HPV L1 Gene DNA in Gardasil and its potential effects

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Conflict of interest
This study was commissioned and sponsored by SANEVAX, Inc. for a future payment not to exceed one U.S. dollar.

Lyme Disease DNA Testing

The Molecular Diagnostics Department of Milford Medical Laboratory is the first, and at present, the only licensed clinical laboratory in the United States to offer a combined DNA Sequencing-based diagnostic test for ***Borrelia burgdorferi,*** the infectious agent for Lyme disease and ***Borrelia miyamotoi*** **[**宮本 健司], the infectious agent of a similar Lyme disease-like illness found recently in the United States.

www.dnalymetest.com

This presentation is based on the laboratory findings reported in the following references

[1] Lee SH. Detection of human papillomavirus (HPV) L1 gene DNA possibly bound to particulate aluminum adjuvant in the HPV vaccine Gardasil®. J Inorg Biochem 2012; 117:85–92.

[2] Lee SH. Detection of human papillomavirus L1 gene DNA fragments in postmortem blood and spleen after Gardasil® vaccination-A case report. Advances in Bioscience and Biotechnology 2012; 3: 1214-1224.

[3] Lee SH. Topological conformational changes of human papillomavirus (HPV) DNA bound to an insoluble aluminum salt – a study by low temperature PCR. Advances in Biological Chemistry 2013; 3: 76-85.

[4] Lee SH. Melting profiles may affect detection of residual HPV L1 gene DNA fragments in Gardasil®. Current Medicinal Chemistry 2013 Sep 30. [Epub ahead of print]

Report of HPV DNA in blood of a 13-year old girl who developed juvenile rheumatoid arthritis after Gardasil vaccination (2011)

Health Genetic Center

2175 Keele St., Toronto, ON M6M 3Z4, Canada
Phone: 1-866-362-0677 Fax: 416-658-2042
Web: www.dna-human.com

Laboratory Report #16941

Report Date: 2011-03-02

Physician:	Dr Fred Hui	Phone	416-443-0811
Clinic:	Chelation Ctr - Don Valley	Fax	416-443-0462
Patient:	[redacted]	Email	

Sample	Individual Tests	Result	Comments
Blood	D144-Human papillomavirus (HPV)	Positive	

GARDASIL®

- **PHARMEUROPA Vol. 21, No. 3, July 2009 p. 418**

The vaccine is produced by the expression of the viral genes coding for the capsid proteins in yeast or in an insect cell/baculovirus vector expression system, purification of the resulting VLPs and the rendering of these particles into an immunogenic preparation.

- **Vaccines and Related Biological Products Advisory Committee (VRBPAC) - Briefing Document 18-May-2006 [Merck submitted to the FDA]**

2.2.1 Chemical and Pharmacological Properties for GARDASIL®
…it contains no viral DNA, and is incapable of causing infection

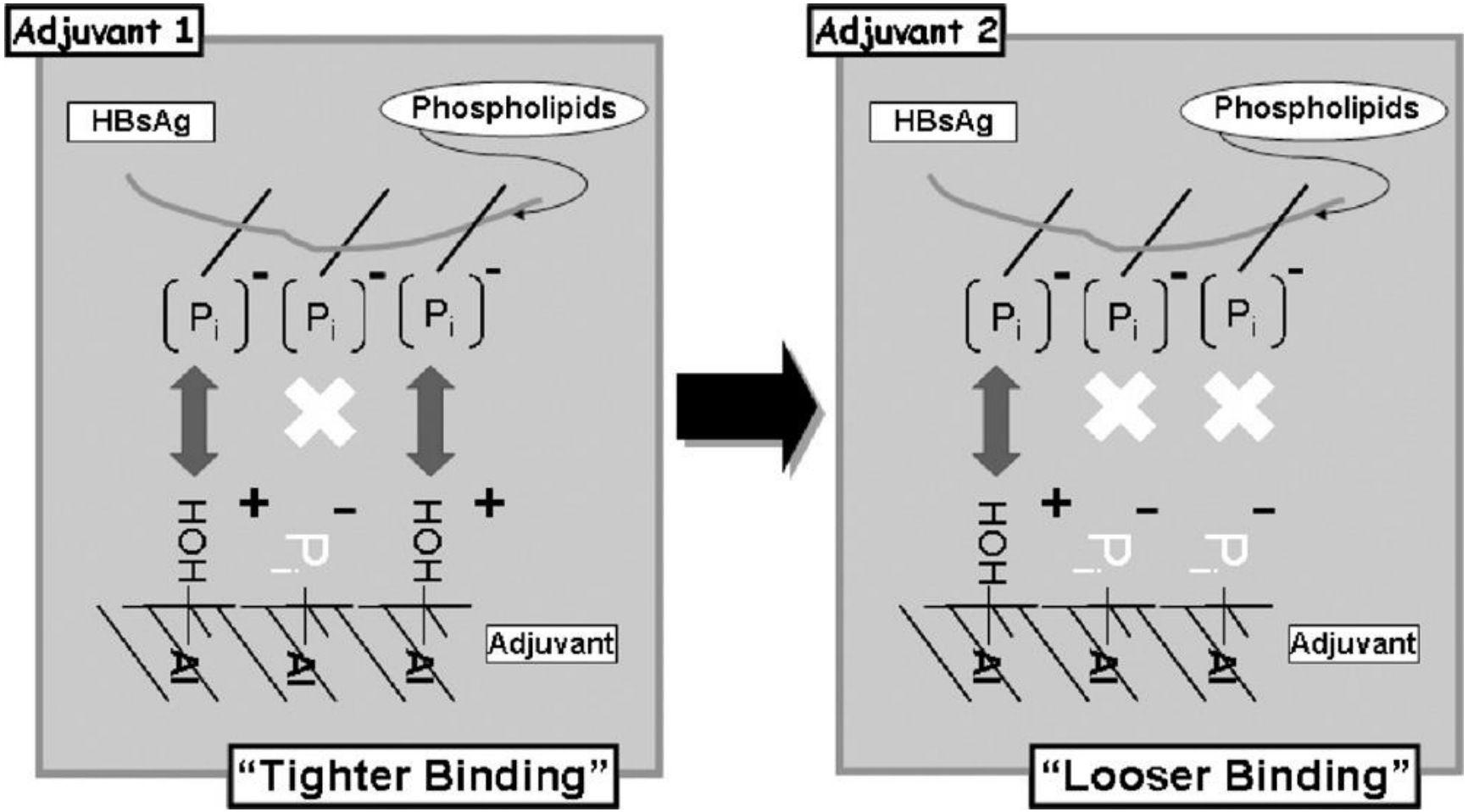
- **Health Canada Summary Basis of Decision (SBD)**

Merck Frosst Canada Ltd. GARDASIL™
Date Issued: 2007/03/16 5

Gardasil™ contains HPV 6, 11, 16, and 18 L1 proteins in addition to the following excipients: amorphous aluminium hydroxyphosphate sulphate adjuvant, sodium chloride, L-histidine, polysorbate 80 (PS-80), sodium borate, and water for injection (WFI). The product contains no preservative or antibiotics. Gardasil™ is not a live virus vaccine. It contains no viral DNA and is not capable of causing infection.

Amorphous aluminum hydroxyphosphate sulfate adjuvant and phosphate ligand exchanges

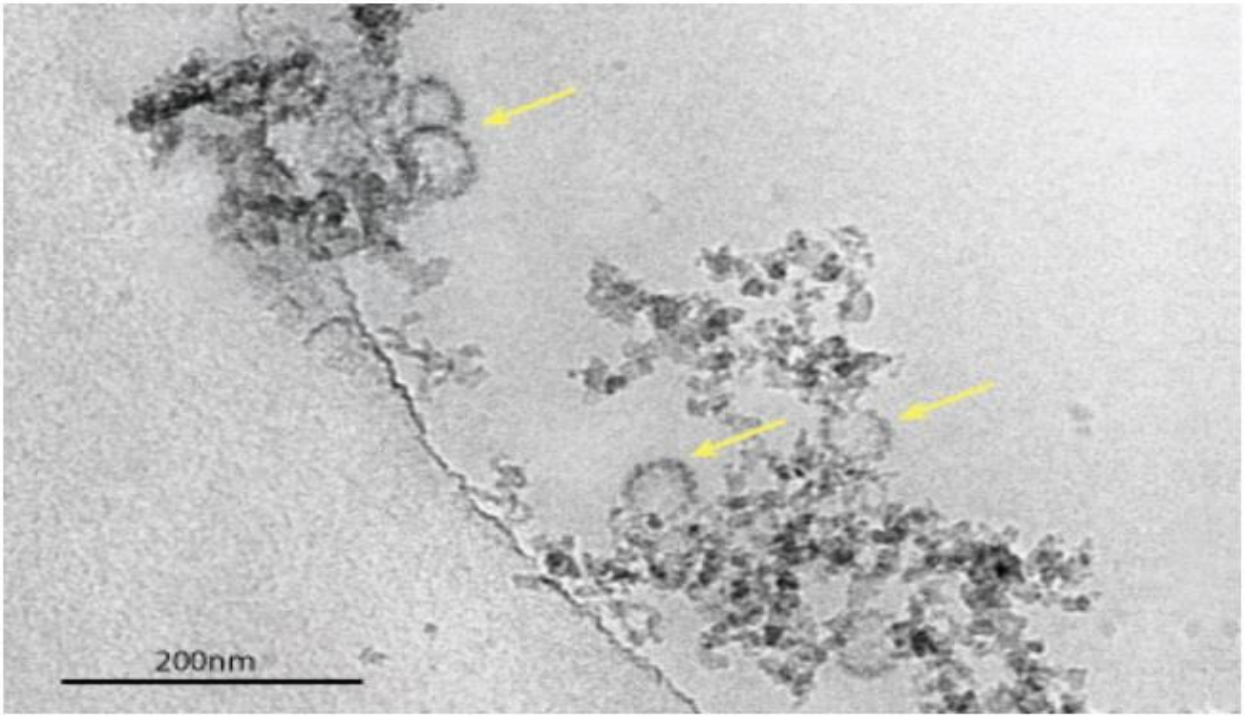
Egan et al on Hep B vaccinesVaccine 27 (2009) 3175–3180



