Physical Mapping of DNA

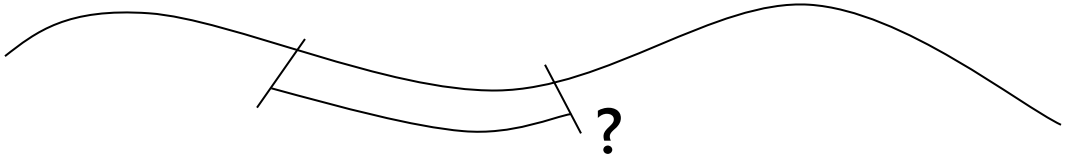
INDRAPRASTHA INSTITUTE *of*
INFORMATION TECHNOLOGY **DELHI**

Dr. Jaspreet Kaur Dhanjal
Assistant Professor, Department for Computational Biology
Email ID: jaspreet@iiitd.ac.in

September 19, 2025

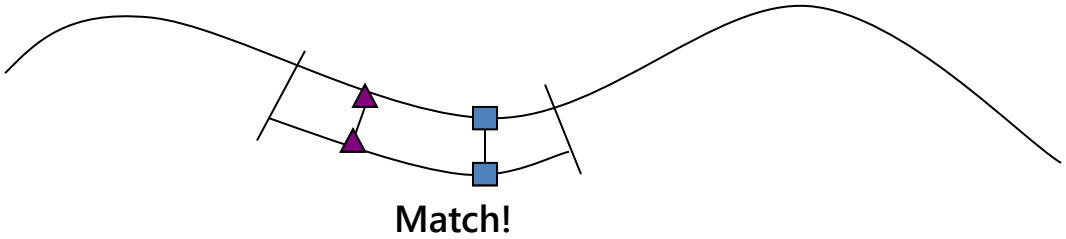
Background

Given a sequence of DNA, how do we figure out where on some larger chromosome the sequence lies?



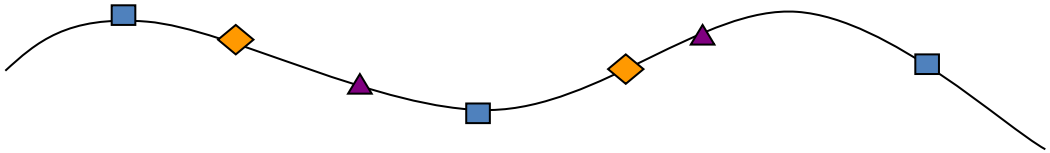
Look for markers that match in both the chromosome and the shorter sequence.

- Markers: Usually short, precisely defined sequences



Creating the Physical Map

How do we create the original map?



Generate fingerprints (markers) with:

- **Restriction site mapping**
- **Hybridization**

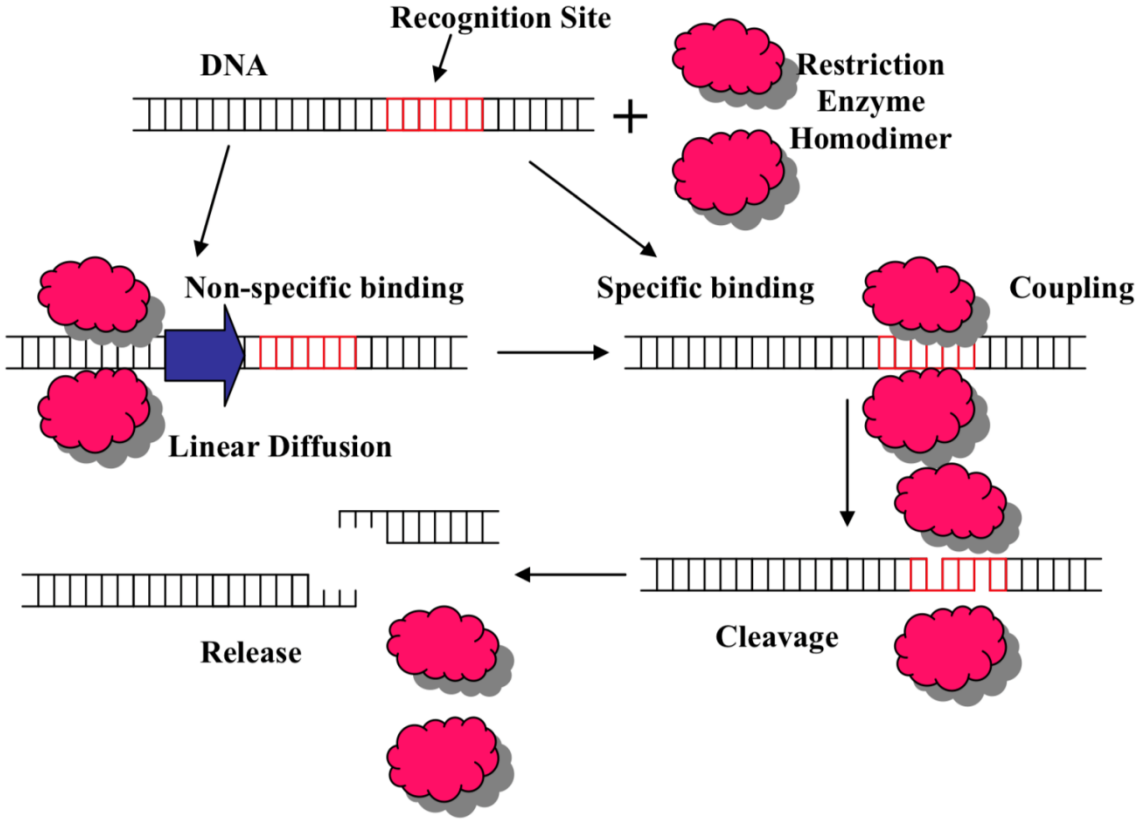
Can't we just expand the sequence assembly techniques we've already learned?
No!

Why not?

- **A chromosome isn't just a few kilo bps long.**
- **Human chromosomes range in length from 51 million to 245 million base pairs.**

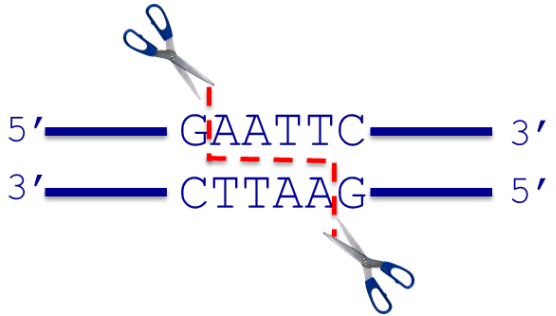
Restriction enzymes

Molecular scissors that cut double stranded DNA molecules at specific points

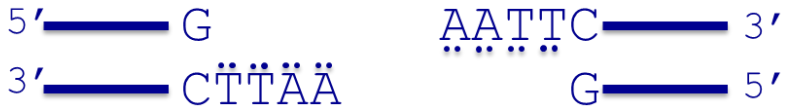


Restriction enzymes

EcoRI recognition site is a palindrome with an axis of symmetry



EcoRI dimer binds sequence and catalyzes double-strand cleavage



Products have “sticky ends” or overhanging bases.

