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Comparação de sequências

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Motivação

Ciccarelli et al, *Science*, 2006

Como foi possível criar tal árvore?

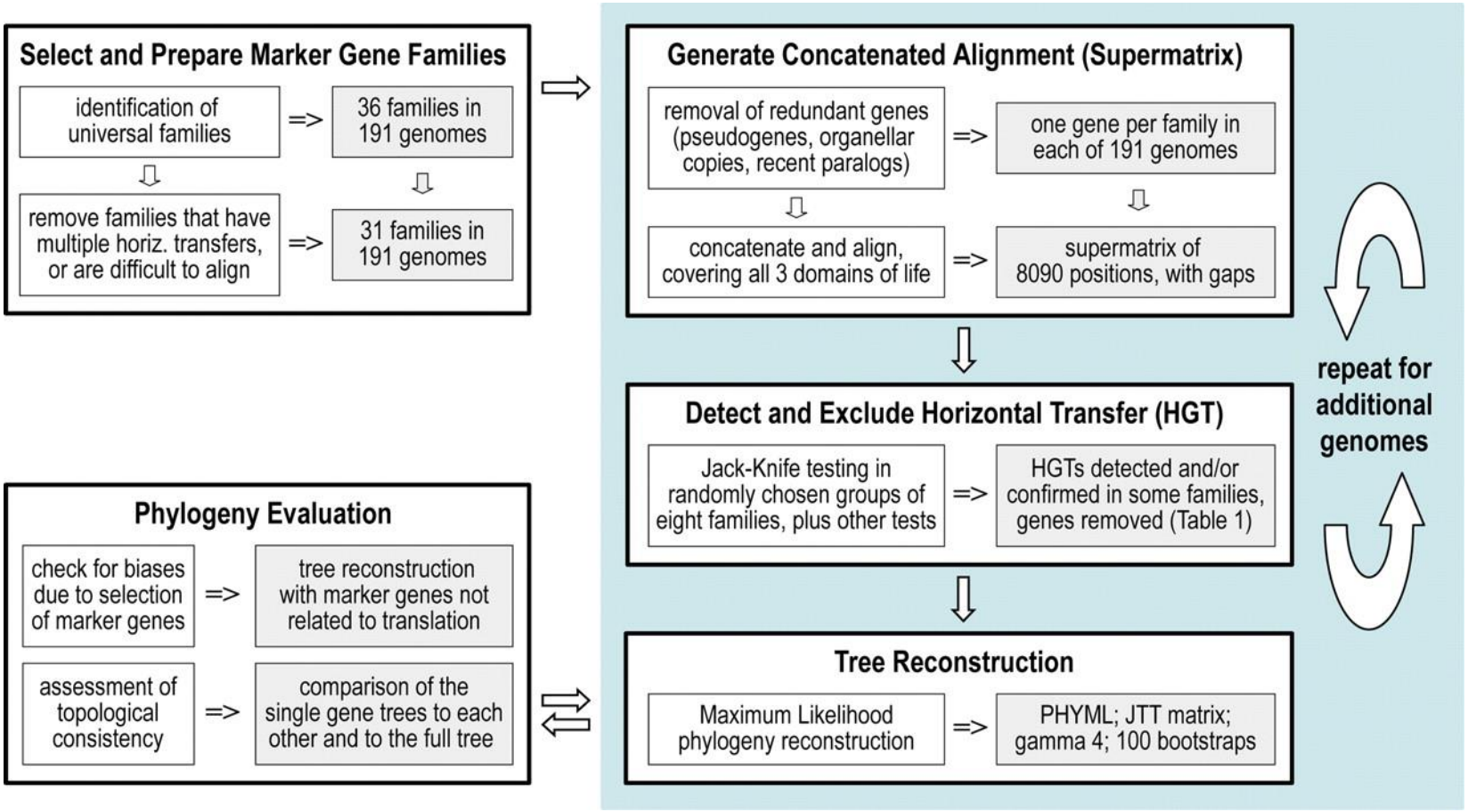
- Determinar genes que são compartilhados por todos os seres vivos
- O que é "compartilhar"?
  - Gene x_1 em organismo *A* tem função α
  - Gene x_2 em organismo *B* é **homólogo** e tem a **mesma função** α
  - Então x_1 e x_2 são "o mesmo gene *x*" e portanto ele é compartilhado por *A* e *B*
- Quais genes são esses?

Orthologous Group	Av. Length	Annotation	Genes in Prok.	Genes in Euk.	Total Genes
COG0012	380	Predicted GTPase, probable translation factor	171	30	201
COG0016	423	Phenylalanine-tRNA synthethase alpha subunit	168	42	210
COG0018†	548	Arginyl-tRNA synthetase	175	45	220
COG0048	137	Ribosomal protein S12	168	48	216
COG0049	182	Ribosomal protein S7	169	41	210
COG0052	240	Ribosomal protein S2	168	79	247
COG0060*	956	Isoleucyl-tRNA synthetase	172	42	214
COG0080	154	Ribosomal protein L11	170	61	231
COG0081	230	Ribosomal protein L1	168	61	229
COG0085†	1138	DNA-directed RNA polymerase, beta subunit	178	60	238
COG0087	288	Ribosomal protein L3	168	54	222
COG0091	157	Ribosomal protein L22	168	75	243
COG0092	240	Ribosomal protein S3	168	30	198
COG0093	130	Ribosomal protein L14	168	41	209
COG0094	182	Ribosomal protein L5	169	36	205
COG0096	131	Ribosomal protein S8	168	55	223
COG0097	177	Ribosomal protein L6P/L9E	168	65	233
COG0098	220	Ribosomal protein S5	168	110	278
COG0099‡	133	Ribosomal protein S13	168	49	217
COG0100	145	Ribosomal protein S11	169	51	220
COG0102	167	Ribosomal protein L13	168	54	222
COG0103	172	Ribosomal protein S9	168	52	220
COG0124*	472	Histidyl-tRNA synthetase	178	31	209
COG0143*†	646	Methionyl-tRNA synthetase	180	35	215
COG0172	442	Seryl-tRNA synthetase	177	37	214
COG0184	154	Ribosomal protein S15P/S13E	168	41	209
COG0186	122	Ribosomal protein S17	170	46	216
COG0197	175	Ribosomal protein L16/L10E	168	54	222
COG0200	166	Ribosomal protein L15	168	70	238
COG0201	445	Preprotein translocase subunit SecY	178	37	215
COG0202	323	DNA-directed RNA polymerase, alpha subunit	171	45	216
COG0256	178	Ribosomal protein L18	168	50	218
COG0495	854	Leucyl-tRNA synthetase	172	43	215
COG0522	199	Ribosomal protein S4 and related proteins	174	46	220
COG0525*‡	880	Valyl-tRNA synthetase	169	37	206
COG0533	375	Metal-dependent proteases with chaperone activity	168	35	203

Questões

- Onde podemos achar as sequências dos genes?
- Como determinar compartilhamento?
- Como preparar esses dados para construir uma árvore?
- Como construir uma árvore?
- Como saber se ela esta correta?

Fig. 1. Overview of the procedure.



F D Ciccarelli et al. Science 2006;311:1283-1287

Respostas

- Onde podemos achar as sequências dos genes?
  - Bancos de dados públicos (NCBI)
  - BLAST
- Como determinar compartilhamento?
  - Através de comparação de sequências
- Como preparar esses dados para construir uma árvore?
  - Alinhamento múltiplo concatenado
- Como construir uma árvore?
  - Métodos de reconstrução filogenética
- Como saber se ela esta correta?
  - Inferência é um termo melhor do que construção
  - Argumentos probabilísticos
  - Transferência Horizontal (Lateral) de Genes

Por que comparar sequências?

- Achar similaridades
  - Dadas 2 sequências, qução parecidas elas são?
  - DNA e proteína
- Buscas em banco de dados
  - Achar quais sequências do banco são parecidas com minha sequência-consulta
  - Consulta (*query*) é tipicamente uma sequência nova
  - “google”

Motivação (cont.)

- Construir famílias de proteínas
  - Saber quais organismos tem membros da família
  - Determinar uma “assinatura” para a família

- Construir filogenias
  - Entender a história evolutiva de genes e organismos

Premissas

- Em geral buscamos sequências “aparentadas”
- Sequências “aparentadas” são similares
- “aparentadas” = **homólogas**
  - Descendem de um mesmo ancestral
- Descendentes sofreram mutações ao longo do tempo

Artigo publicado em agosto/2018

nature ecology & evolution

ARTICLES

https://doi.org/10.1038/s41559-018-0644-x

Integrated genomic and fossil evidence illuminates life's early evolution and eukaryote origin

Holly C. Betts[1], Mark N. Puttick[1,2], James W. Clark[1], Tom A. Williams[1,3], Philip C. J. Donoghue[1] and Davide Pisani[1,3]*

Establishing a unified timescale for the early evolution of Earth and life is challenging and mired in controversy because of the paucity of fossil evidence, the difficulty of interpreting it and dispute over the deepest branching relationships in the tree of life. Surprisingly, it remains perhaps the only episode in the history of life where literal interpretations of the fossil record hold sway, revised with every new discovery and reinterpretation. We derive a timescale of life, combining a reappraisal of the fossil material with new molecular clock analyses. We find the last universal common ancestor of cellular life to have predated the end of late heavy bombardment (>3.9 billion years ago (Ga)). The crown clades of the two primary divisions of life, Eubacteria and Archaebacteria, emerged much later (<3.4 Ga), relegating the oldest fossil evidence for life to their stem lineages. The Great Oxidation Event significantly predates the origin of modern Cyanobacteria, indicating that oxygenic photosynthesis evolved within the cyanobacterial stem lineage. Modern eukaryotes do not constitute a primary lineage of life and emerged late in Earth's history (<1.84 Ga), falsifying the hypothesis that the Great Oxidation Event facilitated their radiation. The symbiotic origin of mitochondria at 2.053–1.21 Ga reflects a late origin of the total-group Alphaproteobacteria to which the free living ancestor of mitochondria belonged.

Métodos

- The dataset consists of 102 species and 29 universally distributed, protein-coding genes

- Proteomes were downloaded from GenBank and putative orthologues were identified using BLAST. The top hits were compiled and aligned into gene-specific files in MUSCLE

Alinhamento de DNA

GTGGTGGCCTACGAAGGT

GTAGTGCCTTCGAAGGGT

Como avaliar um alinhamento?

- Sistema de pontuação
  - Match: +1
  - Mismatch: –1

Pontuação do alinhamento

GTGGTGGCCTACGAAGGT

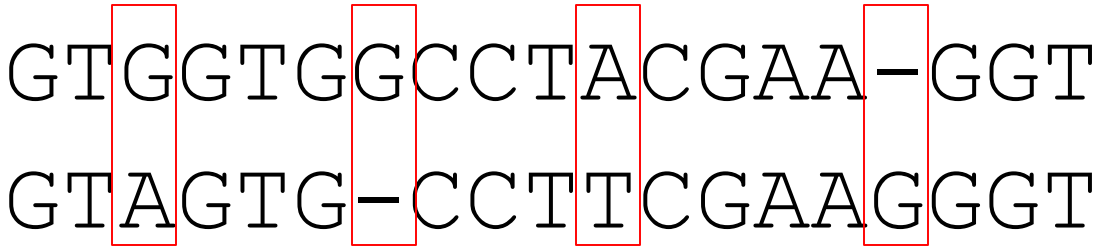
GTAGTGCCTTCGAAGGGT

+1+1-1+1+1+1-1+1-1+1-1-1-1+1-1+1+1+1 = 4

É possível melhorar o alinhamento?

- Sim
- Pela introdução de espaços

Alinhamento com espaços



Sistema de pontuação com espaços

- Match: +1
- Mismatch: –1
- Espaço: –2
- (Buraco: sequência de espaços)
  - Em inglês: *gaps*

Pontuação do alinhamento

GTGGTGGCCTACGAA-GGT

GTAGTG-CCTTCGAAGGGT

+1+1-1+1+1+1-2+1+1+1-1+1+1+1+1-2+1+1+1 = 9

Justificativa para o sistema de pontuação

- Matches tem que ser recompensados (> 0)
- Mismatches e espaços tem que ser penalizados (< 0)
- Mismatches representam substituições
  - Mutações (ocorrem com frequência)
  - Podem não trazer letalidade
- Espaços representam inserções ou remoções
  - Mais prováveis de causarem letalidade
    - Alteram quadro de leitura
  - Ocorrem com muito menos frequência

Alinhamentos ótimos

- São os alinhamentos de pontuação máxima
- similaridade entre duas sequências
  - É o valor da pontuação do alinhamento ótimo
- No exemplo anterior
  - Similaridade = 9

Comparação de sequências de aminoácidos

Pontuação de alinhamento de proteínas

```
H:    IIWGEDTLMEYLENPKKYIPGTKMIFVGIKKKEERADLIAYLKKATNE
C:    VVWTKETLFEYLLNPKKYIPGTKMVFAGLKKADERADLIKYIEVESAKSL
        *   ** *** ********** * * **  ****** *
```

% de identidade é uma medida simples mas válida de similaridade de sequências de proteínas

Aminoácidos se dividem em famílias

- Hidrofóbicos
  - Ala, Val, Phe, Pro, Met, Ile, Leu
- Com carga
  - Asp, Glu, Lys, Arg
- Polares
  - Ser, Thr, Tyr, His, Cys, Asn, Gln
  - Trp
- Gly

Twenty-One Amino Acids

⊕ Positive ⊖ Negative

• Side chain charge at physiological pH 7.4

A. Amino Acids with Electrically Charged Side Chains

Positive

- Arginine (Arg) R
- Histidine (His) H
- Lysine (Lys) K

Negative

- Aspartic Acid (Asp) D
- Glutamic Acid (Glu) E

B. Amino Acids with Polar Uncharged Side Chains

- Serine (Ser) S
- Threonine (Thr) T
- Asparagine (Asn) N
- Glutamine (Gln) Q

C. Special Cases

- Cysteine (Cys) C
- Selenocysteine (Sec) U
- Glycine (Gly) G
- Proline (Pro) P

D. Amino Acids with Hydrophobic Side Chain

- Alanine (Ala) A
- Valine (Val) V
- Isoleucine (Ile) I
- Leucine (Leu) L
- Methionine (Met) M
- Phenylalanine (Phe) F
- Tyrosine (Tyr) Y
- Tryptophan (Trp) W

pKa Data: **CRC Handbook of Chemistry**, v. 2010

Dan Cojocari, Department of Medical Biophysics, **University of Toronto** 2011

Mutações e proteínas

- Substituições que não alteram a estrutura da proteína tendem a ser preservadas durante a evolução
- A troca de um aminoácido de uma família por outro da mesma família em geral cai nessa categoria
- (Indels podem ter consequências mais drásticas)
- Então: como avaliar mismatches?

Matriz de substituição de amino ácidos BLOSUM62

```
#  Matrix made by matblas from blosum62.iij
#  * column uses minimum score
#  BLOSUM Clustered Scoring Matrix in 1/2 Bit Units
#  Blocks Database = /data/blocks_5.0/blocks.dat
#  Cluster Percentage: >= 62
#  Entropy =   0.6979, Expected =  -0.5209
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  B  Z  X  *
A  4 -1 -2 -2  0 -1 -1  0 -2 -1 -1 -1 -1 -2 -1  1  0 -3 -2  0 -2 -1  0 -4
R -1  5  0 -2 -3  1  0 -2  0 -3 -2  2 -1 -3 -2 -1 -1 -3 -2 -3 -1  0 -1 -4
N -2  0  6  1 -3  0  0  0  1 -3 -3  0 -2 -3 -2  1  0 -4 -2 -3  3  0 -1 -4
D -2 -2  1  6 -3  0  2 -1 -1 -3 -4 -1 -3 -3 -1  0 -1 -4 -3 -3  4  1 -1 -4
C  0 -3 -3 -3  9 -3 -4 -3 -3 -1 -1 -3 -1 -2 -3 -1 -1 -2 -2 -1 -3 -3 -2 -4
Q -1  1  0  0 -3  5  2 -2  0 -3 -2  1  0 -3 -1  0 -1 -2 -1 -2  0  3 -1 -4
E -1  0  0  2 -4  2  5 -2  0 -3 -3  1 -2 -3 -1  0 -1 -3 -2 -2  1  4 -1 -4
G  0 -2  0 -1 -3 -2 -2  6 -2 -4 -4 -2 -3 -3 -2  0 -2 -2 -3 -3 -1 -2 -1 -4
H -2  0  1 -1 -3  0  0 -2  8 -3 -3 -1 -2 -1 -2 -1 -2 -2  2 -3  0  0 -1 -4
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4  2 -3  1  0 -3 -2 -1 -3 -1  3 -3 -3 -1 -4
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4 -2  2  0 -3 -2 -1 -2 -1  1 -4 -3 -1 -4
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5 -1 -3 -1  0 -1 -3 -2 -2  0  1 -1 -4
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5  0 -2 -1 -1 -1 -1  1 -3 -1 -1 -4
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6 -4 -2 -2  1  3 -1 -3 -3 -1 -4
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4  7 -1 -1 -4 -3 -2 -2 -1 -2 -4
S  1 -1  1  0 -1  0  0  0 -1 -2 -2  0 -1 -2 -1  4  1 -3 -2 -2  0  0  0 -4
T  0 -1  0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1  1  5 -2 -2  0 -1 -1  0 -4
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1  1 -4 -3 -2 11  2 -3 -4 -3 -2 -4
Y -2 -2 -2 -3 -2 -1 -2 -3  2 -1 -1 -2 -1  3 -3 -2 -2  2  7 -1 -3 -2 -1 -4
V  0 -3 -3 -3 -1 -2 -2 -3 -3  3  1 -2  1 -1 -2 -2  0 -3 -1  4 -3 -2 -1 -4
B -2 -1  3  4 -3  0  1 -1  0 -3 -4  0 -3 -3 -2  0 -1 -4 -3 -3  4  1 -1 -4
Z -1  0  0  1 -3  3  4 -2  0 -3 -3  1 -1 -3 -1  0 -1 -3 -2 -2  1  4 -1 -4
X  0 -1 -1 -1 -2 -1 -1 -1 -1 -1 -1 -1 -1 -1 -2  0  0 -2 -1 -1 -1 -1 -1 -4
* -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4  1
```

Fonte: NCBI

Pontuação leva em conta a matriz

- Match: blosum62(i,i) sempre positivo
- Mismatch: blosum62(i,j) positivo, nulo, negativo
- Espaço: –2

```
H: GDVEKGKKIFIMKCSQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGYSYTAANKNKGIIWG
   GD EKGKK++  +C QCH V+     KTGP LHG+ GR +G   G+ Y+AANKNKG++W
C: GDYEKGKKVYKQRCLQCHVVDSTAT-KTGPTLHGVIGRTSGTVSGFDYSAANKNKGVVWT
```

Como obter alinhamentos ótimos?

- Precisamos de um algoritmo
- Algoritmos são diferentes de programas
  - Algoritmo é um método abstrato
  - Programa é uma encarnação física (numa linguagem de programação) de um algoritmo
  - Um programa pode ser executado num computador
  - Dizemos que um certo algoritmo foi implementado em tal ou qual linguagem
- Quem escrever algoritimo na prova perde 3 pontos!

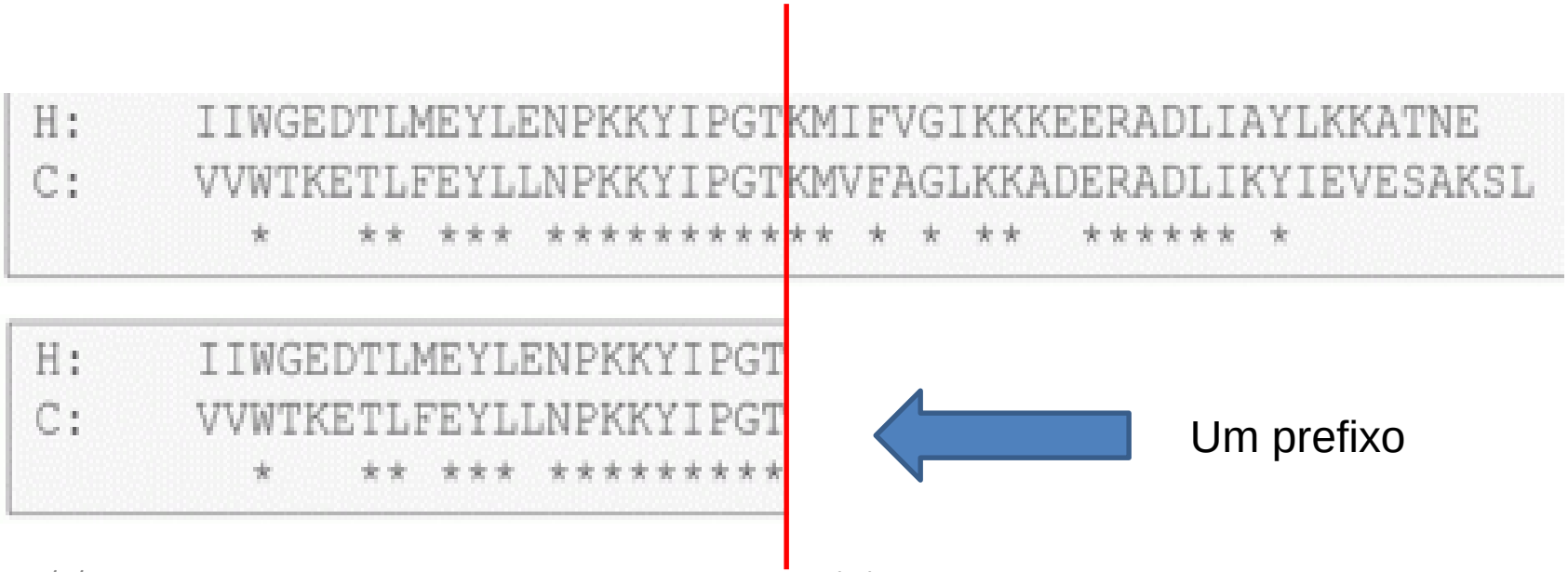
Algoritmo para achar alinhamentos ótimos de DNA

- Desenvolvido com a técnica de Programação dinâmica
- Técnica desenvolvida na década de 1950 por Richard Bellman
- Não é programação e não é dinâmica!

Programação Dinâmica

- PD se usa para problemas que tem uma estrutura de subproblemas
- Num alinhamento com sequências *s* e *t* um subproblema é qualquer alinhamento entre *s'* e *t'* tal que

  s' = um prefixo de *s* e *t'* = um prefixo de *t*



Ideia básica da PD

- Achar soluções de subproblemas e armazená-las numa tabela (matriz)
- Para achar a solução ótima:
  - Ir achando as soluções na direção dos subproblemas menores para os maiores
  - Último elemento da tabela a ser preenchido contém a solução do problema “completo”

	j	0	1	2	3	4
i		t	G	A	T	C
0	s					
1	G					
2	T					
3	C					

Preenchimento da tabela

- Este processo precisa começar com o “menor subproblema possível”. Qual seria?
  - Quando pelo menos uma das sequências é vazia

- Inicialização: Alinhar *s* com cadeia vazia e alinhar *t* com cadeia vazia

	j	0	1	2	3	4
i		t	G	A	T	C
0	s	0	-2	-4	-6	-8
1	G	-2				
2	T	-4				
3	C	-6				

Continuação

- Alinhar caracter *X* com caracter *Y*
- 3 possibilidades
  - *X* com *Y*
    - Aplicar pontuação respectiva, dependendo se for DNA ou proteína
  - *X* com espaço
    - Cobrar -2
  - *Y* com espaço
    - Cobrar -2

Preenchimento de (1,1)

- Significa determinar qual é o melhor alinhamento dos prefixos de *s* e *t* com apenas um caracter cada um
- Alternativas

```
G-      -G      G
-G      G-      G
```

Todos eles usam valores determinados na inicialização

	j	0	1	2	3	4
i		t	G	A	T	C
0	s	0	-2	-4	-6	-8
1	G	-2	1			
2	T	-4				
3	C	-6				

	j	0	1	2	3	4
i		t	G	A	T	C
0	s	0	-2	-4	-6	-8
1	G	-2	1	-1	-3	-5
2	T	-4	-1	0	0	-2
3	C	-6	-3	-2	-1	1

Exercícios

- Inventar duas sequências de DNA curtas e "rodar" (na mão) o algoritmo de PD
- Para se auto-corrigir:

  http://www.codeproject.com/Articles/304772/DNA-Sequence-Alignment-using-Dynamic-Programming-A

  (busque dna sequence alignment code project)

  – Demo parece segura

  – Exige registro

  – Tem código fonte

Complexidade computacional de PD

- Queremos saber quanto tempo gasta o algoritmo
- Mas algoritmos são abstratos, não estão associados a computadores físicos; o que significa “tempo”?
- Então contamos número de operações elementares
  - Operações aritméticas
  - Uso de posições da memória
  - Etc
- Porém queremos apenas algo aproximado
- Uma ordem de grandeza

Complexidade computacional

- Queremos expressar o número de operações como uma função matemática do tamanho das entradas
- Por exemplo
  - n (linear)
  - n^2 (quadrático)
  - $n \log n$
- Vamos desprezar constantes (n e $30n$ dão na mesma)
- Só nos interessa o termo de maior grau (desprezar os demais)
  - $7n^2 + 1400n + 3000 \log n$ vira apenas n^2
- Vamos usar a notação $O(f(n))$ para denotar tudo isso

Complexidade computacional de PD

- A matriz tem tamanho n+1 por m+1
- Todos os elementos da matriz precisam ser preenchidos
- Supondo tempo constante para o preenchimento
  - n+1 × m+1 = $nm + n + m + 1$
  - $O(nm)$
  - Se $n \approx m$, $O(n^2)$
    - Quadrático
- Memória: quadrático também

Algorithm *Similarity*

input: sequences s and t

output: similarity between s and t

$m \leftarrow |s|$

$n \leftarrow |t|$

for $i \leftarrow 0$ **to** m **do**

    $a[i, 0] \leftarrow i \times g$

for $j \leftarrow 0$ **to** n **do**

    $a[0, j] \leftarrow j \times g$

for $i \leftarrow 1$ **to** m **do**

    for $j \leftarrow 1$ **to** n **do**

$$a[i, j] \leftarrow \max(a[i-1, j] + g,\ a[i-1, j-1] + p(i, j),\ a[i, j-1] + g)$$

return $a[m, n]$

FIGURE 3.2

Basic dynamic programming algorithm for comparison of two sequences.

Penalização de espaços pode ser mais sofisticada

```
GTGGTGGCCTACGAAGGT
GTGGTCGC---CGAAGGT
```

```
GTGGTGGCC-ACGAAGGT
GT-GTCGCCTACGA-GGT
```

- No sistema de pontuação apresentado, *k* espaços consecutivos (um buraco ou *gap*) custam o mesmo que *k* espaços separados
- Seria melhor distinguir os dois casos

Penalização de espaços feita por uma função matemática

- k = número de espaços
- $p(k) = a + bk$
- $p(k)$ é subtraído do score
- a = custo para abrir um buraco
- b = custo para continuar um buraco
- Por exemplo: $p(k) = 2 + k$
- 5 espaços consecutivos custam 7
- 5 espaços separados custam 15
- Compare com a função implícita do sistema simples: $p(k) = 2k$

Algoritmo de PD com penalização de buracos por função afim

- O algoritmo é mais complexo
  - São necessárias 3 tabelas ao invés de 1
- Mas a complexidade permanece a mesma (quadrática)
- Algoritmo de Smith-Waterman

Smith-Waterman

$a[i, j]$ = maximum score of an alignment between $s[1..i]$ and $t[1..j]$ that ends in $s[i]$ matched with $t[j]$.

$b[i, j]$ = maximum score of an alignment between $s[1..i]$ and $t[1..j]$ that ends in a space matched with $t[j]$.

$c[i, j]$ = maximum score of an alignment between $s[1..i]$ and $t[1..j]$ that ends in $s[i]$ matched with a space.

The entries (i, j) of these arrays depend on previous entries according to the following formulas, valid for $1 \leq i \leq m$ and $1 \leq j \leq n$:

$$a[i, j] = p(i, j) + \max \begin{cases} a[i-1, j-1] \\ b[i-1, j-1] \\ c[i-1, j-1] \end{cases}$$

$$b[i, j] = \max \begin{cases} -(h+g) + a[i, j-1] \\ -g + b[i, j-1] \\ -(h+g) + c[i, j-1] \end{cases}$$

$$c[i, j] = \max \begin{cases} -(h+g) + a[i-1, j] \\ -(h+g) + b[i-1, j] \\ -g + c[i-1, j]. \end{cases}$$

As before, $p(i, j)$ indicates the score of a matching between $s[i]$ and $t[j]$.

- Se a função for genérica [ex. $p(k) = a + b \log k$)], então a complexidade passa para $O(n^3)$
  - Algoritmo de Needleman-Wunsch

Queremos descobrir sequências aparentadas

- Aparentadas = ancestral comum = homólogas
- Alinhamentos biologicamente relevantes
- Nota máxima por si só não nos informa sobre parentesco
  - Alinhamentos de nota máxima não necessariamente correspondem a alinhamentos biologicamente relevantes
- Como fazer?

Bancos de sequências

- Situação típica
  - Tenho uma sequência consulta
  - Quero saber se existem sequências já publicadas que são “parentes” dela
- Tenho que fazer uma busca em bancos de sequências

Bancos de sequências

- Resultado do sequenciamento em geral é publicado
- “bancos de dados” de sequências
- Na verdade catálogos
- Mais importante: **GenBank**
  - Mantido pelo *National Center for Biotechnological Information*
  - **NCBI**
  - http://www.ncbi.nlm.nih.gov

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II 1 2 3 4 5

Popular Resources

- BLAST
- Bookshelf
- Gene
- Genome
- Nucleotide
- OMIM
- Protein
- PubChem
- PubMed
- PubMed Central
- SNP

NCBI News

New NCBI News Issue

06 Jul 2011

Information on the redesigned PopSet resource, as well as new

Preliminary genomic assemblies from two isolates from the European E. coli outbreak now available

07 Jun 2011

Preliminary genomic assemblies of two isolates are in the

More...

UniProt http://www.uniprot.org/

Estatística de alinhamentos

- Com um banco, temos uma “população” de sequências
- Com essa população, posso criar uma teoria estatística que vai me permitir separar os alinhamentos estatisticamente significativos daqueles obtidos por mero acaso
- Diremos que os alinhamentos estatisticamente significativos são biologicamente relevantes
- A significância estatística precisa ser quantificada: e-value

E-value

- Teoria de Karlin e Altschul
- Calcula o e-value (expect value) de um alinhamento
- $E = Kmne^{-\lambda S}$
- *m* e *n* são os tamanhos das sequências
- *S* é a pontuação
- *K* e λ são parâmetros
- Um banco de sequências pode ser tratado como uma longa sequência de tamanho *n*
- A fórmula dá o **número de alinhamentos** que se esperaria obter com pontuação pelo menos *S* **ao acaso**

e-value

- Não é uma probabilidade
- Pode resultar maior do que 1
- Mas em geral os alinhamentos biologicamente relevantes tem e-value < 10^{-5}
- Para valores assim ou menores, o e-value se comporta como uma probabilidade
- p-values e e-values $P = 1 - e^{-E}$

- E-value depende do tamanho do banco
- Não se pode comparar diretamente e-values obtidos de consultas a bancos diferentes
- Mas existe uma fórmula de conversão
  - Dado o e-value contra banco X, é possível saber qual seria o e-value contra banco Y
  - Essa mesma fórmula pode ser usada para dar o e-value para comparação de apenas duas sequências entre si (supondo que Y seja genBank)

The Statistics of Sequence Similarity Scores

Introduction

To assess whether a given alignment constitutes evidence for homology, it helps to know how strong an alignment can be expected from chance alone. In this context, "chance" can mean the comparison of (i) real but non-homologous sequences; (ii) real sequences that are shuffled to preserve compositional properties [1-3]; or (iii) sequences that are generated randomly based upon a DNA or protein sequence model. Analytic statistical results invariably use the last of these definitions of chance, while empirical results based on simulation and curve-fitting may use any of the definitions.

The statistics of global sequence comparison

Unfortunately, under even the simplest random models and scoring systems, very little is known about the random distribution of optimal global alignment scores [4]. Monte Carlo experiments can provide rough distributional results for some specific scoring systems and sequence compositions [5], but these can not be generalized easily. Therefore, one of the few methods available for assessing the statistical significance of a particular global alignment is to generate many random sequence pairs of the appropriate length and composition, and calculate the optimal alignment score for each [1,3]. While it is then possible to express the score of interest in terms of standard deviations from the mean, it is a mistake to assume that the relevant distribution is normal and convert this *Z*-value into a *P*-value; the tail behavior of global alignment scores is unknown. The most one can say reliably is that if 100 random alignments have score inferior to the alignment of interest, the *P*-value in question is likely less than 0.01. One further pitfall to avoid is exaggerating the significance of a result found among multiple tests. When many alignments have been generated, e.g. in a database search, the significance of the best must be discounted accordingly. An alignment with *P*-value 0.0001 in the context of a single trial may be assigned a *P*-value of only 0.1 if it was selected as the best among 1000 independent trials.