HPV VLPs adsorbed to AAHS nanoparticles

http://www.nanoimagingservices.com/CaseStudies/Characterization-of-Gardasil.aspx.

HPV Particle Morphology Remains Intact When Interacting With Adjuvant:



Direct visualization of VLP-adjuvant complexes in solution can save hundreds of thousands of dollars spent in time and reagents during process development and formulation analysis. NanoImaging Services performs full characterization of VLP-adjuvant complexes in solution using state of the art Molecular Microscopy methods developed specifically to preserve the native structure of your sample and provide a direct measure of VLP-adjuvant complex morphology, aggregation state, and structure.

Gardasil : EPAR European Medicines Agency
www.ema.europa.eu/.../WC500021140.p.

Purification

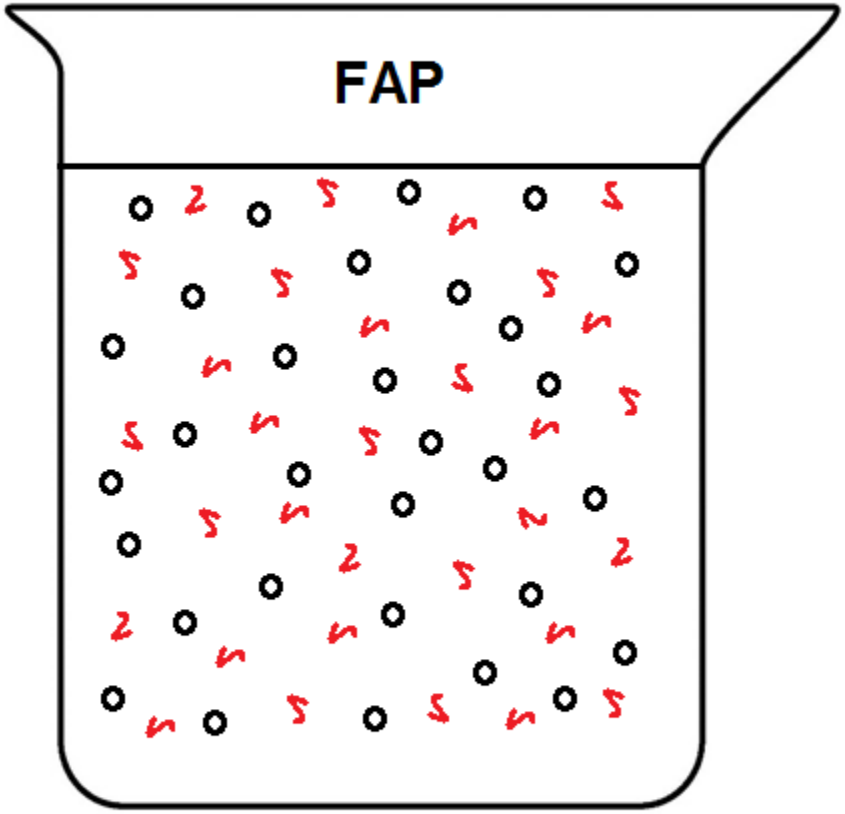
…. The final steps in the **purification process** for all four types are buffer exchange and sterile filtration to produce the final aqueous product **(FAP**). The FAP for each type is then adsorbed onto amorphous aluminium hydroxyphosphate sulfate **(AAHS)** to produce the **MBAP**. The MBAP for each type is then filled into bulk storage containers and stored at 2-8°C.

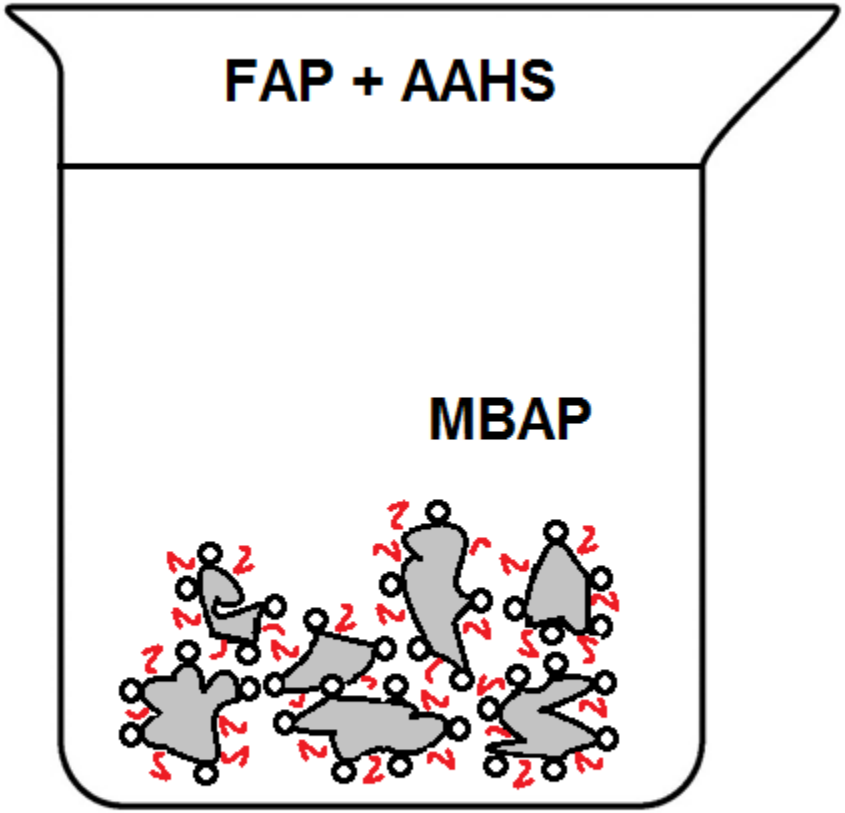
Impurities

Non-L1 protein impurities originated from yeast host cells were analyzed by SDS-PAGE, Western blot, and protease activity. These assays are **typically performed on FAP**. Purity results show successive clearance of protein impurities through the process.

? HPV DNA PCR assays (no mention)-assumed negative!!!

"NO VIRAL DNA" in GARDASIL was based on testing FAP, not MBAP in Merck's manufacturing process



