

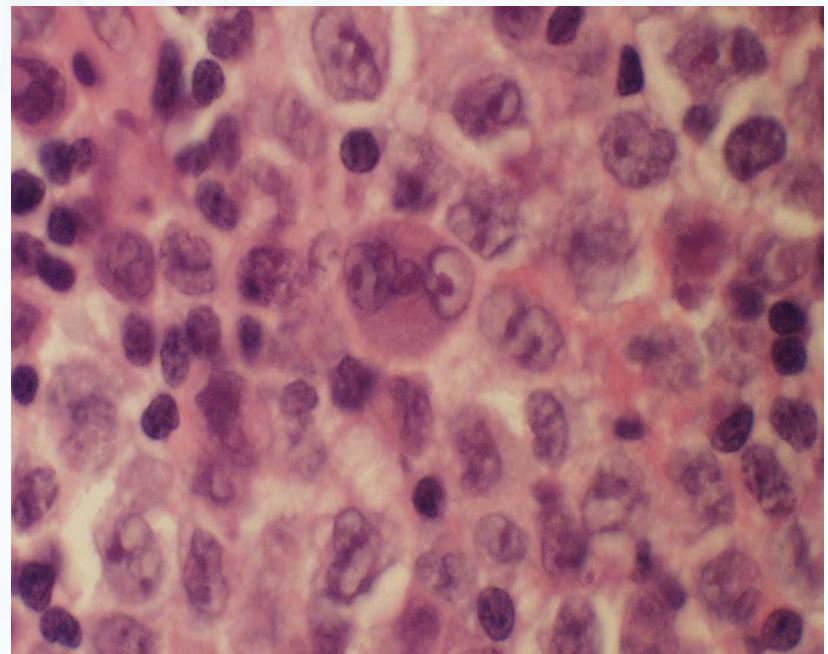
Redundant PCR assays for detection of herpesvirus and polyomavirus genomes

Molecular methods of virus discovery

- Redundant PCR assays
- Subtractive hybridisation
- Rolling circle amplification

Clinical samples v vaccine cell substrates

In Hodgkin lymphoma the tumour cells usually constitute <1% of the total cell population.



Our experiments aim to detect <1000 copies in 1 μ g of sample DNA

Redundant PCR

Redundant PCR

- Primers derived from conserved blocks of **protein** sequence
- Used to identify genes encoding related proteins
- Identification of new members of virus families

Redundant PCR assays

- Herpesvirus
- Polyomavirus
- Retrovirus

Primer design

5'

TA
cloning

Clamp based on
consensus
nucleotide sequence,
modified for Tm

3'

Redundant sequence
based on all possible
codon usages ~14
bases

GTTTGATGCCGACCTTAYGGNTTYACNGG

COnsensus-Degenerate Hybrid Oligonucleotide Primer

<http://blocks.fhcrc.org/codehop.html>

Timothy Rose et al., 2003

Conserved regions in herpesvirus polymerase gene

HSV1	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS
HSV2	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS
VZV	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS
EBV	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS
HCMV	ASLYPSIMQAHNL	FYGFTGV	IYGDTDS
HHV-6	QSLYPSIMQAHNL	VYGVTGA	IYGDTDS
HHV-7	QSLYPSIMQAHNL	VYGVTGA	IYGDTDS
HHV-8	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS

Redundancy

YPSII

IYGDT

Possible codon usage

Y= 2

P=4

S=6

I=3

144

I=3

Y=2

G=4

D=2

48

Primer selection

HSV1	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS
HSV2	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS
VZV	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS
EBV	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS
HCMV	ASLYPSIMQAHNL	FYGFITGV	IYGDTDS
HHV-6	QSLYPSIMQAHNL	VYGVTGA	IYGDTDS
HHV-7	QSLYPSIMQAHNL	VYGVTGA	IYGDTDS
HHV-8	ASLYPSIIQAHNL	VYGFTGV	IYGDTDS

YPSII(M)