Restriction enzymes

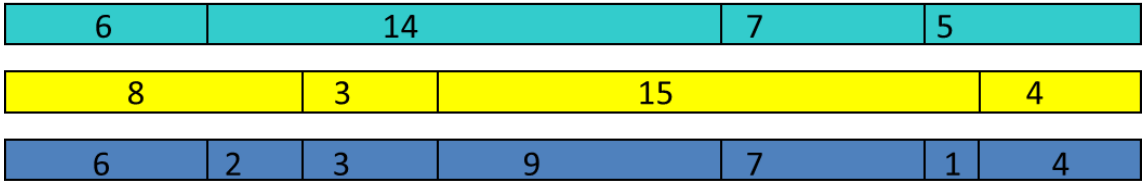
Examples of Restriction Enzymes

Enzyme	Microorganism	Recognition Sequence	Isoschizomers
Alu I	Arthrobacter luteus	AG|CT	
Apa I	Acetobacter pasteurianus	GGGCC|C	Bsp120 I, PspOM I
Bam HI	Bacillus amiloliquifaciens	G|GATCC	
Bgl II	Bacillus globigii	A|GATCT	
Cla I	Caryophanon latum L	AT|CGAT	Bsp DI, Bsc I, BspX I
Dde I	Desulfovibrio desulfuricans	C|TNAG	BstDE I
Dra I	Deinococcus radiophilus	TTT|AAA	
Eco RI	Escherichia coli RY13	G|AATTC	
Eco RV	Escherichia coli J62	GAT|ATC	Eco32 I
Fnu4H I	Fusobacterium nucleatum 4H	GC|NGC	Fsp4H I, Ita I
Hae III	Haemophilus aegyptius	GG|CC	Bsh I, BsuR I, Pal I
Hind II	Haemophilus influenzae Rd	A|AGCTT	
Hinf I	Haemophilus influenzae Rf	G|ANTC	
Kpn I	Klebsiella pnumoniae OK8	GGTAC|C	Acc65 I, Asp718 I
Mbo I	Moraxella bovis	|GATC	Dpn II, Nde II, Sau3A I
Msp I	Morazella sp.	C|CGG	BsiS I, Hap II, Hpa II
Nde I	Neisseria dentrificans	CA|TATG	FauND I
Not I	Nocardia otitidis-caviarum	GC|GGCCGC	CciN I
Pst I	Providencia stuartii 164	CTGCA|G	
Pvu II	Proteus vulgaris	CAG|CTG	
Rsa I	Rhodopseudomonas sphaeroides	GT|AC	
Sma I	Serratio marcescens S	CCC|GGG	Cfr9 I, Psp A I, Xma I
Taq I	Thermus aquaticus YT1	T|CGA	TtaHB8 I
Xba I	Xanthomonas badrii	T|CTAGA	
Xho I	Xanthomonas holcicola	C|TCGAG	PaeR7 I, Sfr274 I, Tli I

Restriction Site Mapping

In this situation, the fingerprint is the length between restriction sites of given enzymes.

Make three copies of target DNA: strings A, B, C.
Apply one enzyme (α) to string A, another (β) to string B, and both (α and β) to string C.
Line up the fragments in A and B so they match C: This is the ***double digestion problem***.



