Stochastic modeling: How good do randomly chosen fragments cover a molecule?

Definition

Let $A : \mathbb{R}_0 \to \mathbb{N}$ a nondecreasing function satisfying $A(0) = 0$, where $A(t)$ describes the number of events until time t. Then we have a poisson process with rate λ, if

(1) $\Pr[A(s+t) - A(s) = n] = \exp(-\lambda \cdot t)\frac{(\lambda \cdot t)^n}{n!}$ *and*

(2) *the distribution of the number of events is stationary, i.e. it depends only on the length but not on the position of a given interval.*

Theorem

Let A be a poisson process.

a) *The expected number $\mathbb{E}[A(t)]$ of events in an interval of length t satisfies $\mathbb{E}[A(t)] = \lambda \cdot t$.*

b) *Let T_n be the time between the $(n-1)$-th and the n-th event. Then*

$$\Pr[T_1 > t] = \Pr[T_n > t] = \exp(-\lambda \cdot t).$$

Model: Assuming fragments of length L are cut from multiple copies of a DNA molecule of length C randomly and independently. Then for i any position of the molecule

$$\Pr[i \text{ is covered by randomly chosen fragment}] = \frac{L}{C}$$

holds and thus

$$\left(1 - \frac{L}{C}\right)^N \approx \exp\left(-\frac{L \cdot N}{C}\right) \tag{1}$$

is the probability that i is not covered by any of the N fragments.

Poisson process?

$$\exp(x) = \lim_{n \to \infty} \left(1 + \frac{x}{n}\right)^n$$

We let $\lambda := \frac{L}{C}$ and ask for the probability of exactly n of the N fragments covering position i. This probability is given by

$$\begin{aligned}\binom{N}{n} \cdot \left(\frac{L}{C}\right)^n \cdot \left(1 - \frac{L}{C}\right)^{N-n} &\approx \frac{N!}{n! \cdot (N-n)!} \lambda^n \cdot \exp(-\lambda \cdot N) \\ &= \frac{N!}{N^n \cdot (N-n)!} \exp(-\lambda \cdot N) \cdot \frac{(\lambda \cdot N)^n}{n!} \\ &\approx \exp(-\lambda \cdot N) \cdot \frac{(\lambda \cdot N)^n}{n!}.\end{aligned}$$

So the number of fragments covering a fixed position is approximately poisson distributed with rate $\lambda = \frac{L}{C}$. (This statement holds for $n \ll N \ll C$ and $L \ll C$.)

The expected number of fragments covering position i is thus $R := \lambda \cdot N = \frac{L \cdot N}{C}$. R is called *redundancy* of the fragment set.

Corollary

The expected number of positions not covered is (approximately) given by

$$\exp\left(-\frac{L \cdot N}{C}\right) \cdot C = \exp(-R) \cdot C.$$

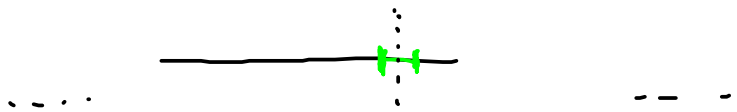
□

Definition

Let $\mathcal{F}$ be a set of fragments of length L and $\Theta \in [0,1]$. We take $\mathcal{F}$ as vertices of a graph and connect two fragments $f_1, f_2 \in \mathcal{F}$ by an indirected edge, if a suffix (prefix) of f_1 of length at least $\Theta \cdot L$ is a prefix (suffix) of f_2 (overlap). We get an undirected graph whose connected components are called Θ-islands.

Intuition: Fragments with only small overlap should not be considered overlapping.

How many Θ-islands are to be expected?



Lemma

Let $\Theta \in [0,1]$ and redundancy R be given and let N be the number of randomly chosen fragments. Then

$$N \cdot \exp(-R \cdot (1-\Theta))$$

is (approximately) the expected number of Θ-islands.