383
382

YGF/VTG

IYGDT

**Nested
analysis**

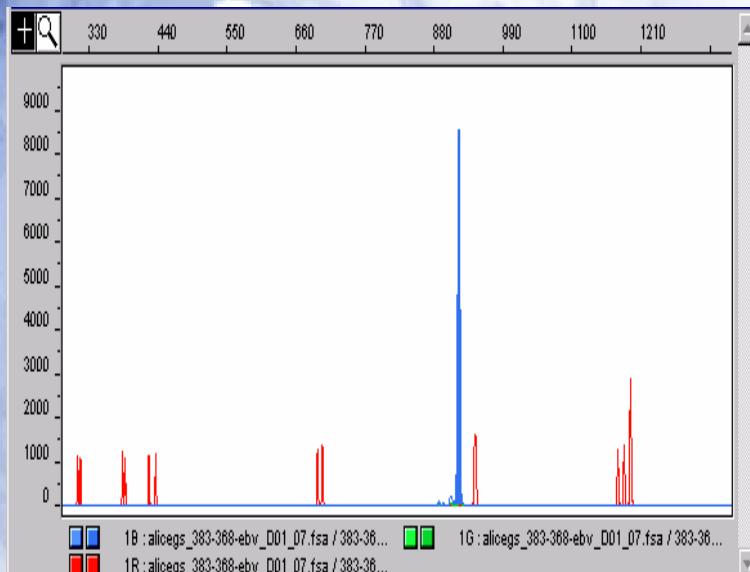
514
515

368
368

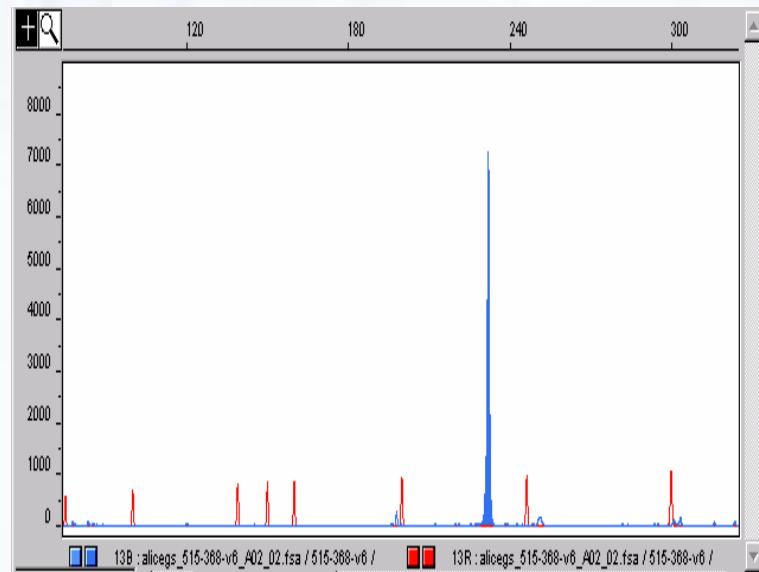
Analyse products using GeneScan

- Improved sensitivity
- Increased size resolution improves interpretation

HHV-6

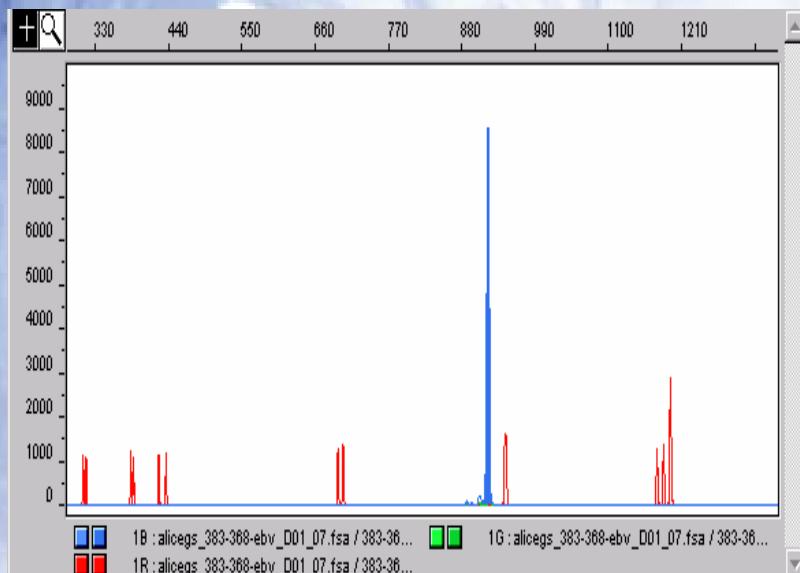


Outer primers

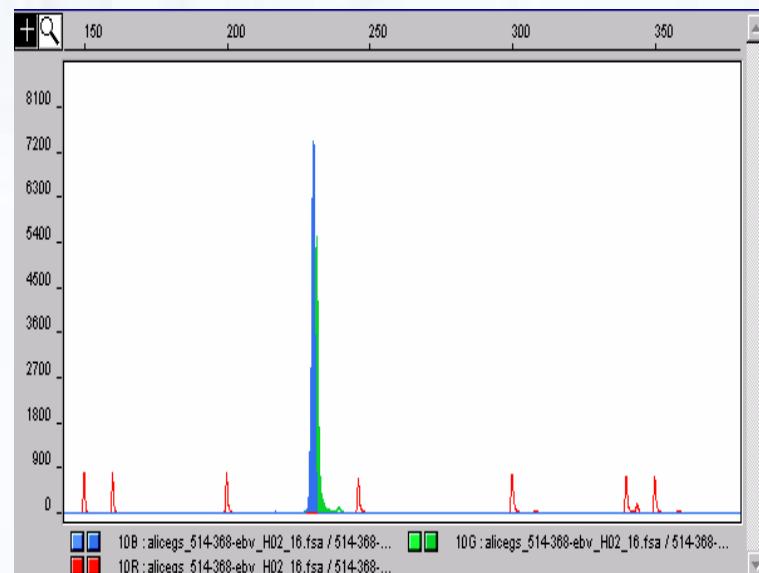


Inner primers

EBV

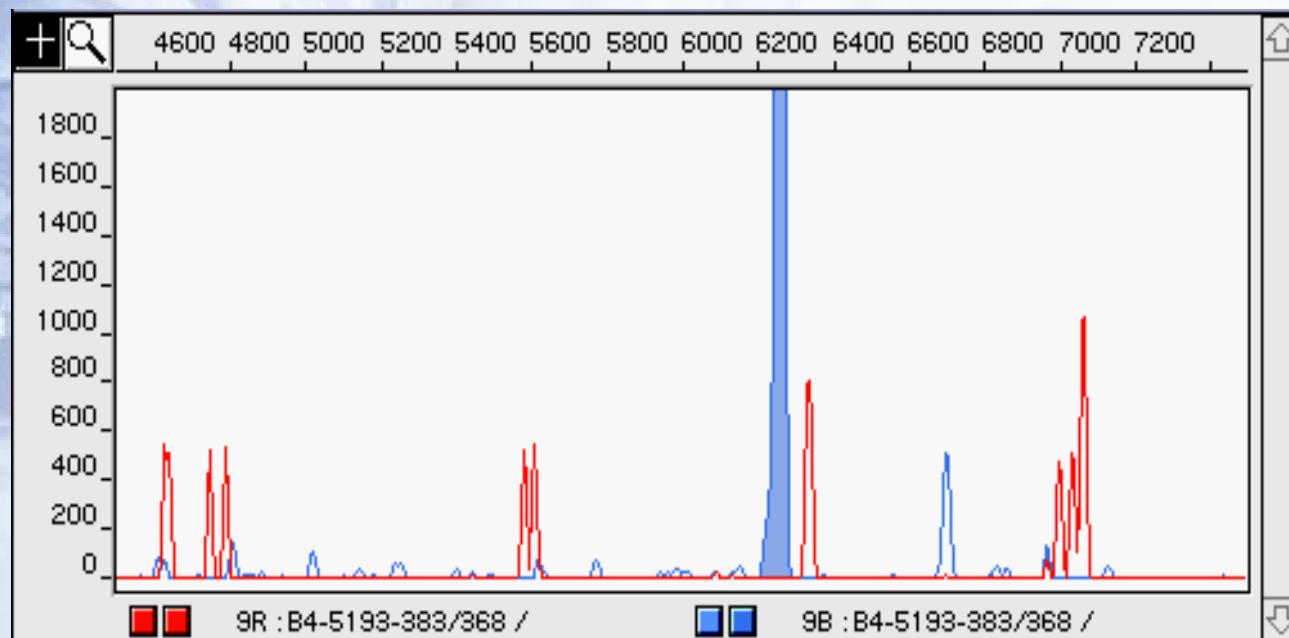


Outer primers

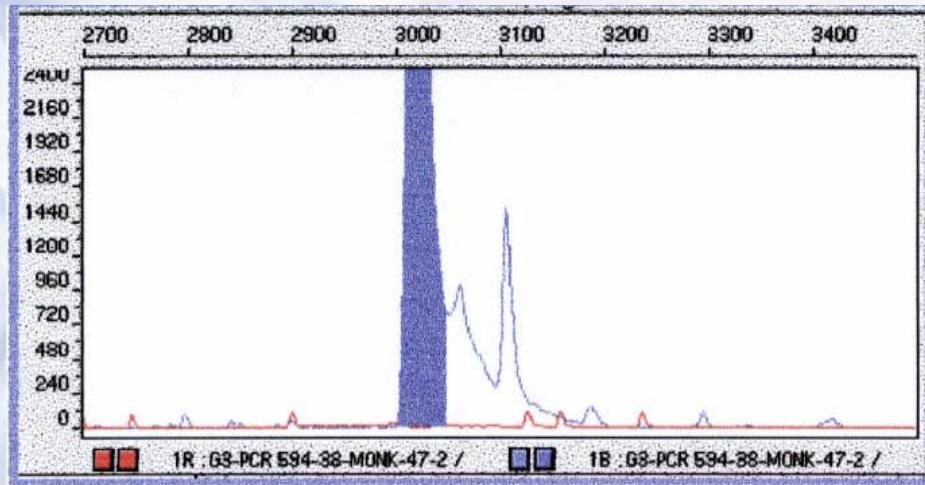


Inner primers

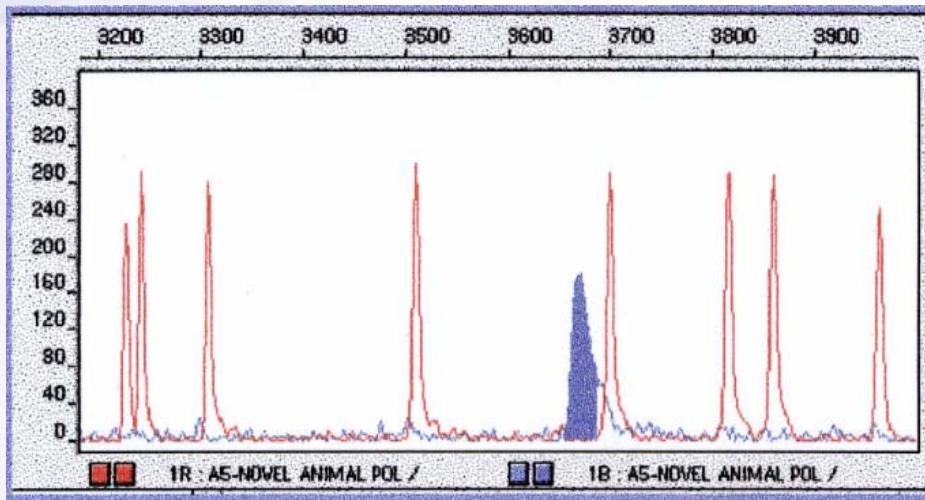
EBV-associated Hodgkin lymphoma



Monkey



Pig

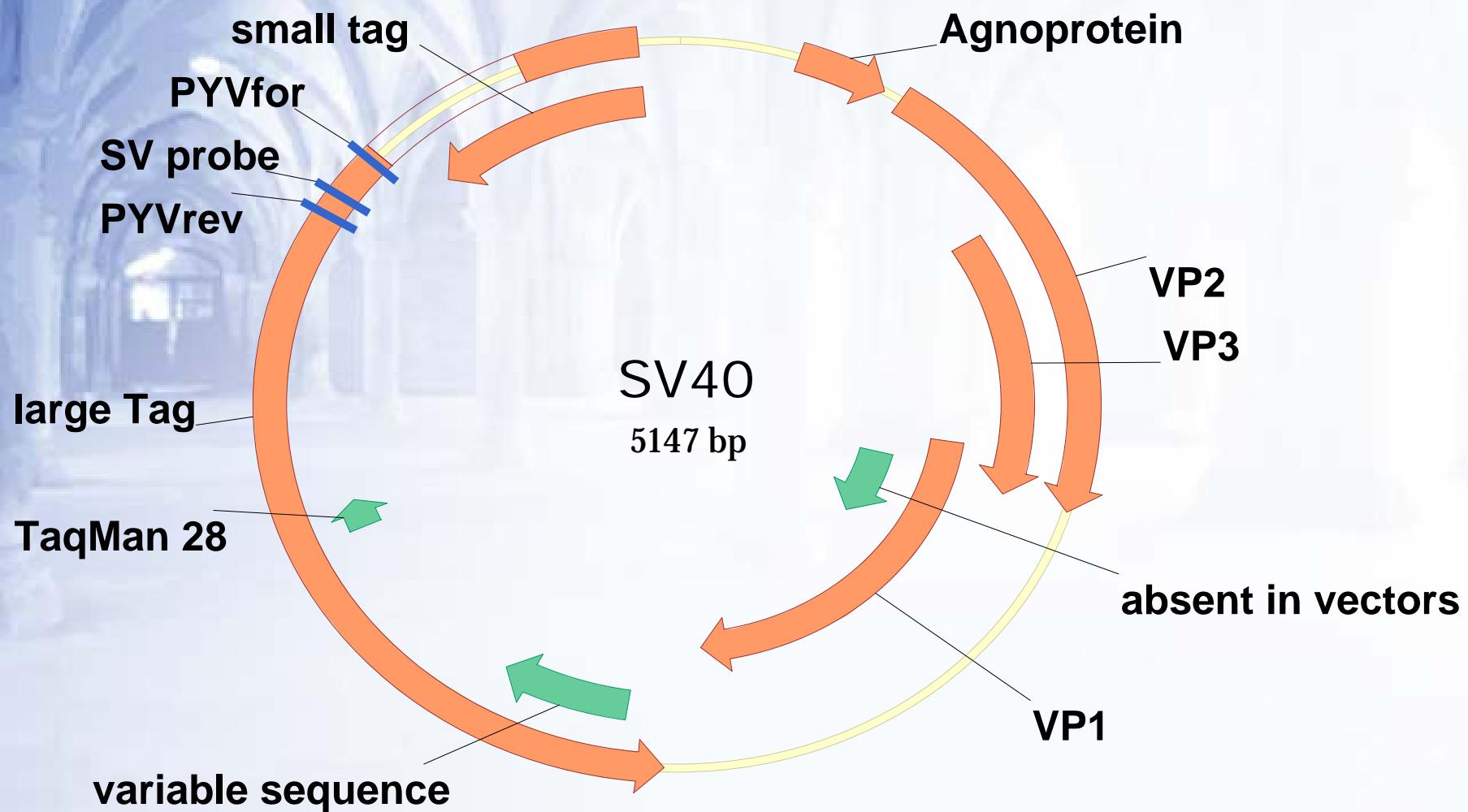


Redundant PCR assays for herpesviruses

- Robust
- Sensitive (<100 copies)
