Métodos de Análise do DNA, de Genes e Genoma I

Genética Molecular
RIB-0102

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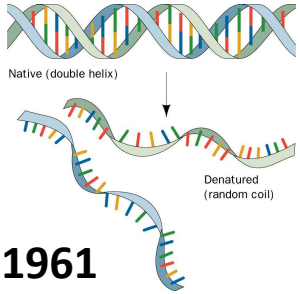
DNA Recombinante …



1961
Marmur e Doty
Renaturação de DNA

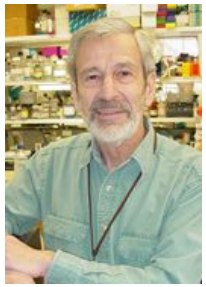
1966
Niremberg, Ochoa e Khorana
Elucidaram o código genético

	T			C			A			G		
T	TTT	Phe	F	TCT	Ser	S	TAT	Tyr	Y	TGT	Cys	C
	TTC			TCC			TAC			TGC		
	TTA	Leu	L	TCA			TAA	STOP		TGA	STOP	
	TTG			TCG			TAG			TGG	Trp	W
C	CTT	Leu	L	CCT	Pro	P	CAT	His	H	CGT	Arg	R
	CTC			CCC			CAC			CGC		
	CTA			CCA			CAA	Gln	Q	CGA		
	CTG			CCG			CAG			CGG		
A	ATT	Ile	I	ACT	Thr	T	AAT	Asn	N	AGT	Ser	S
	ATC			ACC			AAC			AGC		
	ATA			ACA			AAA	Lys	K	AGA	Arg	R
	ATG	Met	M	ACG			AAG			AGG		
G	GTT	Val	V	GCT	Ala	A	GAT	Asp	D	GGT	Gly	G
	GTC			GCC			GAC			GGC		
	GTA			GCA			GAA	Glu	E	GGA		
	GTG			GCG			GAG			GGG		

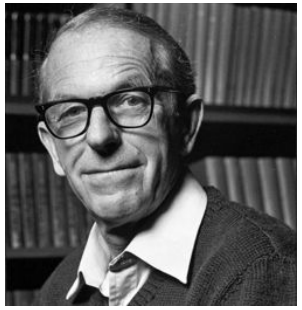
1972
Boyer, Cohen e Berg
Clonagem gênica

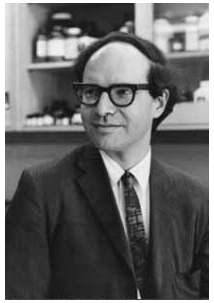
1962
Wener Arber
Enzimas de restrição

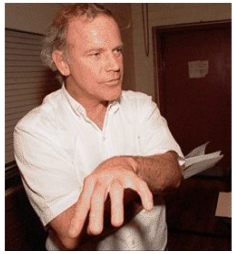
1967
Martin Gellert
DNA ligase



1975-1977
Frederick Sanger e Walter Gilbert
Sequenciamento do DNA







1987

Kary Mullis

Reação em cadeia da polimerase (PCR)

Francis Collins

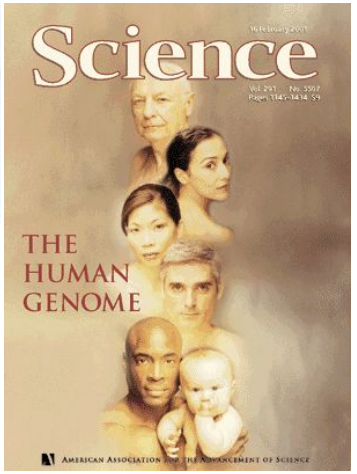
Diretor do "National Human Genome Research Institute"

2001

Seqüenciamento completo do Genoma Humano

Craig Venter

Co-fundador da "Celera Genomics Corporation"

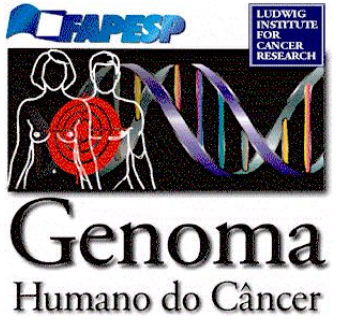


1990

Lançado o Projeto Genoma Humano (HGP)

Esforço de 15 anos coordenado pelo "*Department of Energy* – DOE" e pelo "*National Institutes of Health* – NIH" dos USA para o seqüenciamento completo do genoma humano.

1999



Tecnologia do DNA recombinante

Conceito:

É um conjunto de técnicas de manipulação de DNA em um mesmo organismos ou entre espécies Diferentes.

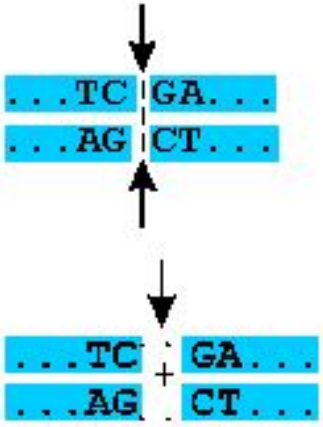
Engenharia genética

Biotecnologia

Tecnologia do DNA recombinante

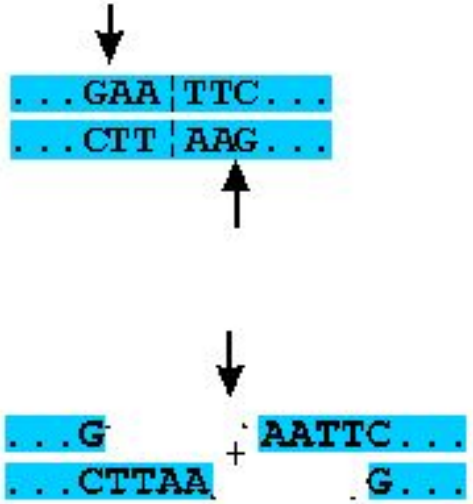
Enzima de restrição:

a) Clivagem no eixo de simetria



Moléculas com extremidades abruptas

b) Clivagem simétricamente situada ao redor do eixo de simetria

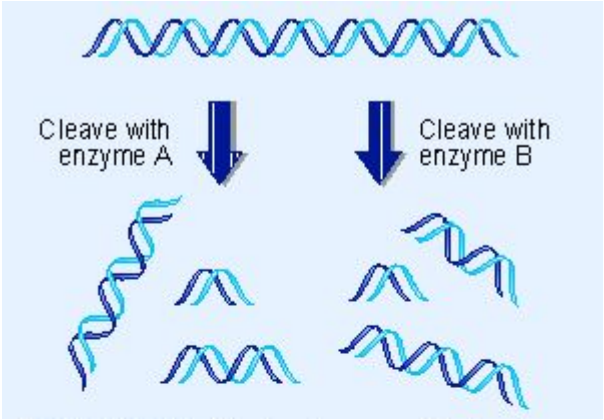


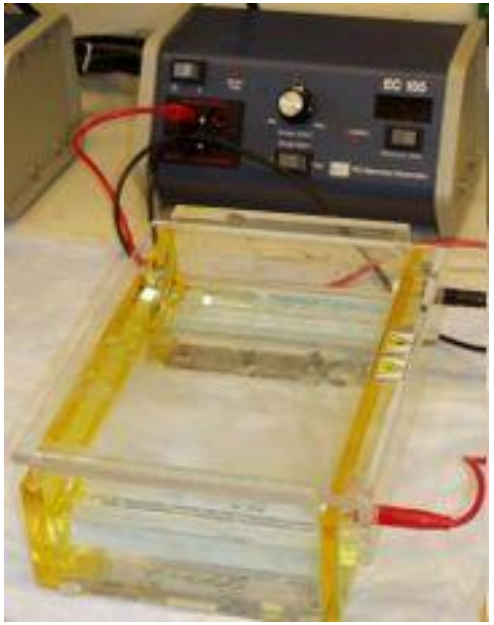
Moléculas com extremidades coesivas

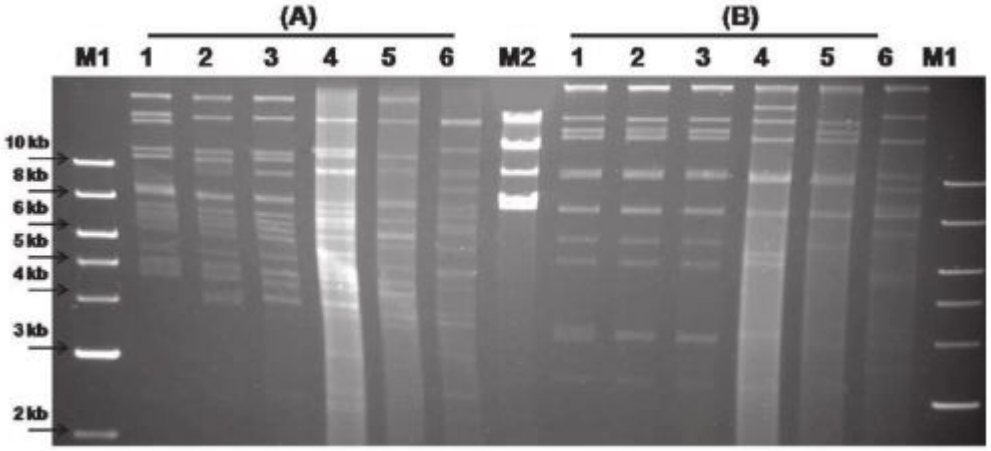
Enzima de restrição:

MICROORGANISM	ENZYME ABBREVIATION	SEQUENCE
Haemophilus aegytius	*Hae*III	5′ . . . G G C C . . . 3′ 3′ . . . C C G G . . . 5′
Thermus aquaticus	*Taq*I	5′ . . . T | C G A . . . 3′ 3′ . . . A G C | T . . . 5′
Haemophilus haemolyticus	*Hha*I	5′ . . . G C G | C . . . 3′ 3′ . . . C | G C G . . . 5′
Desulfovibrio desulfuricans	*Dde*I	5′ . . . C | T *N* A G . . . 3′ 3′ . . . G A *N* T | C . . . 5′
Moraxella bovis	*Mbo*II	5′ . . . G A A G A $(N)_8$ | . . . 3′ 3′ . . . C T T C T $(N)_7$ | . . . 5′
Escherichia coli	*Eco*RV	5′ . . . G A T | A T C . . . 3′ 3′ . . . C T A | T A G . . . 5′
	*Eco*RI	5′ . . . G | A A T T C . . . 3′ 3′ . . . C T T A A | G . . . 5′
Providencia stuarti	*Pst*I	5′ . . . C T G C A | G . . . 3′ 3′ . . . G A | C G T C . . . 5′
Microcoleus	*Mst*II	5′ . . . C C | T *N* A G G . . . 3′ 3′ . . . G G A *N* T | C C . . . 5′
Nocardia otitidis-caviarum	*Not*I	5′ . . . G C | G G C C G C . . . 3′ 3′ . . . C G C C G | G C G . . . 5′

