Computational Biology: Algorithms

Lecture 4

9 October 2008

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Overview: First Half of Lecture

- Clone vectors
- Clone libraries and coverage
- Fingerprints and map building
- Mapping with unique probes

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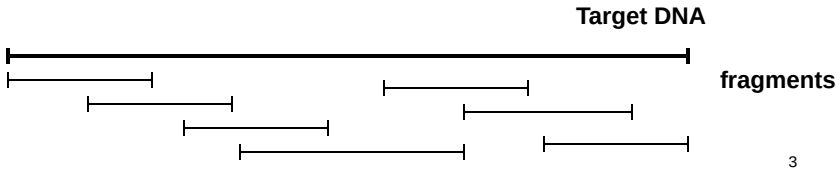
Cloning

Can't deal with DNA at chromosomal lengths (for many organisms)

- Need to process it in pieces
- But need to put it together again
- Hence, capture it as collection (library) of overlapping fragments



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Need to replicate fragments

Insert into some *vector* that a host organism will reproduce

Vector and host determine clone length

- Plasmid 0.1-10 kbp
- λ vector 2-20 kbp
- Cosmid 20-45 kbp
- Bacterial artificial chromosome (BAC) 75-300 kbp
- Yeast artificial chromosome (YAC) 100-1000 kbp

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Clone vectors differ in several ways

- Length of fragment accommodated
- How readily the host takes up the vector
- Copy number per host
- Stability: how faithfully is DNA fragment reproduced
  - Chimerism: fragment splicing or crossover
  - Deletions: of part of fragment
  - Bacterial transposons: “jumping genes”

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Library coverage

Have a library of clones – how much of genome is covered?

Rough estimate

- Assume every cloned fragment has length L
- Assume N clones randomly distributed over genome of length G
- [There are boundary considerations for linear genomes] Assumes a circular genome

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Probability of a base not covered

For particular base b and clone C, probability of base lying in the clone

P(b ∈ C) = L/G $1 - L/G$ $\mathbb{P}(b \notin C)$

Probability that b isn't in any of N clones

$(1 - L/G)^N = (1 - L/G)^{G(N/G)} =$

$((1 - L/G)^G)^{N/G} =$ $(e^{-L})^{N/G} = e^{-LN/G}$ $\left(1 - \frac{a}{d}\right)^d \approx e^{-a}$ gets closer as d grows larger

Number of bases not covered: $G \cdot e^{-LN/G}$

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Example of coverage

Human genome G = 3 gpb

BAC clones L = 200 kbp

Library size N = 25K

LN/G = (25K)(200K)/3 G = $\frac{25 \cdot 200}{30 \phi \phi}$ $\frac{50}{30} \approx 1.67$

3 gbp•$e^{-1.67}$ = $3\text{ gbp} \cdot .188 = 564\text{ Mbp}$

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Physical Mapping

Trying to figure out the order and direction of the clones on the genome

- Generally, without fully sequencing them

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Use “Fingerprints” of Clones

Fingerprint: some property that can be determined for each clone

- Overlapping clones $\rightarrow$ similar fingerprints
- (Hopefully, vice versa)
  - Restriction maps: E. coli, 1987
  - Restriction fragment sizes: Yeast, 1986
  - Hybridization data: Human, 1992

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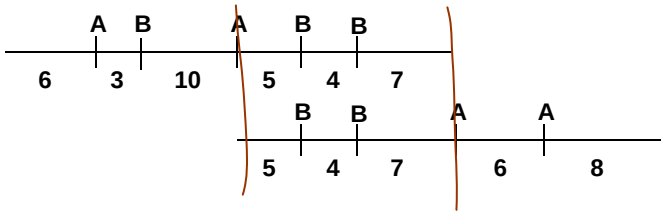
Restriction Map

Derive restriction maps of all clones

Infer overlap if end of one map matches beginning of another (consider reversals)

