Searching (Sub-)Strings

Ulf Leser

This Lecture

- Exact substring search
  - Naïve
  - Boyer-Moore
- Searching with profiles
  - Sequence profiles
  - Ungapped approximate search
  - Statistical evaluation of search results

Searching / Comparing Strings

- Exact matching
  - Given strings p and t: Find all occurrences of s in t
  - Given a set P and t: Find all occurrences of any p∈P in t
- Approximate matching
  - Given p and t: Find all approximate occurrences of p in t
  - Given p and t: Find p', t' such that p' is similar to t' and p' is a substring of p and t' is a substring of t
  - Given p and a set of strings T
    - Find all t∈T that are similar to p
    - Find all t∈T containing a t' similar to a p' contained in p
- Many more variants ...

Applications

- Given strings p and t: Find all occurrences of p in t
  - Restriction enzyme cut positions; fixed patterns in gene structure; seeds for approximate searching
- Given a set P and t: Find all occurrences of any $p \in P$ in t
  - Same with multiple patterns / enzymes
- Given p and t: Find all approximate occurrences of p in t
  - Less conserved patterns; read mapping; TF binding sites
- Given p and t: Find p', t' such that p' similar to t' and p' is a substring of s and t' is a substring of t
  - Local alignment; homologous genes; cross-species searches

Strings

- A string (or sequence) p is an ordered list of characters from an alphabet Σ
  - |s| is the length of p
  - p[i] is the character at position i in p (starting from 1)
  - p[i..j] is the substring from position i to position j in p
  - p[i..j] is an empty string if i > j
  - p[1..i] is a prefix of p ending at position i
  - p[i..] is a suffix of p starting at position i
- Alphabet
  - Usually: Σ={A, C, G, T}
  - Often, we need blanks: Σ'={A, C, G, T, _}
- Lower/upper case: P may denote a set of strings, or a sequence of characters (a string)

Exact Matching

- Given P, T with |P| << |T|
- Find all occurrences of P in T
- Example of application: Restriction enzymes
  - Cut at precisely defined sequence motifs of length 4-10
  - Are used to generate fragments (for later sequencing)
  - Example: Eco RV - GATATC

```
tcagcttactaattaaaaattctttctagtaagtgctaagatcaagaaaataaattaaaaataatggaacatggcacattttcctaaactcttcacagattgctaatgat
tattaattaaagaataaatgttataatttttatggtaacggaatttcctaaaatattaattcaagcaccatggaatgcaaataagaaggactctgttaattggtactat
tcaactcaatgcaagtggaactaagttggtattaatactctttttacatatatatgtagttattttaggaagcgaaggacaatttcatctgctaataaagggattacga
aaaactttttaataacaaagttaaataatcattttgggaattgaaatgtcaaagataattacttcacgataagtagttgaagatagtttaaatttttctttttgtattac
ttcaatgaaggtaacgcaacaagattagagtatatatggccaataaggtttgctgtaggaaaattattctaaggagatacgcgagagggcttctcaaatttattcagaga
tggatgtttttagatggtggtttaagaaaagcagtattaaatccagcaaaactagaccttaggtttattaaagcgaggcaataagttaattggaattgtaaaagatatct
aattcttcttcatttgttggaggaaaactagttaacttcttaccccatgcagggccatagggtcgaatacgatctgtcactaagcaaaggaaaatgtgagtgtagacttt
aaaccattttttattaatgactttagagaatcatgcatttgatgttactttcttaacaatgtgaacatatttatgcgattaagatgagttatgaaaaaggcgaatatatta
ttcagttacatagagattatagctggtctattcttagttataggacttttgacaagatagcttagaaaataagattatagagcttaataaaagagaacttcttggaatta
gctgcctttggtgcagctgtaatggctattggtatggctccagcttactggttaggttttaatagaaaaattccccatgattgctaattatatctatcctattgagaaca
acgtgcgaagatgagtggcaaattggttcattattaactgctggtgctatagtagttatccttagaaagatatataaatctgataaagcaaaatcctggggaaaatattg
ctaactggtgctggtagggtttggggattggattatttcctctacaagaaatttggtgtttactgatatccttataaataatagagaaaaaattaataaagatgatat
```

How to do it?

