2-1. 配列解析基礎

Basic Sequence Analysis

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情報・システム研究機構

ライフサイエンス統合データベース

センター (DBCLS)

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  - –京大化研→理研GSC→埼玉医大→DBCLS
- 専門:
  - –バイオインフォマティクス、とくにDB関係
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この講習の内容

1. 配列解析の歴史
2. 配列、ゲノムデータ記述のフォーマット
   - アクセッション番号
3. 基礎的な配列比較解析の原理と実習
   - アラインメント(DP)
   - データベース検索(BLAST, BLAT,GGRNA)

なぜ相同性じゃなく類似性か

- 遺伝学では、相同性という言葉はタンパク質のアミノ酸配列や遺伝子の塩基配列が共通の祖先をもつときに用いる
- バイオインフォマティクスでは、タンパク質やDNAでの相同性は、配列類似性に基づいて判断される
  - –http://togetter.com/li/307635

1. 配列解析(主に類似性)の歴史

1970	Needleman-Wunsch法
1977	バクテリオファージ(φX174) ゲノム解読(初)
1981	Smith-Waterman法
1988	FASTA論文, NCBI設立
1990	BLAST論文
1995	*H.influenzae* ゲノム解読 (free-living organism初)
1997	BLAST2(Gapped BLAST, PSI-BLAST)論文
2002	BLAT(BLAST Like Alignment Tool)論文
2003	*Homo sapiens* ゲノム解読 (最初のヒトゲノム)
2009	BWA, bowtie論文
2012	GGRNA論文

Needleman-Wunsch

- 配列比較をコンピュータ化
- Global alignment

J. Mol. Biol. (1970) 48, 443–453

A General Method Applicable to the Search for Similarities in the Amino Acid Sequence of Two Proteins

SAUL B. NEEDLEMAN AND CHRISTIAN D. WUNSCH

Department of Biochemistry, Northwestern University, and
Nuclear Medicine Service, V. A. Research Hospital
Chicago, Ill. 60611, U.S.A.

(*Received 21 July 1969*)

Smith-Waterman

- Local alignment

J. Mol. Biol. (1981), **147**, 195–197

Identification of Common Molecular Subsequences

The identification of maximally homologous subsequences among sets of long sequences is an important problem in molecular sequence analysis. The problem is straightforward only if one restricts consideration to contiguous subsequences (segments) containing no internal deletions or insertions. The more general problem has its solution in an extension of sequence metrics (Sellers 1974; Waterman *et al.*, 1976) developed to measure the minimum number of "events" required to convert one sequence into another.

These developments in the modern sequence analysis began with the heuristic

Goad-Kanehisa

- Local alignment

Volume 10 Number 1 1982 Nucleic Acids Research

Pattern recognition in nucleic acid sequences. I. A general method for finding local homologies and symmetries

Walter B.Goad and Minoru I.Kanehisa

Theoretical Biology and Biophysics Group, University of California, Los Alamos National Laboratory, Los Alamos, NM 87545, USA

Received 15 September 1981

ABSTRACT

We present an algorithm--a generalization of the Needleman-Wunsch-Sellers

大域的／局所的アラインメント

- 大域的アラインメント(Global alignment)
  - –配列中の全塩基(アミノ酸)がアラインされるようにしたもの
  - –Needleman-Wunsch algorithm
- 局所的アラインメント(Local alignment)
  - –部分的な類似が見つけられるようにしたもの
  - –Smith-Waterman algorithm
  - –Goad-Kanehisa algorithm
  - –配列類似性検索へ応用

```
Global FTFTALILLAVAV
       F--TAL-LLA-AV

Local  FTFTALILL-AVAV
       --FTAL-LLAAV--
```

http://upload.wikimedia.org/wikipedia/commons/4/4b/Global-local-alignment.png

FASTA

- 配列類似性検索プログラムのハシリ
- FASTA形式に名を残している

Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 2444-2448, April 1988
Biochemistry

Improved tools for biological sequence comparison

(amino acid/nucleic acid/data base searches/local similarity)

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*Department of Biochemistry, University of Virginia, Charlottesville, VA 22908; and †Mathematical Research Branch, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

Communicated by Gerald M. Rubin, December 2, 1987 (received for review September 17, 1987)

ABSTRACT We have developed three computer programs for comparisons of protein and DNA sequences. They can be used to search sequence data bases, evaluate similarity scores, and identify periodic structures based on local sequence similarity. The FASTA program is a more sensitive

FASTP and FASTA ach
tivity in the first step, b
identities or groups of id
acid sequences during t
The *ktup* parameter dete

BLAST

- Basic Local Alignment Search Tool
- 配列類似性検索のデファクトスタンダード

J. Mol. Biol. (1990) **215**, 403–410

Basic Local Alignment Search Tool

Stephen F. Altschul[1], Warren Gish[1], Webb Miller[2]
Eugene W. Myers[3] and David J. Lipman[1]

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National Library of Medicine, National Institutes of Health
Bethesda, MD 20894, U.S.A.

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The Pennsylvania State University, University Park, PA 16802, U.S.A.

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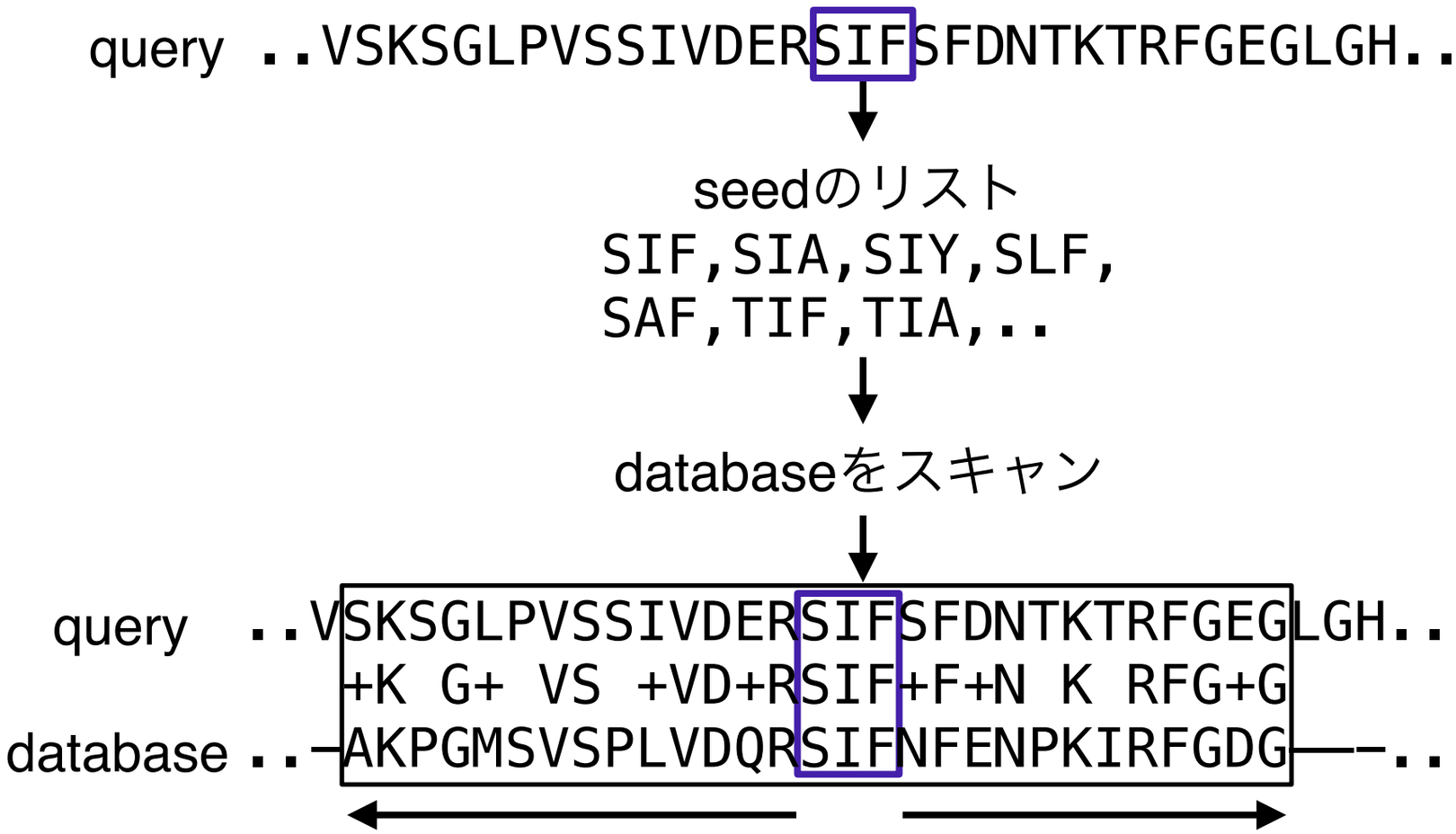
University of Arizona, Tucson, AZ 85721, U.S.A.

(Received 26 February 1990; accepted 15 May 1990)

BLAST algorithm



seedを中心に前後にギャップなしでalignmentを延ばしてHSP(High Scoring Pair)を得る

HSPを組み合わせて統計学的評価が有意なものを順に抽出

配列類似性検索

- query: 質問配列
  - –核酸配列
  - –アミノ酸配列
- DB: 検索対象のDB
  - –核酸配列
  - –アミノ酸配列
- 閾値などのパラメータ
  - –期待値(E)

DB→ query↓	核酸 配列	アミノ酸 配列
核酸 配列	blastn tblastx	blastx
アミノ酸 配列	tblastn	blastp

※blastnだけが核酸配列レベルでの比較。
残り全てはアミノ酸配列レベルの比較

BLAST2

- BLASTでgapを許容するように
- Position-Specific Iterated (PSI:ψ) BLAST

Nucleic Acids Research, 1997, Vol. 25, No. 17 **3389–3402**

Gapped BLAST and PSI-BLAST: a new generation of protein database search programs

Stephen F. Altschul*, Thomas L. Madden, Alejandro A. Schäffer[1], Jinghui Zhang, Zheng Zhang[2], Webb Miller[2] and David J. Lipman

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA, [1]Laboratory of Genetic Disease Research, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA and [2]Department of Computer Science and Engineering, Pennsylvania State University, University Park, PA 16802, USA

Received June 20, 1997; Revised and Accepted July 16, 1997

BLAT

- DBがgenomeに特化した配列類似性検索
  - –genome landing toolとも呼ばれる
- 企業には有償のライセンス
  - –依然としてBLASTを使う例も多く

Resource

BLAT—The BLAST-Like Alignment Tool

W. James Kent

Department of Biology and Center for Molecular Biology of RNA, University of California, Santa Cruz, Santa Cruz, California 95064, USA

ヒトゲノム解読、そしてNGS

- 2003年ヒトゲノム解読 by Sanger sequencer
- Non-Sanger Sequencing (NGS)の開発
  - –pyrosequencing → Roche 454
  - –Solexa(Sequence by synthesis) → Illumina
  - –等々

BWA, bowtie

- queryがNGSの出力(FASTQ)
- DBのindex化
  - –Suffix array
  - –Burrows-Wheeler transform

BIOINFORMATICS ORIGINAL PAPER Vol. 26 no. 5 2010, pages 589–595
doi:10.1093/bioinformatics/btp698

Sequence analysis

Advance Access publication January 15, 2010

Fast and accurate long-read alignment with Burrows–Wheeler transform

Heng Li and Richard Durbin*
Wellcome Trust Sanger Institute, Wellcome Genome Campus, Cambridge, CB10 1SA, UK

Associate Editor: Dmitrij Frishman

ABSTRACT

Motivation: Many programs for aligning short sequencing reads to a reference genome have been developed in the last 2 years. Most of them are very efficient for short reads but inefficient or not applicable for reads >200 bp because the algorithms are heavily and specifically tuned for short queries with low sequencing error rate. However, some sequencing platforms already produce longer reads and others are expected to become available soon. For longer reads, hashing-based software such as BLAT and SSAHA2 remain the only choices. Nonetheless, these methods are substantially slower than short-read aligners in terms of aligned bases per unit time.

no longer than 100 bp. Efficiently aligning long reads against a long reference sequence like the human genome poses a new challenge to the development of alignment tools.