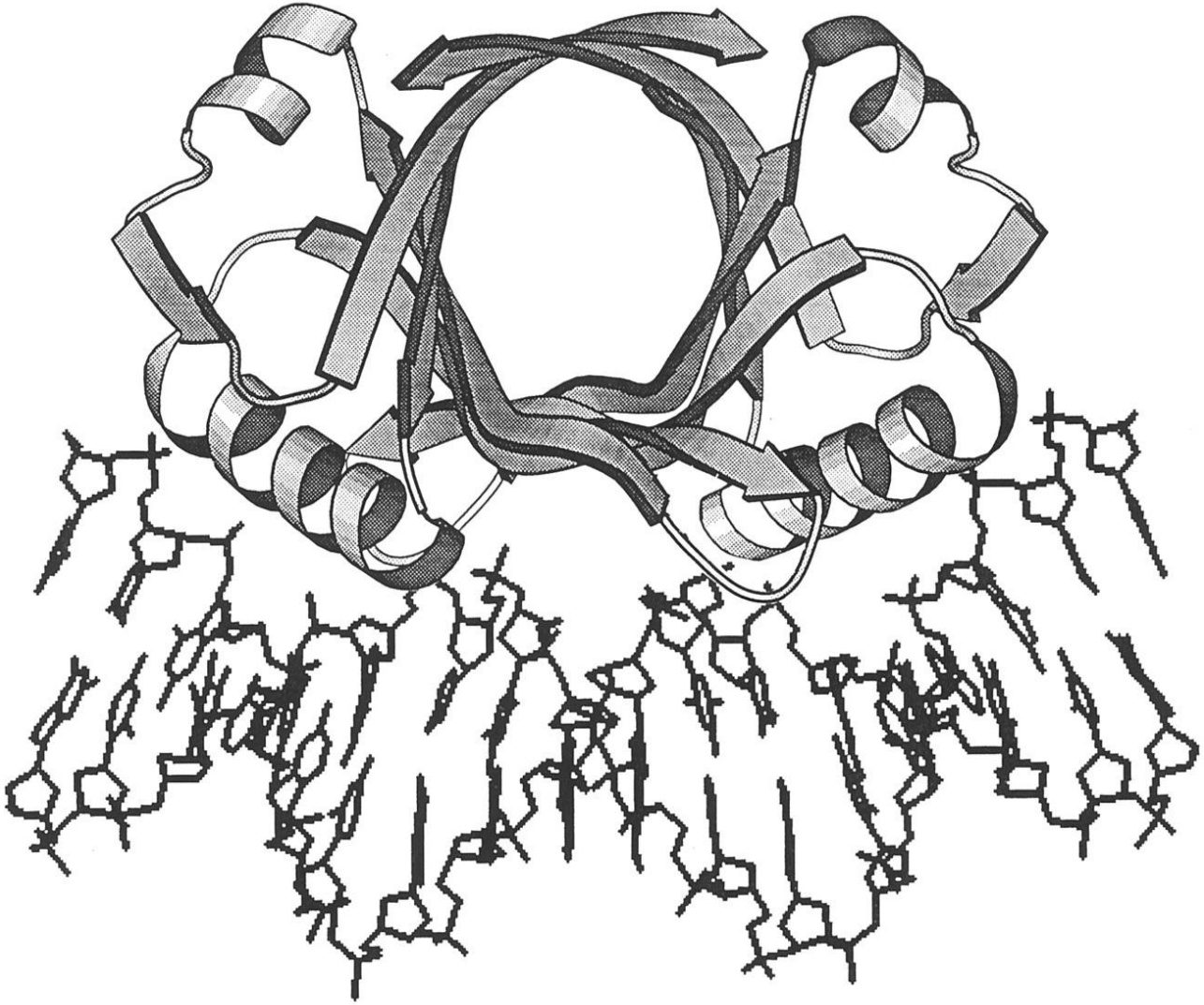


= VLP = HPVL1 gene DNA fragment = AAHS

FAP = final aqueous product

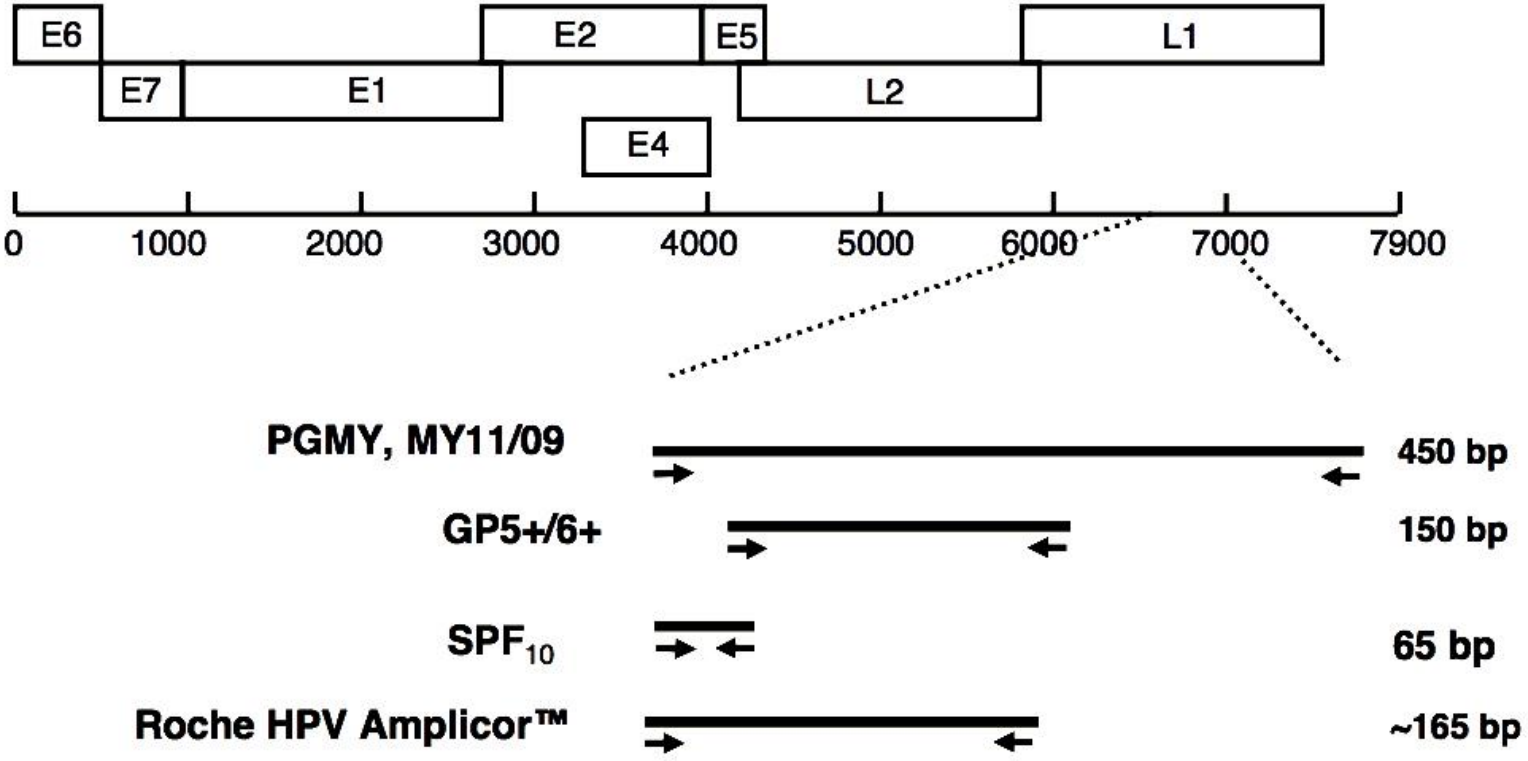
MBAP = Monovalent Bulk Adsorbed Product

Schematic representation of the E2 DNA binding domain from BPV-1 bound to DNA HPV topological non-B Conformation in nature



Lima L M T R , and de Prat-Gay G J. Conformational Changes and Stabilization Induced by Ligand Binding in the DNA-binding Domain of the E2 Protein from Bovine Papillomavirus*Biol. Chem. 1997;272:19295-19303

Common HPV L1 genotyping PCR primers (b=base A,C,G, or T; p=pair)