- The straight-forward way (naïve algorithm)
  - We use two counter: t, p
  - One (outer, t) runs through T
  - One (inner, p) runs through P
  - Compare characters at position T[t+p-1] and P[p]

```
for t = 1 to |T|
        match := true;
        p := 1;
        while ((match) and (p <= |P|))
                if (T[t + p – 1] <> P[p]) then
                        match := false;
                else
                        p := p + 1;
        end while;
        if (match) then
                -> OUTPUT t
end for;
```

Examples

Typical case		Worst case	
T	ctgagatcgcgta	T	aaaaaaaaaaaaaa

```
T   ctgagatcgcgta
P   gagatc
     gagatc
      gagatc
       gagatc
        gagatc
         gatatc
          gatatc
           gatatc
```

```
T   aaaaaaaaaaaaaa
P   aaaaat
     aaaaat
      aaaaat
       aaaaat
           ...
```

- How many comparisons do we need in the worst case?
  - t always runs through T
  - p runs through the entire P for every position in t (worst case)
  - Thus: O(|P|*|T|)
  - A lot: |T|=250M (chromosome), |P|=250 (exon) => ~62E9 ops

Other Algorithms

- Exact substring search has been researched for decades
  - Boyer-Moore, Z-Box, Knuth-Morris-Pratt, Karp-Rabin, Shift-AND, ...
  - All have WC complexity O(|P| + |T|)
  - Real performance depends a lot on size of alphabet and composition of strings (most have strengths in certain settings)
- One simple and popular algorithm: Boyer-Moore
  - We present a simplified form
  - BM is among the fastest algorithms in practice
- Much better performance if T can be preprocessed
  - Best algorithms reach O(|P|)

This Lecture

- Exact substring search
  - Naïve
  - Boyer-Moore
- Searching with profiles
  - Sequence profiles
  - Ungapped approximate search
  - Statistical evaluation of search results

Boyer-Moore Algorithm

- R.S. Boyer /J.S. Moore. „A Fast String Searching Algorithm“, Communications of the ACM, 1977
- Main idea
  - Again, we use two counters (inner loop, outer loop)
  - Inner loop runs from right-to-left
  - If we reach a mismatch, we know
    - The character in T we just haven’t seen
      - This is captured by the bad character rule
    - The suffix in P we just have seen
      - This is captured by the good suffix rule
- Use this knowledge to make longer shifts in T

Bad Character Rule

- Setting 1
  - We are at position t in T and compare right-to-left
  - Let i by the position of the first mismatch in P
    - We saw n-i matches before
  - Let x be the character at the corresponding pos (t-n+i) in T
  - Candidates for matching x in P
    - Case 1: x does not appear in P at all – we can move t such that t-n+i is not covered by P anymore

```
T    xabxfabzzabwzzbzzb        T    xabxfabzzabwzzbzzb
P    abwxyabzz                 P            abwxyabzz
```

What next?

Bad Character Rule 2

- Setting 2
  - We are at position t in T and compare right-to-left
  - Let i by the position of the first mismatch in P
  - Let x be the character at the corresponding pos (t-n+i) in T
  - Candidates for matching x in P
    - Case 1: x does not appear in P at all
    - Case 2: Let j be the right-most appearance of x in P with j<i – we can move t such that j and i align

```
T   xabxkabzzabwzzbzzb
P        abzwyabzz
            ↑  ↑
            j  i
```

```
T   xabxkabzzabwzzbzzb
P           abzwyabzz
                ←
```

What next?

