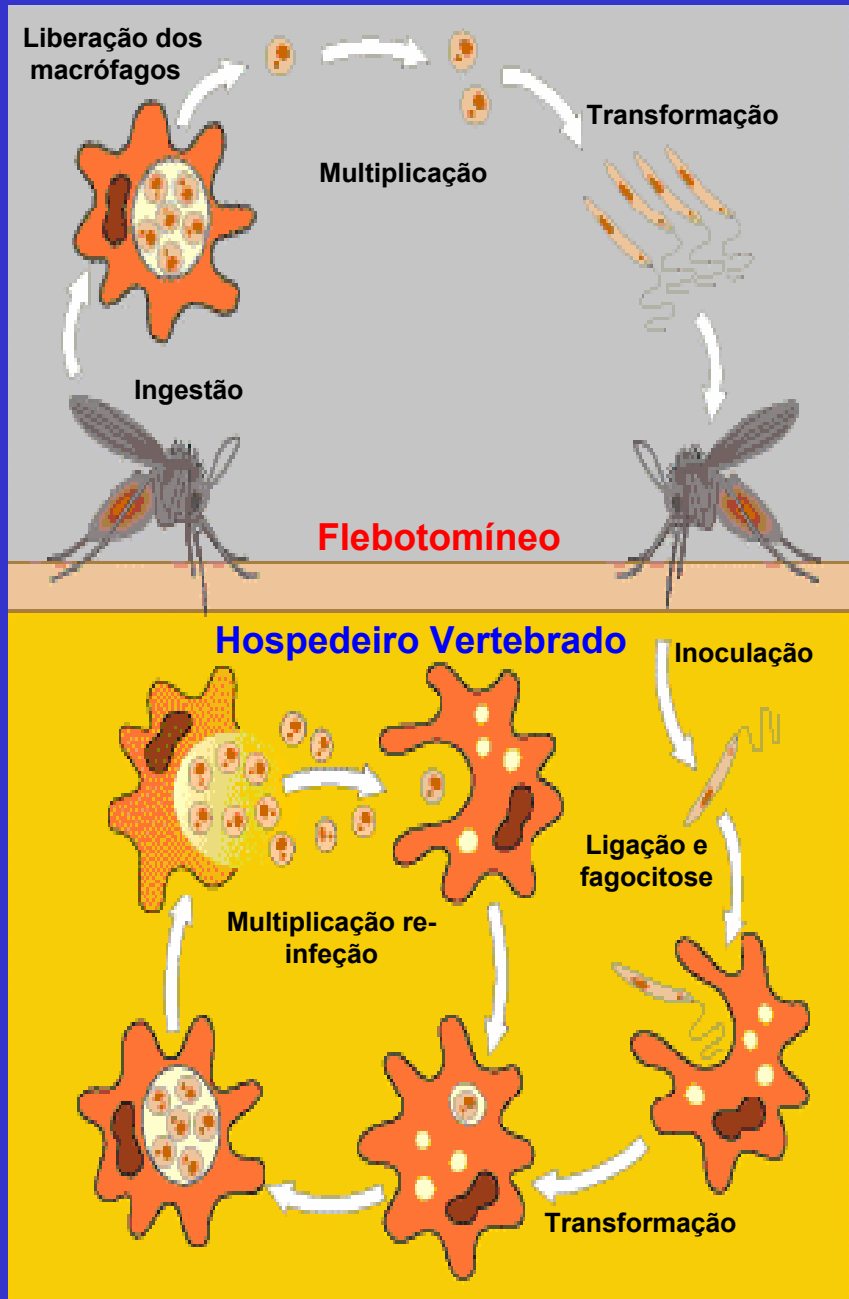
Zhicheng Shen and Marcelo Jacobs-Lorena

**Como achar os genes que codificam as proteínas da matriz
peritrofica de um inseto?**

“By antibody screening of an expression library we have identified and partially characterized a cDNA encoding a putative PM protein, termed *Anopheles gambiae* adult peritrophin 1 (*Ag-Aper1*). *Ag-Aper1* is the first cloned PM gene from a disease vector.”



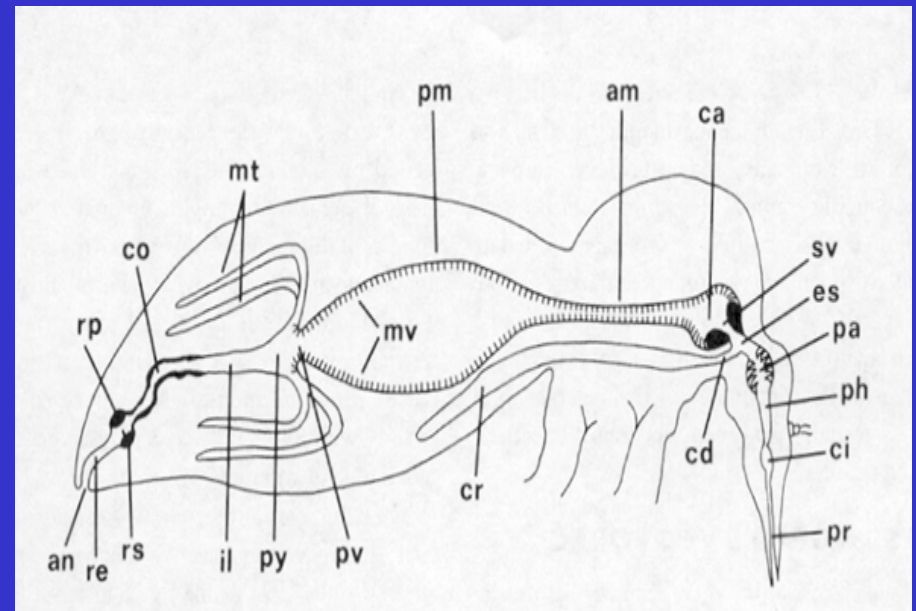
BIBLIOTECAS DE EXPRESSÃO PODEM SER SONDADAS COM ANTICORPOS ESPECÍFICOS

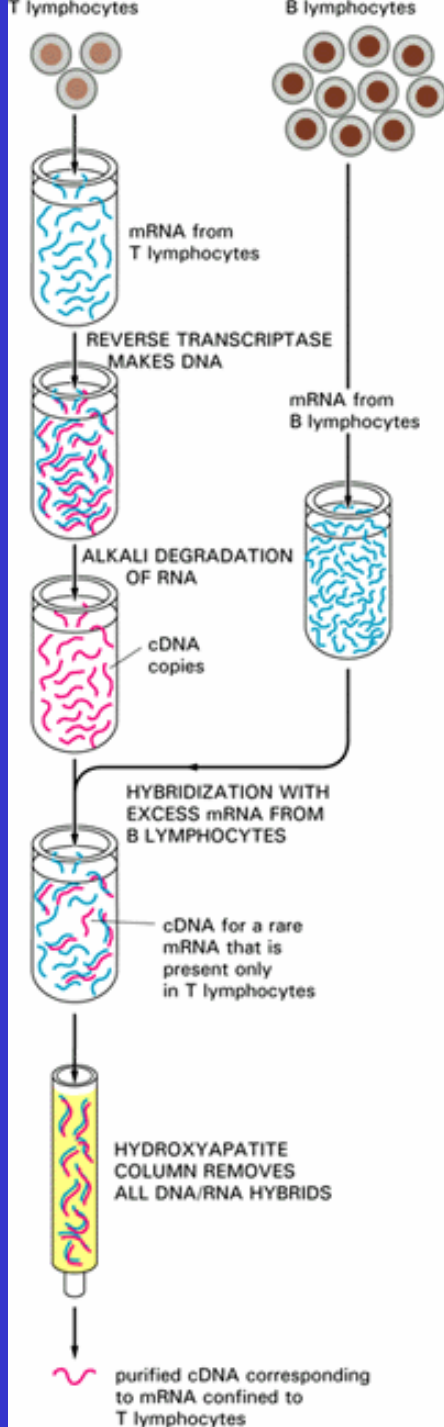




FLEBOTOMÍNEO TRANSMISSOR DA LEISHMANIOSE

ESTUDOS MOLECULARES DO TUBO DIGESTIVO





Hibridização subtrativa, usada para isolar cDNAs raros. Bom para comparar populações de RNAs vindas de tecidos relacionados. Exemplo: tubo digestivo de flebotomíneo alimentado ou não com sangue.

