CSE 527
Computational Biology
Autumn 2007

Lectures 4-5:

BLAST

Alignment score significance

PCR and DNA sequencing

This Week's Plan

- BLAST
- Scoring
- Weekly Bio Interlude: PCR & Sequencing

A Protein Structure: (Dihydrofolate Reductase)

Sequence Evolution

Nothing in Biology Makes Sense Except in the Light of Evolution

– Theodosius Dobzhansky, 1973

- Changes happen at random
- Deleterious/neutral/advantageous changes unlikely/possibly/likely spread widely in a population
- Changes are less likely to be tolerated in positions involved in many/close interactions, e.g.
  - enzyme binding pocket
  - protein/protein interaction surface
  - …

BLAST:

Basic Local Alignment Search Tool

Altschul, Gish, Miller, Myers, Lipman, J Mol Biol 1990

- *The* most widely used comp bio tool
- Which is better: long mediocre match or a few nearby, short, strong matches with the same total score?
  - score-wise, exactly equivalent
  - biologically, later may be more interesting, & is common
  - at least, if must miss some, rather miss the former
- BLAST is a heuristic emphasizing the later
  - speed/sensitivity tradeoff: BLAST may miss former, but gains greatly in speed

BLAST: What

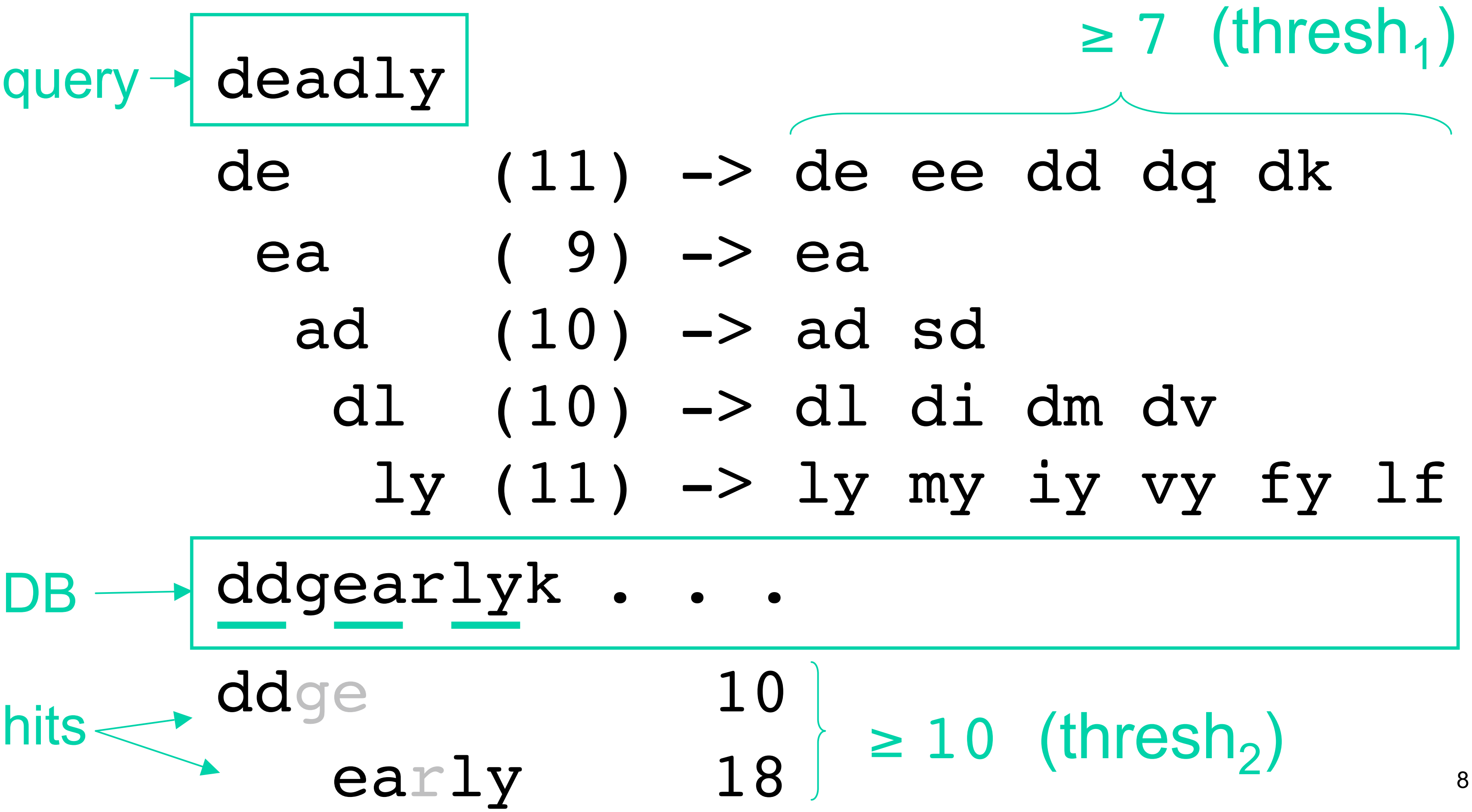
- Input:
  - a query sequence (say, 300 residues)
  - a data base to search for other sequences similar to the query (say, 10^6 - 10^9 residues)
  - a score matrix $\sigma(r,s)$, giving cost of substituting r for s (& perhaps gap costs)
  - various score thresholds & tuning parameters
- Output:
  - "all" matches in data base above threshold
  - "E-value" of each