Bad Character Rule 3

- Setting 3
  - We are at position t in T and compare right-to-left
  - Let i by the position of the first mismatch in P
  - Let x be the character at the corresponding pos (t-n+i) in T
  - Candidates for matching x in P
    - Case 1: x does not appear in P at all
    - Case 2: Let j be the right-most appearance of x in P with $j<i$
    - Case 3: As case 2, but $j>i$ – we need some more knowledge

```
T   xabxkabzzabwzzbzzb
P           abzwyabzz
```

Preprocessing 1

- In case 3, there are some “x” right from position i
  - For small alphabets (DNA), this will almost always be the case
  - Thus, case 3 is the usual one
- These “x” are irrelevant – we need the right-most x left of i
- This can (and should!) be pre-computed
  - Build a two-dimensional array A[|Σ|,|P|]
  - Run through P from left-to-right (pointer i)
  - If character c appears at position i, set all A[c,j]:=i for all j>=i
  - Possible in O(|A|); negligible because P is small
  - Constant lookup time during search

(Extended) Bad Character Rule

- Simple, effective for larger alphabets
- For random DNA, average shift-length is ~2
  - Expected distances to the next match using EBCR
  - Per position in t, the expected length of the match also is ~2
  - Thus, we expect ~ 2*|T|/2 = |T| comparisons
- Worst-Case complexity?

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- Simple, effective for larger alphabets
- For random DNA, average shift-length is ~2
  - Expected distances to the next match using EBCR
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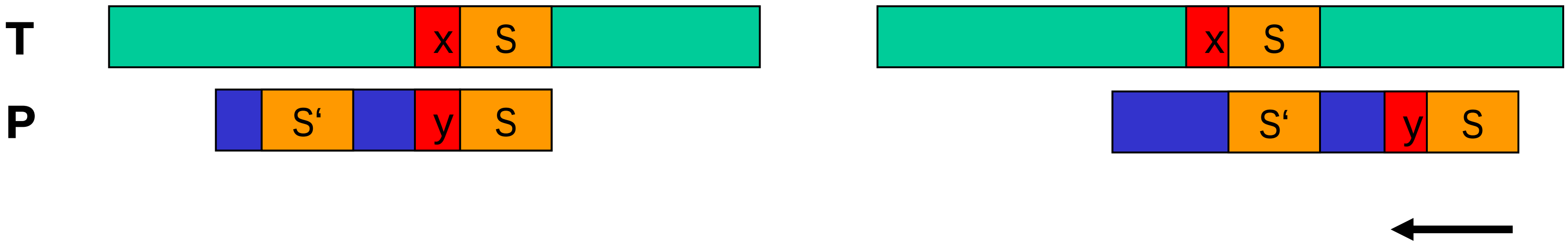
  - Thus, we expect ~ 2*|T|/2 = |T| comparisons
- Worst-Case complexity?

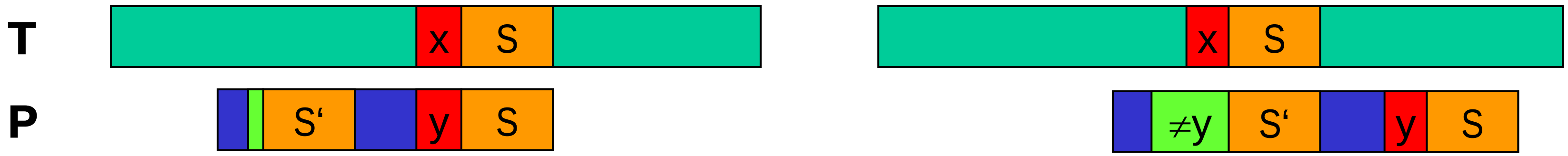
Good-Suffix Rule

- Recall: If we reach a mismatch, we know ...
  - The character in T we just haven't seen
  - The suffix in P we just have seen
- Good suffix rule
  - We have just seen some matches in P; let this suffix be S
  - Where else does S appear in P?
  - If we know the right-most appearance S' of S in P, we can immediately align S' with the current match in T
  - If S does not appear at least twice in P, we shift t by |P|- |S|+1



Good-Suffix Rule – One Improvement

- Actually, we can do a little better
- Not all S‘ are of interest to us



- We only need S‘ whose next character to the left is not y
- Why don‘t we directly require that this character is x?