Long-read alignment has different objectives from short-read alignment. First, in short-read alignment, we would usually like to align the full-length read to reduce the reference bias caused by the mismatches toward the ends of the read. Given this requirement, we can design spaced seed templates (Ma *et al*., 2002) spanning the entire read (Jiang and Wong, 2008; Lin *et al*., 2008; Smith *et al*., 2008), or quickly filter out poor matches, for example, by applying q-gram filtration (Rumble *et al*., 2009; Weese *et al*., 2009)

Software

Ultrafast and memory-efficient alignment of short DNA sequences to the human genome

Ben Langmead, Cole Trapnell, Mihai Pop and Steven L Salzberg

Address: Center for Bioinformatics and Computational Biology, Institute for Advanced Computer Studies, University of Maryland, College Park, MD 20742, USA.

Correspondence: Ben Langmead. Email: langmead@cs.umd.edu

Published: 4 March 2009

Genome ***Biology*** 2009, **10:**R25 (doi:10.1186/gb-2009-10-3-r25)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2009/10/3/R25

Received: 21 October 2008
Revised: 19 December 2008
Accepted: 4 March 2009

GGRNA

- queryは自然言語でも核酸／アミノ酸配列でも
  - –ほぼ一致検索でBLASTよりもはるかに高速に
- DBはRefSeq, INSD(DDBJ他), genome
  - –検索対象の絞込でさらに高速、得たい結果だけをユーザーに

W592–W596 *Nucleic Acids Research, 2012, Vol. 40, Web Server issue* *Published online 28 May 2012*
doi:10.1093/nar/gks448

GGRNA: an ultrafast, transcript-oriented search engine for genes and transcripts

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Received February 25, 2012; Revised April 23, 2012; Accepted April 29, 2012

2. データフォーマット(形式)

ファイルフォーマット	ファイル拡張子
FASTA	.fa .fasta
DDBJ(Genbank)	.dbj (.gbk)
FASTQ	.fq .fastq
SRA/SRA-lite	.sra .lite.sra
SAM/BAM	.sam .bam
GTF(GFF)	.gtf .gff
BED	.bed
VCF	.vcf

.doc は配列データ形式ではない

- 一般にはファイル形式は以下の2つ

1. アスキー(ASCII)形式(テキスト形式)

   後述のデータ形式はほぼこちら。プレーンテキスト

2. バイナリ(binary)形式

   一部のデータ形式がこちら。データの読み込みを早くする、データサイズを小さくする目的で

- 参考

  –バイオインフォマティクス第二版 p38-「2.6 配列は特別なファイル形式でコンピュータに蓄積する」

FASTA

- 配列類似性検索プログラムFASTAで使われる配列データ形式
  - 日本では「ふぁすた」、欧米では「ふぁすと・えー」
- 1行目に“>”で始まる1行のヘッダ行
- 2行目以降に実際のシーケンス文字列

```
>gi|5524211|gb|AAD44166.1| cytochrome b [Elephas maximus maximus]
LCLYTHIGRNIYYGSYLYSETWNTGIMLLLITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTNLV
EWIWGGFSVDKATLNRFFAFHFILPFTMVALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYYTIKDFLG
LLILILLLLLLALLSPDMLGDPDNHMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALFLSIVIL
GLMPFLHTSKHRSMMLRPLSQALFWTLTMDLLTLTWIGSQPVEYPYTIIGQMASILYFSIILAFLPIAGX
IENY
```

参考: http://ja.wikipedia.org/wiki/FASTA

アクセッション番号

- INSD(International Nucleotide Sequence Database)の登録(accession)番号
  - –論文掲載の必須条件
  - –データを他の研究者に再利用してもらうことが研究の価値を高める上でとても大事
- 日本だとDDBJへ
  - –日本語でのやりとり可
- 例: AB016472.1

DDBJ(Genbank)

- INSD(DDBJ/EMBL/GenBank)を記述するフォーマット
- 配列データの基本中の基本

```
LOCUS       AB016472                3508 bp    DNA     linear   PLN 14-FEB-2004
DEFINITION  Arabidopsis thaliana ARR2 gene for ARR2 protein, complete cds.
ACCESSION   AB016472
VERSION     AB016472.1
KEYWORDS    .
SOURCE      Arabidopsis thaliana (thale cress)
  ORGANISM  Arabidopsis thaliana
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; Gunneridae;
            Pentapetalae; rosids; malvids; Brassicales; Brassicaceae;
            Camelineae; Arabidopsis.
REFERENCE   1  (bases 1 to 3508)
  AUTHORS   Sakai,H., Aoyama,T. and Oka,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (22-JUL-1998) to the DDBJ/EMBL/GenBank databases.
            Contact:Hiroe Sakai
            Institute for Chemical Research, Kyoto University, Division of
            Molecular Biology and Information; Gokasho, Uji, Kyoto 611-0011,
            Japan
```

```
REFERENCE   2
  AUTHORS   Sakai,H., Aoyama,T., Bono,H. and Oka,A.
  TITLE     Two-component response regulators from Arabidopsis thaliana
            contain a putative DNA-binding motif
  JOURNAL   Plant Cell Physiol. 39, 1232-1239 (1998)
COMMENT
FEATURES             Location/Qualifiers
     source          1..3508
                     /db_xref="taxon:3702"
                     /ecotype="Columbia"
                     /mol_type="genomic DNA"
                     /organism="Arabidopsis thaliana"
     exon            1..465
     CDS             join(306..465,939..1091,1176..1580,1671..1747,1825..2955,
                     3043..3111)
                     /codon_start=1
                     /gene="ARR2"
                     /note="putative"
                     /product="ARR2 protein"
                     /protein_id="BAA74527.1"
                     /transl_table=1
                     /translation="MVNPGHGRGPDSGTAAGGSNSDPFPANLRVLVVDDDPTCLMILE
                     RMLMTCLYRVTKCNRAESALSLLRKNKNGFDIVISDVHMPDMDGFKLLEHVGLEMDLP
                     VIMMSADDSKSVVLKGVTHGAVDYLIKPVRIEALKNIWQHVVRKKRNEWNVSEHSGGS
                     IEDTGGDRDRQQQHREDADNNSSSVNEGNGRSSRKRKEEEVDDQGDDKEDSSSLKKPR
                     VVWSVELHQQFVAAVNQLGVDKAVPKKILEMMNVPGLTRENVASHLQKYRIYLRRLGG
                     VSQHQGNMNHSFMTGQDQSFGPLSSLNGFDLQSLAVTGQLPPQSLAQLQAAGLGRPTL
                     AKPGMSVSPLVDQRSIFNFENPKIRFGDGHGQTMNNGNLLHGVPTGSHMRLRPGQNVQ
                     SSGMMLPVADQLPRGGPSMLPSLGQQPILSSSVSRRSDLTGALAVRNSIPETNSRVLP
                     TTHSVFNNFPADLPRSSFPLASAPGISVPVSVSYQEEVNSSDAKGGSSAATAGFGNPS
                     YDIFNDFPQHQQHNKNISNKLNDWDLRNMGLVFSSNQDAATATATAAFSTSEAYSSSS
                     TQRKRRETDATVVGEHGQNLQSPSRNLYHLNHVFMDGGSVRVKSERVAETVTCPPANT
                     LFHEQYNQEDLMSAFLKQEGIPSVDNEFEFDGYSIDNIQV"
     intron          466..938
     exon            939..1091
```

```
                     TQRKRRETDATVVGEHGQNLQSPSRNLYHLNHVFMDGGSVRVKSERVAETVTCPPANT
                     LFHEQYNQEDLMSAFLKQEGIPSVDNEFEFDGYSIDNIQV"
     intron          466..938
     exon            939..1091
     intron          1092..1175
     exon            1176..1580
     intron          1581..1670
     exon            1671..1747
     intron          1748..1824
     exon            1825..2955
     intron          2956..3042
     exon            3043..3508
BASE COUNT          983 a         658 c          761 g         1106 t
ORIGIN
        1 attagttcaa cttacttcaa aaaaagaaat gtaacagaga aatccgagct ttcaagctgt
       61 gaaacatagc catgcccttc ataaatcttt ctactaatta ttttttttc acgtaagatt
      121 ctcaaaacaa atcaaaatct catcttcttg gtatcaaaat tttcttactt tttttggttt
      181 tgttatcccg aatttttaga atcaaaaccc acgtcaattc ttttcaaag gcatatattc
      241 tctctgtttc aaactttgtg tctcttcttc tccttctctg atcgttcgtt ttctggacga
      301 gagagatggt aaatccgggt cacggaagag gacccgattc gggtactgct gctggtgggt
      361 caaactccga cccgtttcct gcgaatcttc gagttcttgt cgttgatgat gatccaactt
      421 gtctcatgat cttagagagg atgcttatga cttgtctcta cagaggtaaa attcaatcaa
      481 aatttcgttt taatattaat ctaaaatttt attaatttca aaggtttcat gttgaattat
      541 gatccaattt gtgaaaattt attgtaattt ttgtggttaa ctatggattt taattacggt
      601 ggttgactct gccaaaaata gtaaacagag cttatggaat tgactagaag ttttcattga
      661 ctgttgaatg aatcactgtt agattcagat atatatacat cgcttagttc tctgttctgt
      721 tctaatattg tgatctgctt aaagattctc cttttgaggt ttattttcaa tagatctttt
      781 agttctcaag aaaagtgtga ttcaaatttc ttaaattttg ttcaaaggtt ttaagagtta
      841 gtttgagatt ctggtattta ttttgatcca gttctgtttt cgattatttt caaaattttg
      901 ttttttgatg ctttttgact ctgattctgt tattacagta actaaatgta acagagcaga
      961 gagcgcattg tctctgcttc ggaagaacaa gaatggtttt gatattgtca ttagtgatgt
     1021 tcatatgcct gacatggatg gtttcaagct ccttgaacac gttggtttag agatggattt
     1081 acctgttatc agtacgtatc ttaatcgcta aaaacttatt attaactcta attcgttttc
     1141 ttgagatctg aatgttttga aacctttggt tatagtgatg tctgcggatg attcgaagag
     1201 cgttgtgttg aaaggagtga ctcacggtgc agttgattac ctcatcaaac cggtacgtat
```

FASTQ

- NGS配列データ形式のデファクトスタンダード
  - –プレーンテキスト。ファイル拡張子: .fq .fastq など
  - –1行目に“@”で始まる1行のヘッダ行
  - –2行目に実際の塩基配列
  - –3行目に”+”
  - –4行目に2行目に記述した配列のクオリティ値