Time consuming, lengths not precise



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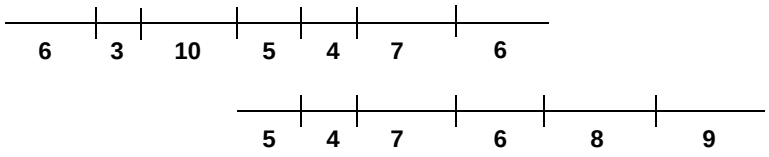
Restriction Fragment Sizes

Generally based on a threshold – certain number or percentage of fragment lengths in common

Faster, more false positives

3, 4, 5, 6, 7, 10

4, 5, 6, 7, 8, 9



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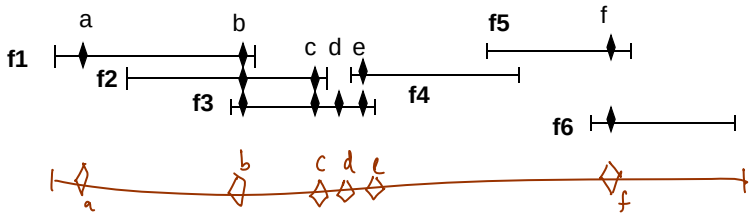
Hybridization Probes



Which probes bind to which fragments

- Unique probes: Sequence Tagged Sites (STS)
- Non-unique probes: short random sequences

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Map Assembly

Combine clones into *contigs*: groups of consistently overlapping clones

Often based on greedy algorithm

- Compute all pairwise overlaps with fingerprinting "strength" of overlap
- Initialize each contig to a single clone
- Repeat: "Greedy"
  - Combine contigs with highest remaining overlap
  - [Make sure consistent with other clones in contig]

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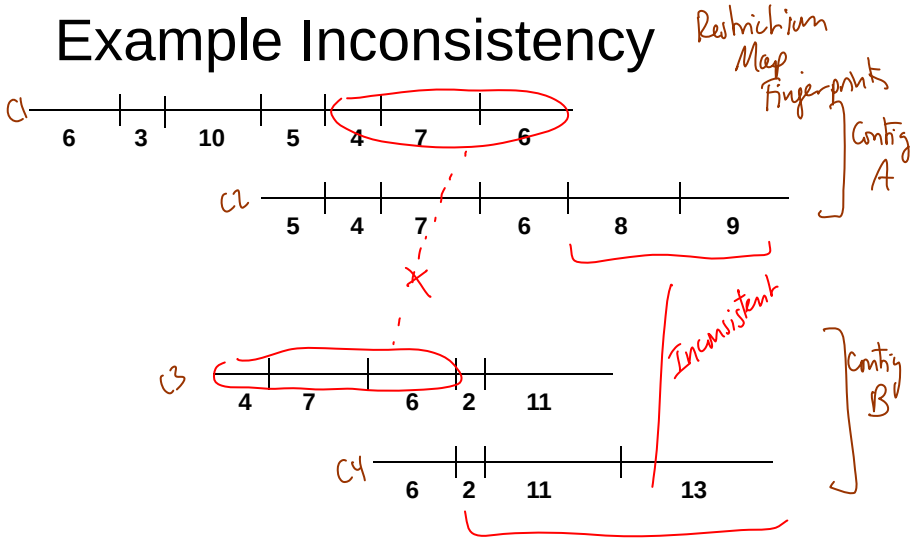
Example Inconsistency



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Mapping with Unique Probes

Given a matrix of which probes are found on which clones:

Can you find an ordering such that in each column, the 1's are consecutive?

	a	b	c	d	e	f	g
f1	1	1					1
f2				1	1	1	
f3				1	1		
f4						1	
f5		1	1		1		
f6		1	1				1

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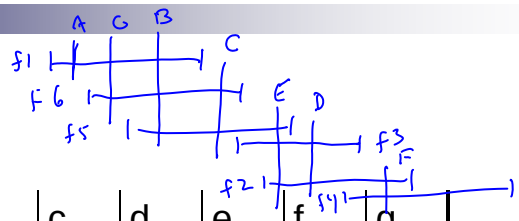
A Solution



	a	b	c	d	e	f	g
f1	1	1					1
f6		1	1				1
f5		1	1		1		
f3				1	1		
f2				1	1	1	
f4						1	