Proof: $C \hat{=}$ length of molecule

$N \hat{=}$ # of fragments

$L \hat{=}$ length of fragment

$\lambda := L/C$

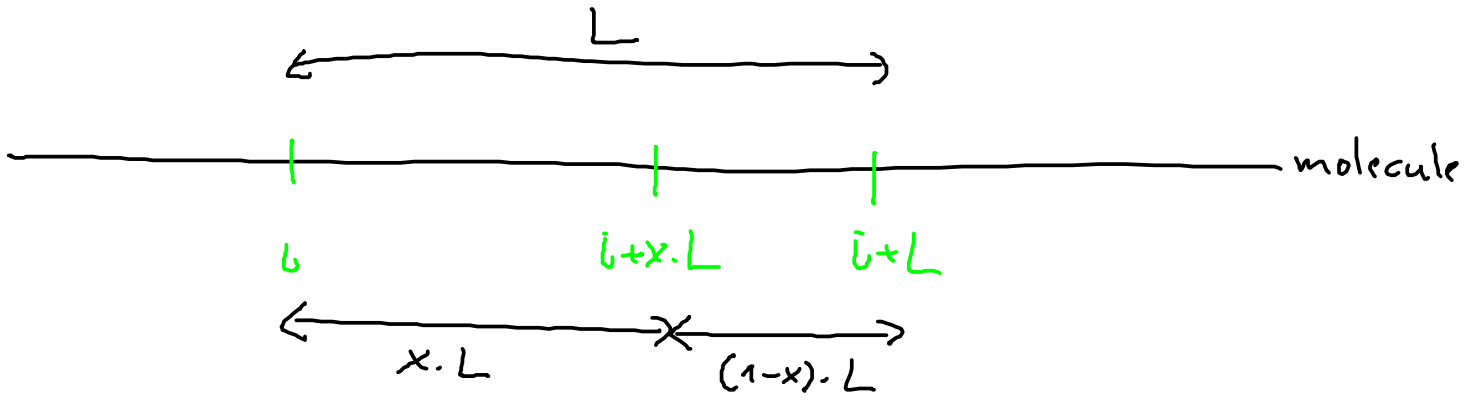
Let i be any position of the molecule

We define

$J(x) := Pr[$positions i and $i+x \cdot L$ are covered by randomly chosen fragment$]$

Claim: $J(x) = \begin{cases} \exp(-R(1-x)) & \text{for } 0 \le x \le 1 \\ 0 & \text{otherwise} \end{cases}$

with $R = \lambda \cdot N = \frac{L \cdot N}{C}$ the redundancy.



$\Longrightarrow$ The number of different locations for a random fragment to cover both positions is given by $(1-x) \cdot L$

↝ P_1[i and $i+x \cdot L$ to be covered by random fragment]

$= \frac{(1-x) \cdot L}{C}$

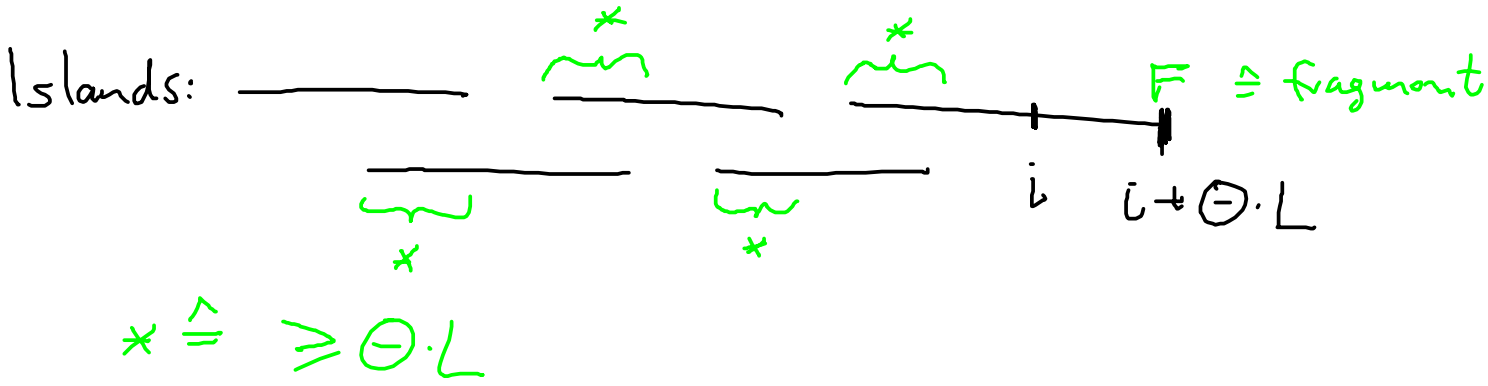
↝ P_1[i and $i+x \cdot L$ are not covered by any of the N fragments]