- Proven++

Polyomaviruses

Assay design



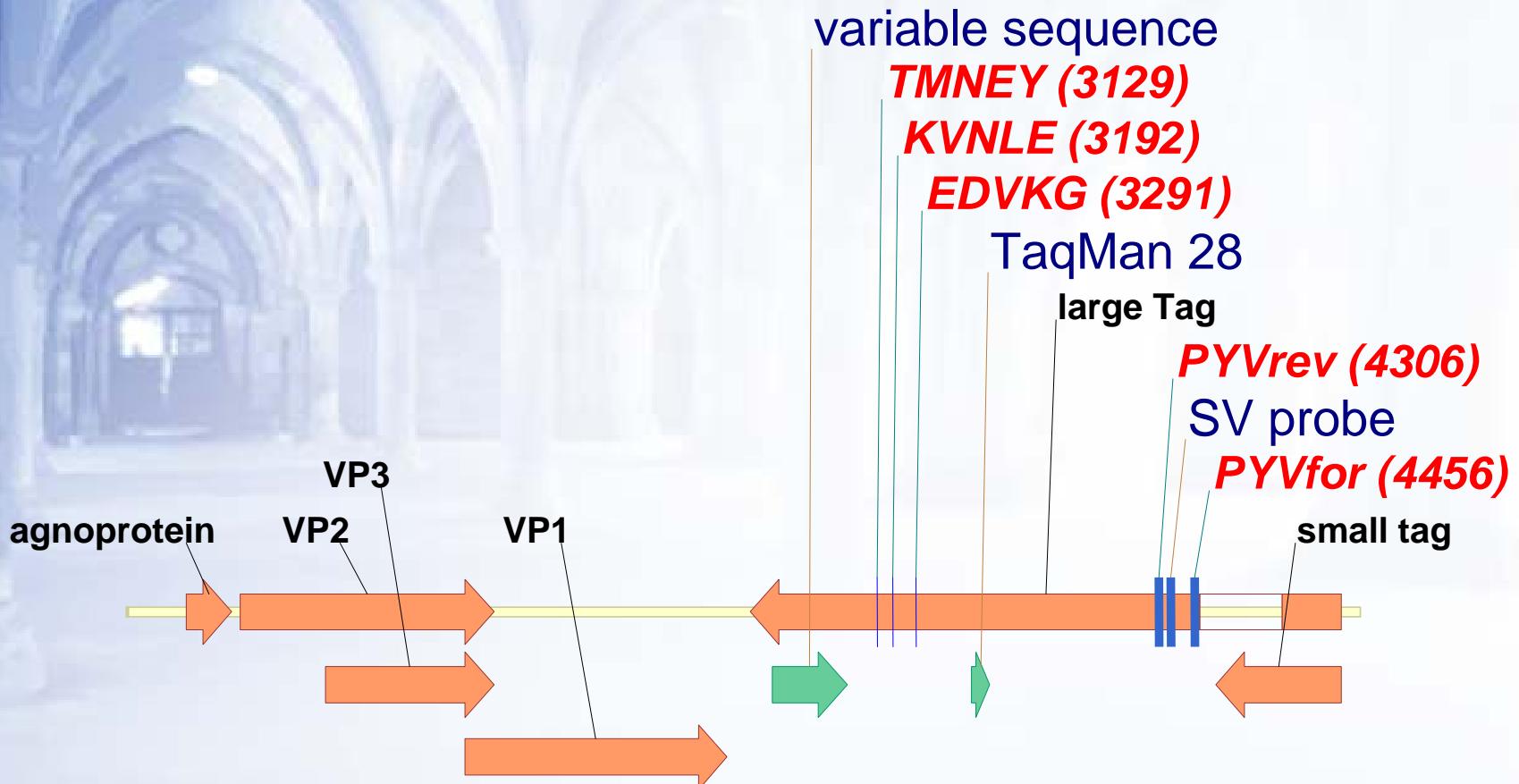
E/DDVKG – 64-fold degeneracy

	(631)	631	640	650	660	672
JC Mad1 large T (461)	ELGVGIDQFMVVF	EDVK	GAE	SRD	LPSGHGISNLDC	LRDYL
BK AS large T (462)	ELGVAIDQYMMVVF	EDVK	GAE	SKD	LPSGHGINNLDS	LRDYL
BK Dunlop large T (462)	ELGVAIDQYMMVVF	EDVK	GAE	SKD	LPSGHGINNLDS	LRDYL
bovine polyoma (425)	ELGMAIDQFTVVF	EDVK	KKSSKSSLQT	GIGFE	NLDNLRDHL	
budgie large T (420)	ELGCAIDQFMVLF	EDVK	TPEPDTNLP	SFGGMVNLDNLRDHL		
strain a3 large T (609)	ELGVAQDQFVVCF	EDVK	QIALNKQLQP	GMGVANLDNLRDYL		
strain a2 large T (607)	ELGVAQDQFVVCF	EDVK	QIALNKQLQP	GMGVANLDNLRTTW		
hamster polyoma large T (582)	ELGVAIDKFVVFE	EDVK	QTGDKRHLQSGLG	INNLDNLRDYL		