Differential Display Reverse Transcriptase - PCR DDRT

mRNAs

————— GAAAAAAAAAAAA

————— TAAAAAAAAAAAA

————— CAAAAAAAAAAAA

Reverse transcription

Anchor primers
H-T_nG
H-T_nC
H-T_nA

←————— CAAAAAAAAAAAA

←————— GTTTTTTTTTTTCGAA

PCR amplification

Anchor primers
H-AP or AP primers
 $\alpha^{33}\text{P-dATP}$

(H-AP1primer)AAGCTTGATTGCC —————→

————— GTTTTTTTTTTTCGAA

(H-AP1primer)AAGCTTGATTGCC —————→

————— GTTTTTTTTTTTCGAA

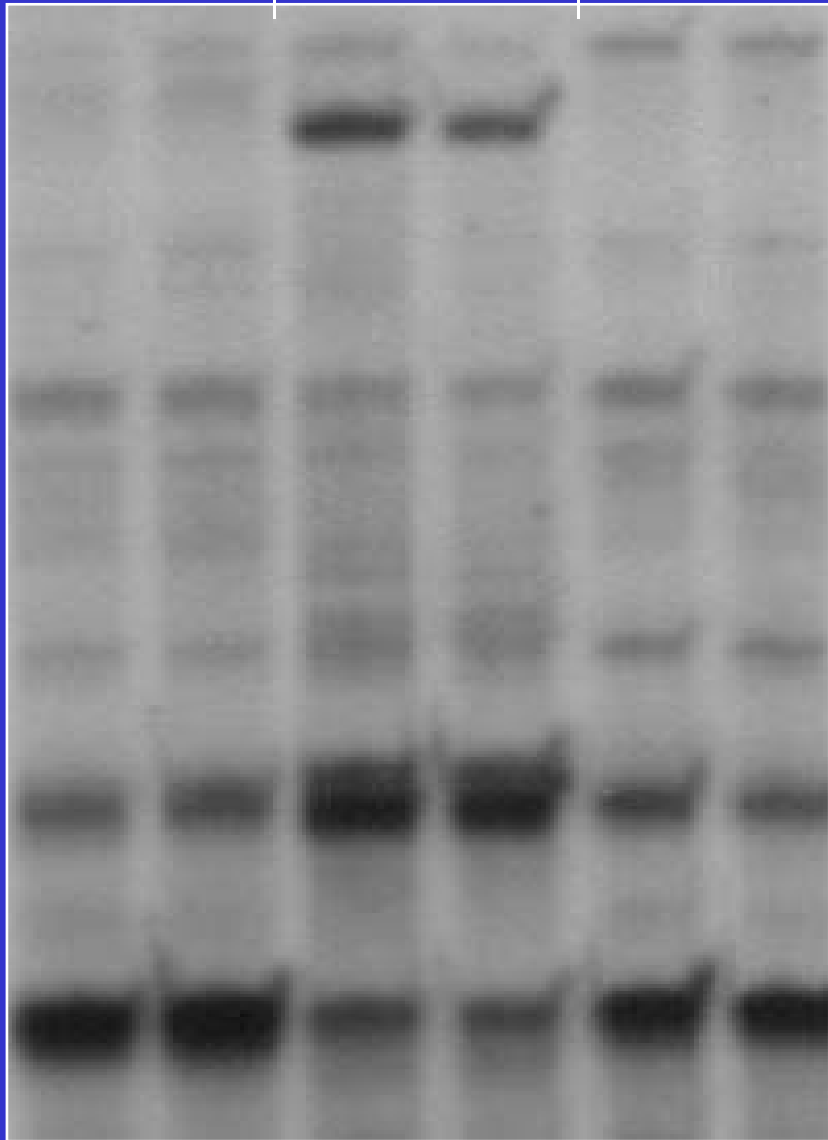
PAGE



GB15h

GB30h

Sugar



DDRT-PCR

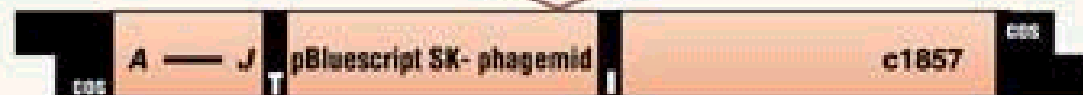
Indication of Differential Display in Polyacrylamide Gel.

**SEQUENCIAMENTO DE ESTs A PARTIR DE
BIBLIOTECAS DE EXPRESSÃO DE TUBO
DIGESTIVO DE FLEBOTOMÍNEO SOB
DIVERSAS CONDIÇÕES:**

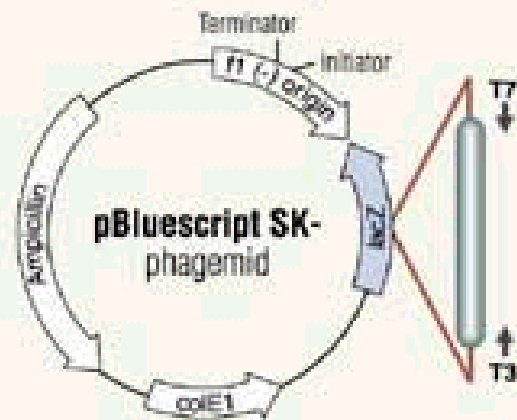
- ALIMENTADO OU NÃO COM SANGUE**
- INFECTADO OU NÃO POR LEISHMANIA**



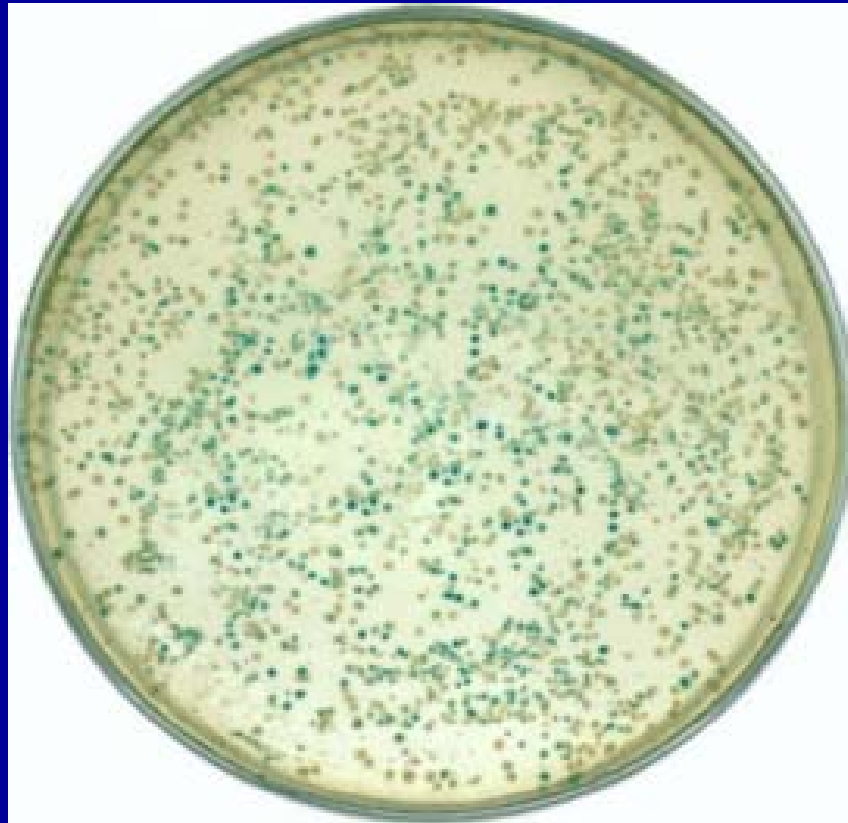
1. Construct DNA library
2. Isolate positive clone



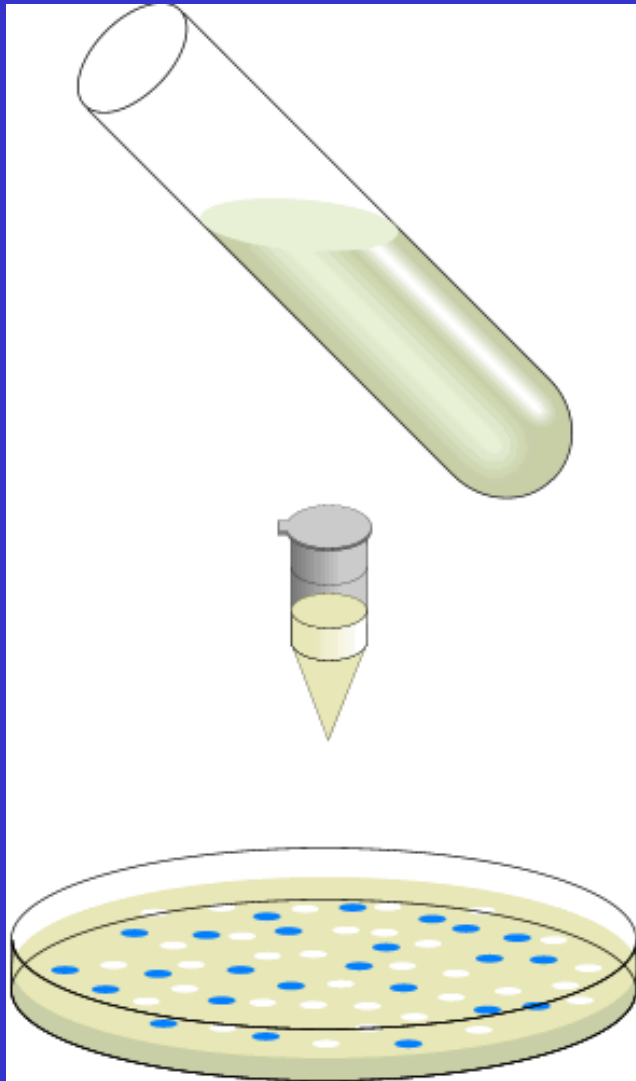
3. Excise the pBluescript plasmid containing the cloned DNA insert by co-infection with helper phage