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

参考: http://ja.wikipedia.org/wiki/Fastq

SRA, SRA-lite

- FASTQ形式の代わりに使われている、NGS配列データ配布フォーマット
  - 配列拡張子: .sra .lite.sra
- SRA-toolkitを使ってFASTQを生成できる
  - http://www.ncbi.nlm.nih.gov/Traces/sra/?view=software

```
fastq-dump -A SRR233129 SRR233129.lite.sra
```

SAM/BAM

- ゲノムマッピングしたときに生成されるアラインメントのフォーマット

–リファレンスゲノム配列に対するアラインメント

- SAMはプレーンテキスト(ASCII)形式なのに対して、BAMはバイナリ(binary)形式

```
1:497:R:-272+13M17D24M      113  1   497   37  37M    15  100338662  0     CGGGTCTGACCTGAGGAGAACTGTGCTCCGCCTTCAG  0;==-==9;>>>>>=>>>>>>>>>>>
19:20389:F:275+18M2D19M     99   1   17644 0   37M    =   17919 314   TATGACTGCTAATAATACCTACACATGTTAGAACCAT      >>>>>>>>>>>>>>>>>>>><<>>><<>>4
19:20389:F:275+18M2D19M     147  1   17919 0   18M2D19M =     17644 -314  GTAGTACCAACTGTAAGTCCTTATCTTCATACTTTGT      ;44999;499<8<8<<<8<<><<<<<
9:21597+10M2I25M:R:-209     83   1   21678 0   8M2I27M  =     21469 -244  CACCACATCACATATACCAAGCCTGGCTGTGTCTTCT      <;9<<5><<<<><<<<><<<<><<9:
```

参考: http://genome.sph.umich.edu/wiki/SAM

GTF(GFF)

- General Transfer Format. GFF(General Feature Format)のversion2
- ゲノムアノテーションのフォーマット
  - –例: ゲノム上のどこに遺伝子があるか

X	Ensembl	Repeat	2419108	2419128	42	.	. hid=trf; hstart=1; hend=21
X	Ensembl	Repeat	2419108	2419410	2502	-	. hid=AluSx; hstart=1; hend=303
X	Ensembl	Repeat	2419108	2419128	0	.	. hid=dust; hstart=2419108; hend=2419128
X	Ensembl	Pred.trans.	2416676	2418760	450.19	-	2 genscan=GENSCAN00000019335
X	Ensembl	Variation	2413425	2413425	.	+	.
X	Ensembl	Variation	2413805	2413805	.	+	.

参考: http://asia.ensembl.org/info/website/upload/gff.html

BED

- ゲノムアノテーションのフォーマット
  - 例: ゲノム上のどこに遺伝子があるか

```
track name=pairedReads description="Clone Paired Reads" useScore=1
chr22 1000 5000 cloneA 960 + 1000 5000 0 2 567,488, 0,3512
chr22 2000 6000 cloneB 900 - 2000 6000 0 2 433,399, 0,3601
```

参考: http://genome.ucsc.edu/FAQ/FAQformat.html#format1

VCF

- Variant Call Format
- 配列の多型を記述するフォーマット

```
##fileformat=VCFv4.0
##fileDate=20110705
##reference=1000GenomesPilot-NCBI37
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS    ID        REF  ALT     QUAL FILTER INFO                              FORMAT      Sample1         Sample2         Sample3
2     4370   rs6057    G    A       29   .      NS=2;DP=13;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:52,51  1|0:48:8:51,51  1/1:43:5:.,.
2     7330   .         T    A       3    q10    NS=5;DP=12;AF=0.017               GT:GQ:DP:HQ 0|0:46:3:58,50  0|1:3:5:65,3    0/0:41:3
2     110696 rs6055    A    G,T     67   PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2    2/2:35:4
2     130237 .         T    .       47   .      NS=2;DP=16;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60  0|0:48:4:56,51  0/0:61:2
2     134567 microsat1 GTCT G,GTACT 50   PASS   NS=2;DP=9;AA=G                    GT:GQ:DP    0/1:35:4        0/2:17:2        1/1:40:3
```

参考: http://en.wikipedia.org/wiki/Variant_Call_Format

RNAseq だけでもこんなにも

データ形式が…

```
SRR001356.1 2023DAAXX:5:1:123:563 length=33
TGTCGGTCCAGCTCGGCCTTGGGCTCCGTTTTC
+SRR001356.1 2023DAAXX:5:1:123:563 length=33
-IIIIIIII8IIIIIIIIIII6IIIIIIIII9I
@SRR001356.2 2023DAAXX:5:1:123:746 length=33
TCTGAACTCGACTCCTTTTGATCGGCCTCGGC
+SRR001356.2 2023DAAXX:5:1:123:47[illegible] length=33
IIIIIII[illegible]
@SRR001356.3 2023DAAXX:5:1:121:746 length=33
GTGGCAGCGTTTTTGGGCCCGCCGCTTGCCGTT
+SRR001356.3 2023DAAXX:5:1:121:746 length=33
IIIII&IIIIIIIIIIIIIIIIHI1IIIIIIII
```

FASTQ

1.tophat (bowtie)

ゲノム

.fa

ゲノムに対する多重配列アラインメント

.bam

2.cufflinks

.gtf

ゲノムアノテーション

遺伝子アノテーション

予測転写単位ごとの（推定）発現量情報

3.cummeRbund

3. 配列解析基礎の演習

- ペアワイズの比較: Dynamic Programming(DP)
  - –dotplot、 Needleman-Wunsch法、 Smith-Waterman法
  - – 【演習1】 EMBOSSのdotplotとneedle,water
- FASTAからBLASTへ、そしてBLAT
  - – 【演習2】 localBLAST
- Suffix Array時代: BWA, bowtie & GGRNA
  - – 【演習3】 GGRNA API

演習1の準備

- 使うプログラムの準備
  - –EMBOSSがインストールされているので済
  - –dottup
  - –needle, water
- 比較する2本の配列の取得
  - –比較したい2本(核酸配列orアミノ酸配列)をFASTA フォーマットで

例1-1：DJ-1(PARK7)

- HsDJ1.pep.fa

```
>gi|31543380|ref|NP_009193.2| protein DJ-1 [Homo sapiens]
MASKRALVILAKGAEEMETVIPVDVMRRAGIKVTVAGLAGKDPVQCSRDVVICPDASLEDAKKEGPYDVV
VLPGGNLGAQNLSESAAVKEILKEQENRKGLIAAICAGPTALLAHEIGFGSKVTTHPLAKDKMMNGGHYT
YSENRVEKDGLILTSRGPGTSFEFALAIVEALNGKEVAAQVKAPLVLKD
```

と

- BmDJ1.pep.fa

```
>gi|350534642|ref|NP_001232899.1| DJ-1 beta [Bombyx mori]
MSKSALVILAQGAEEMETVITVDMLRRGGVTVTLAGLEGSSPVLCSRQVTLVPDKSLTEALAEKQQYDAV
ILPGGLEGSDSLSKSEKVGALLKDHEDNGKIIAAICAAPIAFAAHGVARGRRVTSYPSTRDKLSAGDYTY
VEGERVVVDGNVVTSRGPGTAYWFGLTLIELLTGKEKADQVEKGMLISQY
```

の比較。それぞれ標記のファイル名で保存

配列取得方法

- IDをみると…(FASTAフォーマットの注釈行)
- RefSeqのようなので
  - –NCBIで直接IDで検索
  - –GGRNAでID検索
  - –ググる!
  - –togowsの利用 http://togotv.dbcls.jp/20110425.html
    - http://togows.dbcls.jp/entry/protein/NP_009193.fasta
    - http://togows.dbcls.jp/entry/protein/NP_001232899.fasta

dottup

- とりあえず、実行しましょう

```
dottup -asequence HsDJ1.pep.fa -bsequence BmDJ1.pep.fa -wordsize 4
```

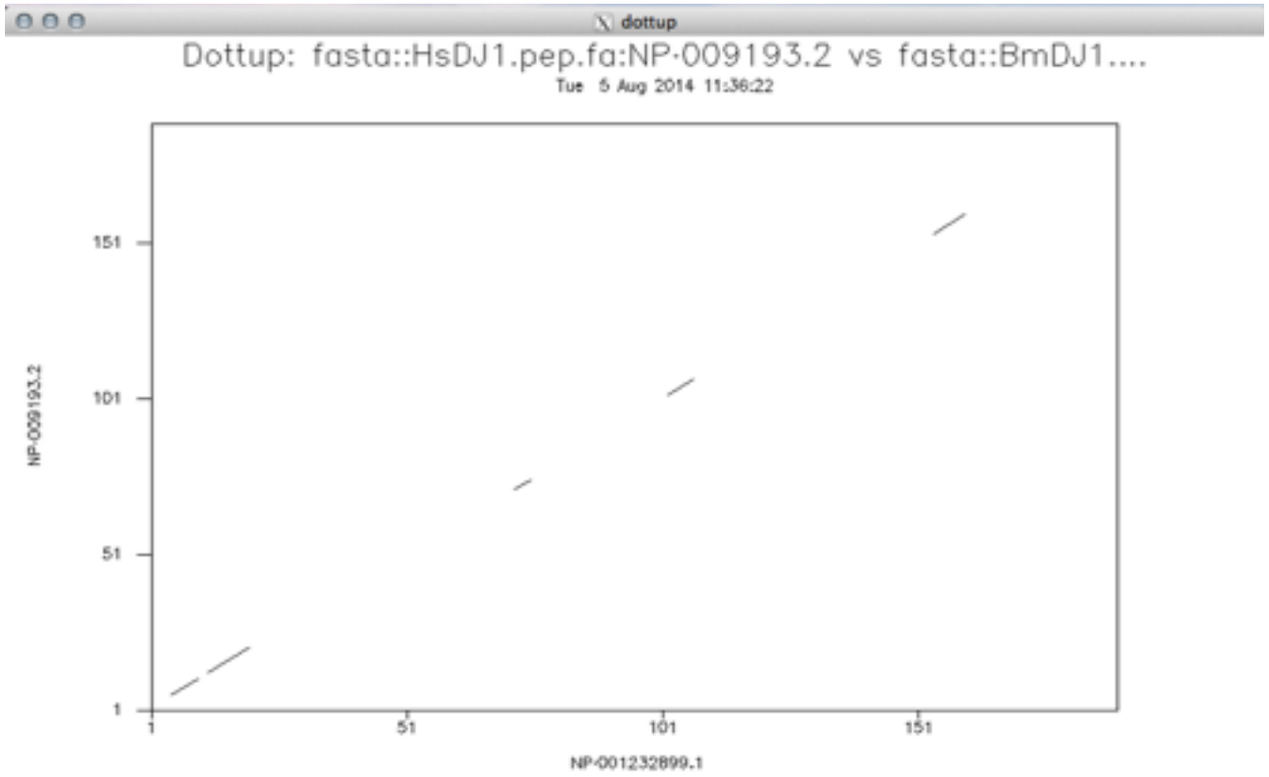
- wordsizeを10にすると?

