

Párování bazí, hybridizace a denaturace DNA/RNA

hraje důležitou roli v přírodě i aplikacích:

- struktura DNA a duplikace genetické informace
- transkripce a translace
- rekombinace DNA
- struktura RNA a její aktivita

- klonování, rekombinantní DNA
- polymerázová reakce (PCR)
- DNA čipy, microarrays
- DNA výpočty

Kombinatorický charakter nukleových kyselin vytváří problémy, které nejsou zvládnutelné bez matematického aparátu a výpočetní techniky

PCR

HRÁČI

primer

templát

DNA polymeráza

CYKLY

denaturace

annealing

polymerizace

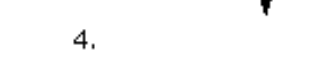
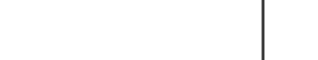
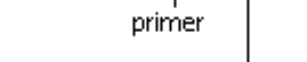
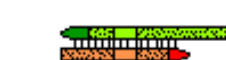
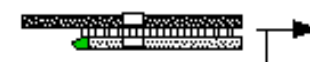
POLYMERASE CHAIN REACTION

DNA region of interest.



primer

1. DNA is denatured. Primers attach to each strand. A new DNA strand is synthesized behind primers on each template strand.

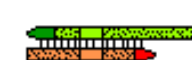


2. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

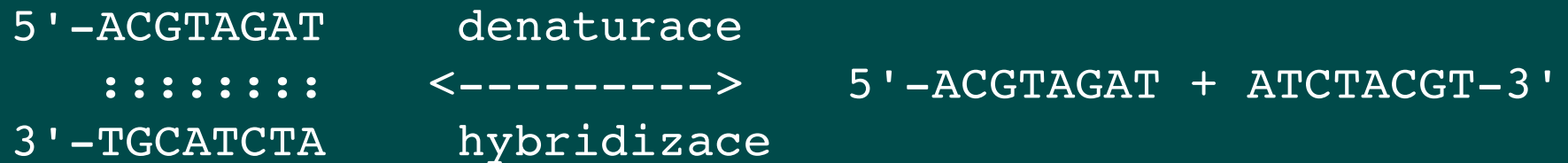
3. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

4. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

5. Continued rounds of amplification swiftly produce large numbers of identical fragments. Each fragment contains the DNA region of interest.



Přibližný výpočet bodu topení oligonukleotidu

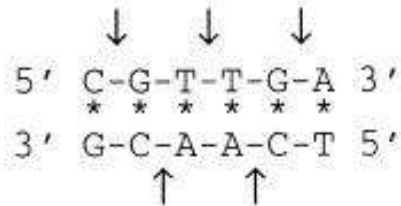


T_m – teplota, při níž je 50% oligonukleotidu denaturováno

$$T_m = 2 * (n(A) + n(T)) + 4 * (n(C) + n(G))$$

$$T_m = 2AT + 4CG$$

Metoda NN (Nearest Neighbor)



$$\begin{aligned}
 \Delta G_{37}^{\circ}(\text{pred.}) &= \Delta G^{\circ}(\text{CG/GC}) + \Delta G^{\circ}(\text{GT/CA}) + \Delta G^{\circ}(\text{TT/AA}) \\
 &\quad + \Delta G^{\circ}(\text{TG/AC}) + \Delta G^{\circ}(\text{GA/CT}) + \Delta G^{\circ}(\text{init.}) \\
 &= -2.17 - 1.44 - 1.00 - 1.45 - 1.30 + 0.98 + 1.03
 \end{aligned}$$