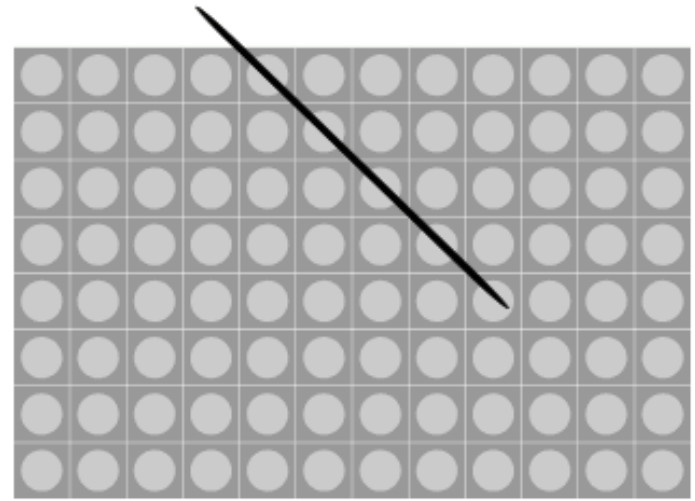
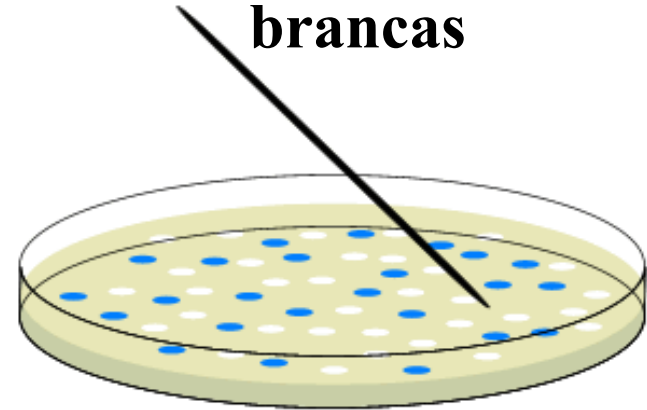
**PLACA CONTENDO COLÔNIAS DE
EXCISÃO DE UMA BIBLIOTECA DE
EXPRESSÃO EM λ ZAP:
colônias claras são recombinantes**



Bibliotecas



Coleta das colônias brancas

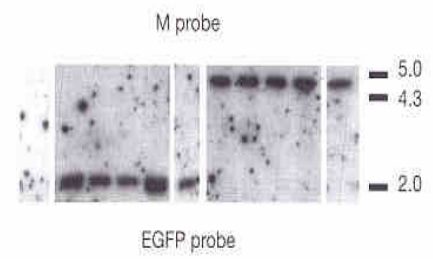
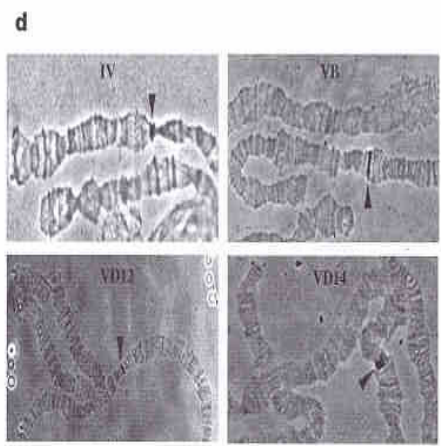
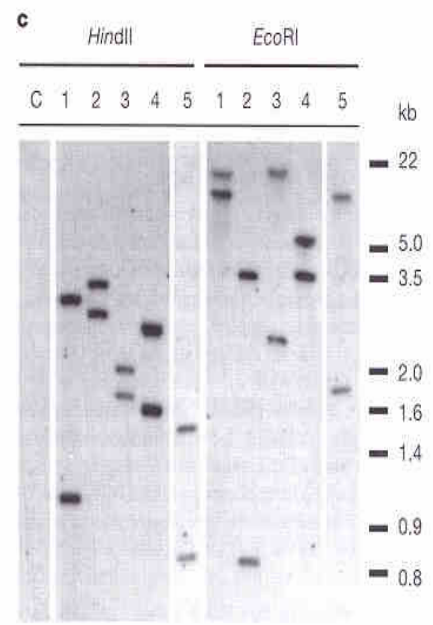
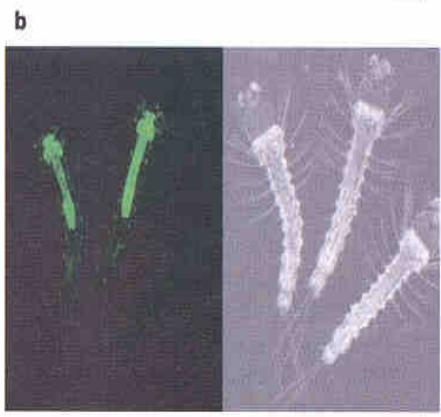
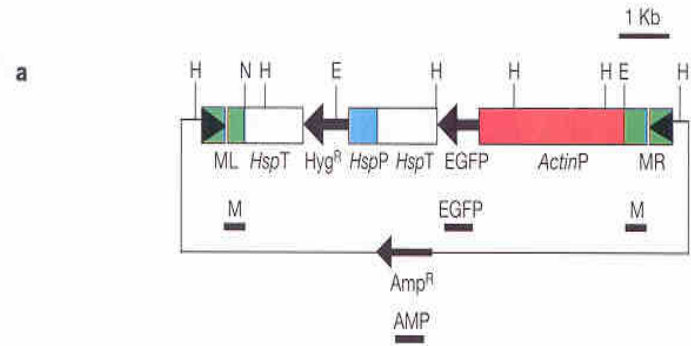


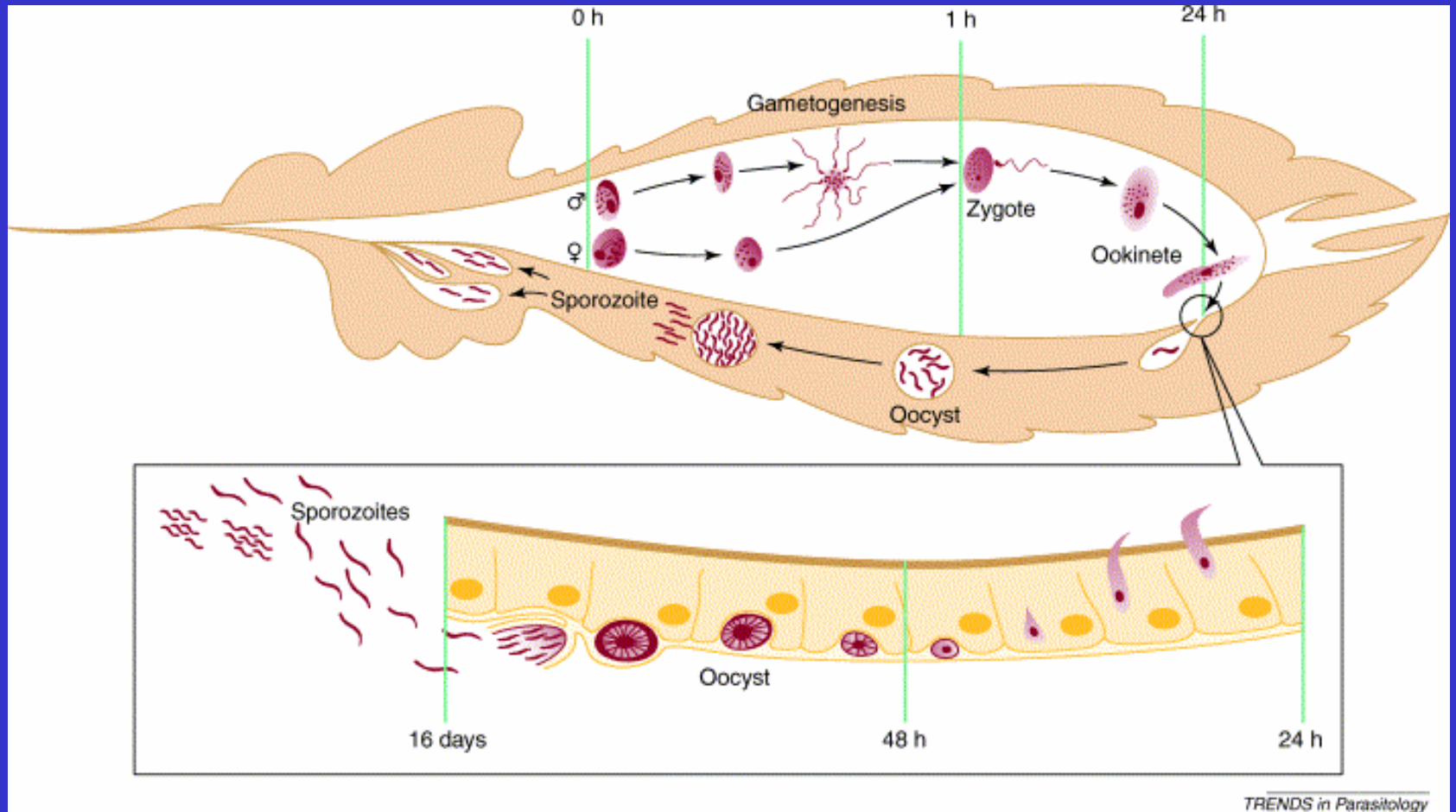
Placas de 96 poços

Tabela IV - ESTs identificadas por sequenciamento

Identificação	Tamanho	Homologia
Y2 (cDNA25)	360 pb	RNAHelicase ATP dependente
Y10 (cDNA28)	60 pb	Proteína Elastina-like
Y10 ¹	330 pb	Sem homologia
Y11 (CDNA11R)	340 pb	Citocromo oxidase C subunidade II
Y27 (CDNA27R)	200 pb	Proteína ribosomal S8
Y30 (cDNA30) peroxisomal	190 pb	Dienoil-CoA isomerase/ Enoil hidratase
Y33	375 pb	Proteína “Zinc finger”
Y35 (cDNA35)	155 pb	Proteína “Zinc finger”
Y39		Citocrome oxidase I
Y41 (cDNA41) ²	200 pb	Enzima de conjug. de ubiquitina / RAD6 homóloga
M4 (19-11cdna4) antígeno	210 pb	Proteína ligadora de célula apresentadora de
M5 ³	140 pb	Possível fator de “splicing” (<i>H. sapiens</i>)
M6	204 pb	Proteína ligadora de DNA
M9 (19-11cdna9) ⁴	190 pb	Similar a <i>A. thaliana</i> F17F8.11
M11 (19-11cdna11) ⁵	179 pb	16S rRNA <i>A. gambiae</i> por Fasta
M13 (19-11cdna13) ⁶	185 pb	NADH <i>A. gambiae</i> por Fasta
M14 (19-11cdna14)	240 pb	Serine hidroximetil-transferase
M17 (19-11cdna17)	386 pb	Anon2 (evolução acelerada)/antena-específico
M18 (19-11cdna18)	305 pb	Proteína ribosomal 60S L7A
M19 (19-11cdna19)	222 pb	Proteína ligadora de guanina (“G binding”)
M22 (19-11cdna22)	330 pb	Fumarilacetoacetase