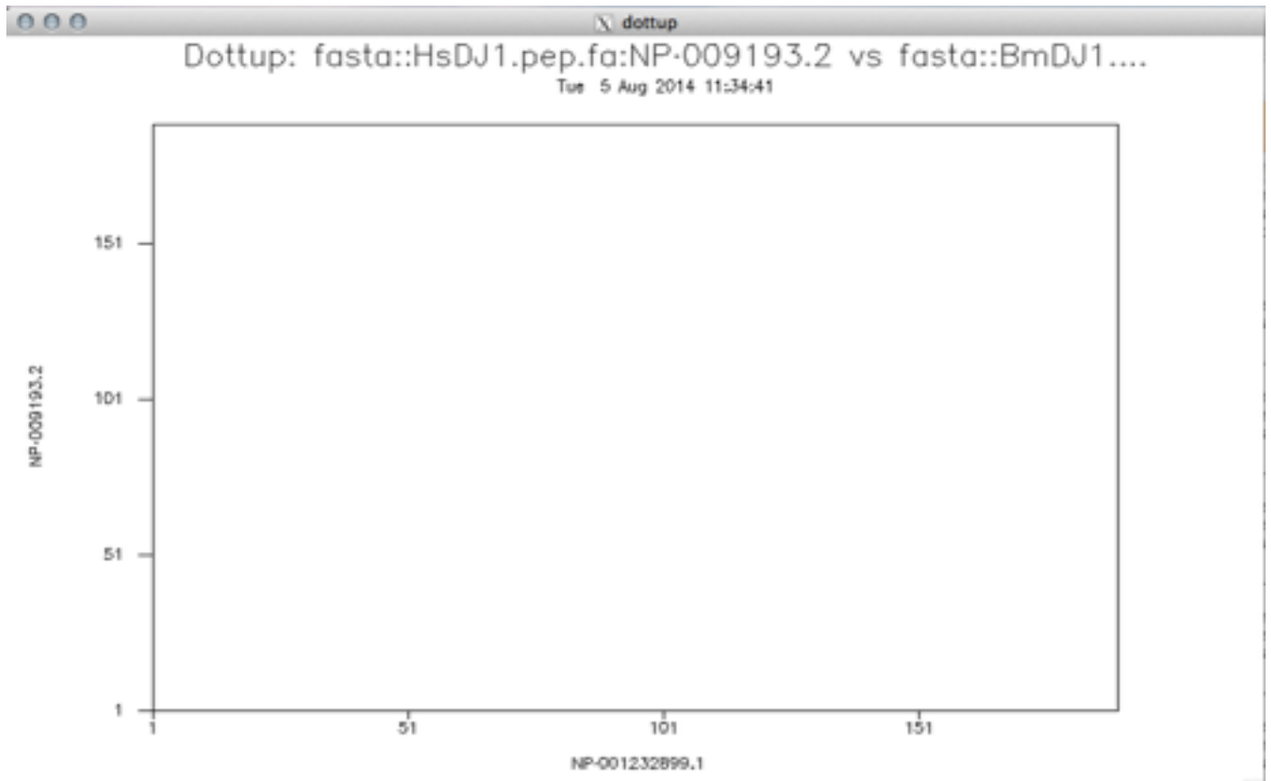
```
dottup -asequence HsDJ1.pep.fa -bsequence BmDJ1.pep.fa -wordsize 10
```

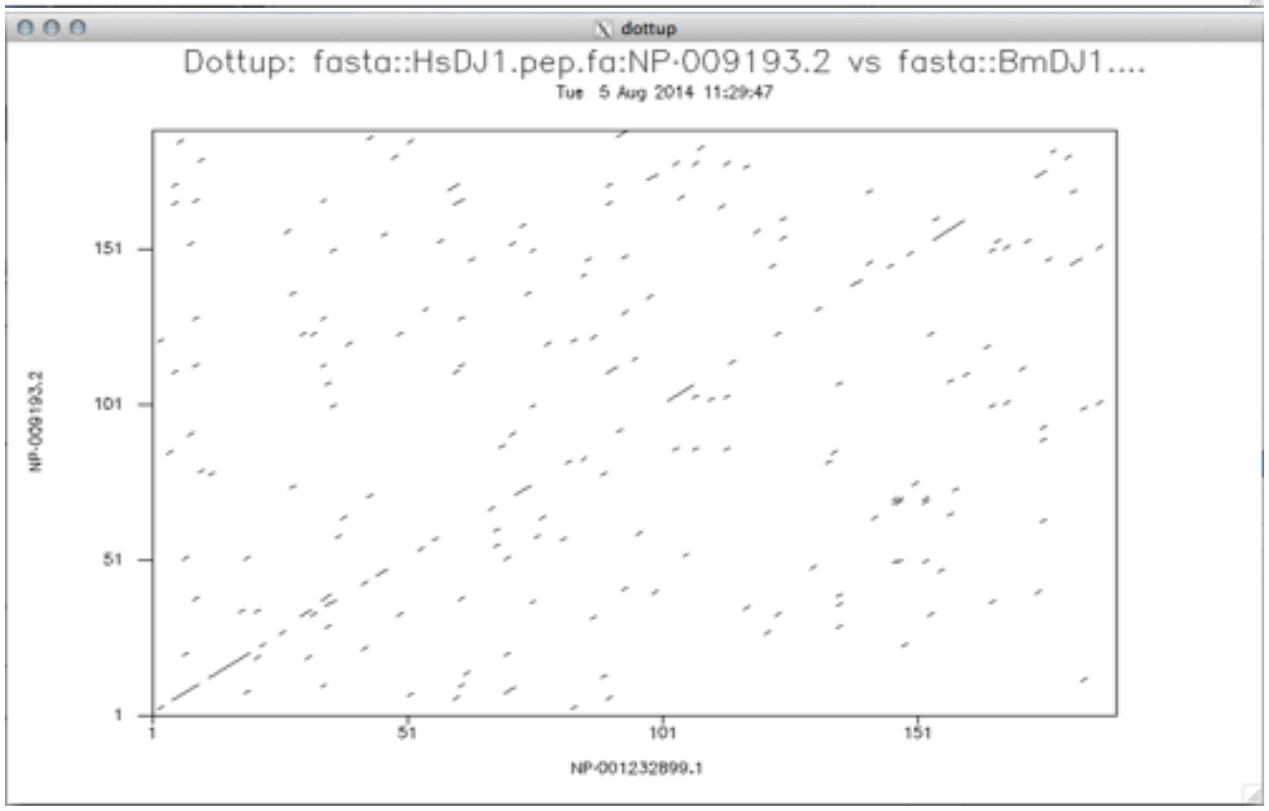
- 逆に2にすると?

```
dottup -asequence HsDJ1.pep.fa -bsequence BmDJ1.pep.fa -wordsize 2
```

dottup出力結果







- 左上: wordsize=4
- 上: wordsize=10
- 左: wordsize=2

needle, water

```
needle HsDJ1.pep.fa BmDJ1.pep.fa
```

```
water HsDJ1.pep.fa BmDJ1.pep.fa
```

- Gap opening penalty [10.0]:
- Gap extension penalty [0.5]:
- 訊かれますが、ともにdefault(何も入力しないでreturnを押す)でOK
- 結果のファイルを見てみましょう...

```
less np_009193.needle
```

```
less np_009193.water
```

needle(左), water(右)の結果

```
########################################
# Program: needle
# Rundate: Sun  3 Aug 2014 15:25:26
# Commandline: needle
#    [-asequence] HsDJ1.pep.fa
#    [-bsequence] BmDJ1.pep.fa
# Align_format: srspair
# Report_file: np_009193.needle
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: NP_009193.2
# 2: NP_001232899.1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 192
# Identity:      97/192 (50.5%)
# Similarity:   129/192 (67.2%)
# Gaps:           5/192 ( 2.6%)
# Score: 457.0
#
#
#=======================================

NP_009193.2         1 MASKRALVILAKGAEEMETVIP
                      .||.||||||:||||||||||
NP_001232899.       1 -MSKSALVILAQGAEEMETVIT
```

```
########################################
# Program: water
# Rundate: Mon  4 Aug 2014 17:42:54
# Commandline: water
#    [-asequence] HsDJ1.pep.fa
#    [-bsequence] BmDJ1.pep.fa
# Align_format: srspair
# Report_file: np_009193.water
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: NP_009193.2
# 2: NP_001232899.1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 187
# Identity:      97/187 (51.9%)
# Similarity:   129/187 (69.0%)
# Gaps:           3/187 ( 1.6%)
# Score: 458.0
#
#
#=======================================

NP_009193.2         3 SKRALVILAKGAEEMETVIPVDVMRRAGIK
                      ||.||||||:||||||||.||::||.|:.
NP_001232899.       2 SKSALVILAQGAEEMETVITVDMLRRGGVT
```

結果の解釈が重要!

- #から始まるコメント行の以下の値を見ましょう
  - # Identity:
  - # Similarity:
  - # Gaps:
  - # Score:
- 次に実際のアラインメントも見比べましょう
  - –どこが違う!?
- それがもっと顕著なのを次の例(EPAS1)で…

スコアはどうやって?

- アミノ酸置換マトリックスを使って計算

	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

ftp://ftp.ncbi.nlm.nih.gov/blast/matrices/BLOSUM62 より抜粋

例1-2: EPAS1(human)

- Ensembl http://www.ensembl.org/ から
  - humanで’EPAS1’を検索
  - geneのページから以下のcDNA配列を取得
    - EPAS1-001
    - EPAS1-007