Degenerate (MY11) and consensus (GP6) PCR primer amplification of HPV L1 DNA

HPV Type	ID Locus	Nucleotide Sequence (3′ – 5′) GP6 site		MY11 site	Total bases	Location (5′ – 3′)
6	AF092932	GAAAAATAAATTGTAAATCA	-----	CCATTGTTATGTCCCTGGGC	181	6723 - 6903
11	EU918768	GAAAAATAAACTGTAAATCA	-----	CCATTGTTATGTCCCTGAGC	181	6707 - 6887
16	FJ006723	GAAAAATAAACTGTAAATCA	-----	CCATTATTGTGGCCCTGTGC	184	6641 - 6824
18	GQ180792	GAAAAATAAACTGCAAATCA	-----	CCATTGTTATGACCCTGTGC	187	6558 - 6744
26	NC_001583	GAAATATAAATTGTAATTCA	-----	CCATTATTATGACCCTGTGC	187	6533 - 6719
31	PPH31A	GAAATATAAATTGTAAATCA	-----	CCATTATTGTGTCCCTGAGC	184	6500 - 6683
32	X74475	GAAATATAAACTGTATATCA	-----	CCATTATTGTGGCCTTGTGC	181	6817 - 6997
33	EU 918766	GAAAAACAAACTGTAGATCA	-----	CCATTATTATGACCTTGTGC	181	6542 - 6722
35	PPH35CG	GAAAAATAAACTGTAAATCA	-----	CCATTATTATGGCCTTGTGC	184	6522 - 6705
39	PPHT39	GAAATATAAATTGTAAATCA	-----	CCATTGTTGTGGCCCTGGGC	187	6585 - 6771
40	X74478	GAAAAATAAACTGCAAATCA	-----	CCATTGTTATGGCCCTGGGC	187	6718 - 6904
45	EF202167	GAAAAATAAACTGTAAATCA	-----	CCATTGTTATGGCCCTGGGC	187	6562 - 6748
51	PPHDNA	GAAAAATAAATTGCAATTCA	-----	CCATTATTGTGACCCTGCGC	184	6466 - 6649
52	GQ472848	GAAAAATAAATTGTAAATCG	-----	CCATTATTGTGGCCCTGCGC	181	6631 - 6811
53	NC 001593	GAAACACAAATTGTAATTCA	-----	CCATTATTATGTCCCTGGGC	181	6594 - 6774
54	AF436129	GAAATATAAATTGTAAATCA	-----	CCATTGTTCTGACCCTGGGC	181	6509 - 6689
55	HPU31791	GAAACATAAACTGTAAGTCA	-----	CCATTATTGTGGCCCTGCGC	187	6625 - 6813
56	X74483	GAAAAACAAATTGTAATTCA	-----	CCATTATTATGGCCTTGGGC	181	6539 - 6719
58	FJ407217	GAAAAACAAACTGTAAGTCA	-----	CCATTGTTATGACCTTGTGC	181	6588 - 6768
59	EU918767	GAAATATAAACTGCAAATCA	-----	CCATTGTTTAAACCCTGAGC	187	6551 - 6737
61	HPU31793	GAAAAATAAATTGCAAATCA	-----	CCATTGTTGTGGCCCTGGGC	184	6712 - 6895
62	AY395706	GAAATATAAATTGCAAATCA	-----	CCATTATTATGGCCCTGCGC	181	6725 - 6905
66	EF177191	GAAACACAAACTGTAGTTCA	-----	CCATTATTATGGCCCTGTGC	181	6590 - 6770
67	D21208	GAAATATAAACTGCAAATCA	-----	CCATTGTTATGACCTTGTGC	181	6564 - 6744
68	EU918769	GAAATATAAATTGCAAATCA	-----	CCATTGTTGTGTCCCTGTGC	187	6453 - 6639
69	AB027020	GAAATATAAACTGTAATTCA	-----	CCATTATTATGACCCTGGGC	187	6489 - 6675
70	HPU21941	GAAATATAAATTGTAAATCA	-----	CCATTATTGTGTCCCTGGGC	187	6529 - 6715
71	AY330623	GAAATATAAATTGCAAATCA	-----	CCATTGTTTGTGCCCTGTGC	181	6791 - 6971
72	X94164	GAAATATAAACTGCAAATCA	-----	CCATTGTTGTGACCCTGGGC	184	6738 - 6921
73	X94165	GAAAAACAAACTGTAAATCA	-----	CCATTATTTTGTCCCTGTGC	190	6433 - 6622
74	AF436130	GAAAAATAAATTGCAAATCA	-----	CCATTATTGTGGCCTTGCGC	190	6524 - 6713
81	AJ620209	GGAAAATAAACTGCAAATCA	-----	CCATTATTATGGCCCTGTGC	181	6809 - 6989
83	AF151983	GCAATATAACCTGTAAGTCA	-----	CCATTATTATGTCCCTGGGC	180	6693 - 6872
84	AF293960	GGAATATAAACTGCAAATCA	-----	CCATTGTTATGACCCTGGGC	181	6619 - 6799
86	AF349909	GAAAAATAAATTGCAAATCA	-----	CTATTATTGTGTCCCTGCGC	181	6615 - 6795
87	AJ400628	GAAAAATAAACTGTAAATCA	-----	CCATTATTGTGGCCCTGGGC	181	6712 - 6892
89	AF436128	GGAATATAAACTGTAGGTCA	-----	CCATTATTATGGCCCTGTGC	180	6706 - 6885
91	AF419318	GAAATATAAACTGTAAATCA	-----	CCGTTATTATGCCCCTGTGC	187	6810 - 6996

HPV PCR Strategy for Gardasil-related HPV L1 gene DNA fragments

HPV Consensus Primers

L1 gene ORF amplification

Primary PCR

MY09 5'-CGTCCMARRGGAWACTGATC-3'

MY11 5'-GCMCAGGGWCATAAYAATGG-3'

Nested PCR

GP5 5'-TTTGTTACTGTGGTAGATAC-3'

GP6 5'-GAAAAATAAACTGTAAATCA-3'

3' ……//……..(MY09)……GP6……..……..//….5'

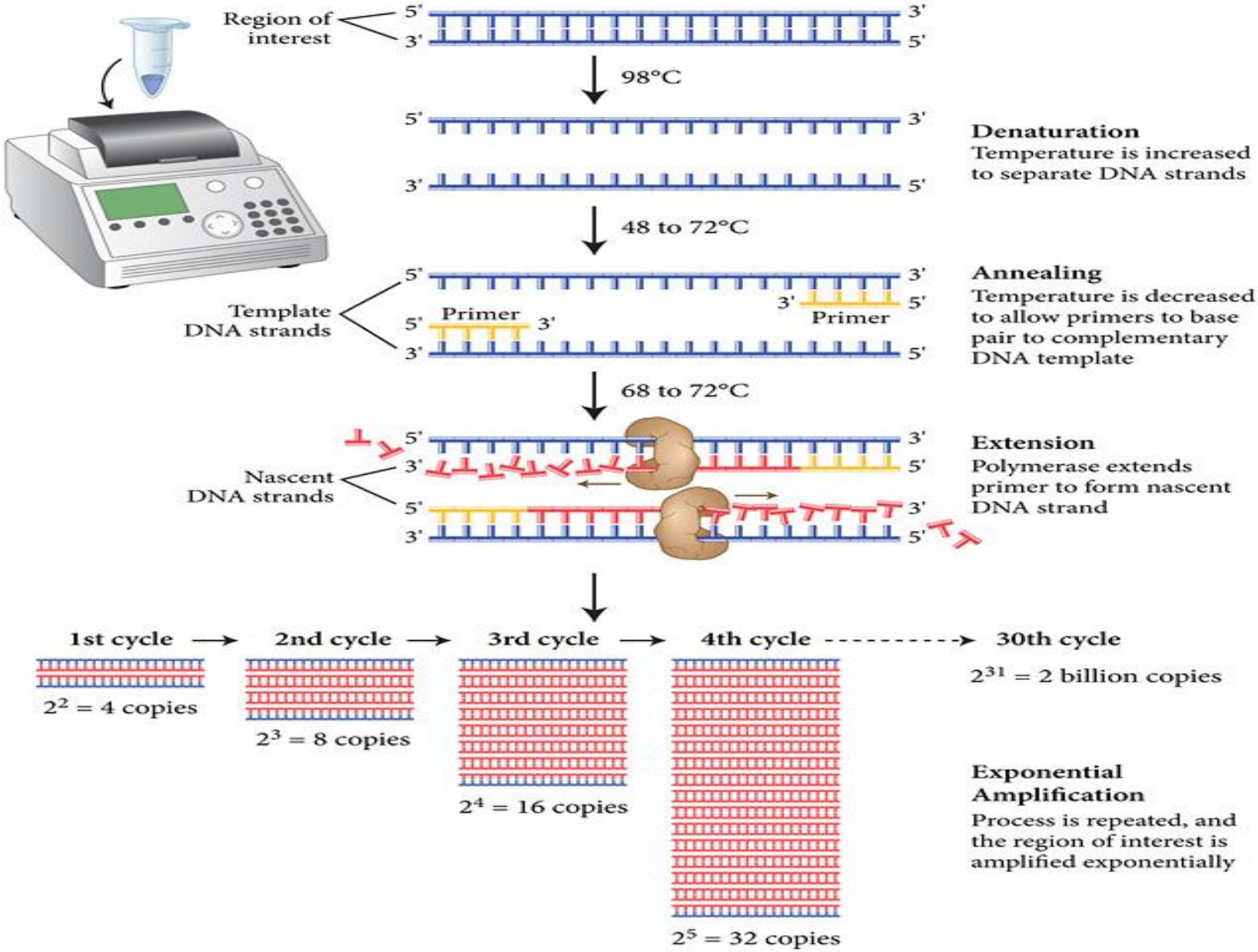
5' ……//……GP5 ……MY11…//... 3'

450bp _____

150 bp GP5/GP6 nested PCR amplicon or

~181-190 bp GP6/MY11 nested PCR amplicon

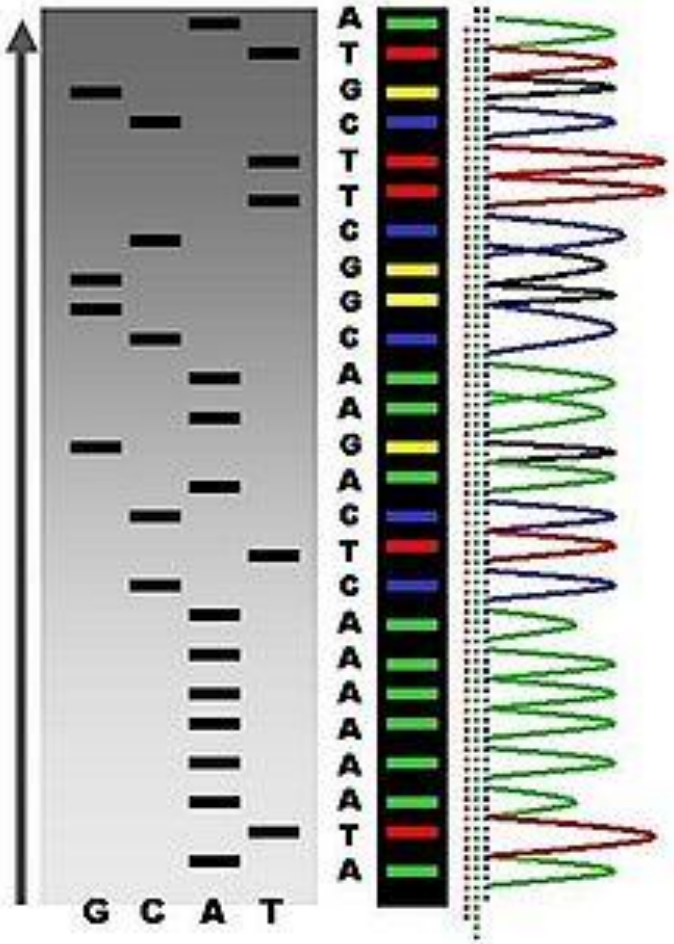
For Direct DNA Sequencing except MY09 binding site