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Another Approach

- Can find consecutive ones in linear time if such an ordering exists
  - Lueker and Booth,1976
  - Not very tolerant of errors
    - Chimerism
    - False negatives
- Are more fault-tolerant algorithms
  - Alizadeh, Karp, Weisser, Zweig, “Physical Mapping of Chromosomes using Unique Probes”
  - In ACM Portal

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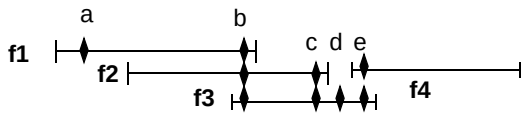
Ordering of Probes

Can tell something about probe order from clone sets

Ca = {f1}
Cb = {f1, f2, f3}
Cc = {f2, f3}
Cd = {f3}
Ce = {f3, f4}



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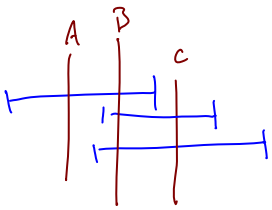
Ordering Information



# of elements (cardinality)

If b is between a and c, then

$|Ca \cap Cb| \geq |Ca \cap Cc|$

$|\{f1\} \cap \{f1,f2,f3\}| \geq |\{f1\} \cap \{f2,f3\}|$

$|\{f1\}| \geq |\emptyset|$

$1 \geq 0$

Isn't always strict. Consider c, d and e

Is d between c and e

$|C_c \cap C_d| \geq |C_c \cap C_e|$

$|\{f3\}| \geq |\{f3\}|$

$=$

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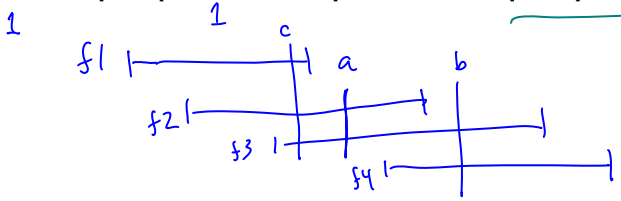
"Closeness" ordering

"Probe a is closer than c to b" if
$|Ca \cap Cb| > |Cc \cap Cb|$
or
$|Ca \cap Cb| = |Cc \cap Cb| \neq \emptyset$ and $|Ca| < |Cc|$



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Neighbors of Probe p

N(p) = set of probes that share some clone with p (excludes p itself)

N(a) = {b, g}	Ca = {f1}
N(b) = {a, c, e, g}	Cb = {f1, f5, f6}
N(c) = {b, e, g}	Cc = {f5, f6}
N(d) = {e, f}	Cd = {f2, f3}
N(e) = {b, c, d, f}	Ce = {f2, f3, f5}
N(f) = {d, e}	Cf = {f2, f4}
N(g) = {a, b, c}	Cg = {f1, f6}

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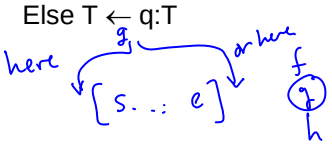
Alizadeh, et al. Algorithm

Build up a probe sequence from a single probe

1. Pick a starting probe p: T = [p]
2. Let T = [s, …, e]. Repeat:
   a. If all of N(e) is in T, T ← reverse(T)
   b. Else, pick a probe q in N(e) not already in T that is closest to e.