Complete Algorithm

```
t := 1;
while (t<=|T|-|P|) do                       \\ outer loop
  p := |P|;
  match  := true;
  while (match and p>=1) do                 \\ inner loop
    if (T[t+p]=P[p]) then p := p-1          \\ matching chars
    else match := false;                    \\ mismatch
  end while;
  if match then print t;                    \\ complete match
  compute shift s1 using BCR(t,p);
  compute shift s2 using GSR(t,p);
  t := t + max(s1, s2);                     \\ shift maximal
end while;
```

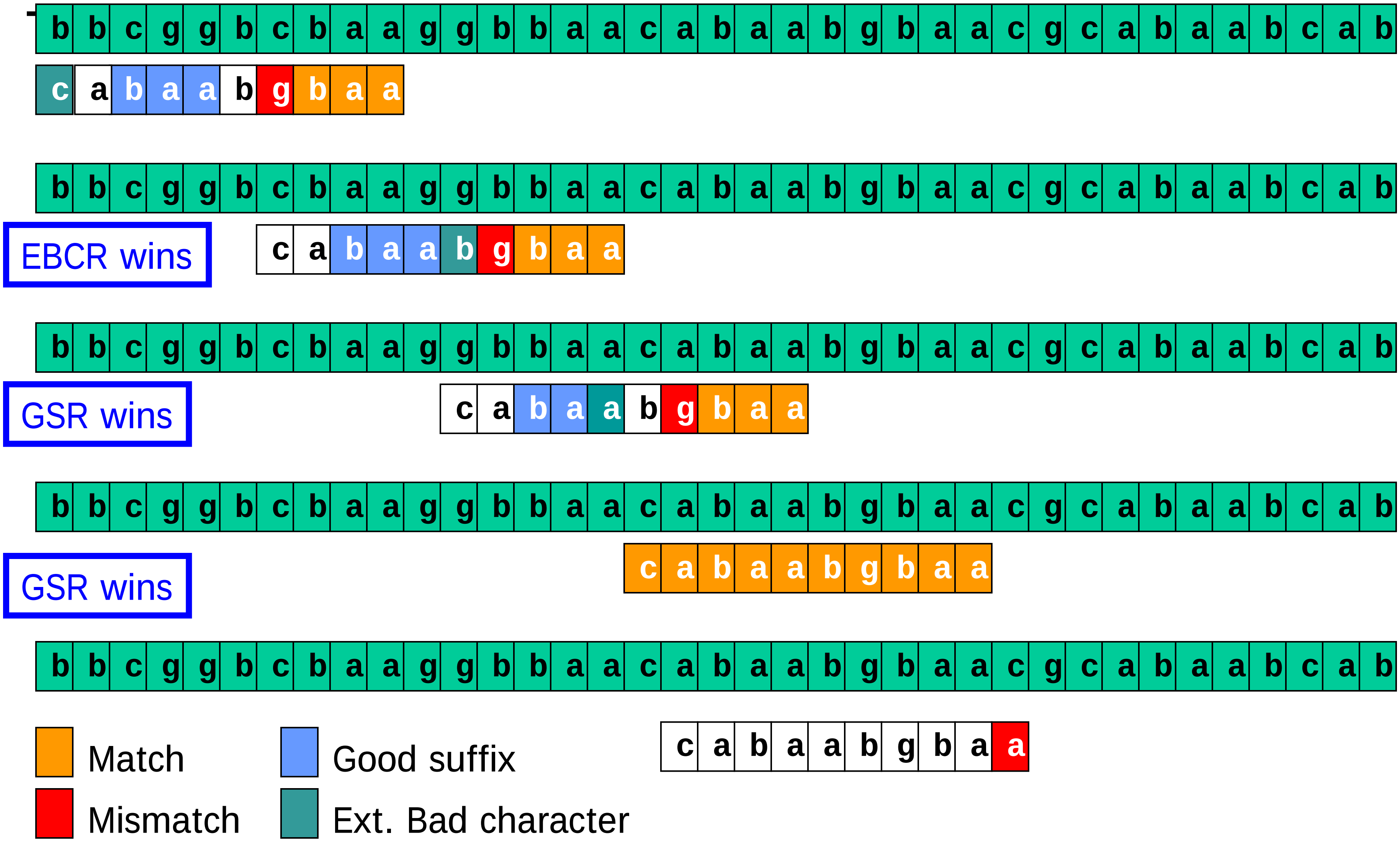
GSR Preprocessing

- We need to find all occurrences of all suffixes of P in P with restrictions on the character left of the suffix
- Could be computed using naïve algorithm for each suffix
- Or, more complicated, in linear time (not this lecture)
- Runtime negligible since we assume P being short

Concluding Remarks

- Worst-case complexity of Boyer-Moore is O(|P|*|T|)
  - WC complexity can be reduced to linear (not this lecture)
- Empirical runtime is sub-linear
  - The larger the alphabet (with roughly equal character frequencies), the faster
- Faster variants
  - Often, using the GSR does not pay off
  - BM-Horspool: Instead of looking at the mismatch character x, always look at the symbol in T aligned to the last position of P
    - Generates longer shifts on average (i is maximal)
- In practice, also naïve algorithm is quite competitive for random strings and non-trivial alphabets (not for DNA)