BLAST: How

Idea: find parts of data base near a good match to some short subword of the query

- Break query into overlapping words w_i of small fixed length (e.g. 3 aa or 11 nt)
- For each w_i, find (empirically, ~50) "neighboring" words v_{ij} with score $\sigma(w_i, v_{ij}) > \text{thresh}_1$
- Look up each v_{ij} in database (via prebuilt index) -- i.e., exact match to short, high-scoring word
- Extend each such "seed match" (bidirectional)
- Report those scoring > thresh_2, calculate E-values

BLAST: Example



≥ 7 (thresh_1)

query → deadly

```
de       (11) -> de ee dd dq dk
 ea      ( 9) -> ea
  ad     (10) -> ad sd
   dl    (10) -> dl di dm dv
    ly   (11) -> ly my iy vy fy lf
```

DB → ddgearlyk . . .

hits

```
ddge            10
   early        18
```

≥ 10 (thresh_2)

BLOSUM 62

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	**4**	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	**5**	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	**6**	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	**6**	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	**9**	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	**5**	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	**5**	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	**6**	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	**8**	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	**4**	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	**4**	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	**5**	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	**5**	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	**6**	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	**7**	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	**4**	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	**5**	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	**11**	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	**7**	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	**4**

BLAST Refinements

- “Two hit heuristic” -- need 2 nearby, nonoverlapping, gapless hits before trying to extend either
- “Gapped BLAST” -- run heuristic version of Smith-Waterman, bi-directional from hit, until score drops by fixed amount below max

- PSI-BLAST -- For proteins, iterated search, using “weight matrix” pattern from initial pass to find weaker matches in subsequent passes

Significance of Alignments

- Is “42” a good score?
- *Compared to what?*

- Usual approach: compared to a specific “null model”, such as “random sequences”

Hypothesis Testing: A Very Simple Example

- Given: A coin, either fair (p(H)=1/2) or biased (p(H)=2/3)
- Decide: which
- How? Flip it 5 times. Suppose outcome D = HHHTH
- Null Model/Null Hypothesis M_0: p(H)=1/2
- Alternative Model/Alt Hypothesis M_1: p(H)=2/3
- Likelihoods:
  - P(D | M_0) = (1/2) (1/2) (1/2) (1/2) (1/2) = 1/32
  - P(D | M_1) = (2/3) (2/3) (2/3) (1/3) (2/3) = 16/243
- Likelihood Ratio: $\frac{p(D \mid M_1)}{p(D \mid M_0)} = \frac{16/243}{1/32} = \frac{512}{243} \approx 2.1$

I.e., alt model is ≈ 2.1x more likely than null model, given data

Hypothesis Testing, II

- Log of likelihood ratio is equivalent, often more convenient
  - add logs instead of multiplying…
- "Likelihood Ratio Tests": reject null if LLR > threshold
  - LLR > 0 disfavors null, but higher threshold gives stronger evidence against
- Neyman-Pearson Theorem: For a given error rate, LRT is as good a test as any (subject to some fine print).