The statistics of local sequence comparison

http://www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html

Programação dinâmica é cara

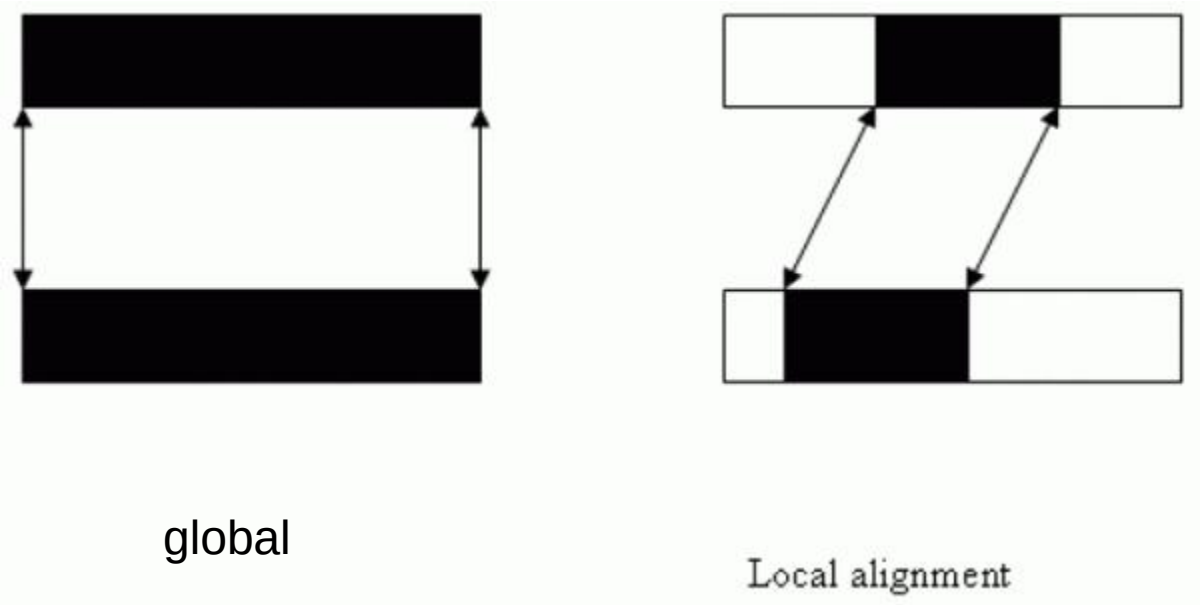
- Especialmente quando
  - Comparação contra muitas sequências
    - Buscas em banco de dados
  - Comparação de muitas sequências entre si
    - Todas contra todas
- Alternativa: BLAST
- Basic Local Alignment Search Tool

BLAST

- Altschul et al., 1990, 1997
- Cerca de 75 mil citações (10/2014)
- Programa mais citado em ciência
  - (mas não são os papers mais citados; o mais citado tem 305 mil citações)
- Heurística
  - Não tem garantia de que sempre consegue achar os alinhamentos de pontuação máxima
  - Sacrifica garantia de otimalidade por velocidade
  - Mas na vasta maioria das vezes tais alinhamentos são de fato encontrados
  - Reporta e-values
  - (É possível fazer cálculo de e-values com PD)

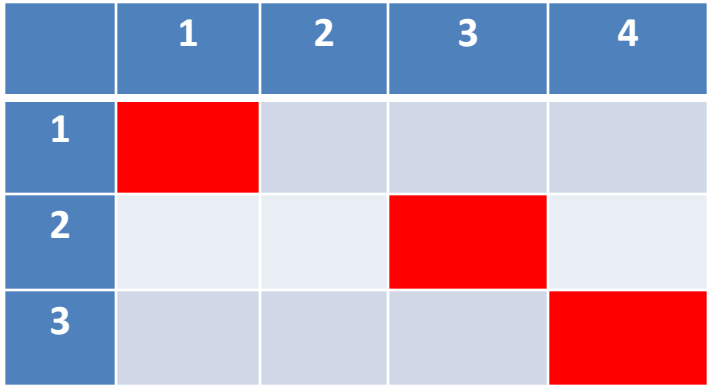
BLAST

- Acha alinhamentos locais

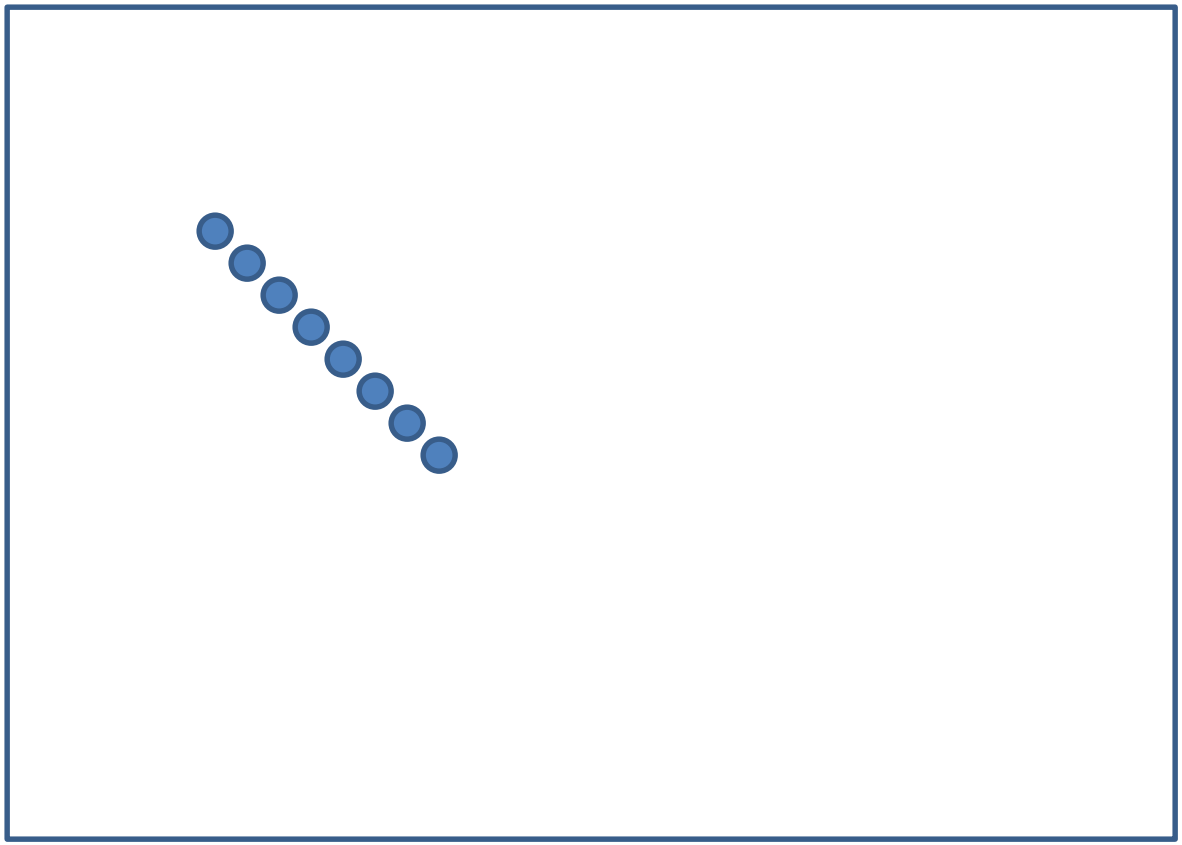


É útil pensar na matriz de PD como se fosse um dotplot

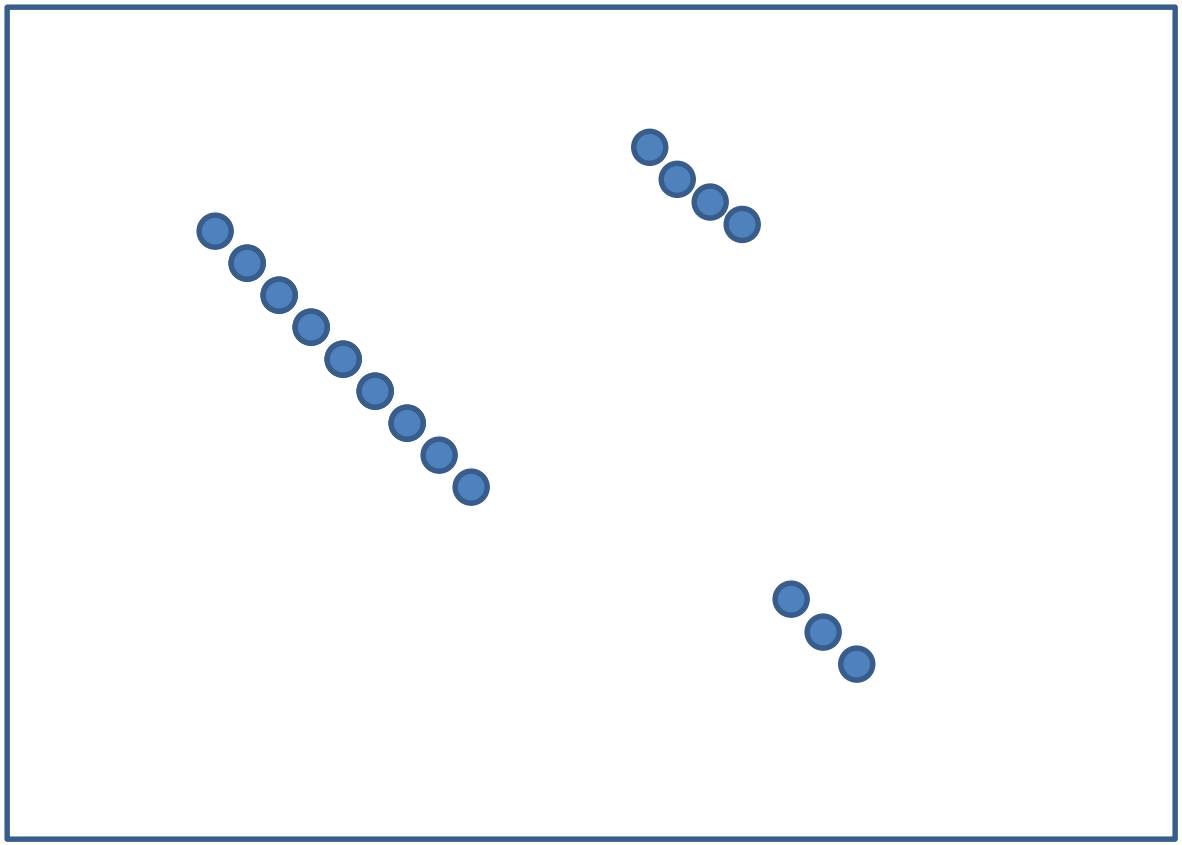
	j	0	1	2	3	4
i		t	G	A	T	C
0	s	0	-2	-4	-6	-8
1	G	-2	1	-1	-3	-5
2	T	-4	-1	0	0	-1
3	C	-6	-3	-2	-1	1



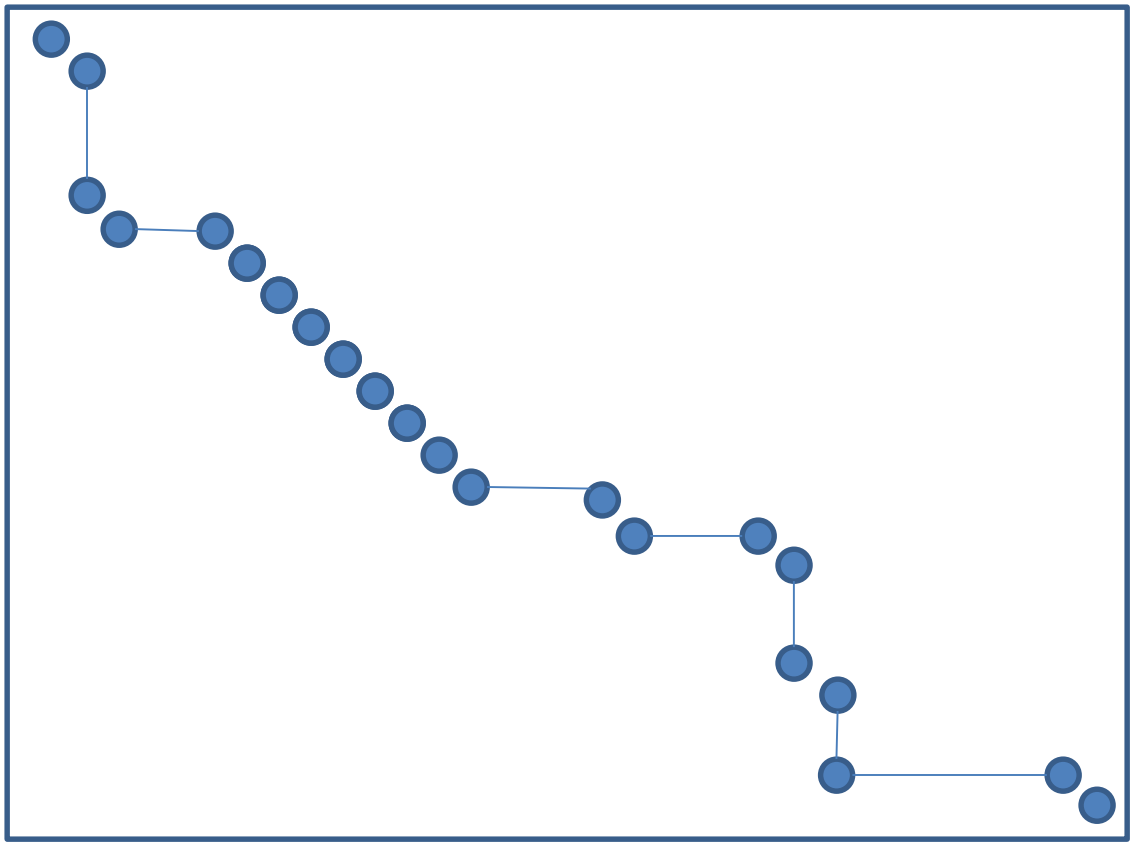
Um alinhamento local é um trecho de células consecutivas num dotplot



Pode haver vários alinhamentos locais



Alinhamento global



Exercício

- Como modificar o algoritmo de PD para que ele encontre o melhor alinhamento local?
- Dica: comece definindo as características do melhor alinhamento local

Alinhamento local com PD

- Um alinhamento global pode ter nota negativa

→ um alinhamento local nunca pode ter nota negativa

- Pois o alinhamento entre sequências vazias tem nota zero
- bons alinhamentos locais são trechos com notas positivas na matriz de PD

Implementação

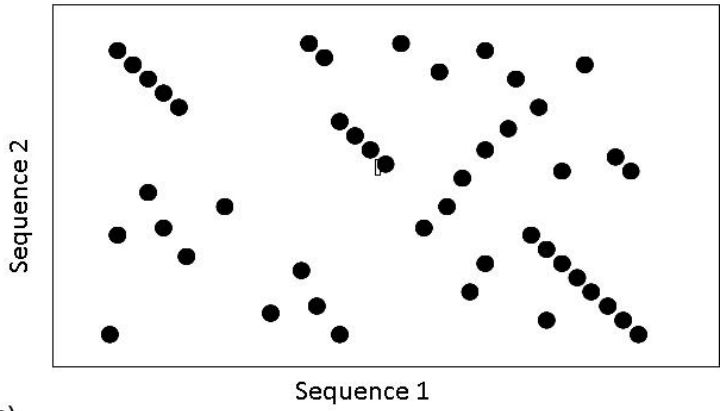
1. Inicialização da coluna zero e da linha zero com zeros
2. Ao preencher um elemento da matriz, fazer a operação de máximo, mas nunca deixar que o valor escolhido seja negativo

   Valor ← max (M[i-1,j], M[i, j-1], M[i-1,j-1], 0)
3. Ao final: procurar o elemento da matriz de valor máximo
4. Para recuperar o alinhamento: percorrer de trás para diante até chegar em zero

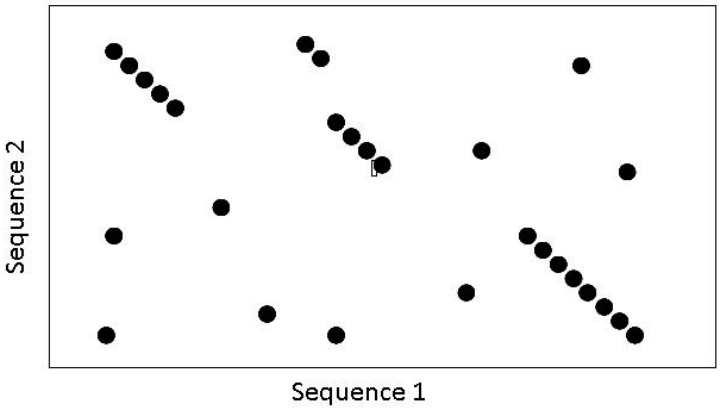
De onde vem a eficiência de BLAST?

- BLAST busca trechos parecidos (*palavras* ou *words*) entre as sequências = alinhamentos-semente
- Para nt, esses alinhamentos tem que ser exatos
- Para aa, esses alinhamentos tem que ter nota positiva
- Estende esses alinhamentos-semente

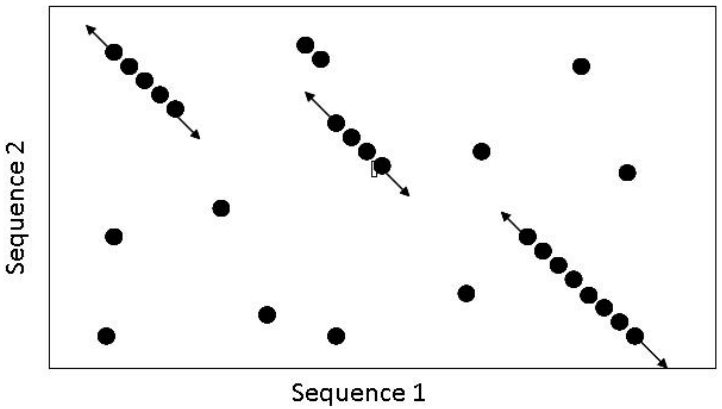
a)



b)



c)



Crédito: I. Lobo

Exemplo de alinhamento ótimo que BLAST não encontraria

- Suponha nt e tamanho mínimo de palavras = 4

```
GTG-TGGCCTA-GAAGCT
|||  |  |  |||  ||  |  |
GTGGTCG-CTACGACG-T
```

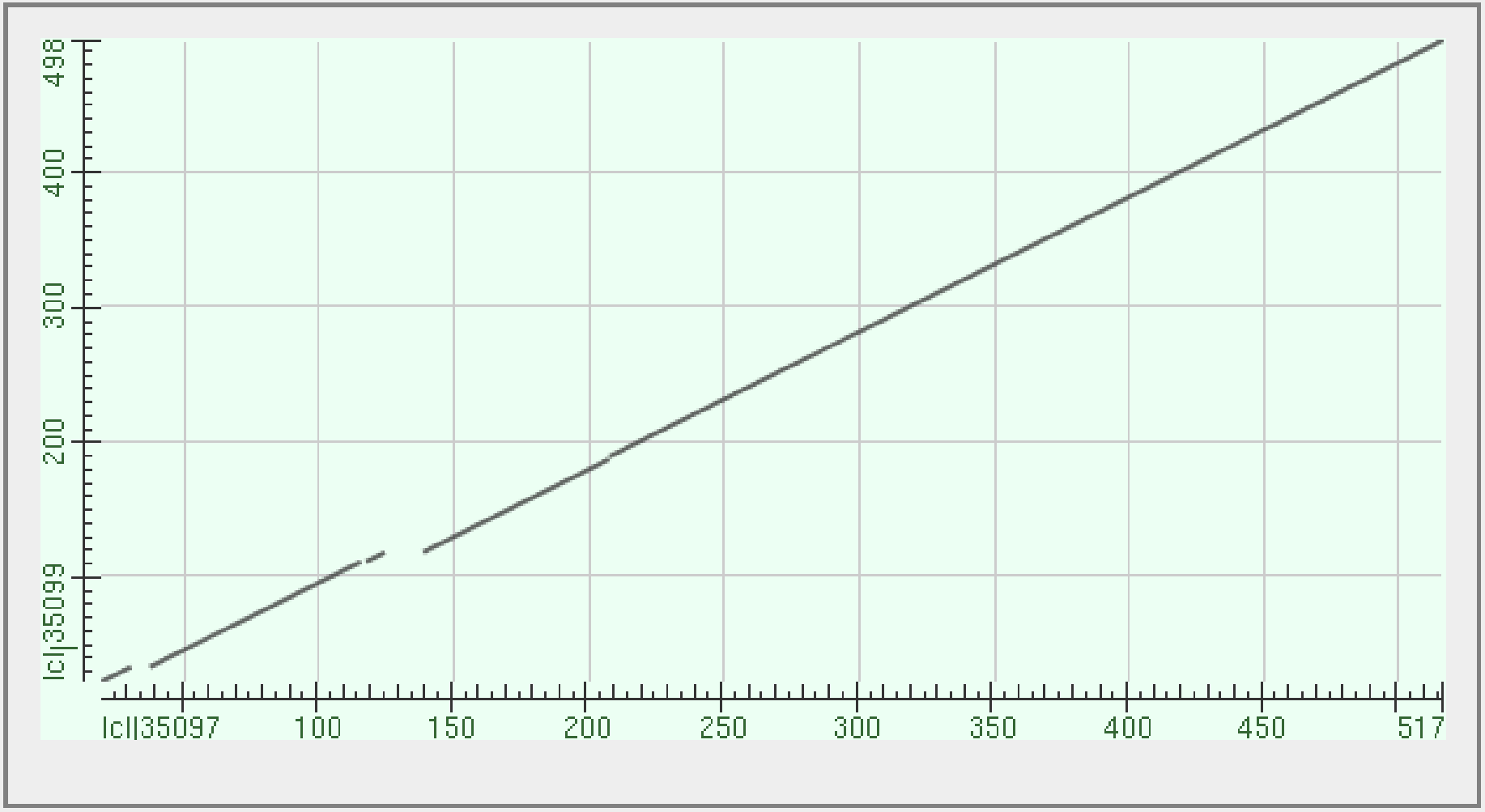
16 posições, 12 identidades (75%)

Características de BLAST

- Tamanho default das palavras
  - DNA: 11 nt
  - Proteínas: 3 aa
- Reporta bit score, raw score, e-value, identidades, positivos, buracos

```
>lcl|35099 t
Length=499

 Score =  604 bits (1558),  Expect = 0.0, Method: Compositional matrix adjust.
 Identities = 301/499 (60%), Positives = 365/499 (73%), Gaps = 25/499 (5%)

Query  21   DAACSEAAGDKSAMMHDALFERFSARLKAQVGPEVYASWFARLKLHTVSKSVVRFTVPTT  80
            DA C E   ++       LF+  S++L+ QVG +VYASWF RLK  +VS ++V  +VPT
Sbjct  23   DARCLETTCEE-------LFKNVSSKLEDQVGSDVYASWFQRLKFRSVSHNIVYLSVPTN  75

Query  81   FLKSWINNRYMDLITSLVQSEDPDVLKVEILVRSASRPVRPAQTEERAQPVQEVGAAPRN  140
            FLK+WI NRY+D IT L Q     +  VEI+VRSA+  + P++T                +
Sbjct  76   FLKAWIKNRYIDTITKLFQESISSIQGVEIIVRSAA--LMPSETS--------------S  119

Query  141  KSFIPSQSATAPAAQPMAAQATLRQGGSGPLFGSPLDTRFTFDTFVEGSSNRVALAAAKT  200
             S I   +A  P            +    P+FGSPLD++F F  F+EG SNRVALAAA T
Sbjct  120  SSAIAHTTAKPPIINTGKISTIQGKQSINPVFGSPLDSKFVFSNFIEGPSNRVALAAAHT  179

Query  201  IAEAGAGA--VRFNPLFIHAGVGLGKTHLLQAIANAAIDSPRNPRVVYLTAEYFMWRFAT  258
            IAE  + +  VRFNPLFIHA VGLGKTHLLQAIANAAI    N RVVYLTAEYFMWRFAT
Sbjct  180  IAEENSSSCTVRFNPLFIHASVGLGKTHLLQAIANAAIKKQNNLRVVYLTAEYFMWRFAT  239

Query  259  AIRDNDALTLKDTLRNIDLLVIDDMQFLQGKMIQHEFCHLLNMLLDSAKQVVVAADRAPW  318
            AIRDN AL  KD LRNIDLL+IDDMQFLQGK+IQHEFCHLLN LLDSAKQ+V AADR P
Sbjct  240  AIRDNYALNFKDCLRNIDLLLIDDMQFLQGKLIQHEFCHLLNSLLDSAKQIVAAADRPPS  299

Query  319  ELESLDPRVRSRLQGGMAIEIEGPDYDMRYEMLNRRMGSARQDDPSFEISDEILTHVAKS  378
            ELESLD R+RSRLQGG+A+ +   D +MR  +L  R+  A++D+P   IS+EIL  VA++
Sbjct  300  ELESLDSRIRSRLQGGVAVPLGAHDIEMRLTILKNRLKMAKKDNPKLYISEEILQRVAQT  359

Query  379  VTASGRELEGAFNQLMFRRSFEPNLSVDRVDELLSHLVGSGEAKRVRIEDIQRIVARHYN  438
            VT SGREL+GAFNQL+FR SFEP L++  VDELLSHLV +GE K++RIEDIQR+V++HYN
Sbjct  360  VTTSGRELDGAFNQLVFRNSFEPVLTIKMVDELLSHLVSAGETKKIRIEDIQRMVSKHYN  419

Query  439  VSRQELVSNRRTRVIVKPRQIAMYLAKMLTPRSFPEIGRRFGGRDHTTVLHAVRKIEDLI  498
            +SR +L+SNRR R IV+PRQIAMYL+K++TPRSFPEIGRRFG RDHTTVLHAVRKIE  +
Sbjct  420  ISRTDLLSNRRVRTIVRPRQIAMYLSKIMTPRSFPEIGRRFGDRDHTTVLHAVRKIEKSM  479

Query  499  SGDTKLGHEVELLKRLINE  517
              DT +  EVELLKRLI+E
Sbjct  480  EKDTVIKKEVELLKRLISE  498
```

Plot of lcl|35097 vs 35099



Buscando no GenBank

t (499 letters)

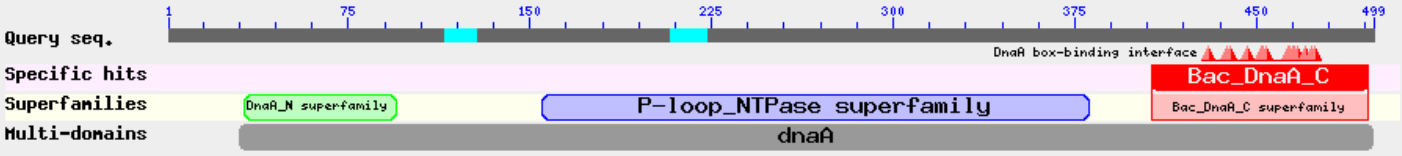
Query ID	lcl|78035	**Database Name**	nr
Description	t	**Description**	All non-redundant GenBank CDS translations+PDB+SwissProt+ environmental samples from WGS projects
Molecule type	amino acid	**Program**	BLASTP 2.2.26+ ▷Citation
Query Length	499		

Other reports: ▷Search Summary [Taxonomy reports] [Distance tree of results] [Multiple alignment]

Graphic Summary

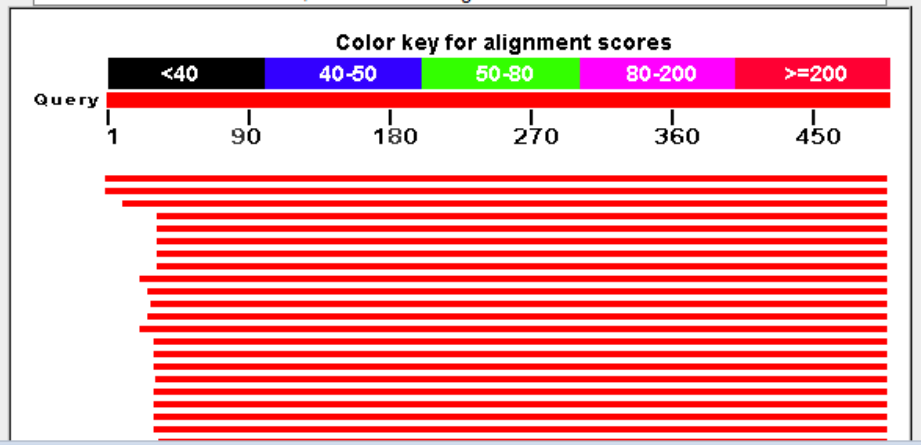
Show Conserved Domains

Putative conserved domains have been detected, click on the image below for detailed results.



Distribution of 100 Blast Hits on the Query Sequence

Mouse over to see the defline, click to show alignments



Lista de hits

Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer PubChem BioAssay

Sequences producing significant alignments:

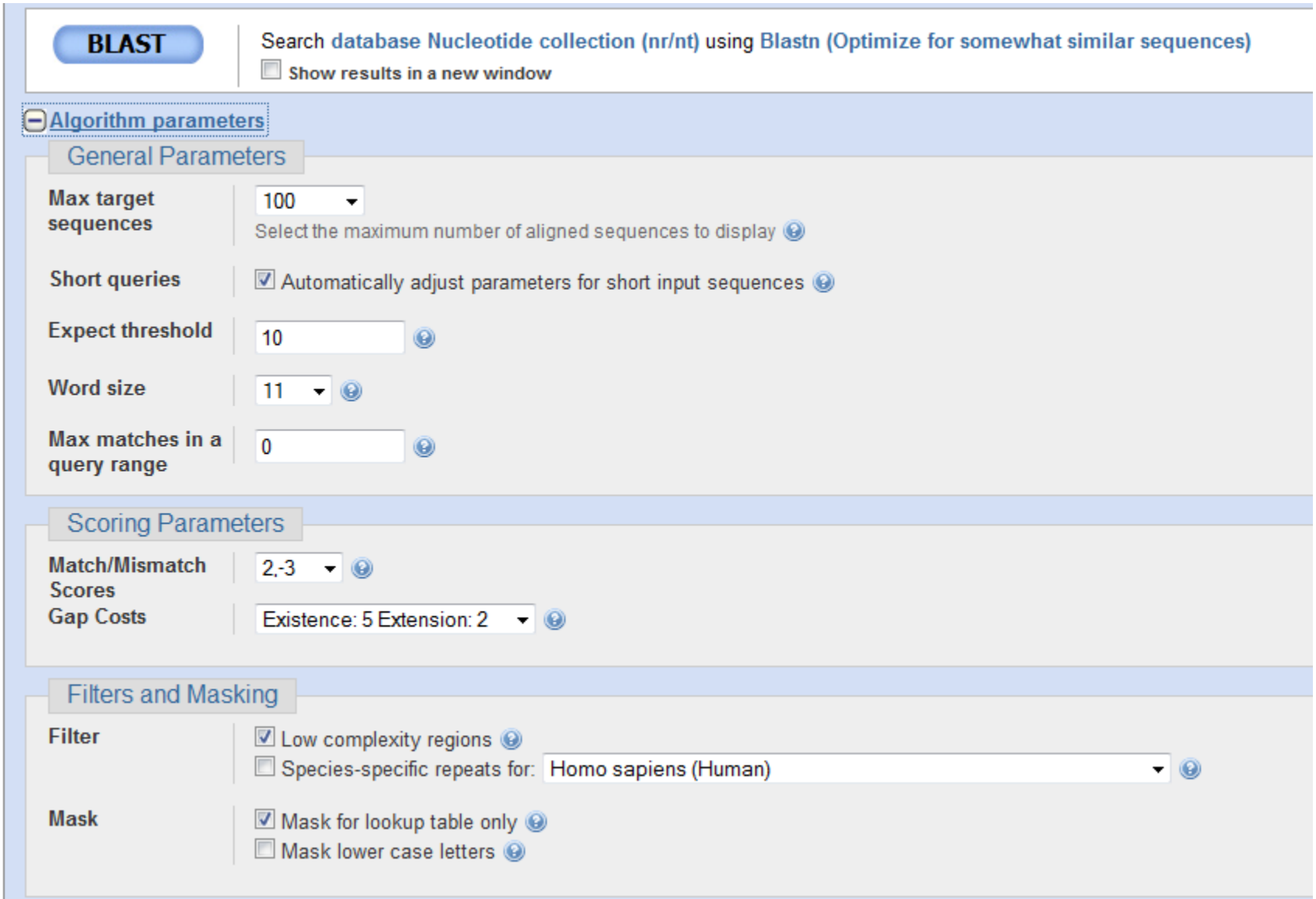
Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
YP_004062317.1	chromosome replication initiator DnaA [Candidatus Liberibacter solanacearum	785	785	99%	0.0	76%	G
YP_003065040.1	dnaA gene product [Candidatus Liberibacter asiaticus str. psy62] >gb|ACT57	755	755	99%	0.0	76%	G
YP_002543179.1	chromosomal replication initiation protein [Agrobacterium radiobacter K84] >g	615	615	97%	0.0	61%	G
YP_765982.1	dnaA gene product [Rhizobium leguminosarum bv. viciae 3841] >sp|Q1MMD6.	613	613	93%	0.0	63%	G
YP_001976569.1	chromosomal replication initiation protein [Rhizobium etli CIAT 652] >gb|ACE8	612	612	93%	0.0	63%	G
YP_002973852.1	dnaA gene product [Rhizobium leguminosarum bv. trifolii WSM1325] >gb|ACS	612	612	93%	0.0	63%	G
YP_467907.1	chromosomal replication initiation protein [Rhizobium etli CFN 42] >gb|ABC891	611	611	93%	0.0	63%	G
YP_002279530.1	dnaA gene product [Rhizobium leguminosarum bv. trifolii WSM2304] >gb|ACI5	608	608	93%	0.0	64%	G
EGP58677.1	chromosomal replication initiation protein [Agrobacterium tumefaciens F2]	607	607	95%	0.0	61%	
EHS51424.1	Chromosomal replication initiator protein dnaA [Rhizobium sp. PDO1-076]	605	605	94%	0.0	62%	
EHH08270.1	chromosomal replication initiation protein [Agrobacterium tumefaciens CCNWC	600	600	93%	0.0	62%	
YP_002548273.1	chromosomal replication initiation protein [Agrobacterium vitis S4] >gb|ACM3	601	601	94%	0.0	61%	G
NP_353356.2	chromosomal replication initiation protein [Agrobacterium tumefaciens str. C5	598	598	95%	0.0	60%	G
ZP_08526429.1	chromosomal replication initiation protein [Agrobacterium sp. ATCC 31749] >s	595	595	93%	0.0	61%	
YP_004277622.1	chromosome replication initiator DnaA [Agrobacterium sp. H13-3] >gb|ADY63	593	593	93%	0.0	61%	G
YP_001325697.1	dnaA gene product [Sinorhizobium medicae WSM419] >gb|ABR58862.1| chro	590	590	93%	0.0	63%	G
ZP_02164856.1	chromosomal replication initiation protein [Hoeflea phototrophica DFL-43] >gb	578	578	93%	0.0	61%	
ZP_05929413.1	chromosomal replication initiator protein dnaA [Brucella abortus bv. 3 str. Tul	578	578	93%	0.0	60%	
P35890.3	RecName: Full=Chromosomal replication initiator protein DnaA	573	573	93%	0.0	62%	
NP_384474.1	chromosomal replication initiation protein [Sinorhizobium meliloti 1021] >ref|Y	574	574	93%	0.0	62%	G
AAA26258.1	dnaA [Sinorhizobium meliloti] >gb|AAA91097.1| dnaA [Sinorhizobium meliloti]	574	574	93%	0.0	62%	
YP_001608612.1	dnaA gene product [Bartonella tribocorum CIP 105476] >emb|CAK00617.1| c	573	573	92%	0.0	59%	G
AFL48605.1	chromosomal replication initiator protein DnaA [Sinorhizobium fredii USDA 257	571	571	93%	0.0	62%	
YP_004547390.1	unnamed protein product [Sinorhizobium meliloti AK83] >gb|AEG51776.1| Chr	573	573	93%	0.0	62%	G
YP_002824558.1	chromosomal replication initiation protein [Sinorhizobium fredii NGR234] >gb|A	571	571	93%	0.0	62%	G
CBI78638.1	chromosomal replication initiator protein DnaA [Bartonella sp. AR 15-3]	572	572	92%	0.0	60%	
YP_002971177.1	chromosomal replication initiator protein DnaA [Bartonella grahamii as4aup] >	571	571	92%	0.0	60%	G
ZP_10237186.1	chromosomal replication initiation protein, partial [Nitratireductor aquibiodomu	569	569	97%	0.0	56%	

Sabores de BLAST

Subject Query	nucleotídeos	aminoácidos
nucleotídeos	BLASTN TBLASTX	BLASTX
aminoácidos	TBLASTN	BLASTP

Também: megablast, psi-blast, phi-blast, delta-blast, etc

Parâmetros de BLASTn



Regiões de baixa complexidade

- Sequências com elementos repetitivos e que aparecem com frequência
- Exemplo em DNA
  - AAAAAAA
- Exemplo em proteína
  - AGNLLGRNVVVVGAG
- Uso do filtro é default
- Pode excluir alinhamentos relevantes

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Low complexity regions in the proteins of prokaryotes perform important functional roles and are highly conserved

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ABSTRACT

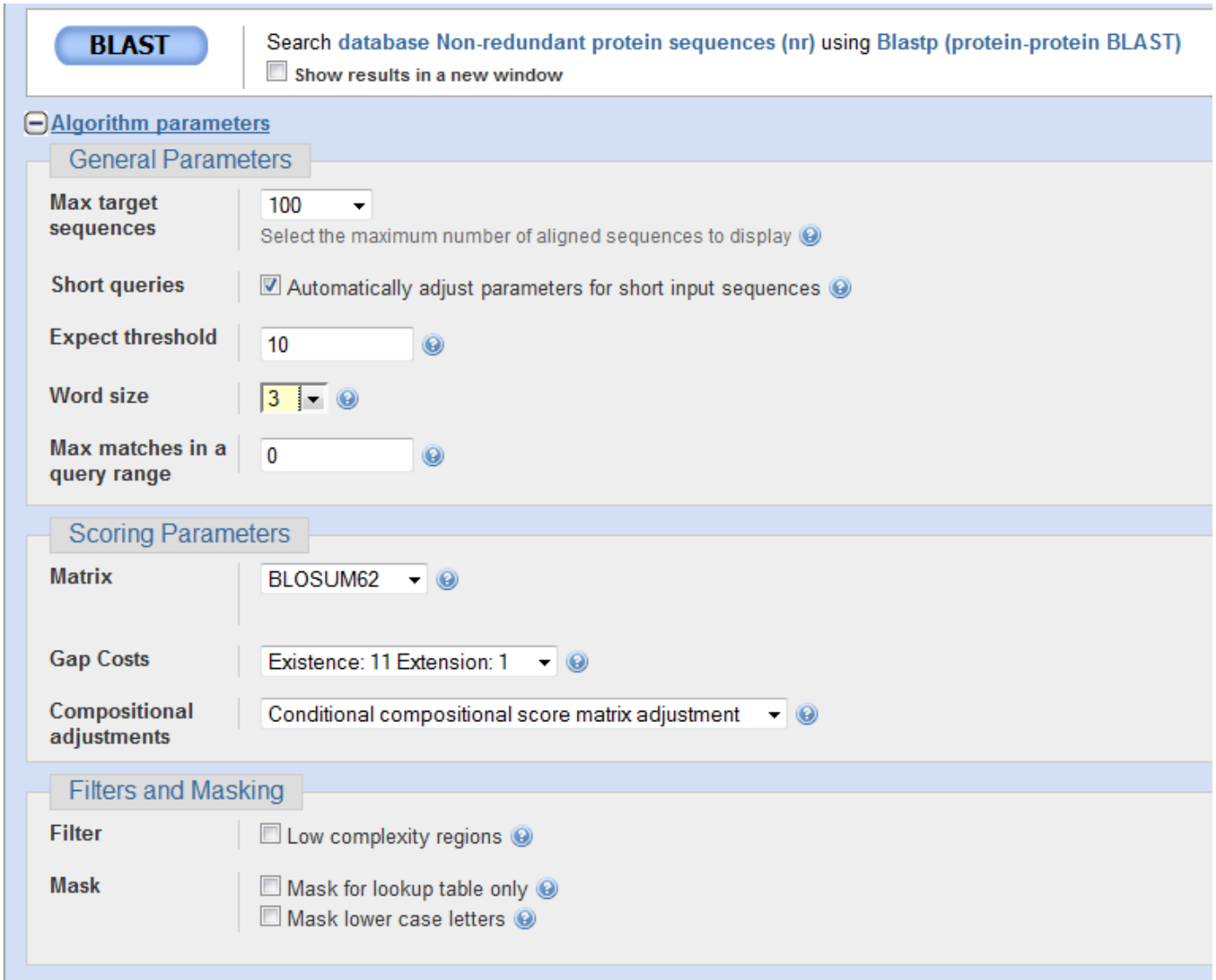
We provide the first high-throughput analysis of the properties and functional role of Low Complexity Regions (LCRs) in more than 1500 prokaryotic and phage proteomes. We observe that, contrary to a widespread belief based on older and sparse data, LCRs actually have a significant, persistent and highly conserved presence and role in many and diverse prokaryotes. Their specific amino acid content is linked to proteins with certain molecular functions, such as the binding of RNA, DNA, metal-ions and polysaccharides. In addition, LCRs have been repeatedly identified in very ancient, and usually highly expressed proteins of the translation machinery. At last, based on the amino acid content enriched in certain categories, we have developed a neural network web server to identify LCRs and accurately predict whether they can bind nucleic acids, metal-ions or are involved in chaperone functions. An evaluation of the tool showed that it is highly accurate for eukaryotic proteins as well.

searching for homologs (5). However, emerging experimental evidence increasingly indicated that LCRs may play important adaptive and conserved roles that are highly relevant to biotechnology, heterologous protein expression, medicine, as well as to our understanding of protein evolution (6–9).