$$\Delta G_{37}^{\circ}(\text{pred.}) = -5.35 \text{ kcal/mol}$$

$$\Delta G_{37}^{\circ}(\text{obs.}) = -5.20 \text{ kcal/mol}$$

$$\Delta G = \Delta H - \Delta S \cdot T$$

ΔG – volna energije

H – entalpije

S – entropije

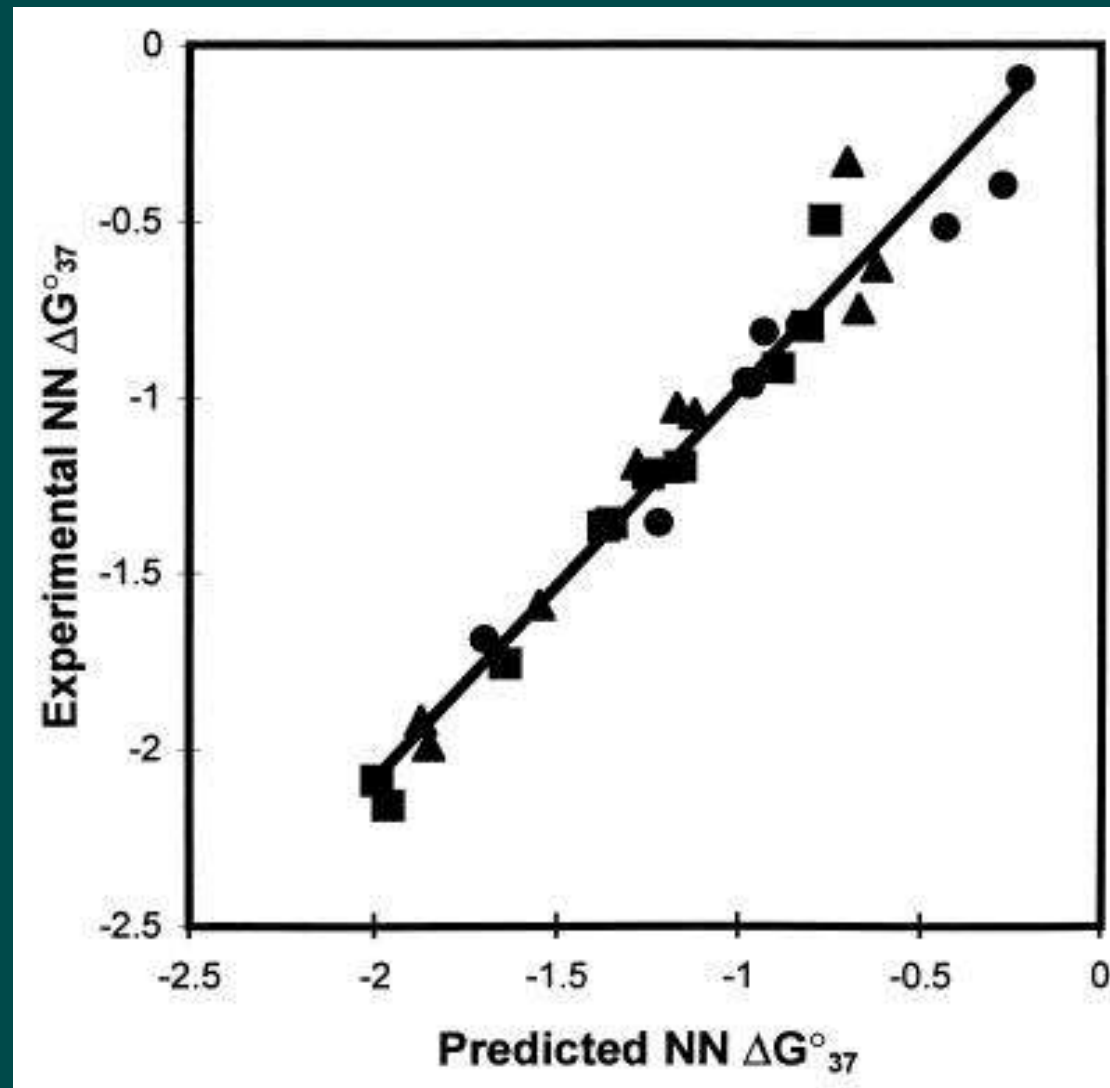
T – temperatura

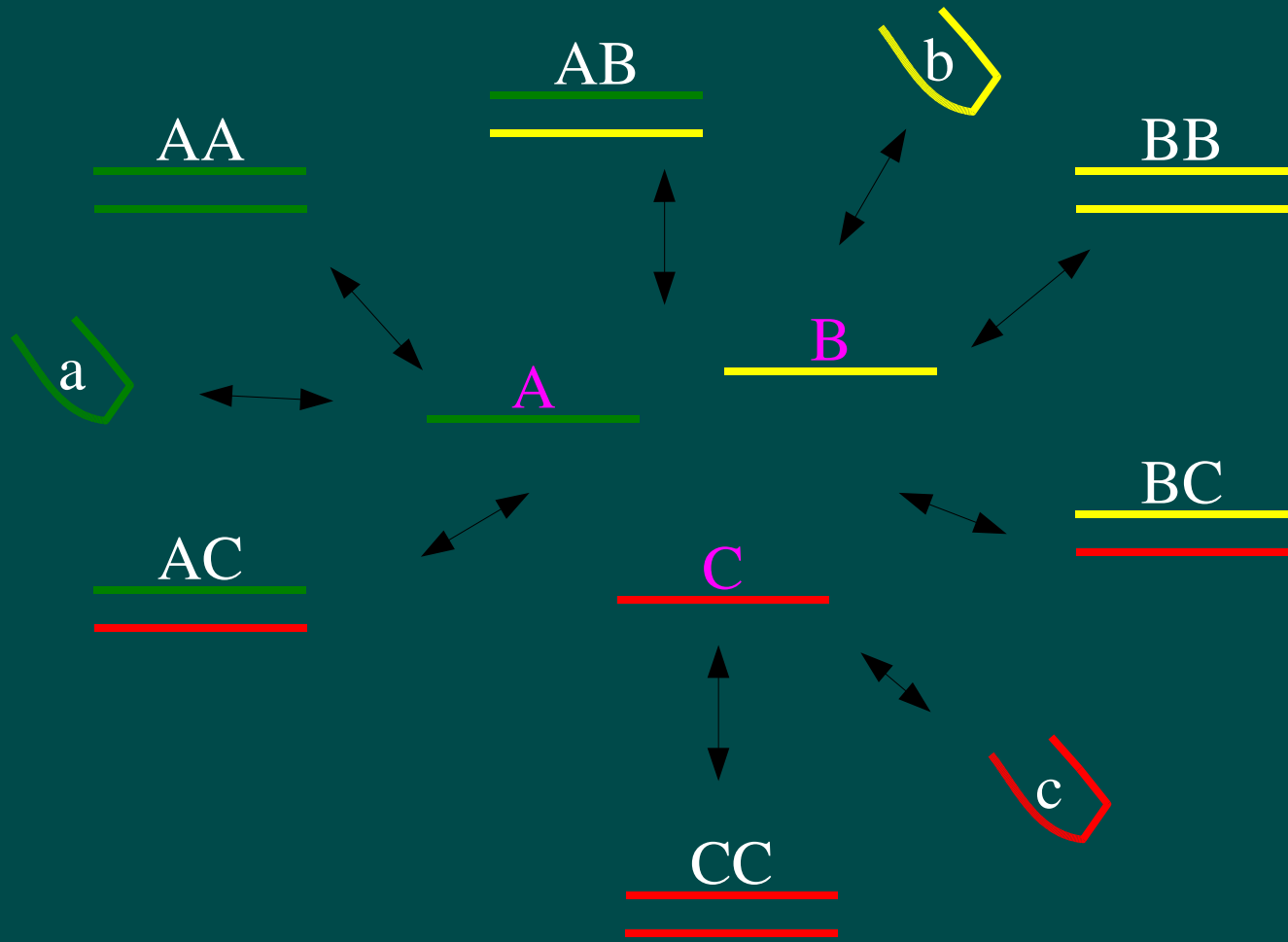
Sequence	Parameter kcal/mol Unified (ref. 22)
AA/TT	-1.00
AT/TA	-0.88
TA/AT	-0.58
CA/GT	-1.45
GT/CA	-1.44
CT/GA	-1.28
GA/CT	-1.30
CG/GC	-2.17
GC/CG	-2.24
GG/CC	-1.84
Average	-1.42
Init. w/term. G·C [*]	0.98
Init. w/term. A·T [*]	1.03
Sodium concentration, M	1.0
Rank of stacking matrix	12