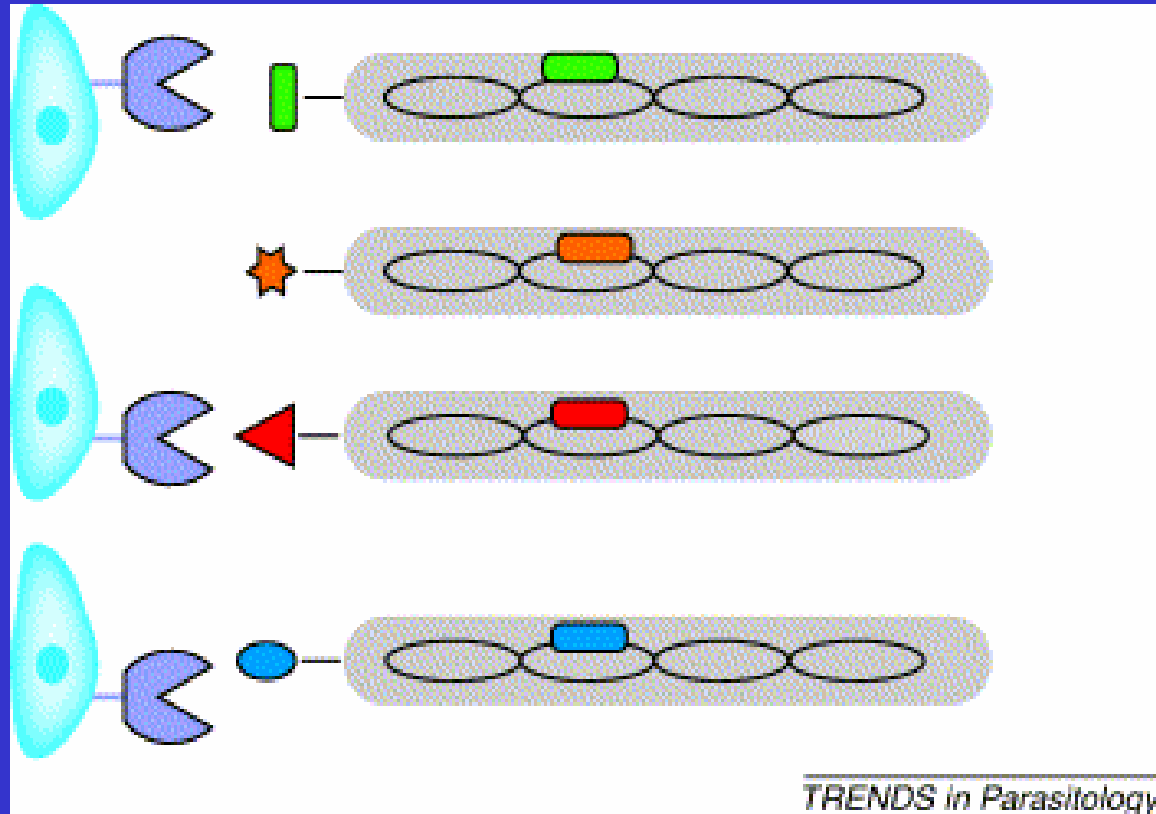
É POSSÍVEL PRODUZIR INSETOS TRANSGÊNICOS INCAPAZES DE TRANSMITIR DOENÇAS





PERGUNTA: COMO IMPEDIR A TRANSMISSÃO DA MALÁRIA PELO MOSQUITO?

BIBLIOTECA DE PHAGE DISPLAY



Fago mostrando DNA e a sequencia nucleotídica randômica que codifica um peptídeo. Células da glândula salivar expressam receptor. Neste caso o peptídeo vermelho encaixa no receptor, permitindo a seleção deste fago.

Ghosh A, Moreira LA, Jacobs-Lorena M

Targeting *Plasmodium* ligands on mosquito salivary glands and midgut with a phage display peptide library. *Proc. Natl Acad. Sci. U.S.A.* 98 (2001), pp. 13278–13281.

MAIS UMA RAZÃO PORQUE O CONHECIMENTO DE PROMOTORES É IMPORTANTE

Robust gut-specific gene expression in transgenic *Aedes aegypti* mosquitoes

Luciano A. Moreira,^{*} Marten J. Edwards,[†] Faisal Adhami,^{*} Nijole Jasinskiene,[‡] Anthony A. James,[‡] and Marcelo Jacobs-Lorena^{*§}

^{*} Department of Genetics, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-4955; [†] Department of Zoology, Ohio Wesleyan University, Delaware, OH 43015; and [‡] Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697-3900

Abstract

Genetic modification of the vectorial capacity of mosquito vectors of human disease requires promoters capable of driving gene expression with appropriate tissue and stage specificity.

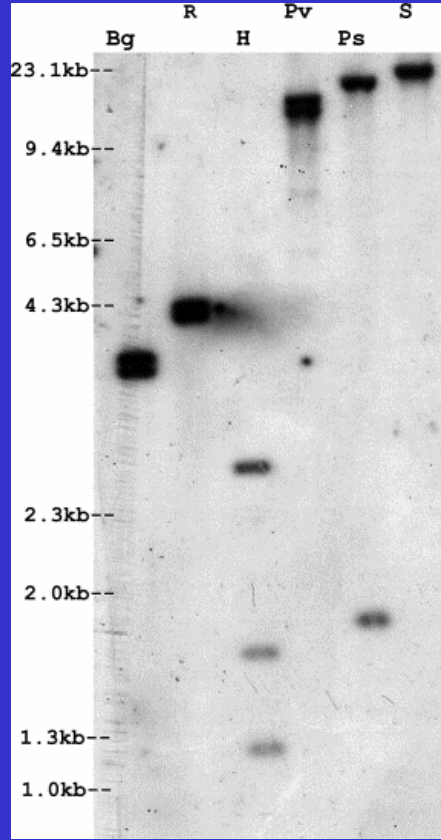
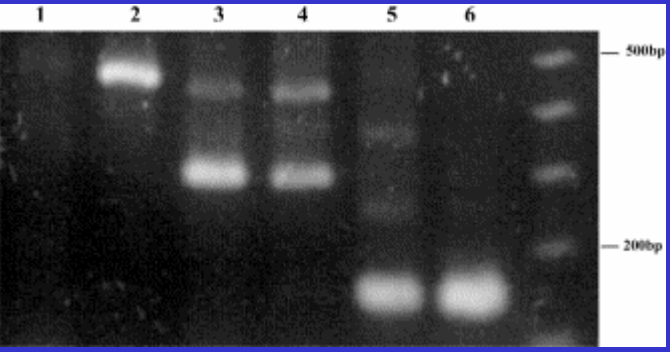
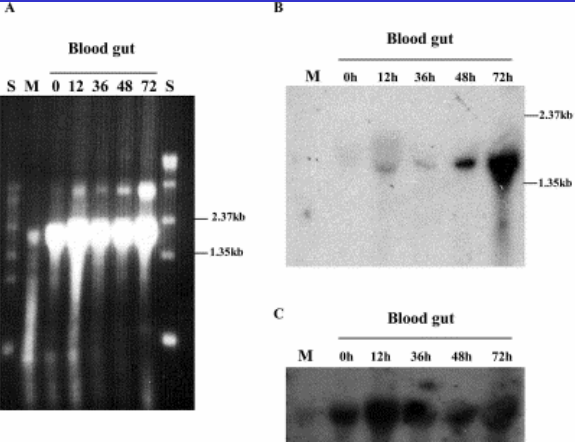
We report on the characterization in transgenic *Aedes aegypti* of two mosquito gut-specific promoters. A 1.4-kb DNA fragment adjacent to the 5' end of the coding region of the *Ae. aegypti* carboxypeptidase (*AeCP*) gene and a corresponding 3.4-kb DNA fragment at the 5' end of the *Anopheles gambiae* carboxypeptidase (*AgCP*) gene were linked to a firefly luciferase reporter gene and introduced into the *Ae. aegypti* germ line by using *Hermes* and *mariner* (*Mos1*) transposons. ..

Molecular characterization of Lchit1, a midgut chitinase cDNA from the leishmaniasis vector *Lutzomyia longipalpis*.

