I Conferencia

Nociones Básicas en Biología Molecular, Genética y Genómica

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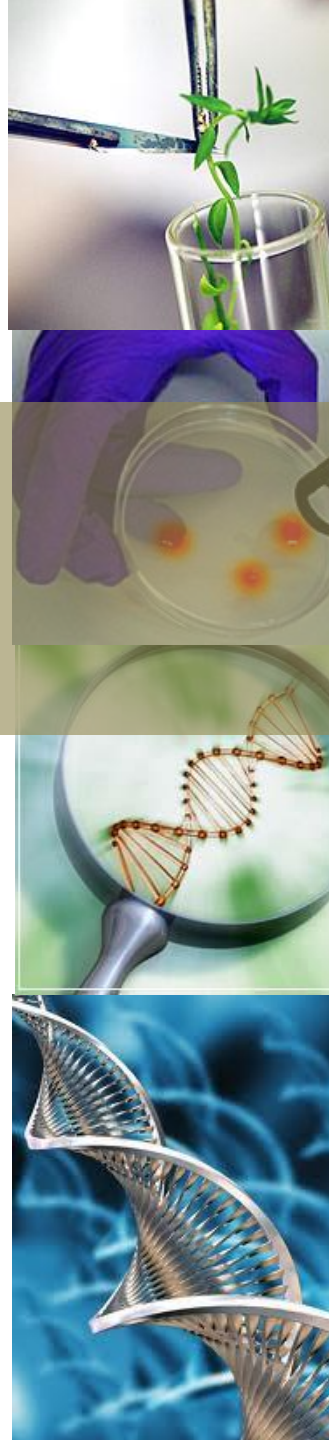
CONTENIDO

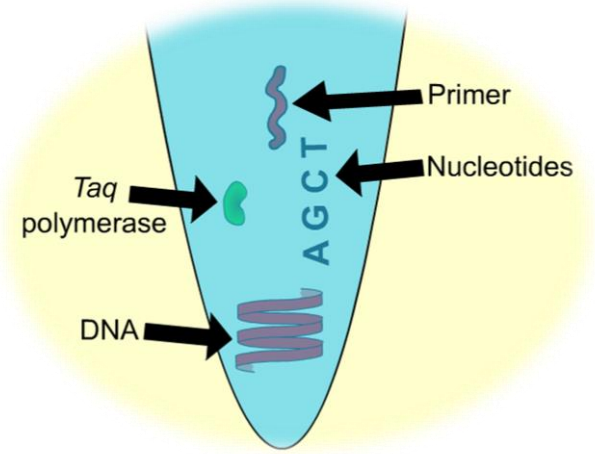
Técnicas básicas empleadas en Biología Molecular: PCR - Electroforesis

Clonamiento y expresión de genes por tecnología del ADN recombinante

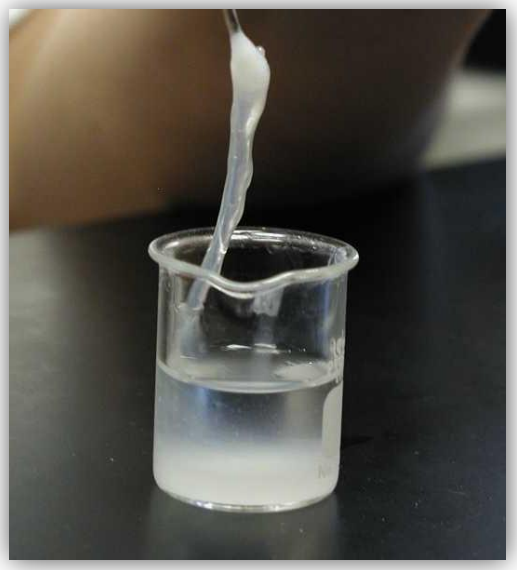
Knockout de proteínas transportadoras de membrana del ácido pirazinoico en M. smegmatis



EXTRACCIÓN DE ADN



REACCIÓN EN CADENA DE LA POLIMERASA



ELEMENTOS

Solución tampón o Buffer de PCR

5 - 10 mM Tris (pH 7 - 8).

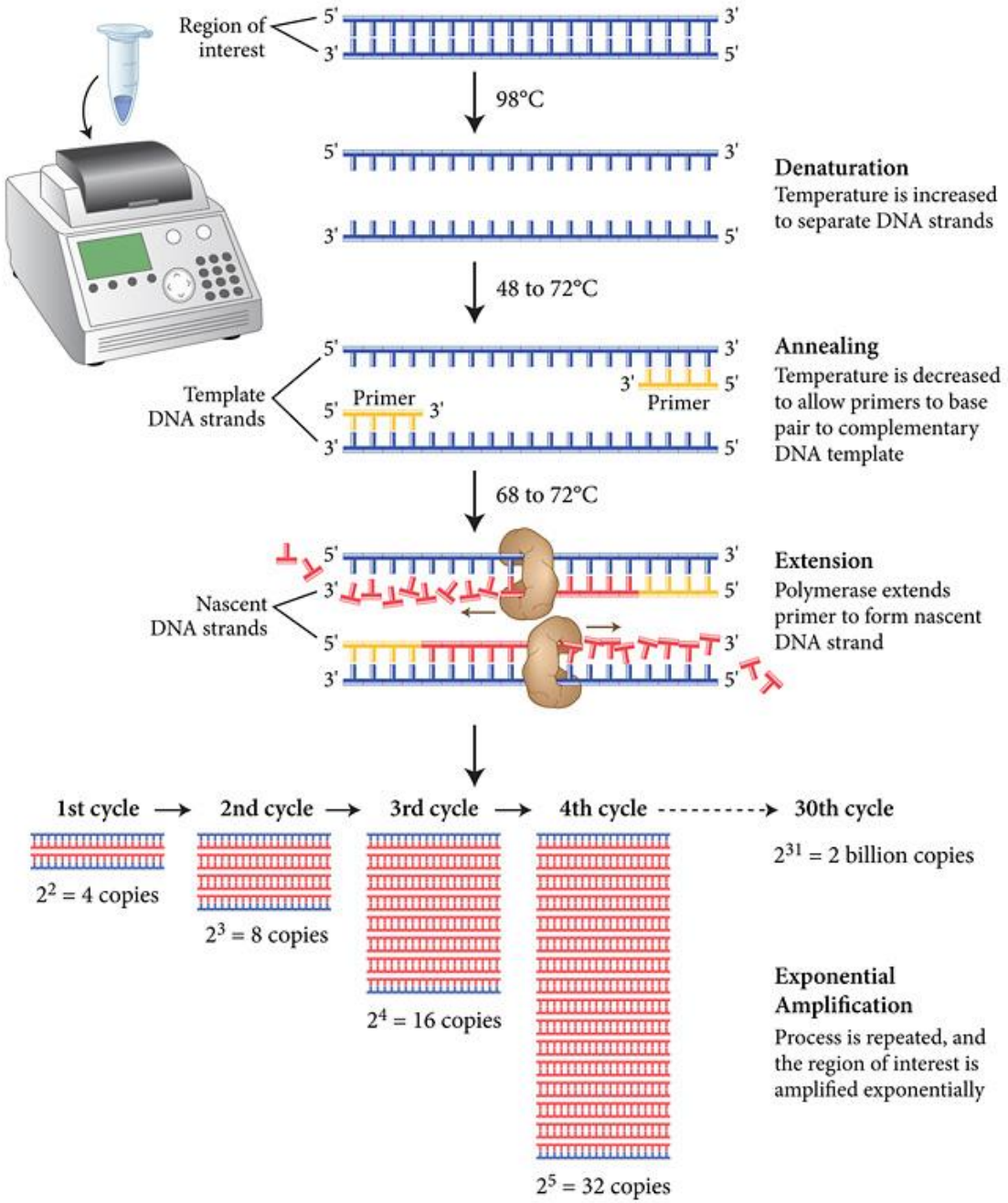
dNTPs

Cloruro de Magnesio

Cebadores (Primers)

ADN polimerasa

ADN molde



ELECTROFORESIS

- La electroforesis es una técnica básica en biología molecular y biotecnología que consiste en la separación de macromoléculas bajo un campo eléctrico, de acuerdo a su tamaño, carga y ***forma***.

- Nos permite la separación de moléculas cargadas como ADN, ARN y/o proteínas.

- La matriz más empleada es la ***Agarosa***.

Identificación *in silico*, caracterización molecular y análisis de expresión de la proteína de filamento para flagelar PFR3 del *Tripanosoma brucei*

Silico identification, molecular characterization and expression analysis of the Trypanosoma brucei paraflagellar rod protein PFR3

Morell M, García-Pérez JL, Thomas MC, López MC*

Departamento de Biología Molecular, Instituto de Parasitología y Biomedicina «López Neyra», CSIC. Granada – SPAIN. * Corresponding author e-mail: mclopez@ipb.csic.es

RESUMEN

En el presente artículo se describen la identificación y el aislamiento del gen codificante para la proteína PFR3 del *T. brucei*. La secuencia deducida de aminoácidos produce una proteína de 592 residuos con un punto isoeléctrico de 5,14 y presenta una identidad de secuencia del 68,9% con la proteína PFR3 del *T. cruzi*. Sin embargo, el porcentaje de homología entre la proteína PFR3 de *T. brucei* y otras secuencias disponibles de PFRs de *T. brucei* y *T. cruzi* es inferior al 22%. En contraste con lo descrito para los miembros de la familia de proteínas de filamento paraflagelar, la mayor divergencia entre las proteínas PFR3 de *T. cruzi* y *T. brucei* se encuentra en la región central de la proteína, con una similitud del 38% en 200 aminoácidos. Estimamos que existen dos copias de la proteína PFR3 de *T. brucei* por genoma haploide. El gen se transcribe como mARN de aproximadamente 3,6 kb de longitud, presente con la misma abundancia en formas parasitarias procíclicas y del torrente sanguíneo.