p-values

- the *p-value* of such a test is the probability, assuming that the null model is true, of seeing data as extreme or more extreme that what you actually observed
- e.g., we observed 4 heads; p-value is prob of seeing 4 or 5 heads in 5 tosses of a fair coin
- Why interesting? It measures probability that we would be making a mistake in rejecting null.
- Usual scientific convention is to reject null only if p-value is < 0.05; sometimes demand p << 0.05
- can analytically find p-value for simple problems like coins; often turn to simulation/permutation tests for more complex situations; as below

A Likelihood Ratio Test for Alignment

- Defn: two proteins are *homologous* if they are alike because of shared ancestry; similarity by descent

- suppose among proteins overall, residue x occurs with frequency p_x
- then in a random alignment of 2 random proteins, you would expect to find x aligned to y with prob $p_x p_y$
- suppose among *homologs*, x & y align with prob p_{xy}
- are seqs X & Y homologous? Which is more likely, that the alignment reflects chance or homology? Use a *likelihood ratio test.*

$$\sum_i \log \frac{p_{x_i y_i}}{p_{x_i} p_{y_i}}$$

Non-*ad hoc* Alignment Scores

- Take alignments of homologs and look at frequency of x-y alignments vs freq of x, y overall
- Issues
  - biased samples
  - evolutionary distance

- BLOSUM approach
  - large collection of trusted alignments (the BLOCKS DB)
  - subsetted by similarity, e.g. BLOSUM62 => 62% identity

$$\frac{1}{\lambda}\log_2 \frac{p_{x\ y}}{p_x p_y}$$

ad hoc Alignment Scores?

- Make up any scoring matrix you like
- Somewhat surprisingly, under pretty general assumptions[**], it is *equivalent* to the scores constructed as above from some set of probabilities p_{xy}, so you might as well understand what they are

[**] e.g., average scores should be negative, but you probably want that anyway, otherwise local alignments turn into global ones, and some score must be > 0, else best match is empty

BLOSUM 62

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	**4**	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	**5**	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	**6**	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	**6**	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	**9**	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	**5**	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	**5**	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	**6**	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	**8**	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	**4**	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	**4**	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	**5**	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	**5**	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	**6**	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	**7**	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	**4**	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	**5**	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	**11**	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	**7**	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	**4**

Overall Alignment Significance, I
A Theoretical Approach: EVD

Let X_i, $1 \le i \le N$, be indp. random variables drawn from some (non-pathological) distribution

Q. what can you say about distribution of y = sum{ X_i }?

A. y is approximately *normally* distributed

Q. what can you say about distribution of y = max{ X_i }?

A. it's approximately an *Extreme Value Distribution (EVD)*

$$P(y \le z) \cong \exp(-KNe^{-\lambda z}) \qquad (*)$$

For ungapped local alignment of seqs x, y, N ~ |x|*|y|

λ, K depend on scores, etc., or can be estimated by curve-fitting random scores to (*). (cf. reading)

EVD Pro/Con

- Pro:
  - gives p-values for alignment scores
- Con:
  - It’s only approximate
  - parameter estimation
  - theory may not apply. E.g., it is NOT known to hold for gapped alignments (although empirically it seems to work pretty well).