Ramalho-Ortigão JM, Traub-Cseko YM.

```

Llongipalpis  -----KTVVETLPLG--ILAVLSTLGGVSTGKLTGKGGKSLKLEKLEK
Agambiae-AgCh11  MVKGGVILAVAAALTEPEPHASAPGKVCVITWAVRPGNGKSLKLEKLEK
Pcochleriae      --MRNQLSSECVLLE---SISVLSGKVCVSTWAVRPGNGKSLKLEKLEK
Gmorsitans-GChit1  MNRKPKLIGLIFVTLRQGYIDANADKLVVYQVWAVRPGNGKSLKLEKLEK
AaegyptiCHIT1    -----KVVLTALVLPF-----LGGQGFARVYVLSKVAHYRFPVSTLTPGKSL
Llongipalpis  THILVFFPGS-SGSGRTIDPGLLDLNNKLSLRSNENKRVNPKRQKSLKAGGNKGG
Agambiae-AgCh11  THILVFFPGN-EGSGRTIDPGLLDLNNKLSLRSNENKRVNPKRQKSLKAGGNKGG
Pcochleriae      THILVFFPGS-H-SGSGRTIDPGLLDLNNKLSLRSNENKRVNPKRQKSLKAGGNKGG
Gmorsitans-GChit1  THILVFFPGS-VVYVSGSGRTIDPGLLDLNNKLSLRSNENKRVNPKRQKSLKAGGNKGG
AaegyptiCHIT1    THILVFFPGS-LGDSNYKSLIDPGLLDLNNKLSLRSNENKRVNPKRQKSLKAGGNKGG
Llongipalpis  SVTFRSDVLSGKQNTKNSKLSLKYKFGKLDLWYVLAQKQGN-QEGRDLSLITD
Agambiae-AgCh11  SVTFRSDVLSGKQNTKNSKLSLKYKFGKLDLWYVLAQKQGN-QEGRDLSLITD
Pcochleriae      SVTFRSADVLSKTLANNKLSLQVWQKQGLDWEVLSLSSNYTLEKDLKSL
Gmorsitans-GChit1  SINSMSAADNMSRSLKSLKSLRPHGKFGKLDLWYVLAQKQGN-ASAGVYKTEPL
AaegyptiCHIT1    GMLRSLVWVWVLDLSDPGLLDLWYVLAQKQGN-ASAGVYKTEPL
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Llongipalpis  NNAPLVLSGKQTE-LEKQNVKSLRKYVLSKSLKSLKGLVFRGGKSLVNVNPKP
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Pcochleriae      NNAPLVLSGKQTE-LEKQNVKSLRKYVLSKSLKSLKGLVFRGGKSLVNVNPKP
Gmorsitans-GChit1  NNAPLVLSGKQTE-LEKQNVKSLRKYVLSKSLKSLKGLVFRGGKSLVNVNPKP
AaegyptiCHIT1    NNAPLVLSGKQTE-LEKQNVKSLRKYVLSKSLKSLKGLVFRGGKSLVNVNPKP
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AaegyptiCHIT1    LQVHLS-----KQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLS
Llongipalpis  VSPFNQWVLDLSSKLSLNNKSLNLSLGGKSLNLSLQVHLSLQVHLSLQVHLSLQVHLS
Agambiae-AgCh11  VSPFNQWVLDLSSKLSLNNKSLNLSLGGKSLNLSLQVHLSLQVHLSLQVHLSLQVHLS
Pcochleriae      VSPFNQWVLDLSSKLSLNNKSLNLSLGGKSLNLSLQVHLSLQVHLSLQVHLSLQVHLS
Gmorsitans-GChit1  VSPFNQWVLDLSSKLSLNNKSLNLSLGGKSLNLSLQVHLSLQVHLSLQVHLSLQVHLS
AaegyptiCHIT1    VSPFNQWVLDLSSKLSLNNKSLNLSLGGKSLNLSLQVHLSLQVHLSLQVHLSLQVHLS
Llongipalpis  SLVNA-----LNNHTWVLS--LSTNNNENKVV-----
Agambiae-AgCh11  SLVNA-----LNNHTWVLS--LSTNNNENKVV-----
Pcochleriae      SLVNA-----LNNHTWVLS--LSTNNNENKVV-----
Gmorsitans-GChit1  SLVNA-----LNNHTWVLS--LSTNNNENKVV-----
AaegyptiCHIT1    SLVNA-----LNNHTWVLS--LSTNNNENKVV-----
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Pcochleriae      LQVHLS-----LQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLS
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AaegyptiCHIT1    LQVHLS-----LQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLS
Llongipalpis  LQVHLS-----LQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLS
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Pcochleriae      LQVHLS-----LQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLS
Gmorsitans-GChit1  LQVHLS-----LQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLS
AaegyptiCHIT1    LQVHLS-----LQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLSLQVHLS
    
```



APESAR DO GENE DE QUITINASE DE

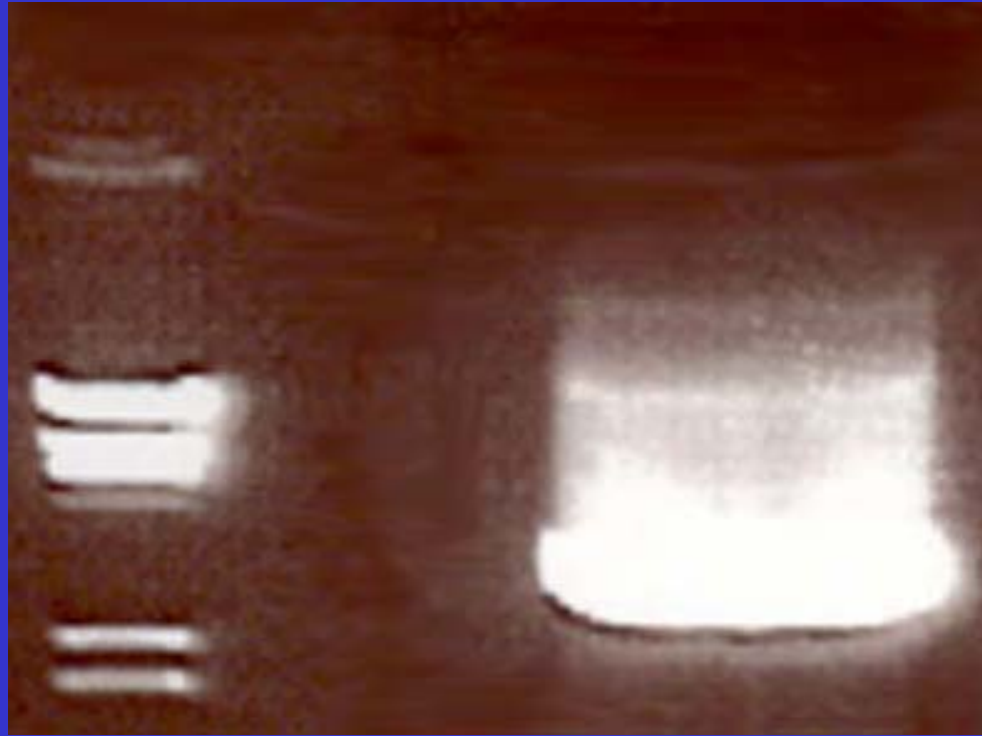
Lutzomyia longipalpis

TER +/- 1300 PB,

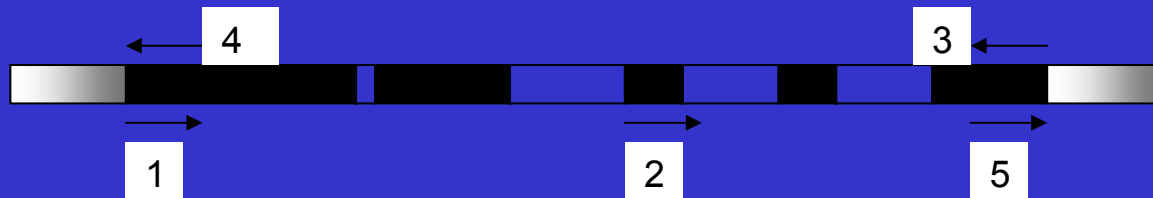
O PCR DO CLONE GENÔMICO DEU ORIGEM A UM

FRAGMENTO DE QUASE 4000 PB

PORQUE?



> 3.000PB



Esquema do gene de quitinase e dos oligonucleotídeos utilizados nos primeiros sequenciamentos do gene.