JCM
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Identification of Infectious Agents in Onychomycoses by PCR-Terminal Restriction Fragment Length Polymorphism

Julie Verrier,[a] Marina Pronina,[b] Corinne Peter,[c] Olympia Bontems,[a] Marina Fratti,[a] Karine Salamin,[a] Stéphanie Schürch,[d] Katia Gindro,[d] Jean-Luc Wolfender,[e] Keith Harshman,[b,c] and Michel Monod[a]

Department of Dermatology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland[a]; Centre Intégratif de Génomique (CIG), Génopode-UNIL, Lausanne, Switzerland[b]; Lausanne Genomic Technologies Facility, Génopode-UNIL, Lausanne, Switzerland[c]; Mycology Group, Agroscope Changins-Wädenswil ACW, Nyon, Switzerland[d]; and Phytochemistry, Pharmaceutical Sciences, Université de Genève, Geneva, Switzerland[e]

A fast and reliable assay for the identification of dermatophyte fungi and nondermatophyte fungi (NDF) in onychomycosis is essential, since NDF are especially difficult to cure using standard treatment. Diagnosis is usually based on both direct microscopic examination of nail scrapings and macroscopic and microscopic identification of the infectious fungus in culture assays. In the last decade, PCR assays have been developed for the direct detection of fungi in nail samples. In this study, we describe a PCR-terminal restriction fragment length polymorphism (TRFLP) assay to directly and routinely identify the infecting fungi in nails. Fungal DNA was easily extracted using a commercial kit after dissolving nail fragments in an Na_2S solution. *Trichophyton* spp., as well as 12 NDF, could be unambiguously identified by the specific restriction fragment size of 5′-end-labeled amplified 28S DNA. This assay enables the distinction of different fungal infectious agents and their identification in mixed infections. Infectious agents could be identified in 74% (162/219) of cases in which the culture results were negative. The PCR-TRFLP assay described here is simple and reliable. Furthermore, it has the possibility to be automated and thus routinely applied to the rapid diagnosis of a large number of clinical specimens in dermatology laboratories.

¿Y para qué amplificar el DNA?

Aplicaciones Biomédicas

Oncogenes

Enfermedades genéticas

Paternidad

Terapia génica

Ciencias Forenses

Genetic Fingerprinting

Ciencias de los Alimentos

Determinación de especies animales

Investigación

Mutagenésis dirigda

Clonamiento

Filogenia

Expresión génica

ARTICLE

doi:10.1038/nature12394

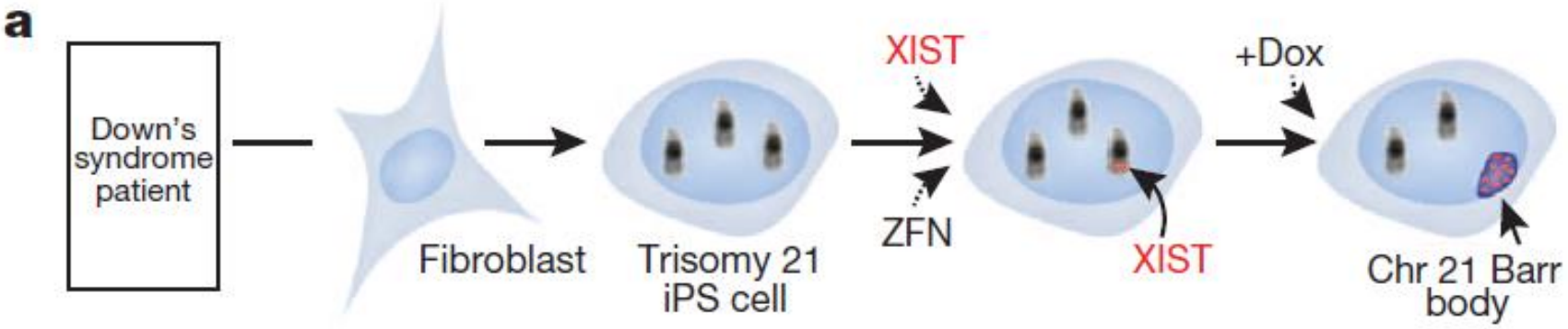
Translating dosage compensation to trisomy 21

Jun Jiang[1], Yuanchun Jing[1], Gregory J. Cost[2], Jen-Chieh Chiang[1], Heather J. Kolpa[1], Allison M. Cotton[3], Dawn M. Carone[1], Benjamin R. Carone[1], David A. Shivak[2], Dmitry Y. Guschin[2], Jocelynn R. Pearl[2], Edward J. Rebar[2], Meg Byron[1], Philip D. Gregory[2], Carolyn J. Brown[3], Fyodor D. Urnov[2], Lisa L. Hall[1] & Jeanne B. Lawrence[1]

Down's syndrome is a common disorder with enormous medical and social costs, caused by trisomy for chromosome 21. We tested the concept that gene imbalance across an extra chromosome can be ***de facto*** corrected by manipulating a single gene, ***XIST*** (the X-inactivation gene). Using genome editing with zinc finger nucleases, we inserted a large, inducible ***XIST*** transgene into the ***DYRK1A*** locus on chromosome 21, in Down's syndrome pluripotent stem cells. The ***XIST*** non-coding RNA coats chromosome 21 and triggers stable heterochromatin modifications, chromosome-wide transcriptional silencing and DNA methylation to form a 'chromosome 21 Barr body'. This provides a model to study human chromosome inactivation and creates a system to investigate genomic expression changes and cellular pathologies of trisomy 21, free from genetic and epigenetic noise. Notably, deficits in proliferation and neural rosette formation are rapidly reversed upon silencing one chromosome 21. Successful trisomy silencing ***in vitro*** also surmounts the major first step towards potential development of 'chromosome therapy'.

Dr. Lawrence's talk was *spellbinding*, and her idea *to silence the third chromosome 21 of Down syndrome is perhaps the most brilliant idea* I've heard in my many years of writing about genetics.

RESEARCH ARTICLE



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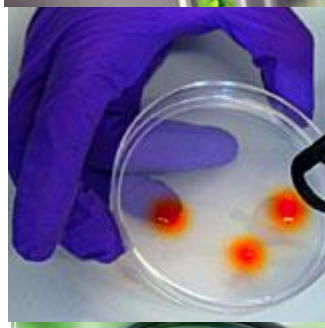
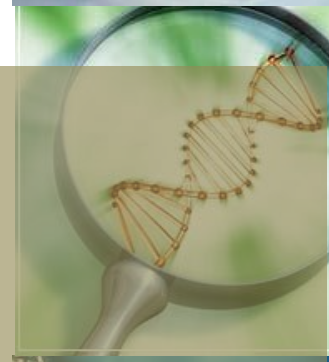
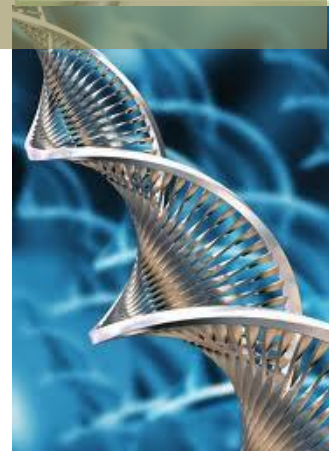
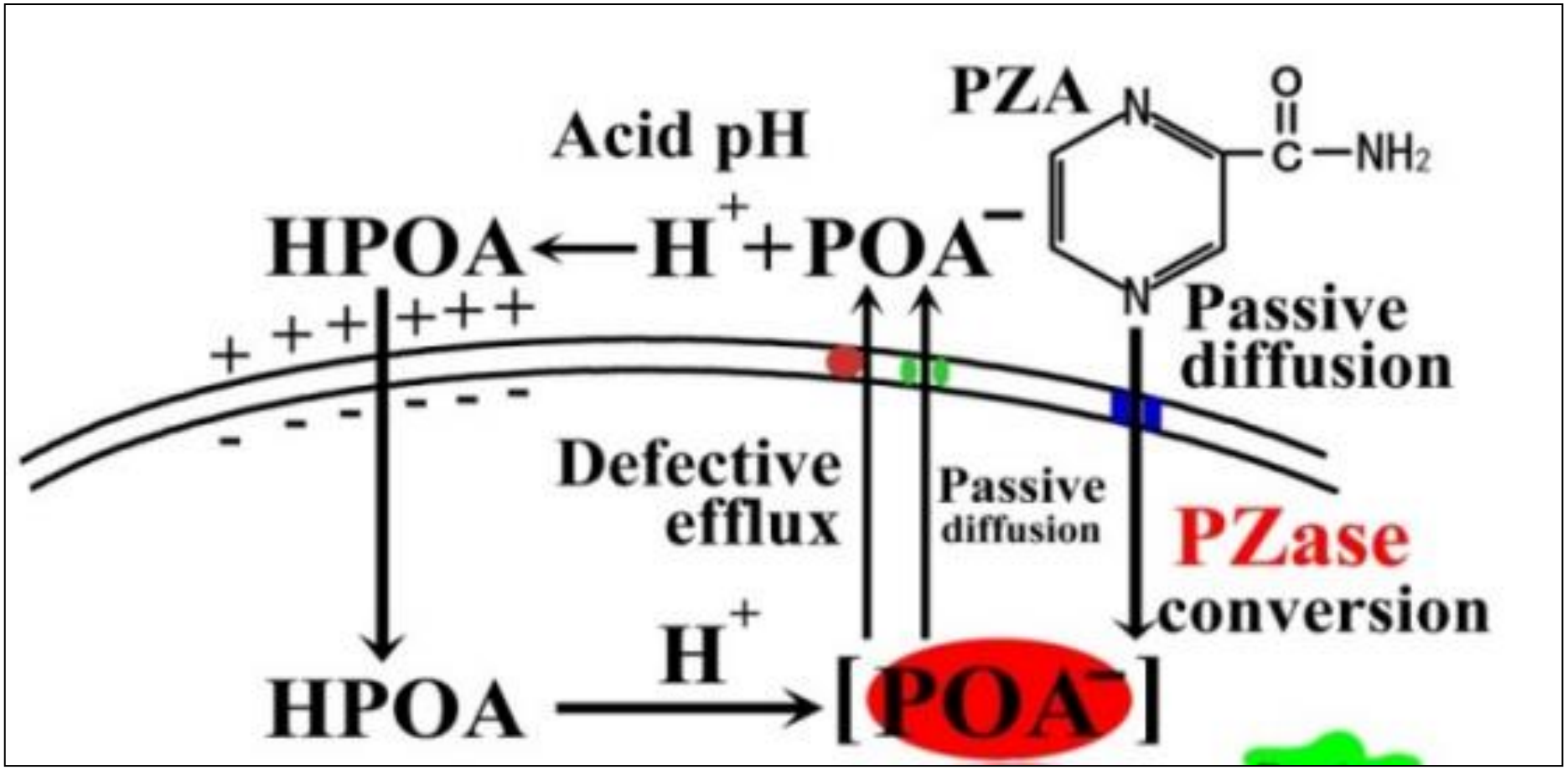