monkey LPV large T (523)	ELGCAIDQFCVLI	DDVK	QTILNKHILQP	GQGVNNLDNLRDHL		
SV40 large T (460)	ELGVAIDQFLVVF	EDVK	TGGESRDLP	SQGINNLDNLRDYL		
LPV large T (523)	ELGCAIDQLCVLI	DDVK	QTILNKHILQP	GQGVNNLDNLRDHL		
JC large T (461)	ELGVGIDQFMVVF	EDVK	GAE	SRD	LPSGHGISNLDC	LRDYL
BK large T (461)	ELGVAIDQYMMVVF	EDVK	GAE	SKD	LPSGHGINNLDS	LRDYL
Consensus (631)	ELGVAIDQFMVVF	EDVK	GAE	SRD	LPSGHGISNLDC	LRDYL
Section 17						
	(673)	673	680	690	700	714
JC Mad1 large T (503)	DGSV	KVNLERKHKQNKRT	QVFFPGIV	TMNEY	SVPRTLQARFVR	
BK AS large T (504)	DGSV	KVNLEKKHLNKRT	QIFPPGLV	TMNEY	PVPKTTLQARFVR	
BK Dunlop large T (504)	DGSV	KVNLEKKHLNKRT	QIFPPGLV	TMNEY	PVPKTTLQARFVR	
bovine polyoma (467)	DGAV	PVNLERKHKQNKVT	QIFPPGIV	TCNEY	DIPLTVKIRMYQ	