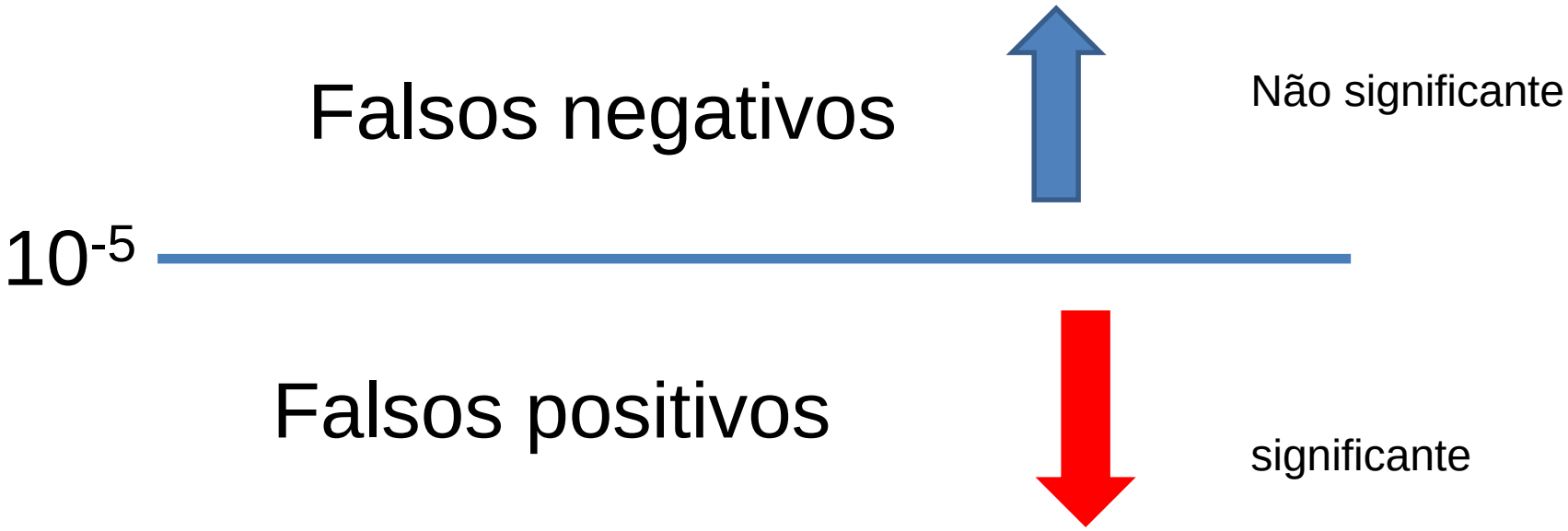
One of the established methodologies to identify LCRs is by measuring their Shannon entropy (1,10). The lower the value of the calculated entropy, the more homogeneous the region is in terms of amino acid content. Several computational tools have been developed to detect LCRs (11–17) (see especially (17) for a very recent and extended review on this topic). A subset of LCRs are single amino acid repeats (SARs) or tandem or interspersed repeats of short period (2–5 amino acids). Furthermore, repetition of large amino acid segments of >10 amino acids, or even motif or domain repeats are also categorized as protein repeats (9,18,19), but are not the subject of the current study.

The LCRs of eukaryotic proteins have been the focus of many past and recent studies, due to their involvement in human diseases (20), especially neurodegenerative ones. For example, hydrophobic LCRs tend to form amyloids in humans and other eukaryotes (21). From a mechanistic point of view, LCRs were originally proposed to be unstructured

Parâmetros de BLASTp



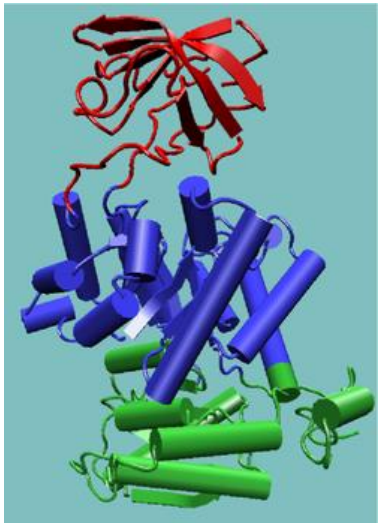
Posso acreditar nos resultados do BLAST?



1) Nem todos os alinhamentos estatisticamente significativos são biologicamente relevantes
2) Nem todos os alinhamentos que não são estatisticamente significantes não são relevantes

Exemplo do caso (1)

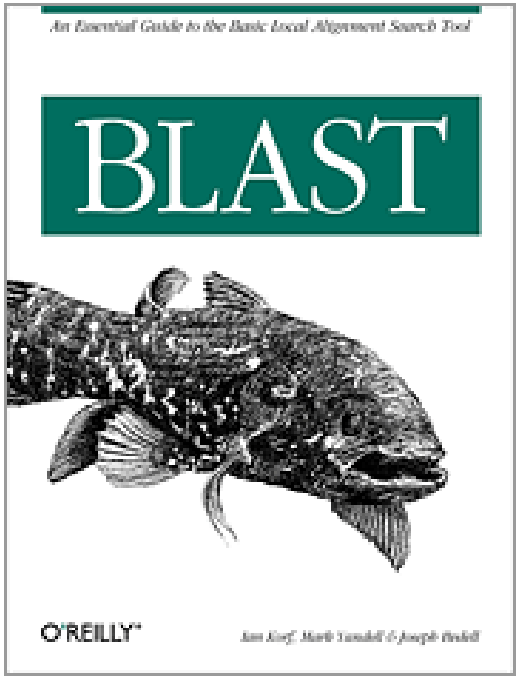
- Duas proteínas podem compartilhar um domínio e não serem relacionadas
  - falsos positivos de BLAST
- Acontece quando as proteínas tem múltiplos domínios



Pyruvate kinase

http://en.wikipedia.org/wiki/File:1pkn.png

Referências



Ian Korf, Mark Yandell, Joseph Bedell

Artigo por Ingrid Lobo (Write Science Right) © 2008 Nature Education

http://www.nature.com/scitable/topicpage/basic-local-alignment-search-tool-blast-29096

Updates

- New post in Eyes on Environment: Unique and Alone On the EDGE of Existence
- New topic in Women in Science: The First Woman to Win a Fields Medal: Maryam Mirzakhani
- New post in Viruses101: Ebola Outbreak Declared an International Public Health Emergency
- New post in Accumulating Glitches: Does Biology Have Laws?
- New post in Accumulating Glitches: The Language of DNA

Topic Rooms

Within this Subject (21)

- Comparative Genomics (5)
- Functional Genomics (4)
- Genome Sequencing and Annotation (6)
- Translational Genomics (6)

Other Topic Rooms

- Genetics
  - Gene Inheritance and Transmission
  - Gene Expression and Regulation
  - Nucleic Acid Structure and Function
  - Chromosomes and Cytogenetics
  - Evolutionary Genetics
  - Population and Quantitative

ADVANCED

GENOMICS | Lead Editor: Michael Goldman, Christopher D. Smith

Basic Local Alignment Search Tool (BLAST)

By: Ingrid Lobo, Ph.D. (*Write Science Right*) © 2008 Nature Education

Citation: Lobo, I. (2008) Basic Local Alignment Search Tool (BLAST). *Nature Education* 1(1):215

Awash in a sea of data, how do scientists identify the function of a newly cloned gene? Online resources like the Basic Local Alignment Search Tool (BLAST) provide a helping hand.

Aa Aa Aa

Since the discovery of the genetic code, biological research has undergone a sea of change in the way it is performed. Until the early twentieth century, biology focused on the processes of living organisms and almost always involved experiments in laboratories and in the field. The growth of molecular biology during the twentieth century moved research into the test tube, where biological systems could be painstakingly dissected and reassembled. Then, beginning in the 1970s, scientists started accumulating DNA and protein sequence data at an exponential rate; in fact, researchers currently have approximately 97 billion bases sequenced and over 93 million records. Amazingly, this sequence data doubles every 18 months!

But how do investigators make sense of this massive amount of data? How can they identify the functions of newly cloned genes? And is it possible to estimate the evolutionary relationships between genes or proteins just by examining their nucleotide or amino acid sequences? To address these important issues, researchers must first tease out the relationships between different species that are descended from a common ancestor. Any sequence similarity can then be used to infer function and evolutionary relationships. In fact, one common method for examining and comparing genes is to search for similarities between newly sequenced DNA and databases of gene sequences that have already been described. By identifying related genes or gene families with known functions, scientists can infer the functions and evolutionary relationships of newly cloned genes or even whole genomes.

As gene and protein sequence databases grew at the end of the twentieth century, scientists turned to computers to help analyze this abundant and ever-growing amount of data. Today, one of the most common tools used to examine DNA and protein sequences is the Basic Local Alignment Search Tool, also known as BLAST (Altschul *et al.*, 1990). BLAST is a computer algorithm that is available for use online at the National Center for Biotechnology Information (NCBI) website, as well as many other sites. BLAST can rapidly align and compare a query DNA sequence with a database of sequences, which makes it a critical tool in ongoing genomic research. In fact, the initial paper describing the program, published in the *Journal of Molecular Biology* and entitled "Basic Local Alignment Search Tool," was the most highly cited publication of the 1990s (Taubs, 2000). In recent years, the parallel development of large-scale sequencing projects and bioinformatic tools like BLAST has enabled scientists to study the genetic blueprint of life across many species, and it has also helped connect biology and computer science in the maturing field of bioinformatics.

Alignment Theory

Although the computer science principles behind BLAST have been around for some time, prior to BLAST, they had not been applied to biology. Before BLAST, alignment programs used dynamic programming algorithms, such as the Needleman-Wunsch and Smith-Waterman algorithms, that required long processing times and the use of supercomputers or parallel computer processors (Collins & Coulson, 1984; Gotoh & Tagashira, 1986; Smith & Waterman, 1981).

Novos programas

- Usearch [Edgar 2010]
  - Até 400x mais rápido do que BLAST
  - Com algum sacrifício de precisão
- Pauda [Huson e Xie, 2014]
  - Blastx “dos pobres”
  - 10.000x mais rápido do que blastx!
  - Com mais sacrifício de precisão
- Diamond [Buchfink, Xie, Huson 2014]
  - 20.000x mais rápido do que blastx!
  - Sem sacrifício de precisão!

Alinhamento múltiplo

- Queremos alinhar mais do que $n = 2$ sequências
- n pode variar de 3 a milhares
- Por que haveria interesse em fazer tais alinhamentos?

Motivação mais geral

- Representante da situação em que semelhança com 2 casos pode ser apenas coincidência
- Mas semelhança com 10 ou 20 ou 100 casos é muito mais difícil que seja coincidência

Motivação mais concreta

- Para construir filogenias é necessário criar AMs

Alinhamento múltiplo

```
C:      ----SDIPAGDYEKGKKVYKQRCLQCHVVDSTAT-KTGPTLHGVIGRTSGTVSGFDYSAA
Y:      ----TEFKAGSAKKGATLFKTRCLQCHTVEKGGPHKVGPNLHGIFGRHSGQAEGYSYTDA
A:      MASFDEAPPGNPKAGEKIFRTKCAQCHTVEKGAGHKQGPNLNGLFGRQSGTTPGYSYSAA
D:      -----GVPAGDVEKGKKLFVQRCAQCHTVEAGGKHKVGPNLHGLIGRKTGQAAGFAYTDA
H:      ---------GDVEKGKKIFIMKCSQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGYSYTAA
M:      ---------GDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQAAGFSYTDA
                 *. : * .::  .* ***.*:  .  * **.*:*::** :* . *: *: *
```

```
C:      NKNKGVVWTKETLFEYLLNPKKYIPGTKMVFAGLKKADERADLIKYIEVESAKSL
Y:      NIKKNVLWDENNMSEYLTNPKKYIPGTKMAFGGLKKEKDRNDLITYLKKACE---
A:      NKSMAVNWEEKTLYDYLLNPKKYIPGTKMVFPGLKKPQDRADLIAYLKEGTA---
D:      NKAKGITWNEDTLFEYLENPKKYIPGTKMIFAGLKKPNERGDLIAYLKSATK---
H:      NKNKGIIWGEDTLMEYLENPKKYIPGTKMIFVGIKKKEERADLIAYLKKATNE--
M:      NKNKGITWGEDTLMEYLENPKKYIPGTKMIFAGIKKKGERADLIAYLKKATNE--
        *    : * :..: :** ********** * *:**  :* *** *::
```

Como dar notas para alinhamentos múltiplos?

Soma de pares (aminoácidos)

- Numa coluna, podemos separar todos os pares de aminoácidos
  - 1 com 2, 1 com 3, 1 com 4, etc
  - 2 com 3, 2 com 4, etc
  - A cada par corresponde uma nota na matriz BLOSUM62
  - A soma de todas as notas dos pares dá a nota da coluna
  - A soma das notas das colunas dá a nota do alinhamento

Coluna de um alinhamento

L

I

V

V

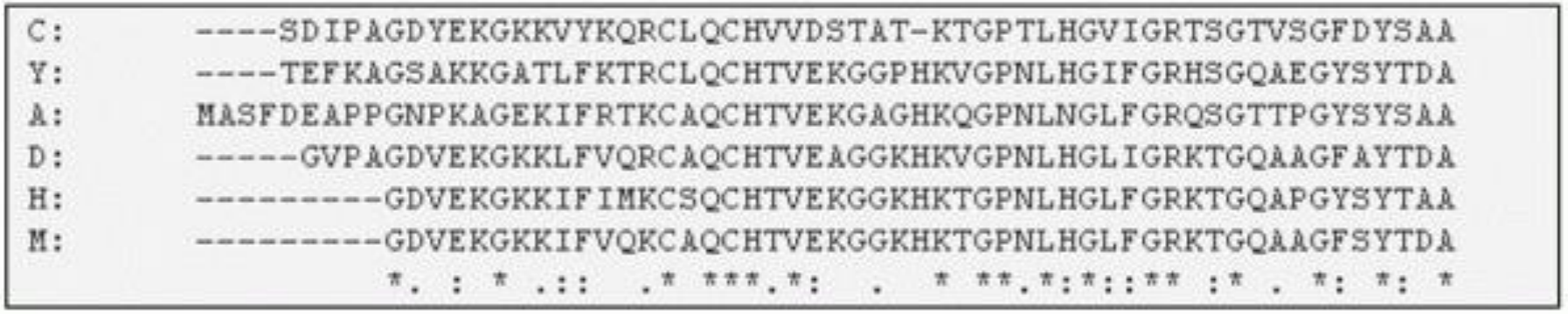
I

BLOSUM62

```
L/L:  4
L/I:  2
L/V:  1
I/I:  4
I/V:  3
V/V:  4
```

	L	I	V	V	I	
L		2	1	1	2	**6**
I			3	3	4	**10**
V				4	3	**7**
V					3	**3**
I						**26**

Nota da coluna



What do the consensus symbols mean in the alignment?

An * (asterisk) indicates positions which have a single, fully conserved residue

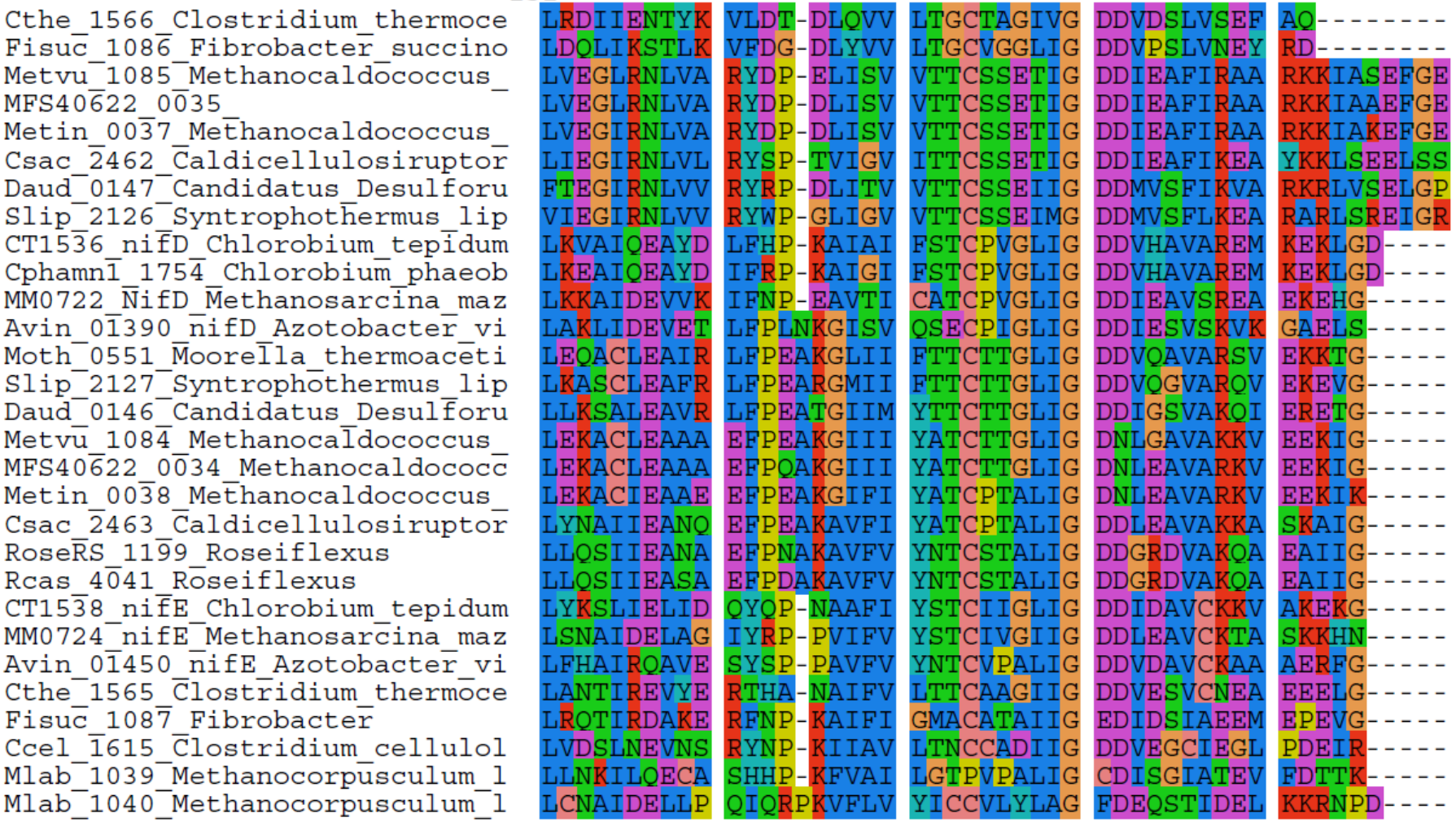
A : (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix

A . (period) indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix

Não existe padrão universalmente aceito para avaliar AMs

- Ou seja, não existe o equivalente de e-values em BLAST
- Diferentes programas produzem diferentes notas

Multiple Sequence Alignment



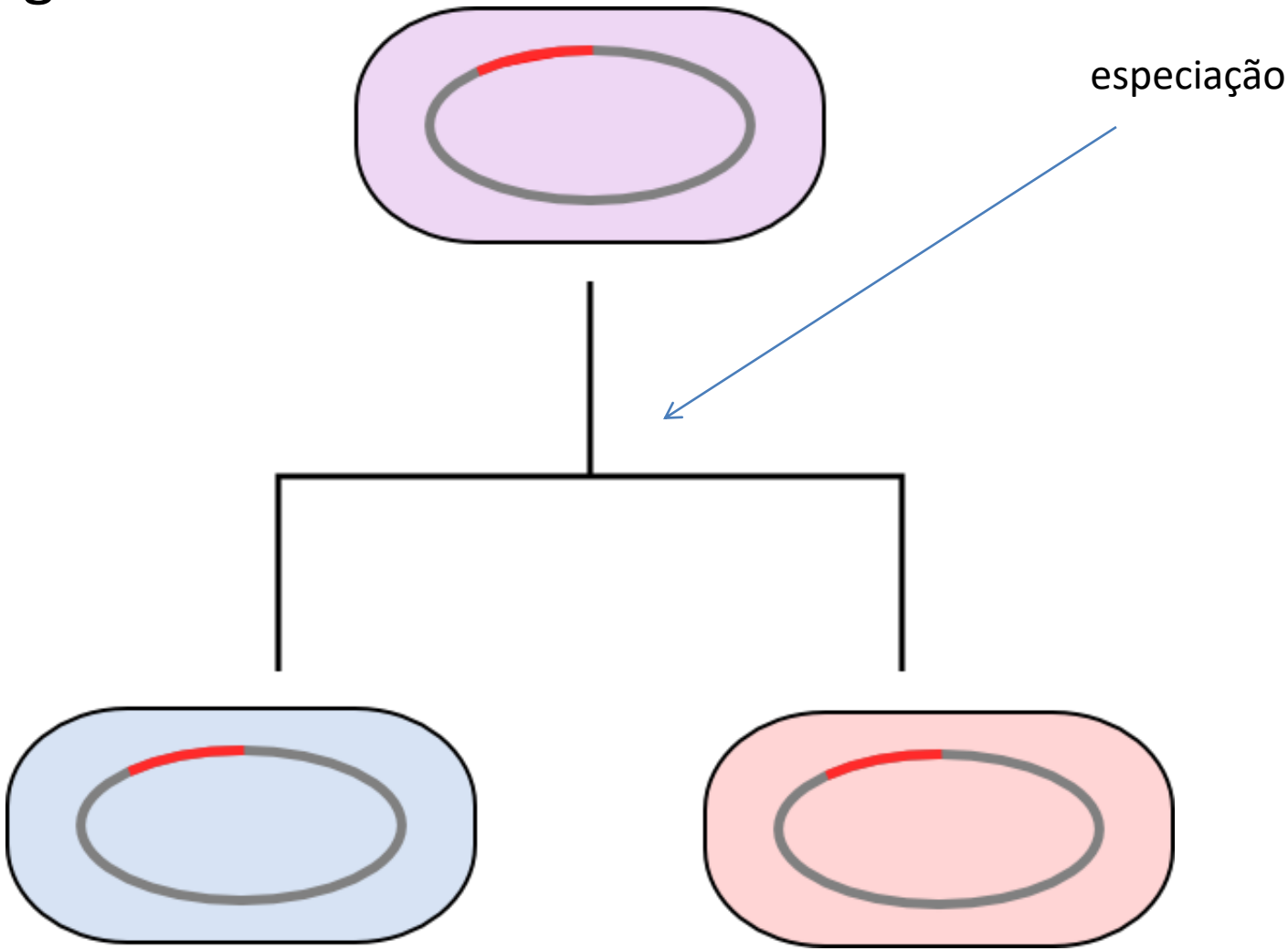
Sequências de entrada

- Dois conceitos importantes
  - Homologia
  - Família

Homologia

- Dois genes que tem um mesmo ancestral são homológos
- Freq. usado erroneamente com o sentido de similar
- Similaridade não implica necessariamente em homologia
  - Asas: morcêgo e insetos (convergência)
- Às vezes a similaridade é (ou parece) baixa mas mesmo assim existe homologia
  - Barbatana de baleia e braços em humanos
- Dois tipos de homologia
  - Ortologia e paralogia

Ortólogos



parálogos

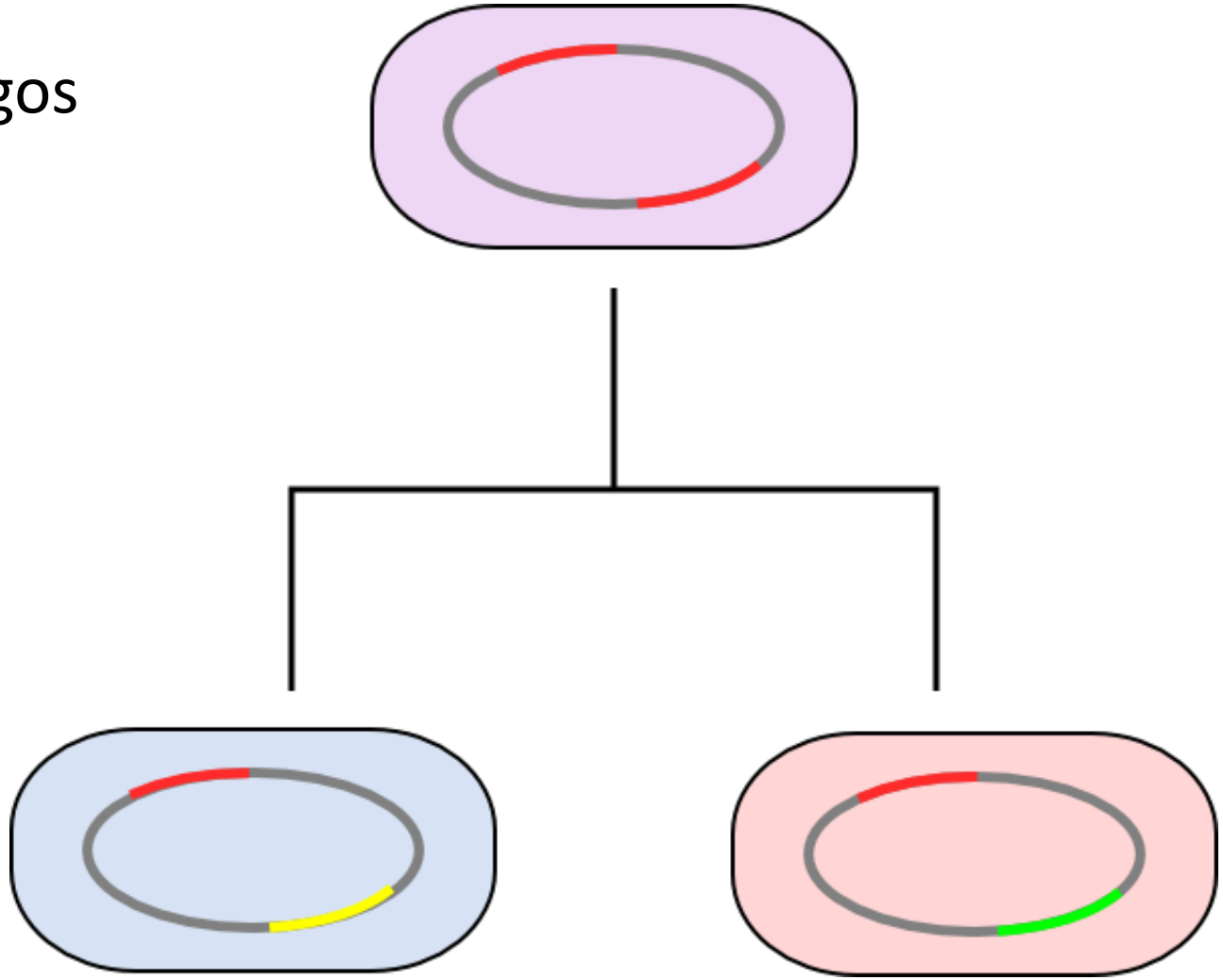


Figure by C. Lasher

In-parálogos

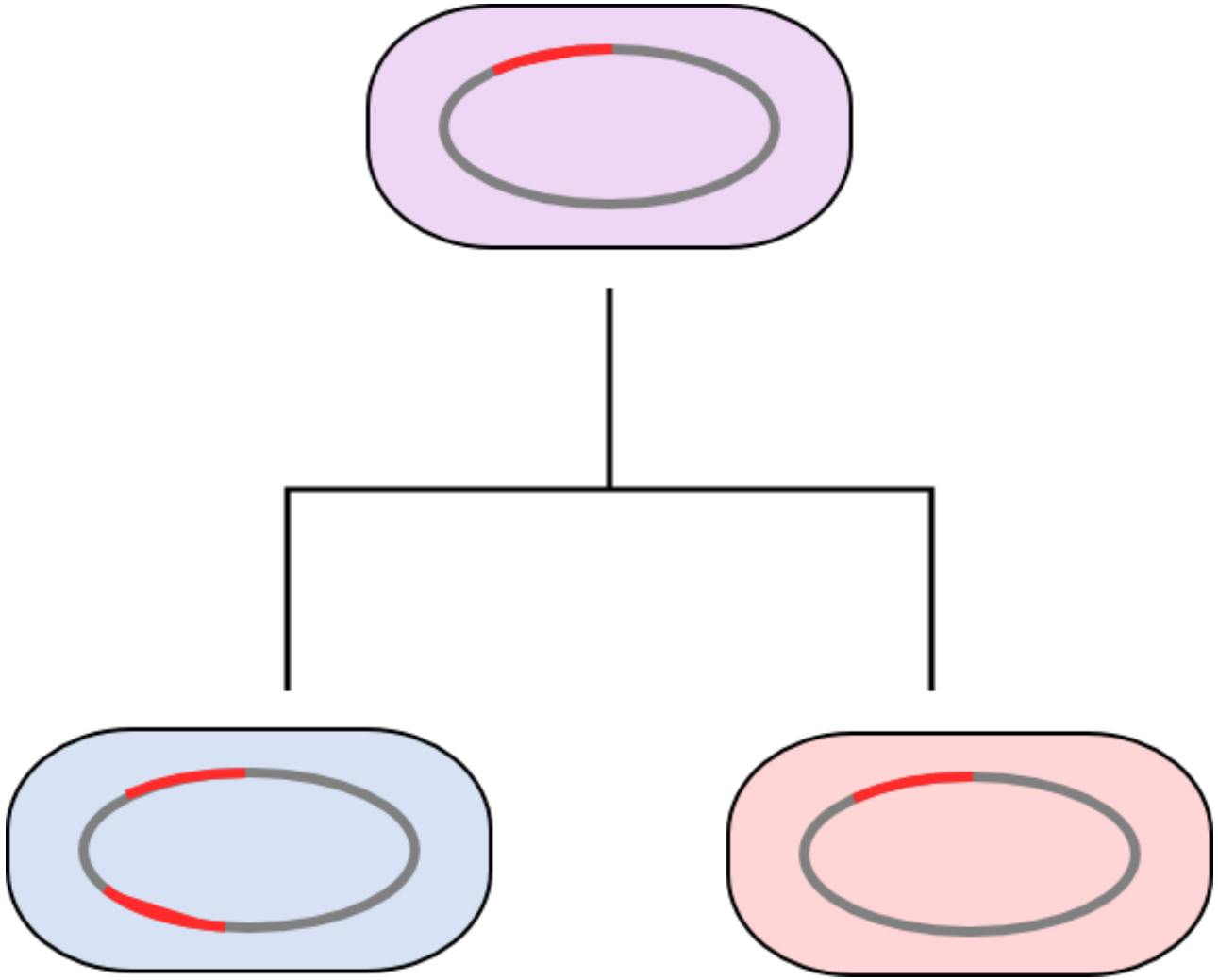


Figure by C. Lasher

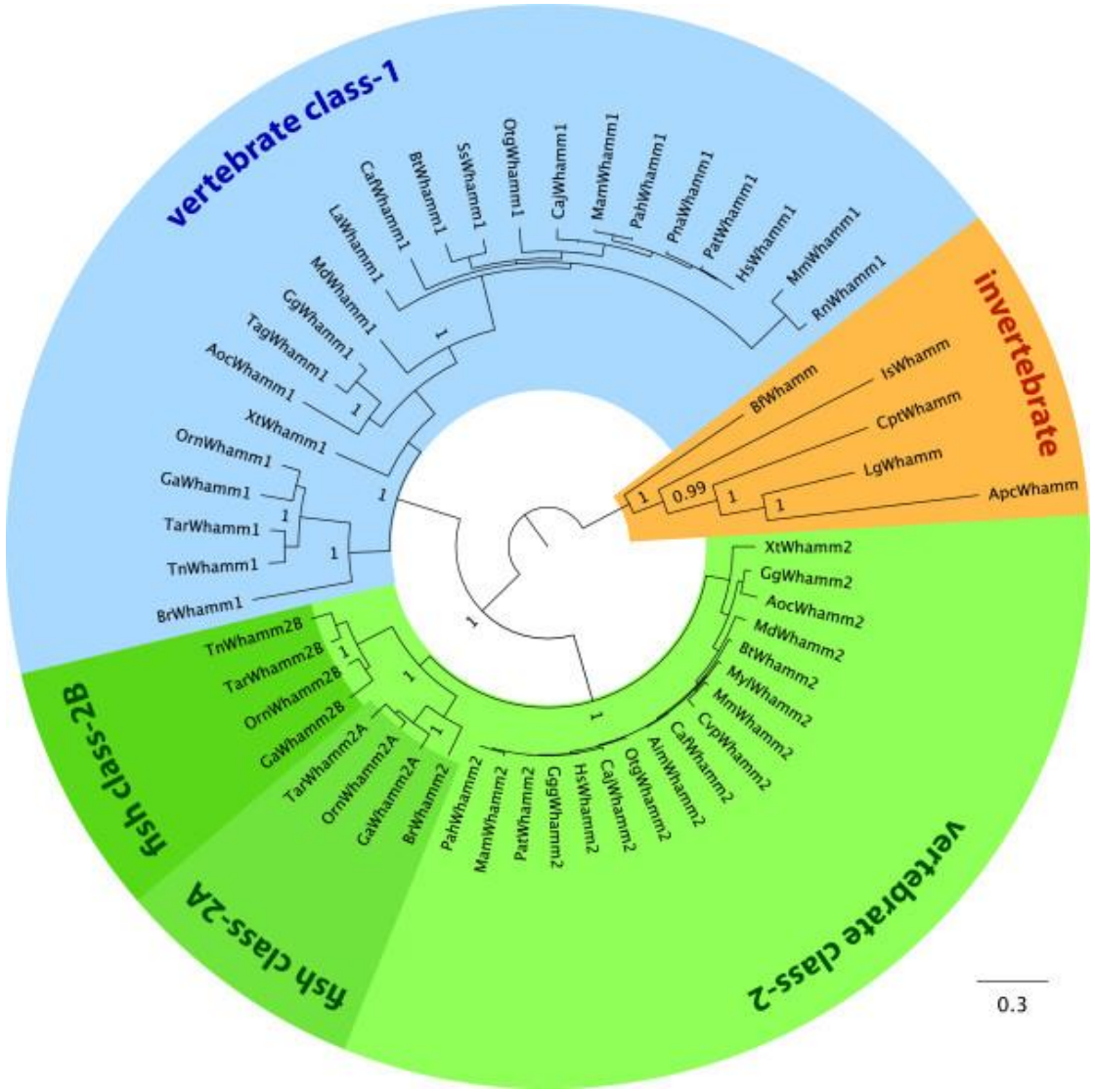
Homologia e função

- Seria bom se proteínas homólogas tivessem mesma função
- Geralmente é o caso; mas nem sempre
- Parálogos estão mais sujeitos a desenvolver novas funções
  - Neo-funcionalização
- Na prática
  - Membros de uma mesma família de proteínas são homólogos e em geral tem mesma função
  - Superfamílias e subfamílias

Família de proteínas

- Definição operacional
  - Duas proteínas estão na mesma família se seus genes são homólogos
- ou (mais exigente)
  - Duas proteínas estão na mesma família se seus genes são ortólogos

- Falar em proteínas homólogas é um certo abuso de linguagem: são os genes que são homólogos



Phylogenetic tree of the WHAMM proteins

Kollmar *et al. BMC Research Notes* 2012 **5**:88 doi:10.1186/1756-0500-5-88

Colunas num AM devem ser homólogas

- O gene ancestral comum das sequências no AM também tinha aquela posição

Alinhar DNA ou aminoácidos?