      If e is closer than s to q, T ← T:q

      Else T ← q:T



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T = [c]

$C_c \cap C_b = \{f5, f6\}$ 2

$C_c \cap C_e = \{f5\}$ 1

$C_c \cap C_g = \{f6\}$ 1

N(a) = {b, g}	Ca = {f1}
N(b) = {a, c, e, g}	Cb = {f1, f5, f6}
N(c) = {b, e, g}	Cc = {f5, f6}
N(d) = {e, f}	Cd = {f2, f3}
N(e) = {b, c, d, f}	Ce = {f2, f3, f5}
N(f) = {d, e}	Cf = {f2, f4}
N(g) = {a, b, c}	Cg = {f1, f6}

T = [c b]

{a, e, g}

$C_b \cap C_a = \{f1\}$ 1

$C_b \cap C_e = \{f5\}$ 1

$C_b \cap C_g = \{f1, f6\}$ 2

g go at beginning or end?

$C_g \cap C_b = \{f1, f6\}$

$C_g \cap C_c = \{f6\}$

T = [c b g]

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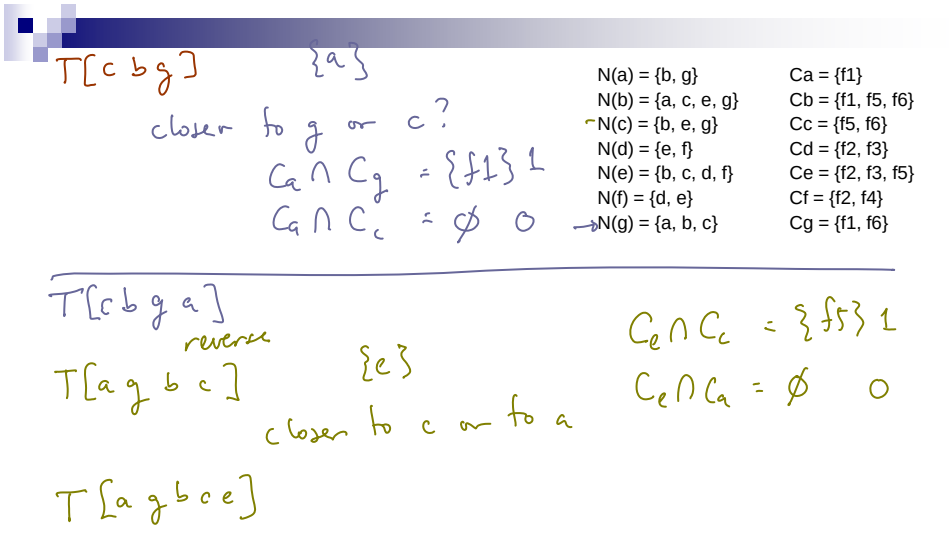
T[c b g]
{a}
closer to g or c?
Ca ∩ Cg = {f1} 1
Ca ∩ Cc = ∅ 0
N(a) = {b, g}
N(b) = {a, c, e, g}
N(c) = {b, e, g}
N(d) = {e, f}
N(e) = {b, c, d, f}
N(f) = {d, e}
N(g) = {a, b, c}
Ca = {f1}
Cb = {f1, f5, f6}
Cc = {f5, f6}
Cd = {f2, f3}
Ce = {f2, f3, f5}
Cf = {f2, f4}
Cg = {f1, f6}
T[c b g a]
reverse
T[a g b c]
{e}
closer to c or to a
Ce ∩ Cc = {f5} 1
Ce ∩ Ca = ∅ 0
T[a g b c e]