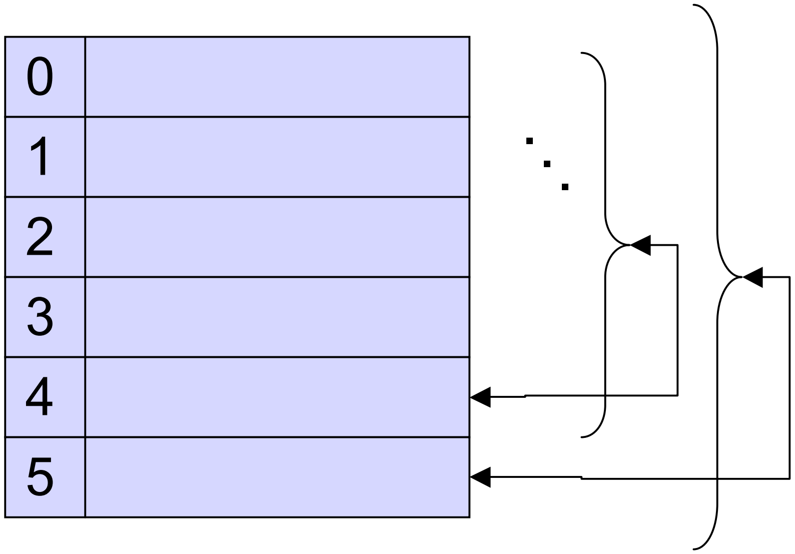
Overall Alignment Significance, II Empirical (via randomization)

- generate N random sequences (say N = 10^3 - 10^6)
- align x to each & score
- if k of them have better score than alignment of x to y, then the (empirical) probability of a chance alignment as good as observed x:y alignment is k/N

- How to generate "random" sequences?
  - Alignment scores often sensitive to sequence composition
  - so uniform 1/20 or 1/4 is a bad idea
  - even background p_i can be dangerous
  - Better idea: *permute* y N times

Generating Random Permutations

```
for (i= n-1; i>0; i--){
    j = random(0..i);
    swap X[i]<-> X[j];
}
```



Permutation Pro/Con

- Pro:
  - Gives empirical p-values for alignments with characteristics like sequence of interest, e.g. residue frequencies
- Con:
  - Can be inaccurate if your method of generating random sequences is unrepresentative
  - E.g., probably better to preserve di-, tri-residue statistics and/or other higher-order characteristics, but increasingly hard to know exactly what to model & how
  - Slow
  - Especially if you want to assess low-probability p-values

p-values & multiple testing

- Above give “p-values”: probability of a score more extreme than observed if the target sequence were random
- must be careful whether p-value means wrt comparison to one other random protein, or best of a database of n random proteins
- E.g., suppose p-value for x:y match is 10^{-3} , then you’d expect to see a score that good only one time in a thousand among non-homologous sequences
- Sounds good
- What if you *found* y by picking best match among 10^4 proteins?
- Sounds not so good

E-values

- “p-value”: probability of a score more extreme than observed in a given random target data base
- E-value: expected number of matches that good or better in a random data base of the given size & composition
- Related: P = 1 - exp(-E)
  - E = 5 <--> P = .993
  - E = 10 <--> P = .99995
  - E = .01 <--> $P = E - E^2/2 + E^3/3! \ \ldots \approx E$
- both equally valid; E-value is perhaps a more intuitively interpretable quantity, & perhaps makes role of data base size more explicit

Issues

- What if the model is wrong?
- E.g., are adjacent positions really independent?

Summary

- BLAST is a highly successful search/alignment heuristic. It looks for alignments anchored by short, strong, ungapped "seed" alignments
- Assessing statistical significance of alignment scores is crucial to practical applications
  - score matrices derived from "likelihood ratio" test of trusted alignments vs random "null" model
  - for gapless alignments, Extreme Value Distribution (EVD) is theoretically justified for overall significance of alignment scores; empirically seems ok for gapped alignments, too
  - permutation tests are a simple (but brute force) alternative