budgie large T (462)	EGSV	PVNLERKHKQNKVS	QIFPPGI	ITMNNY	VLPHTIQARART	
strain a3 large T (651)	DGSV	KVNLEKKHNSKRSQL	FPPCVCT	TMNEY	LIPQTVWARFHM	
strain a2 large T (649)	NGSV	KVNLEKKHNSKRSQL	FPPCVCT	TMNEY	LIPQTVWARFHM	
hamster polyoma large T (624)	DGSV	KVNLEKKHVNKRSQI	FPPCIV	TANEY	FFPQTLYARFH	
monkey LPV large T (565)	DGTI	KVNLEKKHVNKRSQI	FPPVIM	TMNEY	LLPPTIGVRFAL	
SV40 large T (502)	DGSV	KVNLEKKHLNKRT	QIFPPGIV	TMNEY	SVPKTTLQARFVK	
LPV large T (565)	DGTI	KVNLEKKHVNKRSQI	FPPVIM	TMNEY	LLPRTIGVRFAL	
JC large T (503)	DGSV	KVNLERKHKQNKRT	QVFFPGIV	TMNEY	SVPRTLQARFVR	
BK large T (503)	DGSV	KVNLEKKHLNKRT	QIFPPGLV	TMNEY	PVPKTTLQARFVR	
Consensus (673)	DGSV	KVNLEKKHLNKRT	QIFPPGIV	TMNEY	VPKTLQARF R	