Region of interest
5'
3'
3'
5'
98°C
5'
3'
3'
5'
Denaturation
Temperature is increased to separate DNA strands
48 to 72°C
5'
3'
Template DNA strands
5'
Primer
3'
3'
Primer
5'
3'
5'
Annealing
Temperature is decreased to allow primers to base pair to complementary DNA template
68 to 72°C
5'
3'
Nascent DNA strands
3'
5'
5'
3'
3'
5'
Extension
Polymerase extends primer to form nascent DNA strand
1st cycle
2nd cycle
3rd cycle
4th cycle
30th cycle
$2^2 = 4$ copies
$2^3 = 8$ copies
$2^4 = 16$ copies
$2^5 = 32$ copies
$2^{31} = 2$ billion copies
Exponential Amplification
Process is repeated, and the region of interest is amplified exponentially

DNA sequencing termination reaction

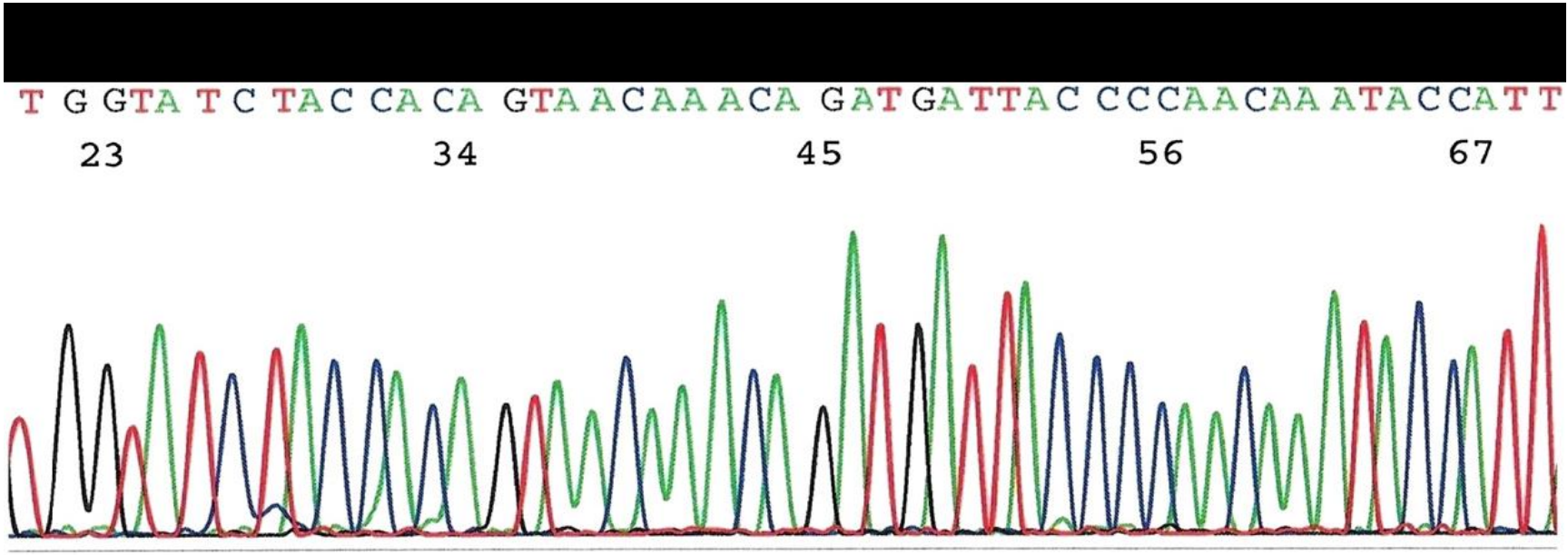
Gel:

G	GCGAATGCGTCCACAACGCTACAGGTG
T	GCGAATGCGTCCACAACGCTACAGGT
G	GCGAATGCGTCCACAACGCTACAGG
G	GCGAATGCGTCCACAACGCTACAG
A	GCGAATGCGTCCACAACGCTACA
C	GCGAATGCGTCCACAACGCTAC
A	GCGAATGCGTCCACAACGCTA
T	GCGAATGCGTCCACAACGCT
C	GCGAATGCGTCCACAACGC
G	GCGAATGCGTCCACAACG
C	GCGAATGCGTCCACAAC
A	GCGAATGCGTCCACAA
A	GCGAATGCGTCCACA
C	GCGAATGCGTCCAC
A	GCGAATGCGTCCA
C	GCGAATGCGTCC
C	GCGAATGCGTC
T	GCGAATGCGT
G	GCGAATGCG
C	GCGAATGC
G	GCGAATG
T	GCGAAT



Synthetic construct of HPV-11 DNA detected in Gardasil® AAHS adjuvant.

Lee SH. J Inorg Biochem117 (2012) 85–92

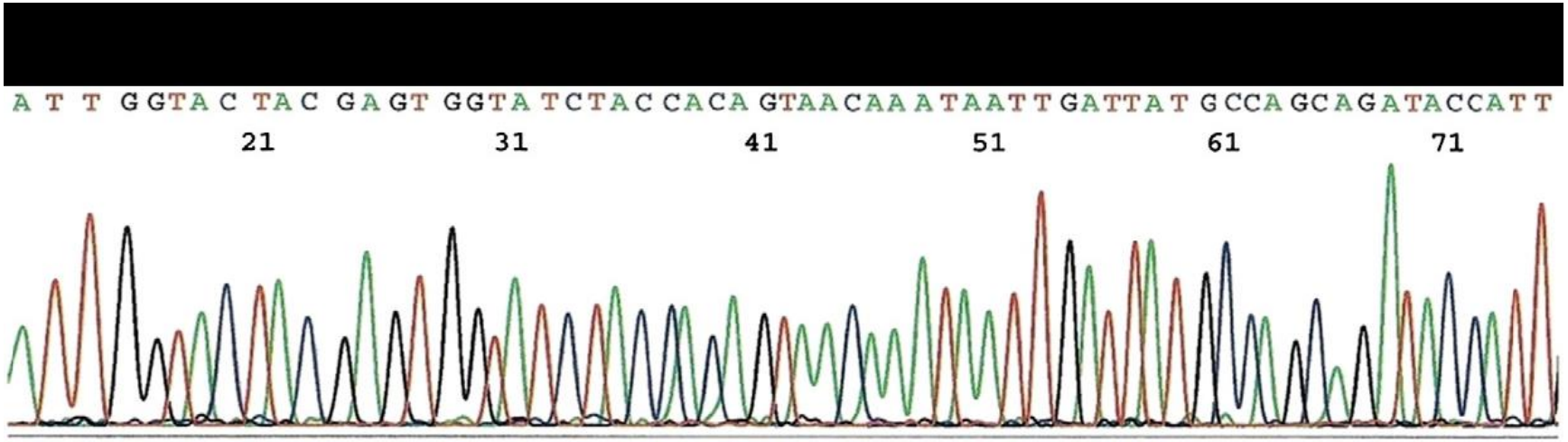



```
>  gb|U55993.1|SCU55993  Synthetic HPV11 major capsid protein L1
gene, complete cds
Length=1506

 Score = 97.6 bits (49),  Expect = 5e-18
 Identities = 49/49 (100%), Gaps = 0/49 (0%)
 Strand=Plus/Minus
```