ATGAGCTTGGGCCGTGTCAGCTCCCCAGCTGTC

Alu 1 → AGCT

Alu 1 : 5, 15, 8, 5
Apa 1: 12, 21

Both: 5, 7, 8, 8, 5

Restriction Site Mapping

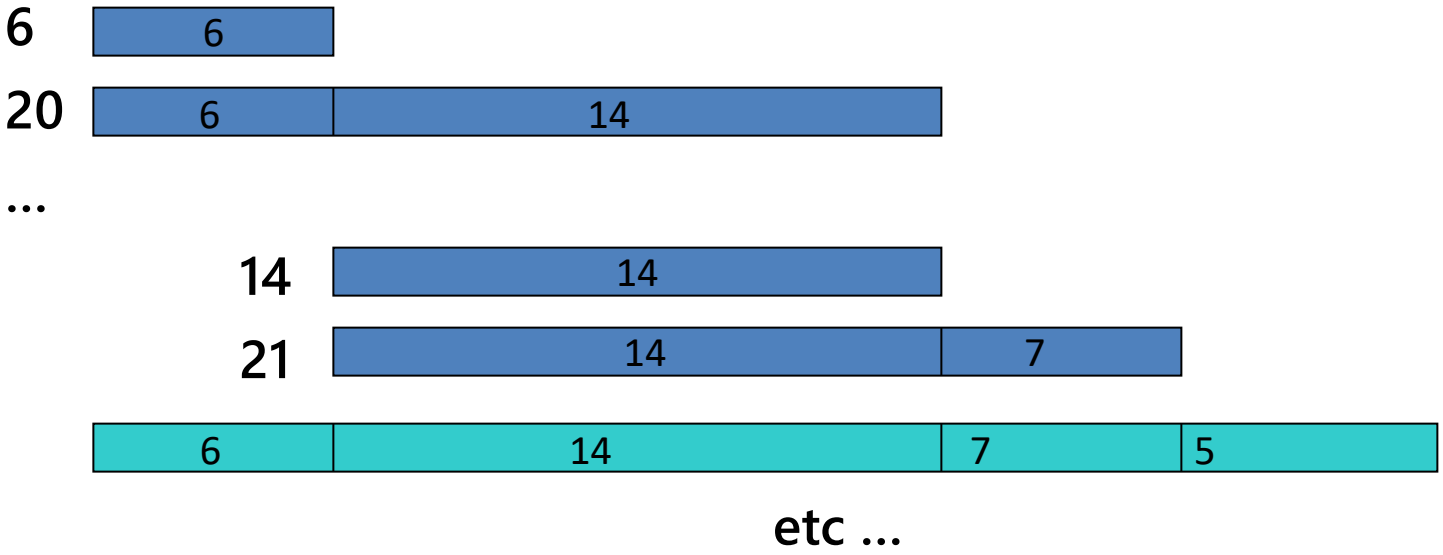
A variant is the ***partial digestion approach***:

Use only one enzyme, but allow it to act for different time periods.

Different restriction sites will be recognized.

Fragment sites: 6, 20, 27, 32;
14, 21, 26;
7, 12;
and 5

Restriction Site Mapping



Fragment sites: 6, 20, 27, 32; 14, 21, 26; 7, 12; and 5

Restriction site model

Back to the double digest problem:

Enzyme A: 1000, 2100, 1400, 500

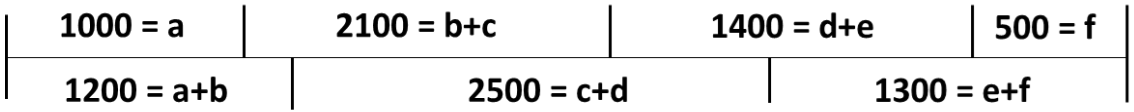
Enzyme B: 1200, 2500, 1300

Enzyme A+B: 1000, 200, 1900, 600, 800, 500

Find permutations of A and B such that there is a one-to-one correspondence between all the subintervals and C.

A+B:	1000	200	1900	600	800	500
	a	b	c	d	e	f

A: 1000= a, 2100= b+c, 1400= d+e, 500=f B: 1200= a+b, 2500=c+d, 1300= e+f



Restriction site models

Limitations:

- This double digestion problem is NP-complete.
- Between 2 sites cut by A, there are three sites – b1, b2, b3 cut by enzyme B. It will be difficult to tell the order of the fragments [b1,b2] and [b2,b3].
- The number of solutions is k! for k = number of restriction sites.

Enhanced Double Digestion Problem

- The Enhanced Double Digest (EDD) problem is NP-hard in the general case, but if the lengths of fragments in C (the string acted upon by both types of enzymes) are distinct, it can be solved in linear time!
- We have the multisets A and B.

  A = {6, 14, 7, 5}

  B = {8, 3, 15, 4}
- Take the actual fragments corresponding to each member of the either set (since the sets are only lengths). Apply the other enzyme to the fragment (i.e. apply enzyme β to fragments from A, and vice versa) to create subfragments.
- AB_i is the multiset of subfragments created by applying enzyme β to fragments from A;
- BA_j from applying enzyme α to fragments of B.