–それぞれのtranscriptのページ(タブ)で左のカラム下の’Export data’をクリックして出てくるページで’cDNA’にチェックすると取得可能

具体的には

- EPAS1-001.nt.fa (ENST00000263734)

```
>ENST00000263734 cdna:KNOWN_protein_coding
GCTTTACACTCGCGAGCGGACCGCCACACGGGTCCGGTGCCCGCTGCGCTTCCGCCCCAGCGCTCCTGAGGCGGCCGTACAATCCTCGGCAGTGTCCTGAGACTGTATGGTCAGCTCAGCCCGGCCTCCGACTCCTTCCGACTCCCAGCATTCGAGCCACTT
TTTTTTTTCTTTGAAAACTCAGAAAAGTGACTCCTTTTCCAGGGAAAAAGGAACTTGGGTTCCCTTCTCTCCGTCCTCTTTTCGGGTCTGACAGCCTCCACCCACTCCTTCCCCGGACCCCGCCTCCGCGCGCAGGTTCCTCCCAGTCACCTTTCTCCACCC
CCGCCCCCGCACCTAGCCCGCCGCGCGCCACCTTCCACCTGACTGCGCGGGGCGCTCGGGACCTGCGCGCACCTCGGACCTTCACCACCCGCCCGGGCCGCGGGGAGCGGACGAGGGCCACAGCCCCCCACCCGCCAGGGAGCCCAGGTGCTCGGCGTCTGA
ACGTCTCAAAGGGCCACAGCGACAATGACAGCTGACAAGGAGAAGAAAAGGAGTAGCTCGGAGAGGAGGAAGGAGAAGTCCCGGGATGCTGCGCGGTGCCGGCGGAGCAAGGAGACGGAGGTGTTCTATGAGCTGGCCCATGAGCTGCCTCTGCCCCACAGT
GTGAGCTCCCATCTGGACAAGGCCTCCATCATGCGACTGGCAATCAGCTTCCTGCGAACACACAAGCTCCTCTCCTCAGTTTGCTCTGAAAACGAGTCCGAAGCCGAAGCTGACCAGCAGATGGACAACTTGTACCTGAAAGCCTTGGAGGGTTTCATTGCC
GTGGTGACCCAAGATGGCGACATGATCTTTCTGTCAGAAAACATCAGCAAGTTCATGGGACTTACACAGGTGGAGCTAACAGGACATAGTATCTTTGACTTCACTCATCCCTGCGACCATGAGGAGATTCGTGAGAACCTGAGTCTCAAAAATGGCTCTGGT
TTTGGGAAAAAAAGCAAAGACATGTCCACAGAGCGGGACTTCTTCATGAGGATGAAGTGCACGGTCACCAACAGAGGCCGTACTGTCAACCTCAAGTCAGCCACCTGGAAGGTCTTGCACTGCACGGGCCAGGTGAAAGTCTACAACAACTGCCCTCCTCAC
AATAGTCTGTGTGGCTACAAGGAGCCCCTGCTGTCCTGCCTCATCATCATGTGTGAACCAATCCAGCACCCATCCCACATGGACATCCCCCTGGATAGCAAGACCTTCCTGAGCCGCCACAGCATGGACATGAAGTTCACCTACTGTGATGACAGAATCACA
GAACTGATTGGTTACCACCCTGAGGAGCTGCTTGGCCGCTCAGCCTATGAATTCTACCATGCGCTAGACTCCGAGAACATGACCAAGAGTCACCAGAACTTGTGCACCAAGGGTCAGGTAGTAAGTGGCCAGTACCGGATGCTCGCAAAGCATGGGGGCTAC
GTGTGGCTGGAGACCCAGGGGACGGTCATCTACAACCCTCGCAACCTGCAGCCCCAGTGCATCATGTGTGTCAACTACGTCCTGAGTGAGATTGAGAAGAATGACGTGGTGTTCTCCATGGACCAGACTGAATCCCTGTTCAAGCCCCACCTGATGGCCATG
AACAGCATCTTTGATAGCAGTGGCAAGGGGGCTGTGTCTGAGAAGAGTAACTTCCTATTCACCAAGCTAAAGGAGGAGCCCGAGGAGCTGGCCCAGCTGGCTCCCACCCCAGGAGACGCCATCATCTCTCTGGATTTCGGGAATCAGAACTTCGAGGAGTCC
TCAGCCTATGGCAAGGCCATCCTGCCCCCGAGCCAGCCATGGGCCACGGAGTTGAGGAGCCACAGCACCCAGAGCGAGGCTGGGAGCCTGCCTGCCTTCACCGTGCCCCAGGCAGCTGCCCCGGGCAGCACCACCCCCAGTGCCACCAGCAGCAGCAGCAGC
TGCTCCACGCCCAATAGCCCTGAAGACTATTACACATCTTTGGATAACGACCTGAAGATTGAAGTGATTGAGAAGCTCTTCGCCATGGACACAGAGGCCAAGGACCAATGCAGTACCCAGACGGATTTCAATGAGCTGGACTTGGAGACACTGGCACCCTAT
ATCCCCATGGACGGGGAAGACTTCCAGCTAAGCCCCATCTGCCCCGAGGAGCGGCTCTTGGCGGAGAACCCACAGTCCACCCCCCAGCACTGCTTCAGTGCCATGACAAACATCTTCCAGCCACTGGCCCCTGTAGCCCCGCACAGTCCCTTCCTCCTGGAC
AAGTTTCAGCAGCAGCTGGAGAGCAAGAAGACAGAGCCCGAGCACCGGCCCATGTCCTCCATCTTCTTTGATGCCGGAAGCAAAGCATCCCTGCCACCGTGCTGTGGCCAGGCCAGCACCCCTCTCTCTTCCATGGGGGGCAGATCCAATACCCAGTGGCCC
CCAGATCCACCATTACATTTTGGGCCCACAAAGTGGGCCGTCGGGGATCAGCGCACAGAGTTCTTGGGAGCAGCGCCGTTGGGGCCCCCTGTCTCTCCACCCCATGTCTCCACCTTCAAGACAAGGTCTGCAAAGGGTTTTGGGGCTCGAGGCCCAGACGTG
CTGAGTCCGGCCATGGTAGCCCTCTCCAACAAGCTGAAGCTGAAGCGACAGCTGGAGTATGAAGAGCAAGCCTTCCAGGACCTGAGCGGGGGGGACCCACCTGGTGGCAGCACCTCACATTTGATGTGGAAACGGATGAAGAACCTCAGGGGTGGGAGCTGC
CCTTTGATGCCGGACAAGCCACTGAGCGCAAATGTACCCAATGATAAGTTCACCCAAAACCCCATGAGGGGCCTGGGCCATCCCCTGAGACATCTGCCGCTGCCACAGCCTCCATCTGCCATCAGTCCCGGGGAGAACAGCAAGAGCAGGTTCCCCCCACAG
TGCTACGCCACCCAGTACCAGGACTACAGCCTGTCGTCAGCCCACAAGGTGTCAGGCATGGCAAGCCGGCTGCTCGGGCCCTCATTTGAGTCCTACCTGCTGCCCGAACTGACCAGATATGACTGTGAGGTGAACGTGCCCGTGCTGGGAAGCTCCACGCTC
CTGCAAGGAGGGGACCTCCTCAGAGCCCTGGACCAGGCCACCTGAGCCAGGCCTTCTACCTGGGCAGCACCTCTGCCGACGCCGTCCCACCAGCTTCACTCTCTCCGTCTGTTTTTGCAACTAGGTATTTCTAACGCCAGCACACTATTTACAAGATGGACT
TACCTGGCAGACTTGCCCAGGTCACCAAGCAGTGGCCTTTTTCTGAGATGCTCACTTTATTATCCCTATTTTTAAAGTACACAATTGTTTTACCTGTTCTGAAATGTTCTTAAATTTTGTAGGATTTTTTTCCTCCCCACCTTCAATGACTTCTAATTTATA
TTATCCATAGGTTTCTCTCCCTCCTTCTCCTTCTCACACACAACTGTCCATACTAACAAGTTTGGTGCATGTCTGTTCTTCTGTAGGGAGAAGCTTTAGCTTCATTTTACTAAAAAGATTCCTCGTTATTGTTGTTGCCAAAGAGAAACAAAAATGATTTTG
CTTTCCAAGCTTGGTTTGTGGCGTCTCCCTCGCAGAGCCCTTCTCGTTTCTTTTTTAAACTAATCACCATATTGTAAATTTCAGGGTTTTTTTTTTTTGTTTAAGCTGACTCTTTGCTCTAATTTTGGAAAAAAAGAAATGTGAAGGGTCAACTCCAACGT
ATGTGGTTATCTGTGAAAGTTGCACAGCGTGGCTTTTCCTAAACTGGTGTTTTTCCCCCGCATTTGGTGGATTTTTTATTATTATTCAAAAACATAACTGAGTTTTTTAAAAGAGGAGAAAATTTATATCTGGGTTAAGTGTTTATCATATATATGGGTACT
TTGTAATATCTAAAAACTTAGAAACGGAAATGGAATCCTGCTCACAAAATCACTTTAAGATCTTTTCGAAGCTGTTAATTTTTCTTAGTGTTGTGGACACTGCAGACTTGTCCAGTGCTCCCACGGCCTGTACGGACACTGTGGAAGGCCTCCCTCTGTCGG
CTTTTTGCCATCTGTGATATGCCATAGGTGTGACAATCCGAGCAGTGGAGTCATTCAGCGGGAGCACTGCGCGCTATCCCCTCACATTCTCTATGTACTATGTATGTATGTATTATTATTATTGCTGCCAAGAGGGTCTGATGGCACGTTGTGGGGTCGGGG
GGTGGGGCGGGGAAGTGCTCTAACTTTTCTTAAGGTTTTGTTGCTAGCCCTTCAAGTGCACTGAGCTATGTGACTCGGATGGTCTTTCACACGGCACATTTGGACATTTCCAGAACTACCATGAGATGGTTTAGACGGGAATTCATGCAAATGAGGGGTCAA
AAATGGTATAGTGACCCCGTCCACGTCCTCCAAGCTCACGACCTTGGAGCCCCGTGGAGCTGGACTGAGGAGGAGGCTGCACAGCGGGAGAGCAGCTGGTCCAGACCAGCCCTGCAGCCCCCACTCAGCCGGCAGCCAGATGGCCCCGCAAGGCCTCCAGGG
ATGGCCCCTAGCCACAGGCCCTGGCTGAGGTCTCTGGGTCGGTCAGTGACATGTAGGTAGGAAGCACTGAAAATAGTGTTCCCAGAGCACTTTGCAACTCCCTGGGTAAGAGGGACGACACCTCTGGTTTTTCAATACCAATTACATGGAACTTTTCTGTAA
TGGGTACAATGAAGAAGTTTCTAAAAACACACACAAAGCACATTGGGCCAACTATTTAGTAAGCCCGGATAGACTTATTGCCAAAAACAAAAAATAGCTTTCAAAAGAAATTTAAGTTCTATGAGAAATTCCTTAGTCATGGTGTTGCGTAAATCATATTTT
AGCTGCACGGCATTACCCCACACAGGGTGGCAGAACTTGAAGGGTTACTGACGTGTAAATGCTGGTATTTGATTTCCTGTGTGTGTTGCCCTGGCATTAAGGGCATTTTACCCTTGCAGTTTTACTAAAACACTGAAAAATATTCCAAGCTTCATATTAACC
CTACCTGTCAACGTAACGATTTCATGAACGTTATTATATTGTCGAATTCCTACTGACAACATTATAACTGTATGGGAGCTTAACTTTATAAGGAAATGTATTTTGACACTGGTATCTTATTAAAGTATTCTGATCCTA
```