- DNA: mais difícil garantir homologia nas colunas
- DNA é mais sensível, mas a 3a base de codons não é informativa
- Comparação com aminoácidos permite que proteínas mais distantes possam ser incluídas
  - Há casos em que não dá para alinhar DNA (muita divergência)
- DNA é indicado quando as proteínas são todas idênticas ou quase idênticas
  - Ex: cepas de uma bactéria

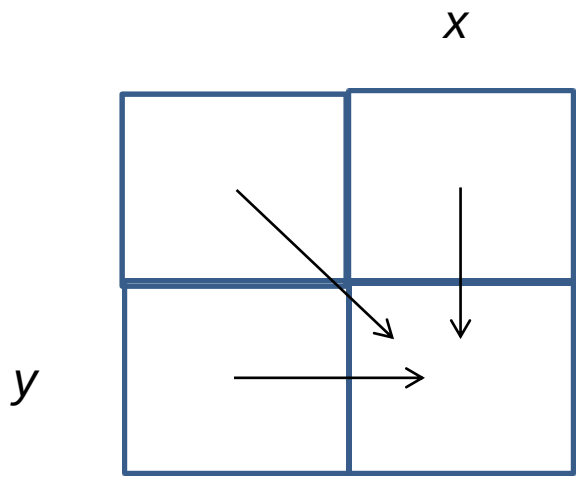
Algoritmo para alinhamento múltiplo de sequências

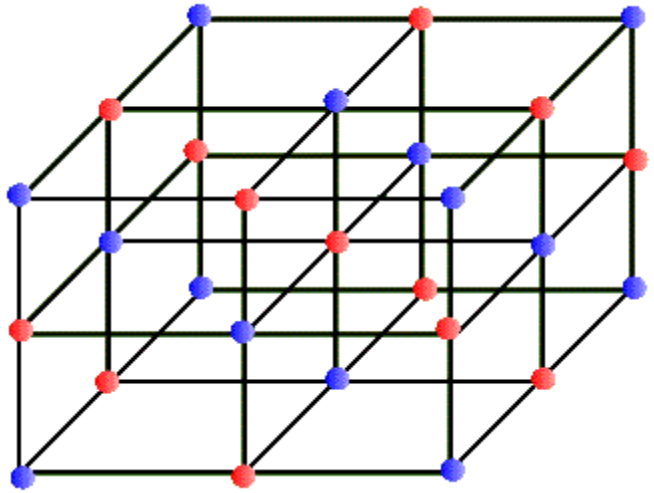
- Programação dinâmica
- Generalização de alinhamento 2-a-2

Generalização de PD para AM

2 sequências

3 sequências





$O(n^2)$

$O(n^3)$

$\Omega(2^k n^k)$

Consequência

- Se PD para alinhamentos 2-a-2 já é caro...
- ...para AM é ainda mais caro!
- Portanto todos os programas práticos para AM são heurísticas
  - Não tem garantia de otimalidade (produzem aproximações)

Mesmo sendo heurísticas esses programas tem limitações

- As sequências de entrada:
  - Não muito longas (até 10 kb?)
  - Não muitas (até 1000?)
  - esses números variam dependendo do programa e do computador

Alinhamento progressivo

- Ideia: combinar alinhamentos de pares, iniciando com o par mais similar entre si
- Ir juntando os pares
- Dois estágios
  1. constrói-se uma árvore-guia que determina a hierarquia de similaridade entre os pares
  2. as sequências são adicionadas ao alinhamento num processo guiado pela árvore
- Seria melhor que AM e árvore fossem feitos simultaneamente
  - Muito mais complicado de fazer com rigor

Programas para AM

- **Muscle**
  - Edgar, R.C. (2004) *Nucleic Acids Res*. **32**(5):1792-1797
  - www.drive5.com/muscle
- **MAFFT**
  - Katoh, Misawa, Kuma, Miyata 2002 (*Nucleic Acids Res.* **30**:3059-3066)
  - mafft.cbrc.jp/alignment/software/
- ClustalW/X (antigos) **Clustal Omega** (novo)
  - Sievers et al. Molecular Systems Biology (2011) 7:539
  - http://www.clustal.org/omega/
  - http://www.ebi.ac.uk/Tools/msa/clustalo/
- Outros: Probcons, Cobalt (NCBI), T-coffee

A Comprehensive Benchmark Study of Multiple Sequence Alignment Methods: Current Challenges and Future Perspectives

Julie D. Thompson, Benjamin Linard, Odile Lecompte, Olivier Poch

Published: March 31, 2011 • DOI: 10.1371/journal.pone.0018093

Abstract

Multiple comparison or alignmentof protein sequences has become a fundamental tool in many different domains in modern molecular biology, from evolutionary studies to prediction of 2D/3D structure, molecular function and inter-molecular interactions etc. By placing the sequence in the framework of the overall family, multiple alignments can be used to identify conserved features and to highlight differences or specificities. In this paper, we describe a comprehensive evaluation of many of the most popular methods for multiple sequence alignment (MSA), based on a new benchmark test set. The benchmark is designed to represent typical problems encountered when aligning the large protein sequence sets that result from today's high throughput biotechnologies. We show that alignmentmethods have significantly progressed and can now identify most of the shared sequence features that determine the broad molecular function(s) of a protein family, even for divergent sequences. However,we have identified a number of important challenges. First, the locally conserved regions, that reflect functional specificities or that modulate a protein's function in a given cellular context,are less well aligned. Second, motifs in natively disordered regions are often misaligned. Third, the badly predicted or fragmentary protein sequences, which make up a large proportion of today's databases, lead to a significant number of alignment errors. Based on this study, we demonstrate that the existing MSA methods can be exploited in combination to improve alignment accuracy, although novel approaches will still be needed to fully explore the most difficult regions. We then propose knowledge-enabled, dynamic solutions that will hopefully pave the way to enhanced alignment construction and exploitation in future evolutionary

Subject Areas

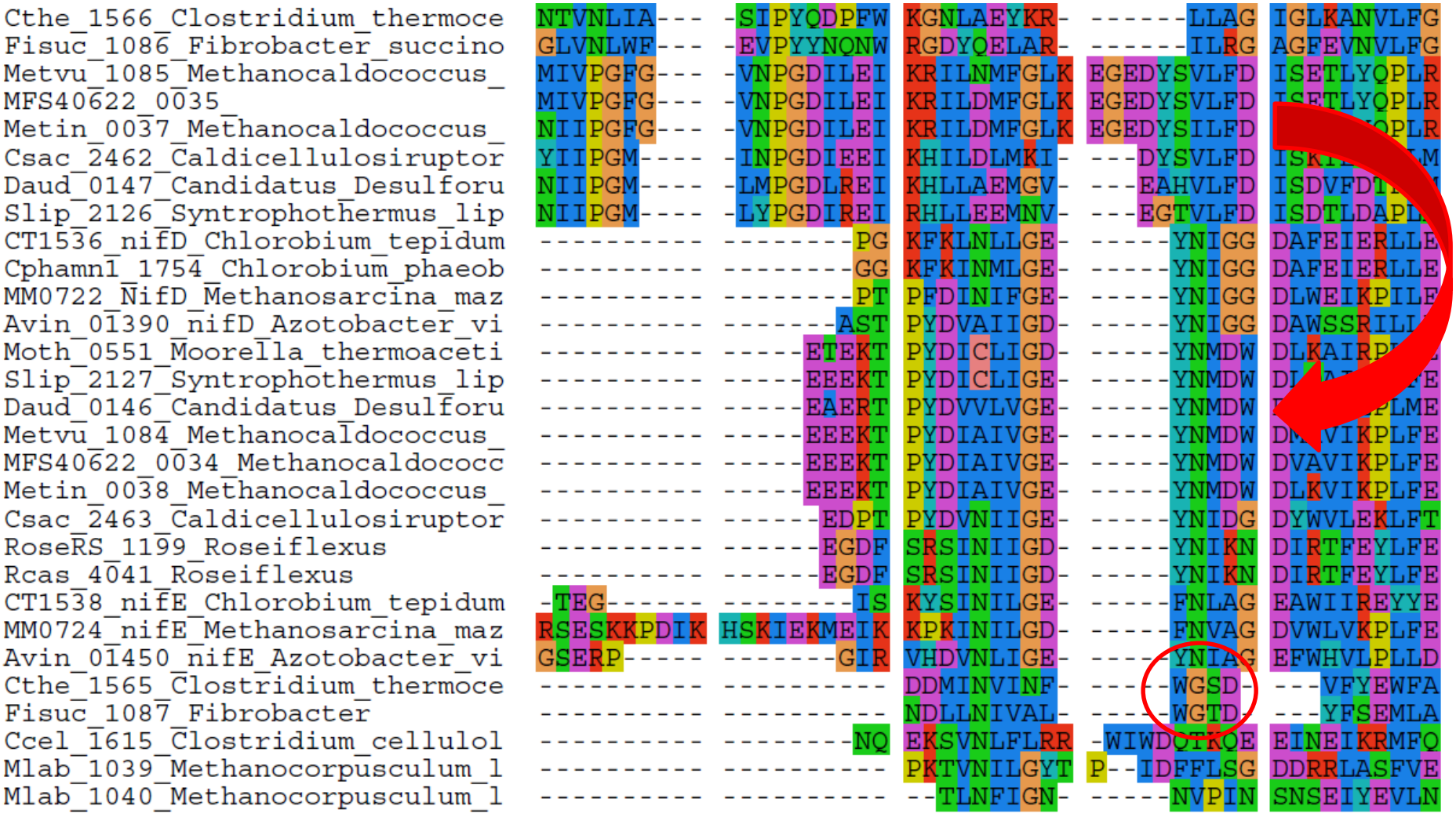
- Computer software
- Database searching
- Genome evolution
- Multiple alignment c...
- Sequence alignment
- Sequence analysis
- Sequence databases
- Sequence motif anal...

Figure 1: An example benchmark alignment.
(A) Reference alignment of representative sequences of the p53/p63/p73 family, with the domain organization shown above
(B) the alignment (AD: activation domain, Oligo: oligomerization, SAM: sterile alpha motif). Colored blocks indicate conserved
(C) regions. The grey regions correspond to sequence segments that could not be reliably aligned and white regions indicate
(D) gaps in the alignment. (B) Different MSA programs produce different alignments, especially in the N-terminal region (boxe
(E) d in red in A) containing rare motifs and a disordered proline-rich domain.

Esquema comparativo de notas

Credit: R. Dixon

Edição de alinhamentos



Edição de alinhamentos

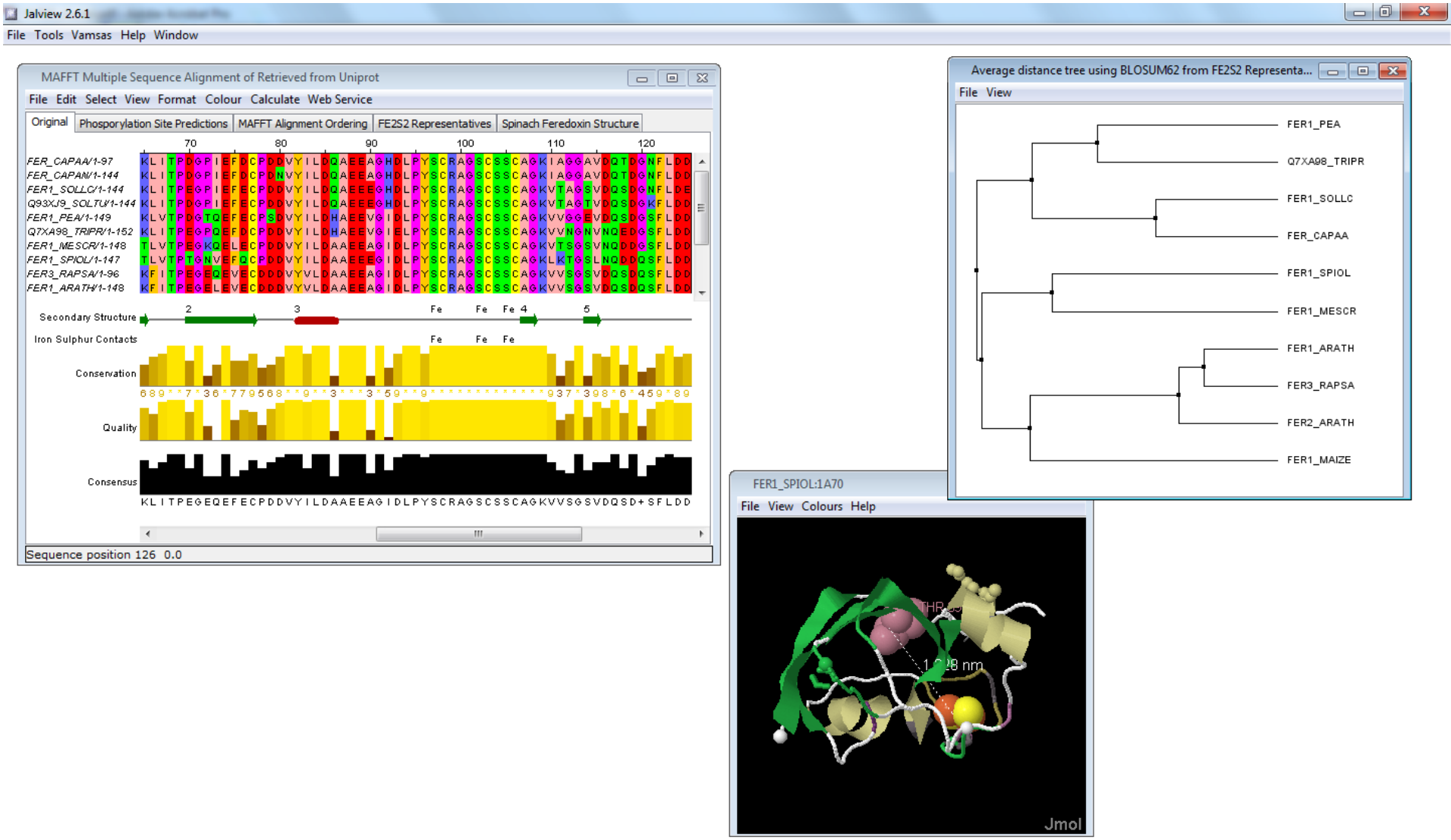
- Algumas colunas podem não ser informativas
- No olho às vezes é possível ver alinhamentos locais melhores
- Edição manual
- Edição automática

Edição manual de AMs

- **Jalview**
  - **www.jalview.org**
  - Waterhouse et al. *Bioinformatics* 2009 **25** (9) 1189-1191
- **Seaview**
  - http://pbil.univ-lyon1.fr/software/seaview.html
    - Gouy M., Guindon S. & Gascuel O. (2010) *Molecular Biology and Evolution* **27(2)**:221-224

JALVIEW

http://www.jalview.org/



SeaView

Version 4.4.2

NEW: seaview drives the **Gblocks** program to select blocks of conserved sites.
NEW: seaview drives the **Clustal Ω** program to perform multiple sequence alignment.

SeaView is a multiplatform, graphical user interface for multiple sequence alignment and molecular phylogeny.

- SeaView reads and writes various file formats (NEXUS, MSF, CLUSTAL, FASTA, PHYLIP, MASE, Newick) of DNA and protein sequences and of phylogenetic trees.
- SeaView drives programs muscle or Clustal Omega for multiple sequence alignment, and also allows to use any external alignment algorithm able to read and write FASTA-formatted files.
- Seaview drives the Gblocks program to select blocks of evolutionarily conserved sites.
- SeaView computes phylogenetic trees by
  - parsimony, using PHYLIP's dnapars/protpars algorithm,
  - distance, with NJ or BioNJ algorithms on a variety of evolutionary distances,
  - maximum likelihood, driving program PhyML 3.0.
- SeaView prints and draws phylogenetic trees on screen, SVG, PDF or PostScript files.
- SeaView allows to download sequences from EMBL/GenBank/UniProt using the Internet.

Screen shots of the main alignment and tree windows. On-line help document. Old seaview version 3.2

Download SeaView

Mac Universal **MacOS X**	**32-bit Linux on x86** **64-bit Linux on x86_64**
MS Windows **self-extractible archive**	**Solaris on SPARC**
source code	

Note for Linux/Unix users: The downloaded archives contain the seaview executable itself, an example data file, a .html file, and 4 other programs (muscle, clustalo, phyml, Gblocks) that seaview drives. These 4 programs and the .html file can either be left in the same directory as seaview, or be put in any directory of your PATH.

Edição automática de AMs

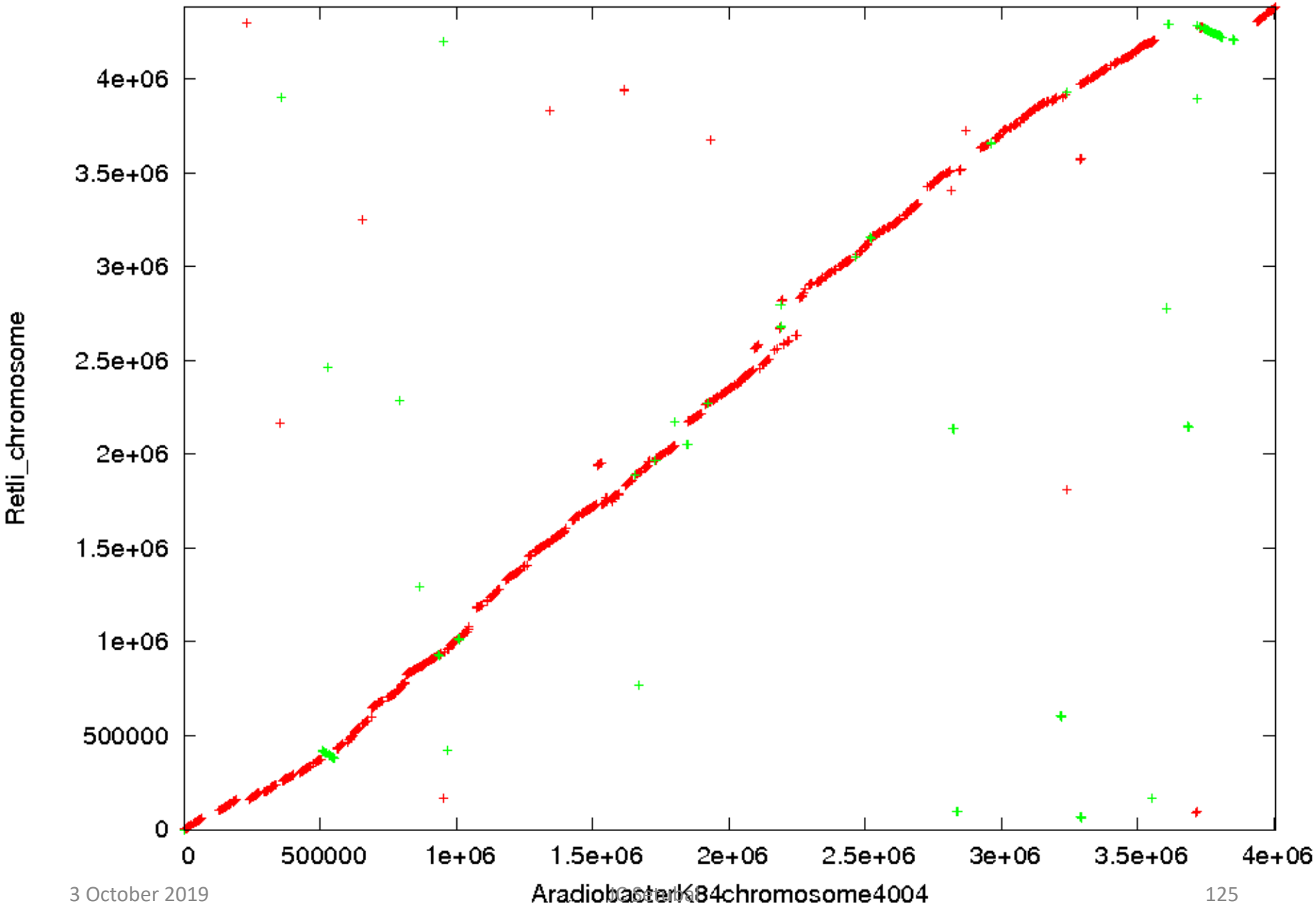
- GBLOCKS
  - http://molevol.cmima.csic.es/castresana/Gblocks_server.html
  - Castresana, J. (**2000**) **Molecular Biology and Evolution** 17, 540-552
- GUIDANCE
  - http://guidance.tau.ac.il/index.html
  - Penn, O., Privman, E., Ashkenazy, H., Landan, G., Graur, D. and Pupko, T. (2010). **GUIDANCE: a web server for assessing alignment confidence scores.** *Nucleic Acids Research, 2010 Jul 1; 38 (Web Server issue):W23-W28*; doi: 10.1093/nar/gkq443

Formatos de saída

- clustal, FASTA, MSF, NEXUS, PHYLIP
- **http://molecularevolution.org/resources/fileformats/converting**

Alinhamento entre sequências longas

- Cromossomos inteiros
- O cromossomo típico de uma bactéria tem 4 Mbp
- Cromossomo de humanos: 300 Mbp
- Cromossomos e plasmídeos: replicons


Retli_chromosome
0
500000
1e+06
1.5e+06
2e+06
2.5e+06
3e+06
3.5e+06
4e+06
Aradiobacter_K84chromosome4004

BLAST não serve

- Computadores mesmo com dezenas de GB de RAM não dão conta de rodar BLAST para essas entradas
- Problema não é tempo; é memória RAM
- Outras abordagens são necessárias

O programa MUMmer

- Delcher AL, Phillippy A, Carlton J, Salzberg SL. Fast algorithms for large-scale genome alignment and comparison. *Nucleic Acids Res.* 2002 Jun 1;30(11):2478-83.
- Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. Versatile and open software for comparing large genomes. *Genome Biol.* 2004;5(2):R12
- http://mummer.sourceforge.net

Como MUMmer funciona

- It finds Maximal Unique Matches
- These are exact matches above a user-specified threshold that are unique
- Exact matches found are clustered and extended (using dynamic programming)
  - Result is approximate matches
- Data structure for exact match finding: suffix tree
  - Difficult to build but very fast
- Nucmer and promer
  - Both very fast
  - O(*n* + #MUMs), *n* = genome lengths

Árvore de sufixos para GTATCTAGG

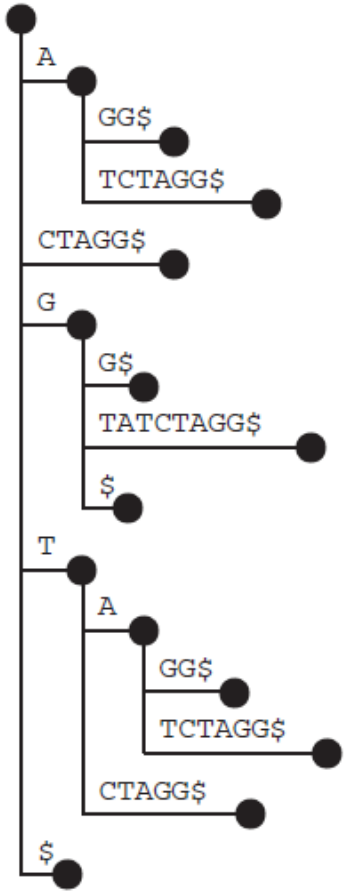


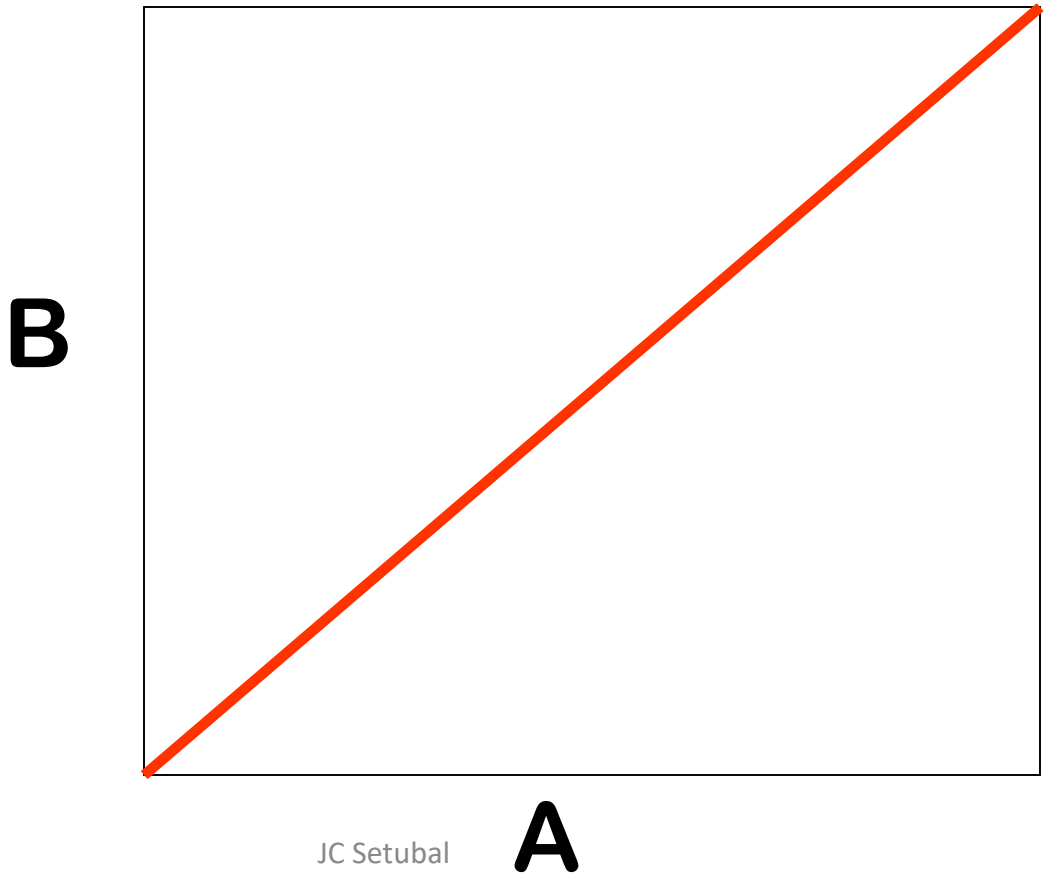
FIGURE 3.19

An example of a suffix tree for string GTATCTAGG. *A dollar sign marks the end of the string.*

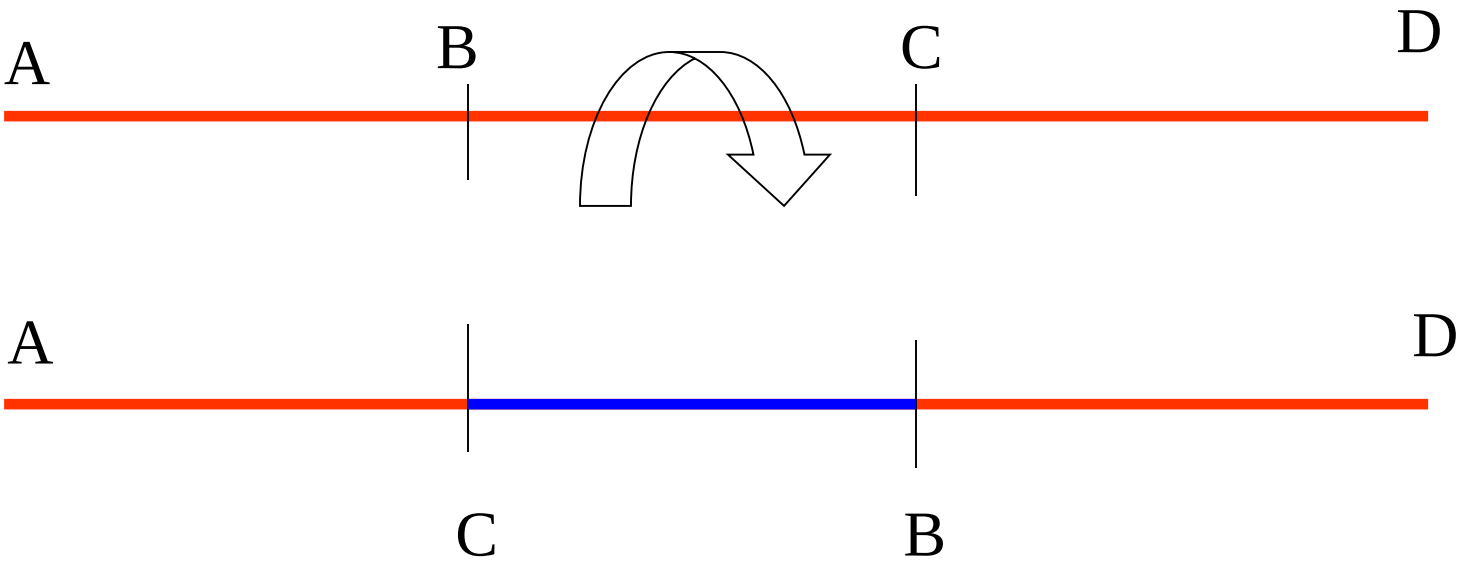
- Alinhamentos de replicons inteiros revelam rearranjos

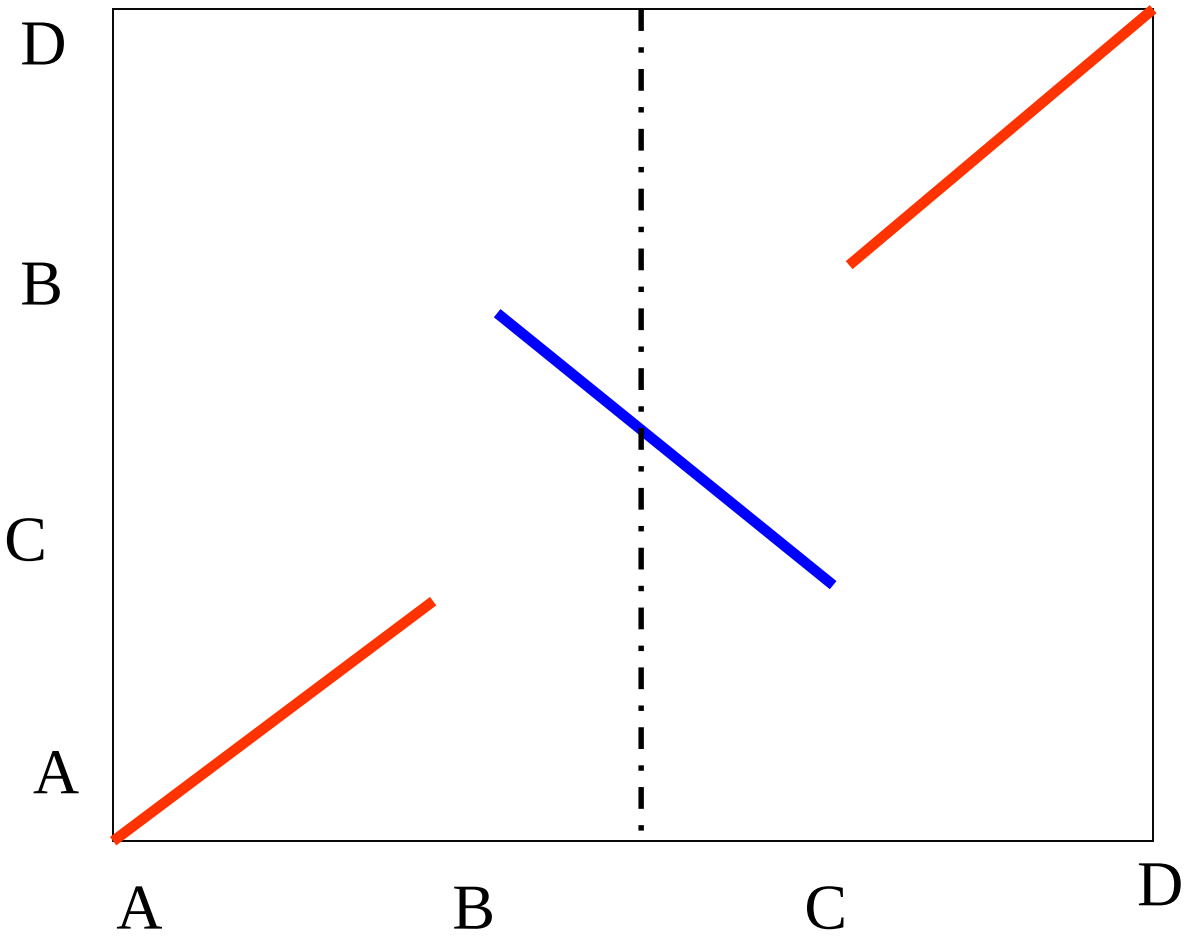
Alinhamentos de pares de replicons completos