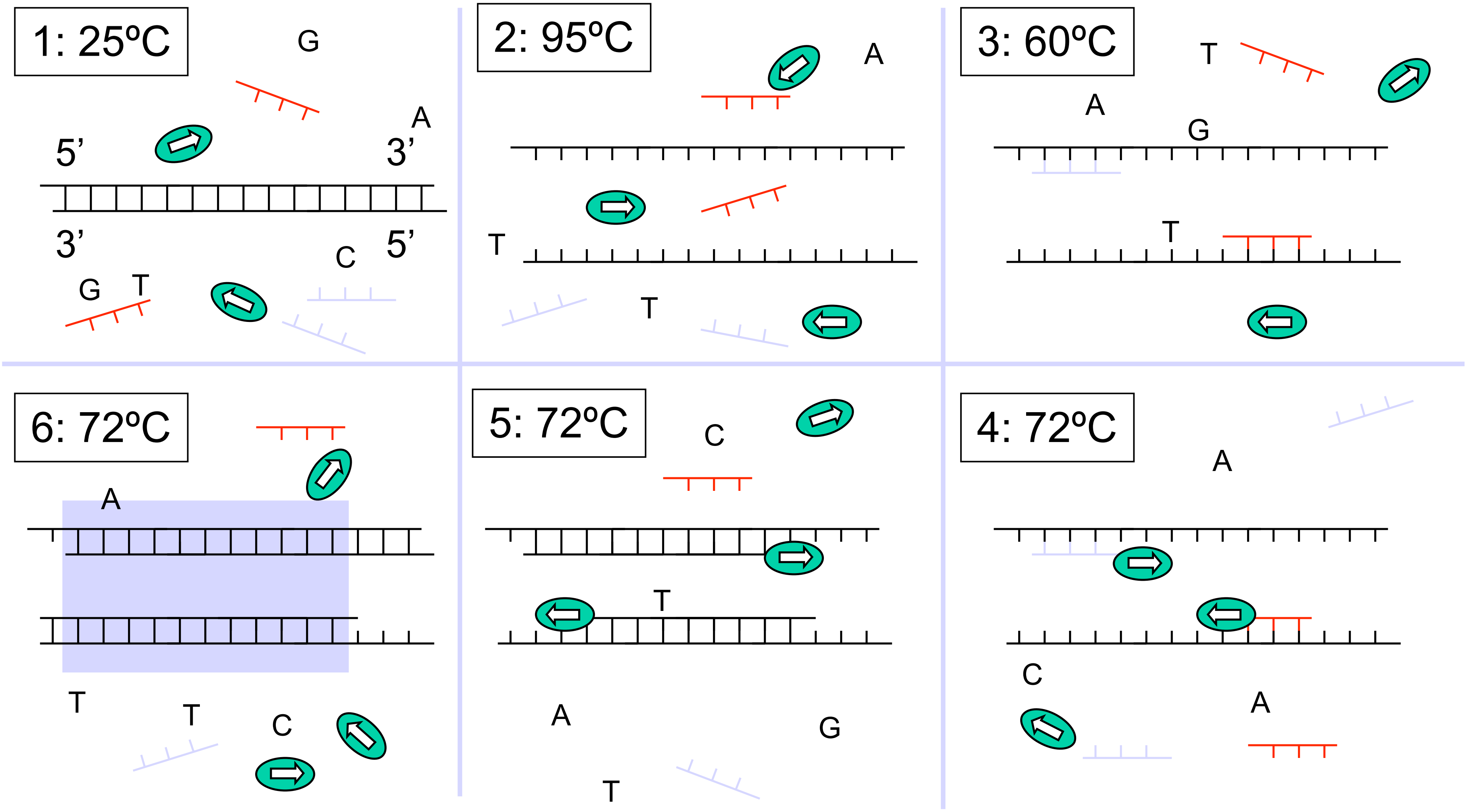
Weekly Bio(tech) Interlude

2 Nobel Prizes:

PCR: Kary Mullis, 1993

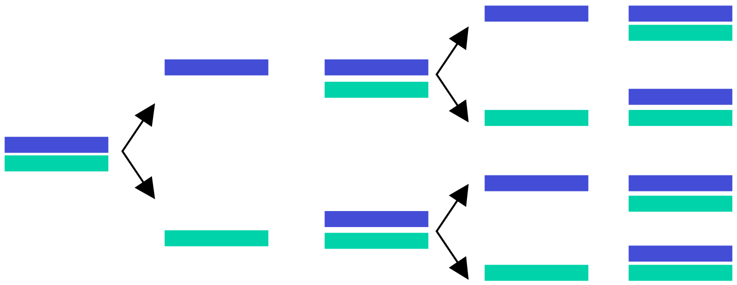
DNA Sequencing: Frederick Sanger, 1980

PCR


1: 25ºC
G
A
5’
3’
3’
5’
C
G
T
2: 95ºC
A
T
T
3: 60ºC
T
A
G
T
6: 72ºC
A
T
T
C
5: 72ºC
C
T
A
G
T
4: 72ºC
A
C
A

Hot spring, near Great Fountain Geyser, Yellowstone National Park

PCR



- Ingredients:
  - many copies of deoxy nucleotide triphosphates
  - many copies of two primer sequences (~20 nt each)
    - readily synthesized
  - many copies of Taq polymerase (*Thermus aquaticus*),