KVNLE – 128-fold
PVNLE – 256-fold

TMNEY – 16-fold

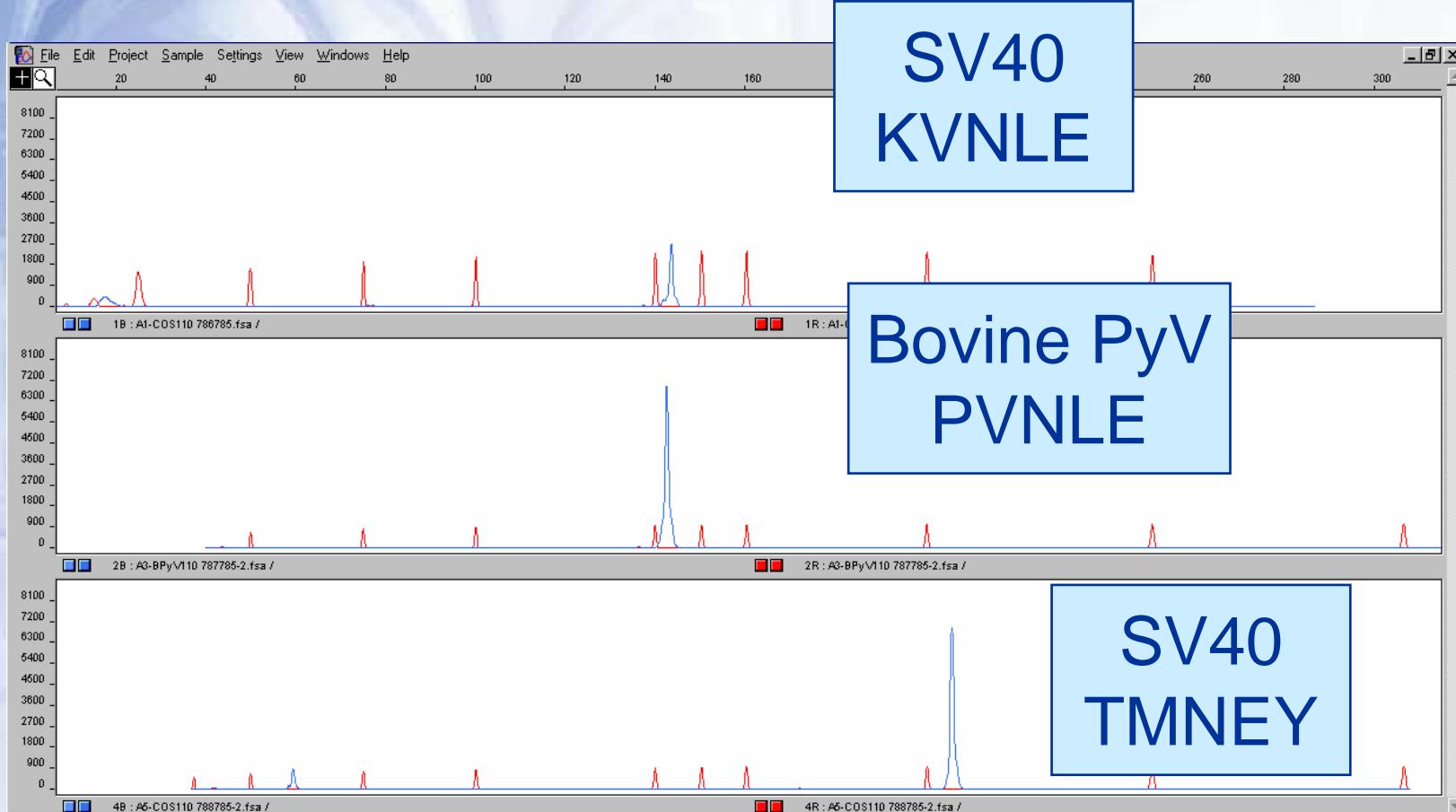
Polyomavirus primers



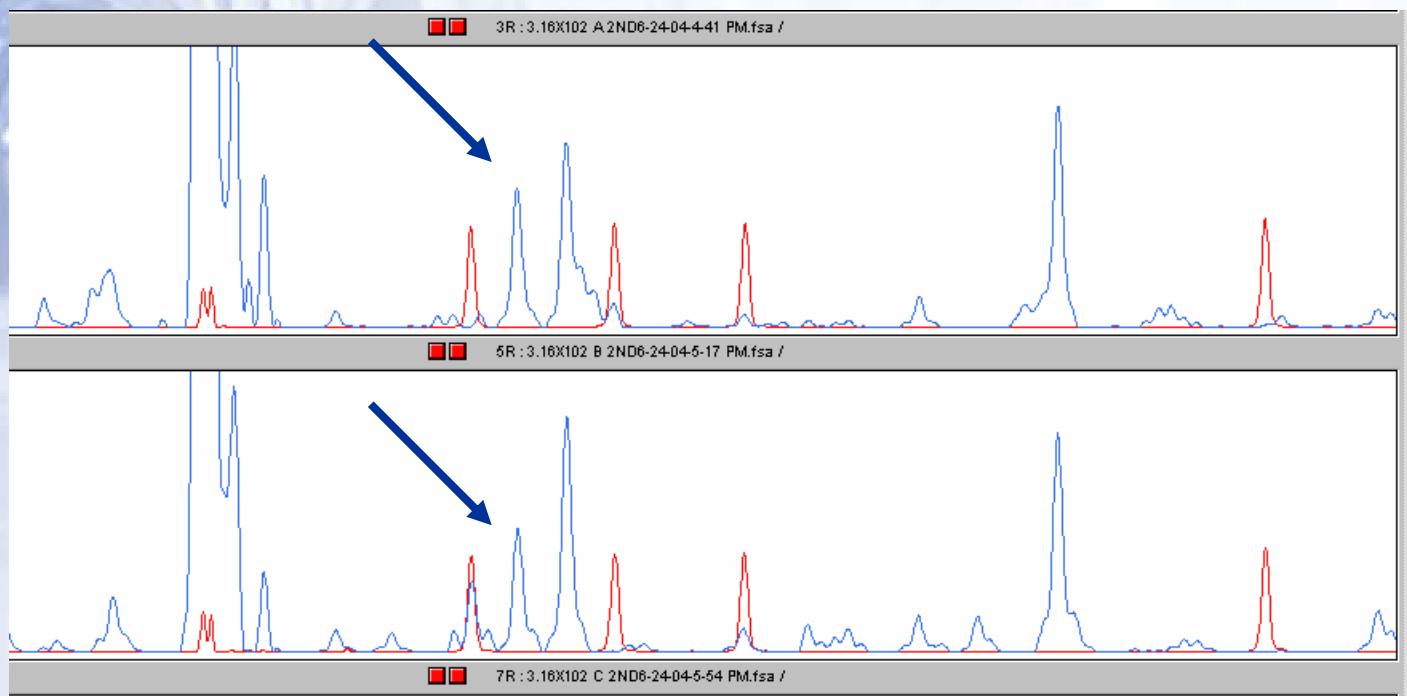
SV40 degen primers

5147 bp

Polyomavirus assays



Detection of $10^{2.5}$ copies SV40 in 1 μ g DNA



Validation

- Predicted specificities
- Sensitivity

TMNEY – 100 copies

KVNLE – 10^3 to 5×10^3 copies

Polyomaviruses & human lymphoma

- TaqMan for SV40, BKV and JCV