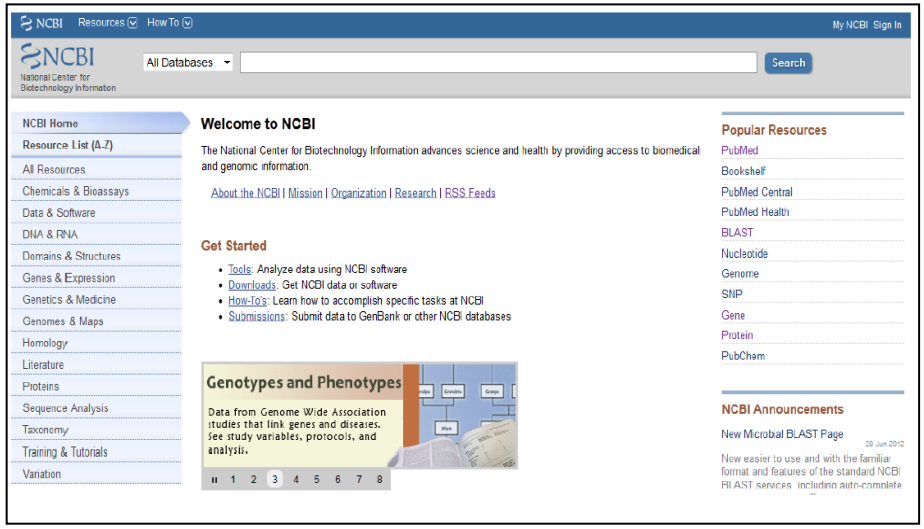
CONOCIMIENTOS EN BIOINFORMÁTICA

1

BÚSQUEDA DE SECUENCIAS Y DISEÑO DE PRIMERS

¿Qué clonamos?





Display Settings: FASTA

Send:

Showing 561 bp region from base 2288681 to 2289241.