- EPAS1-007.nt.fa (ENST00000449347)

```
>ENST00000449347 cdna:KNOWN_protein_coding
AGATCACACTGGGGAACCAGACTGACTTCTCCAATTCTGAACTCGCCCCGGCCTCGGGCGGCTCAAAGGGCCTCCTCTGCCGCATCCCCGCCAAAACCAAACCGCCTGGCACAAGCCGCCCGCCGCGCGCCACCTTCCACCTGACTGCGCGGGGCGCTCGGG
ACCTGCGCGCACCTCGGACCTTCACCACCCGCCCGGGCCGCGGGGAGCGGACGAGGGCCACAGCCCCCCACCCGCCAGGGAGCCCAGGTGCTCGGCGTCTGAACGTCTCAAAGGGCCACAGCGACAATGACAGCTGACAAGGAGAAGAAAAGGAGTAGCTCG
GAGAGGAGGAAGGAGAAGTCCCGGGATGCTGCGCGGTGCCGGCGGAGCAAGGAGACGGAGGTGTTCTATGAGCTGGCCCATGAGCTGCCTCTGCCCCACAGTGTGAGCTCCCATCTGGACAAGGCCTCCATCATGCGACTGGCAATCAGCTTCCTGCGAACA
CACAAGCTCCTCTCCTCAGTTTGCTCTGAAAACGAGTCCGAAGCCGAAGCTGACCAGCAGATGGACAACTTGTACCTGAAAGCCTTGGAGGGTTTCATTGCCGTGGTGACCCAAGATGGCGACATGATCTTTCTGTCAGAAAACATCAGCAAGTTCATGGGA
CTTACACAGGTGGAGCTAACAGGACATAGTATCTTTGACTTCACTCATCCCTGCGACCATGAGGAGATTCGTGAGAACCTGAGTCTCAAAAATGGCTCTGGTTTTGGGAAAAAAAGCAAAGACATGTCCACAGAGCGGGACTTCTTCATGAGGATGAAGTGC
ACGGTCACCAACAGAGGCCGTACTGTCAACCTCAAGTCAGCCACCTGGAAGGTCTTGCACTGCACGGGCCAGGTGAAAGTCTACAACAACTGCCCTCCTCACAATAGTCTGTGTGGCTACAAGGAGCCCCTGCTGTCCTGCCTCATCATCATGTGTGAACCA
ATCCAGCACCCATCCCACATGGACATCCCCCTGGATAGCAAGACCTTCCTGAGCCGCCACAGCATGGACATGAAGTTCACCTACTGTGATGACAG
```

dottup

- とりあえず、実行しましょう

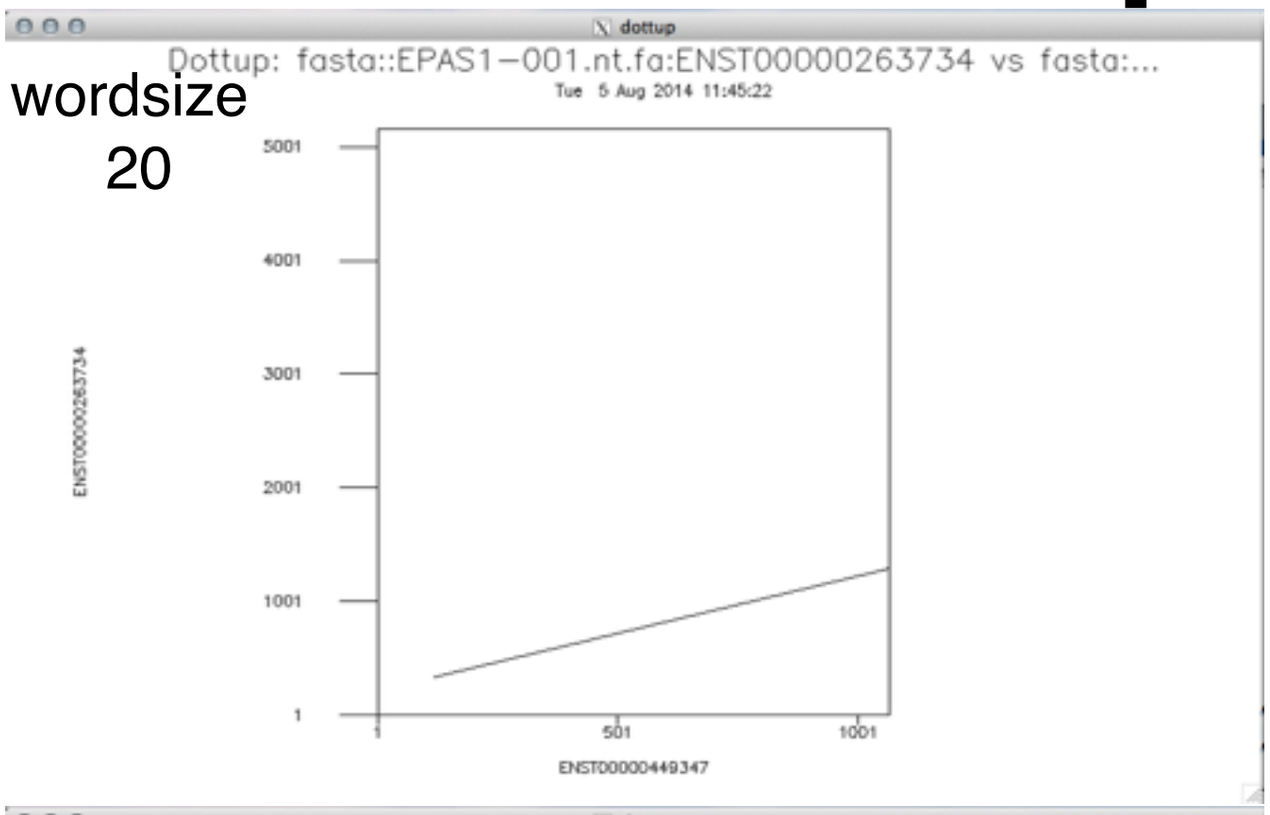
```
dottup -asequence EPAS1-001.nt.fa -bsequence EPAS1-007.nt.fa
```

  - wordsize訊いてきますが、defaultの10 で

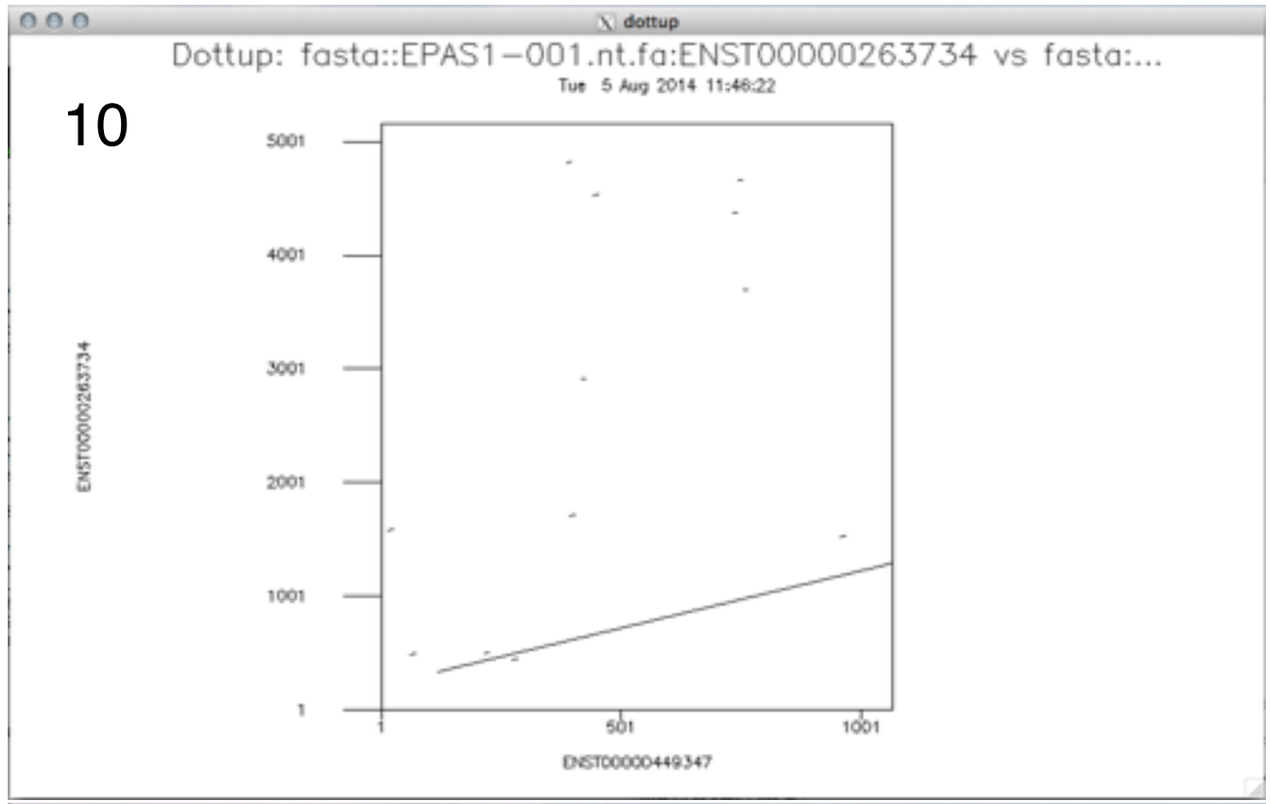
- 同じコマンドでwordsizeを変えてみましょう
  - 20では?
  - 6では?
  - 3では?