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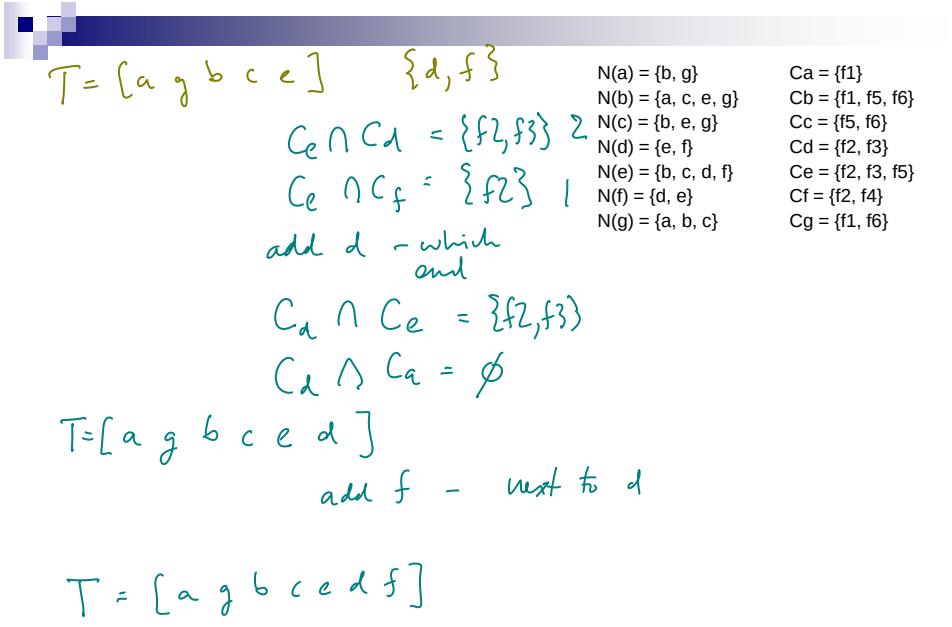
T=[a g b c e]
{d, f}
Ce ∩ Cd = {f2, f3} 2
Ce ∩ Cf = {f2} 1
add d - which and
Cd ∩ Ce = {f2, f3}
Cd ∩ Ca = ∅
T=[a g b c e d]
add f - next to d
T = [a g b c e d f]
N(a) = {b, g}
N(b) = {a, c, e, g}
N(c) = {b, e, g}
N(d) = {e, f}
N(e) = {b, c, d, f}
N(f) = {d, e}
N(g) = {a, b, c}
Ca = {f1}
Cb = {f1, f5, f6}
Cc = {f5, f6}
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Ce = {f2, f3, f5}
Cf = {f2, f4}
Cg = {f1, f6}

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N(a) = {b, g}	Ca = {f1}
N(b) = {a, c, e, g}	Cb = {f1, f5, f6}
N(c) = {b, e, g}	Cc = {f5, f6}
N(d) = {e, f}	Cd = {f2, f3}
N(e) = {b, c, d, f}	Ce = {f2, f3, f5}
N(f) = {d, e}	Cf = {f2, f4}
N(g) = {a, b, c}	Cg = {f1, f6}

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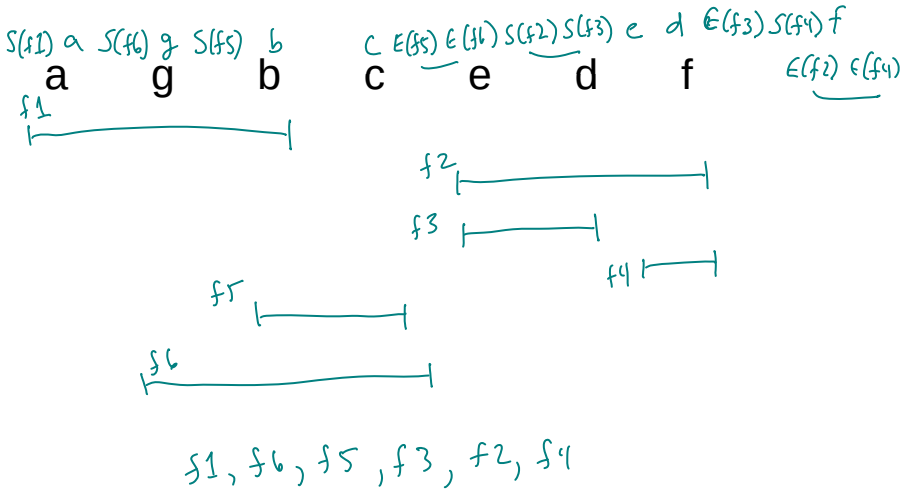
From Probe Order to Map



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