$= \left(1 - \frac{(1-x) \cdot L}{C}\right)^N = \left(1 - \frac{(1-x) \cdot R}{N}\right)^N$

$R = \frac{L \cdot N}{C}$

$N \to \infty$

$= \exp(-(1-x) \cdot R)$



Observation: F is the only fragment which covers positions i and $i+\Theta \cdot L$ since otherwise it would not be the rightmost element of the considered island.

$$\mathbb{E}[\#\Theta\text{-islands}] = \mathbb{E}[\#\text{rightmost fragments}]$$

↪ we are looking for

$$\mathbb{E}[\#\text{fragments covering positions } i \text{ and } i+\Theta L \text{ exclusively}]$$

$$= N \cdot J(\Theta) \approx N \cdot \exp(-R(1-\Theta))$$

Since $J(\Theta) \hat{=} \Pr[$random fragment does not overlap the rightmost fragment$]$

$\hat{=} \Pr[$fragment is the last of an island$]$

□

Example: We consider the case of a molecule with 10^8 bases to be mapped. We assume that a library of 10000 fragments has been created, each around 50000 bases long. In this case $R = \frac{5 \cdot 10^4 \cdot 10^4}{10^8} = 5$ and for Θ small enough $N \cdot \exp(-R \cdot (1 - \Theta)) \approx 10^4 \cdot \exp(-5) = 67.37946999\ldots$ many islands are to be expected.

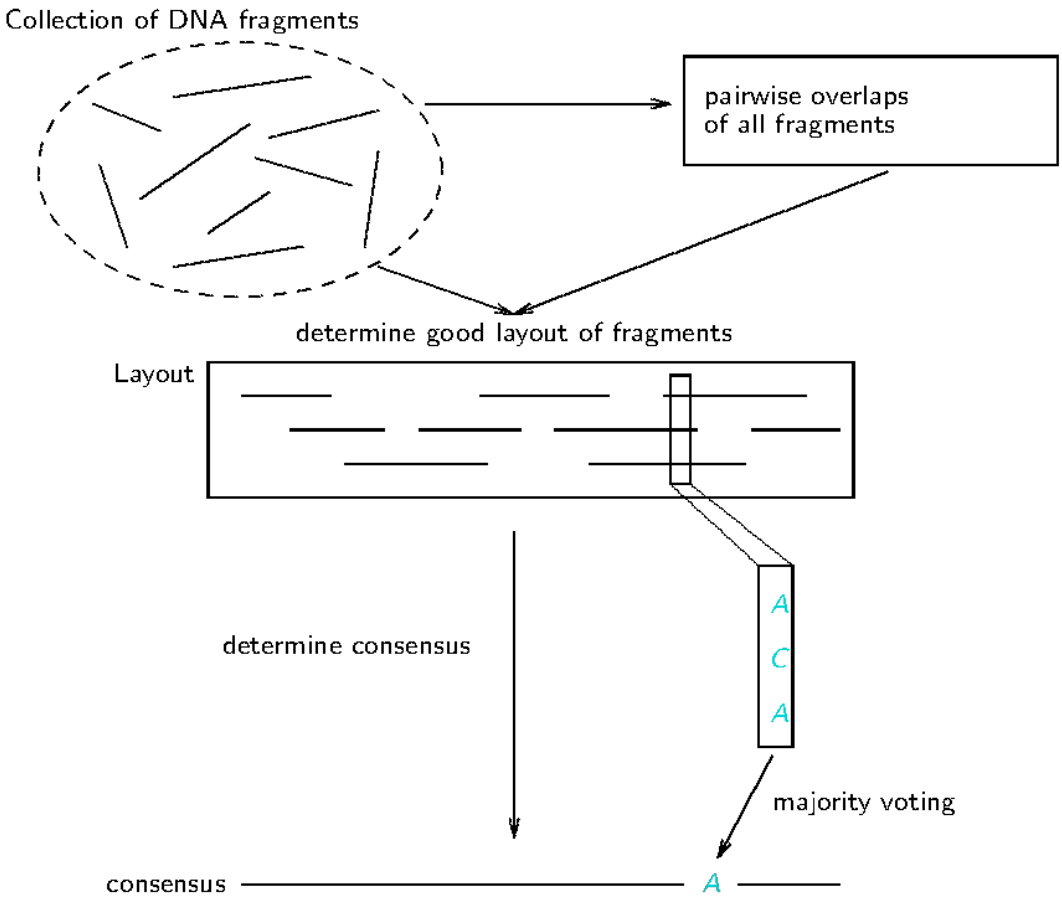
Shotgun sequencing and fragment assembly:

Definition

Let D be a DNA molecule to be sequenced and $S = \{s_1, \ldots, s_n\}$ the set of words (fragment sequences), observed at a shotgun sequencing of D. Then the fragment assembly problem *is to determine (algorithmically) the arrangement of the words from S corresponding to their original positions in D.*

Solution of the fragment assembly problem:

1. **Overlap:** In this phase the possible overlaps of pairs of words from S are determined. They do not have to be exact prefix-suffix pairs but alignments with great similarity may also be used.
2. **Layout:** From the result of the first phase now an arrangement (similar to a semiglobal alignment) of the words from S is designed, representing the arrangement of the fragments in D. The structure resulting from the overlaps is called *Layout*.
3. **Consensus:** As the layout usually contains several fragments overlapping at a given position of D in the final step it has to be decided, which symbol is chosen. This can e.g. be done by majority voting.



Sources of errors and problems:

- sequencing errors,
- creation of chimeras,
- uncovered parts (toxic effect of a fragments wrt. host),
- orientation (s_i or complement reverse to s_i?),
- repeats (of (almost) identical substrings) (overlap or not?).

Shortest superstrings

We create as layout an overlapping of the fragments that implies a sequence for D as short as possible.
Formally we are looking for a word w_S containing all $s_i \in S$ (superstring for S) for $S = \{s_1, \ldots, s_n\}$ a subword free set of words. We call this problem the *shortest common superstring problem* (SCSP).

Another view at the problem is given by the notion of compression:

Definition

Let w_S a superstring for set $S = \{s_1, \ldots, s_n\}$. The compression *of w_S is defined by*

$$comp(w_S) := \left(\sum_{1 \leq i \leq n} |s_i| \right) - |w_S|.$$

Intuitively $comp(w_S)$ is the number of symbols that w_S saves compared to the trivial superstring $s_1 \cdot s_2 \cdots s_n$.

Correspondingly the maximum compression common superstring problem (MCCSP) is the problem of finding algorithmically a superstrings for S with maximal compression.

Obviously: Optimal solutions for SCSP and MCCSP are the same.

But: Performance guarantees can not be exchanged between the problems.

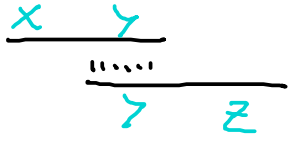
Definition

Given a set $S := \{s_1, s_2, \ldots s_n\}$, $n > 0$, of words. If there are decompositions of $s_i, s_j \in S$ satisfying

- $s_i = xy$,
- $s_j = yz$,
- $x \neq \varepsilon$ *and* $z \neq \varepsilon$,
- $|y|$ *maximal with these properties,*

y is called overlap *of s_i* and *s_j (notation $Ov(s_i, s_j)$). The* Merge *$< s_i, s_j >$* of *s_i* and *s_j then is the word*

$$< s_i, s_j > := xyz,$$



and we call x prefix of the merge $< s_i, s_j >$ (notation $Pref(s_i, s_j)$).

