Pam + Nicola

BLURB re HIV / DIAGNOSTICS / USE OF PCR

INSTRUCTIONS

- OPEN NCBI
- TYPE ACC # INTO SEARCH
- PULL OUT SECTION OF SEQUENCE WHICH CONTAINS RELEVANT BIT FOR PCR (~ 500 bases ?)

NOW YOU HAVE TO DESIGN PRIMERS FOR THE PCR REACTON TO WORK SUCCESSFULLY.

aaGgcgt-.-.-.-.-
Sequence

NOTE: NO NUMBERS JUST A STRING OF LETTERS.

OF COURSE DNA IS DOUBLE-STRANDED

aatgcgt.....
ttacgca.....
double-stranded sequence

ONCE THIS IS DONE SOMEHOW HIGHLIGHT AREA THAT MUST BE CONTAINED IN THE PCR PRODUCT

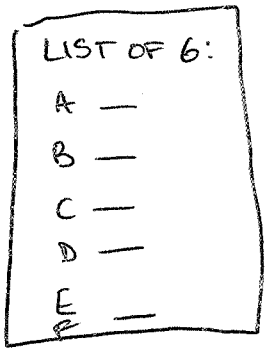
- CHOOSE LEFT PRIMER (← MATCH BY CLICK AND DRAG ?)

LIST OF 6 :
A —
B —
C —
D —
E —
F —

DECIDE WHICH IS BEST.
(see basic rules of primer design)

YOUR LEFT PRIMER IS INCORRECT ! CLUE.....

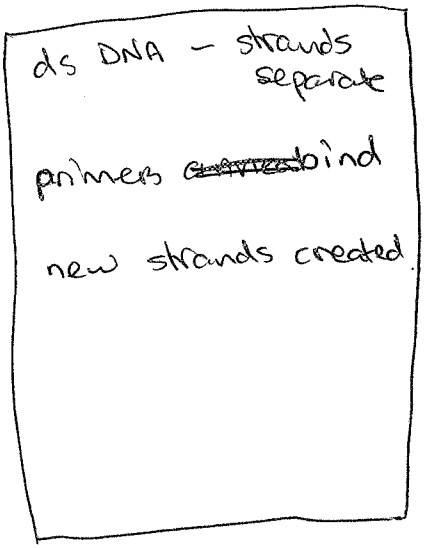
- CHOOSE RIGHT PRIMER



PRIMERS ARE CHOSEN BUT ARE THEY UNIQUE

- OPEN NCBI
- SEARCH FOR PRIMER SEQUENCE
- HOPEFULLY UNIQUE TO HIV!

END RESULT OF THIS BIT



Basic rules of primer design:

- length — 20–30 bases long

Sequence — avoid long runs of a single letter
eg Actgggggggcgggca

- have c or g at last letter

- avoid self annealing

- must be unique to DNA sequence

GC content - 40–60%

T_m — between 50 and 65°c

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