0/152 NHL & 0/35 HL samples
- Consensus assay
0/128 NHL & 0/18 HL samples
- Redundant PCR
0/35 cHL samples

Conclusions – redundant PCR

- Robust
- Relatively sensitive
- Suitable for screening
- Dependent on well conserved sequences
- Usual PCR contamination issues

Subtractive hybridisation

Isolates differences
between two nucleic acid
samples

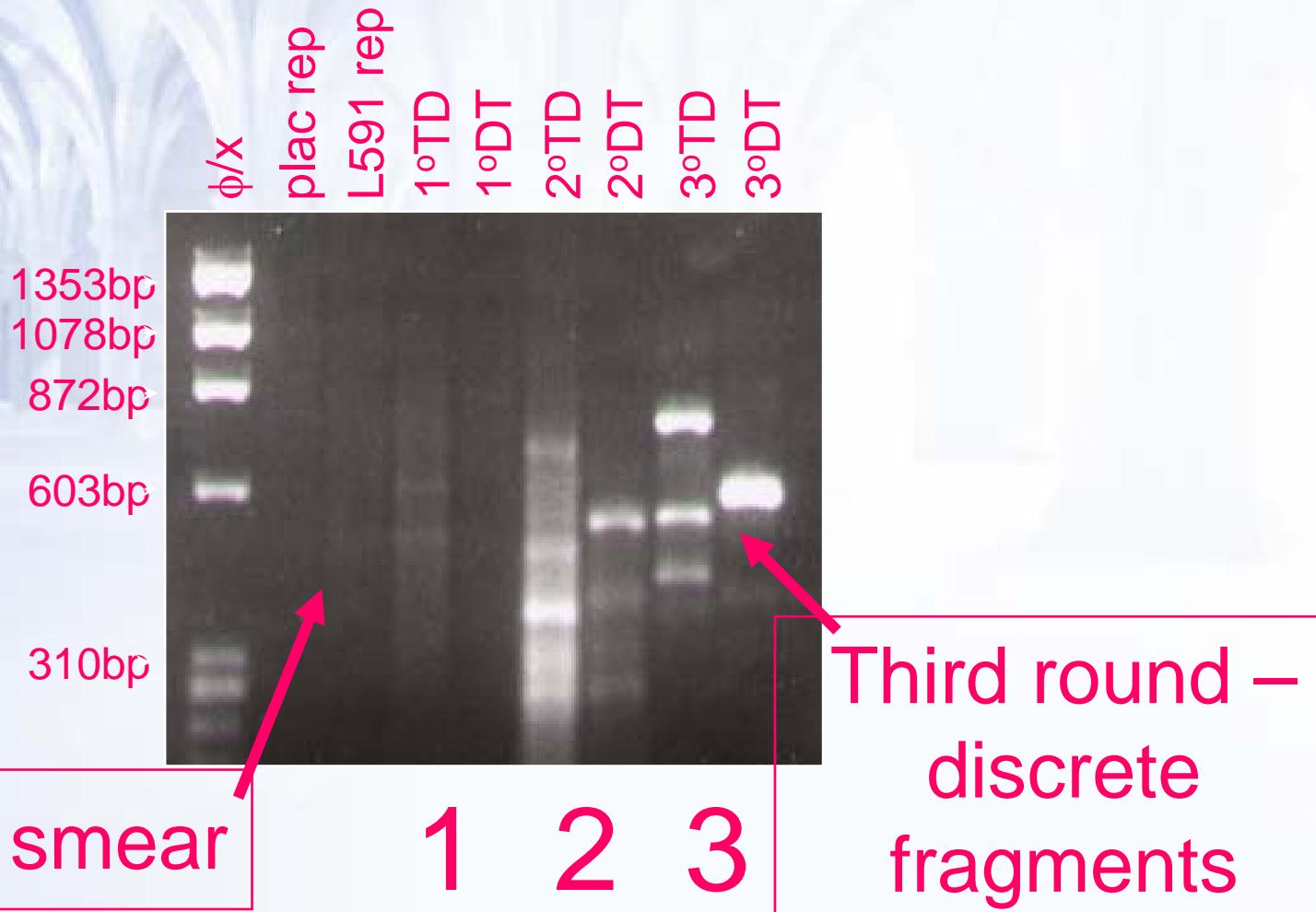
Oncogenic viruses in vaccine cell substrates