Tecnologia do DNA recombinante

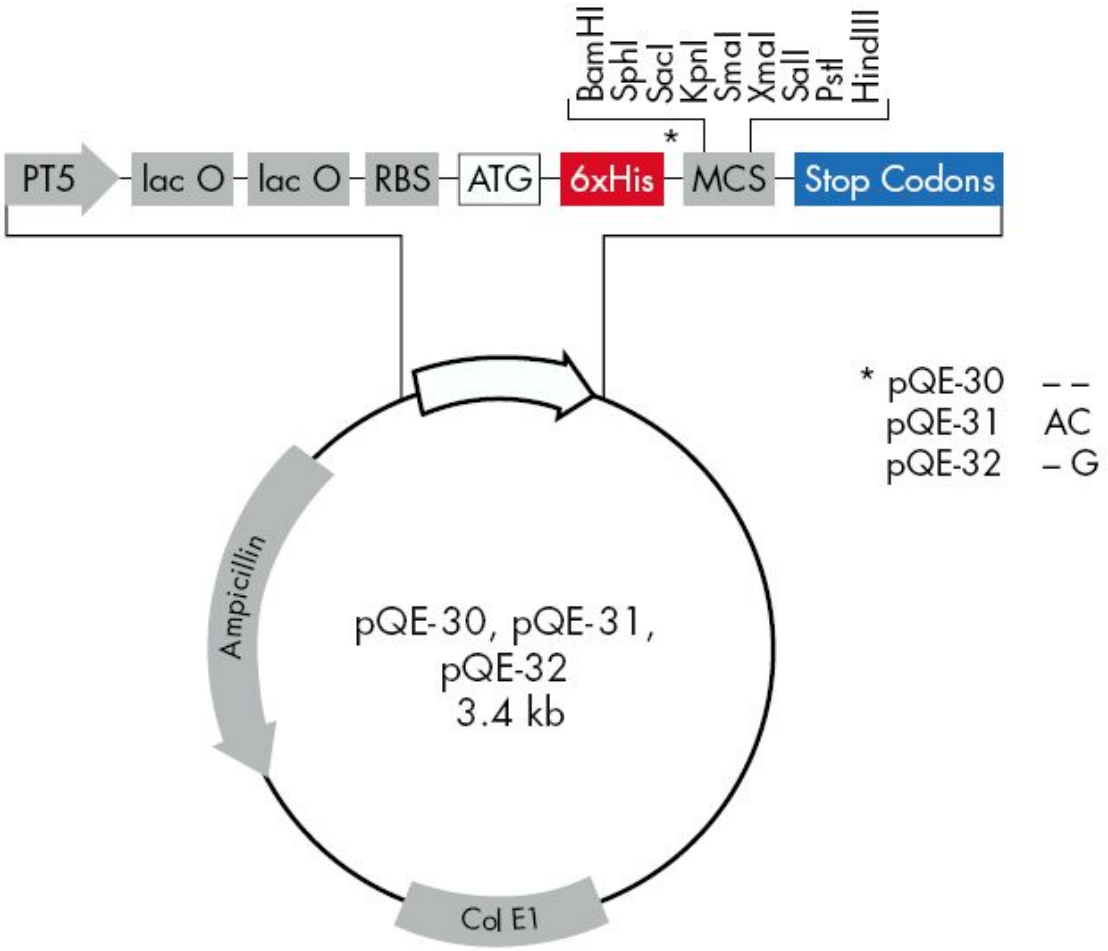






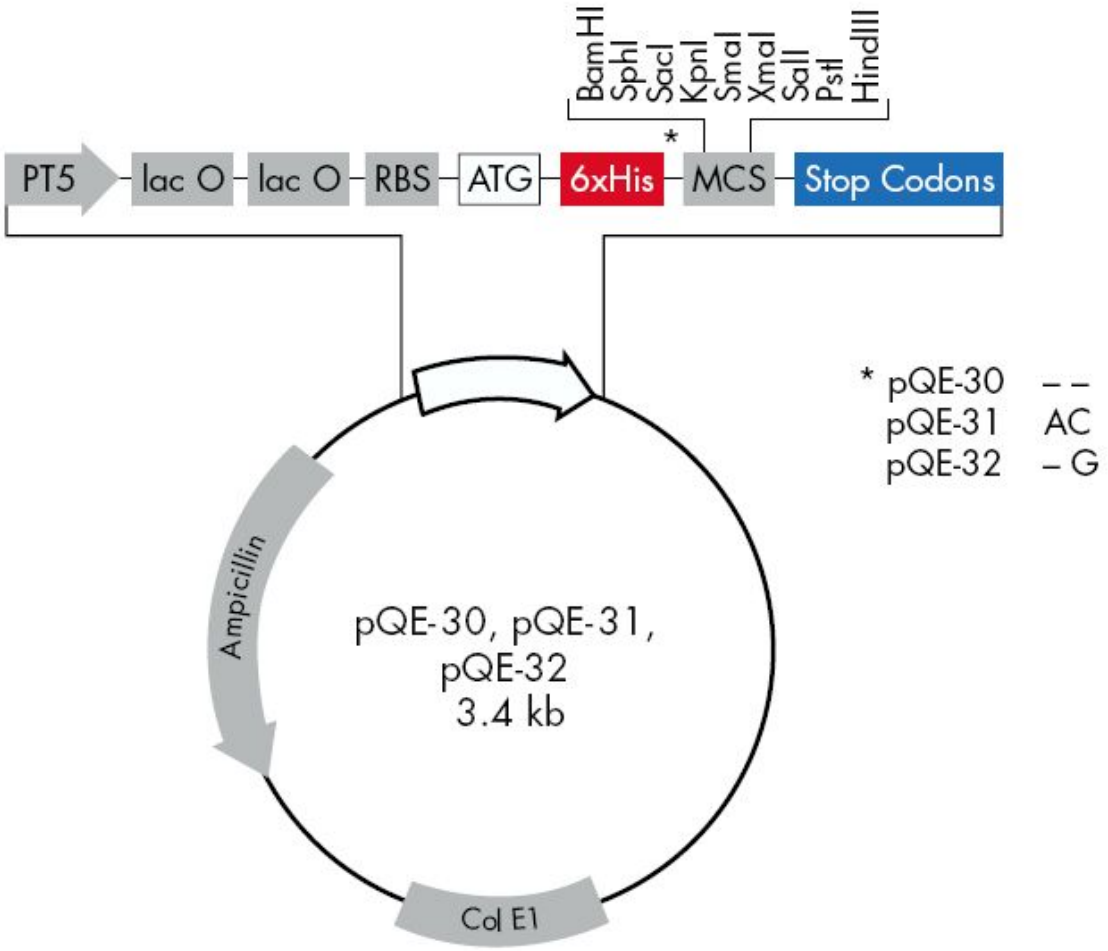
Tecnologia do DNA recombinante

Vetores:



Tecnologia do DNA recombinante

Vetores:

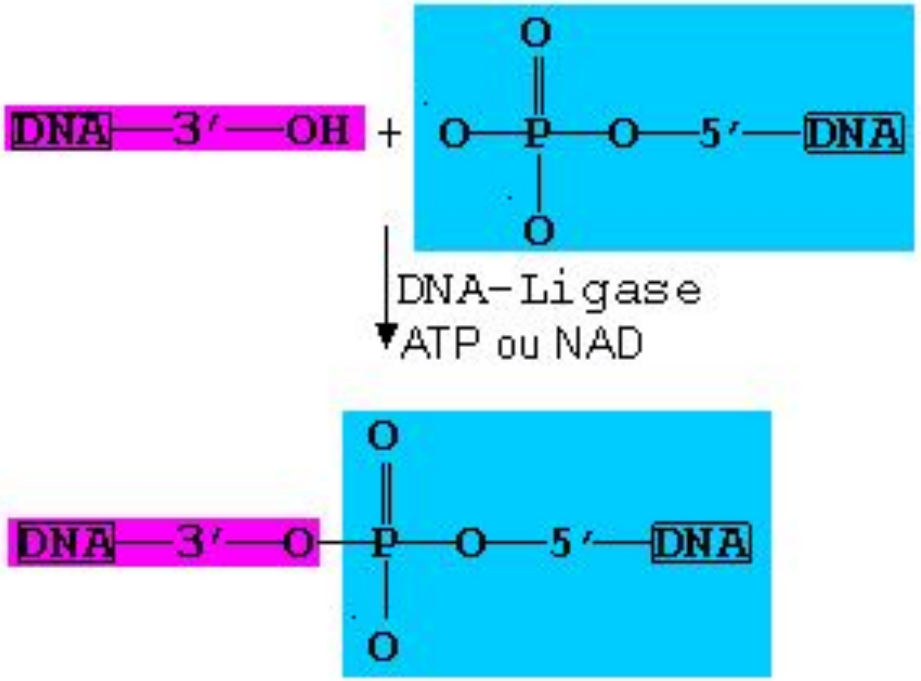


Outros vetores:

Cosmídeos
BACs
YACs
Bacteriófagos

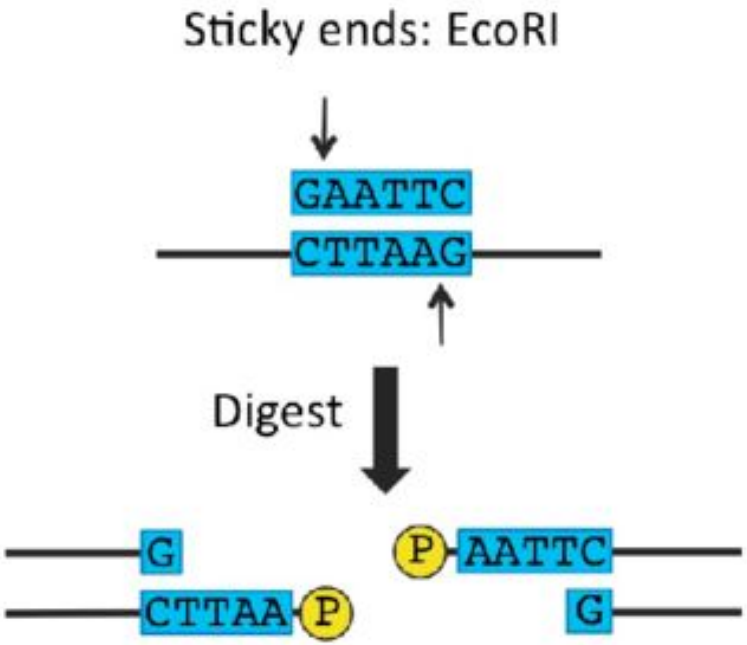
Tecnologia do DNA recombinante

DNA ligase:

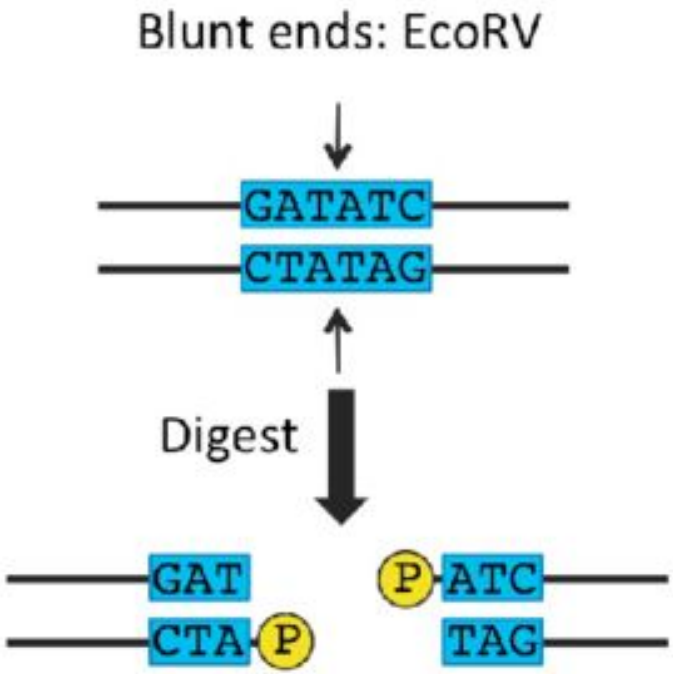


Tecnologia do DNA recombinante

Digestão com extremidades coesivas:

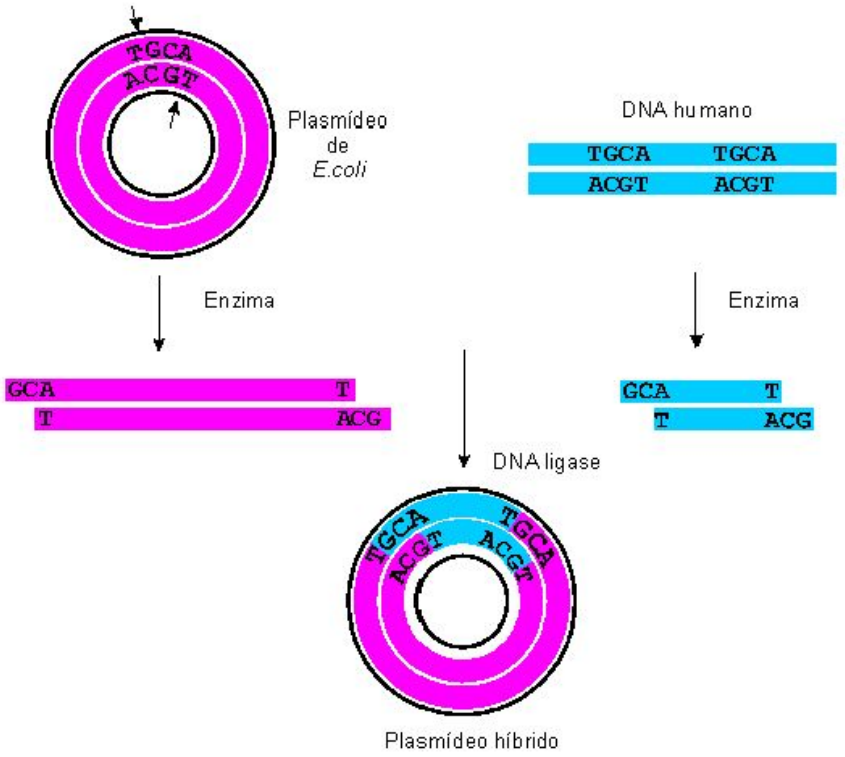


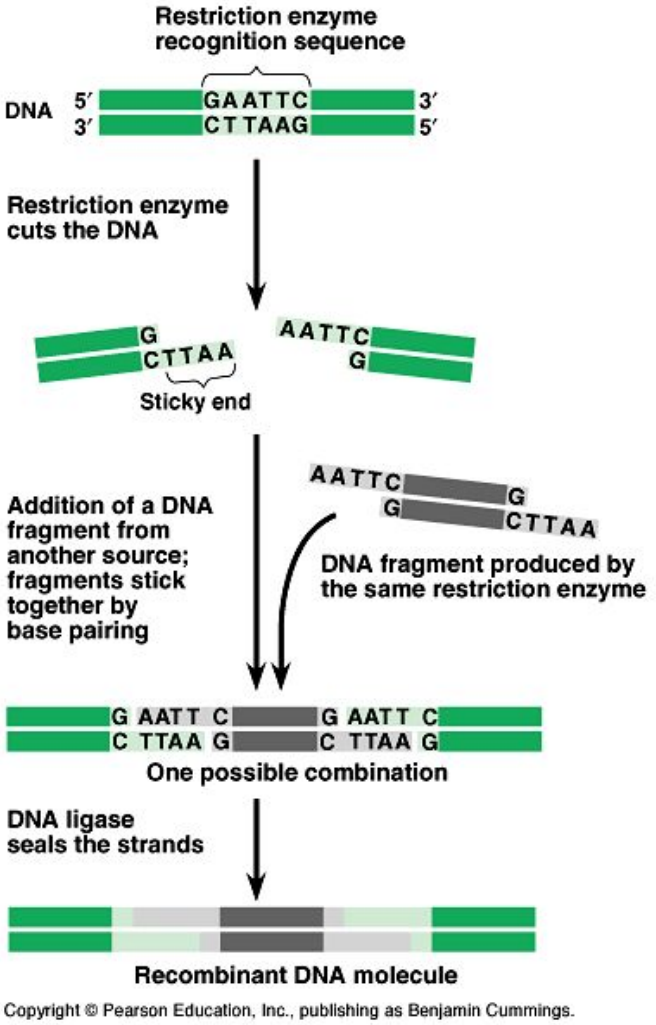
Digestão com extremidades não coesivas:



Tecnologia do DNA recombinante

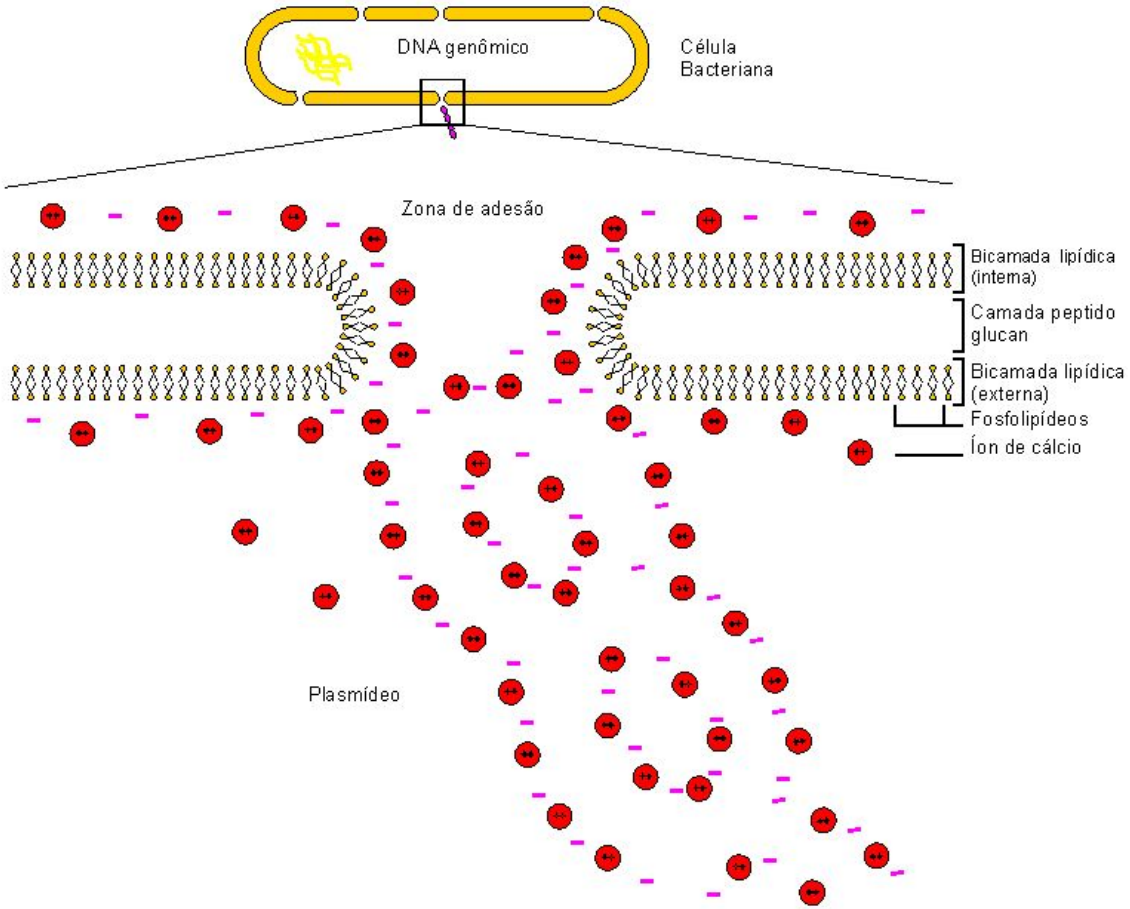
Clonagem:



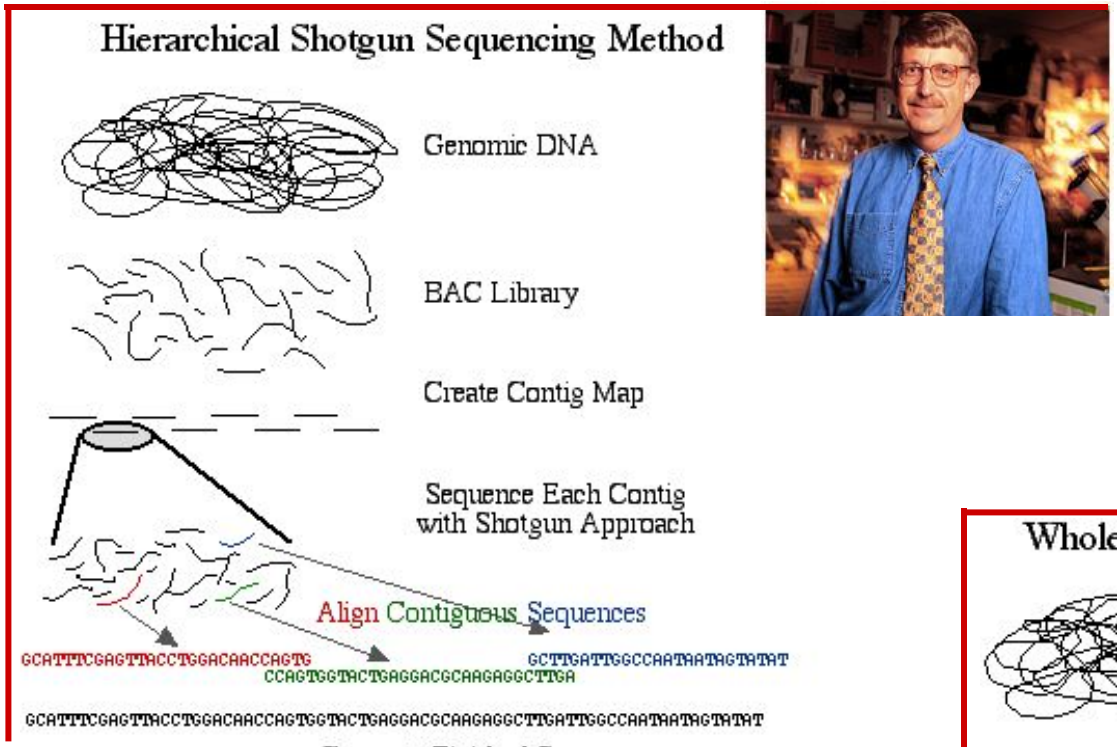


Tecnologia do DNA recombinante

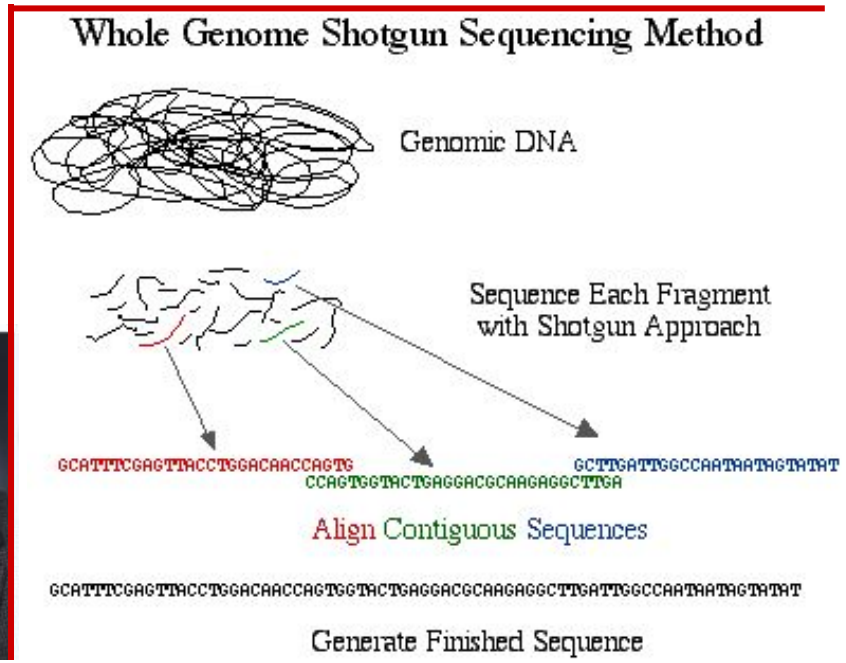
Transformação:



Biblioteca Genômica



Francis Collins
Diretor do "National Human Genome Research Institute"