Mycobacterium tuberculosis H37Rv chromosome, complete genome

NCBI Reference Sequence: NC_000962.2

GenBank Graphics

```
>gi|57116681:2288681-2289241 Mycobacterium tuberculosis H37Rv chromosome,
complete genome
TCAGGAGCTGCAAACCAACTCGACGCTGGCGGTGCGCATCTCCTCCAGCGCGGCGACGGTGGTATCGGCC
GACACACCCGCTGTCAGGTCCACCAGCACCCTGGTGGCCAAGCCATTGCGTACCGCGTCCTCGGCCGTCT
GGCGCACACAATGATCGGTGGCAATACCGACCACATCGACCTCATCGACGCCGCGTTGCCGCAGCCAATT
CAGCAGTGGCGTGCCGTTCTCGTCGACTCCTTCGAAGCCGCTGTACGCTCCGGTGTAGGCACCCTTGTAG
AACACCGCCTCGATTGCCGACGTGTCCAGACTGGGATGGAAGTCCGCGCCGGGAGTACCGCTGACGCAAT
GCGGTGGCCACGACGAGGAATAGTCCGGTGTGCCGGAGAAGTGGTCACCCGGGTCGATGTGGAAGTCCTT
GGTTGCCACGACGTGATGGTAGTCCGCCGCTTCGGCCAGGTAGTCGCTGATGGCGCGGGCCAGCGCGGCG
CCACCGGTTACCGCCAGCGAGCCACCCTCGCAGAAGTCGTTCTGCACGTCGACGATGATCAACGCCCGCA
T
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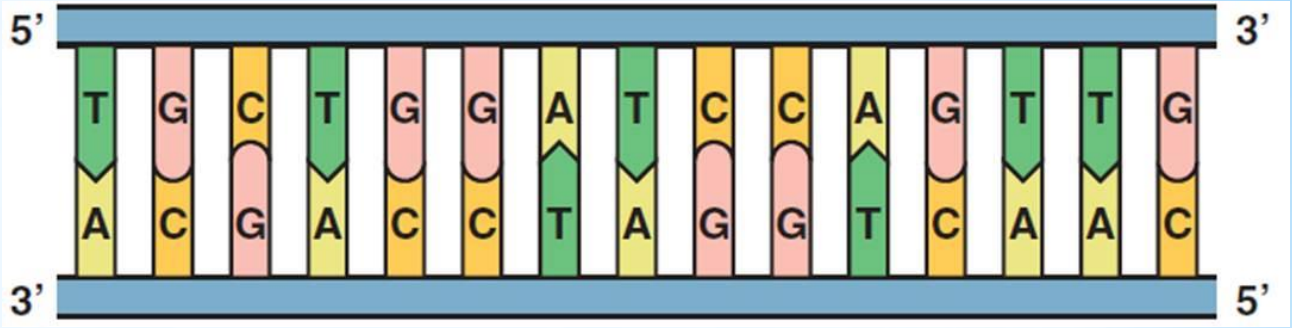


Diseño de primers

Cadena +

Fw

gen

Rev

5′-AGTCTCGAGATGCGTAGAGCTC--810bp--CCGATCGATCGCTAACGTCGTCG-3′

La Taq Pol tiene dirección 5'->3' e inicia la síntesis desde el 3'-OH del primer

Cadena +

Fw

Rev

5′-AGTCTCGAGATGCGTAGAGCTC--810bp-- -CCGATCGATCGCTAACGTCGTCG-3′

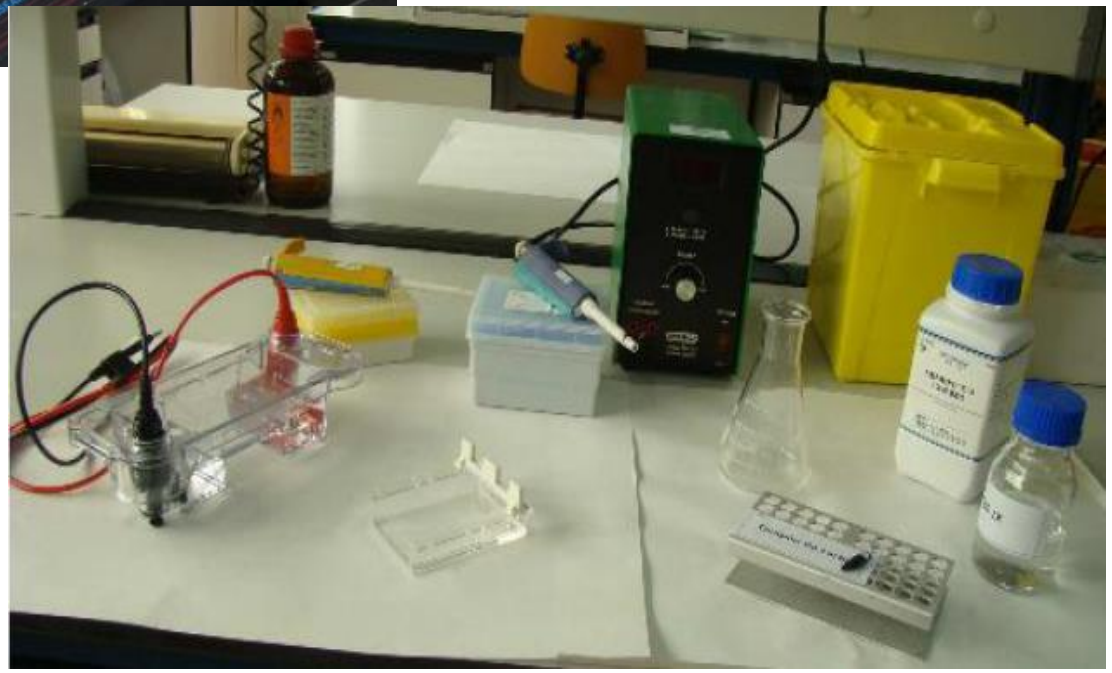