Se as sequências fossem idênticas veríamos:

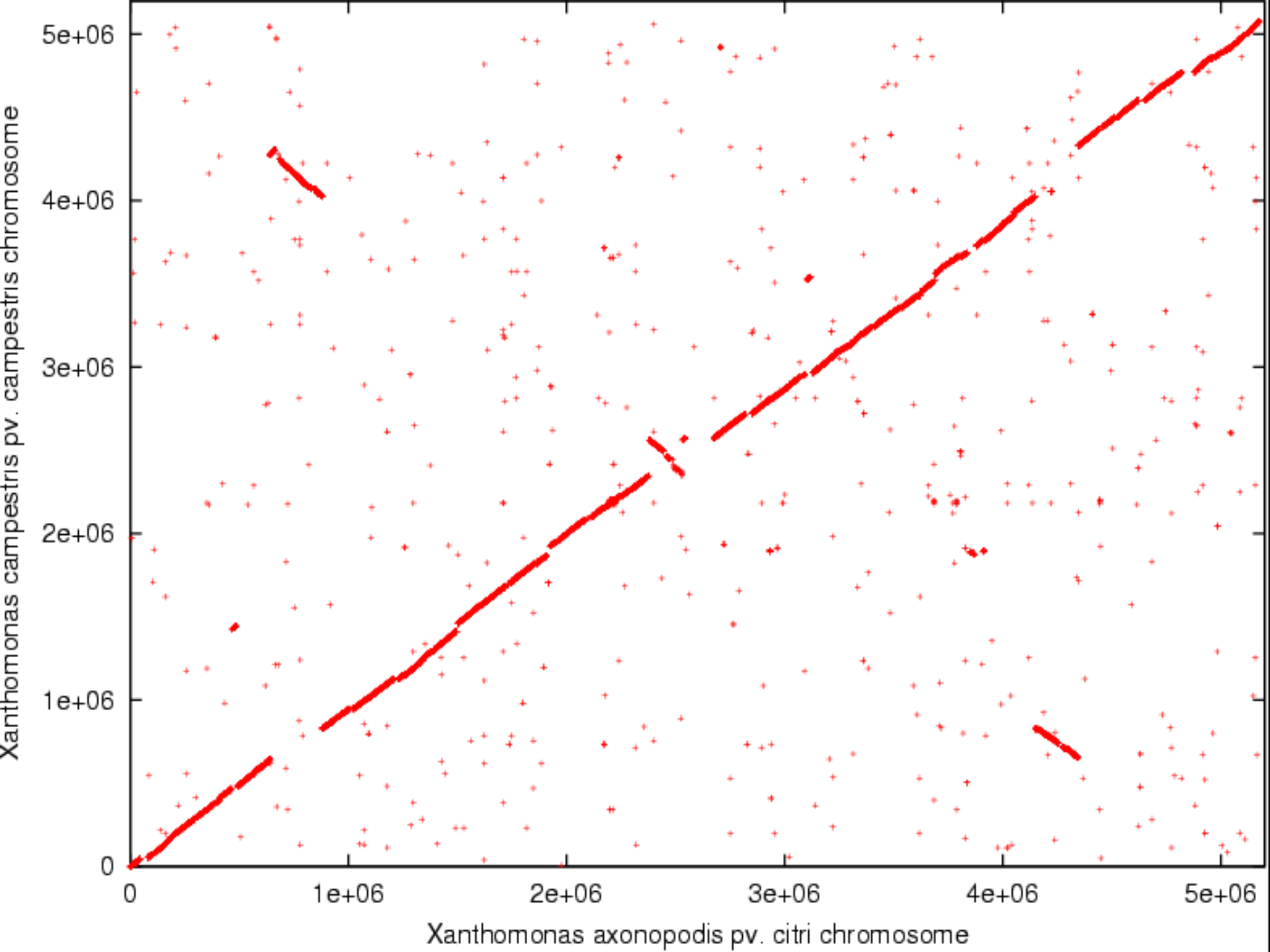


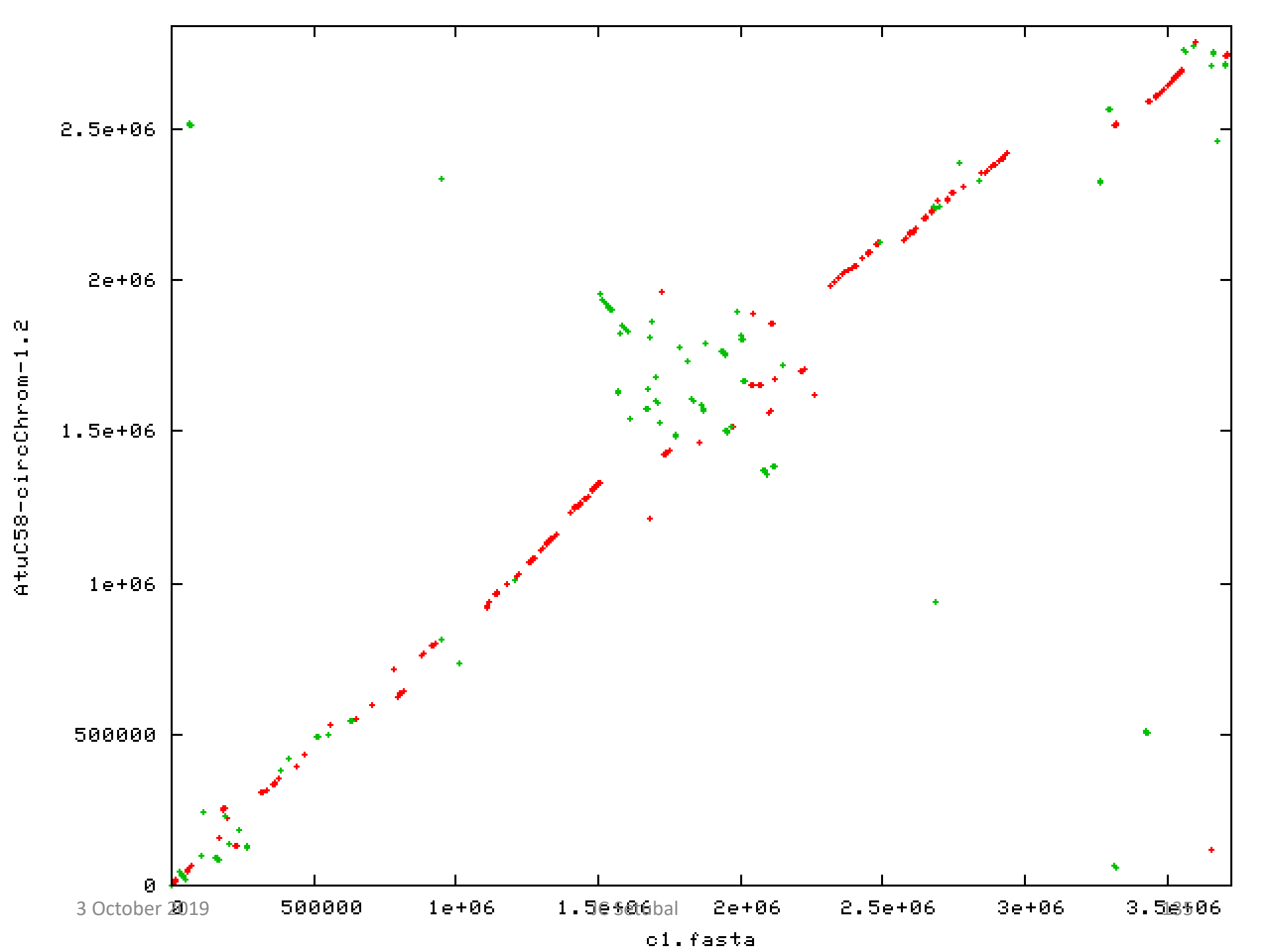
uma *inversão*

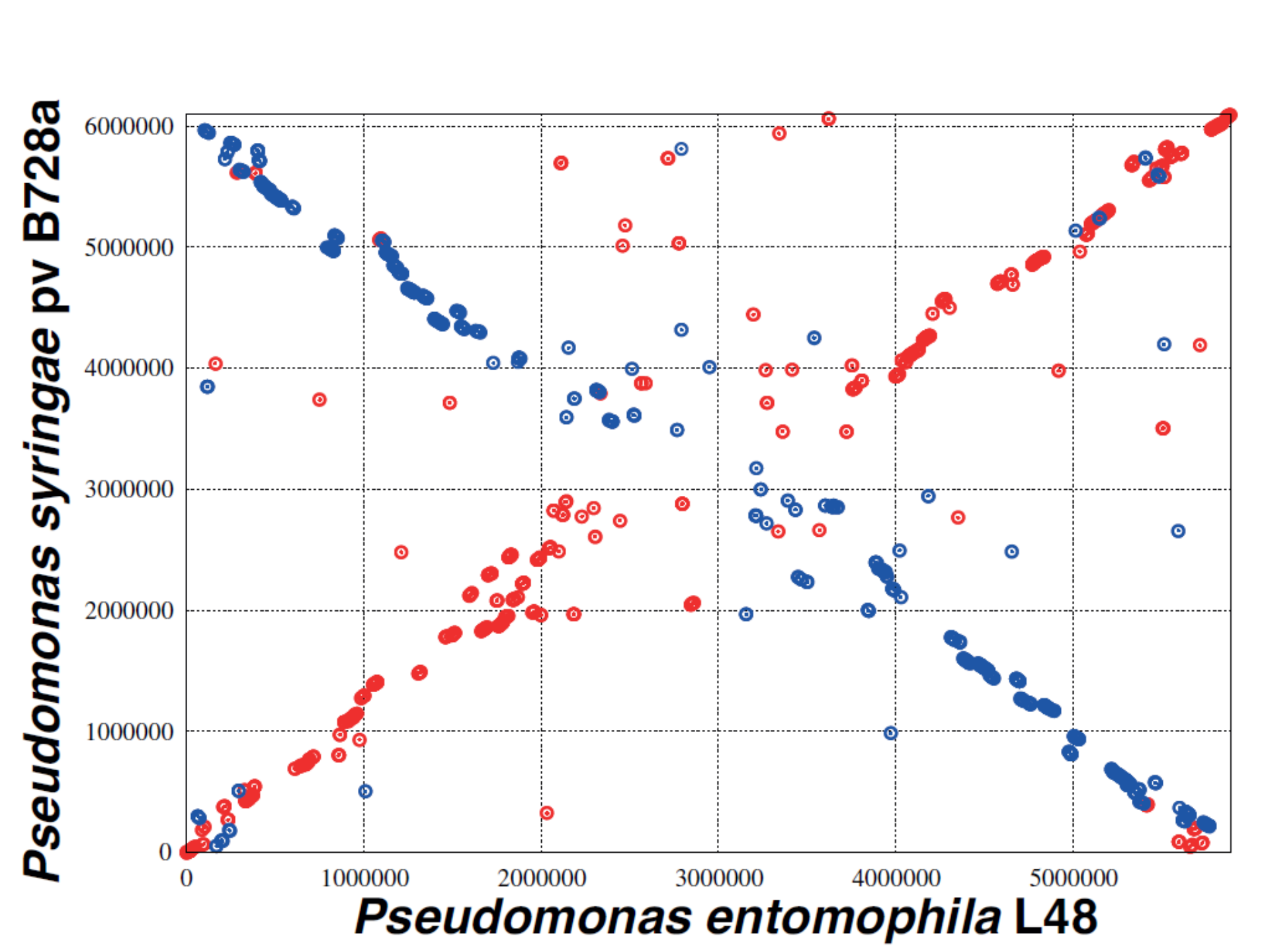




Such inversions seem to happen around the *origin* or *terminus of replication*

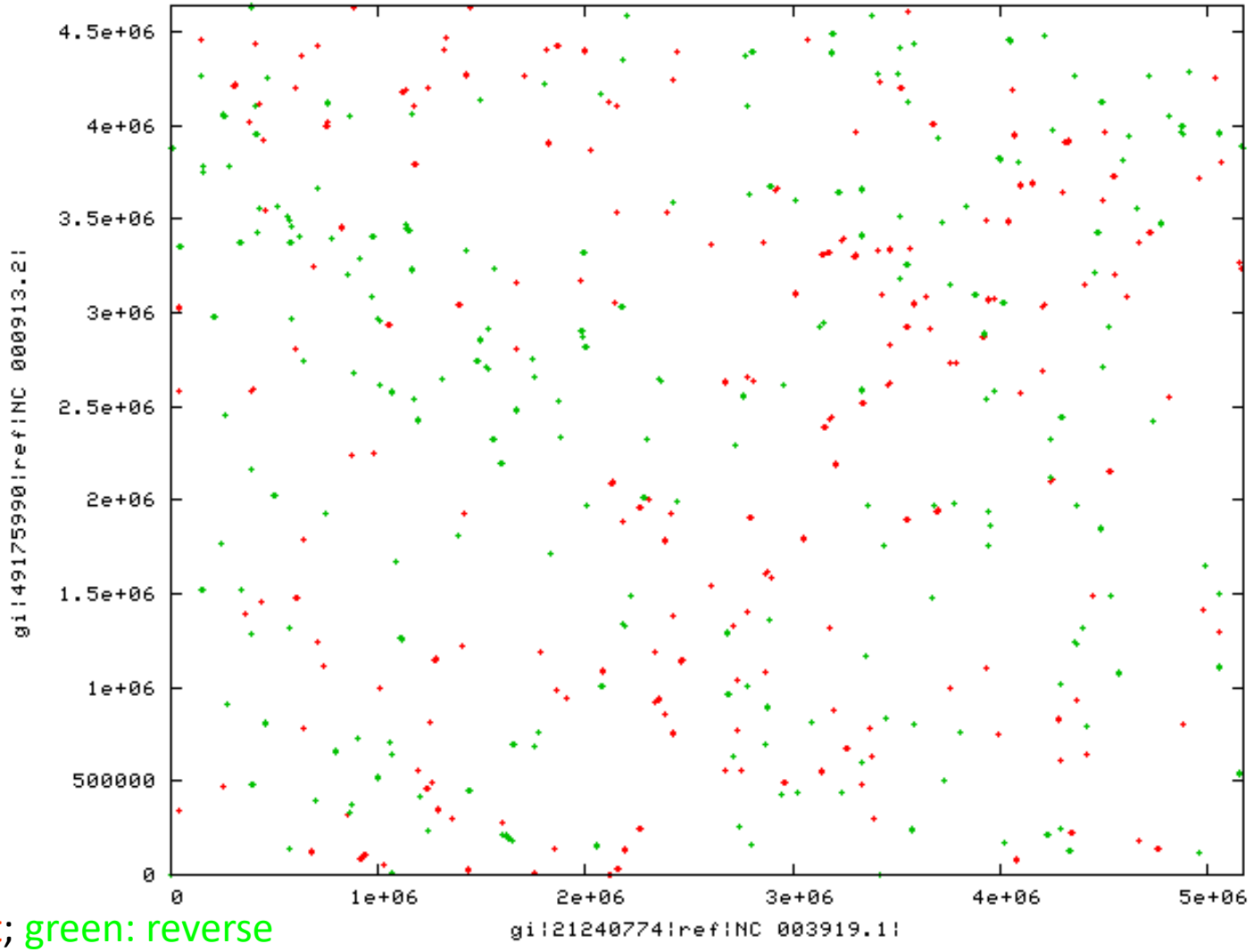

Xanthomonas campestris pv. campestris chromosome
5e+06
4e+06
3e+06
2e+06
1e+06
0
0
1e+06
2e+06
3e+06
4e+06
5e+06
Xanthomonas axonopodis pv. citri chromosome


AtuC58-circChrom-1.2
c1.fasta
0
500000
1e+06
1.5e+06
2e+06
2.5e+06
3e+06
3.5e+06


Pseudomonas syringae pv B728a
6000000
5000000
4000000
3000000
2000000
1000000
0
0
1000000
2000000
3000000
4000000
5000000
Pseudomonas entomophila L48

E. coli K12

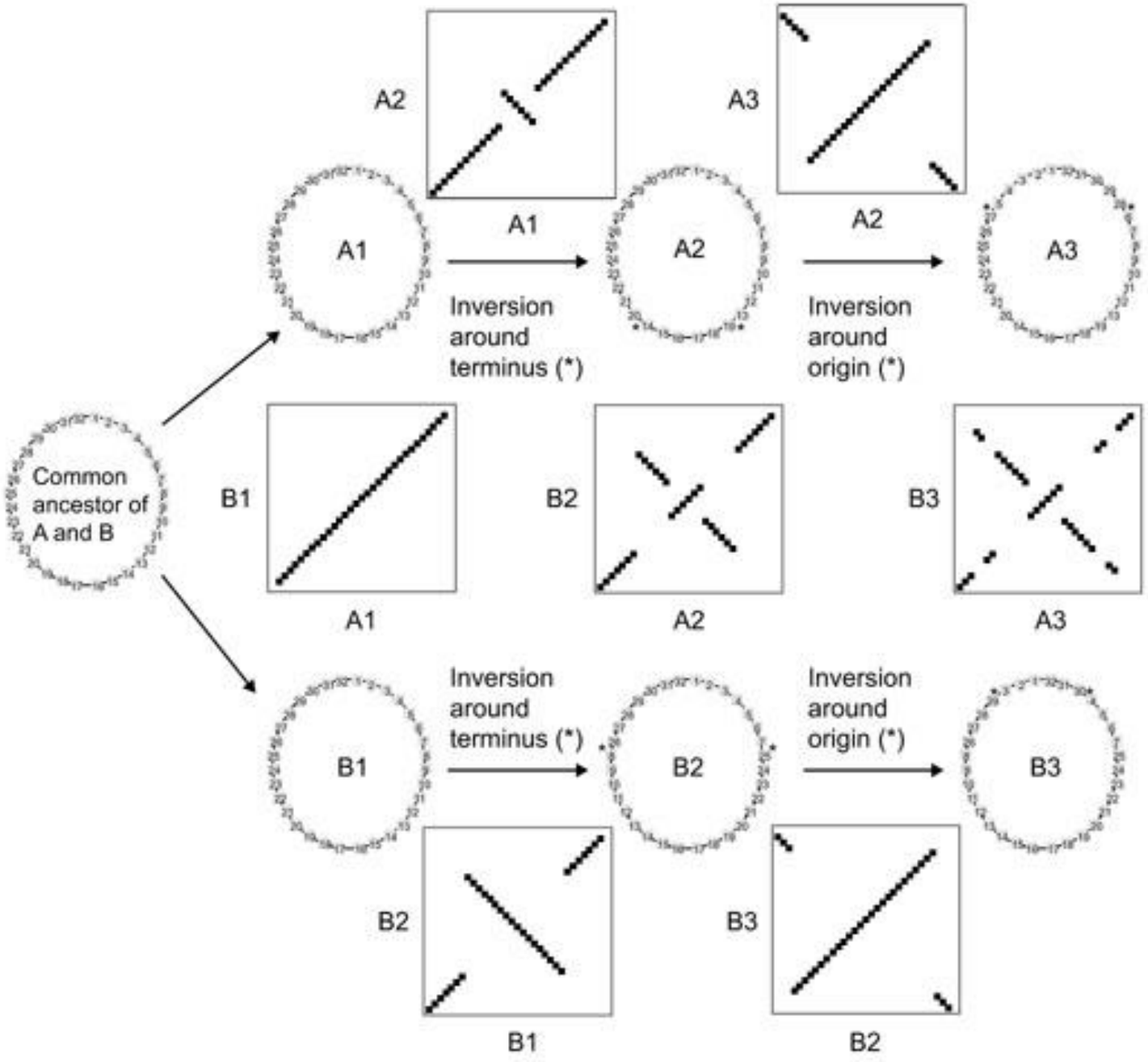
Promer alignment



Red: direct; green: reverse

Both are γ proteobacteria!

Xanthomonas axonopodis* pv *citri



Eisen JA, Heidelberg JF, White O, Salzberg SL. Evidence for symmetric chromosomal inversions around the replication origin in bacteria. *Genome Biol.* 2000;1(6):RESEARCH0011

Alinhamento múltiplo de sequências longas

- O programa MAUVE
- Darling AC, Mau B, Blattner FR, Perna NT. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res. 2004 Jul;14(7):1394-403.

How MAUVE works

- Seed-and-extend hashing
- Seeds/anchors: Maximal Multiple Unique Matches of minimum length *k*
- Result: Local collinear blocks (LCBs)
- O($G^2n + Gn \log Gn$), G = # genomes, *n* = average genome length

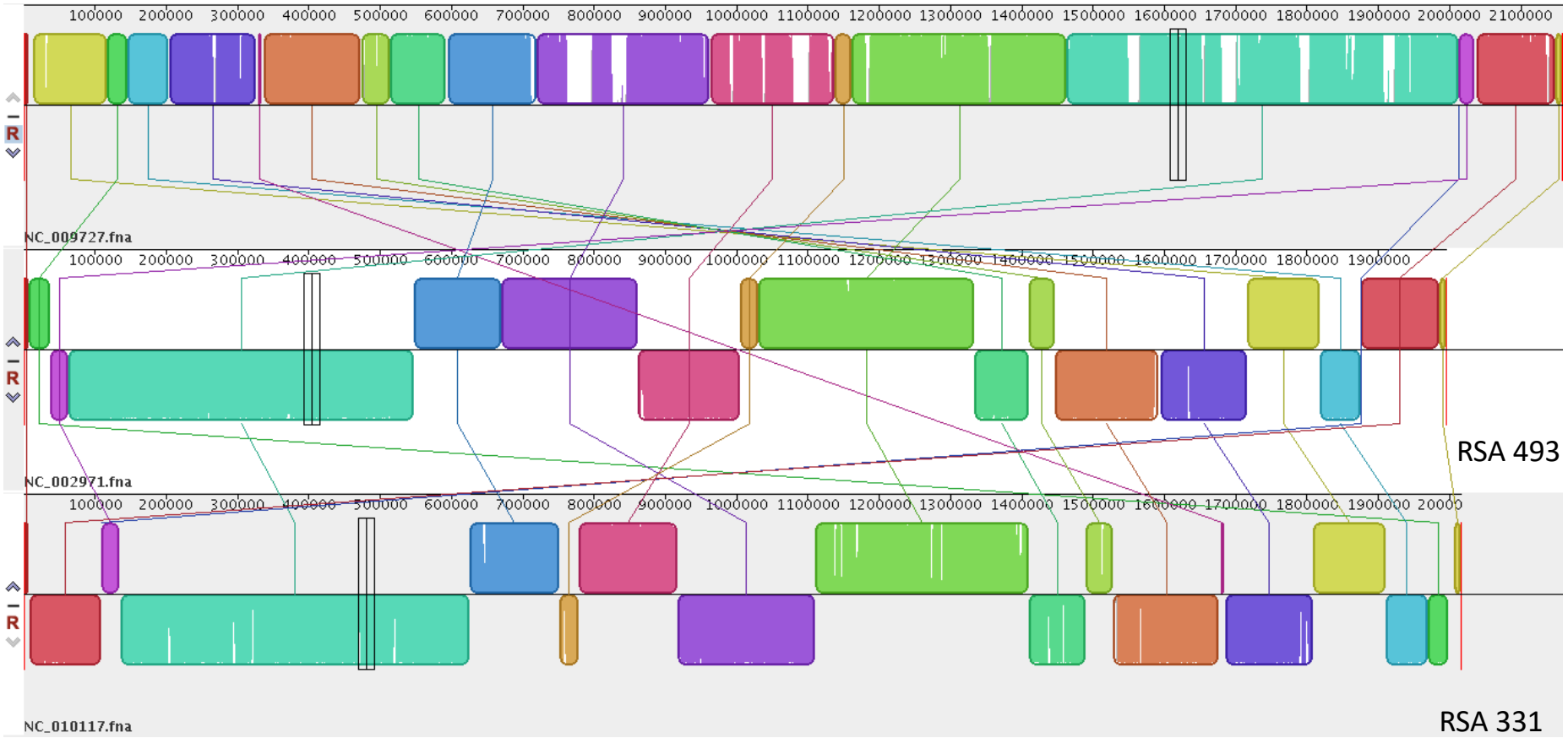
Alignment algorithm

1. Find Multi-MUMs
2. Use the multi-MUMs to calculate a phylogenetic guide tree
3. Find LCBs (subset of multi-MUMs; filter out spurious matches; requires *minimum weight*)
4. Recursive anchoring to identify additional anchors (extension of LCBs)
5. Progressive alignment (CLUSTALW) using guide tree

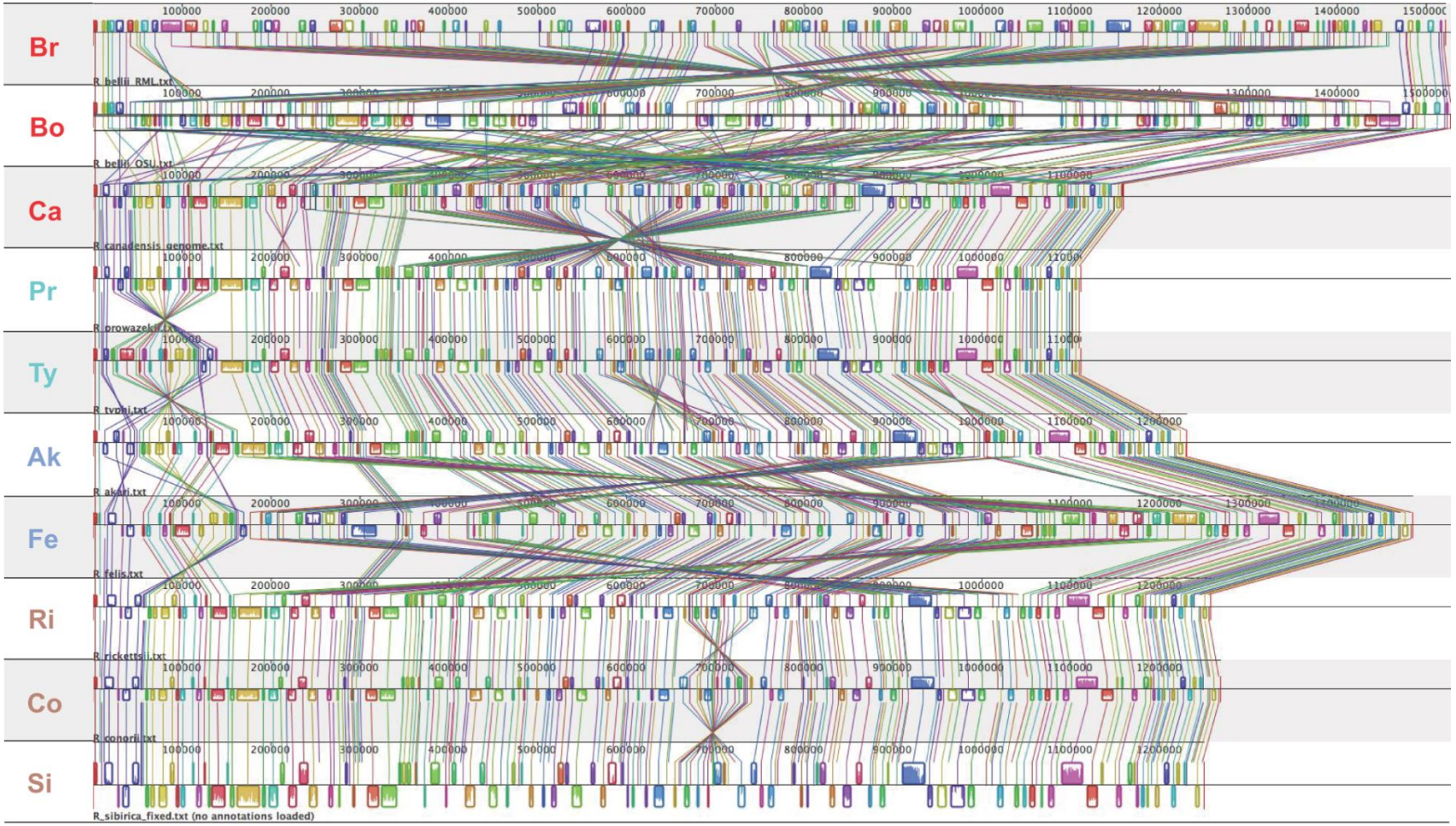
Brucella: Main chromosome alignment

Brucella: Chromosome 2 alignment

Coxiella: Chromosome alignment



Rickettsia


Br
Bo
Ca
Pr
Ty
Ak
Fe
Ri
Co
Si
R_sibirica_fixed.txt (no annotations loaded)

Sumário de comparação de sequências

	Sequências curtas	**Sequências longas**
2-a-2	Prog. Dinâmica	Mummer
2-a-2 muitas vezes	BLAST, usearch, diamond	Mummer
múltiplo	Muscle, MAFFT	Mauve, MUGSY

Distância genômica

- Ao comparar genomas, muitas vezes é útil poder expressar essa comparação por meio de um único número
  - Quando se comparam pares de replicons
    - Que podem ser “replicons” concatenados
- Distância pode ser entendida como o inverso da similaridade

Distância e similaridade

- São conceitos muito parecidos
- Em particular *distância de edição*
- Como transformar sequência *s* em sequência *t*
- Operações
  - Substituição do caracter *a* por *b* (custo = 1)
  - Inserção ou Remoção de um caracter (custo = 2)
- O algoritmo de PD já visto resolve esse problema

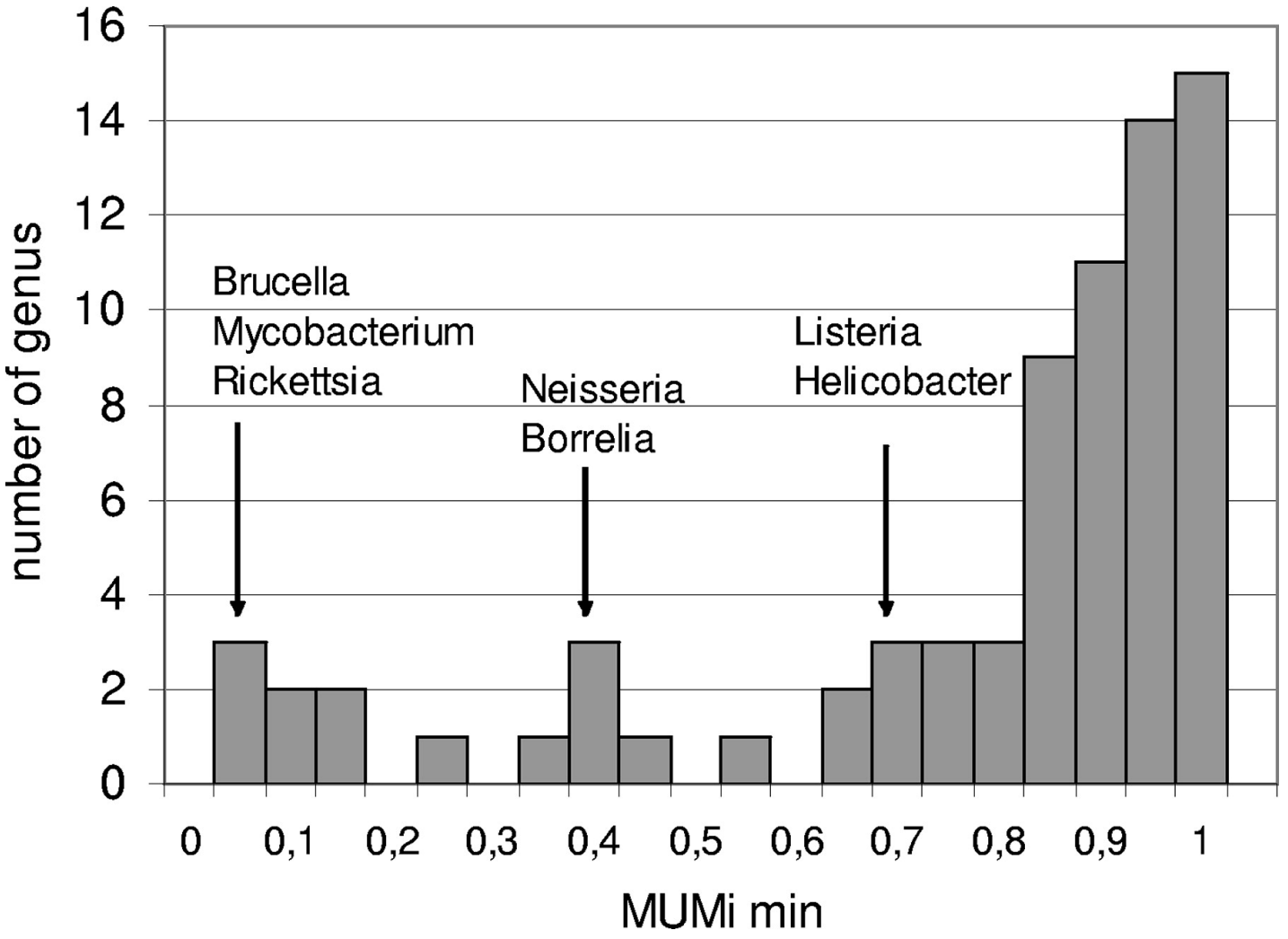
Uma fórmula de distância genômica

- MUMi = MUM index
- Baseado em MUMmer
- Deloger et al. 2009
- MUMi = $1 - L_{mum}/L_{av}$
- L_{mum} = soma dos comprimentos de todos os MUMs que não tem sobreposição
- L_{av} = comprimento médio dos 2 genomas sendo comparados
- Para obter MUMi, basta rodar MUMmer com um script perl desses autores

How MUMi works

- Identical sequences: zero
- Totally different sequences: 1
- The boundary between **genus** and **species** is around 0.8

Distribution of all minimal MUMi values per genus.



Marc Deloger et al. J. Bacteriol. 2009;191:91-99

Journal of Bacteriology

Conclusão

- Não dá para comparar distâncias MUMi entre diferentes gêneros

Uma matriz de distâncias genômicas em Brucella

Strain	MUMi value[b]					
	83–13	BO2	NF 2653	BO1	*B. suis*	*B. microti*
B. neotomae 5K33	0.145	0.168	0.146	0.168	0.017	0.022
Brucella sp. strain 83–13		0.172	0.009	0.175	0.147	0.150
Brucella sp. strain BO2			0.168	0.107	0.169	0.169
Brucella sp. strain NF 2653				0.172	0.147	0.146
B. inopinata BO1					0.169	0.167
B. suis biovar 3 686						0.020

↵*a* Only the sequence of *B. microti* was complete, and its two chromosome sequences were concatenated. Concatenation was done by inserting a string with 100 nucleotides between contigs.

↵*b* The minimum MUMi value is 0, and the maximum MUMi value is 1.