HPV-18 DNA fragment detected in Gardasil® AAHS adjuvant

Lee SH. J Inorg Biochem117 (2012) 85–92



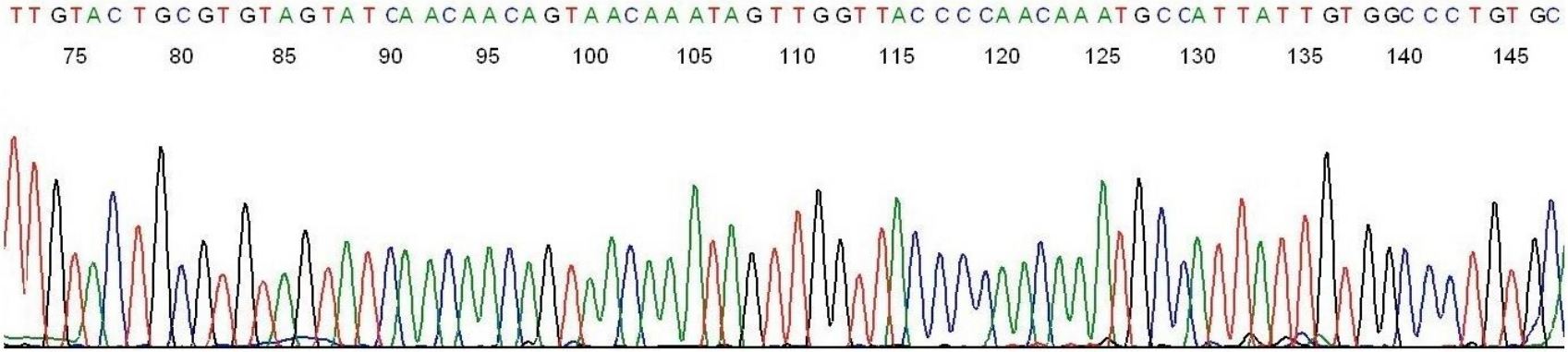

```
>□ gb|EF202155.1|  Human papillomavirus type 18 isolate Qv04924, complete
genome Length=7824

 Score =  125 bits (63),  Expect = 3e-26
 Identities = 63/63 (100%), Gaps = 0/63 (0%)
 Strand=Plus/Minus

Query  1     ATTGGTACTACGAGTGGTATCTACCACAGTAACAAATAATTGATTATGCCAGCAGATACC  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  6609  ATTGGTACTACGAGTGGTATCTACCACAGTAACAAATAATTGATTATGCCAGCAGATACC  6550
```

Table 1 – Gardasil® lot numbers, countries of origin and HPV L1 gene DNA found J Inorg Biochem 2012; 117:85–92.

Lot #	Country/Source	Genotype
1437Z vial	**USA**, Connecticut	HPV-11 HPV-18
1511Z prefilled syringe	**USA**, New York	HPV-18
0553AA vial	**USA**, New Jersey	HPV-11 HPV-18
NL35360 prefilled syringe	**France**	HPV-11 HPV-18
NP23400 prefilled syringe	**Spain**, Valencia	HPV-11 HPV-18
NN33070 prefilled syringe	**Spain**, Valencia	HPV-11 HPV-18
NM25110 prefilled syringe	**Australia**, Sydney	HPV-11 HPV-18
NL01490 prefilled syringe	**New Zealand**, Tauranga	HPV-18
NK16180 prefilled syringe	**New Zealand**, Northland	HPV-18
NK00140 prefilled syringe	**New Zealand**, Tauranga	HPV-11 HPV-18
NM08120 prefilled syringe	**New Zealand**, Christchurch	HPV-11 HPV-18
NL13560 prefilled syringe	**New Zealand**, Wellington	HPV-11 HPV-18
NL39620 prefilled syringe	**Poland**	HPV-11
NN28160 vial	**Russia**	HPV-11 HPV-18
NL49190 prefilled syringe	**Bulgaria**	HPV-11 HPV-18
NM29390 prefilled syringe	**India**	HPV-18



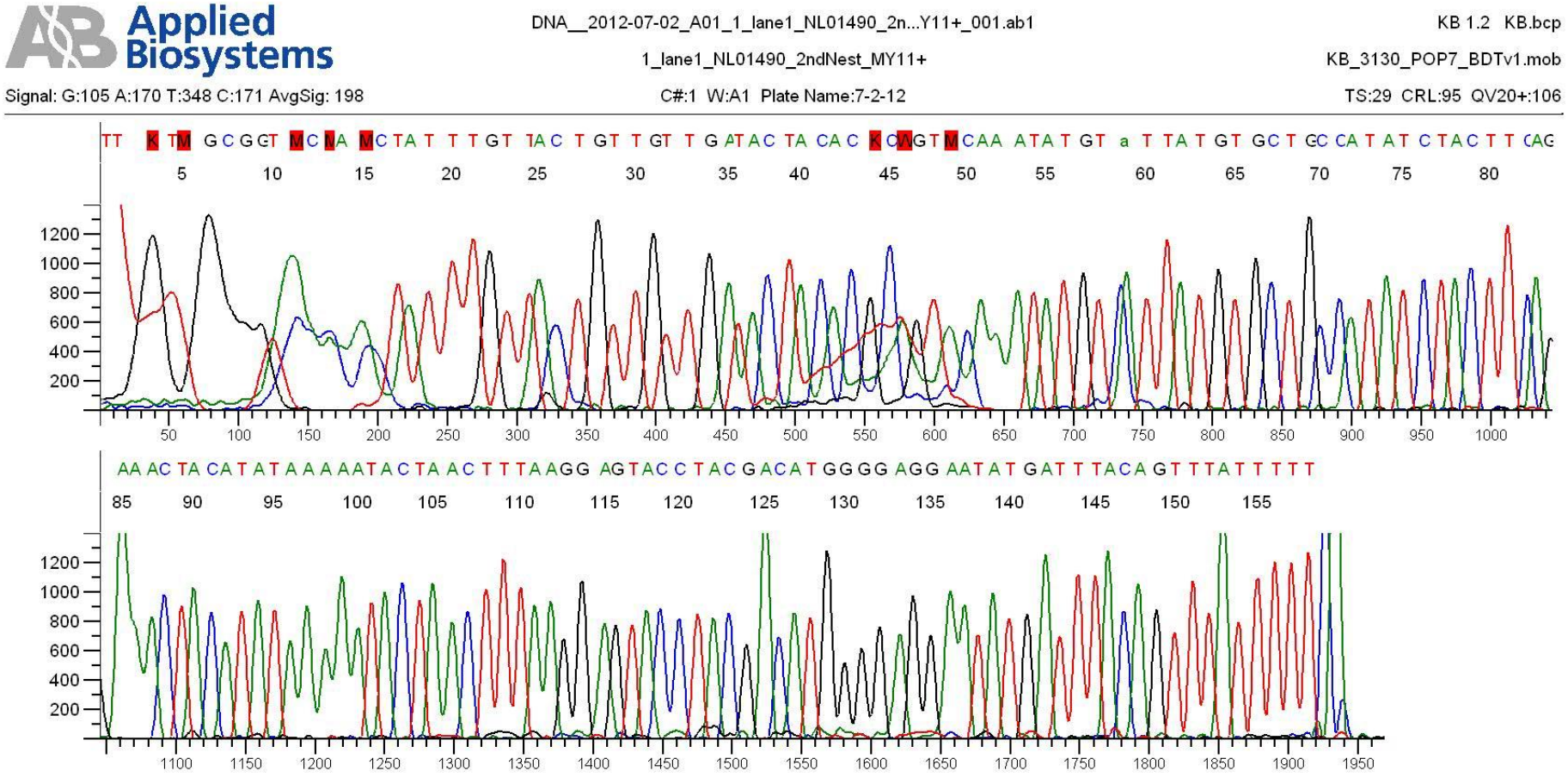

```
>gb|HQ644299.1|  Human papillomavirus type 16 isolate Z122, complete genome
Length=7905

 Score =  138 bits (152),  Expect = 4e-30
 Identities = 76/76 (100%), Gaps = 0/76 (0%)
 Strand=Plus/Minus

Query  1     TTGTACTGCGTGTAGTATCAACAACAGTAACAAATAGTTGGTTACCCCAACAAATGCCAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  6658  TTGTACTGCGTGTAGTATCAACAACAGTAACAAATAGTTGGTTACCCCAACAAATGCCAT  6599

Query  61    TATTGTGGCCCTGTGC  76
             ||||||||||||||||
Sbjct  6598  TATTGTGGCCCTGTGC  6583
```

HPV-16 L1 gene DNA in non-B conformation found in postmortem blood and spleen of an 18 years old female who died in sleep 6 months after Gardasil vaccination in New Zealand



Specific DNA sequence of HPV-16 L1 gene DNA in non-B conformation mixed with HPV-18 L1 DNA in Gardasil lot #NL01490 was amplified with a modified MY11+ primer

Collection Date/Time:Jul 02,2012 13:51:31 EDT
Inst Model/Name:3130/LEE3130-18320-036