Enhanced Double Digestion Problem

Example:

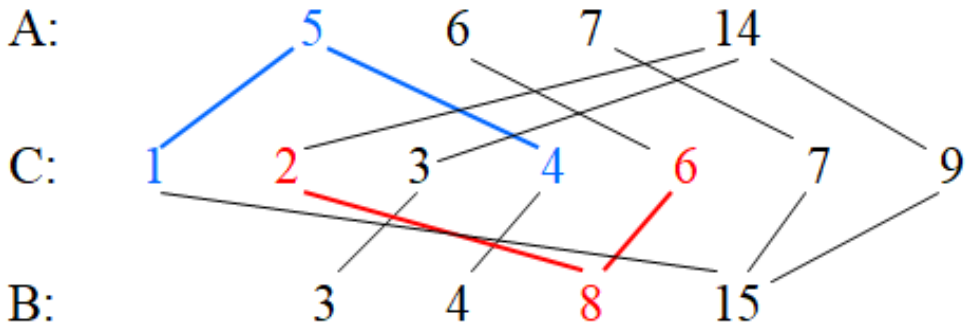
A={5, 6, 7, 14}

B={3, 4, 8, 15}

AB_1={1,4}, AB_2={6}, AB_3={7}, AB_4={2,3,9}

BA_1={3}, BA_2={4}, BA_3={2,6}, BA_4={1,7,9}

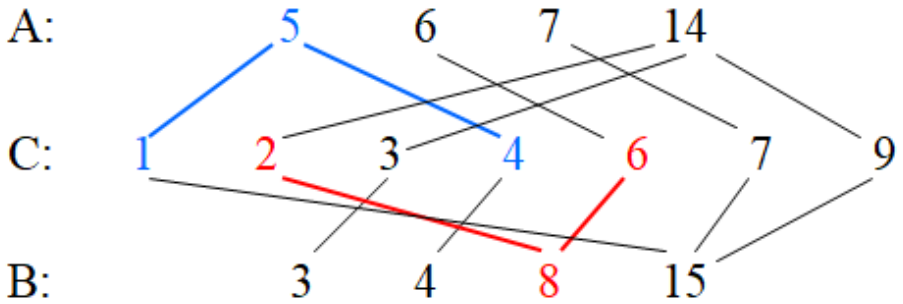
Given A, B, AB_i and BA_j for all i, j, construct an undirected graph that connects each element of A and B to its corresponding AB/BA. Note that all elements in C will be covered



Enhanced Double Digestion Problem

Create a spanning tree:

Starting at random node, follow all paths from the node without repeating the edges.



$$6^{A}-6^{C}-8^{B}-2^{C}-14^{A}-9^{C}-15^{B}-1^{C}-5^{A}-4^{C}-4^{B}$$

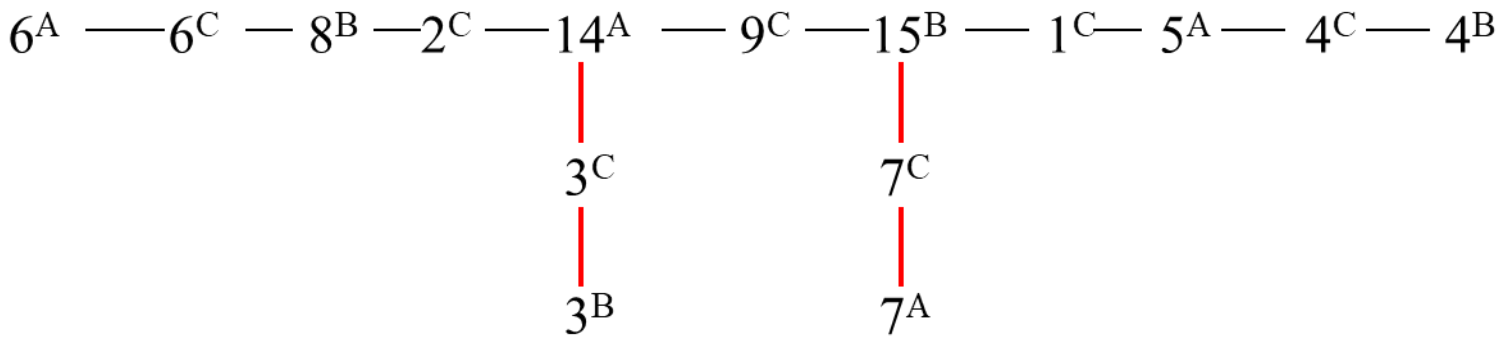
$$3^{C}-3^{B}$$

$$7^{C}-7^{A}$$

Enhanced Double Digestion Problem

- The graph (G) will always be connected, and every node in A and B will only be adjacent to nodes from C. Each node from C connects to only one node each from A and B.