Exon 

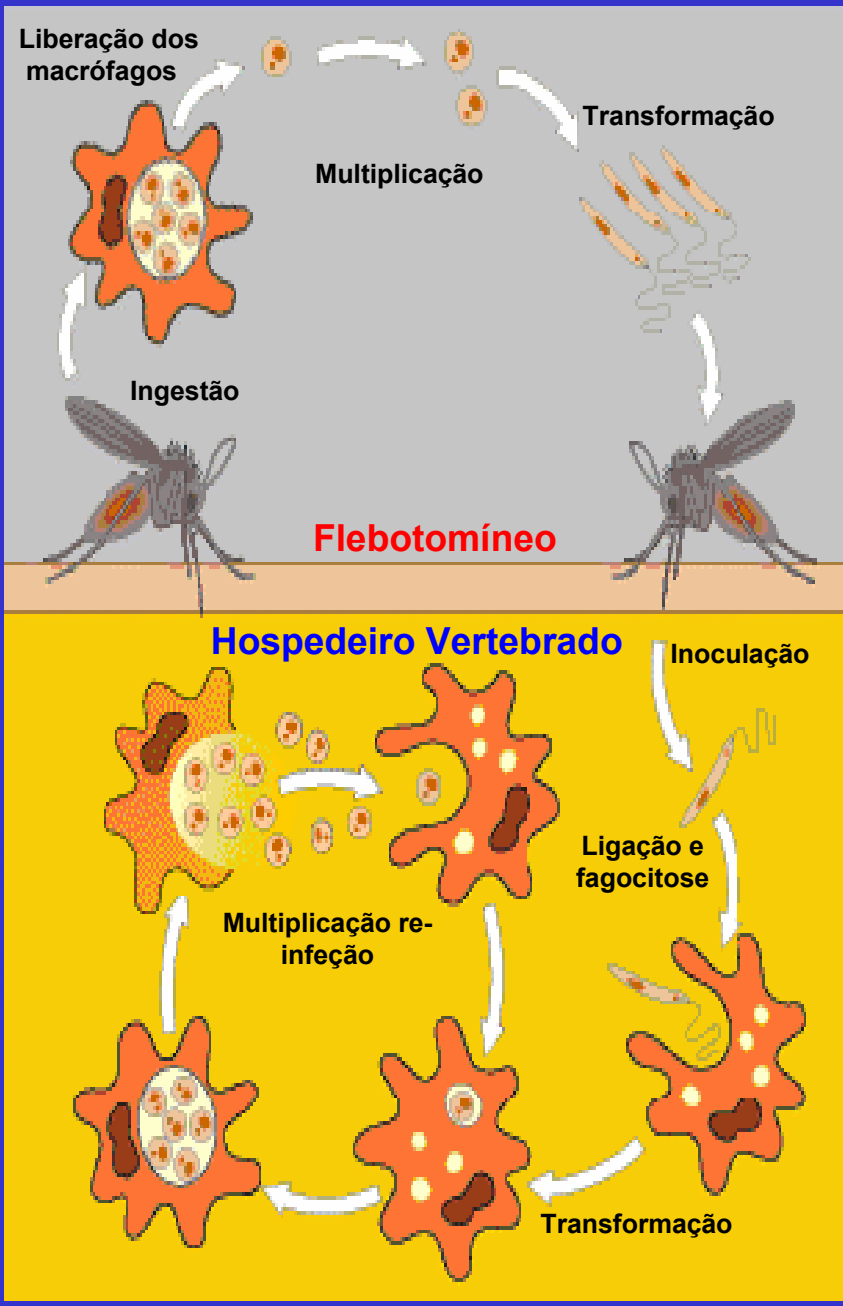
Intron 

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IRILDOPYLDLDENYGLGNIRKFNELKKVNPKLKTIAGVGGWNEG
SVTFSQ cagggtcagtgaaacagatctcacgatttttaagaagat
ttcatTTTTGAGACTTTTTCTCTATTAG (*)
VVNDPRKRQNFVKNSLEFLKKYNFDGLDVDWEYPAQRGGNQEKD
KEAYTLLLKELSEFLHPKGYLSAAVASAEFSAKISYNIAEVS
K
taagttcaaaggaatTTTTtagggaagattgggctaaactattc
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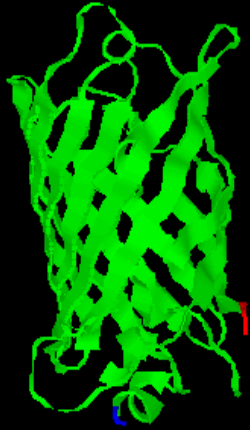
CLONE cDNA