  - Empirical results much better than worst-case estimations

Example


b b c g g b c b a a g g b b a a c a b a a b g b a a c g c a b a a b c a b
c a b a a b g b a a
b b c g g b c b a a g g b b a a c a b a a b g b a a c g c a b a a b c a b
EBCR wins
c a b a a b g b a a
b b c g g b c b a a g g b b a a c a b a a b g b a a c g c a b a a b c a b
GSR wins
c a b a a b g b a a
b b c g g b c b a a g g b b a a c a b a a b g b a a c g c a b a a b c a b
GSR wins
c a b a a b g b a a
b b c g g b c b a a g g b b a a c a b a a b g b a a c g c a b a a b c a b
c a b a a b g b a a
Match
Mismatch
Good suffix
Ext. Bad character

This Lecture

- Exact substring search
- Searching with profiles
  - Splicing
  - Position Specific Weight Matrices
  - Likelihood scores

Approximate Search (First Step)

- Requiring an exact match is too strict in most bioinformatics applications
  - Sequencing errors, mutations, individual differences, ...
- More often, one is interested in matches similar to P
- Many definitions of “similar” are possible
- Now: Position Specific Weight Matrices (PSWM)
  - Also called profiles
  - Powerful tool with many bioinformatics applications
  - We develop the idea using an example taken from Spang et al. “Genome Statistics”, Lecture 2004/2005, FU Berlin

Splicing

- Not all DNA of a “gene” is translated into amino acid
- Splicing: Removal of introns
- Alternative splicing: Removal of some exons