- Cells – DNA genomes
- Supernatant – DNA or RNA genomes

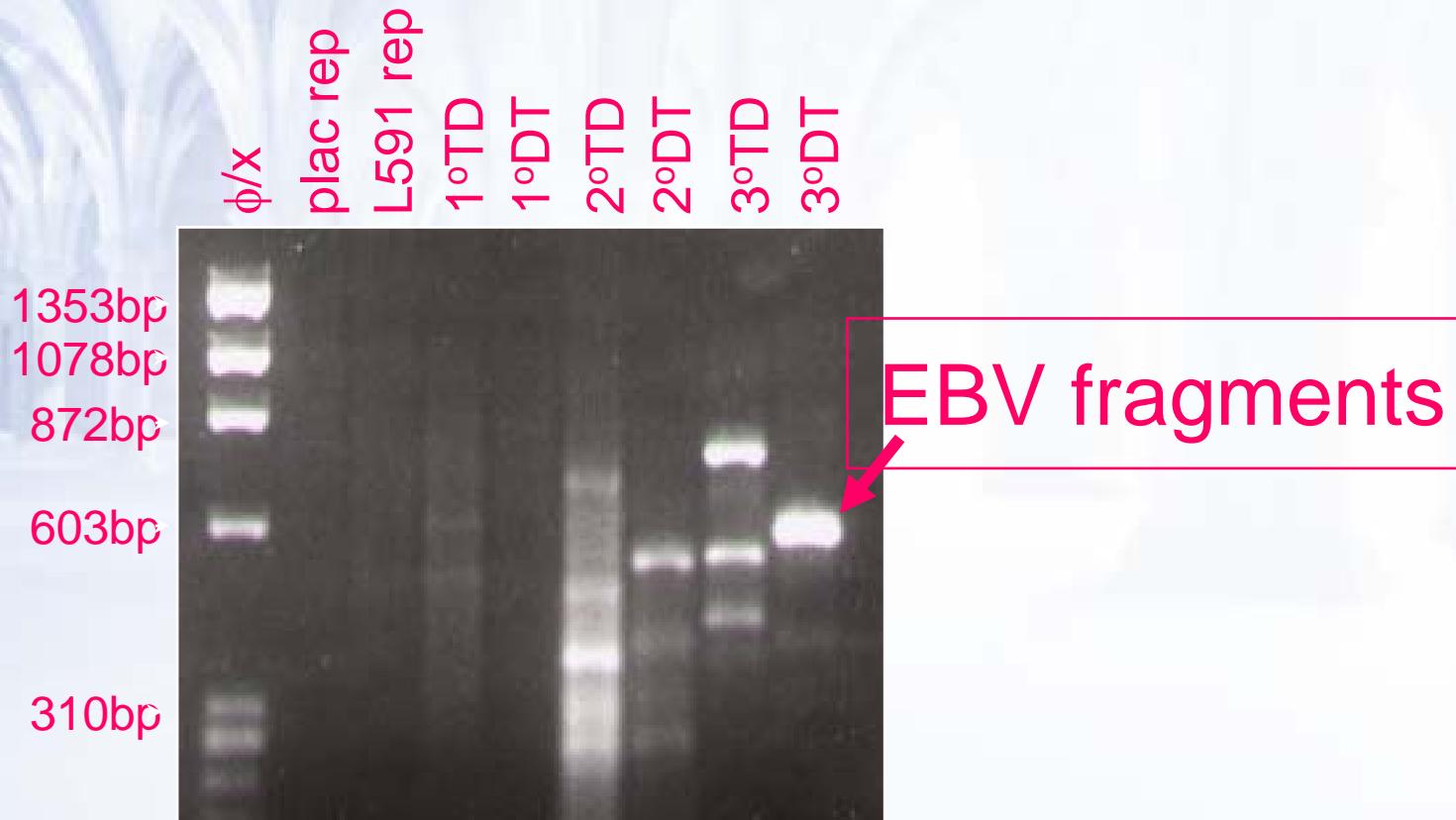
Representational difference analysis (RDA)

- Designed to isolate differences between two DNA samples
- Uses representation of DNA genome to reduce problems associated with complexity

BamHI RDA of EBV-infected HL-derived cell line, L591



BamHI RDA of EBV-infected cell line, L591



>1 million fold amplification

RDA – pros

- No *a priori* knowledge required
- Does detect genomes at single copy level
- Track record – HHV-8!

RDA – cons

- May require >1 representation for virus discovery
 - herpesvirus 1 representation
 - adenovirus 2 representations
 - retrovirus 3-4 representations
- Relatively time-consuming and technically demanding
- High index of suspicion required!

Rolling circle amplification

- Technique for amplifying closed circular DNA molecules
- Adapted for identification of viruses with ds circular DNA genomes
- ?identification of polyomavirus genomes

Acknowledgements

- Alice Gallagher
- Jane MacKenzie
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