    - readily available commercialy
  - as little as 1 strand of template DNA
  - a programmable “thermal cycler”
- Amplification: million to billion fold
- Range: up to 2k bp routinely; 50k with other enzymes & care
- *Very widely used*; forensics, archeology, cloning, sequencing, …

DNA Forensics

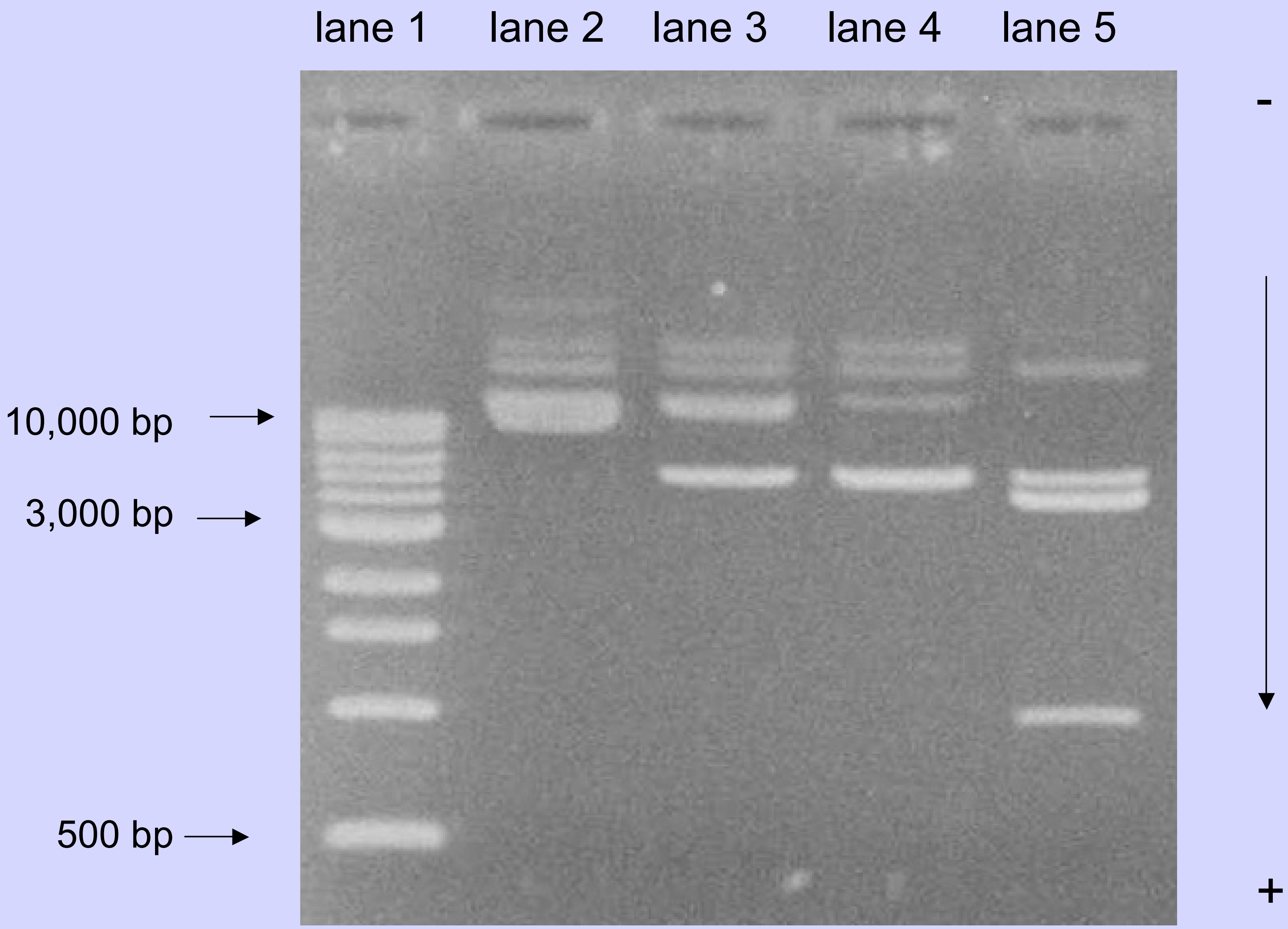
- E.g. FBI “CODIS” (combined DNA indexing system) data base
- pick 13 short, variable regions of human genome
- amplify each from, e.g., small spot of dried blood
- measure product lengths (next slides)

