

Virology - Is It Practical?

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Or

Lessons learned when practicals
don't work as expected

Or

Computer Simulations of Hepatitis
B Virus in Patients

Introduction

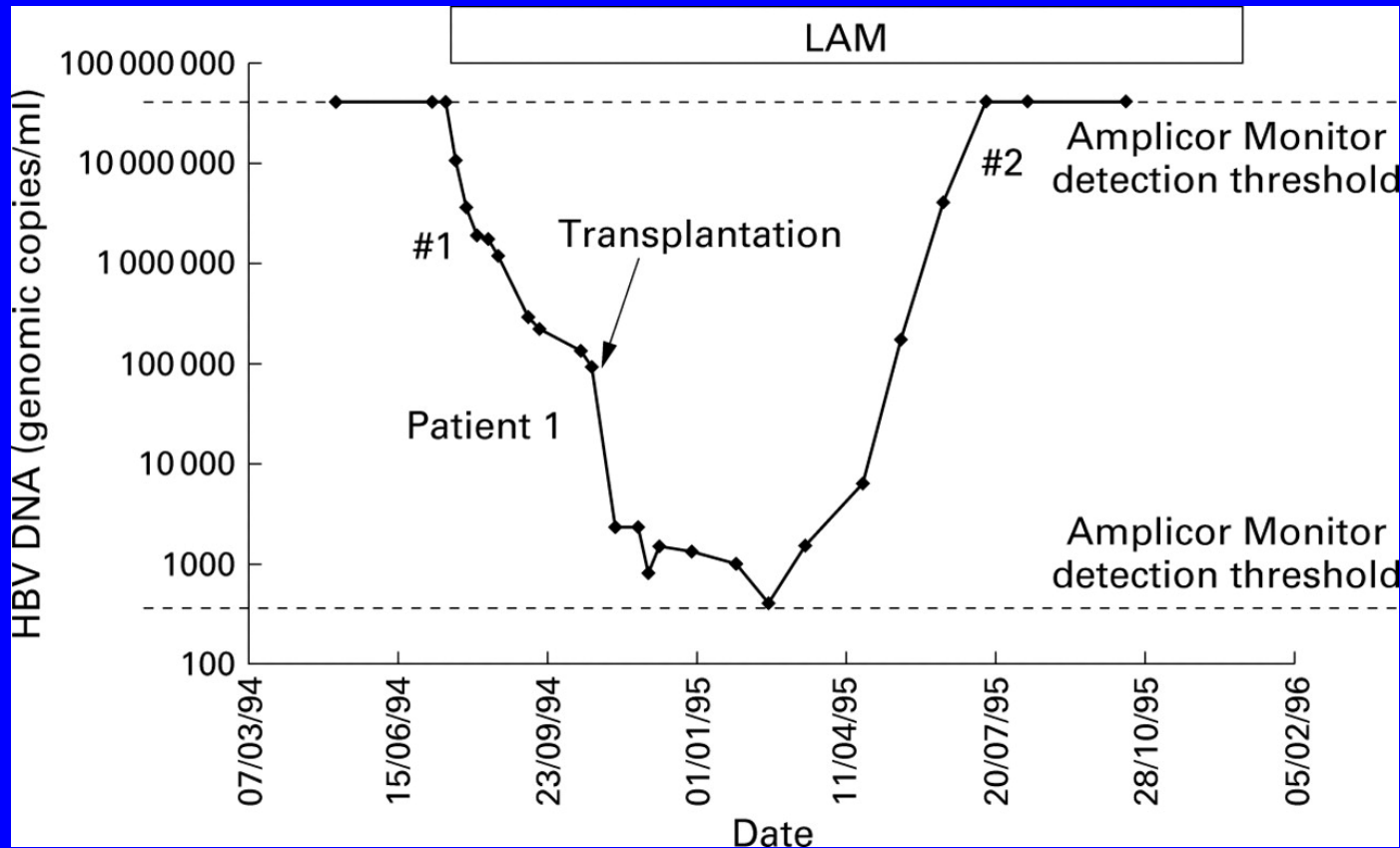
- What led to the practical
- Background on HBV
- The setting
- The practical
- Outcomes