Porovnání metod AT2CG4 a NN

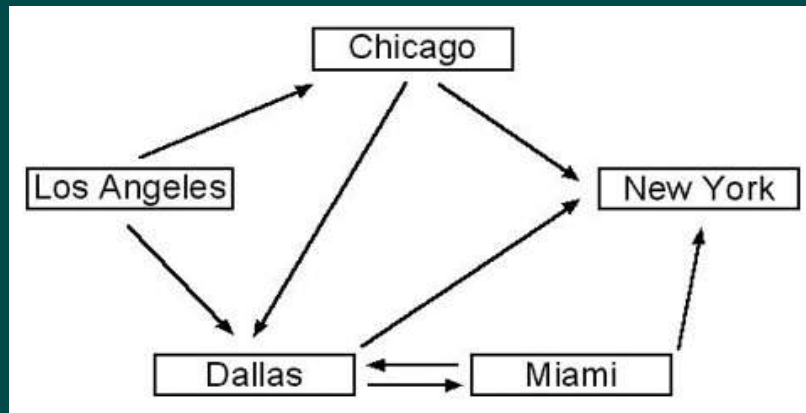
Primer Name	Sequence	Melting Tm (AT=2,CG=4)	Melting Tm Nearest Neighbor
KS	5'- CGAGGTCGACGGTATCG	56 °C	57 °C
M13 (-20) (Universal)	5'-GTAAAACGACGGCCAGT	52 °C	55 °C
M13 (-40)	5'-GTTTTCCCAGTCACGAC	52 °C	53 °C
M13 forward	5'-GTTTTCCCAGTCACGACGTTG	64 °C	59 °C
M13 reverse	5'-TGAGCGGATAACAATTTACACAG	68 °C	58 °C
M13 Reverse	5'-CAGGAAACAGCTATGAC	50 °C	50 °C
M13 Reverse (-40)	5'-GTTGTGTGGAATTGTG	46 °C	48 °C
Neomycin forward	5'-AGGATCTCCTGTCATCTCACCTTGCTCCTG	92 °C	66 °C
Neomycin reverse	5'-AAGAACTCGTCAAGAAGGCGATAGAAGGCG	90 °C	65 °C