5′-GAGATGCGTAGAGCT-3′-->

<--3′-CTAGCTAGCGATTG-5′

3′-TCAGAGCTCTACGCATCTCGAG---810bp-- -GGCTAGCTAGCGATTGCAGCAGC-5

Cadena -

Los primers se escriben siempre 5′–3′



*PURIFICACIÓN DEL AMPLICÓN

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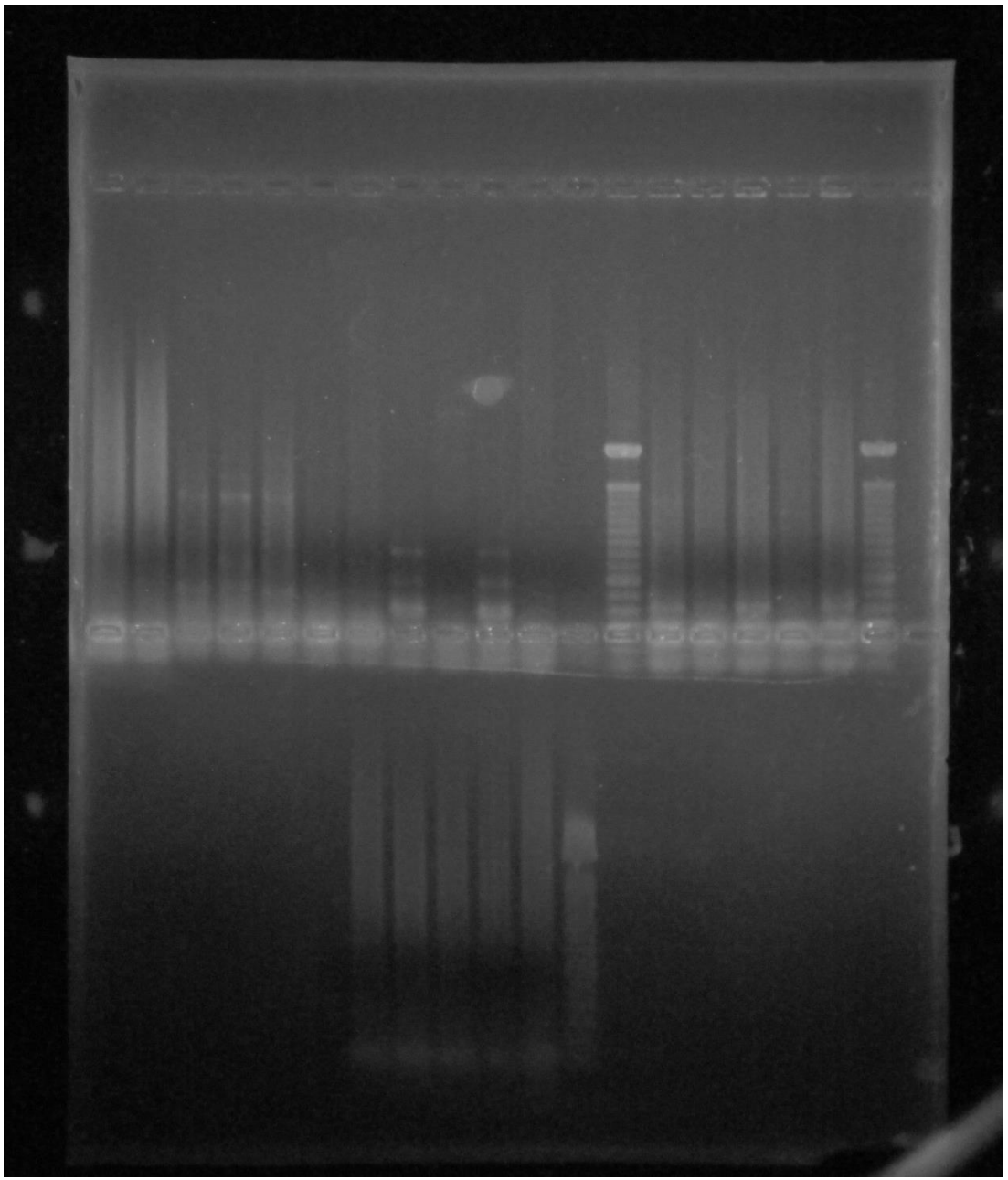
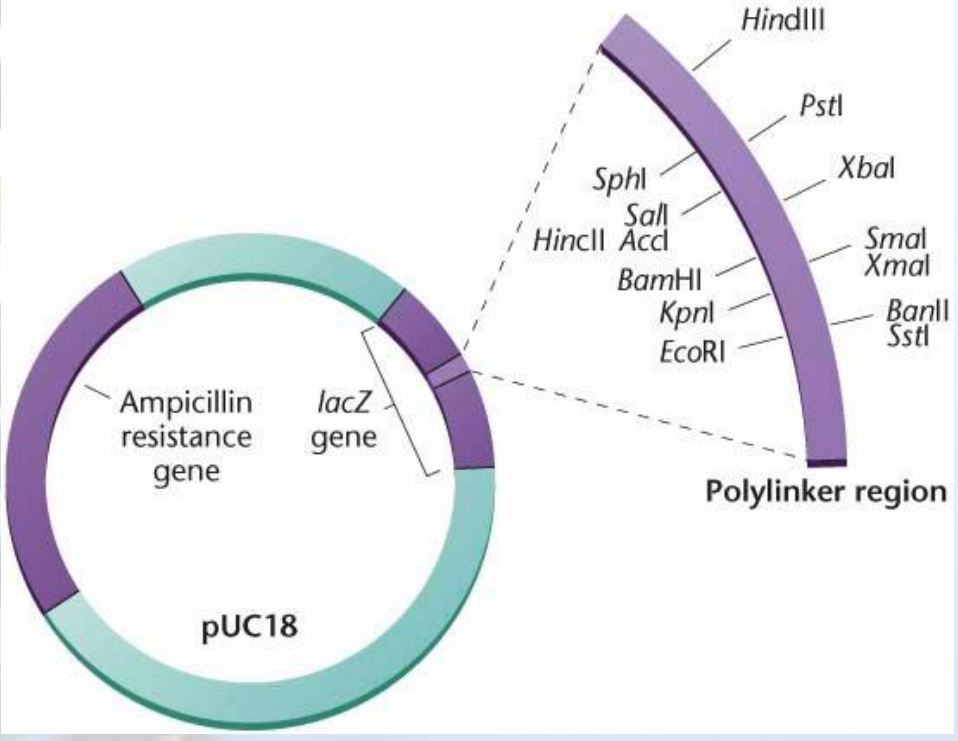
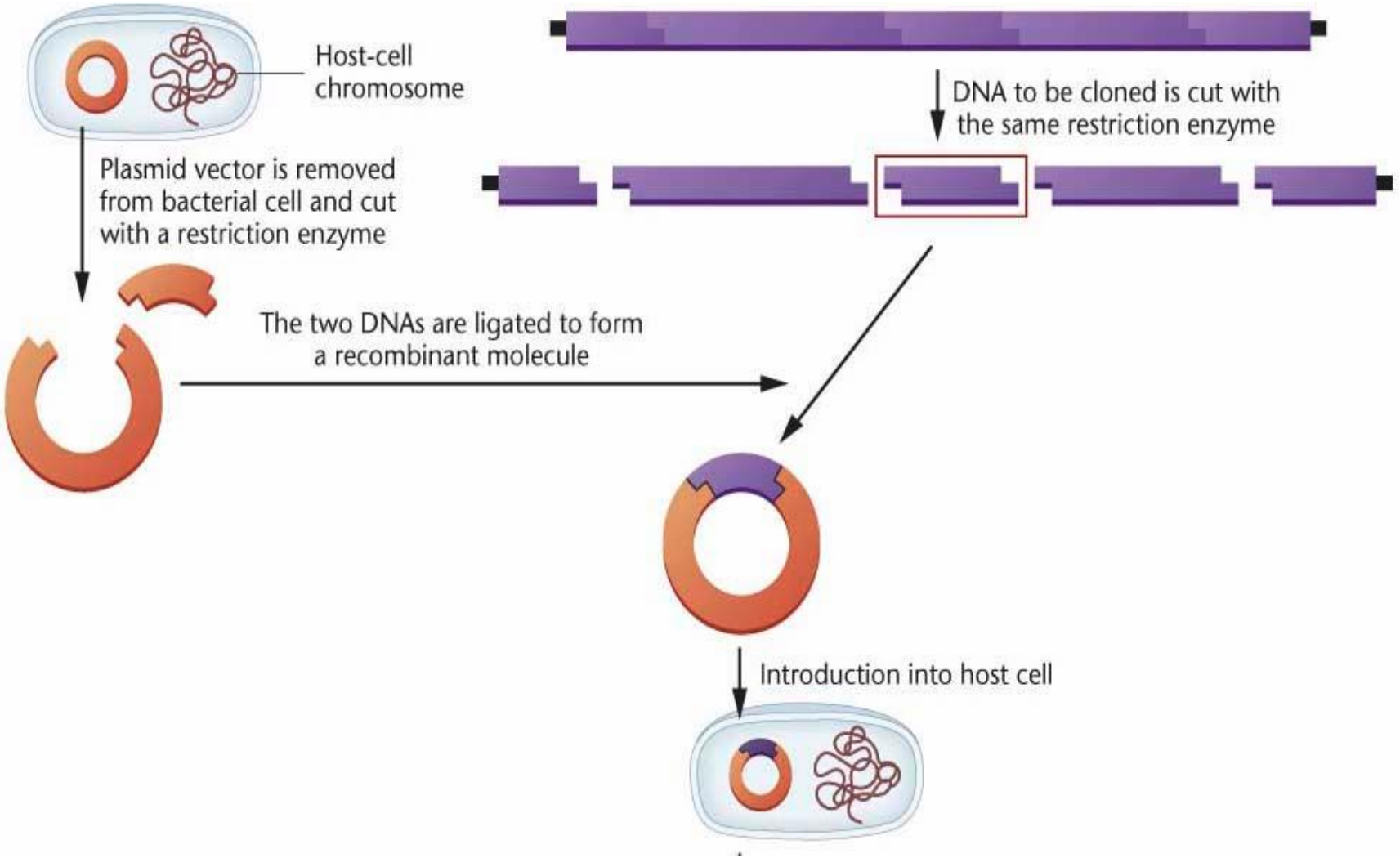


3 VECTORIZACIÓN



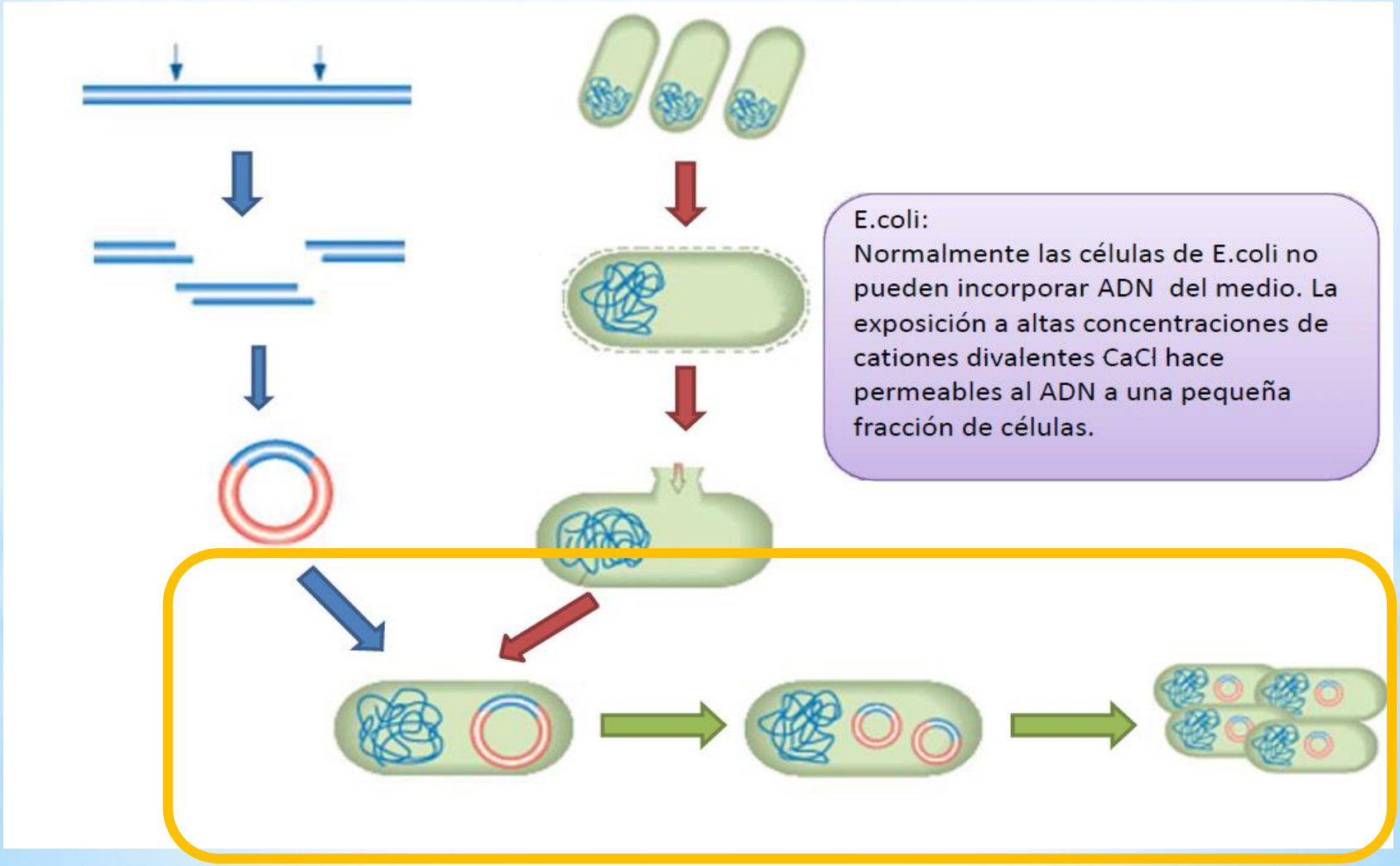
DIGESTIÓN ENZIMÁTICA Y LIGACIÓN

REACCIÓN DE LIGACIÓN



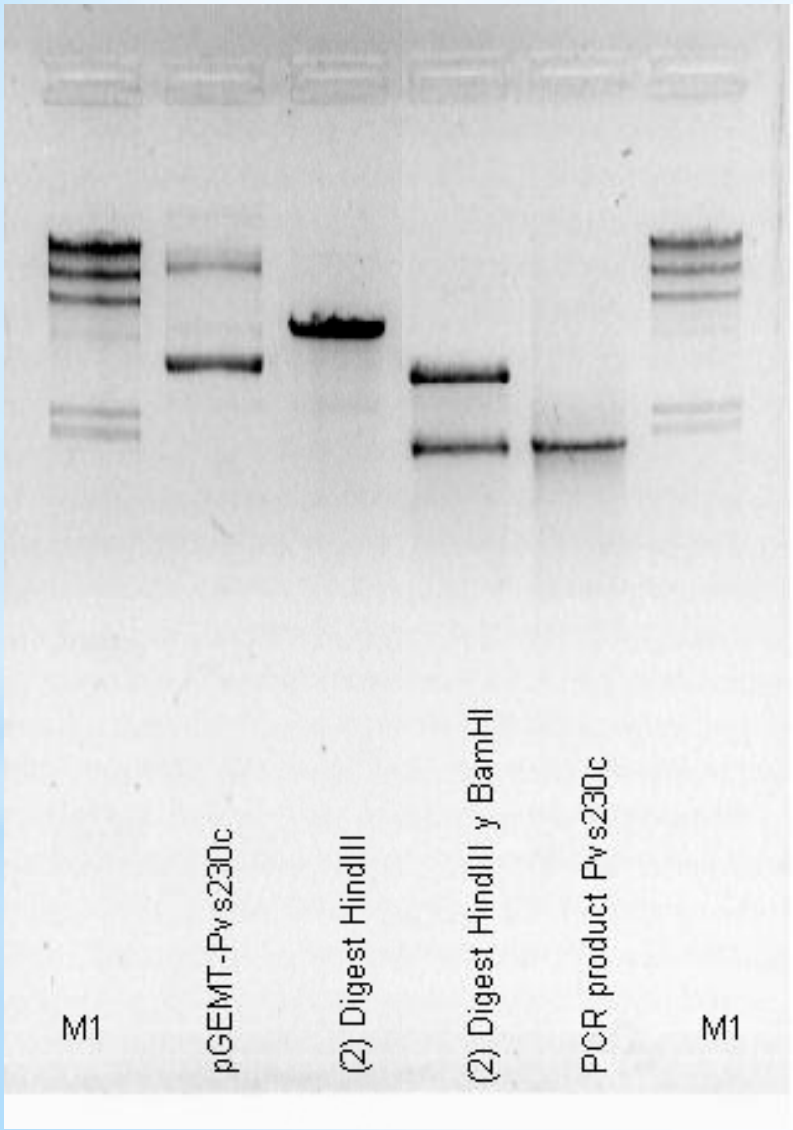
4

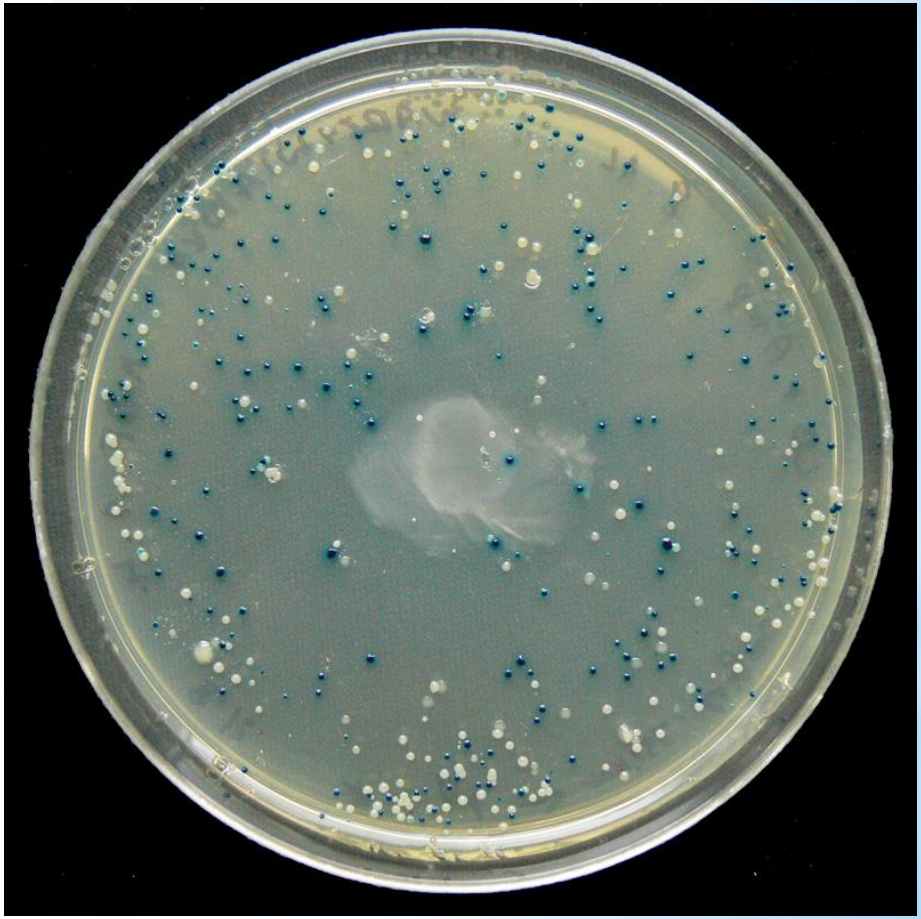
Transformación En Células de clonación



E.coli:
Normalmente las células de E.coli no pueden incorporar ADN del medio. La exposición a altas concentraciones de cationes divalentes CaCl hace permeables al ADN a una pequeña fracción de células.

Verificación de presencia de inserto (gen pncA) en el vector



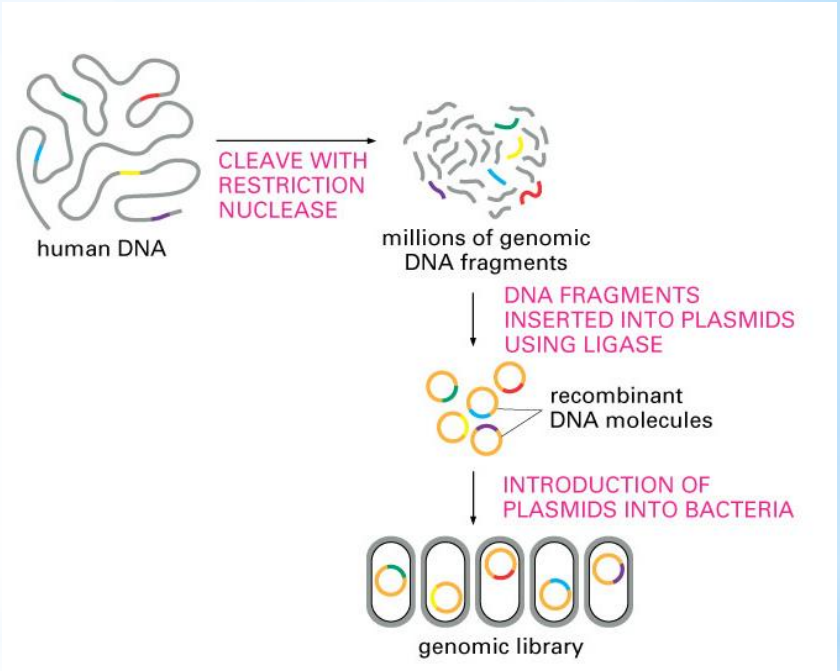


Molecular analysis of DNA and construction of genomic libraries of Mycobacterium leprae.

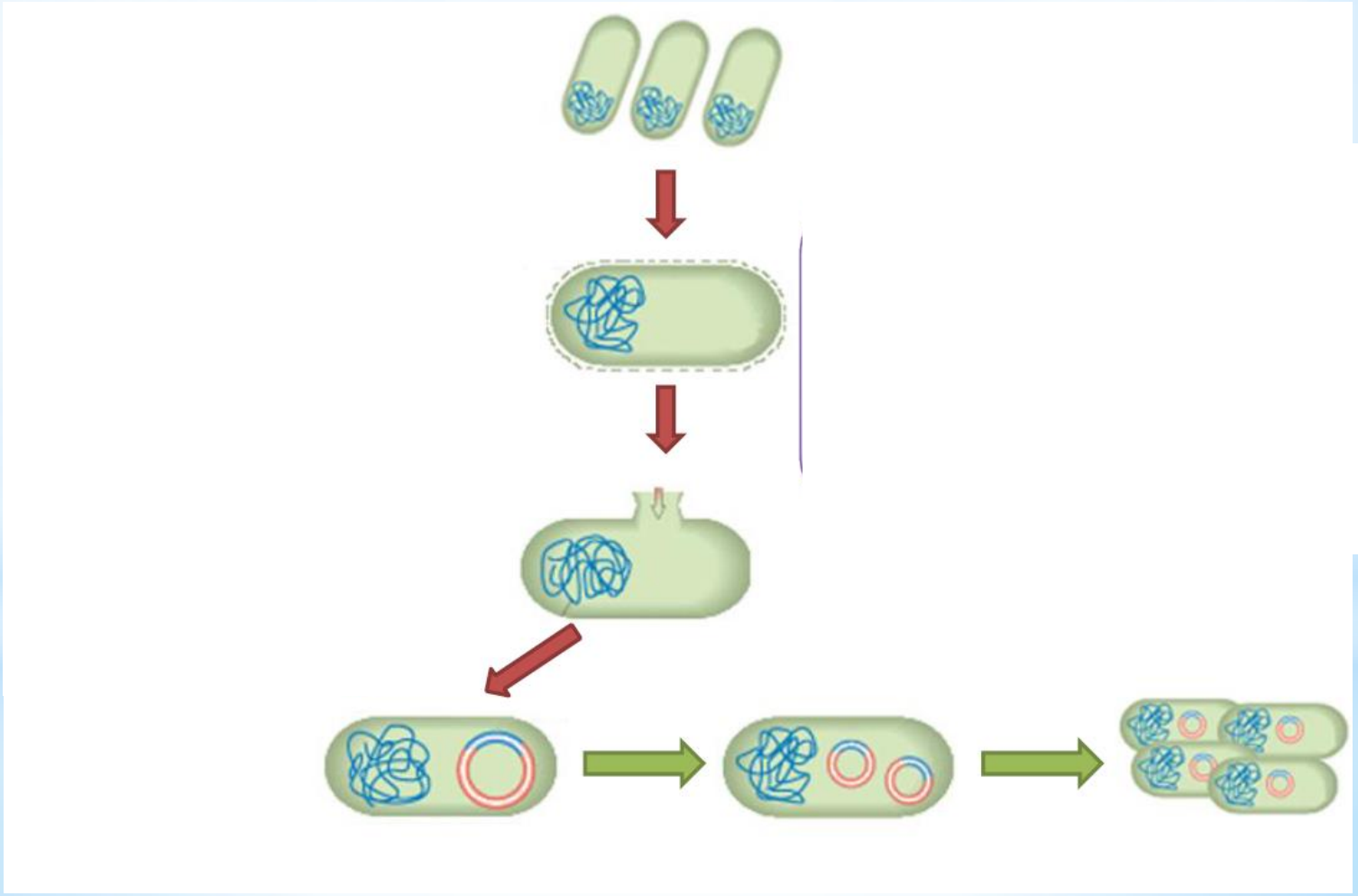
J E Clark-Curtiss, W R Jacobs, M A Docherty, L R Ritchie and R Curtiss 3rd

ABSTRACT