Hepatitis B virus in Liver Transplantation

- HBV is a common cause of end-stage liver disease leading to transplantation
- Virus will infect graft unless cleared
- HBV+ patients awaiting liver transplant are often treated with Lamivudine (3TC)
- Lamivudine treatment is continued post-transplant
- Nearly all patients initially respond well
- BUT up to 20% of these patients relapse with high loads of Lamivudine-resistant HBV

Hepatitis B virus in Liver Transplantation

A 'typical' course of infection



Mutimer, D et al. Gut 2000;46:107-113

Heriot-Watt 2005

Leeds September 2005

HBV Uses Reverse Transcriptase

- HBV uses reverse transcriptase
- HBV has Tyr Met Asp Asp (YMDD) in the active site of reverse transcriptase (encoded in pol gene)
- HBV is susceptible to Lamivudine

How does HBV pol sequence mutate in response to Lamivudine?

- A consistent pattern of mutations seen, e.g.:

Tyr Met Asp Asp

TAT ATG GAT GAT

TAT **G**TG GAT GAT

Tyr **Val** Asp Asp

Data Analysis

- Standard bioinformatics software from the University of Wisconsin Genetics Computer Group (GCG)
- Used software to compare and present sequence information and search for mutations

Output

TGTCTGGCTTTCAGTTATATGGATGATGTGGTTTTGGGGGCCAAGTCTGTACAACATCTT

721 -----+-----+-----+-----+-----+-----+ 780

ACAGACCGAAAGTCAATATACCTACTACACCAAACCCCGGTTTCAGACATGTTGTAGAA

a Pol	C	L	A	F	S	<u>Y</u>	<u>M</u>	<u>D</u>	<u>D</u>	V	V	L	G	A	K	S	V	Q	H	L	-
b sAg	V	W	L	S	V	<u>I</u>	<u>W</u>	<u>M</u>	<u>M</u>	W	F	W	G	P	S	L	Y	N	I	L	-
c	S	G	F	Q		<u>L</u>	<u>Y</u>	<u>G</u>	*	C	G	F	G	G	Q	V	C	T	T	S	* -

- HBV Pol and s Antigen genes overlap
- Mutations in pol gene often result in an effect on s Antigen
- Only certain mutations tolerated

The Setting

- Practical forms part of Final Year BMedSc Virology module at University of Birmingham
- 5 week module, 'lectures' and practical

The Objective

- To enable students to analyze and interpret sequence data use a standard bioinformatics software package
 - Since we were using *GCG* for the research project, used *GCG* to teach the course

The Tasks

Problem-based learning using a standard set of programs (in *GCG*) to analyze data and make judgments

This is the problem we give the students:

Determine the ORF which contains the YXDD motif and translate that sequence. Also translate in the other forward reading frames and use FASTA to determine if these code for any other HBV gene(s).

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What would you predict might happen if Lamivudine treatment were stopped?

How it Looks for the Students

PRETTY of: hbvseq.msf{*} December 1, 2004 12:03 ..

	201				250
hbvseq.msf{ae0}	TTTCCCCC	TGTTTGGCTT	TCAGCTATAT	GGATGATGTG	GTATTGGGGG
hbvseq.msf{ae50}	TTTCCCCC	TGTTTGGCTT	TCAGCTATAT	GGATGATGTG	GTATTGGGGG
hbvseq.msf{ae150}	TTTCCCCC	TGTTTGGCTT	TCAGCTATAT	GGATGATGTG	GTATTGGGGG
hbvseq.msf{ae413}	TTTCCCCC	TGTTTGGCTT	TCAGCTATGT	GGATGATGTG	GTATTGGGGG
hbvseq.msf{ae450}	TTTCCCCC	TGTTTGGCTT	TCAGCTATGT	GGATGATGTG	GTATTGGGGG
hbvseq.msf{ae500}	TTTCCCCC	TGTTTGGCTT	TCAGCTATGT	GGATGATGTG	GTATTGGGGG