Valores MUMi em Xanthomonas

XacA628	0.4017	0.0176	0.0172	0.0090	0.0193		0.0084	0.0083	0.0069	0.0606	0.0523	0.0558	0.0536	0.0536	0.0535	0.0536	0.0482	0.0538	0.0169	0.0169	0.0149	0.015(
XacA636	0.4030	0.0177	0.0182	0.0088	0.0238	0.0084		0.0085	0.0116	0.0624	0.0550	0.0584	0.0560	0.0560	0.0560	0.0560	0.0511	0.0561	0.0179	0.0180	0.0169	0.016
XacA654	0.4033	0.0131	0.0136	0.0036	0.0251	0.0083	0.0085		0.0119	0.0625	0.0557	0.0592	0.0568	0.0568	0.0568	0.0568	0.0512	0.0569	0.0133	0.0133	0.0180	0.018
XacA828	0.4040	0.0208	0.0211	0.0123	0.0178	0.0069	0.0116	0.0119		0.0646	0.0558	0.0598	0.0565	0.0565	0.0565	0.0565	0.0518	0.0566	0.0210	0.0210	0.0181	0.018
XacAS270	0.4073	0.0656	0.0655	0.0629	0.0707	0.0606	0.0624	0.0625	0.0646		0.0131	0.0067	0.0603	0.0600	0.0603	0.0600	0.0352	0.0601	0.0654	0.0654	0.0639	0.063
XacAS8	0.4031	0.0590	0.0588	0.0558	0.0633	0.0523	0.0550	0.0557	0.0558	0.0131		0.0085	0.0541	0.0538	0.0541	0.0538	0.0291	0.0539	0.0588	0.0588	0.0561	0.056
XacAS9	0.4045	0.0621	0.0619	0.0593	0.0661	0.0558	0.0584	0.0592	0.0598	0.0067	0.0085		0.0558	0.0555	0.0558	0.0555	0.0304	0.0556	0.0619	0.0619	0.0578	0.057
XacAW13	0.4074	0.0584	0.0581	0.0567	0.0637	0.0536	0.0560	0.0568	0.0565	0.0603	0.0541	0.0558		0.0001	0.0001	0.0001	0.0611	0.0004	0.0578	0.0578	0.0554	0.055
XacAW14	0.4074	0.0581	0.0578	0.0568	0.0633	0.0536	0.0560	0.0568	0.0565	0.0600	0.0538	0.0555	0.0001		0.0001	0.0001	0.0611	0.0004	0.0575	0.0575	0.0551	0.055
XacAW15	0.4074	0.0584	0.0581	0.0567	0.0637	0.0535	0.0560	0.0568	0.0565	0.0603	0.0541	0.0558	0.0001	0.0001		0.0001	0.0610	0.0004	0.0578	0.0578	0.0554	0.055
XacAW16	0.4074	0.0581	0.0578	0.0568	0.0633	0.0536	0.0560	0.0568	0.0565	0.0600	0.0538	0.0555	0.0001	0.0001	0.0001		0.0611	0.0004	0.0575	0.0575	0.0551	0.055
XacAs1682	0.4007	0.0599	0.0597	0.0518	0.0643	0.0482	0.0511	0.0512	0.0518	0.0352	0.0291	0.0304	0.0611	0.0611	0.0610	0.0611		0.0613	0.0594	0.0594	0.0574	0.057
XacAw12879	0.4074	0.0583	0.0579	0.0568	0.0636	0.0538	0.0561	0.0569	0.0566	0.0601	0.0539	0.0556	0.0004	0.0004	0.0004	0.0004	0.0613		0.0576	0.0577	0.0553	0.055
XacBL18	0.4094	0.0007	0.0003	0.0125	0.0222	0.0169	0.0179	0.0133	0.0210	0.0654	0.0588	0.0619	0.0578	0.0575	0.0578	0.0575	0.0594	0.0576		0.0000	0.0063	0.006
XacFB19	0.4094	0.0007	0.0003	0.0125	0.0222	0.0169	0.0180	0.0133	0.0210	0.0654	0.0588	0.0619	0.0578	0.0575	0.0578	0.0575	0.0594	0.0577	0.0000		0.0063	0.006
XacGD2	0.4077	0.0057	0.0063	0.0171	0.0194	0.0149	0.0169	0.0180	0.0181	0.0639	0.0561	0.0578	0.0554	0.0551	0.0554	0.0551	0.0574	0.0553	0.0063	0.0063		0.000
XacGD3	0.4077	0.0058	0.0063	0.0171	0.0194	0.0150	0.0169	0.0181	0.0182	0.0639	0.0561	0.0579	0.0557	0.0553	0.0557	0.0553	0.0575	0.0555	0.0064	0.0064	0.0005	
XacJX4	0.4077	0.0065	0.0070	0.0168	0.0196	0.0146	0.0173	0.0177	0.0178	0.0641	0.0563	0.0579	0.0557	0.0554	0.0557	0.0554	0.0572	0.0556	0.0068	0.0071	0.0024	0.002
XacJX5	0.4077	0.0065	0.0070	0.0167	0.0196	0.0146	0.0172	0.0177	0.0179	0.0640	0.0561	0.0578	0.0556	0.0553	0.0556	0.0553	0.0571	0.0555	0.0071	0.0071	0.0024	0.002
XacLMG941	0.4125	0.1430	0.1429	0.1354	0.1467	0.1324	0.1345	0.1348	0.1348	0.1380	0.1364	0.1365	0.1535	0.1535	0.1535	0.1535	0.1309	0.1537	0.1427	0.1427	0.1388	0.138
XacMF20	0.4094	0.0007	0.0003	0.0125	0.0222	0.0170	0.0180	0.0133	0.0210	0.0652	0.0587	0.0617	0.0578	0.0575	0.0578	0.0575	0.0595	0.0577	0.0000	0.0000	0.0063	0.006
XacMN10	0.4076	0.0056	0.0062	0.0168	0.0195	0.0144	0.0171	0.0176	0.0177	0.0638	0.0560	0.0576	0.0556	0.0553	0.0556	0.0553	0.0570	0.0555	0.0062	0.0062	0.0024	0.002
XacMN11	0.4075	0.0052	0.0058	0.0169	0.0186	0.0146	0.0172	0.0177	0.0182	0.0639	0.0559	0.0577	0.0560	0.0557	0.0560	0.0557	0.0569	0.0559	0.0059	0.0058	0.0026	0.002
XacMN12	0.4076	0.0054	0.0060	0.0166	0.0186	0.0144	0.0169	0.0175	0.0177	0.0638	0.0560	0.0577	0.0556	0.0553	0.0556	0.0553	0.0569	0.0555	0.0060	0.0061	0.0023	0.002
XacNT17	0.4095	0.0005	0.0001	0.0125	0.0221	0.0171	0.0180	0.0135	0.0209	0.0656	0.0589	0.0621	0.0580	0.0577	0.0580	0.0577	0.0596	0.0578	0.0002	0.0002	0.0062	0.006
XacUI6	0.4077	0.0059	0.0065	0.0171	0.0190	0.0149	0.0177	0.0181	0.0182	0.0644	0.0566	0.0583	0.0558	0.0555	0.0558	0.0555	0.0572	0.0557	0.0063	0.0065	0.0025	0.002
XacUI7	0.4076	0.0055	0.0060	0.0173	0.0190	0.0150	0.0177	0.0182	0.0183	0.0644	0.0567	0.0583	0.0557	0.0554	0.0557	0.0554	0.0571	0.0556	0.0061	0.0061	0.0014	0.001
XacX18	0.3933	0.1462	0.1461	0.1379	0.1488	0.1348	0.1368	0.1378	0.1364	0.1522	0.1446	0.1455	0.1543	0.1543	0.1543	0.1543	0.1399	0.1544	0.1459	0.1459	0.1422	0.142
XacX20	0.4086	0.1559	0.1559	0.1476	0.1590	0.1447	0.1469	0.1476	0.1462	0.1417	0.1337	0.1351	0.1471	0.1471	0.1471	0.1471	0.1367	0.1472	0.1557	0.1557	0.1519	0.151
XagCFBP7119	0.4154	0.1611	0.1608	0.1537	0.1650	0.1507	0.1531	0.1535	0.1521	0.1552	0.1469	0.1488	0.1544	0.1544	0.1544	0.1544	0.1453	0.1545	0.1606	0.1606	0.1574	0.157
XalfaCFBP3836	0.1448	0.3896	0.3893	0.3831	0.3930	0.3808	0.3822	0.3826	0.3832	0.3924	0.3882	0.3888	0.3951	0.3952	0.3952	0.3951	0.3839	0.3952	0.3892	0.3893	0.3867	0.386
XalfaF1	0.1413	0.3926	0.3923	0.3876	0.3965	0.3852	0.3866	0.3870	0.3868	0.3951	0.3905	0.3916	0.3980	0.3980	0.3980	0.3980	0.3871	0.3980	0.3921	0.3921	0.3900	0.390
Xalfacm1510	0.1390	0.3895	0.3892	0.3829	0.3935	0.3807	0.3810	0.3824	0.3822	0.3910	0.3864	0.3875	0.3942	0.3942	0.3942	0.3942	0.3822	0.3943	0.3891	0.3891	0.3871	0.387
Xalfacm1637	0.1398	0.3903	0.3901	0.3832	0.3932	0.3814	0.3810	0.3831	0.3823	0.3938	0.3888	0.3901	0.3960	0.3960	0.3960	0.3960	0.3853	0.3961	0.3899	0.3899	0.3874	0.387
XauB11122	0.4191	0.2843	0.2842	0.2767	0.2833	0.2726	0.2749	0.2762	0.2709	0.2837	0.2811	0.2829	0.2919	0.2919	0.2919	0.2919	0.2792	0.2919	0.2841	0.2841	0.2808	0.280
XauB1561	0.4264	0.2948	0.2946	0.2872	0.2984	0.2854	0.2857	0.2874	0.2867	0.2949	0.2925	0.2941	0.3021	0.3021	0.3021	0.3021	0.2902	0.3022	0.2945	0.2945	0.2914	0.291
XauC10535	0.4164	0.2877	0.2876	0.2800	0.2912	0.2777	0.2784	0.2798	0.2798	0.2912	0.2853	0.2876	0.2962	0.2962	0.2962	0.2962	0.2823	0.2962	0.2874	0.2874	0.2842	0.284
XauC1559	0.4412	0.3166	0.3166	0.3101	0.3201	0.3085	0.3079	0.3104	0.3100	0.3216	0.3156	0.3178	0.3255	0.3255	0.3256	0.3255	0.3126	0.3256	0.3164	0.3164	0.3135	0.313
XauC1609	0.4354	0.3036	0.3034	0.2974	0.3070	0.2951	0.2959	0.2972	0.2969	0.3081	0.3021	0.3039	0.3130	0.3130	0.3130	0.3129	0.2991	0.3130	0.3033	0.3033	0.3004	0.300
XauC535	0.4241	0.2953	0.2953	0.2875	0.2989	0.2854	0.2861	0.2872	0.2871	0.2984	0.2919	0.2941	0.3041	0.3041	0.3041	0.3041	0.2890	0.3041	0.2951	0.2951	0.2921	0.292
XauC763	0.4109	0.2807	0.2806	0.2729	0.2843	0.2702	0.2710	0.2722	0.2721	0.2844	0.2783	0.2805	0.2894	0.2894	0.2894	0.2894	0.2750	0.2895	0.2804	0.2804	0.2772	0.277

XacMN12	0.4076	0.0054	0.0060	0.0166	0.0186	0.0144	0.0169	0.0175	0.0177	0.063
XacNT17	0.4095	0.0005	0.0001	0.0125	0.0221	0.0171	0.0180	0.0135	0.0209	0.065
XacUI6	0.4077	0.0059	0.0065	0.0171	0.0190	0.0149	0.0177	0.0181	0.0182	0.064
XacUI7	0.4076	0.0055	0.0060	0.0173	0.0190	0.0150	0.0177	0.0182	0.0183	0.064
XacX18	0.3933	0.1462	0.1461	0.1379	0.1488	0.1348	0.1368	0.1378	0.1364	0.152
XacX20	0.4086	0.1559	0.1559	0.1476	0.1590	0.1447	0.1469	0.1476	0.1462	0.141
XagCFBP7119	0.4154	0.1611	0.1608	0.1537	0.1650	0.1507	0.1531	0.1535	0.1521	0.155
XalfaCFBP3836	0.1448	0.3896	0.3893	0.3831	0.3930	0.3808	0.3822	0.3826	0.3832	0.392
XalfaF1	0.1413	0.3926	0.3923	0.3876	0.3965	0.3852	0.3866	0.3870	0.3868	0.395
Xalfacm1510	0.1390	0.3895	0.3892	0.3829	0.3935	0.3807	0.3810	0.3824	0.3822	0.391
Xalfacm1637	0.1398	0.3903	0.3901	0.3832	0.3932	0.3814	0.3810	0.3831	0.3823	0.393
XauB11122	0.4191	0.2843	0.2842	0.2767	0.2833	0.2726	0.2749	0.2762	0.2709	0.283
XauB1561	0.4264	0.2948	0.2946	0.2872	0.2984	0.2854	0.2857	0.2874	0.2867	0.294
XauC10535	0.4164	0.2877	0.2876	0.2800	0.2912	0.2777	0.2784	0.2798	0.2798	0.291
XauC1559	0.4412	0.3166	0.3166	0.3101	0.3201	0.3085	0.3079	0.3104	0.3100	0.321
XauC1609	0.4354	0.3036	0.3034	0.2974	0.3070	0.2951	0.2959	0.2972	0.2969	0.308
XauC535	0.4241	0.2953	0.2953	0.2875	0.2989	0.2854	0.2861	0.2872	0.2871	0.298
XauC763	0.4109	0.2807	0.2806	0.2729	0.2843	0.2702	0.2710	0.2722	0.2721	0.284

Largest distance: **0.4506** (*X. perforans* 91-118 and *X. fuscans aurantifolii* C 1559)

For comparison: distance between *Stenotrophomonas maltophilia* and Xac 306: **0.912**

ANI

- Average Nucleotide Identity [Goris et al. 2007]
- Baseado em BLAST
- one-way ANI (best hits)
- two-way ANI (reciprocal best hits)
- Typically, 2 species will have ANI ≥ 95%
- < 75% : not to be trusted
- http://enve-omics.ce.gatech.edu/ani/

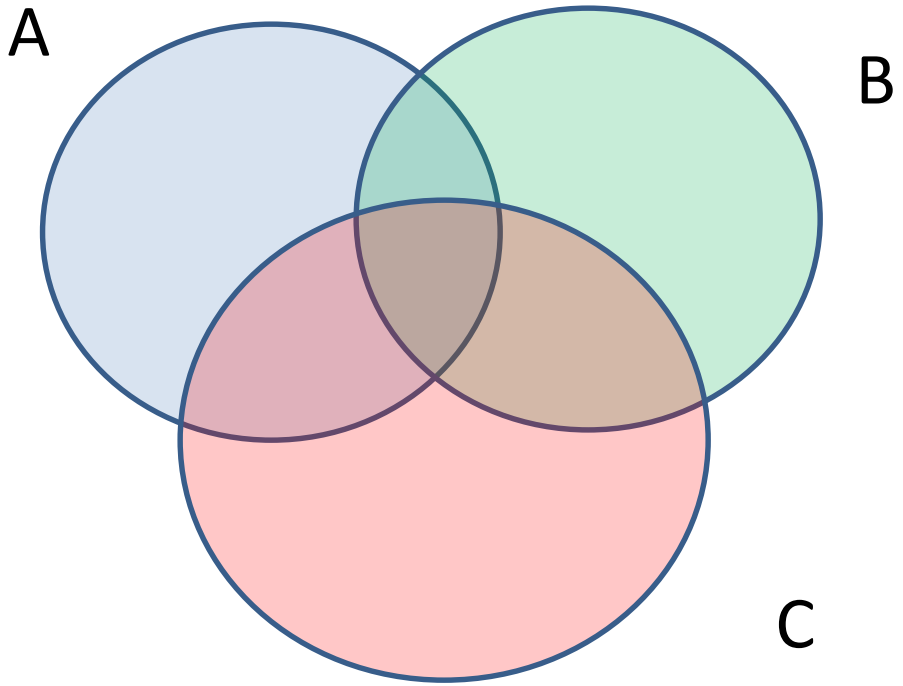
GGDC

- Genome-to-genome distance calculation
- rigorous *in silico* replacement for DNA-DNA hybridization wet-lab experiments
- http://ggdc.dsmz.de
- Meier-Kolthoff, J.P., et al., *Genome sequence-based species delimitation with confidence intervals and improved distance functions.* BMC Bioinformatics, 2013. 14: p. 60

Comparação de conjuntos de genes

- Given a set of genomes, represented by their ‘proteomes’ or sets of protein sequences
- Given homologous relationships (as given for example by orthoMCL)
  - Which genes are shared by genomes *X* and *Y*?
  - Which genes are unique to genome *Z*?
  - Venn or extended Venn diagrams

3-way genome comparison



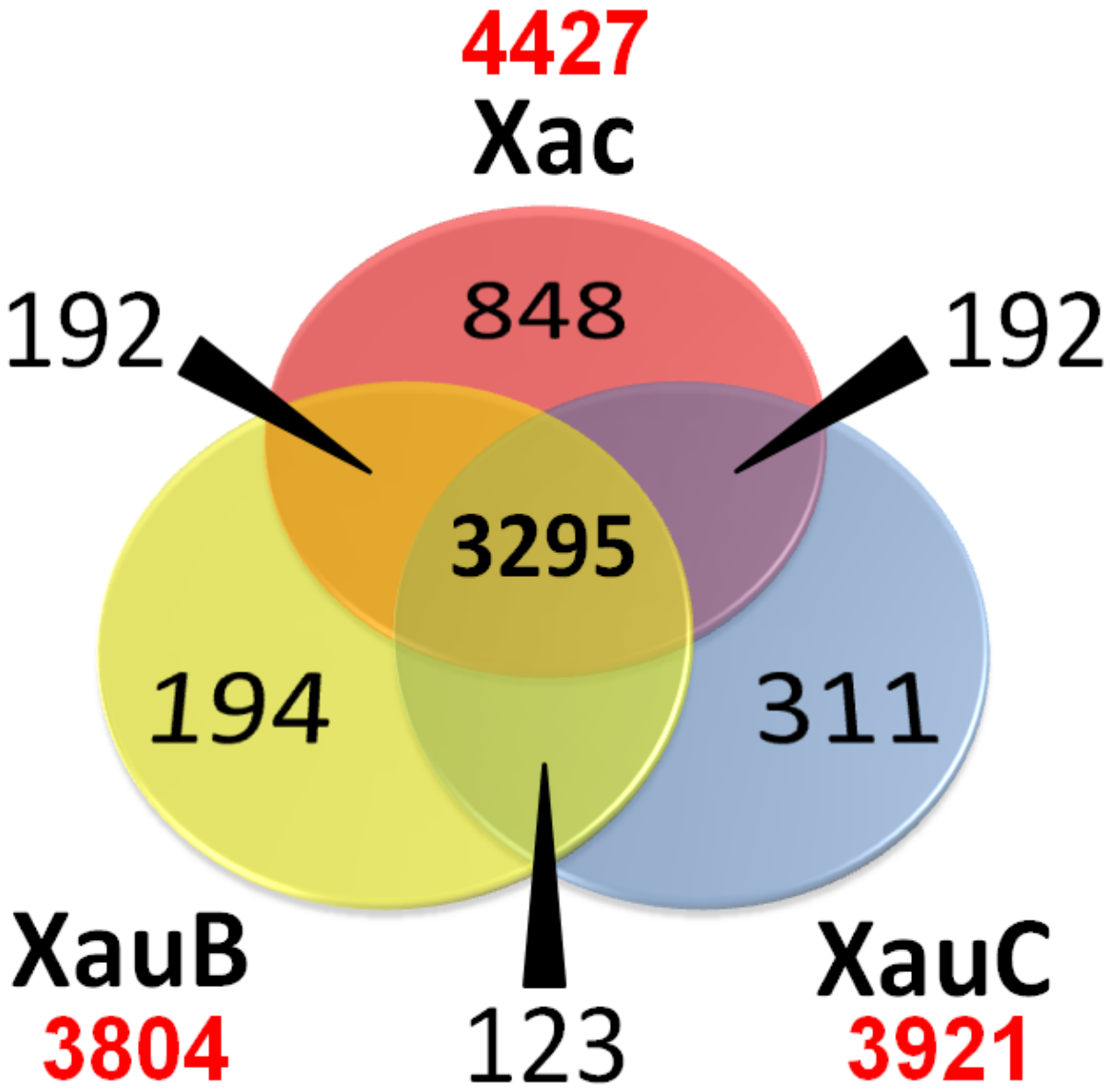
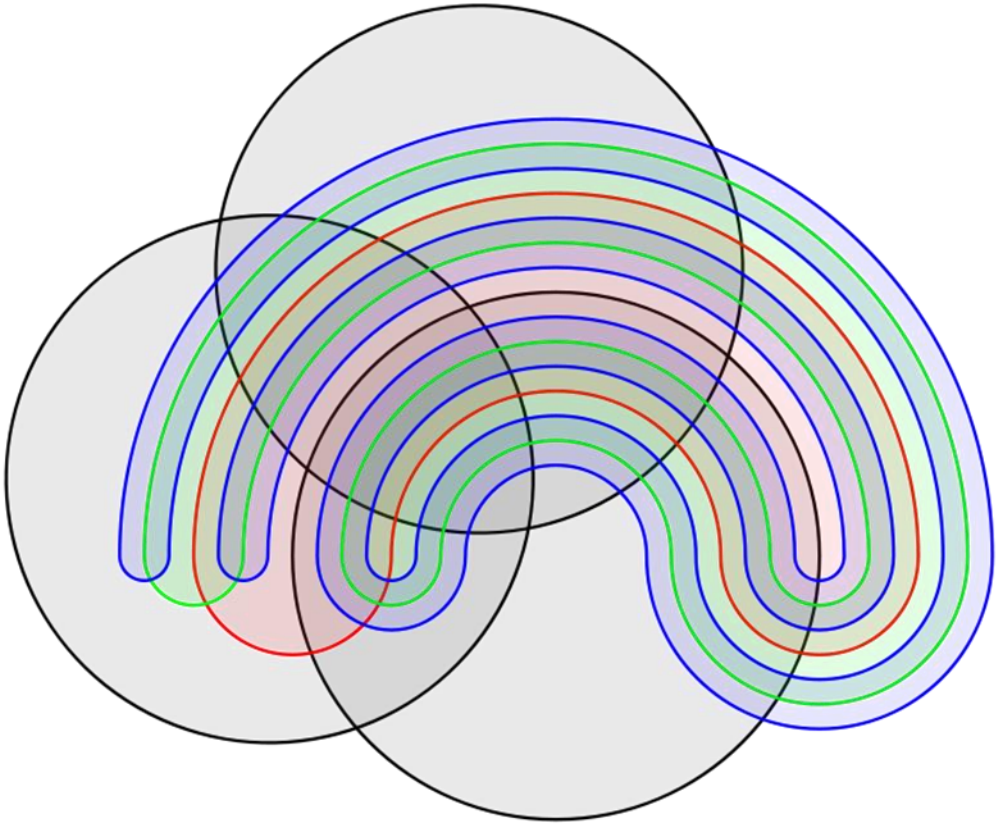

4427
Xac
848
192
192
3295
194
311
XauB
3804
123
XauC
3921

Diagrama de Venn para n = 6



Número de comparações é quadrático em *n*
Número de regiões num diagrama de Venn = 2^n

Source: wikipedia

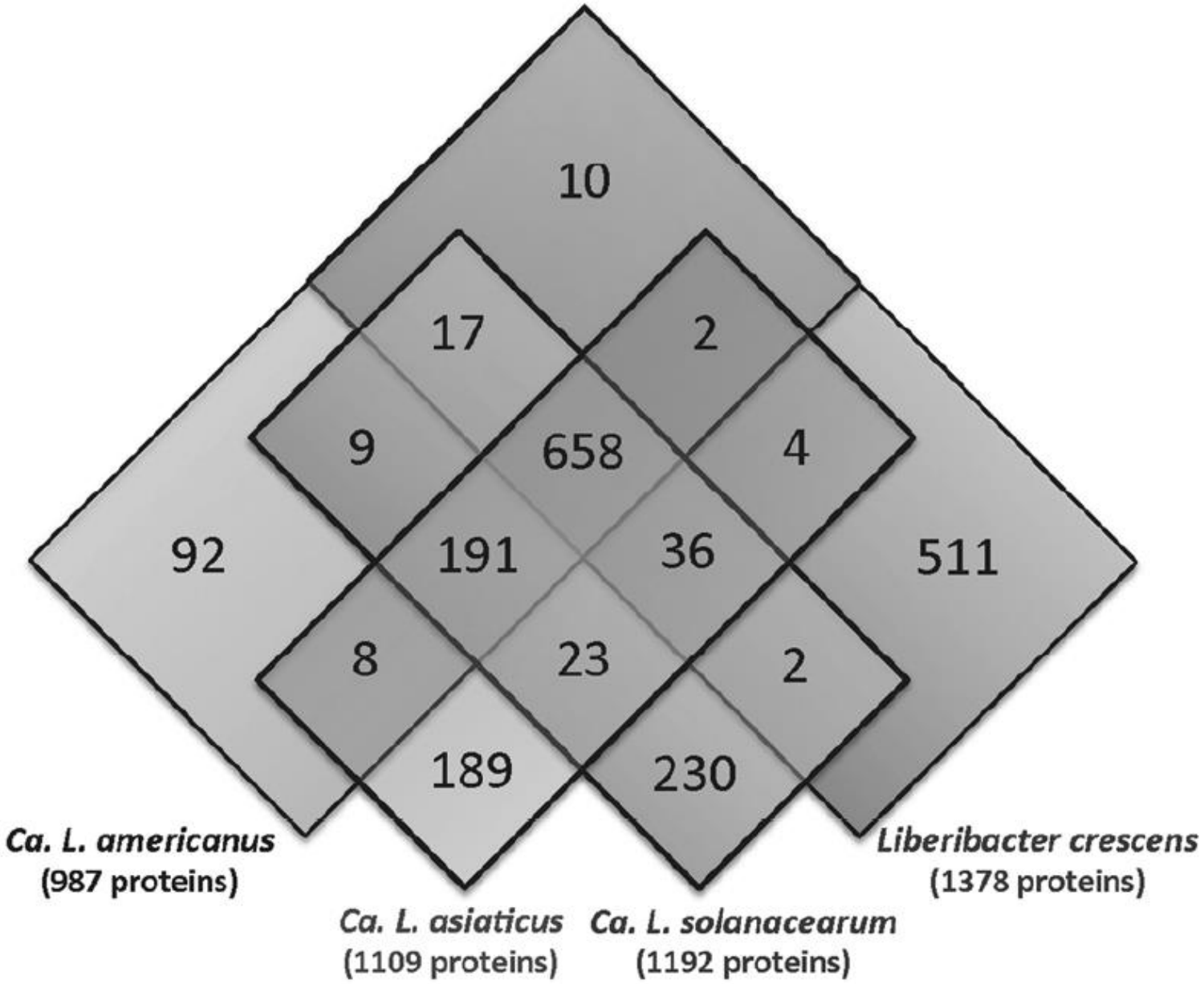


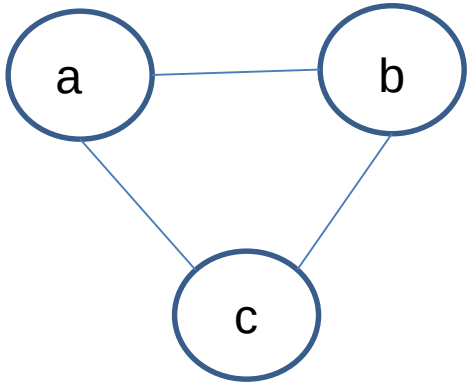
Fig. 2. Venn diagram showing protein-coding gene sharing among the four Liberibacter genomes. Numbers below each species are the total number of predicted protein-coding genes for that genome.

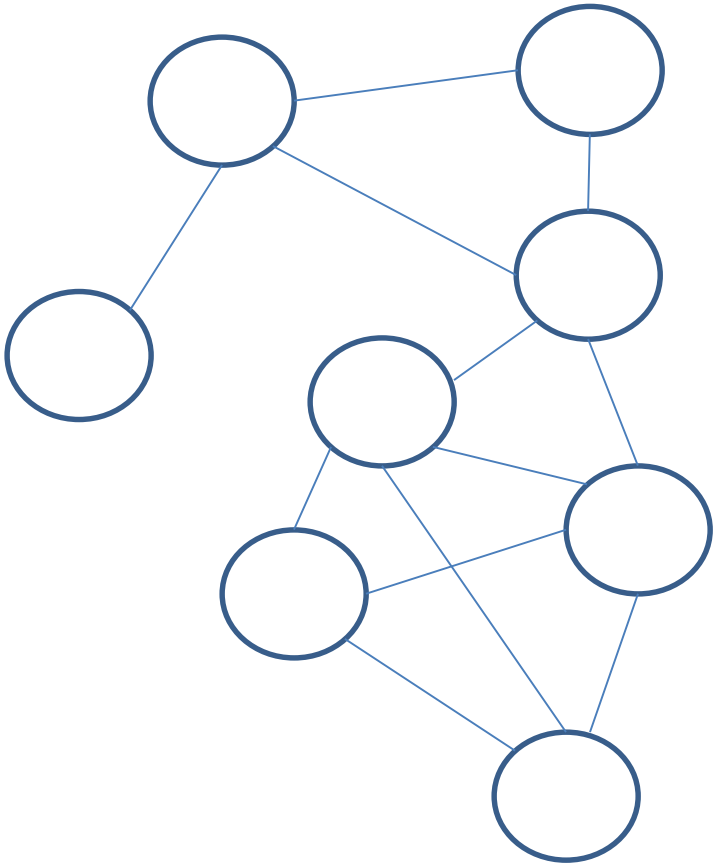
Wulff et al. MPMI Vol. 27, No. 2, 2014, pp. 163–176 http://dx.doi.org/10.1094/MPMI-09-13-0292-R.

Cômputo de famílias de proteínas

1. Verificar as similaridades entre as sequências
   a) Usando (por exemplo) BLAST + critérios
2. Representar as similaridades num grafo
3. Aplicar um algoritmo de clusterização sobre o grafo

Clusterização é necessária porque o grafo pode ser complexo



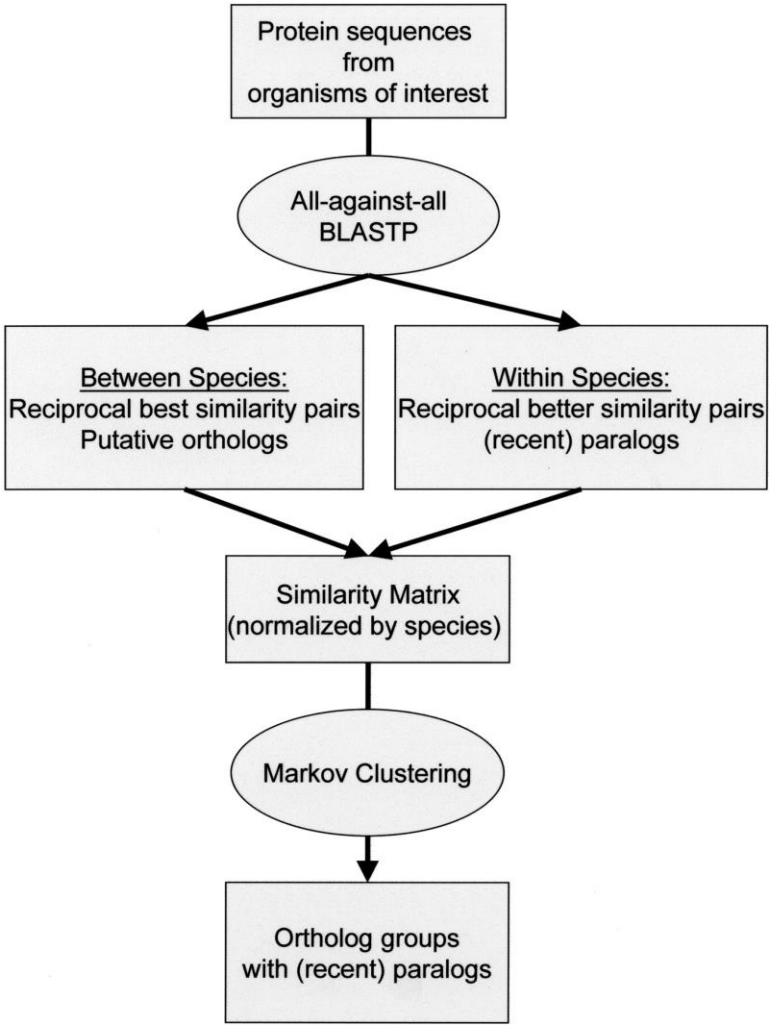


Resultado da clusterização

- Matriz com genes nas colunas (g_j), genomas nas linhas (O_i)
- Cada coluna representa uma família de genes homólogos

	g1	g2	g3	g4	…
O1	✓		✓	✓	
O2		✓	✓		
O3	✓		✓		
O4			✓	✓	✓
O5	✓		✓		✓
O6			✓		
…			✓		

orthoMCL pipeline



Li Li et al. Genome Res. 2003; 13: 2178-2189

NEWS

Mar 31, 2011
OrthoMCL-DB Version 5 is released. We have included 150 genomes in this release.

May 31, 2010
OrthoMCL-DB Version 4 is released. There are 138 genomes included in version 4.

Oct 9, 2009
OrthoMCL-DB Version 3 is released. The new dataset includes 128 genomes. New web site features include: (1) a tool to assign your proteins to OrthoMCL groups (see the new tools menu); (2) a mapping from Version 3 groups to Version 2 and 1 is available for searching, allowing you to track changes across versions; (3) the phyletic pattern in the groups result page is configurable, so you can tailor it to the clades you are interested in.

Sep 22, 2009
OrthoMCL-DB pipeline re-engineered. We have completely overhauled how we produce the database, encoding the entire process in a pipeline system. This should dramatically improve our ability to deliver new versions of the database.

Feb 28, 2008

Welcome to OrthoMCL DB

Ortholog Groups of Protein Sequences from Multiple Genomes!