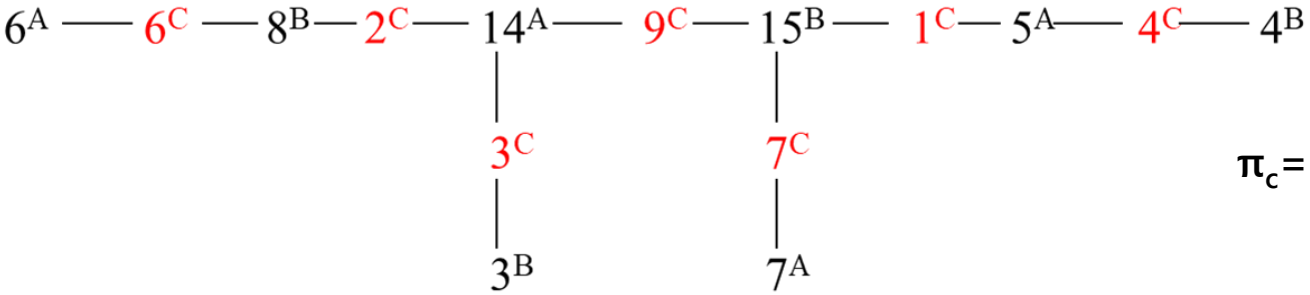
- If the problem can be solved: G will be a spanning tree, and any subtree that "hangs" on the longest path will be a 2-node length path (dangler).



Enhanced Double Digestion Problem

- If the graph G is not a spanning tree, and not all subtrees hanged off the longest path are danglers, then there is no valid permutation.
- Perform Dangler-first search on the graph G…

- Traverse G starting at one end of a path S with the largest number of edges, reading only the nodes from C. Whenever reaching a node with degree greater than 2 (must have a dangler), read the nodes in C from the hanging danglers first, then continue to traverse S. This sequence is πc.

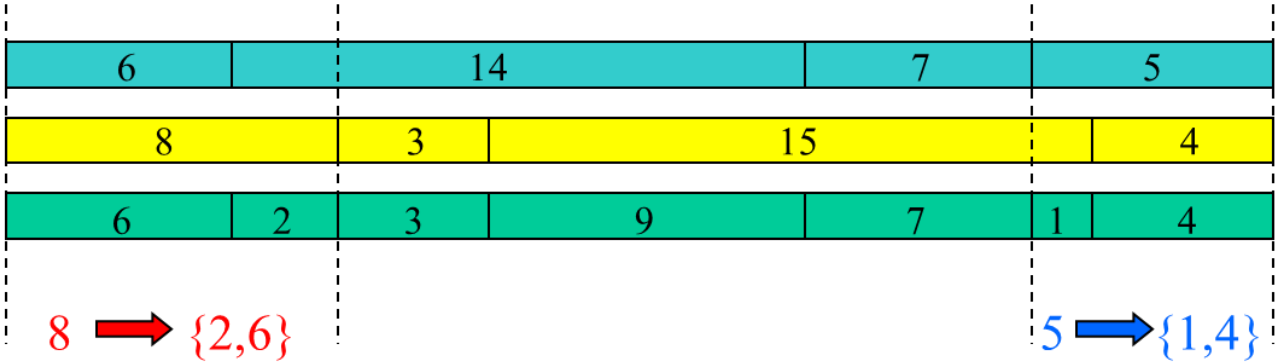


π_c = 6, 2, 3, 9, 7, 1, 4

Enhanced Double Digestion Problem

The elements in each AB_i form a consecutive subsequence in π_c. Likewise, the elements in each BA_j also form a consecutive subsequence in π_c.