dottup出力結果

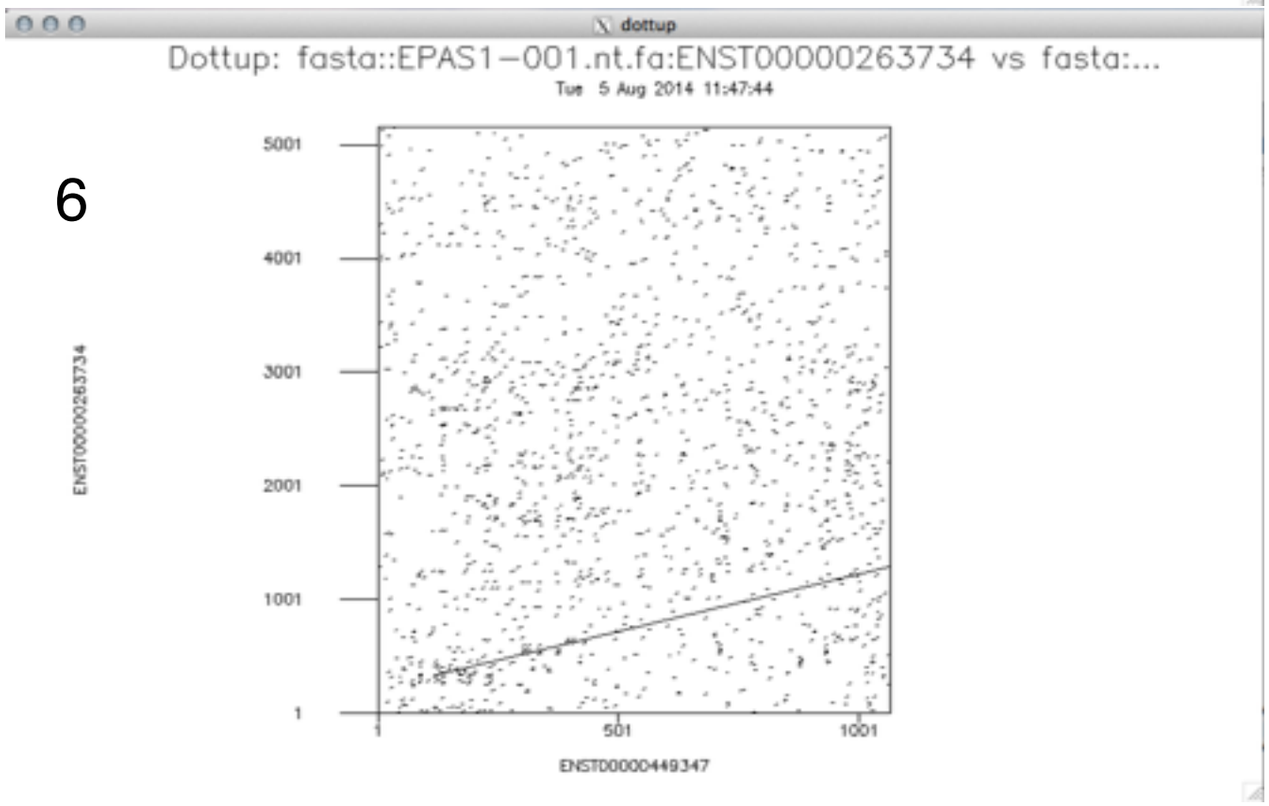
wordsize
20



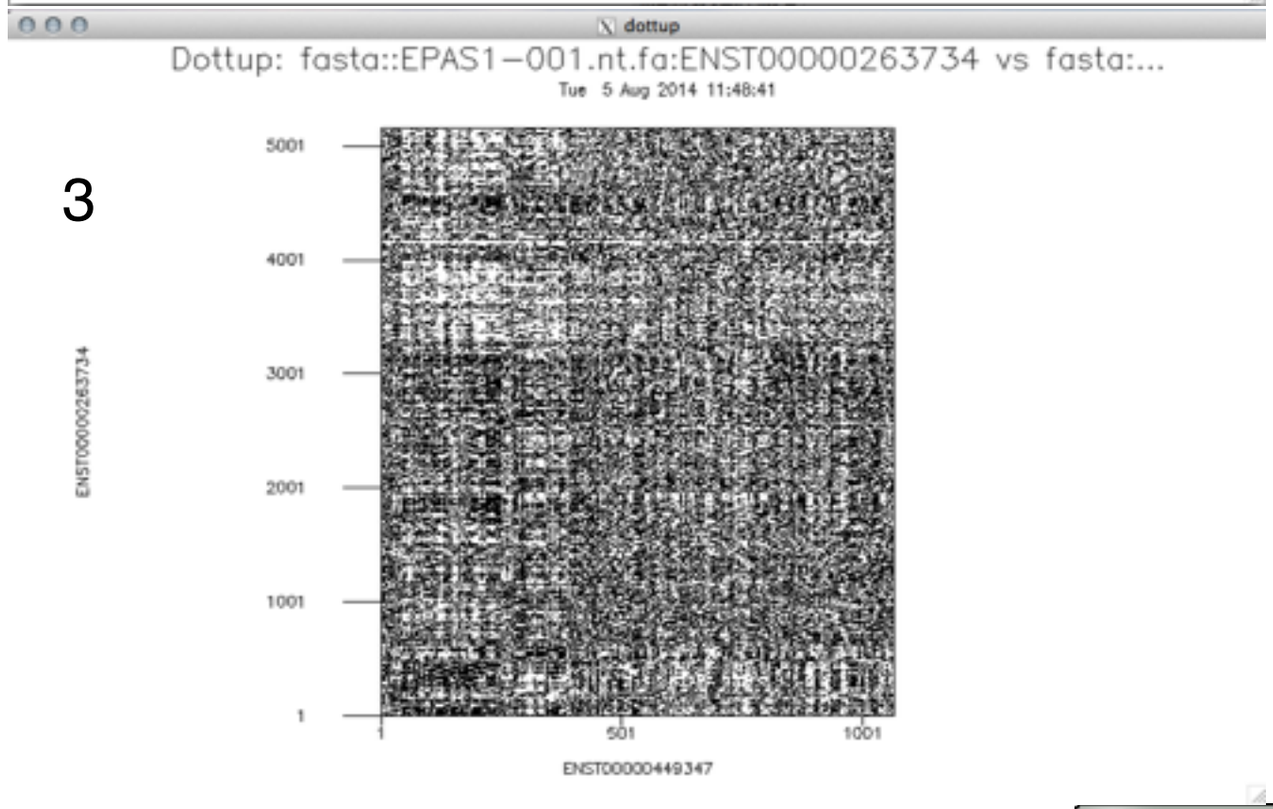
10



6



3



needle, water

```
needle EPAS1-001.nt.fa EPAS1-007.nt.fa
```

```
water EPAS1-001.nt.fa EPAS1-007.nt.fa
```

- Gap opening penalty [10.0]:
- Gap extension penalty [0.5]:
- 訊かれますが、ともにdefault(何も入力しないでreturnを押す)でOK
- 結果のファイルを見てみましょう...

```
less enst00000263734.needle
```

```
less enst00000263734.water
```

needle(左), water(右)の結果

```
########################################
# Program: needle
# Rundate: Sun  3 Aug 2014 15:56:56
# Commandline: needle
#    [-asequence] EPAS1-001.nt.fa
#    [-bsequence] EPAS1-007.nt.fa
# Align_format: srspair
# Report_file: enst00000263734.needle
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: ENST00000263734
# 2: ENST00000449347
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 5185
# Identity:    1024/5185 (19.7%)
# Similarity:  1024/5185 (19.7%)
# Gaps:        4143/5185 (79.9%)
# Score: 4851.0
#
#
#=======================================

ENST000002637      1 GCTTTACACTCGCGAGCGGACCG

ENST000004493      0 -----------------------
```

```
########################################
# Program: water
# Rundate: Sun  3 Aug 2014 15:57:08
# Commandline: water
#    [-asequence] EPAS1-001.nt.fa
#    [-bsequence] EPAS1-007.nt.fa
# Align_format: srspair
# Report_file: enst00000263734.water
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: ENST00000263734
# 2: ENST00000449347
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 1105
# Identity:    1015/1105 (91.9%)
# Similarity:  1015/1105 (91.9%)
# Gaps:          75/1105 ( 6.8%)
# Score: 4854.0
#
#
#=======================================

ENST000002637    215 ACTTGGGTTCC-------CTTCTCTCC
                     |||.|||..||       ||||||
ENST000004493      8 ACTGGGGAACCAGACTGACTTCTC---
```

DNA用の置換マトリックス

```
#
# This matrix was created by Todd Lowe   12/10/92
#
# Uses ambiguous nucleotide codes, probabilities rounded to
#  nearest integer
#
# Lowest score = -4, Highest score = 5
#
   A   T   G   C   S   W   R   Y   K   M   B   V   H   D   N
A  5  -4  -4  -4  -4   1   1  -4  -4   1  -4  -1  -1  -1  -2
T -4   5  -4  -4  -4   1  -4   1   1  -4  -1  -4  -1  -1  -2
G -4  -4   5  -4   1  -4   1  -4   1  -4  -1  -1  -4  -1  -2
C -4  -4  -4   5   1  -4  -4   1  -4   1  -1  -1  -1  -4  -2
S -4  -4   1   1  -1  -4  -2  -2  -2  -2  -1  -1  -3  -3  -1
W  1   1  -4  -4  -4  -1  -2  -2  -2  -2  -3  -3  -1  -1  -1
R  1  -4   1  -4  -2  -2  -1  -4  -2  -2  -3  -1  -3  -1  -1
Y -4   1  -4   1  -2  -2  -4  -1  -2  -2  -1  -3  -1  -3  -1
K -4   1   1  -4  -2  -2  -2  -2  -1  -4  -1  -3  -3  -1  -1
M  1  -4  -4   1  -2  -2  -2  -2  -4  -1  -3  -1  -1  -3  -1
B -4  -1  -1  -1  -1  -3  -3  -1  -1  -3  -1  -2  -2  -2  -1
V -1  -4  -1  -1  -1  -3  -1  -3  -3  -1  -2  -1  -2  -2  -1
H -1  -1  -4  -1  -3  -1  -3  -1  -3  -1  -2  -2  -1  -2  -1
D -1  -1  -1  -4  -3  -1  -1  -3  -1  -3  -2  -2  -2  -1  -1
N -2  -2  -2  -2  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
```

ftp://ftp.ncbi.nlm.nih.gov/blast/matrices/NUC.4.4 より抜粋

演習2の準備

- 使うプログラムの準備
  - NCBI BLASTがインストールされているので済
- 検索に使うquery配列とDB配列の取得
  - 使いたい核酸配列orアミノ酸配列を(multi)FASTAフォーマットで
    - 出芽酵母の全遺伝子配列(核酸配列とアミノ酸配列両方)がすでに取得済み
    - それらが検索可能なようにインデックスされている必要あり

DBの準備

- DBのあるディレクトリに移動
- アミノ酸配列をBLAST用にフォーマット
- ゲノム(塩基)配列をBLAST用にフォーマット

```
cd /home/admin1409/genome
makeblastdb -in yeast.aa -dbtype prot -hash_index
makeblastdb -in yeast.nt -dbtype nucl -hash_index
```

- 使うDBごとにmakeblastdbコマンドを実行する必要あり

例2-1: DJ-1でyeastのアミノ酸配列全体を検索

- query: アミノ酸配列(HsDJ1.pep.fa)
- DB: 酵母のアミノ酸配列(yeast.aa)
- → blastp コマンドを使用

```
blastp -query ~/HsDJ1.pep.fa -db yeast.aa
-num_threads 4 > HsDJ1vsyeast.aa.txt
```

- 注意
  - home directoryにHsDJ1.pep.faがある想定
  - BLASTがCPU4つまで使うのを許可

HsDJ1vsyeast.aa.txt の中身

```
BLASTP 2.2.29+


Reference: Stephen F. Altschul, Thomas L. Madden, Alejandro A.
Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J.
Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of
protein database search programs", Nucleic Acids Res. 25:3389-3402.


Reference for composition-based statistics: Alejandro A. Schaffer,
L. Aravind, Thomas L. Madden, Sergei Shavirin, John L. Spouge, Yuri
I. Wolf, Eugene V. Koonin, and Stephen F. Altschul (2001),
"Improving the accuracy of PSI-BLAST protein database searches with
composition-based statistics and other refinements", Nucleic Acids
Res. 29:2994-3005.



Database: yeast.aa
           6,298 sequences; 2,974,038 total letters



Query= gi|31543380|ref|NP_009193.2| protein DJ-1 [Homo sapiens]

Length=189
                                                                  Score     E
Sequences producing significant alignments:                      (Bits)  Value

  gi|6324388|ref|NP_014458.1| Similar to ferric reductases Fre1p ...  28.5    0.73
  gi|6320742|ref|NP_010822.1| Ydr533cp                                26.6    2.6
```

```
> gi|6324388|ref|NP_014458.1| Similar to ferric reductases Fre1p
and Fre2p; Fre4p
Length=719

 Score = 28.5 bits (62),  Expect = 0.73, Method: Composition-based stats.
 Identities = 11/22 (50%), Positives = 14/22 (64%), Gaps = 0/22 (0%)

Query  88   VKEILKEQENRKGLIAAICAGP  109
            V+EIL E  N  G +A +C GP
Sbjct  666  VEEILNESVNHSGSLAVVCCGP  687


> gi|6320742|ref|NP_010822.1| Ydr533cp
Length=237

 Score = 26.6 bits (57),  Expect = 2.6, Method: Compositional matrix adjust.
 Identities = 38/168 (23%), Positives = 65/168 (39%), Gaps = 36/168 (21%)

Query  1    MASKRAL--------VILAKGAEE----METVIPVDVMRRAGIKVTV---AGLAGKDPVQ  45
            MA K+ L         V  + GA+     +E + P +  R+ G +V       G  G D
Sbjct  1    MAPKKVLLALTSYNDVFYSDGAKTGVFVVEALHPFNTFRKEGFEVDFVSETGKFGWDEHS  60

Query  46   CSRDVVICPDAS---LED-------AKKEGP-------YDVVVLPGGNLGAQNLSESAAV  88
             ++D +   D +    +D        AK + P        Y +     G+    +  ++  +
Sbjct  61   LAKDFLNGQDETDFKNKDSDFNKTLAKIKTPKEVNADDYQIFFASAGHGTLFDYPKAKDL  120

Query  89   KEILKEQENRKGLIAAICAGPTALLAHEIGFGSKVTTHPLAKDKMMNG  136
            ++I  E     G++AA+C GP         G   K T  PL + K + G
Sbjct  121  QDIASEIYANGGVVAAVCHGPAIF----DGLTDKKTGRPLIEGKSITG  164


> gi|6325167|ref|NP_015235.1| Ribosomal protein S6A (S10A) (rp9)
(YS4); Rps6ap
Length=236
```