Current Release:
Version: 5
Number of Genomes: 150
Number of Protein Sequences: 1398546
Number of Ortholog Groups: 124740

Search for Groups

- by IDs, Keyword, or PFam domain
- by Phyletic Pattern
- by Phyletic Pattern - Advanced
- by Group Properties
- Query History - Groups

Search for Sequences

- by IDs, Keyword, Taxonomy or PFam domain
- by BLAST Search
- Query History - Sequences

Tools

- Assign your proteins to OrthoMCL groups

OrthoMCL Software

Database Download

Xanthomonas fuscans str. *aurantifolii* Genome Project

Choose genomes to see OrthoMCL families containing genes from them

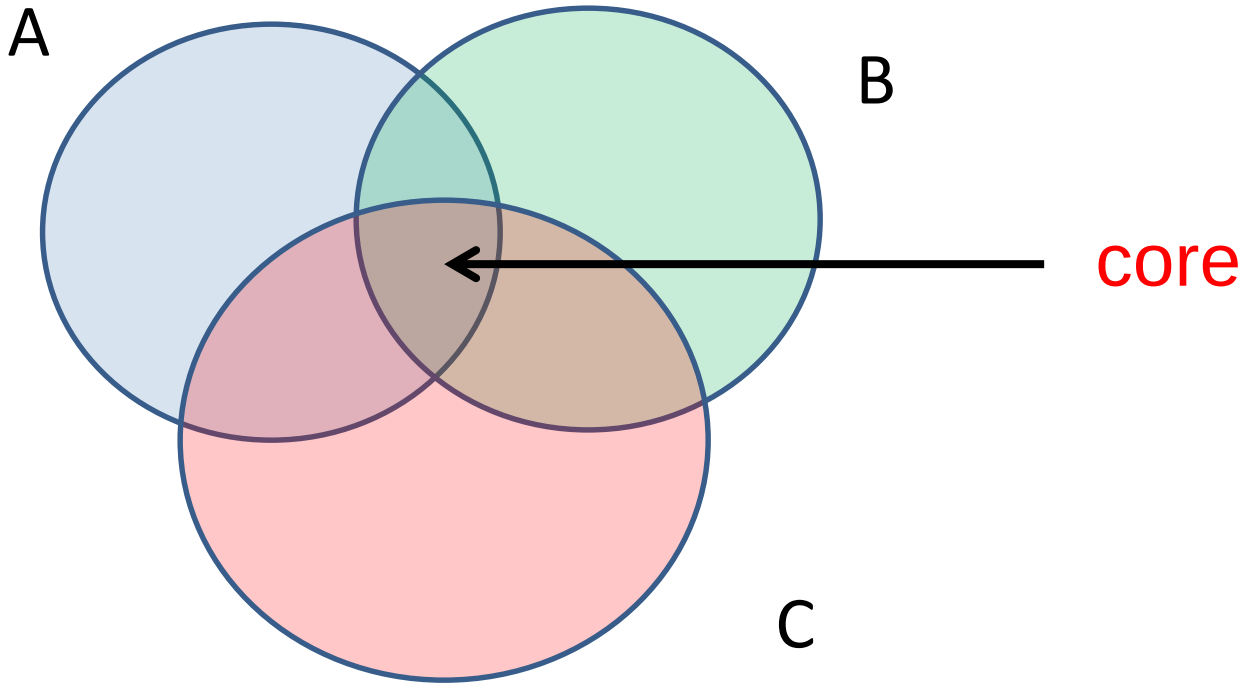
Include	Exclude	I don't care	
○	○	◉	Xanthomonas fuscans subsp. aurantifolii str. 11122 (B)
○	○	◉	Xanthomonas fuscans subsp. aurantifolii str. 10535 (C)
○	○	◉	Xanthomonas campestris pv. campestris str. 8004
○	○	◉	Xanthomonas campestris pv. campestris str. ATCC 3391
○	○	◉	Xanthomonas campestris pv. campestris B100
○	○	◉	Xanthomonas campestris pv. vesicatoria str. 85-10
○	○	◉	Xanthomonas axonopodis pv. citri str. 306
○	○	◉	Xanthomonas oryzae pv. oryzae KACC10331
○	○	◉	Xanthomonas oryzae pv. oryzae MAFF 311018
○	○	◉	Xanthomonas oryzae pv. oryzae PXO99A
○	○	◉	Xanthomonas albilineans GPE PC73
○	○	◉	Xylella fastidiosa 9a5c
○	○	◉	Xylella fastidiosa M12
○	○	◉	Xylella fastidiosa M23 plasmid pXFAS01
○	○	◉	Xylella fastidiosa Temecula1

○ check all ○ check all ○ check all(see all families)

Submit Query Reset

```
========================================
>Family 5286  -- Gblocks 331/384 (86%)
21243924              nr   alkanesulfonate transporter substrate-binding subunit [Xanthomonas axonopodis pv. citri str. 306]
9810940               nr   - nitrate transport protein [Xanthomonas fuscans subsp. aurantifolii str. 11122 (B)]
9929350               nr   - nitrate transport protein [Xanthomonas fuscans subsp. aurantifolii str. 10535 (C)]

count_ = 3
========================================
>Family 5287  -- Gblocks 346/346 (100%)
21243925              nr   oxidoreductase [Xanthomonas axonopodis pv. citri str. 306]
9810930               nr   - oxidoreductase [Xanthomonas fuscans subsp. aurantifolii str. 11122 (B)]
9929360               nr   - oxidoreductase [Xanthomonas fuscans subsp. aurantifolii str. 10535 (C)]

count_ = 3
========================================
>Family 5288  -- Gblocks 442/442 (100%)
21243926              nr   nitrilotriacetate monooxygenase component A [Xanthomonas axonopodis pv. citri str. 306]
9810920               nr   - nitrilotriacetate monooxygenase component A [Xanthomonas fuscans subsp. aurantifolii str. 11122 (B)]
9929370               nr   - nitrilotriacetate monooxygenase component A [Xanthomonas fuscans subsp. aurantifolii str. 10535 (C)]

count_ = 3
========================================
>Family 5289  -- Gblocks 356/356 (100%)
21243950              nr   avirulence protein [Xanthomonas axonopodis pv. citri str. 306]
9814680               nr   - type III secretion system hopXl-like protein [Xanthomonas fuscans subsp. aurantifolii str. 11122 (B)]
9900040               nr   - type III secretion system hopXl-like protein [Xanthomonas fuscans subsp. aurantifolii str. 10535 (C)]

count_ = 3
========================================
>Family 5290  -- Gblocks 291/297 (97%)
21243956              nr   hypothetical protein XAC3230 [Xanthomonas axonopodis pv. citri str. 306]
9826830               nr   - conserved hypothetical protein [Xanthomonas fuscans subsp. aurantifolii str. 11122 (B)]
9923780               nr   - conserved hypothetical protein [Xanthomonas fuscans subsp. aurantifolii str. 10535 (C)]

count_ = 3
========================================
>Family 5291  -- Gblocks 203/373 (54%)
21243959              nr   transposase [Xanthomonas axonopodis pv. citri str. 306]
9803570               nr   - transposase [Xanthomonas fuscans subsp. aurantifolii str. 11122 (B)]
9900450               nr   - transposase [Xanthomonas fuscans subsp. aurantifolii str. 10535 (C)]

count_ = 3
========================================
>Family 5292  -- Gblocks 163/171 (95%)
21243960              nr   hypothetical protein XAC3234 [Xanthomonas axonopodis pv. citri str. 306]
9820110               nr   - conserved hypothetical protein [Xanthomonas fuscans subsp. aurantifolii str. 11122 (B)]
```

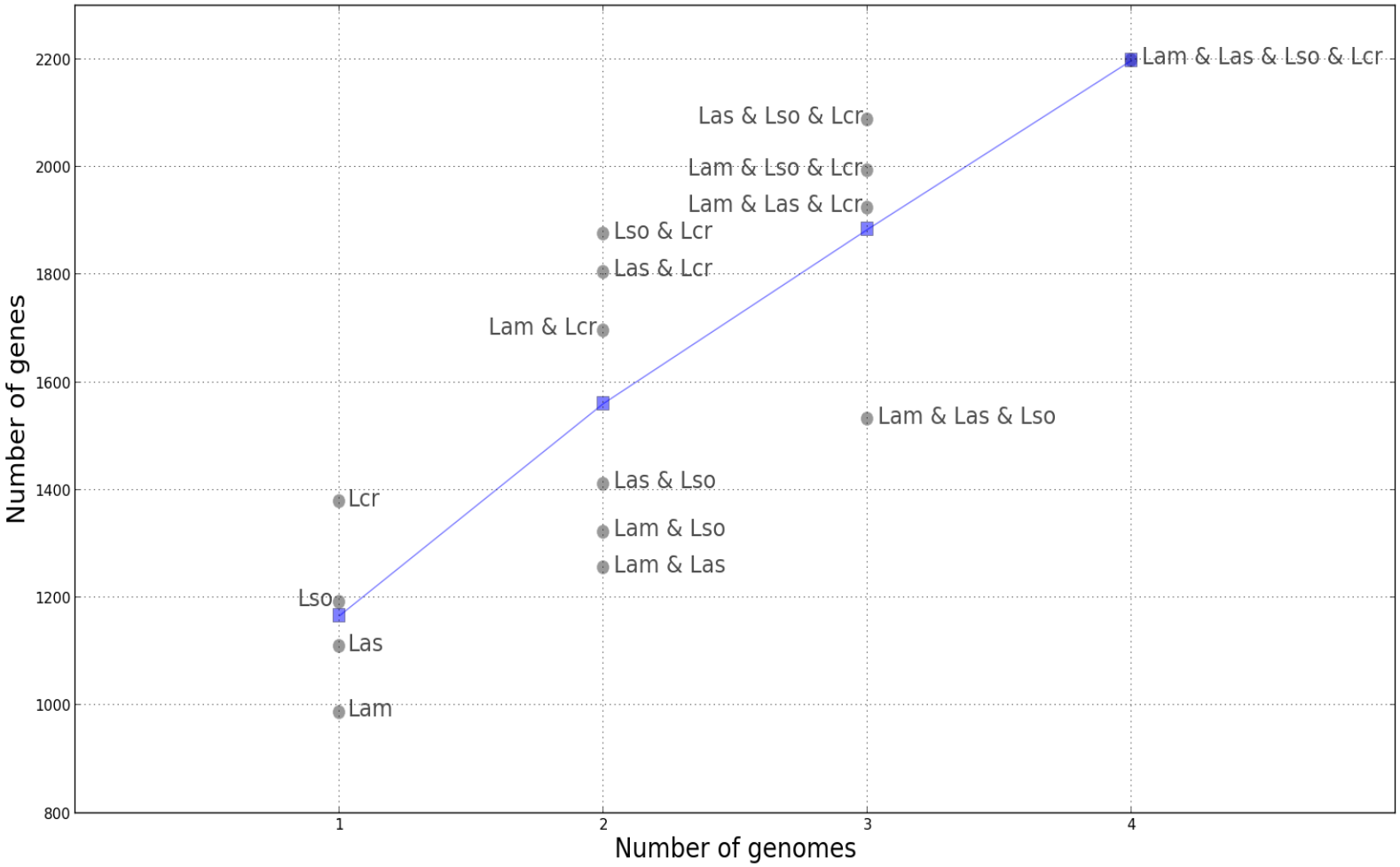
Pan genoma; genoma core e genoma acessório



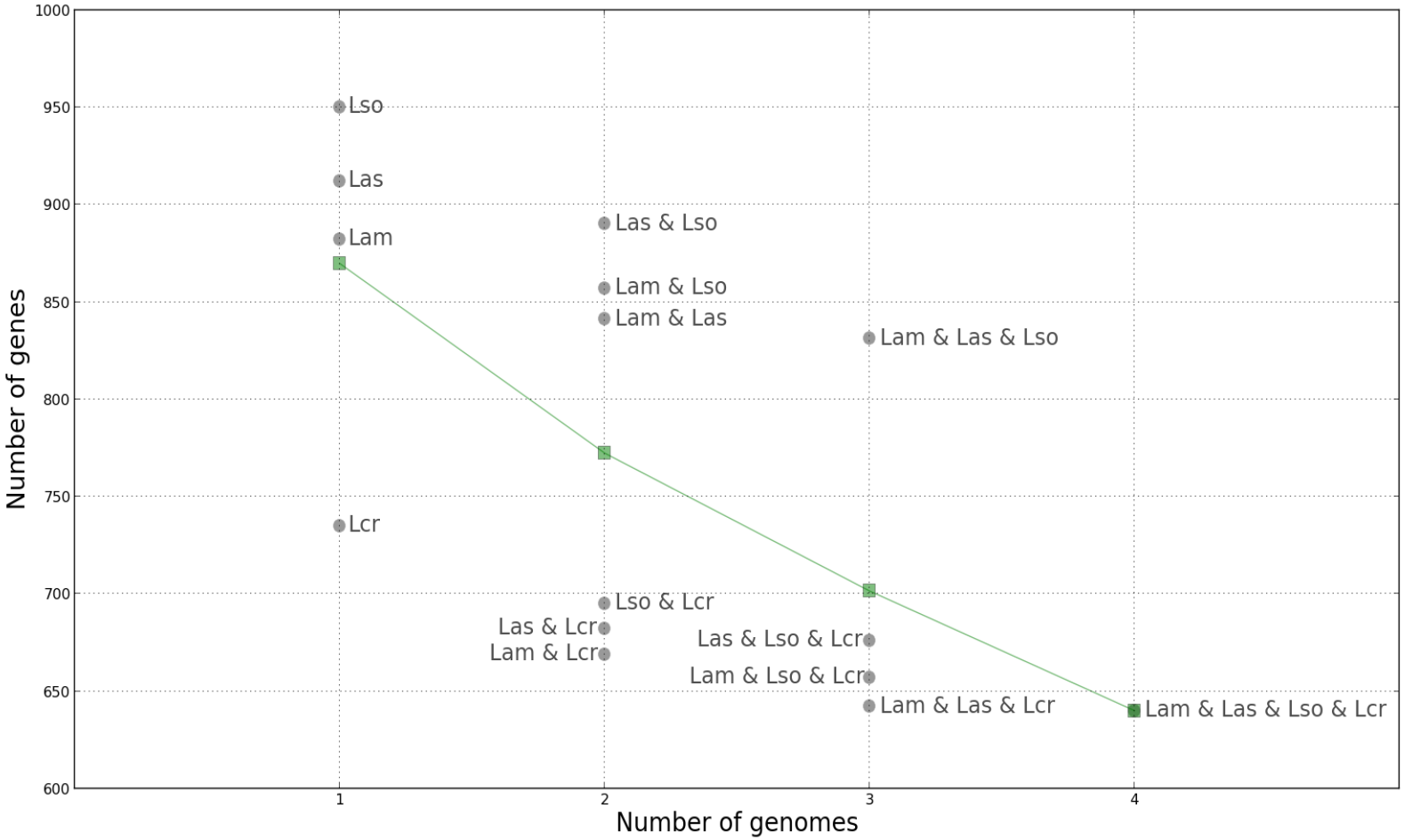
pan: A U B U C

acessório: pan - core

Curva de pan-genoma (n = 4)



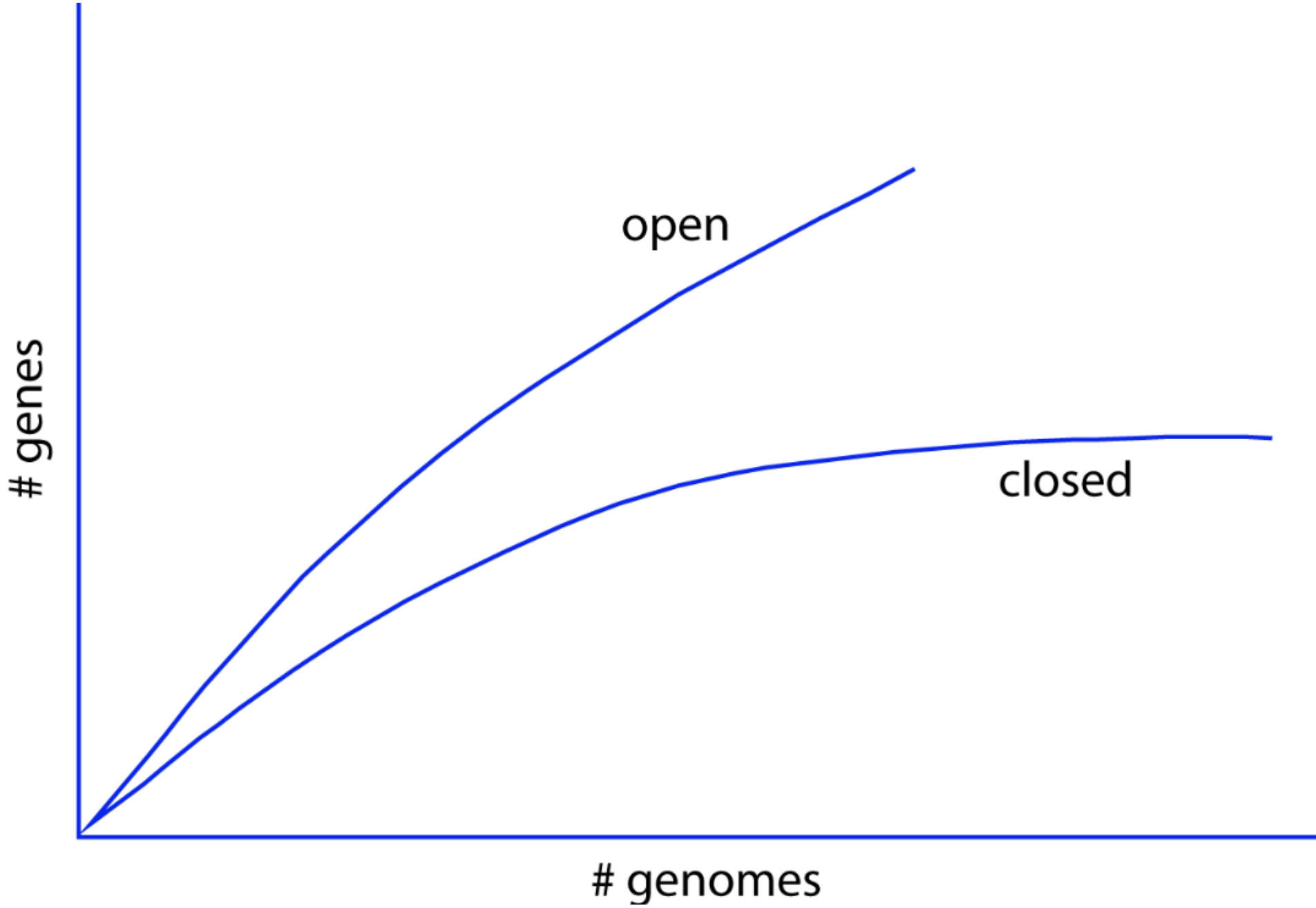
Curva de core genoma (n = 4)



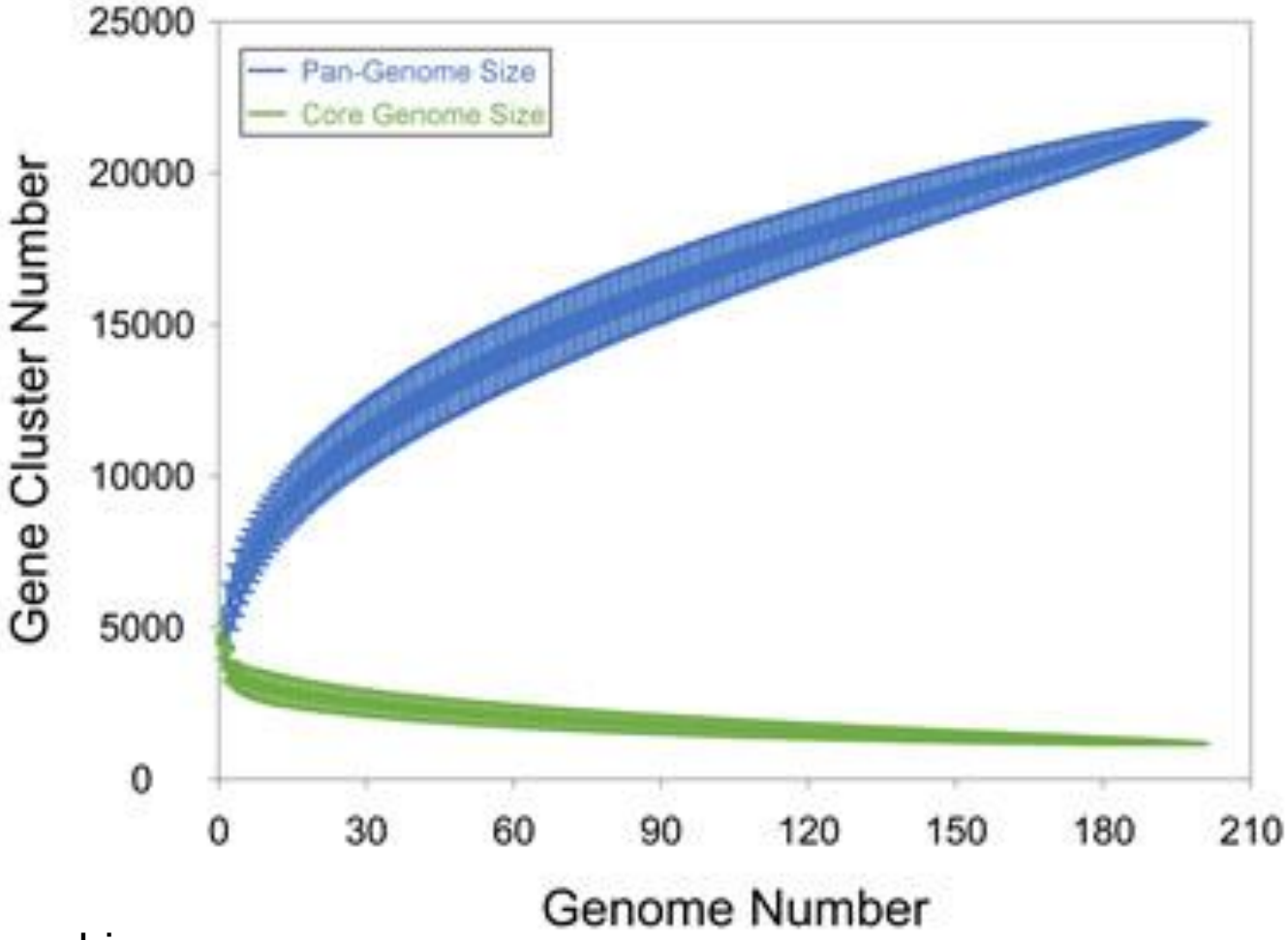
O número de genes para x=1 não é o mesmo do gráfico anterior pois os singletons são descartados

Genomas fechados e abertos

- Fechado
  - O número de genes/famílias do pan-genoma atinge um platô
- Aberto
  - O número de genes/famílias não atinge um platô; cresce sempre que se acrescentam novos genomas


open
closed
genes
genomes

O genoma de *E. coli* é aberto



Fonte: pangp.big.ac.cn

Bacillus anthracis

- Genoma fechado
- Cerca de 3.000 genes

Ferramentas para análise pan/core

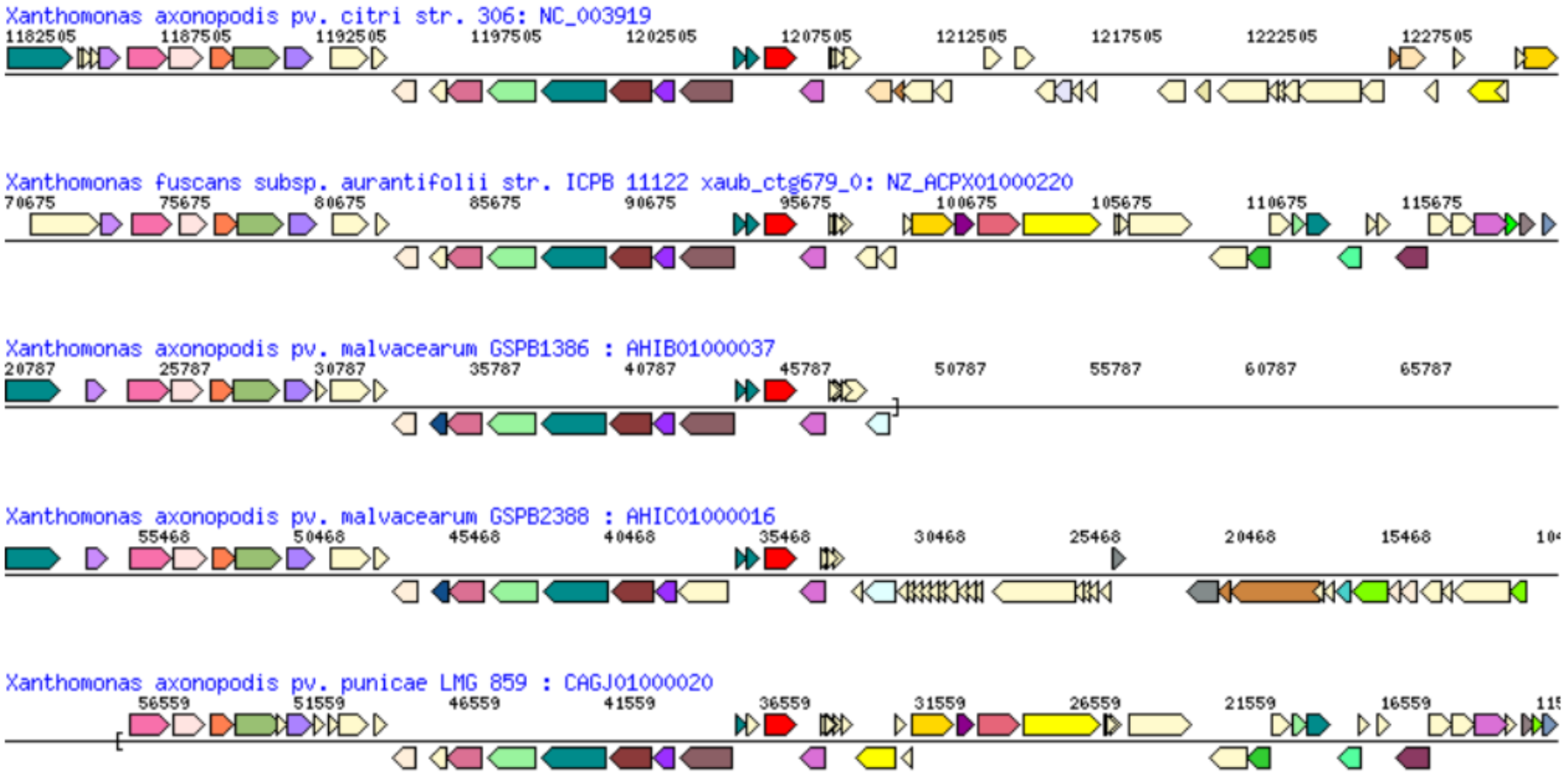
- Get_Homologues
- Contreras-Moreira, B. and P. Vinuesa, *GET_HOMOLOGUES, a versatile software package for scalable and robust microbial pangenome analysis.* Appl Environ Microbiol, 2013. 79(24): p. 7696-701

- Roary
- Page, A.J., et al., *Roary: rapid large-scale prokaryote pan genome analysis.* Bioinformatics, 2015. 31(22): p. 3691-3

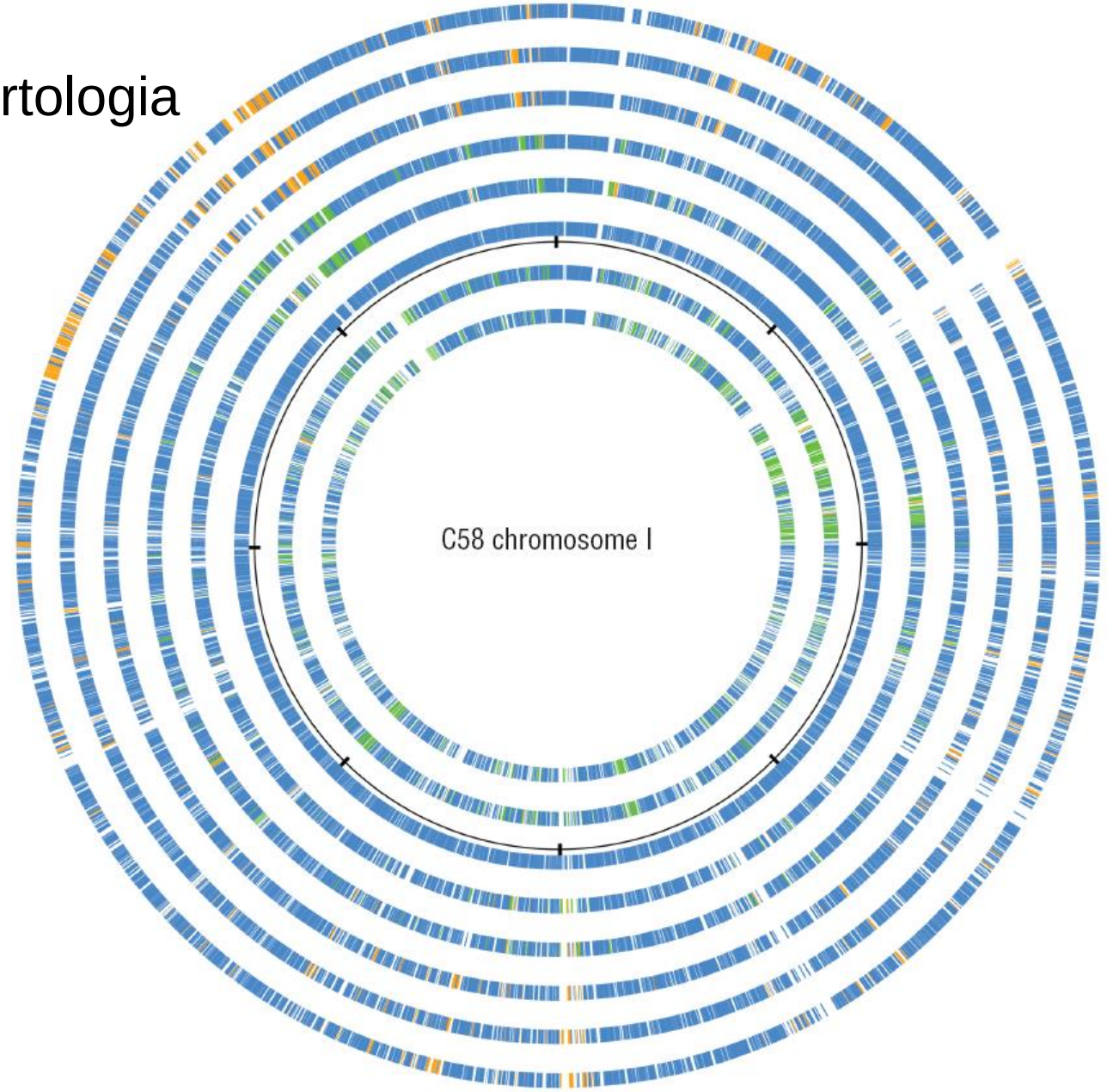
Conjuntos + contexto

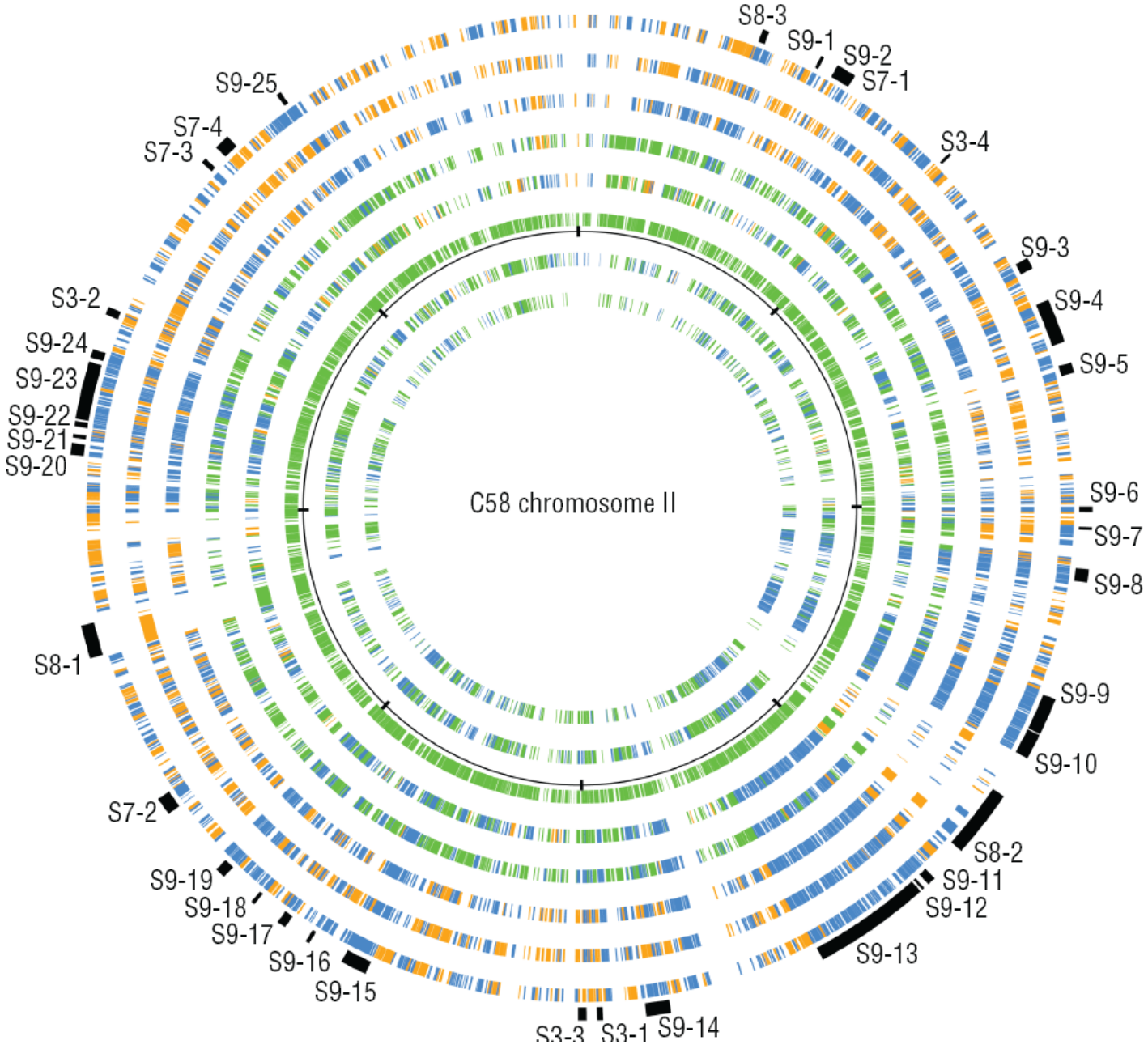
- Como genes compartilhados aparecem em seus respectivos genomas?
- **Filogenômica**
- Busca de sintenia = preservação de ordem
- Basta fazer um alinhamento
  - Os “caracteres” a serem alinhados são os genes
  - alinhamento de ortólogos

Alinhamento de ortólogos obtido no IMG/JGI



Roda da ortologia




C58 chromosome II
S8-3
S9-1
S9-2
S7-1
S3-4
S9-3
S9-4
S9-5
S9-6
S9-7
S9-8
S9-9
S9-10
S8-2
S9-11
S9-12
S9-13
S9-14
S3-1
S3-3
S9-15
S9-16
S9-17
S9-18
S9-19
S7-2
S8-1
S9-20
S9-21
S9-22
S9-23
S9-24
S3-2
S9-25
S7-3
S7-4

Sumário - 1

- Comparação de sequências “curtas” 2-a-2
  - Alinhamento
  - Sistemas de pontuação
  - Matrizes de substituição
  - Programação dinâmica
  - Significância estatística de alinhamentos
  - BLAST

Sumário - 2

- Comparação de várias sequências ao mesmo tempo
  - Alinhamento múltiplo
  - Programas
- Comparação de sequências “longas”
  - 2-a-2
  - Alinhamento múltiplo

Sumário - 3

- Distâncias genômicas
- Comparação entre conjuntos de genes
- Pan/Core
- Sintenia

Protein family resources

Clusters of orthologous groups (COG, KOG, eggNOG)

KEGG orthologs

wellcome trust sanger institute

HOME | SEARCH | BROWSE | FTP | HELP | ABOUT

Pfam 26.0 (November 2011, 13672 families)

The Pfam database is a large collection of protein families, each represented by **multiple sequence alignments** and **hidden Markov models (HMMs)**. More...

Pfam

keyword search Go

QUICK LINKS	YOU CAN FIND DATA IN PFAM IN VARIOUS WAYS...
SEQUENCE SEARCH	Analyze your protein sequence for Pfam matches
VIEW A PFAM FAMILY	View Pfam family annotation and alignments
VIEW A CLAN	See groups of related families
VIEW A SEQUENCE	Look at the domain organisation of a protein sequence
VIEW A STRUCTURE	Find the domains on a PDB structure
KEYWORD SEARCH	Query Pfam by keywords
JUMP TO	enter any accession or ID Go Example

Enter any type of accession or ID to jump to the page for a Pfam family or clan, UniProt sequence, PDB structure, etc.