Basecall Date/Time:2012-07-02 14:13:21 -05:00
Sequence Scanner v1.0

Printed on: Jul 02,2012 14:31:57 EDT
Electropherogram Data Page 1 of 1

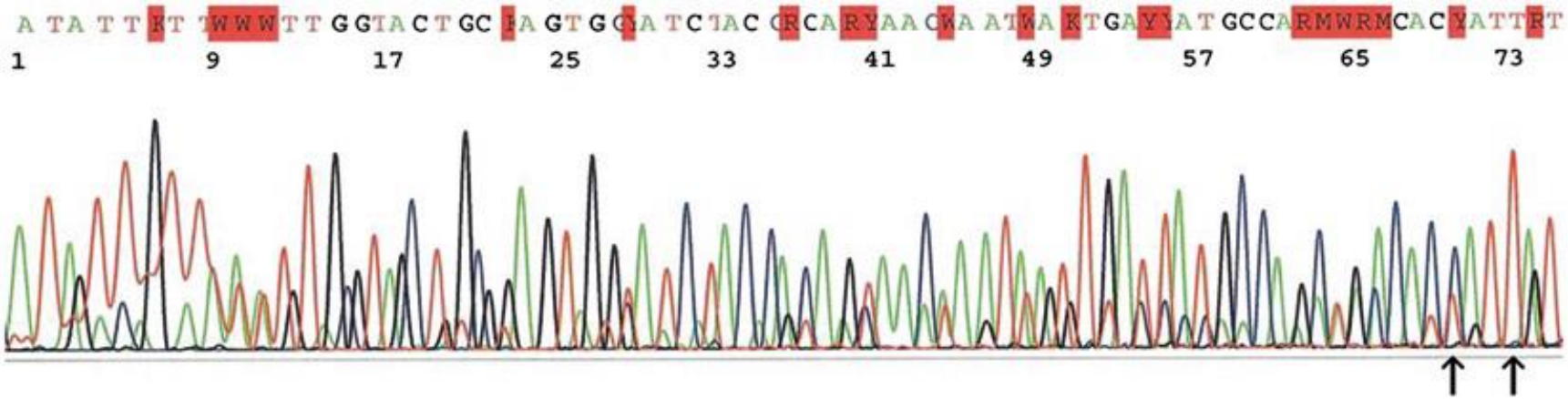
Five Gardasil vaccine samples from New Zealand physicians -all tested positive for HPV L1 gene DNA

Lot #	Country/Source	Results
NL01490	**New Zealand**, Tauranga	HPV-18 HPV-16
NK16180	**New Zealand**, Northland	HPV-18 HPV-16
NK00140	**New Zealand**, Tauranga	HPV-11 HPV-18 HPV-16
NM08120	**New Zealand**, Christchurch	HPV-11 HPV-18 HPV-16
NL13560	**New Zealand**, Wellington	HPV-11 HPV-18 HPV-16

DNA sequencing of mixed HPV-16 and HPV-18 L1 genes in B conformation

The size of the GP6/MY11 PCR amplicon of HPV-18 is 3-bp longer than that of HPV16. Therefore, one end of the primer sequence 3'CCATT---5' of the MY11 primer site is positioned ahead of the other, as shown in this mixed HPV DNA sequence tracing. The first T of each end segment is indicated by an arrow.

Lee SH. Methods Mol Biol 2012; 903:65-101.



非ウイルス型遺伝子キャリアとしてのリン酸カルシウムおよび水酸化アルミニウム†
—DNA-無機塩複合体の形態と DNA トランスフェクション効率—

松澤有希子[1††]・恵美宣彦[2]・神戸俊夫[3]

化学工学論文集

Calcium Phosphate and Aluminum Hydroxide as Non-Virus Gene Carrier: The Morphology of DNA-salt Complex and the Effects It on DNA Transfection *KAGAKU KOGAKU RONBUNSHU* 2003; 29:.680-684.

We also investigated the complex between DNA and aluminum hydroxide, which already used as an adjuvant. The extent of DNA uptake was also affected by the size of the DNA-aluminum hydroxide complex. The dramatic changes in DNA conformation are discussed in relation to the efficiency of gene transfection.

Transfection: process of deliberately introducing nucleic acids into eukaryotic cells by non-viral methods

Double Edged Sword of DNA/Aluminum Complexes

- Host DNA/aluminum adjuvant complex, phagocytized by APCs, activates pathways that increase their ability to interact productively with antigen-specific CD4 T cells [1,2], ^ host immune responses.
- **What does viral DNA/aluminum complex do?**

(a) Free HPV-16 L1 gene DNA injected into mice induces a strong CD8 T cell response. [3]

(b) Viral DNA activates the macrophages through the interferon regulatory transcription factor IRF3 (TLR) to release cytokines, including TNF.

(c) TNF is a myocardial depressant and promotes inflammation.

Macrophages know: host DNA harmless; viral DNA harmful, thus react differently.

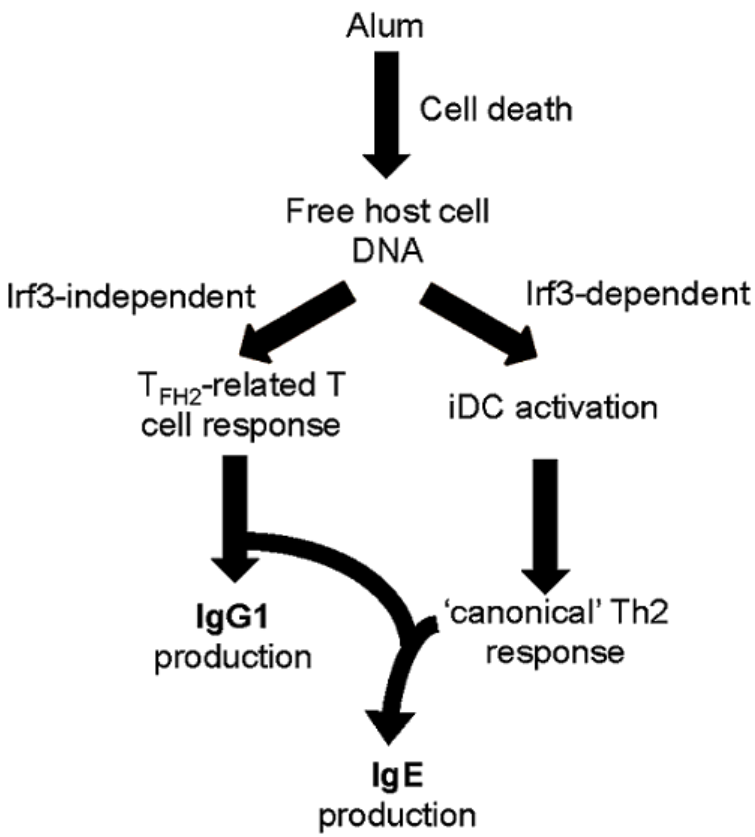
References

[1] Marichal T et al. Nat. Med. 2011; 17: 996-1002.

[2] McKee AS et al. Proc. Natl. Acad. Sci. U.S.A. 2013; 110: E1122-E1131.

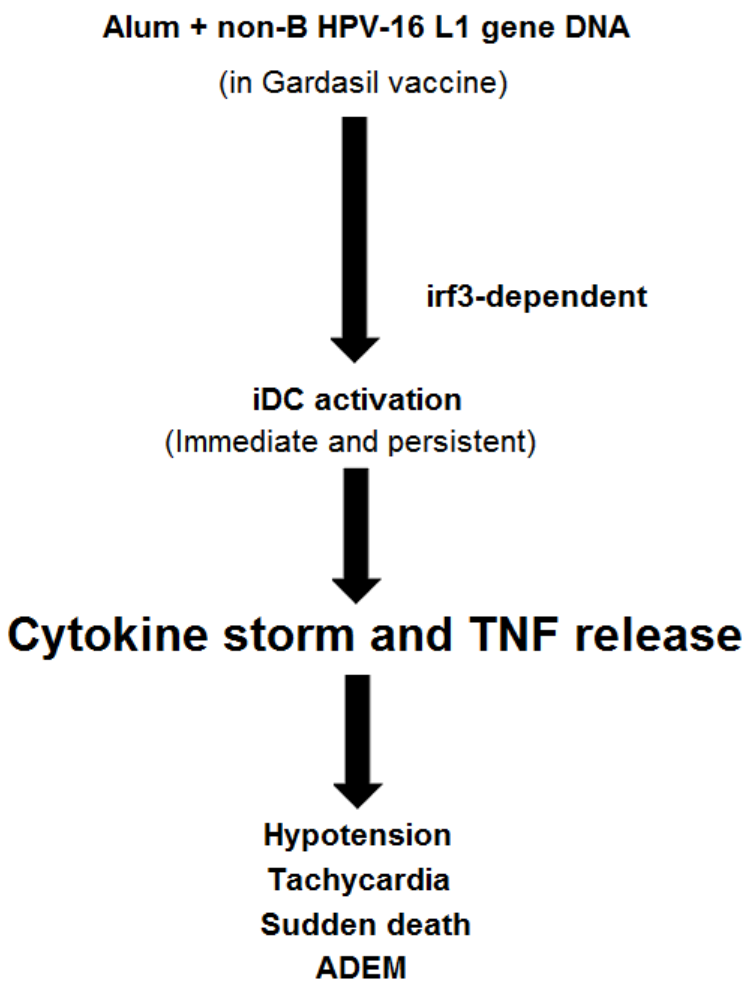
[3] Caulfield MJ et al. Human Vaccines 2007; 3: 139-145.