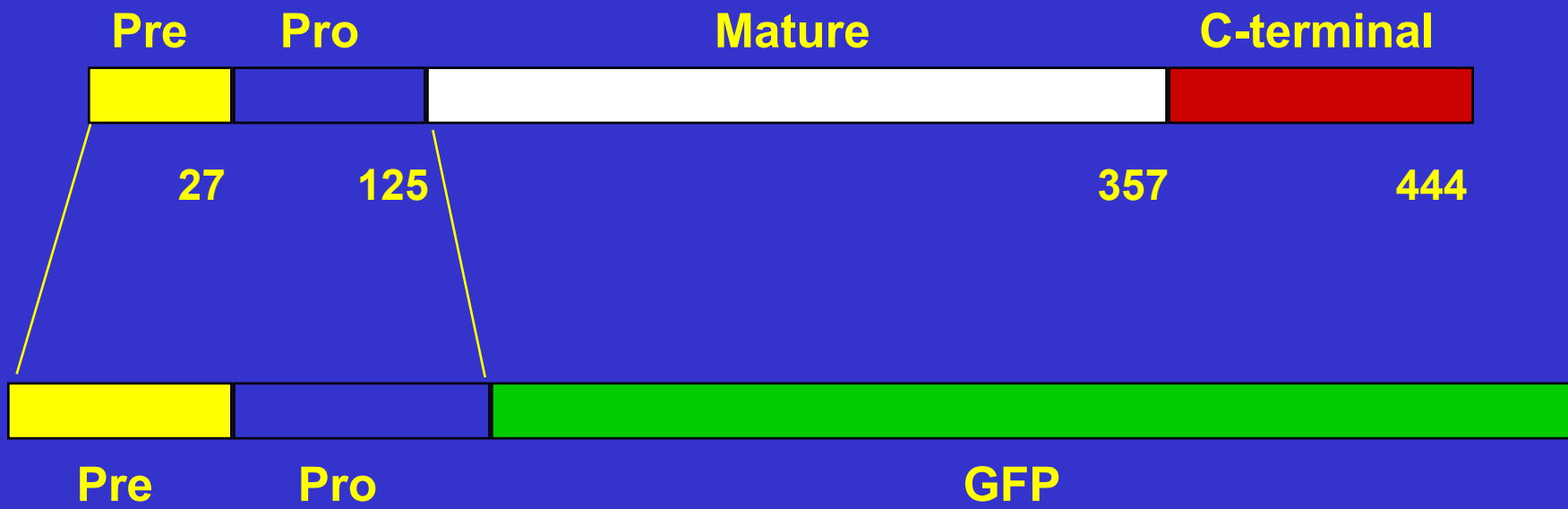
CLONE GENÔMICO

introns

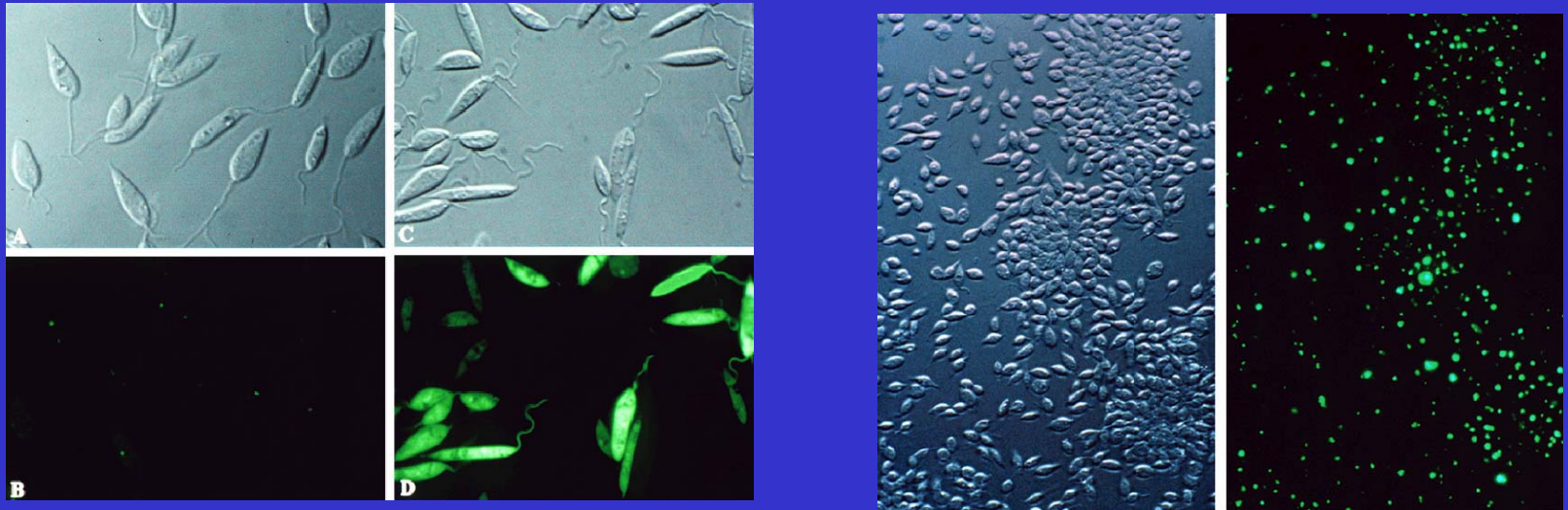


**PERGUNTA: COMO UMA
CISTEÍNA PROTEINASE DE
LEISHMANIA CHEGA AO
LISOSOMA??**





Endereçamento da cisteína proteinase Lpcys2 ao lisosoma em leishmania



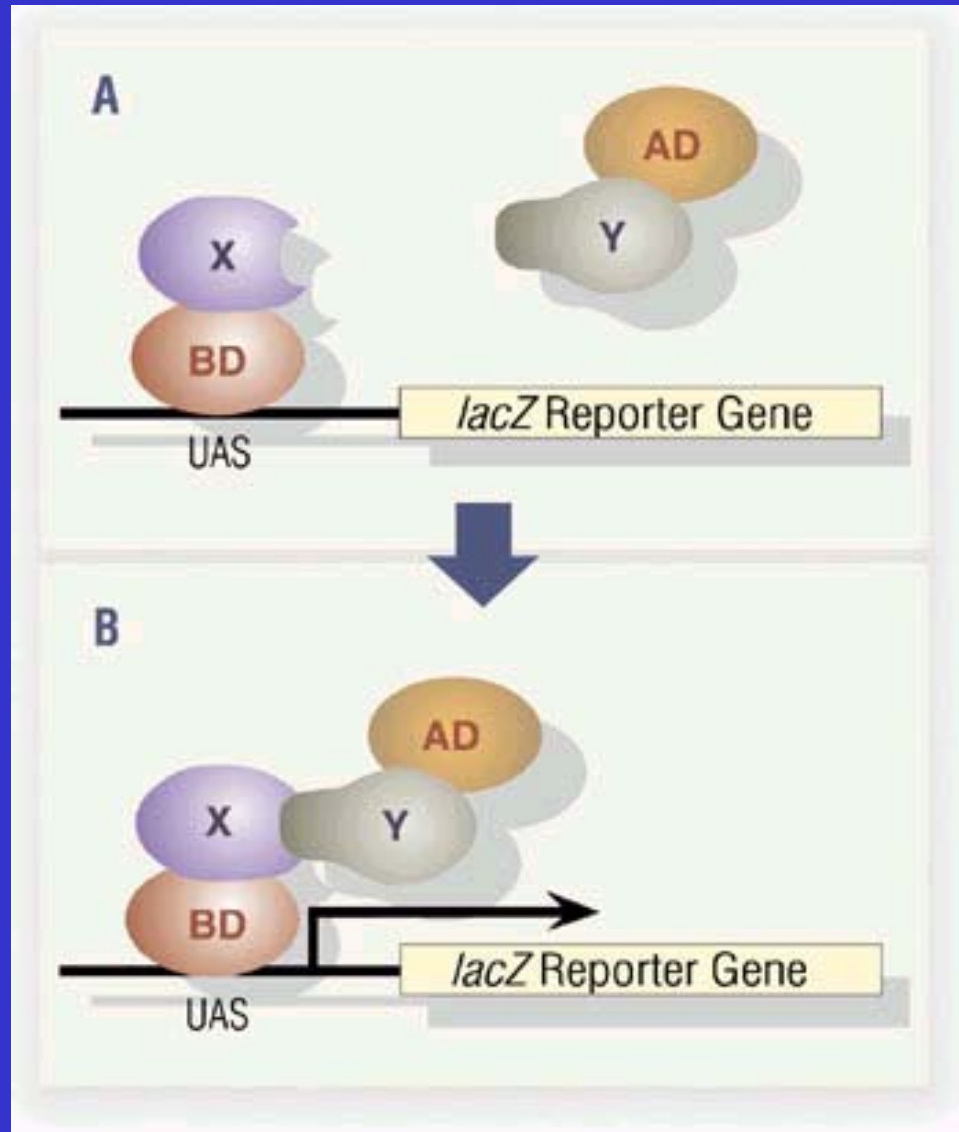
Costa-Pinto D, Trindade LS, McMahon-Pratt D, Traub-Cseko YM. Cellular trafficking in trypanosomatids: a new target for therapies? *Int J Parasitol* 2001 May 1;31(5-6):536-43

Existe um sinal de endereçamento no domínio pró de Lpcys2 responsável pelo direcionamento.

**COMO IDENTIFICAR UMA PROTEÍNA
QUE INTERAGE COM ESSE DOMÍNIO?**

SISTEMA DE DOIS HÍBRIDOS DE LEVEDURA

“ YEAST TWO HYBRID SYSTEM ”



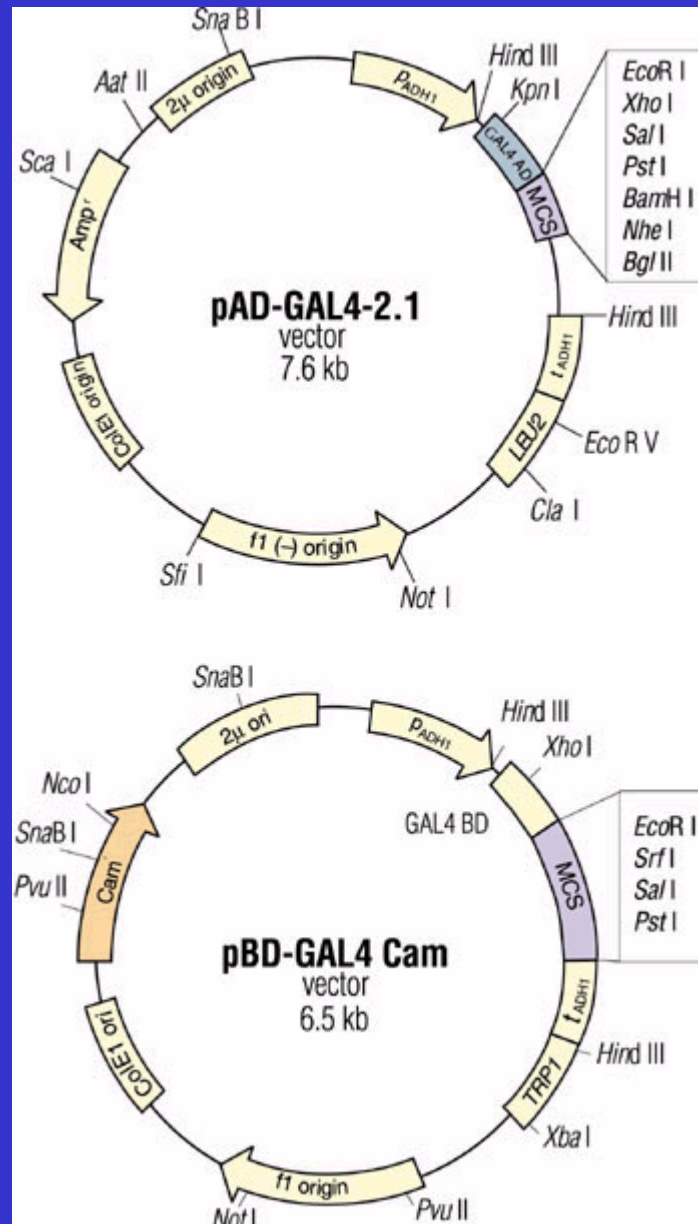
Proteína “isca”