Molecular analysis of DNA from Mycobacterium leprae, "Mycobacterium lufu," and Mycobacterium vaccae has demonstrated that the G + C (guanine plus cytosine) contents of the DNAs are 56, 61, and 65%, respectively, and that the genome sizes are 2.2 X 10(9), 3.1 X 10(9), and 3.1 X 10(9) daltons, respectively. Because of the significant differences in both G + C content and genome size among M. leprae, "M. lufu," and M. vaccae DNAs, these species are not related, although hybridization experiments under nonstringent conditions, with two separate cloned M. leprae DNA inserts as probes, indicate that there are some conserved sequences among the DNAs. The G + C content of Dasypus novemcinctus (armadillo, the animal of choice for cultivating M. leprae) DNA was determined to be 36%. Genomic libraries potentially representing more than 99.99% of each genome were prepared by cloning into the cosmid vector, pHC79, in Escherichia coli K-12. A genomic library representing approximately 95% of the genome of M. vaccae was prepared in pBR322. M. leprae DNA was subcloned from the pHC79::M. leprae library into an expression vector, pYA626. This vector is a 3.8-kilobase derivative of pBR322 in which the promoter region of the asd (aspartate semialdehyde dehydrogenase) gene from Streptococcus mutans has been inserted



6 Transformación En Células de Expresión



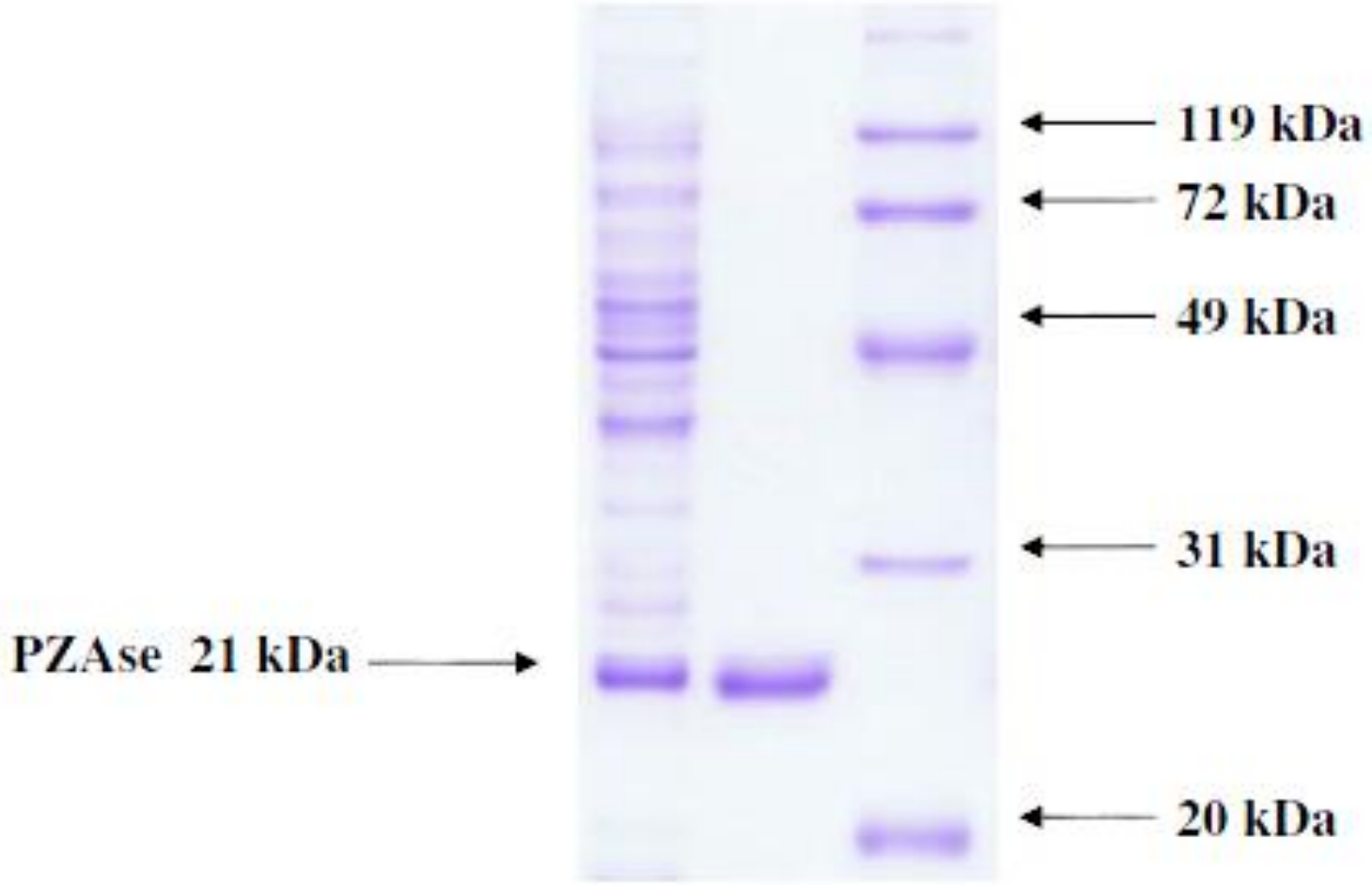


Figure 1. Coomassie blue SDS-PAGE of recombinant pyrazinamidase from *Mycobacterium tuberculosis* wild type H37Rv strain.

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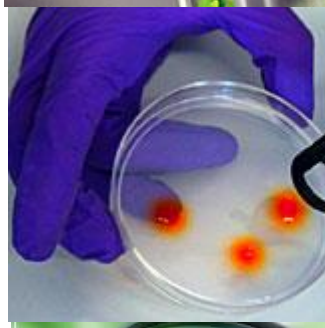

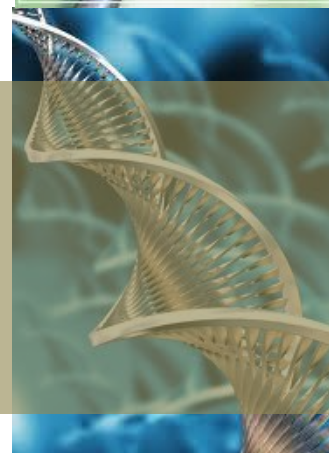

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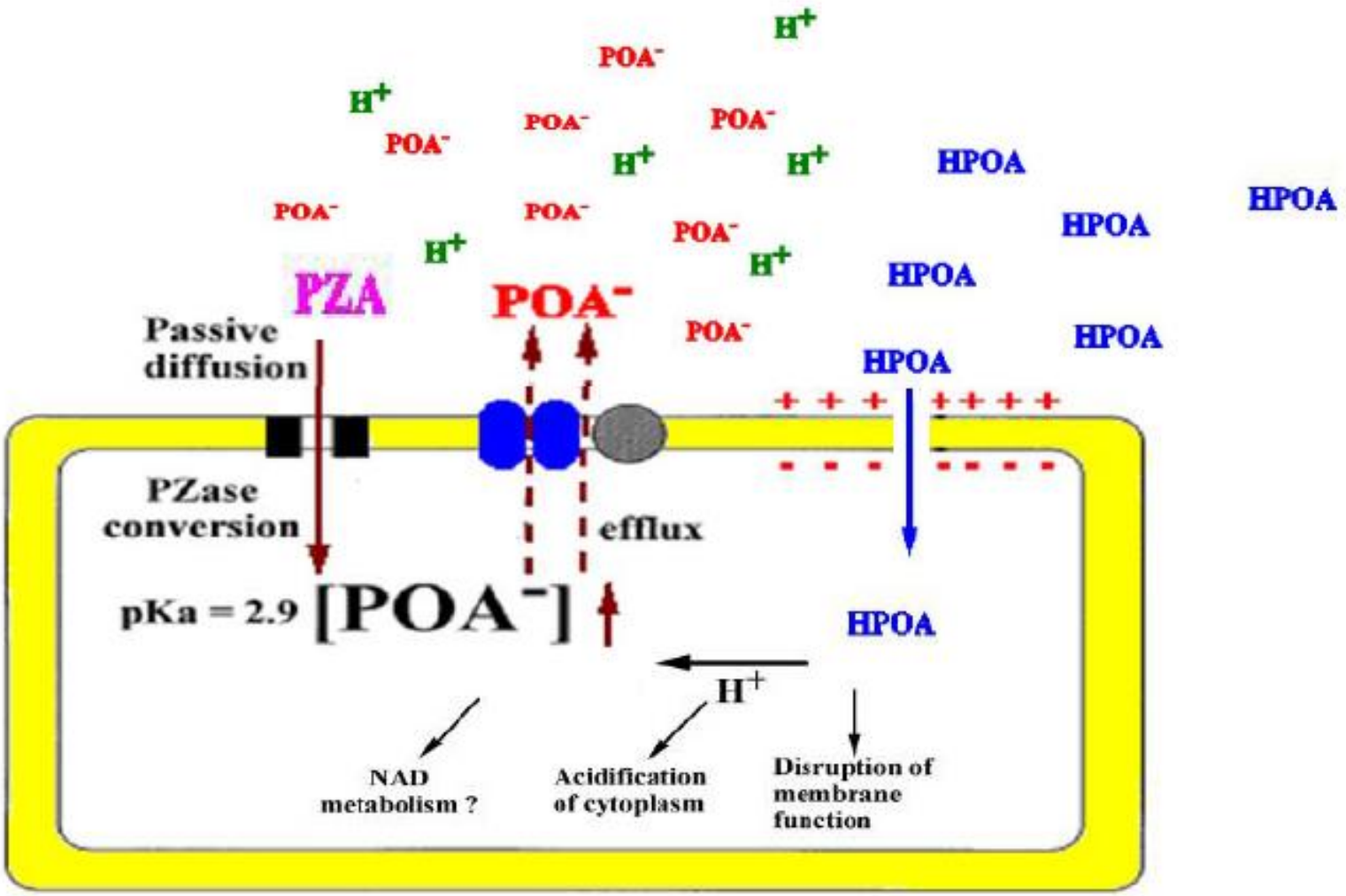


Vectores de clonaje

Vectores de expresión

Vectores Integrativos

Shuttle vectors

Vector Suicida

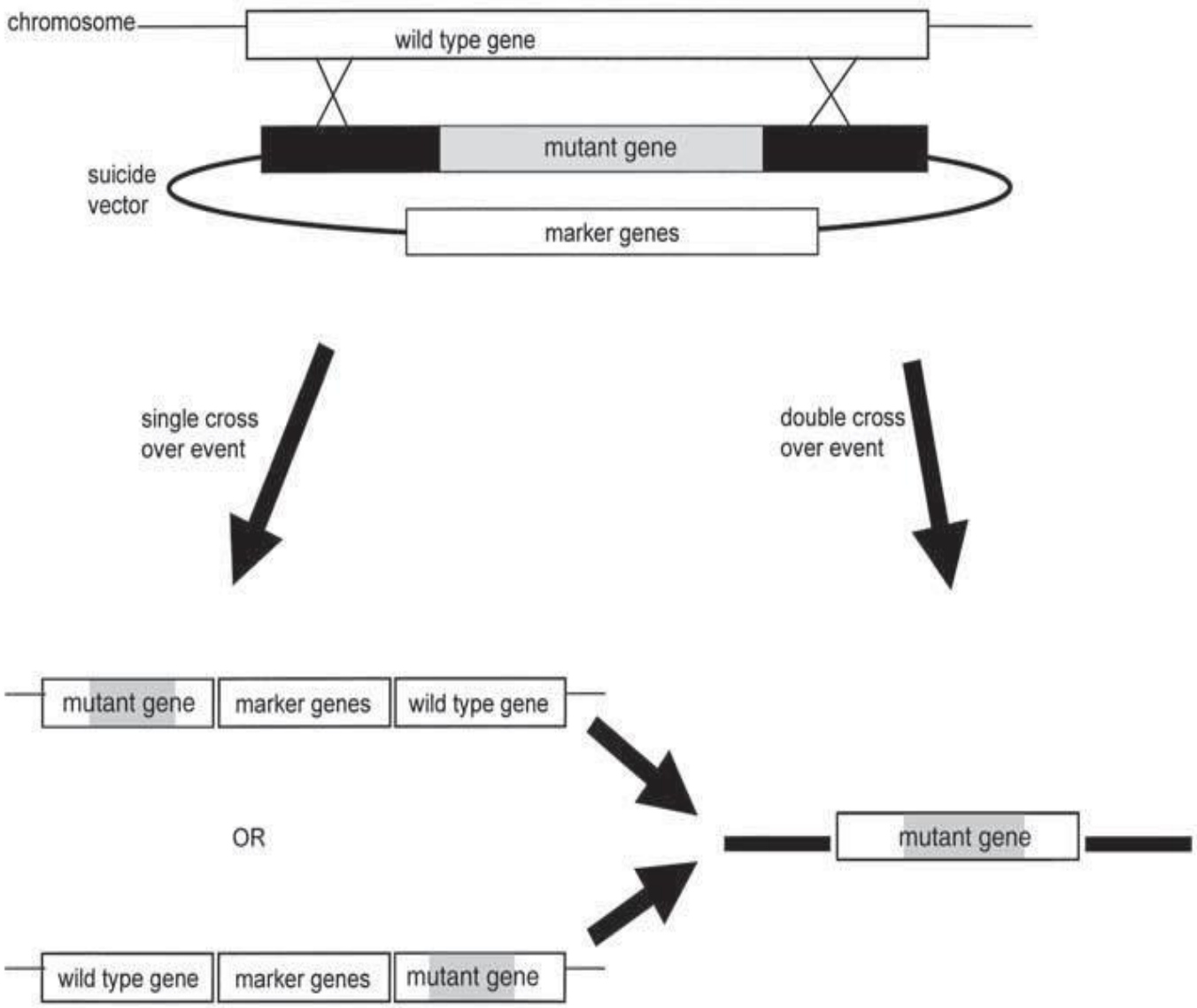
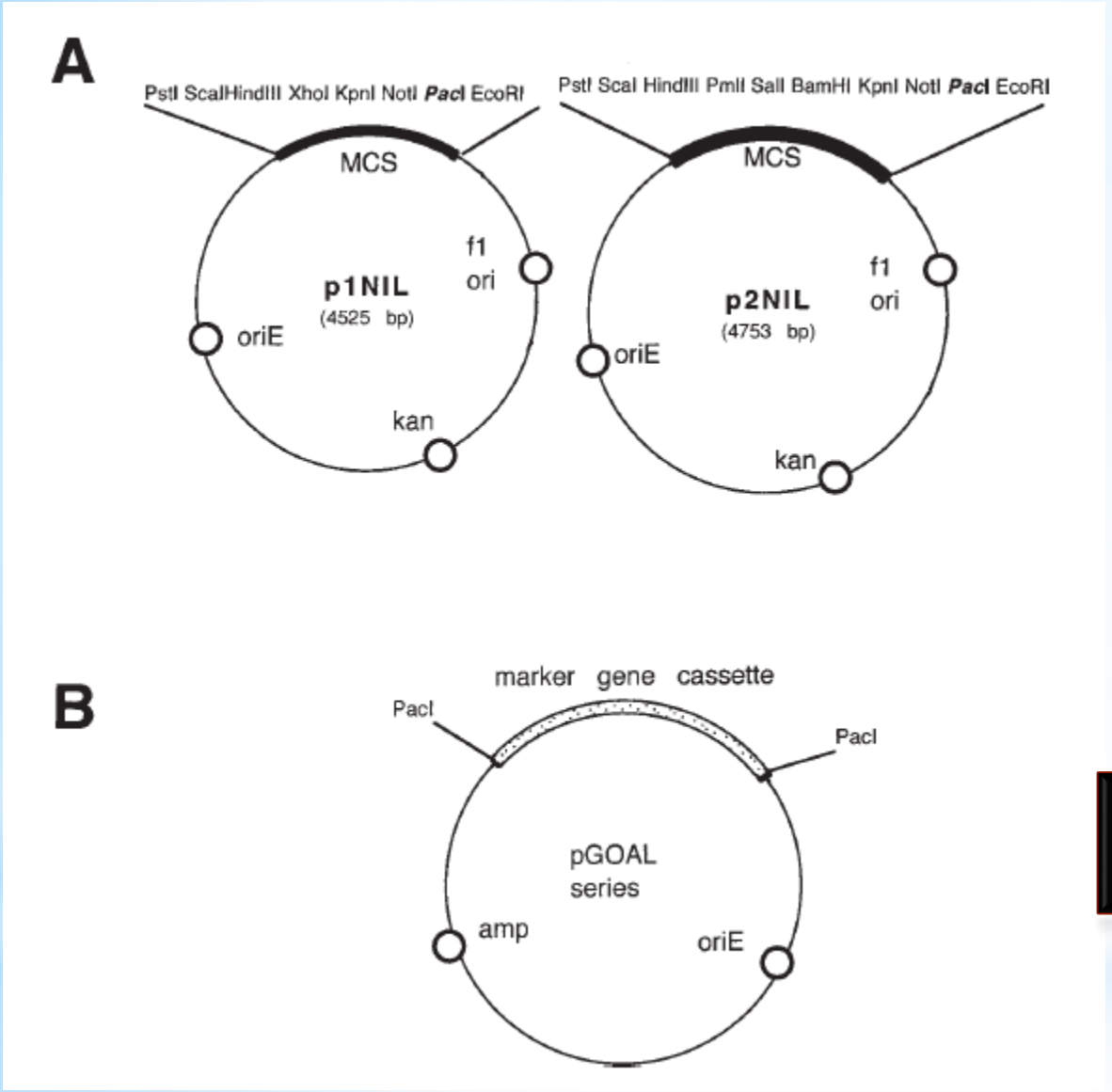


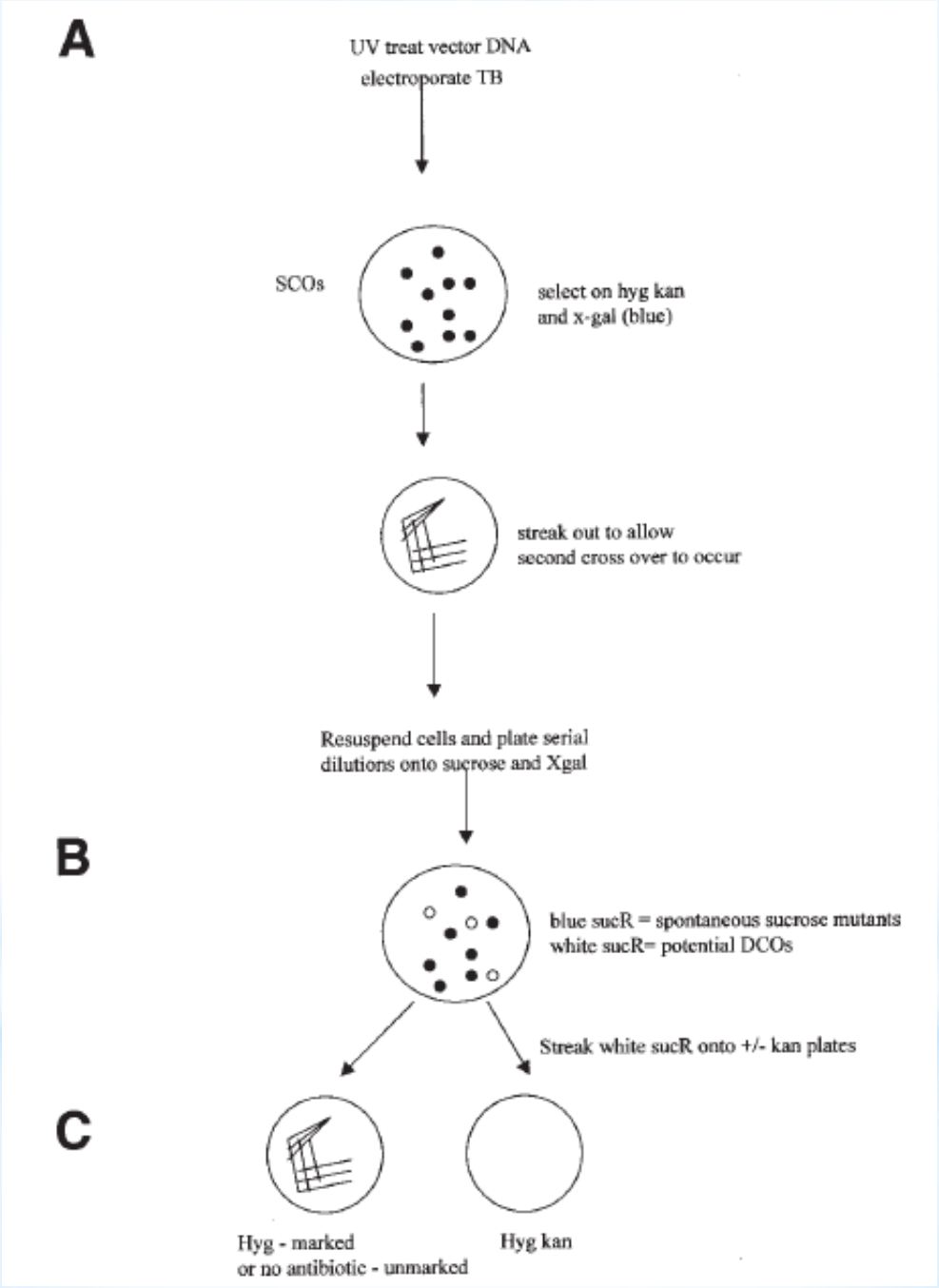
TABLE 1. Some counterselectable markers and their mechanisms of toxicity

Counterselectable marker	Description
sacB	*B. subtilis* gene encoding levansucrase that converts sucrose to levans, which is harmful to the bacteria (14)
rpsL(*strA*)	Encodes the ribosomal subunit protein (S12) target of streptomycin (10)