"Half-Truth"



Proposed model for the adjuvant effect of host cell DNA upon alum immunization.
Marichal T et al. Nat. Med. 2011; 17: 996-1002.

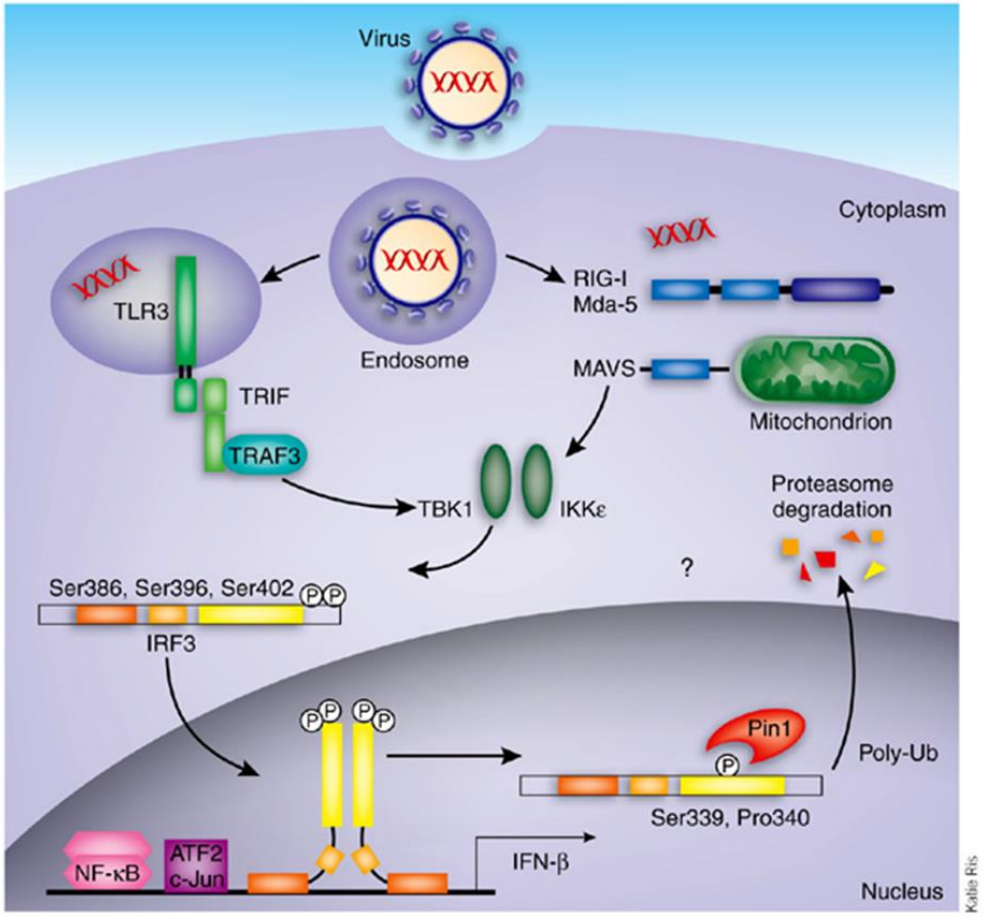
"In Real Life"

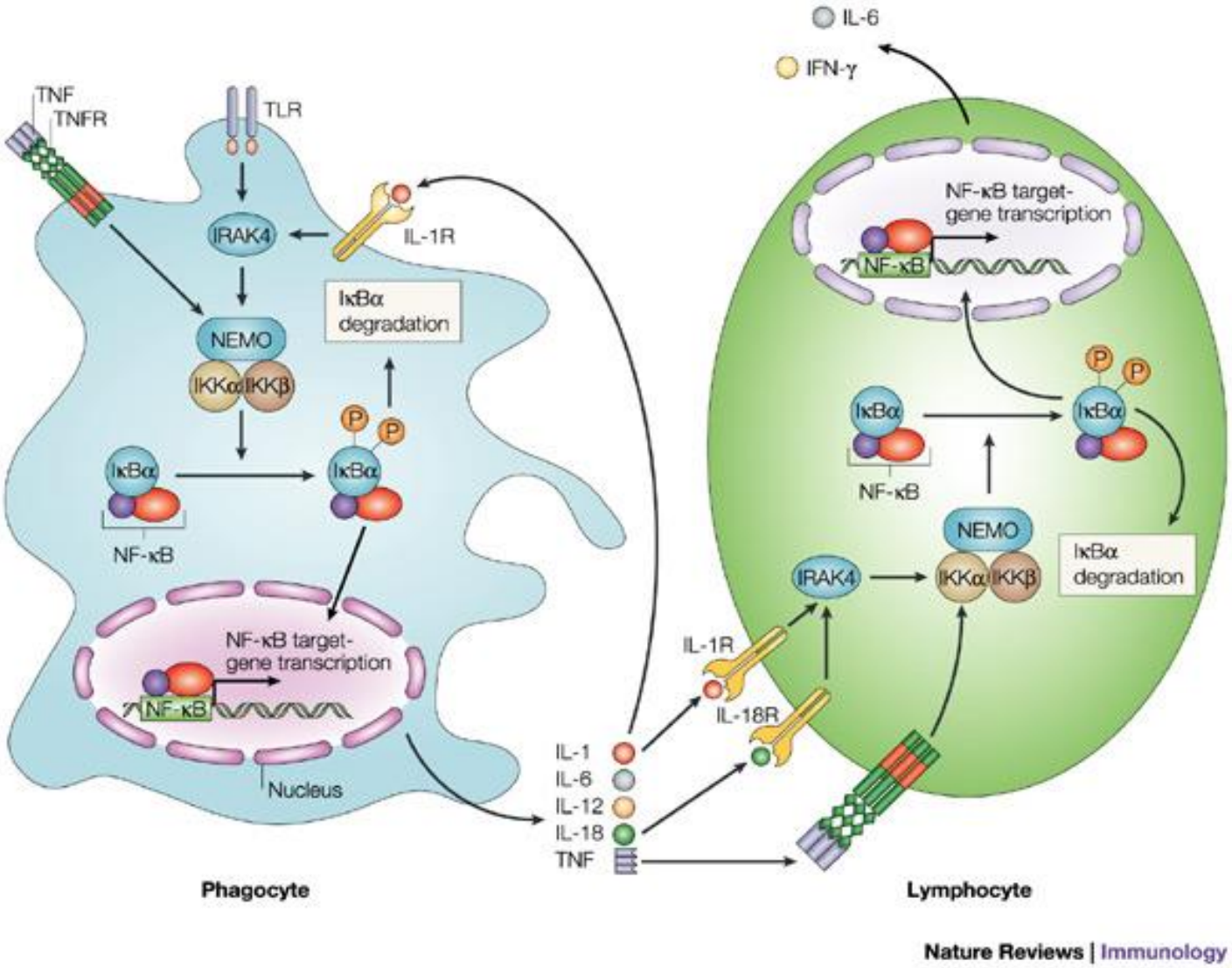


Proposed model for side effects Alum/viral DNA upon Gardasil vaccination.

Viral DNA, Toll-like Receptor (IRF3-dependent) activation

Goutagny N, Severa M, Fitzgerald KA. Nat Immunol. 2006 Jun;7(6):555-7.





J. Casanova, L. Abel. The human model: a genetic dissection of immunity to infection in natural conditions. Nature Reviews Immunology 2004; 4, 55-66.

HPV Vaccination may prevent 70%HPV infections with a 95% self-clearance rate

HPV vaccines	**INITIATION**	**PROMOTION**	**PROGRESSION**	
Age 9-12	HPV test	Pap smears	Biopsy/Cone	Hysterectomy at Age>50
Vaccines	**HPV infection ⇔**	**HPV persistent* ⇔**	**CIN ⇒**	**Invasive cancer**
Reactions	168 days median	(5%+/-)	▲	**>10 years**

E6, E7 transcription

integration, multiparity, OC use, smoking,

inflammation*, viral variant, micronutrients

*A tumor promoter- macrophage-mediated immune response causing:

cell death inhibition, genomic instability, fibroblast

activation, changed matrix metabolism, angiogenesis

Final Notes to the HPV vaccination policy makers

- Cervical Cancer is a serious disease of the grandmothers, but it is:

[1] Rare in Japan and in the USA;
[2] Predictable by periodic Pap smears and reliable HPV testing;
[3] Preventable by ablative procedures of the precancerous lesions;
[4] Treatable if invasive lesion is found by hysterectomy (> age 50).

- Adverse reactions after HPV vaccination affect teenagers and are:

[1] Rare, but (5-20) X other school-based vaccination programs;
[2] Unpredictable;
[3] Not preventable:
[4] Difficult to treat.

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