Or view the help pages for more information

Recent Pfam blog posts

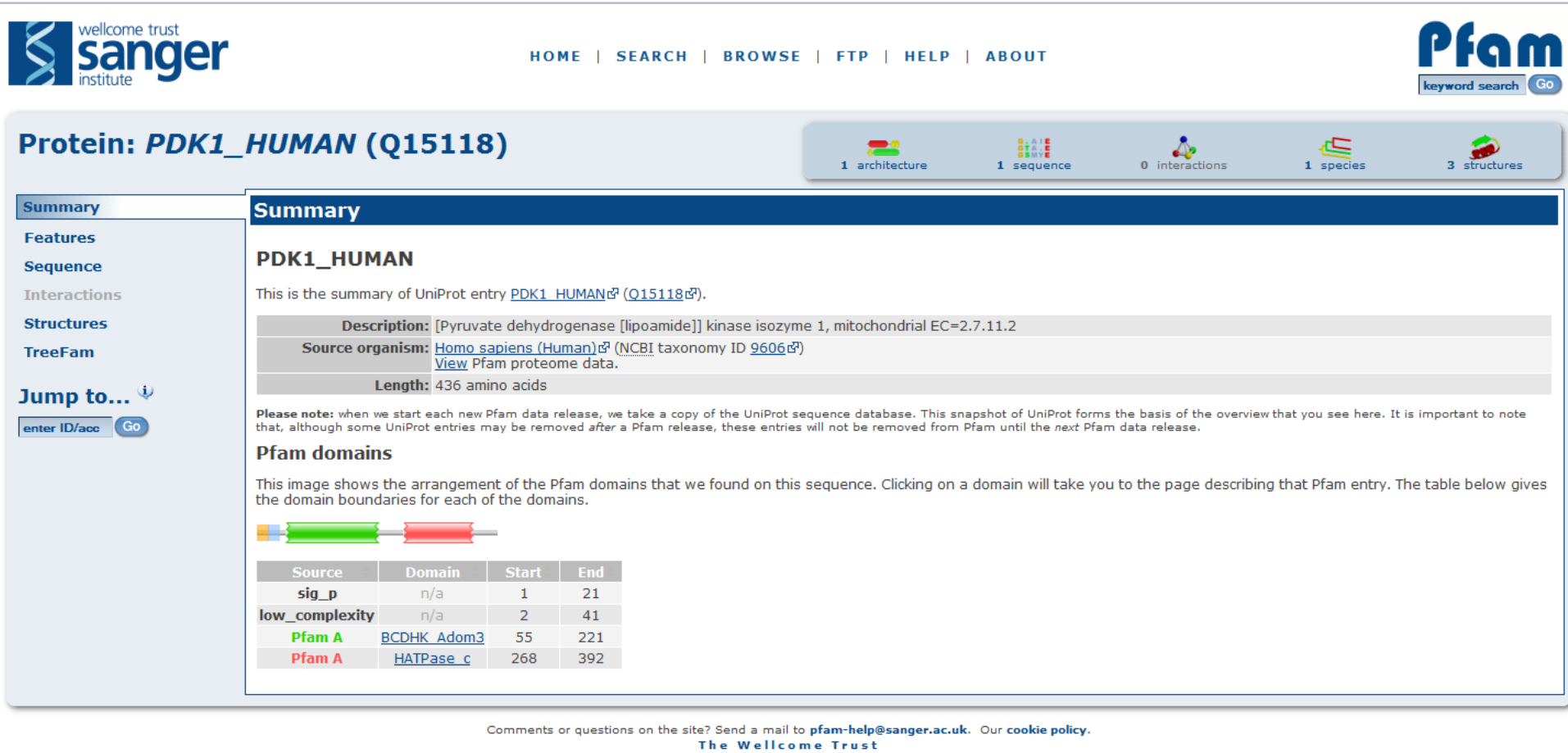
☒Hide th

Does my family of interest have a determined 3D protein structure? (posted 9 May 2012)

Two related questions that we are often asked via the Pfam helpdesk is 'Which families have a known three-dimensional structure?' and 'Why is a particular a PDB structure not found in Pfam'. You may thir that there are obvious answers to these questions – but as with many things in life the answer is not [...]

TreeFam is back with a new release ! (posted 27 March 2012)

Query by accession



KEYWORD SEARCH

All Go

SEQUENCE SEARCH

Enter a protein sequence:

Sequence query limits: Protein - 50kb

Submit

The PANTHER (**P**rotein **AN**alysis **TH**rough **E**volutionary **R**elationships) Classification System is a unique resource that **classifies genes by their functions**, using published scientific experimental evidence and evolutionary relationships to predict function even in the absence of direct experimental evidence. Proteins are **classified by expert biologists** according to:

- Gene families and subfamilies, including annotated phylogenetic trees
- Gene Ontology classes: molecular function, biological process, cellular component
- PANTHER Protein Classes
- Pathways, including diagrams

PANTHER is part of the Gene Ontology Reference Genome Project.

PANTHER is supported by a research grant from the National Institute of General Medical Sciences [grant GM081084] and maintained by the Thomas lab at the University of Southern California.

Quick links

- Whole genome function views
- Gene expression tools
- cSNP tools
- Upload multiple gene IDs
- Community Curation
- My Workspace
- HMM scoring
- Downloads
- Genome statistics
- Site map

Newsletter subscription

Enter your Email:

Subscribe

What can I do on the PANTHER site?
Guide to getting started

News

(March 16, 2012)

PANTHER 7.2 is released.

Click for additional info.

Publications

How to cite PANTHER

"PANTHER version 7: improved phylogenetic trees, orthologs and collaboration with the Gene Ontology Consortium." Mi, et al.

"Applications for protein sequence-function evolution data: mRNA/protein expression analysis and coding SNP scoring tools." Thomas, et al.

"PANTHER: a library of protein families and subfamilies indexed by function." Thomas, et al.

PANTHER HMM SEQUENCE SCORING RESULTS

The top scoring HMM is reported, along with the E-value (the number of expected false-positive hits expected). If the E-value is less than 1e-3, no hits are reported.

PANTHER Hit: PROLINE SYNTHETASE CO-TRANSCRIBED BACTERIAL HOMOLOG PROTEIN (PTHR10146)
HMM E-value score: 2.2e-113

```
Sequence Domain  seq-f seq-t    hmm-f hmm-t      score  E-value
-------- ------- ----- -----    ----- -----      -----  -------
sequence   1/1       1   234 []    11   253 ..   387.5 2.2e-113

Alignments of top-scoring domains:
sequence: domain 1 of 1, from 1 to 234: score 387.5, E = 2.2e-113
                     *->lgvaanLakVlerikaaaakagRdppavrLvAVSKTkPaelileayd
                        ++va+nL++V++++++a+ak+ R   + +LvAVSKTkP+e+++eay+
    sequence       1    MAVAKNLLAVRAKVAEAVAKSARQ-QQCTLVAVSKTKPVEDLQEAYE 46

                     aGqRhFGENYvQElleKaplLpdlcpdikWHFIGhLQsNKvkkll.gvpn
                     a qRhFGENY+QEl++KaplLp    d+kWH+IGh+QsNK+k+l+++vpn
    sequence      47 ADQRHFGENYIQELVQKAPLLPK---DVKWHYIGHVQSNKAKPLVrDVPN 93

                     ldmvhsvDslklAdklnkaaaklkglgkplkvlvQVNtsGEesKsGvppe
                     l++v++vDs+k+A++lnka  ++  ++++l+v+vQVNts Ee+KsG++ +
    sequence      94 LFVVETVDSIKIANALNKASGEF--RSEKLNVMVQVNTSEEEQKSGIDAD 141

                     Elpellkhvlkkcpn LellGLMTIGpfdgdlekgpnpdFalLaklrkevc
                       +el++h+++ c++L+l GLMTIG++++  ++     F +L+++rk+v+
    sequence     142 GSVELAQHIVSSCEHLNLTGLMTIGRYGDTTSE----CFDRLVACRKRVA 187

                     kklglnpkllELSMGMSgDfelAIeaGsTlVRvGsaIFGeRdypkkp<-*
                     +++g  +  l LSMGMSgDfelAI  GsT+VRvGs+IFG+R+y +k+
    sequence     188 EAIGKAETDLDLSMGMSGDFELAISCGSTHVRVGSTIFGARNYANKE    234
```

Search

PANTHER families

Go

Quick links

- Whole genome function views
- Gene expression tools
- cSNP tools
- Upload multiple gene IDs
- Community Curation
- My Workspace
- HMM scoring
- Downloads
- Genome statistics
- Site map

PANTHER FAMILY INFORMATION

Family:	PROLINE SYNTHETASE CO-TRANSCRIBED BACTERIAL HOMOLOG PROTEIN (PTHR10146)
Subfamilies:	1
PANTHER Links:	Tree MSA
GO Molecular Function:	catalytic activity
GO Biological Process:	metabolic process ↳ primary metabolic process ↳ cellular amino acid and derivative metabolic process ↳ cellular amino acid metabolic process
GO Cellular Component:	
PANTHER protein class:	
Pathway Categories:	No pathway information available
Genes:	45
HMM Length	273
Downloads:	HMM (HMMER format)

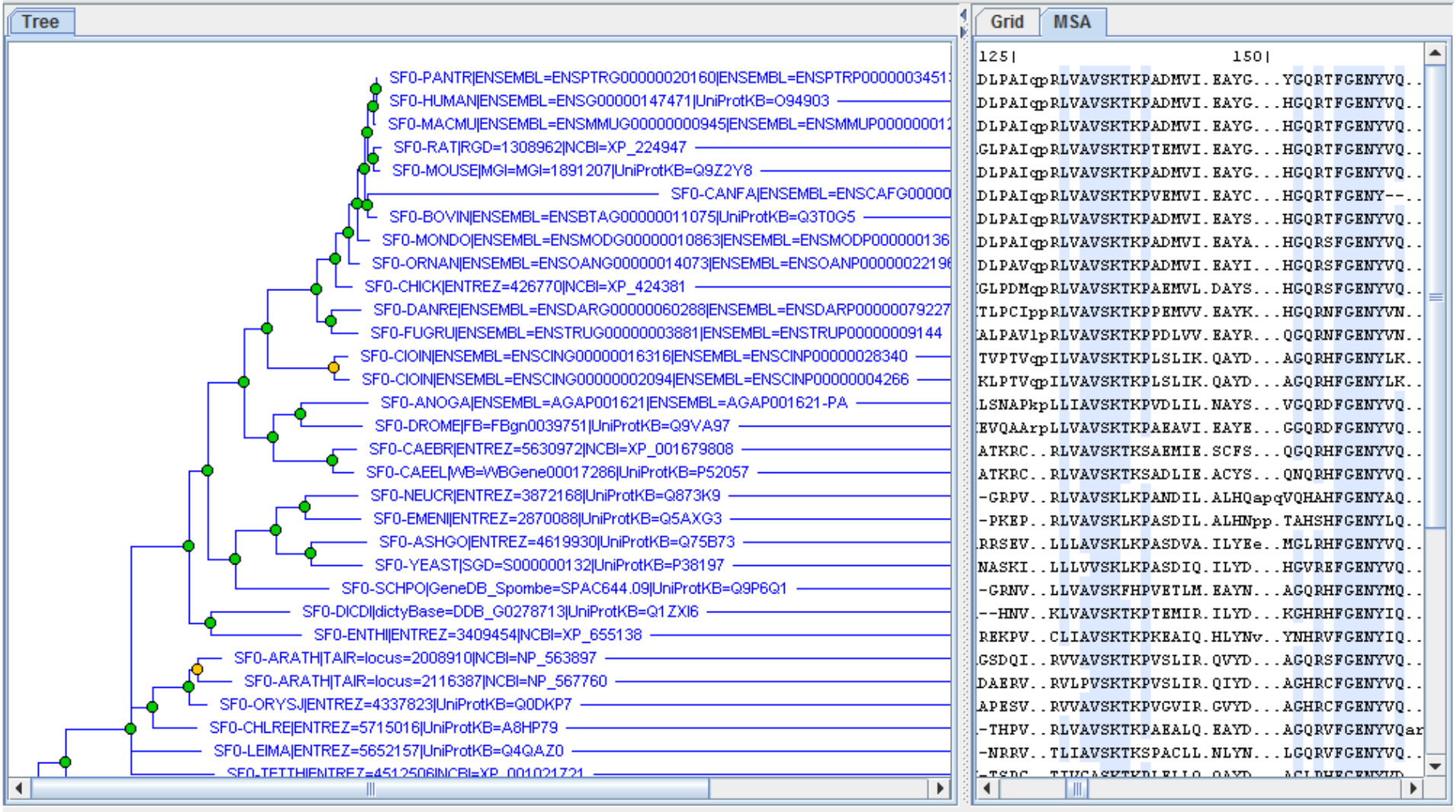
GENES ASSIGNED TO THIS FAMILY

Species	Count
Anopheles gambiae	1
Aquifex aeolicus vf5	1
Arabidopsis thaliana	2

PANTHER TREE VIEWER • close window

Family Name : PROLINE SYNTHETASE CO-TRANSCRIBED BACTERIAL HOMOLOG PROTEIN (PTHR10146)

Tree **MSA**



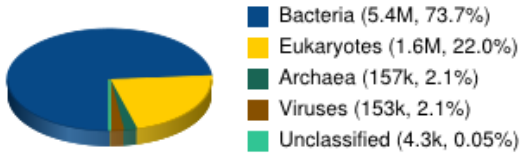
PhyloFacts 3.0.2

PhyloFacts release PF3.0.2 contains 7,337,238 protein sequences from 99,254 unique taxa (including strains) across 92,800 families (25,446 grouped by PFAM domain and 67,354 grouped by multi-domain architecture agreement). More ...

SEQUENCE ACCESSION SEARCH	Query PhyloFacts by UniProt accession or identifier
ORTHOLOG IDENTIFICATION	PhyloFacts Orthology Group: phylogenetic orthologs
JUMP TO PHYLOFACTS FAMILY	View PhyloFacts family alignments, trees, and annotations
PHYLOFACTS-PFAM SEARCH	Query PhyloFacts by Pfam accession (PhyloFacts-Pfam Project)
GENOME COVERAGE	View coverage of key species in PhyloFacts
STATISTICS	View PhyloFacts coverage statistics
DOWNLOADS	Download PhyloFacts data
CITING PHYLOFACTS	How to cite PhyloFacts

PhyloFacts statistics

7.3M unique proteins across the Tree of Life



PhyloFacts is funded by a grant from the Department of Energy, Division of Biological and Environmental Research (details).

Phylofacts
query by sequence search

PHOG0274269_00186 – Proline synthetase co-transcribed bacterial protein

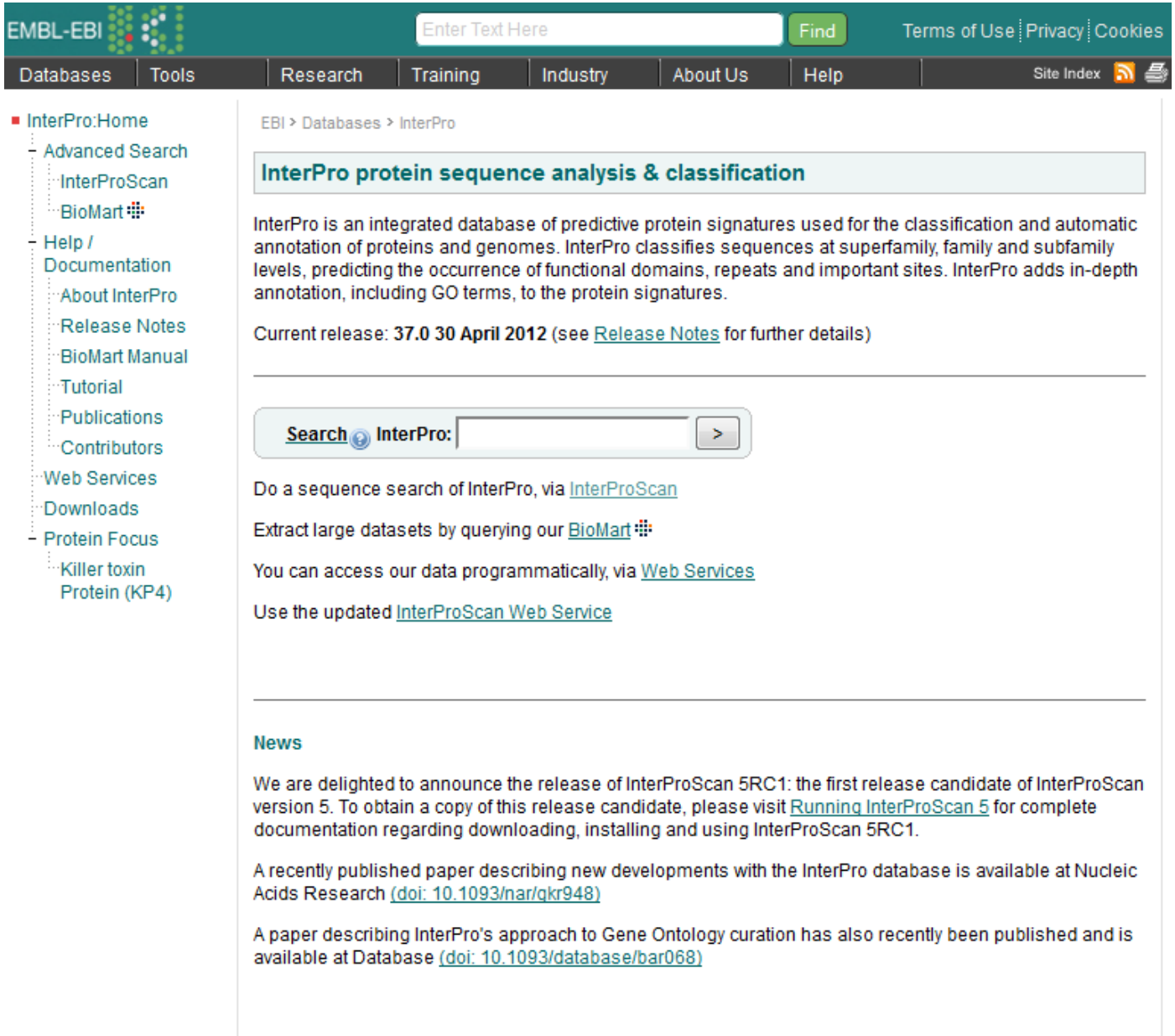
PHOG tree:	View tree
Pfam domains:	Ala_racemase_N
Taxonomic distribution:	stramenopiles
PhyloFacts family:	bpg0243724
Alignment:	Global
Number of sequences:	4
Alignment length:	296

PhyloFacts Orthology Group Members

Gene ID	Species	Description	Swiss	GO	EC	KEGG	Lit.
D8LNZ4	Ectocarpus siliculosus (Brown alga)	Putative uncharacterized protein					
B7GC89	Phaeodactylum tricornutum (strain CCAP 1055/1)	Predicted protein					
D0MS28	Phytophthora infestans T30-4	Proline synthetase co-transcribed bacterial protein					
B8BUT1	Thalassiosira pseudonana (Marine diatom)	Predicted protein					

Download As CSV

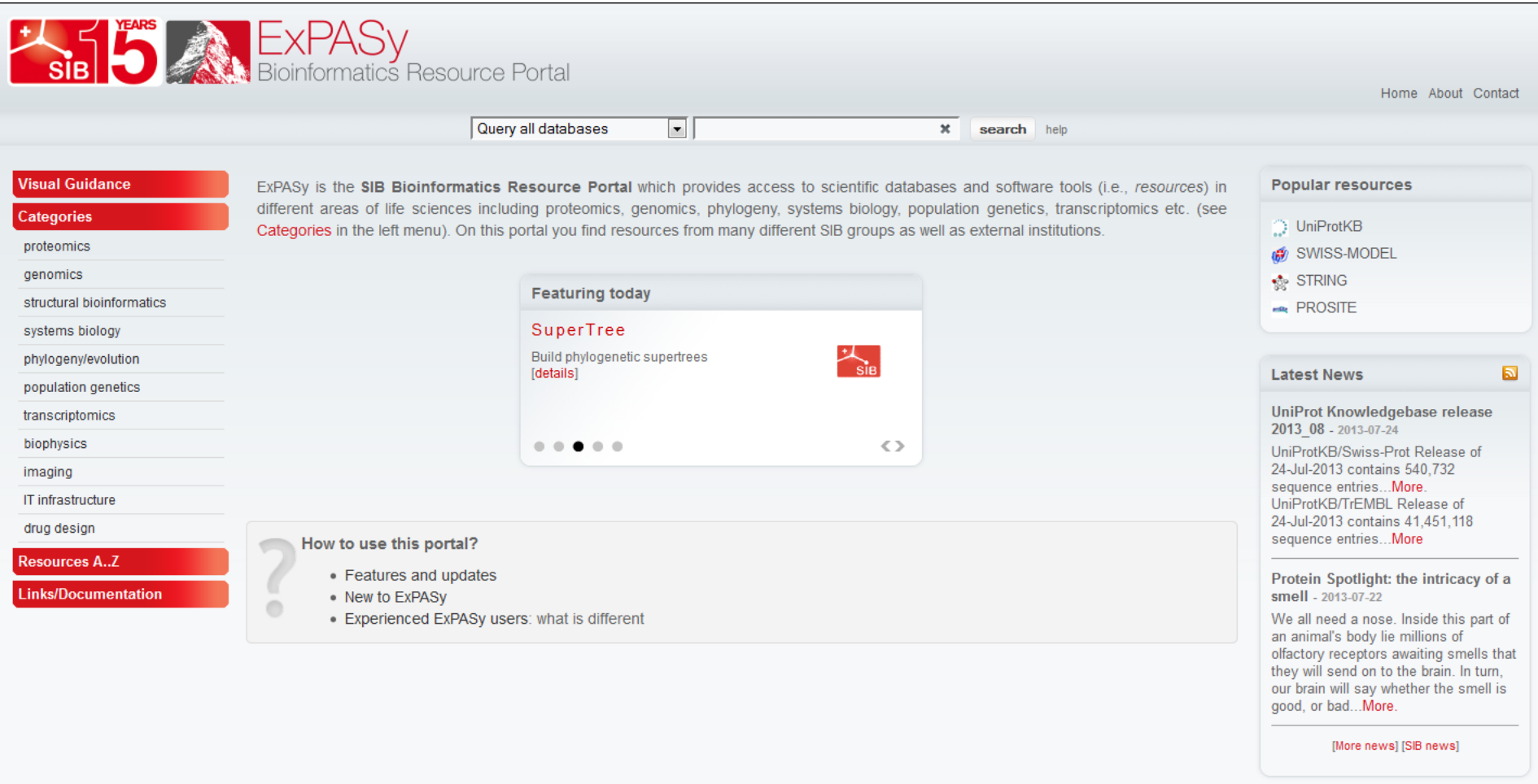
Resource federation: InterPro



Not as easy as it may sound…

- Specific protein families may not be consistent across resources
- Most families (MSAs, trees, HMMs) in these resources are not manually curated
  - Domains in Pfam-A are curated
  - TIGRfams are curated
  - HAMAP families are curated

http://www.expasy.org



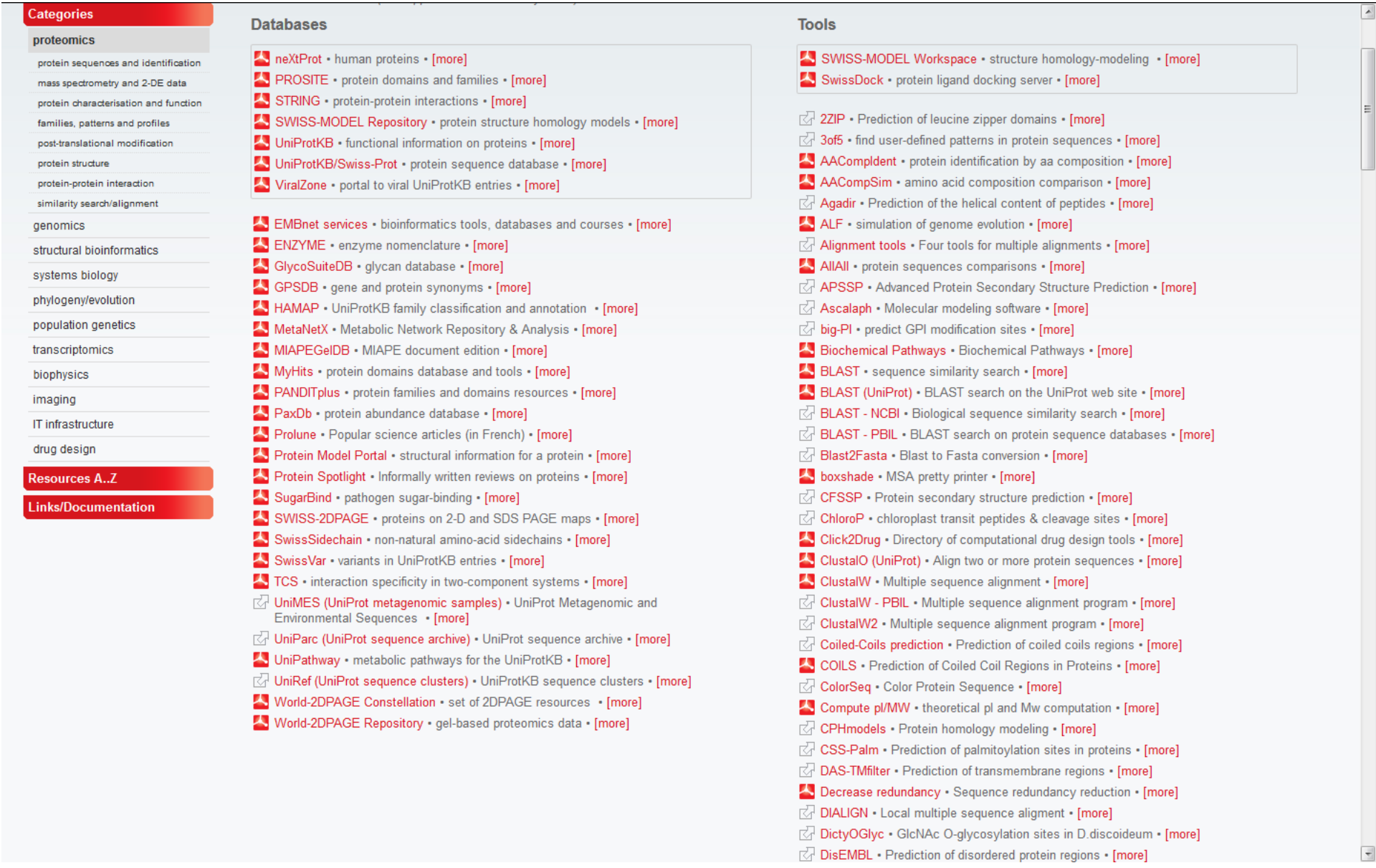
Visual Guidance
Categories
proteomics
genomics
sequence alignment
similarity search
characterisation/annotation
structural bioinformatics
systems biology
phylogeny/evolution
population genetics
transcriptomics
biophysics
imaging
IT infrastructure
drug design
Resources A..Z
Links/Documentation

SIB resources
External resources - *(No support from the ExPASy Team)*

Tools

- Alignment tools • Four tools for multiple alignments • [more]
- boxshade • MSA pretty printer • [more]
- ClustalW • Multiple sequence alignment • [more]
- ClustalW - PBIL • Multiple sequence alignment program • [more]
- ClustalW2 • Multiple sequence alignment program • [more]
- Codon Suite • codon-based sequence analysis • [more]
- Decrease redundancy • Sequence redundancy reduction • [more]
- DIALIGN • Local multiple sequence aligment • [more]
- GENIO/logo • RNA/DNA & Amino Acid Sequence Logos • [more]
- Kalign - EBI • Fast and accurate multiple sequence alignment • [more]
- Kalign - SBC • Fast and accurate multiple sequence alignment • [more]
- LALIGN • Pairwise alignment • [more]
- MADAP • clustering for genome annotation data • [more]
- MAFFT - CBRC • Multiple sequence aligment • [more]
- MAFFT - EBI • Multiple sequence aligment • [more]
- MaxAlign • Gap removal from alignments • [more]
- Multialin • Multiple sequence alignment • [more]
- MUSCLE • Multiple alignment server • [more]
- Newick Utilities • high-throughput phylogenetic tree processing • [more]
- Phylogibbs • regulatory sites discovery • [more]
- SIBsim4 • spliced sequence alignment • [more]
- T-Coffee • sequence and structure multiple alignments • [more]
- T-Coffee - EBI • Multiple sequence alignment program • [more]
- T-Coffee - WUR • Multiple sequence alignment program • [more]
- WebLogo • Sequence logos • [more]

proteômica



Categories

proteomics

- protein sequences and identification
- mass spectrometry and 2-DE data
- protein characterisation and function
- families, patterns and profiles
- post-translational modification
- protein structure
- protein-protein interaction
- similarity search/alignment

genomics

structural bioinformatics

systems biology

phylogeny/evolution

population genetics

transcriptomics

biophysics

imaging

IT infrastructure

drug design

Resources A..Z

Links/Documentation

Databases

- neXtProt • human proteins • [more]
- PROSITE • protein domains and families • [more]
- STRING • protein-protein interactions • [more]
- SWISS-MODEL Repository • protein structure homology models • [more]
- UniProtKB • functional information on proteins • [more]
- UniProtKB/Swiss-Prot • protein sequence database • [more]
- ViralZone • portal to viral UniProtKB entries • [more]

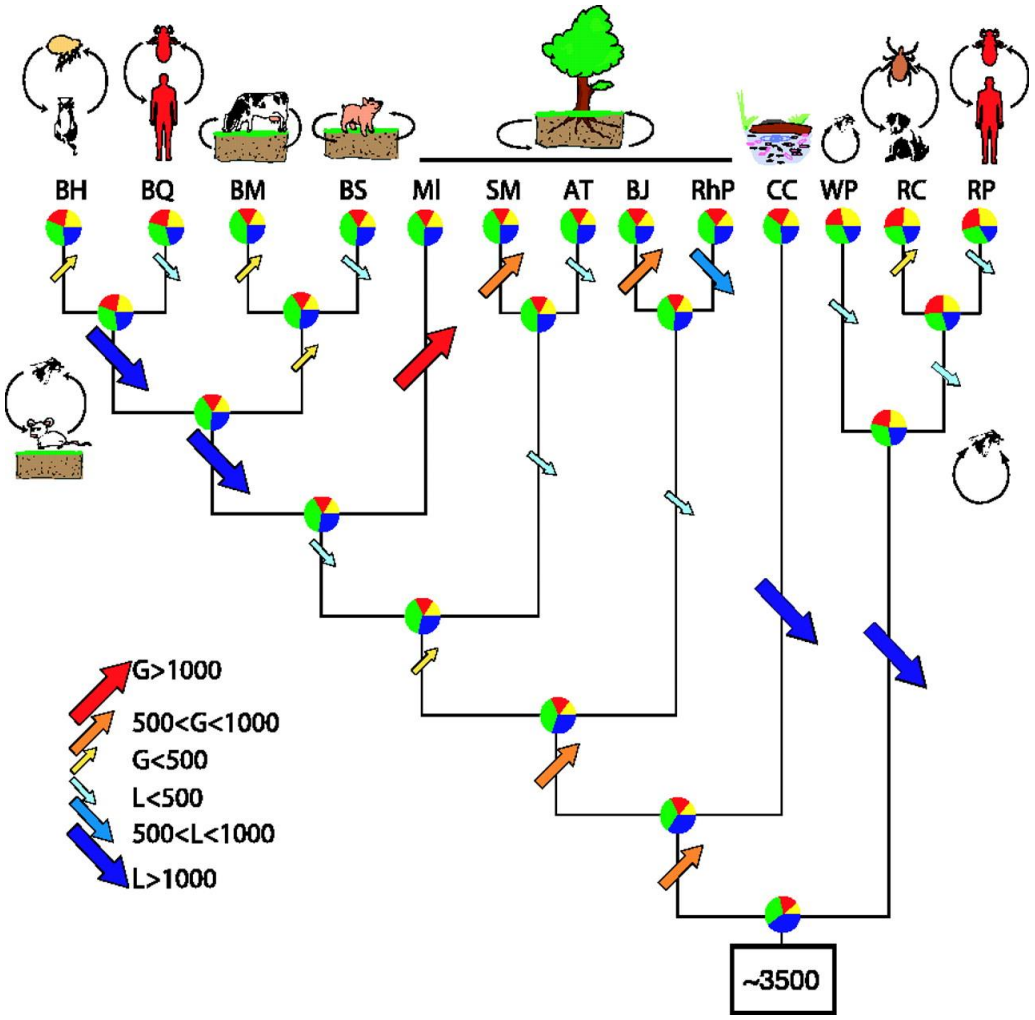
- EMBnet services • bioinformatics tools, databases and courses • [more]
- ENZYME • enzyme nomenclature • [more]
- GlycoSuiteDB • glycan database • [more]
- GPSDB • gene and protein synonyms • [more]
- HAMAP • UniProtKB family classification and annotation • [more]
- MetaNetX • Metabolic Network Repository & Analysis • [more]
- MIAPEGelDB • MIAPE document edition • [more]
- MyHits • protein domains database and tools • [more]
- PANDITplus • protein families and domains resources • [more]
- PaxDb • protein abundance database • [more]
- Prolune • Popular science articles (in French) • [more]
- Protein Model Portal • structural information for a protein • [more]
- Protein Spotlight • Informally written reviews on proteins • [more]
- SugarBind • pathogen sugar-binding • [more]
- SWISS-2DPAGE • proteins on 2-D and SDS PAGE maps • [more]
- SwissSidechain • non-natural amino-acid sidechains • [more]
- SwissVar • variants in UniProtKB entries • [more]
- TCS • interaction specificity in two-component systems • [more]
- UniMES (UniProt metagenomic samples) • UniProt Metagenomic and Environmental Sequences • [more]
- UniParc (UniProt sequence archive) • UniProt sequence archive • [more]
- UniPathway • metabolic pathways for the UniProtKB • [more]
- UniRef (UniProt sequence clusters) • UniProtKB sequence clusters • [more]
- World-2DPAGE Constellation • set of 2DPAGE resources • [more]
- World-2DPAGE Repository • gel-based proteomics data • [more]

Tools

- SWISS-MODEL Workspace • structure homology-modeling • [more]
- SwissDock • protein ligand docking server • [more]

- 2ZIP • Prediction of leucine zipper domains • [more]
- 3of5 • find user-defined patterns in protein sequences • [more]
- AACompIdent • protein identification by aa composition • [more]
- AACompSim • amino acid composition comparison • [more]
- Agadir • Prediction of the helical content of peptides • [more]
- ALF • simulation of genome evolution • [more]
- Alignment tools • Four tools for multiple alignments • [more]
- AllAll • protein sequences comparisons • [more]
- APSSP • Advanced Protein Secondary Structure Prediction • [more]
- Ascalaph • Molecular modeling software • [more]
- big-PI • predict GPI modification sites • [more]
- Biochemical Pathways • Biochemical Pathways • [more]
- BLAST • sequence similarity search • [more]
- BLAST (UniProt) • BLAST search on the UniProt web site • [more]
- BLAST - NCBI • Biological sequence similarity search • [more]
- BLAST - PBIL • BLAST search on protein sequence databases • [more]
- Blast2Fasta • Blast to Fasta conversion • [more]
- boxshade • MSA pretty printer • [more]
- CFSSP • Protein secondary structure prediction • [more]
- ChloroP • chloroplast transit peptides & cleavage sites • [more]
- Click2Drug • Directory of computational drug design tools • [more]
- ClustalO (UniProt) • Align two or more protein sequences • [more]
- ClustalW • Multiple sequence alignment • [more]
- ClustalW - PBIL • Multiple sequence alignment program • [more]
- ClustalW2 • Multiple sequence alignment program • [more]
- Coiled-Coils prediction • Prediction of coiled coils regions • [more]
- COILS • Prediction of Coiled Coil Regions in Proteins • [more]
- ColorSeq • Color Protein Sequence • [more]
- Compute pI/MW • theoretical pI and Mw computation • [more]
- CPHmodels • Protein homology modeling • [more]
- CSS-Palm • Prediction of palmitoylation sites in proteins • [more]
- DAS-TMfilter • Prediction of transmembrane regions • [more]
- Decrease redundancy • Sequence redundancy reduction • [more]
- DIALIGN • Local multiple sequence aligment • [more]
- DictyOGlyc • GlcNAc O-glycosylation sites in D.discoideum • [more]
- DisEMBL • Prediction of disordered protein regions • [more]

Fig. 4. Net gene loss or gain throughout the evolution of the {alpha}-proteobacterial species



Boussau, Bastien et al. (2004) Proc. Natl. Acad. Sci. USA 101, 9722-9727