Domínio de ligação

Domínio
ativador

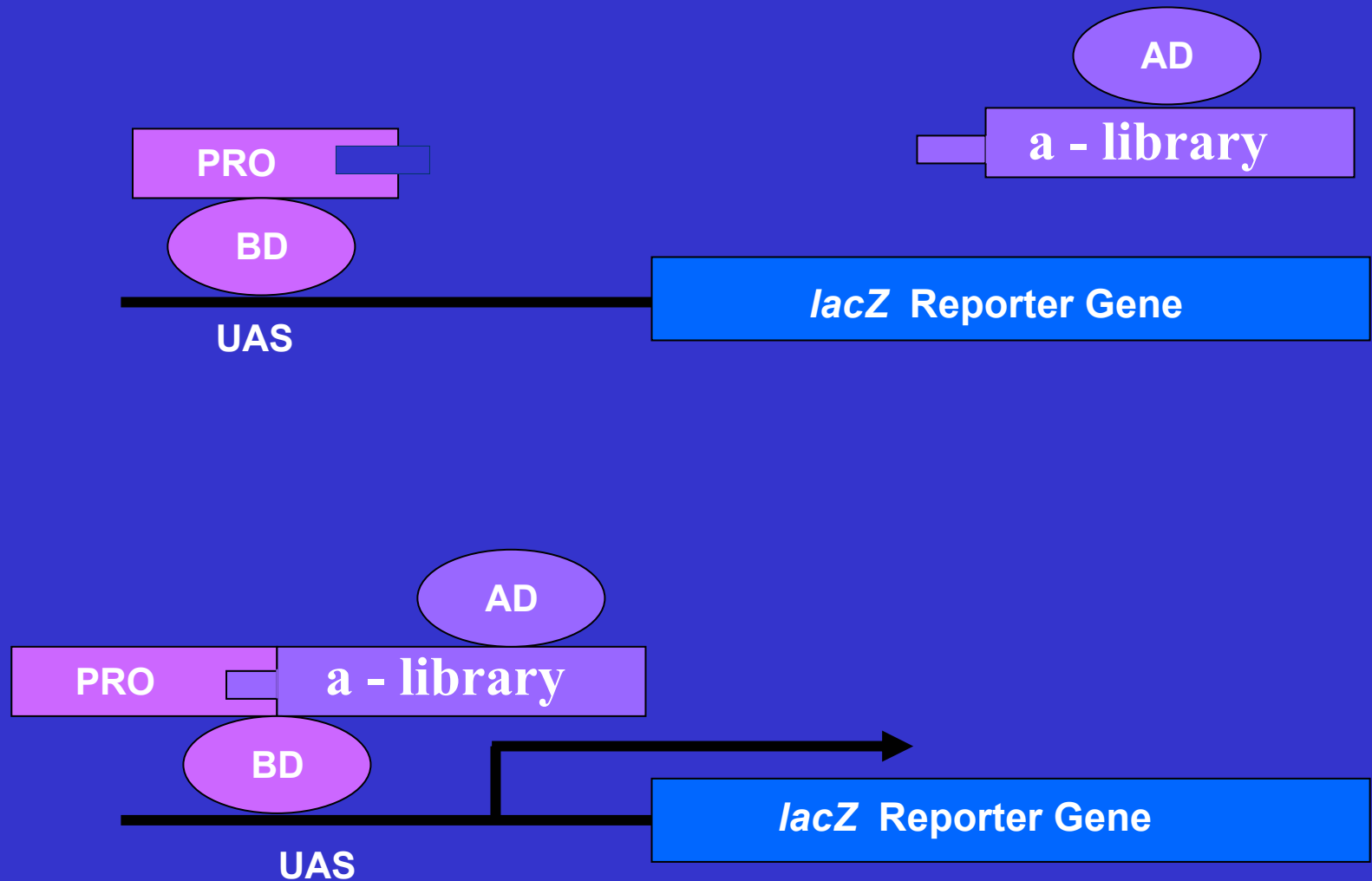
Proteína
desconhecida

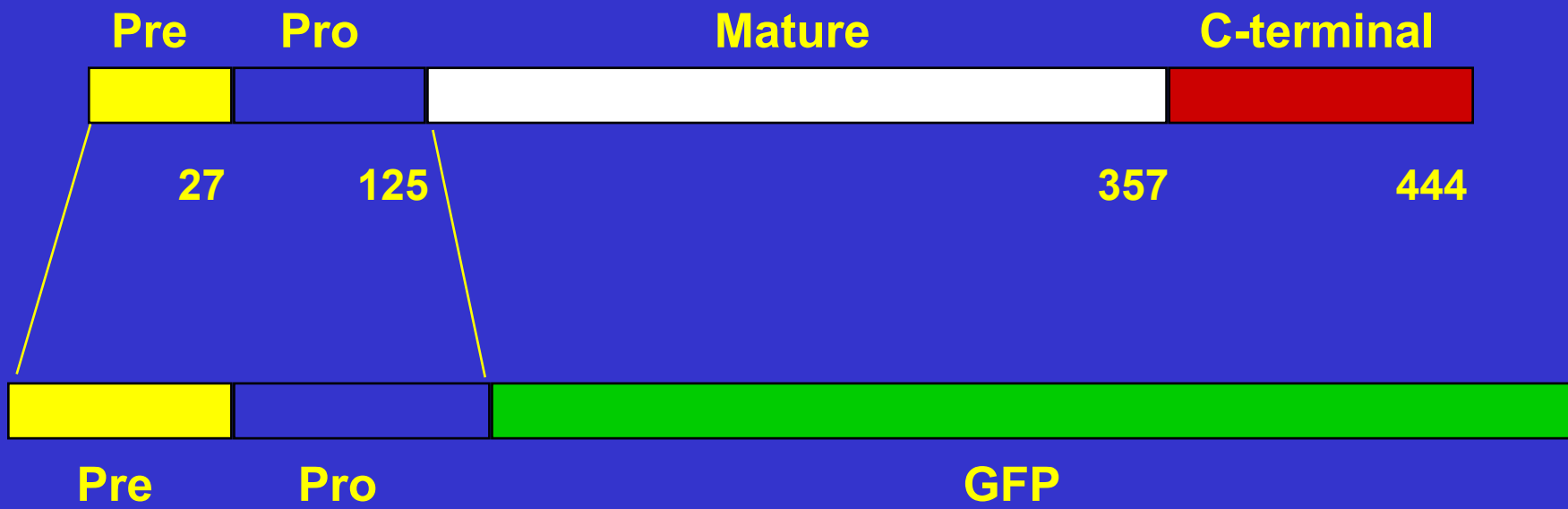
Plasmídio para clonagem da biblioteca



Plasmídio para clonagem da “isca”

YEAST TWO HYBRID SYSTEM





FINALMENTE...

Isto é só parte da história, existem outras maneiras de “clonar” e produzir proteínas recombinantes.

Exemplo: produção de proteína C, ou outras proteínas envolvidas em coagulação.

- Hemofílicos : fator VIII, fator IX.
- Erro inatos de metabolismo na produção de prot. C (no controle de coagulação), e também em certos tipos de cirurgia.
- Proteína ativadora de plasminogênio (que dissolve coágulos), para pacientes de derrames e ataques cardíacos.
- Anti-tripsina alfa-1 para certas formas de enfisema.

PROBLEMA...

- PRESENTES EM SANGUE DE DOADORES EM QUANTIDADES ÍNFIMAS.
- Portanto muito caras: tratamento de hemofilia com fator VIII (normalmente apenas quando o paciente está sangrando) custa milhares de dólares por ano. O custo do fornecimento constante da proteína custaria mais que US\$ 100.000,00.

ANTES

- sangue humano doado, processado em grandes quantidades.
- ou de vasto número de células crescidas em bioreatores.

ALTERNATIVA?

Animais transgênicos como fábricas de drogas

VANTAGENS?

- BENEFÍCIOS ECONÔMICOS.
- RISCOS MENORES DE CONTAMINAÇÃO (HIV, HEPATITE C, DOENÇA DE CREUZFELDT-JAKOB).

COMO? Histórico:

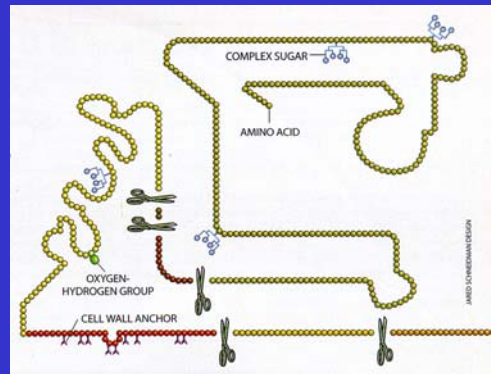
- 1980, Gordon JW, Universidade de Yale: embriões de camundongo capazes de incorporar DNA estranho.
- 1987, Wagner TE, Universidade de Ohio: DNA de coelho incorporado em camundongos-, Hennighasen e Clark na Escócia, ativaram genes estranhos na glândula mamária de camundongos, com o produto sendo secretado no leite: ativador de plasminogênio humano; beta-galactoglobulina de carneiro;
- Em seguida carneiros: fator X.

EXPRESSÃO EM PORCOS (Genie). **Porque?**

- período de gestação e gerações curtas.
- ninhada grande.
- 300 litros de leite por ano/animal.

Biologicamente ativo?

Modificações pós-traducionais corretas?
(processamento e modificações?)

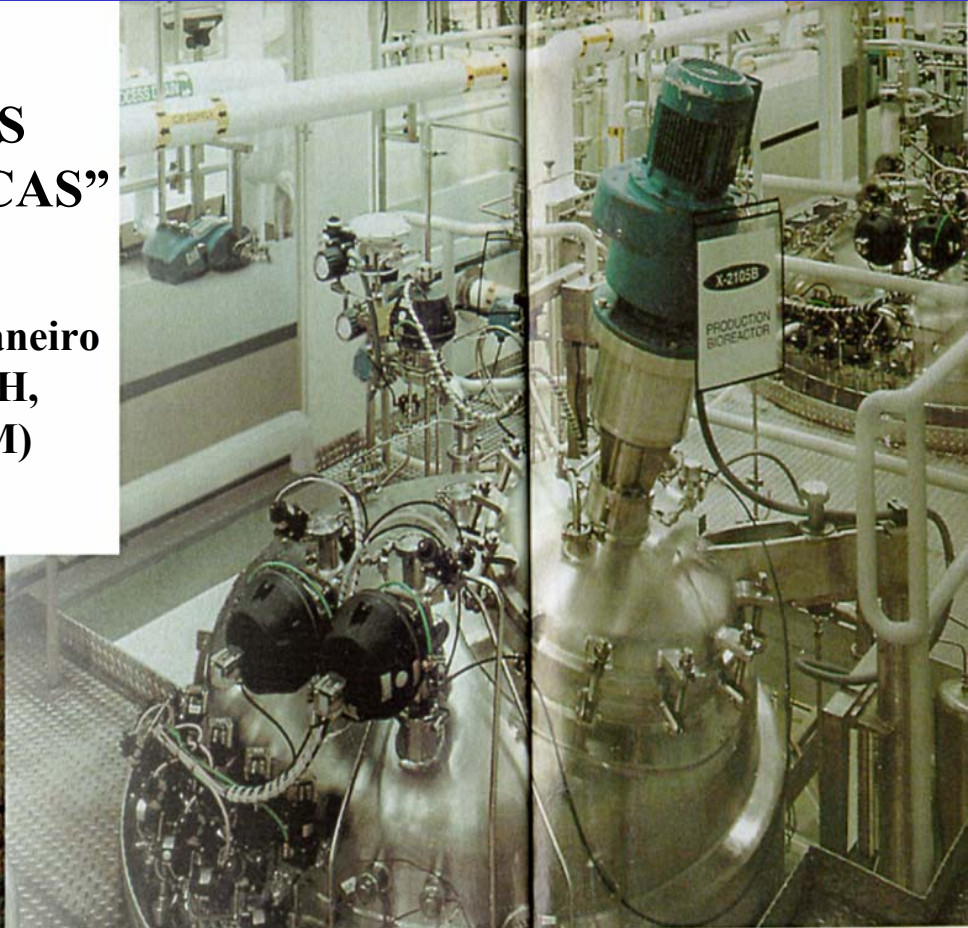


- 1/3 da proteína C ativa.
- ausência de furina para processar
- 1995 em camundongo: transgênico duplo

Direções futuras?

ANIMAIS TRANSGÊNICOS COMO “FÁBRICAS” DE DROGAS

Scientific American janeiro
de 1997 (Velandar, WH,
Lubon H, Drohan WM)





BIBLIOTECA DE OXFORD