- PCR is important in that sample size is reduced from grams of tissue to a few cells

Gel Electrophoresis

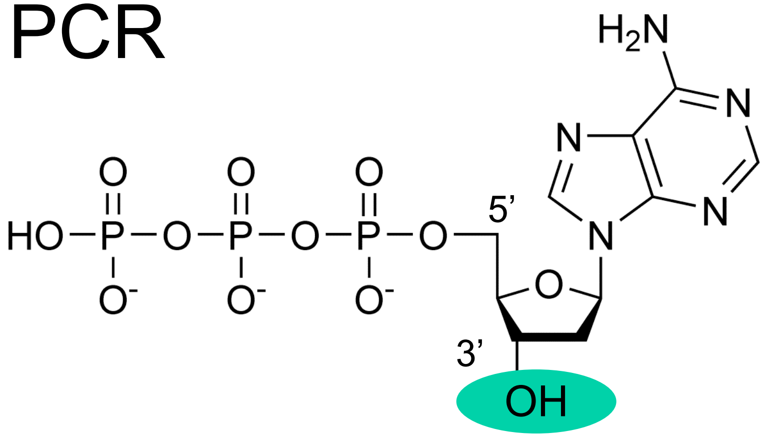
- DNA/RNA backbone is negatively charges
- Molecules moves slowly in gels under an electric field
  - agarose gels for large molecules
  - polyacrylamide gels for smaller ones
- Smaller molecules move faster