Spolehlivost metody NN

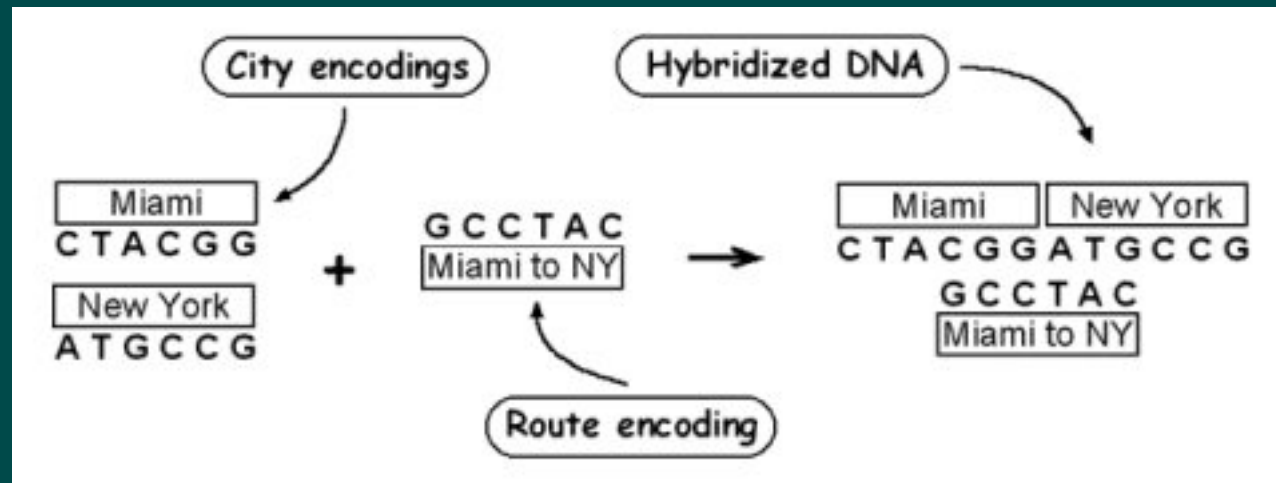




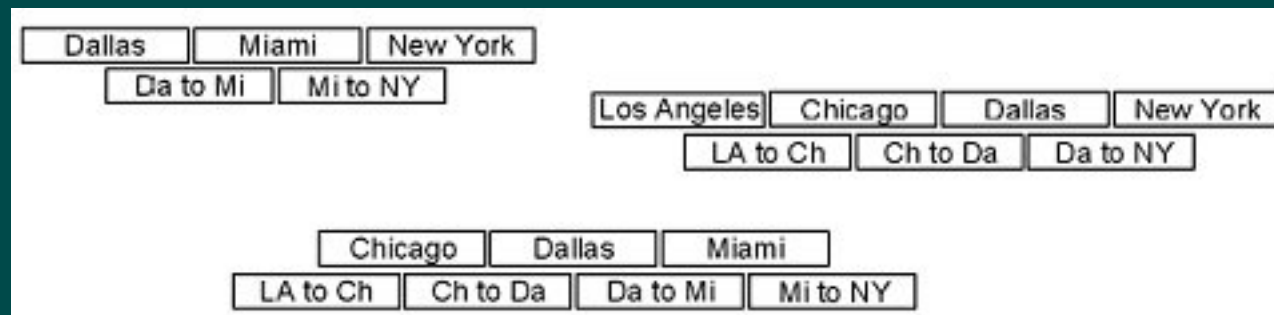
DNA computing – DNA-based computing



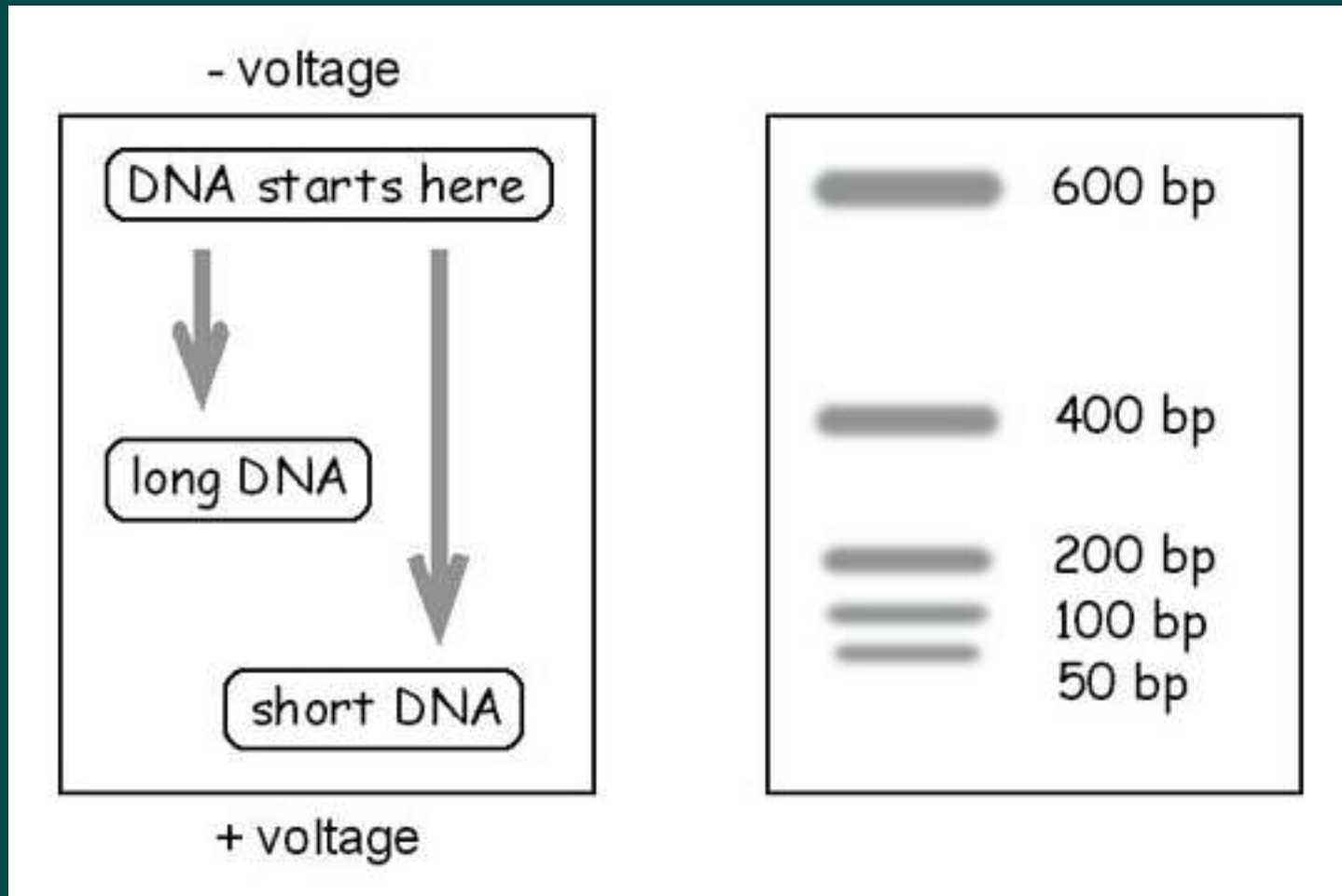
Los Angeles	GCTACG
Chicago	CTAGTA
Dallas	TCGTAC
Miami	CTACGG
New York	ATGCCG