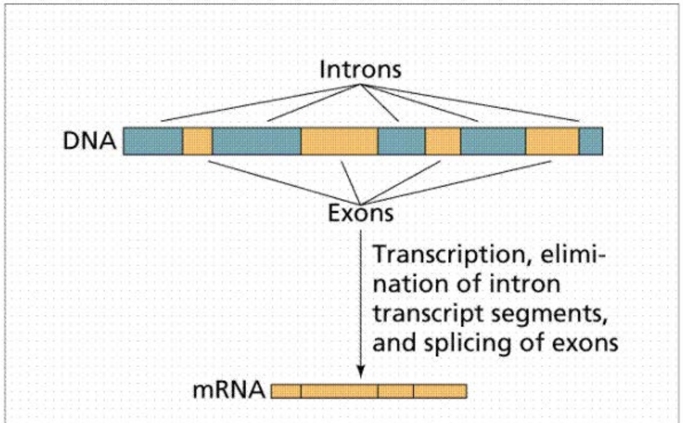
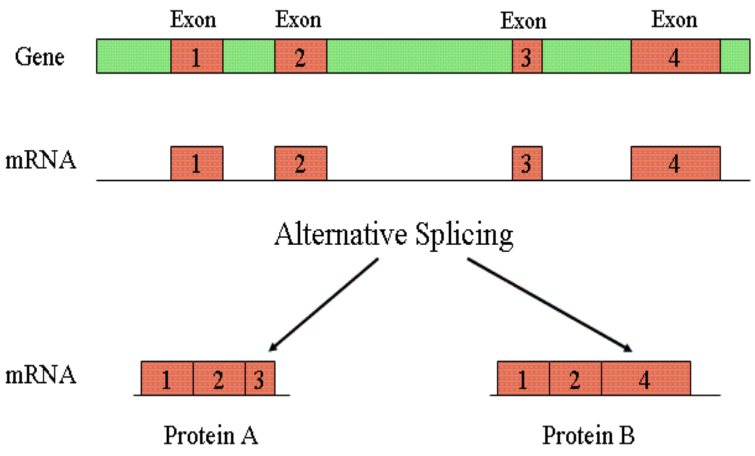
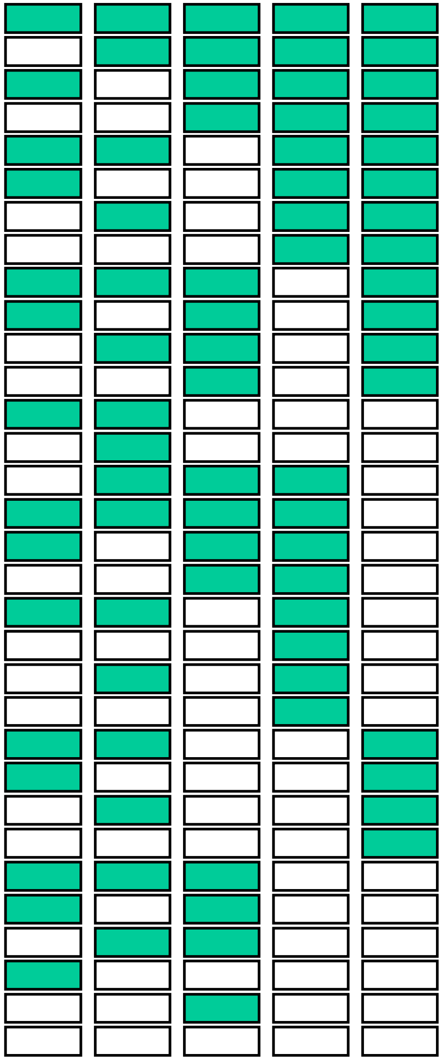


Figure 13.3



Diversity



- From a gene with n exons, alternative splicing can create 2^n-1 proteins
- Example: Troponin T (muscle protein)
  - 18 exons
  - 64 different known isoforms
  - 10 exons present in all isoforms

- Source: Eurasnet, „Alternative Splicing“

Recognizing Splice Sites

- A special enzyme (spliceosome) very precisely recognizes exon-intron boundaries in mRNA
- Spliceosome recognizes certain sequence motifs
- How are these motifs characterized? Can we find them?
  - Very often, introns start with GT and end with AG
  - But that is not specific enough - why?
  - In random sequences, we expect a GT (AG) at every 16^{th} position
  - Thus, the average distance between a GT and an AG is 16, and we find such pairs very often
  - But: Introns typically are larger than 100 bases

Context of a Splice Site

CTCCGAAGTAGGATT	CTCCGAAGTAGGATT
TCAGAAGGTGAGGGC	TCAGAAGGTGAGGGC
TTGGAAGGTTCGCAG	TTGGAAGGTTCGCAG
TACTCAGGTACTCAC	TACTCAGGTACTCAC
CGCCCAGGTGACCGG	CGCCCAGGTGACCGG
AGAAAGAGTAAGCTC	AGAAAGAGTAAGCTC
CAATGCTGTATGTGT	CAATGCTGTATGTGT
GGTCTCGGTAACTGC	GGTCTCGGTAACTGC
CCTGCTGGTAAGGCC	CCTGCTGGTAAGGCC
TGTTGCGGTAGGTCC	TGTTGCGGTAGGTCC

- Observing real splice sites, we find no crisp context
- But: columns are not composed at random
- How can we capture and quantify this knowledge?