How it Looks for the Students

PRETTY of: hbvprot.msf{*} December 2, 2004 14:12 ..

```

1
hbvprot.msf{mm0}  ~L~LYQTFGR KLHLYSHPII LGFRKIPMGV GLSPFLLAQF TSAICSVVRR
hbvprot.msf{mm70} ~L~LYQTFGR KLHLYSHPII LGFRKIPMGV GLSPFLLAQF TSAICSVVRR
hbvprot.msf{mm305} LLLLYQTFGR KLHLYSHPII LGFRKIPMGV GLSPFLMAQF TSAICSVVRR
hbvprot.msf{mm333} LLLLYQTFGR KLHLYSHPII LGFRKIPMGV GLSPFLMAQF TSAICSVVRR
hbvprot.msf{mm361} LLLLYQTFGR KLHLYSHPII LGFRKIPMGV GLSPFLMAQF TSAICSVVRR

51
hbvprot.msf{mm0}  AFPHCLAFSY MDDVVLGAKS VQHLESLEFTA VTNFLL~~~~ ~~~~~~
hbvprot.msf{mm70} AFPHCLAFSY MDDVVLGAKS VQHLESLEFTA VTNFLLSLGI HLNPKNKTKRG
hbvprot.msf{mm305} AFPHCLAFSY VDDVVLGAKS VQHLEALFTA VTNFLLSLGI HLNPKNKTK~~
hbvprot.msf{mm333} AFPHCLAFSY VDDVVLGAKS VQHLEALFTA VTNFLLSLGI HLNPKNKTK~~
hbvprot.msf{mm361} AFPHCLAFSY VDDVVLGAKS VQHLEALFTA VTNFLLSLGI HLNPKNKTK~~
100
```


An Unexpected Outcome

- Students correctly identify pol ORF
- They then run FASTA searches to find the sAg ORF - but have trouble
- Why?...

Reason

Many sequences in protein databases identified as HBV sAg are in wrong reading frame

Codons Around Motif of Interest

TGTCTGGCTTTCAGTTATATGGATGATGTGGTTTTGGGGGCCAAGTCTGTACAACATCTT

721 -----+-----+-----+-----+-----+-----+-----+ 780

ACAGACCGAAAGTCAATATACCTACTACACCAAACCCCGGTTTCAGACATGTTGTAGAA

a Pol	C	L	A	F	S	<u>Y</u>	<u>M</u>	<u>D</u>	<u>D</u>	V	V	L	G	A	K	S	V	Q	H	L	-
b sAg	V	W	L	S	V	<u>I</u>	<u>W</u>	<u>M</u>	<u>M</u>	W	F	W	G	P	S	L	Y	N	I	L	-
c	S	G	F	Q		<u>L</u>	<u>Y</u>	<u>G</u>	*	C	G	F	G	G	Q	V	C	T	T	S	* -

•HBV Pol and s Antigen genes overlap

A Genuine Match to HBV Pol

Q9wp64 hepatitis b virus p protein

```
                                10      20      30
0frame2                                LMLLYKTYGWLHLYSHPIVLGFRKIPMGV
                                |||
Q9WP64    RLSSNSRINNNQYGTMQNLHDSCSRQLYVSLMLLYKTYGWLHLYSHPIVLGFRKIPMGV
            420      430      440      450      460      470

                40      50      60      70      80
0frame2    GLSPFLLAQFTSAICSVVRRAFPCLAFSYMDDVVLGAKSVQHREALYTAVTNFLLS
            |||
Q9WP64    GLSPFLLAQFTSAICSVVRRAFPCLAFSYMDDVVLGAKSVQHREALYTAVTNFLLSLGI
            480      490      500      510      520      530

Q9WP64    HLNPNKTKRWGYSLNFMGYIIGSWGTLPQDHIVQKIKHCFRKLVPVNRPIDWKVCQRIVGL
            540      550      560      570      580      590
```

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b sAg	V	W	L	S	V	<u>I</u>	<u>W</u>	<u>M</u>	<u>M</u>	W	F	W	G	P	S	L	Y	N	I	L	-
c	S	G	F	Q		<u>L</u>	<u>Y</u>	<u>G</u>	*	C	G	F	G	G	Q	V	C	T	T	S	* -

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a Pol	C	L	A	F	S	<u>Y</u>	<u>M</u>	<u>D</u>	<u>D</u>	V	V	L	G	A	K	S	V	Q	H	L	-
b sAg	V	W	L	S	V	<u>I</u>	<u>W</u>	<u>M</u>	<u>M</u>	W	F	W	G	P	S	L	Y	N	I	L	-
c	S	G	F	Q		<u>L</u>	<u>Y</u>	<u>G</u>	*	C	G	F	G	G	Q	V	C	T	T	S	* -

How has this arisen?