- So, you can *separate DNAs & RNAs by size*


lane 1
lane 2
lane 3
lane 4
lane 5
-
10,000 bp
3,000 bp
500 bp
+

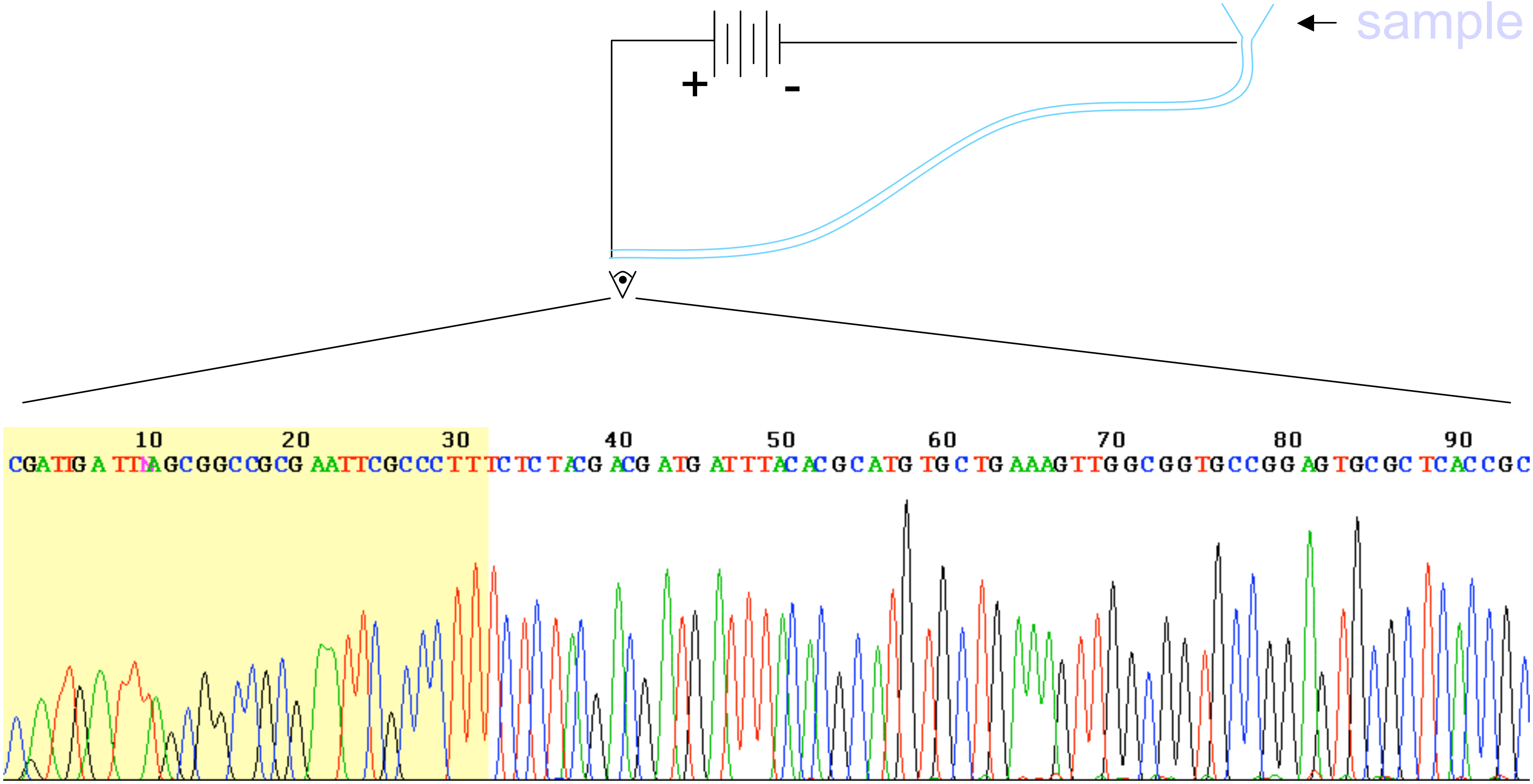
DNA Sequencing

- Like one-cycle, one-primer PCR
- Suppose 0.1% of A's:
  - are *di*-deoxy adenosine's; backbone can't extend
  - carry a green florescent dye



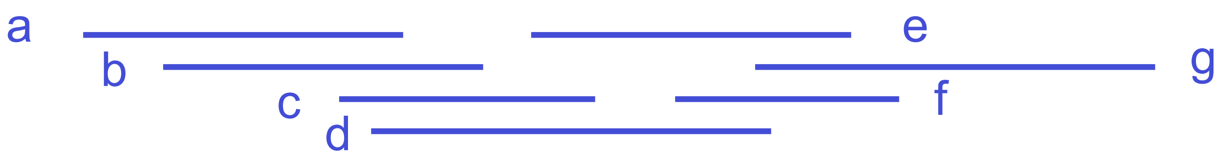
- Separate by capillary gel electrophoresis
- If frags of length 42, 49, 50, 55 … glow green, those positions are A's
- Ditto C's (blue), G's (yellow), T's (red)

DNA Sequencing


+
-
sample
10
20
30
40
50
60
70
80
90
CGATTGATTNAGCGGCCGCGAATTCGCCCTTTCTCTACGACGATGATTTACACGCATGTGCTGAAAGTTGGCGGTGCCGGAGTGCGCTCACCGC

DNA Sequencing

- Highly automated
- Typically can “read” about 600 nt in one run
- “Whole Genome Shotgun” approach:
  - cut genome randomly into ~ G / 600 x 10 fragments
  - sequence each
  - reassemble by computer



- Complications: repeated region, missed regions, sequencing errors, chimeric DNA fragments, …
- But overall accuracy ~10^{-4}, if careful

Summary

- PCR allows simple *in vitro* amplification of minute quantities of DNA (having pre-specified boundaries)
- Sanger sequencing uses
  - a PCR-like setup with modified chemistry to generate varying length prefixes of a DNA template with the last nucleotide of each color-coded
  - gel electrophoresis to separate DNA by size, giving sequence
- Sequencing random overlapping fragments allows genome sequencing