Vizualization: Sequence Logos

- Very popular
- Based on information content of each base at each position
  - Which, in turn, is based on the entropy of the columns

CTCCGAAGTAGGATT
TCAGAAGGTGAGGGC
TTGGAAGGTTCGCAG
TACTCAGGTACTCAC
CGCCCAGGTGACCGG
AGAAAGAGTAAGCTC
CAATGCTGTATGTGT
GGTCTCGGTAACTGC
CCTGCTGGTAAGGCC
TGTTGCGGTAGGTCC

Position-Specific Weight Matrices

\# DONOR FREQUENCY MATRIX from http://genomic.sanger.ac.uk/spldb/SpliceDB.html

	1	2	3	4	5	6	7	8	9
A	34.08	60.36	9.14	0.00	0.00	52.57	71.26	7.08	15.98
C	36.24	12.90	3.27	0.00	0.00	2.82	7.56	5.50	16.46
G	18.31	12.48	80.34	100.00	0.00	41.94	11.76	81.35	20.90
T	11.38	14.25	7.24	0.00	100.00	2.55	9.29	5.88	46.16

- Count in every column the frequencies of all bases
- Store the relative frequencies in an array of size |P|*|Σ|
  - With |P| being the size of the context around the splice sites
- At “GT”, all values except one are 0% and one is 100%
  - Actually, GT is not perfectly conserved in real sequences
- In random sequences, all values should be 25%

Scoring with a PSWM

- Eventually, we want to find potential splice sites in a genome G (e.g. to do gene prediction)
- We need a way to decide, given a sequence S and a PSWM A (both of the same length): Does S match A?
  - We devise a function assigning a score to S given A
  - With this function, we score all subsequences of length |A| in G
  - Subsequences above a given threshold are considered candidates
- We give this question a probabilistic interpretation
  - Assume, for each column, a dice which four faces; each face is thrown with probability equal to the relative frequencies as given in the PSWM A for this column
  - What is the probability that this dice generates S?

Examples

- In random sequences, all values in A are 25%, and all possible S would get the same probability: $\frac{1}{4}^{|S|}$
- But

	1	2	3	4	5	6	7	8	9
A	34.08	60.36	9.14	0.00	0.00	52.57	71.26	7.08	15.98
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  – P(AAGGTAAGT) ~ 0.3*0.6*0.8*1*1*0.5*0.7*0.8*0.5 ~ 0.023
  – P(CCCGTCCCC) ~ 0.4*0.1*0.03*1*1*0.02*0.08*0.05*0.2 ~ 3E-8
  – P(AGTCTGAAG) ~ 0.3*0.1*0.1*0*1*0.4*0.7*0.07*0.2 = 0

  – 1st sequence matches A much better than the second
  – 3rd sequence hints towards overfitting

This Lecture

- Exact substring search
- Searching with profiles
  - Splicing
  - Position Specific Weight Matrices
  - Likelihood scores

I am not Convinced (yet)

- Is S actually a match for A?
- We need to quantify the “goodness” of a score
  - By comparing it to other / best / worst scores
- Observations
  - The first match on the previous slide is about as good as it can get: Best possible sequence has a score of 0.025 (compared to 0.023)
  - If match S is not a splice site, it is an “ordinary” sequence. How likely is it that S is generated under this zero model (Z)?
    - “Zero model” often means: Equal probability for all bases
      - Could include species bias, coding region bias, CpG island bias, ...
    - $p(S|\text{"zero"}) = \frac{1}{4}^9 \sim 3.8E\text{-}6$
  - Thus, is it much more likely (app. 6000 times more likely) that S was generated under the A model than that is was generated under the Z model