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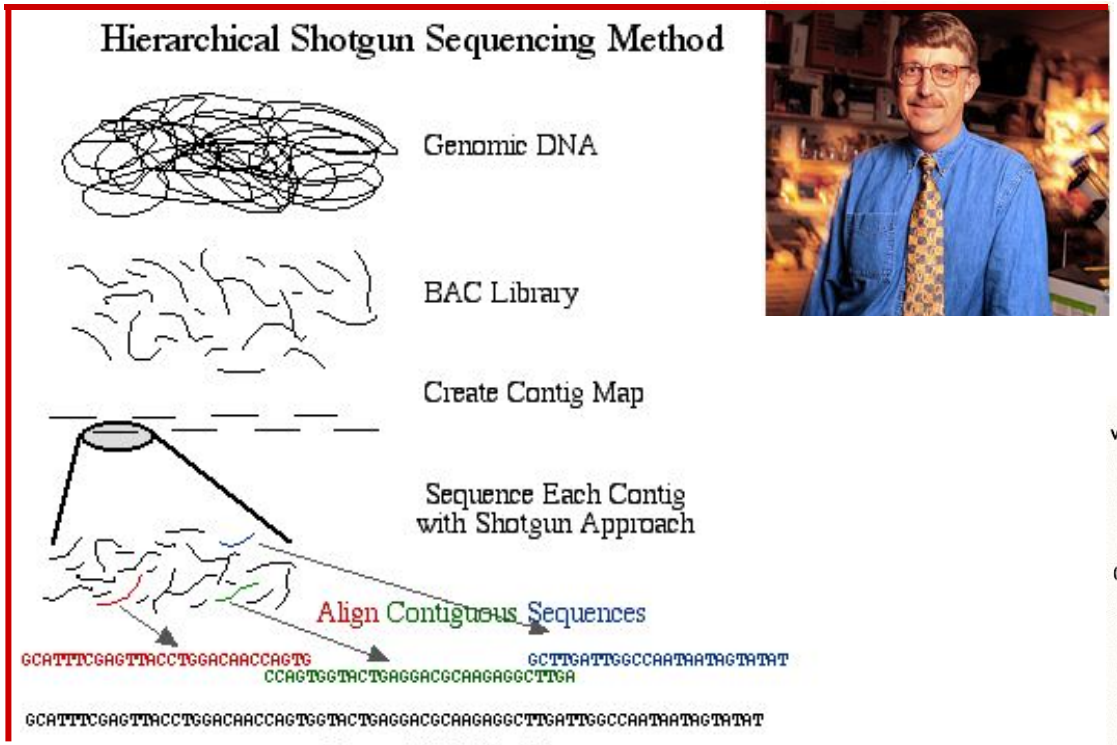
Biblioteca Genômica



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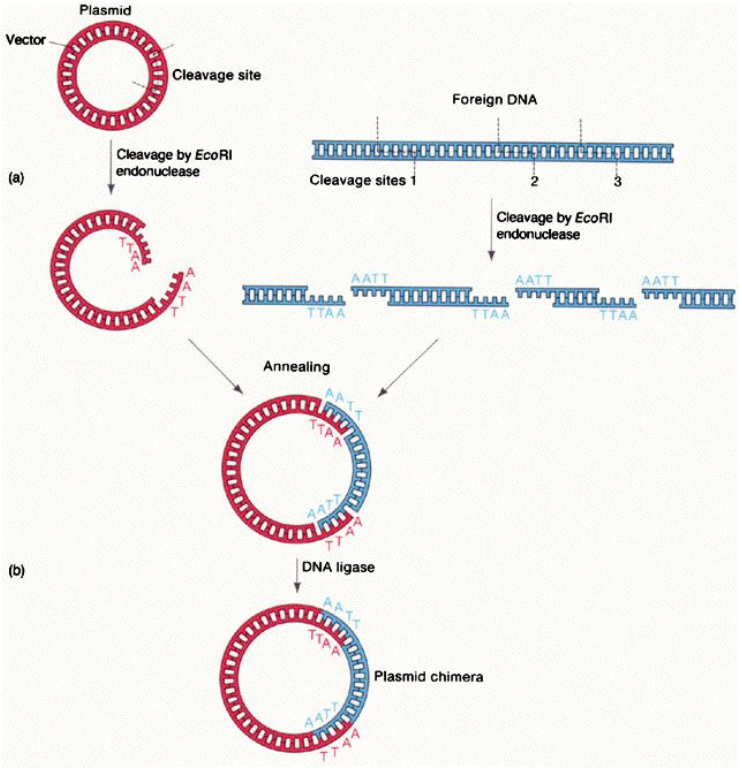
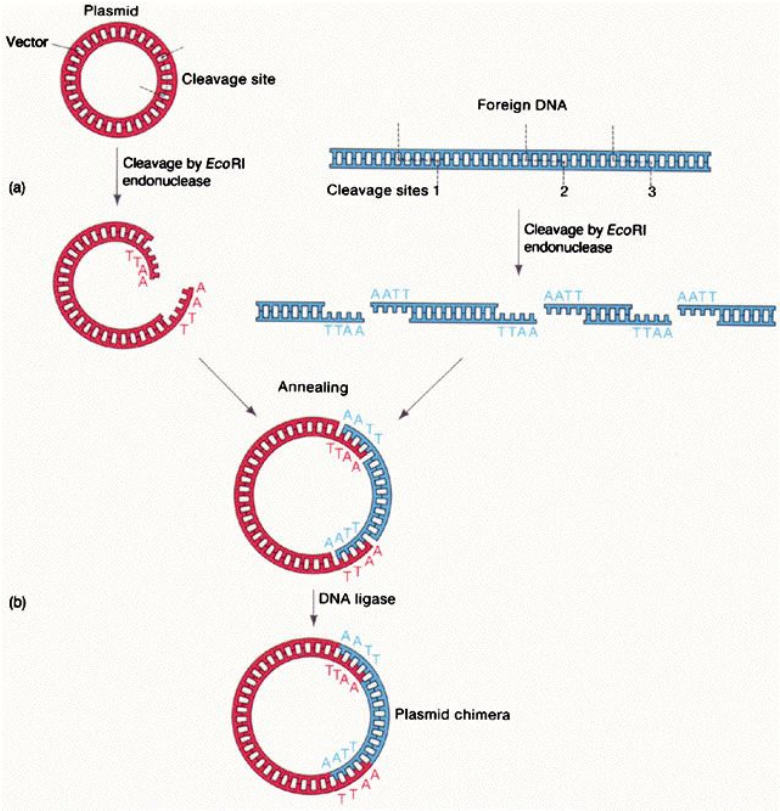