Definition

Let $S = \{s_1, \ldots, s_n\}$ *be a set of words and* G *the digraph with vertices* S *and edges* $S \times S$. *The* overlap graph $OG(S)$ *of* S *is the digraph we get from marking* G *according to*

$$ov : (S \times S) \to \mathbb{N}_0 : ov(s_i, s_j) := |Ov(s_i, s_j)|.$$

If we mark G *according to*

$$pr : (S \times S) \to \mathbb{N}_0 : pr(s_i, s_j) := |Pref(s_i, s_j)|,$$

we get the distance graph *of* S *(notation* $DG(S)$*).*

Example: $S = \{aabca, aacab, aaddd, ababaa, caba\}$, table shows edge weights $ov(x, y) \mid pr(x, y)$:

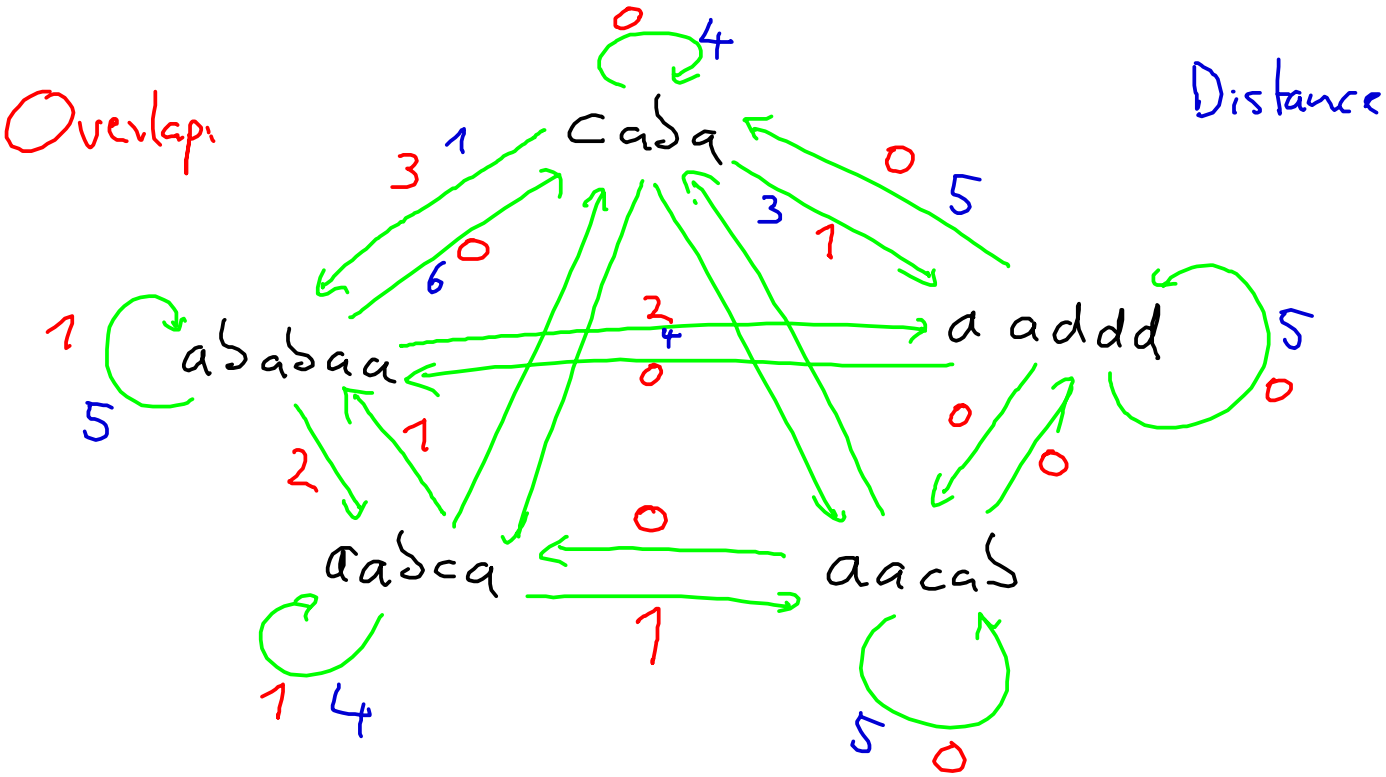
Greedy:

aa caba

	y				
x	$aabca$	$aacab$	$aaddd$	$ababaa$	$caba$
$aabca$	1 | 4	1 | 4	1 | 4	1 | 4	2 | 3
$aacab$	0 | 5	0 | 5	0 | 5	2 | 3	3 | 2
$aaddd$	0 | 5	0 | 5	0 | 5	0 | 5	0 | 5
$ababaa$	2 | 4	2 | 4	2 | 4	1 | 5	0 | 6
$caba$	1 | 3	1 | 3	1 | 3	3 | 1	0 | 4