tetAR	Confers resistance to tetracycline but sensitivity to lipophilic compounds (fusaric and quinalic acids) (27)
pheS	Encodes the α subunits of Phe-tRNA synthetase, which renders bacteria sensitive to *p*-chlorophenylalanine, a phenylalanine analog (21)
thyA	Encodes thymidilate synthetase, which confers sensitivity to trimethoprim and related compounds (47)
lacY	Encodes lactose permease, which renders bacteria sensitive to t-*o*-nitrophenyl-β-D-galactopyranoside (32)
gata-1	Encodes a zinc finger DNA-binding protein which inhibits the initiation of bacterial replication (53)
ccdB	Encodes a cell-killing protein which is a potent poison of bacterial gyrase (3)



Vector Suicida

Cassetes de Contraselección


A
UV treat vector DNA
electroporate TB
SCOs
select on hyg kan
and x-gal (blue)
streak out to allow
second cross over to occur
Resuspend cells and plate serial
dilutions onto sucrose and Xgal
B
blue sucR = spontaneous sucrose mutants
white sucR= potential DCOs
Streak white sucR onto +/- kan plates
C
Hyg - marked
or no antibiotic - unmarked
Hyg kan

Biopharm

Laboratorio de Bioinformática y Farmacogenética

GRACIAS POR SU GENTIL ATENCIÓN!!

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bit.ly/1jaeI07