Biblioteca Genômica



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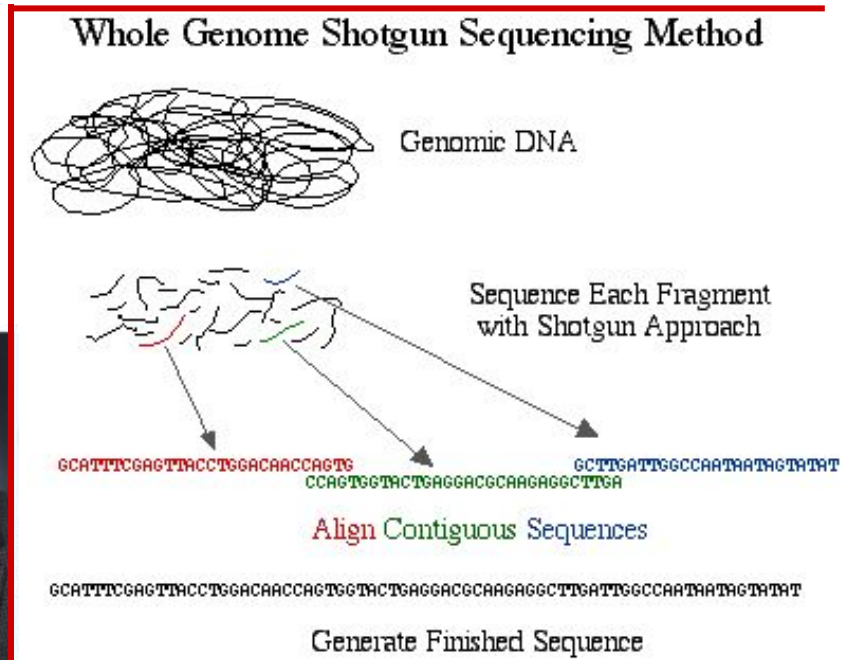
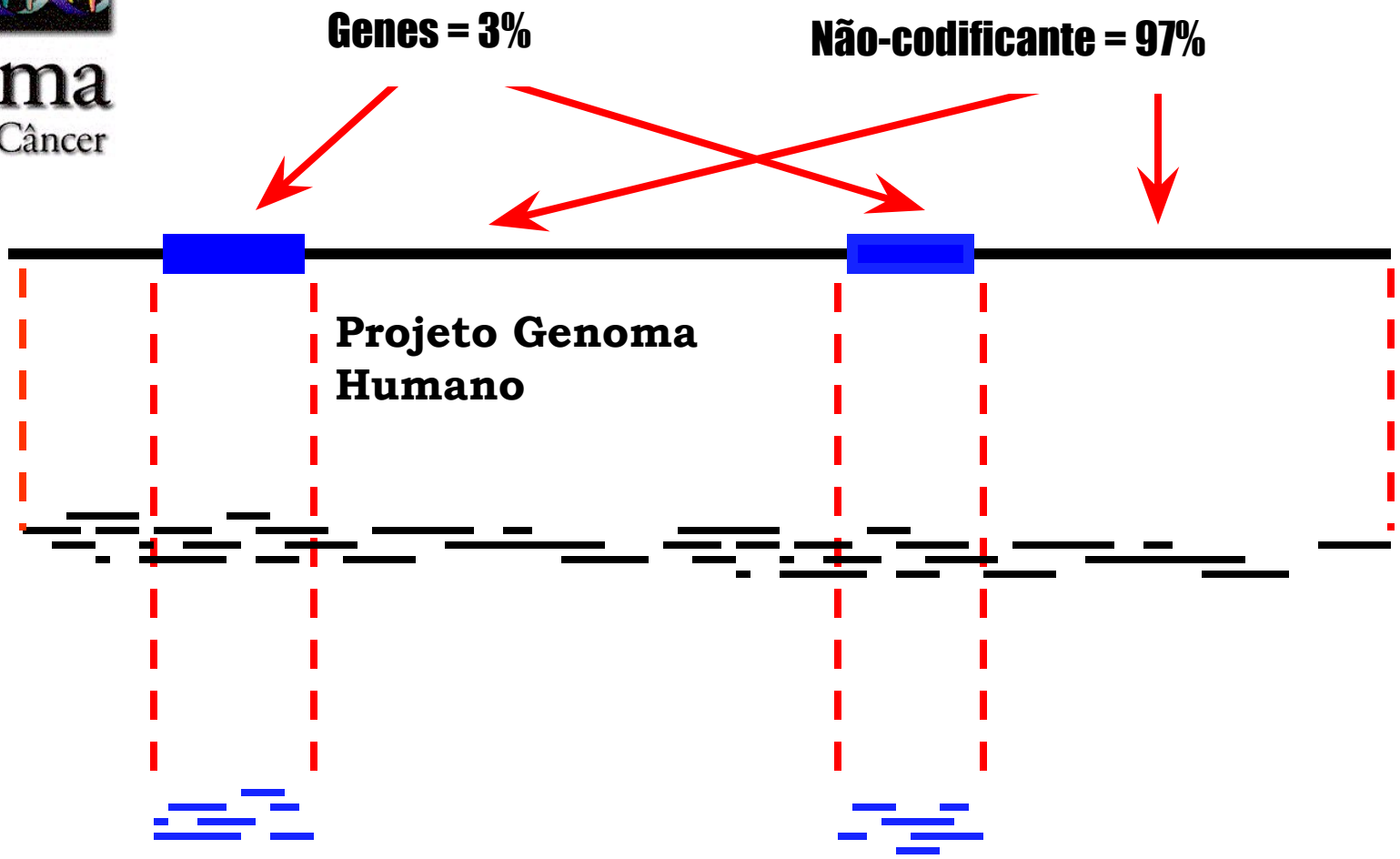


Biblioteca de cDNA

Genoma
Humano do Câncer



Orestes FAPESP-Ludwig