- Presumably DNA sequence translated without thinking!
- But by this time students are able (with help) to check this themselves against genuine sAg sequences

Outcomes

- Students correctly identify HBV protein sequences
- Reinforces message that can't always trust such volatile and developing resources as sequence databases

Outcomes (3)

Have had:

- Mini-rebellions



Outcomes (4)

- Students in tears



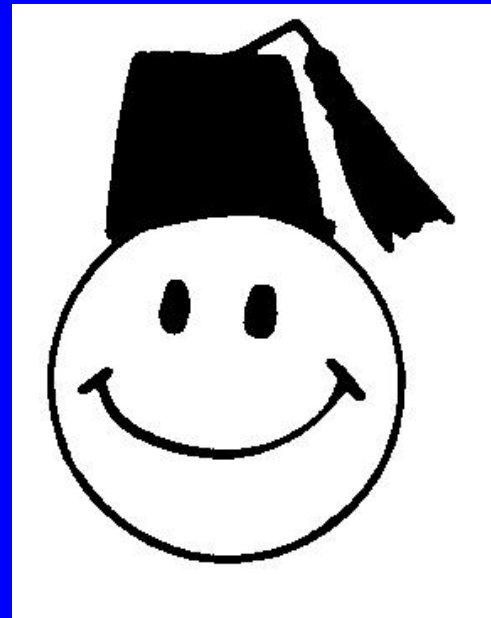
Outcomes (5)

- Students walking out



Outcomes (6)

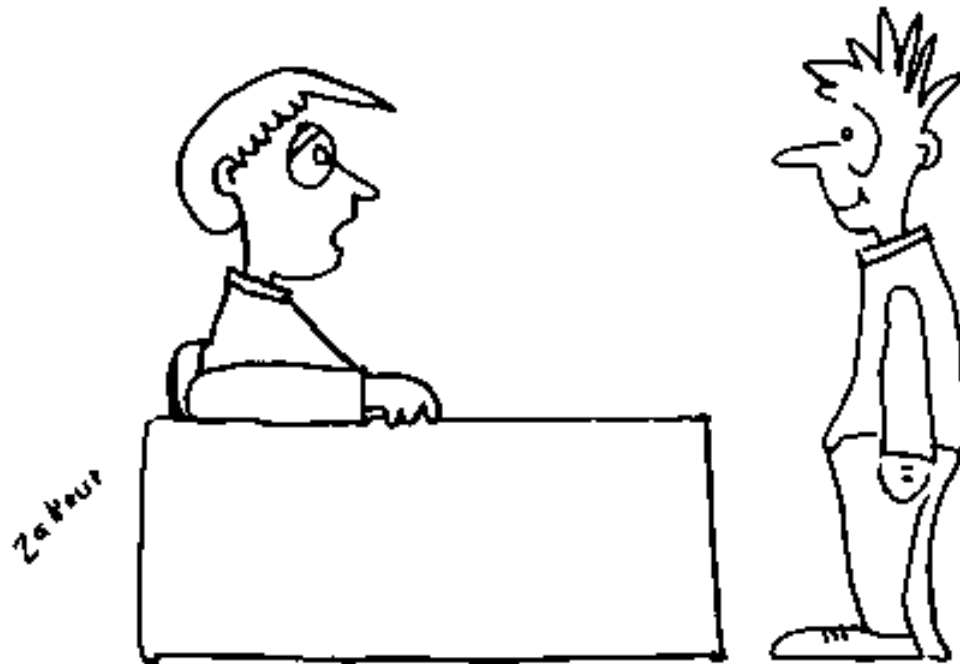
- BUT...
- All students complete the exercise
- Nearly all admit they felt a sense of satisfaction and achievement



Acknowledgments

- Andrew Simpson
- Julia Lodge
- Steve Minchin
- Julian Parkhill
- Carole Sparke
- Phil McLeish
- Google images
- My son for 'crying' image

GUIDANCE



You really should have a better reason for a career in the sciences than, "lab coats are cool...."

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