Overlap

Distance



Using our method from the exercises (9. Task) to find all pairwise overlaps of words in S we can create the overlap graph for S in time $\mathcal{O}(N \cdot (\log(N) + |\Sigma| + n))$, assuming S is a set of words over Σ and $N := \sum_{1 \leq i \leq n} |s_i|$.

Using $pr(s_i, s_j) = |Pref(s_i, s_j)| = |s_i| - |Ov(s_i, s_j)|$ we get the same running time for creating the distance graph.

Note that $n^2 = \mathcal{O}(N \cdot n)$ and even $n^2 \leq n \cdot N$ holds as by assumption ε being a substring of every word can't be an element of S.

Edge $s_i \rightarrow s_j$ in the overlap or distance graph can be identified with merge $< s_i, s_j >$.
$\Rightarrow$ Path corresponds to series of merges of the words represented by the nodes. E.g. for path $s_1, s_2, \ldots, s_k$

$$< s_1, s_2, \ldots, s_k >:= Pref(s_1, s_2) \cdot Pref(s_2, s_3) \cdots Pref(s_{k-1}, s_k) \cdot s_k.$$

A superstring for S then corresponds to a path through the graph visiting each node exactly once, a minimal superstring (i.e. a solution for SCSP or MCCSP) to a path with optimal weight (for the distance graph this is the minimal weight).

$\Rightarrow$ Correspondence of our problems and TSP!

Theorem

Dec-SCSP is NP-complete.

□

Approximation algorithm:

```
while |S|>1 do
    Determine s_i, s_j ∈ S, s_i ≠ s_j, with ...
    ...maximal overlap of all pairs in S.
    Set s' :=< s_i, s_j > and S = S \ {s_i, s_j} ∪ {s'}.
return (s ∈ S) //the only word remaining in S
```

This way the algorithm creates a hamiltonian cycle in the overlap graph.

Example: $S = \{aabca, aacab, aaddd, ababaa, caba\}$ (Graph see previous example)
First choice: Two alternatives ($(caba, ababaa)$ and $(aacab, caba)$).
We take $(caba, ababaa) \Rightarrow$ merge $< caba, ababaa >= cababaa$ and $S = \{cababaa, aabca, aaddd, aacab\}$.

Second step: Combine $aacab$ and $cababaa$ to get $aacababaa$ and $S = \{aacababaa, aabca, aaddd\}$.

Third step: Pair $(aacababaa, aaddd)$ has maximal overlap.
$\Rightarrow S = \{aacababaaddd, aabca\}$.

Last step: Output $aabcaacababaaddd$ of length 16 (being a shortest superstring for the input considered).

Theorem

Assuming a constant size of the alphabet above greedy algorithm given input $S = \{s_1, \ldots, s_n\}$ takes running time in $\mathcal{O}(N \cdot (n + \log(N)))$, $N = \sum_{1 \le i \le n} |s_i|$.

Proof:

Constant alphabet size $\rightsquigarrow$ compute all pairwise overlaps in time $\mathcal{O}(N \cdot (n + \log(N)))$ [suffix trees]

Sort the n^2 edges in time $\mathcal{O}(n^2 + N)$ $= \mathcal{O}(n \cdot N)$ (according to label) using distribution counting (labels are bounded above by length

of the longest input sequence and U is N)

Graph: time $O(n^2)$ adjacency matrix.

UNION-FIND (to prevent cycles):

Array $R[1:n]$ with $R[i]$ = name of partition i belongs to

Partition: linear list (storing it length)

Initialize: $\Theta(n)$; n singletons $\{S_i\}$

$\Longrightarrow$ initialization $O(N \cdot (n + \log(N))$