Likelihood (Odds) Ratios

- Given two models A, Z. The likelihood ratio score of a sequence S is the ratio of p(S|A) / p(S|Z)
  - score(AAGGTACGT) ~ 6000
  - score(CCCGTCCCC) ~ 1/140
  - score(CTGGTCCGA) ~ 3
  - score(TCCGTCCCC) < 1

	1	2	3	4	5	6	7	8	9
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1. P (AAGGTACGT) ≈ 0.34*0.6*0.8*1*1*0.53*0.71*0.81*0.46 =0.023
2. P (CCCGTCCCC) ≈ 0.36*0.13*0.03*1*1*0.03*0.08*0.05*0.16 =2.7e-08
3. P (CTGGTCCGA) ≈ 0.36*0.14*0.8*1*1*0.03*0.08*0.81*0.16 =1.25e-05
4. P (TACCTCCGT) = 0

- Also called odds score
  - This is just one (popular) method for computing a “goodness”

Matching with a PSWM

- Given genome G, models A and Z, and a threshold t: Find all S in G with likelihood(S)>t
- Method: For all S with |S|=|A|, compute likelihood (S)
  - This requires ~|G|*|A| divisions and multiplications
  - Divisions can be saved easily (how?)

Numeric trick

- Values get quite small (close to 0) for longer A
- This yields problems with numeric stability in programs
- Better: Compute log-likelihood score s'=$\log_2$(score(...))
  - Also faster: Replaces multiplication with addition
  - Pre-compute divisions

$$s'(S) = \log\left(\frac{p(S\mid A)}{p(S\mid Z)}\right) = \log\left(\frac{p(S_1\mid A_1)*\ldots*p(S_n\mid A_n)}{p(S_1\mid Z_1)*\ldots*p(S_n\mid Z_n)}\right)$$

$$= \log\left(\frac{p(S_1\mid A_1)}{p(S_1\mid Z_1)}\right) + \ldots + \log\left(\frac{p(S_n\mid A_n)}{p(S_n\mid Z_n)}\right)$$

Beware

- Assume a highly conserved motif A of length 8
  - The chance that an arbitrary S, |S|=8, matches A is only 0.000015
  - But: |G|=3.000.000.000
  - Only by change, we will have ~45,000 perfect matches
  - This applies even if we set the threshold at maximum
  - Help: For |A|=16, we expect less than 1 match by chance
- Generally: Number of false hits depend on the threshold t
  - Higher t: Stricter search, less false hits, but may incur misses
  - Lower t: Less strict, less misses, but more false hits
- Note: A match is an hypothesis calling for further analysis
  - By additional knowledge (e.g.: is S part of a gene?)
  - By experimentation (e.g.: can we find an isoform spliced at S)?

Pattern Matching

- We discussed exact matching and matching with a PSWM
- But motifs also may look quite differently
  - Motifs (domains) in protein sequences
  - Some important positions and much “glue” of unspecified length
  - Pattern here may be: [AV].*FGKG[SIV]^2.*[LI]...
  - Which positions in S should we compare to which columns in P?
  - How can we derive a specific pattern P from S_1-S_6?

```
S1: M---AIDE----NKQKALAAALGQ--KQFGKGSIMRLGEDR-SMDVETISTGSLSLDI
S2: MSDN--------KKQQALELALKQI-KQFGKGSIMKLGDG-ADHSIEAIPSGSIALDI
S3: M----AINTDTSGKQKALTMVLNQIERSFGKGVIMRLGDA-TRMRVETISTGALTLDL
S4: M-----------DRQKALEAAVSQ--RAFGKGSIM-LGGKD---ETEVVSTRILGLDV
S5: M------DE---NKKRALAAALGQI-KQFGKGVSMRMGDHE-RQAIPAISTGSLGLDI
S6: MD---------------------K-EKSFGKGSIMRMGEE-VVEQVEVIPTGSIA---
```

Further Reading

- On string matching algorithms
  - Gusfield
- On sequence logos and TFBS-identification
  - Christianini & Hahn, chapter 10
  - Merkl & Waack, chapter 10