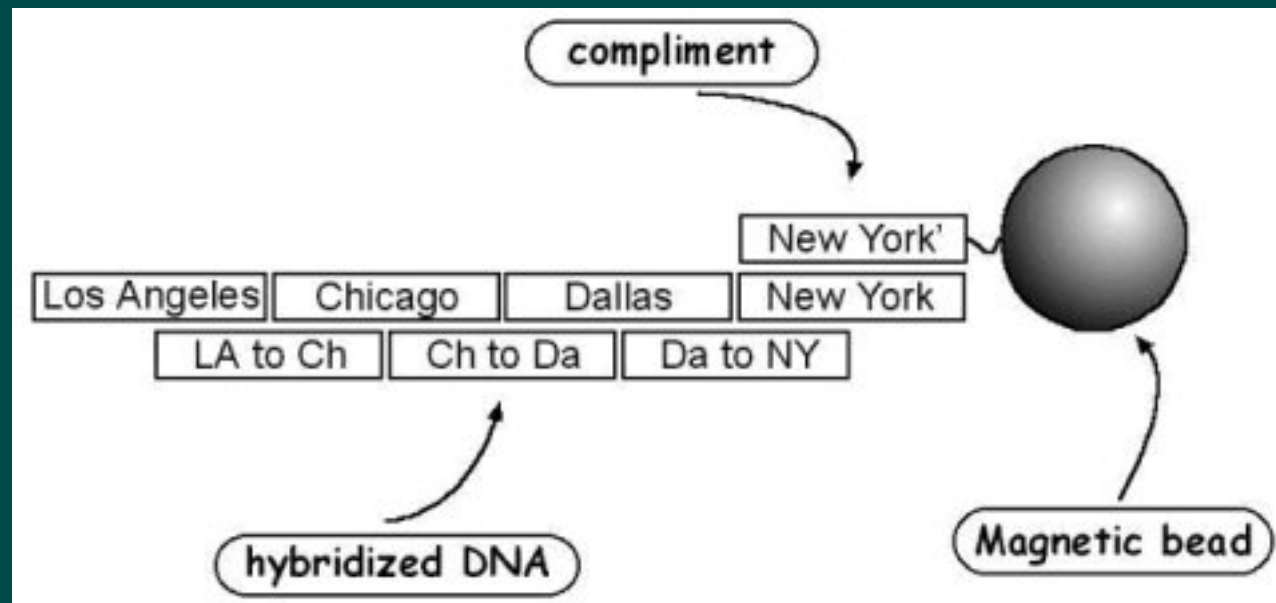
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