オプションを変えてみる

- E-valueがdefault10なのでそれを厳し目に

```
blastp -query ~/HsDJ1.pep.fa -db yeast.aa
-num_threads 4 -evalue 1
```

- 出力フォーマットをタブ区切りに

```
blastp -query ~/HsDJ1.pep.fa -db yeast.aa
-num_threads 4 -outfmt 6
```

- オプションの種類と説明は...

```
blastp -help | less
```

例2-2: EPAS1でyeastゲノム配列を翻訳しながら検索(tblastx)

- query: 核酸配列(EPAS1-001.nt.fa)
- DB: 酵母のゲノム配列(yeast.nt)
- → tblastx コマンドを使用

```
tblastx -query ../EPAS1-001.nt.fa -db yeast.nt
-num_threads 4 -evalue 1|less
```

- 注意
- home directoryにEPAS1-001.nt.faがある想定
- BLASTがCPU4つまで使うのを許可

tblastxの計算結果

```
TBLASTX 2.2.29+


Reference: Stephen F. Altschul, Thomas L. Madden, Alejandro A.
Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J.
Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of
protein database search programs", Nucleic Acids Res. 25:3389-3402.



Database: yeast.nt
           17 sequences; 12,155,026 total letters


Query= ENST00000263734 cdna:KNOWN_protein_coding

Length=5160
                                                                   Score     E
Sequences producing significant alignments:                       (Bits)  Value  N

  gi|7839148|ref|NC_001136.2| Saccharomyces cerevisiae chromosome...  31.3    0.028  2
  gi|6322016|ref|NC_001141.1| Saccharomyces cerevisiae chromosome...  35.4    0.29   1


> gi|7839148|ref|NC_001136.2| Saccharomyces cerevisiae chromosome
IV, complete chromosome sequence
Length=1531929

 Score = 31.3 bits (62),  Expect(2) = 0.028
 Identities = 14/41 (34%), Positives = 20/41 (49%), Gaps = 0/41 (0%)
```

例2-3: 興味のあるquery,DBで BLAST検索を実行しよう

- query
  –NCBIやその他のサイトで興味あるものを検索
- DB
  –UCSC Genome Browser
    - http://hgdownload.soe.ucsc.edu/downloads.html
  –Ensembl
    - http://www.ensembl.org/info/data/ftp/
  –NCBI
    - http://www.ncbi.nlm.nih.gov/guide/genomes-maps/

例2: 番外編 自分のマシンに BLASTをインストールしよう

- Local BLASTの使い方

使い倒し系チャンネル 統合TV

  - –Windows http://togotv.dbcls.jp/20110119.html
    - 導入・準備編
    - 検索実行・オプション編
  - –MacOSX http://togotv.dbcls.jp/20110420.html
    - 導入・準備編
    - 検索実行・オプション編
- AJACS名古屋
  - –http://motdb.dbcls.jp/?AJACS32%2Fbono

演習3の準備

- Perlプログラミングの準備
  - –昨日までみっちりやっているので済
- 検索するキーワード/配列の準備
  - –実はこれが重要
  - –今回使用する例:
    1. エストロゲン応答エレメント: AGGTCAnnnTGACCT
    2. PEST配列: PEST

DBCLS Database Center for Life Science

統合遺伝子検索 GGRNA ver.2

Help | Advanced search | English　旧バージョン

検索　Zoo (All organisms in RefSeq)

遺伝子をGoogleのように検索できるサイトです。 NCBI RefSeq の transcript を全文検索します。

検索例:

- 「homeobox」「claudin」..... フリーワード検索
- 「"RNA interference"」..... ダブルクオートで囲ってフレーズ検索
- 「Argonaute "PAZ dor
- 「NM_001518」「105
- 「symbol:VIM」.....
- 「ref:Naito」..... 文献
- 「1552311_a_at」.....
- 「aa:KDEL」..... アミ
- 「caagaagagattg」....
- 「comp:caagaagagatt
- 「iub:aggtcannrtgacct
- 詳細な使い方

新着情報:

- 2014-07-20 データベー
- 2013-07-24 ソースを公
- 2013-07-08 GGRNA v
- 2012-05-29 下記論文の
- 2012-05-29 GGRNAの
- 過去の新着情報

検索結果へのリンク:

- http://GGRNA.dbcls.jp/species/query+string[.format][.download]
  - species → 生物種の学名の頭文字。hs, mm, etc. 省略時は全生物種 (zoo)
  - query+string → URLエンコードしたクエリ文字列
  - format → html, txt, json。省略時は html
  - download → URLの最後に付加すると検索結果をファイルとしてダウンロードできる
- 指定できる species および format の種類など、詳細は下記を参照:
  - http://GGRNA.dbcls.jp/api.txt
- 使用例:
  - http://GGRNA.dbcls.jp/NM_001518.txt
    「NM_001518」を検索してタブ区切りテキストで取得
  - http://GGRNA.dbcls.jp/mm/homeobox.txt
    マウスで「homeobox」を検索してタブ区切りテキストで取得
  - http://GGRNA.dbcls.jp/dm/%22RNA+interference%22.json
    ショウジョウバエで「"RNA interference"」を検索してJSONで取得
    URLでは " は %22、スペースは + にエンコードしている点に注意
  - http://GGRNA.dbcls.jp/caagaagagattg.json
    全生物種で「caagaagagattg」を検索してJSONで取得

例3-1: エストロゲン応答エレメントを持つ遺伝子の抽出

http://ggrna.dbcls.jp/hs/iub%3aAGGTCAnnnTGACCT.txt

```
# [ GGRNA.v2 | 2014-08-05 13:53:59 ]
#
# iub:AGGTCAnnnTGACCT	33
# [INTERSECTION]	33
#
# accession	version	gi	length	symbol	synonym	geneid	division	source	definition	nt_position	aa_position
NR_073388	NR_073388.1	410110941	1007	ALG1L9P		285407	RefSeq	Homo sapiens (human)	Homo sapiens asparagine-linked glycosylation 1-like 9, pseudogene (ALG1L9P), transcript variant 1, non-coding RNA.	853
XM_006715925	XM_006715925.1	578814108	2140	TPK1	HTPK1; PP20; THMD5	27010	RefSeq	Homo sapiens (human)	PREDICTED: Homo sapiens thiamin pyrophosphokinase 1 (TPK1), transcript variant X7, mRNA.	1042

XM_005249974	XM_005249974.1	530386953	2205	TPK1	HTPK1; PP20; THMD5	27010	RefSeq	Homo sapiens (human)	PREDICTED: Homo sapiens thiamin pyrophosphokinase 1 (TPK1), transcript variant X5, mRNA.	1107

NM_001042482	NM_001042482.1	110227856	2302	TPK1	HTPK1; PP20; THMD5	27010	RefSeq	Homo sapiens (human)	Homo sapiens thiamin pyrophosphokinase 1 (TPK1), transcript variant 2, mRNA.	1194
XM_005249970	XM_005249970.1	530386945	2417	TPK1	HTPK1; PP20; THMD5	27010	RefSeq	Homo sapiens (human)	PREDICTED: Homo sapiens thiamin pyrophosphokinase 1 (TPK1), transcript variant X1, mRNA.	1319

XM_005249971	XM_005249971.2	578814104	2439	TPK1	HTPK1; PP20; THMD5	27010	RefSeq	Homo sapiens (human)	PREDICTED: Homo sapiens thiamin pyrophosphokinase 1 (TPK1), transcript variant X2, mRNA.	1341

NM_022445	NM_022445.3	110227855	2449	TPK1	HTPK1; PP20; THMD5	27010	RefSeq	Homo sapiens (human)	Homo sapiens thiamin pyrophosphokinase 1 (TPK1), transcript variant 1, mRNA.	1341
```

以上の情報からコード書く

- ggrnaapi.pl

```
#!/usr/bin/perl

my $url = "http://ggrna.dbcls.jp/hs/iub%3aAGGTCAnnnTGACCT.txt";

open(FILE, "curl -s $url |") or die "$!\n";
#open(FILE, "wget -O - $url |") or die "$!\n";
while(<FILE>) {
        chomp;
        next if(/^\#/);
        my @words = split(/\t/);
        print $words[4]."\n";
}
close FILE;
```

variantで複数遺伝子出てくるのを sort &uniq

- 普通に実行すると…

```
perl ggrnaapi.pl | less
```

- 同じ遺伝子名が複数回出てくるので、

```
perl ggrnaapi.pl | sort | uniq | less
```

- とすると…

```
ALG1L9P
KANSL3
RAB5B
SETD5
SIN3A
TPK1
```

複数遺伝子出てくるのをcount

- sort & uniqしないでそれの回数を数えるには

```
#!/usr/bin/perl
while(<>) {
        my($word) = split;
        $num{$word}++;
}
foreach (sort keys %num) {
        print "$_\t$num{$_}\n";
}
```

count.pl

- のように要素の数を数えるコードを書いて

```
perl ggrnaapi.pl | perl count.pl
```

- さらにその数が多いもの順にするのに

```
perl ggrnaapi.pl | perl count.pl | sort -rn -k2
```

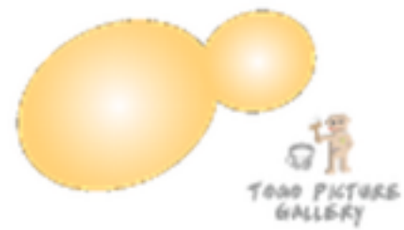
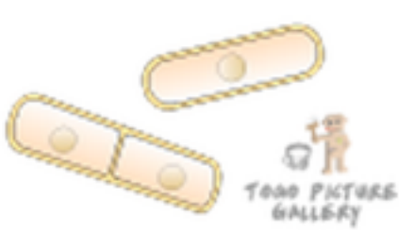
例3-2: PEST配列

- 同様にPEST配列で
- 基本$urlを以下のように書き換えるだけ
  - http://ggrna.dbcls.jp/hs/aa%3aPEST.txt
- ‘hs'を別のものに変えることで別の生物の結果も計算してみましょう

生物種指定の変更

生物種の指定:

- GGRNAはデフォルトでRefSeqに収録された全生物種を同時に検索します。
- 下記のモデル生物は専用のURLを用意しています。

ヒト (hs) 	マウス (mm) 	ラット (rn) 	ニワトリ (gg) 
ツメガエル (xt) 	ゼブラフィッシュ (dr) 	ホヤ (ci) 	ショウジョウバエ (dm) 
線虫 (ce) 	シロイヌナズナ (at) 	イネ (os) 	
出芽酵母 (sc) 	分裂酵母 (sp) 		

http://ggrna.dbcls.jp/help.html より

例3-3: 興味のある配列パターンで

- 同様にして興味のある配列パターンで
- 以下の基本$urlを書き換えて
  - http://ggrna.dbcls.jp/hs/aa%3aPEST.txt
- 生物種も別のものに変え、さまざまな生物の結果も計算してみましょう
  - 例えば
    - E-box: CACGTG
    - 小胞体保留シグナル: KDEL配列