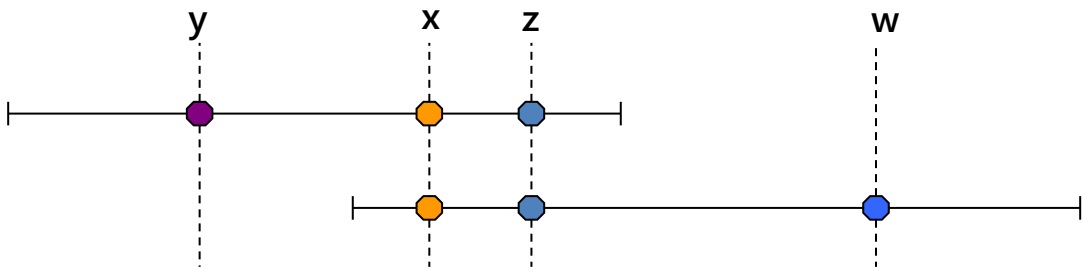
This permutation is a valid permutation… meaning: we have the answer!



Hybridization Mapping

- Check whether specific small sequences (called probes) bind (hybridize) to fragments (clones)
- The fingerprint is the subset of probes that successfully hybridize to the clone.
- If some portion of one clone's fingerprint matches another, they are likely to be from overlapping regions of the target.

- Probes x, y, z, bound to clone A; x, w and z bound to clone B… overlap in x and z.



Hybridization mapping model

- Consecutive Ones Property Model (C1P)
- This can be solved in linear time!
- Assumptions:
  - The probes are unique.
  - There are no errors.
  - All of the correspondences of clones and probes have been found.

Hybridization mapping model

- Build a matrix (n x m), n = number of clones, m = number of probes.
- Entry i,j is a binary code for whether probe j hybridized to clone i.

Probes →

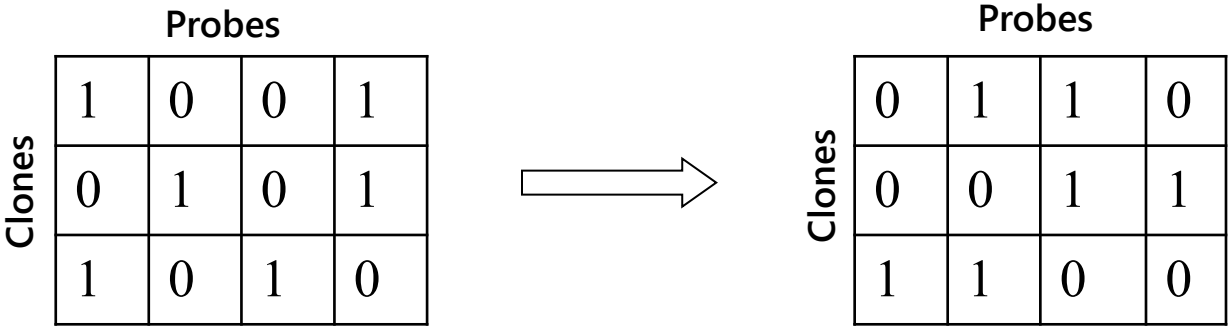
Clones →

1	1	0	1
0	1	0	1
1	0	1	1

Here, probe 1 hybridized to clone 1, probe 2 hybridized to clone 1, probe 1 hybridized to clone 3, probe 4 hybridized to clone 3.

Hybridization mapping model

- Find a permutation of the columns (probes) such that all the 1s in each row (clone) are consecutive.



Hybridization mapping model

- This algorithm can be run in linear time!
- Unfortunately, the assumption that there are no errors isn't useful because biology isn't a mathematical model. Probes may not bind; DNA may be replicated incorrectly.