



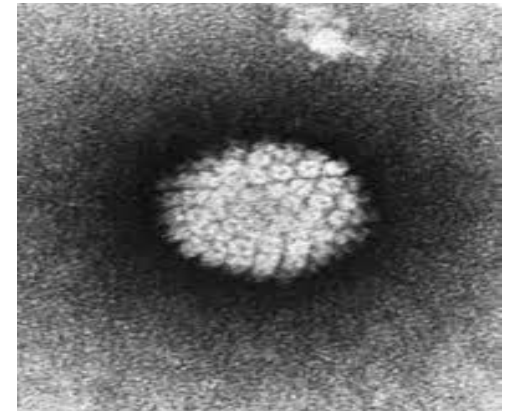
# HPV λοίμωξη: φυσική ιστορία, μοριακή διάγνωση και έρευνα

**Χριστίνα Κοτταρίδη**

**Επίκουρη Καθηγήτρια, τμήμα Βιολογίας ΑΠΘ**

# Ο ιός των ανθρωπίνων θηλωμάτων HPV (Human papillomavirus)

- ✓ Ένας κοινός σεξουαλικά μεταδιδόμενος ιός που μπορεί να μολύνει το δέρμα και τον βλεννογόνο του τραχήλου της μήτρας
- ✓ Περισσότερο από 150 τύποι έχουν πλήρως ταυτοποιηθεί
- ✓ Περίπου 15 HPV τύποι θεωρούνται υψηλού κίνδυνου και μπορεί να προκαλέσουν Καρκίνο Τραχήλου Μήτρας



# Ποια η διαφορετικότητα του HPV?

- ✓ Ο HPV χαρακτηρίζεται από την ικανότητά του να διαφεύγει από το φυσικό μηχανισμό ανοσιακής απάντησης
  - Δεν προκαλεί ιαιμία (πχ Ηπατίτιδα)
  - Δεν καταστρέφει άμεσα τα κύτταρα του ξενιστή
  - Καθυστερεί τη φλεγμονώδη απάντηση μέσω κυτοκινών

Margaret Stanley. Immune responses to human papillomavirus. Vaccine 24S1 (2006) S1/16-S1/22.

Stanley, Lowy, Frazer. Chapter 12: Prophylactic HPV vaccines: Underlying mechanisms. Vaccine 24S3 (2006) S3/106-S3/113.

Munoz, Castellsagué, Berrington de González, Gissmann. Chapter 1: HPV in the etiology of human cancer. Vaccine 24S3 (2006) S3/1-S3/10.

# Papillomaviridae

Ετερογενής ομάδα ιών (θηλαστικά, πτηνά και ερπετά)  
περισσότεροι από 150 τύποι έχουν ανιχνευτεί στον άνθρωπο  
(Human Papillomavirus-HPV, Bernard et al., 2010)

**Richard Edwin Shope 1933**, PV απομονώθηκαν για πρώτη  
φορά από κουνέλια-Shope Papillomavirus ή  
CRPV (cottontail rabbit papillomavirus)

## INFECTIOUS PAPILLOMATOSIS OF RABBITS

By RICHARD E. SHOPE, M.D.

WITH A NOTE ON THE HISTOPATHOLOGY

By E. WESTON HURST, M.D.

(From the Department of Animal and Plant Pathology of The Rockefeller Institute for  
Medical Research, Princeton, N. J.)

PLATES 33 to 35

(Received for publication, July 19, 1933)

Our attention was recently called to a disease occurring in wild cottontail rabbits in northwestern Iowa.<sup>1</sup> Rabbits shot there by hunters were said to have numerous horn-like protuberances on the skin over various parts of their bodies. The animals were referred to popularly as "horned" or "warty" rabbits.

Warts from a naturally occurring case of the disease in Iowa were obtained and sent to the laboratory in sterile 50 per cent glycerol. These glycerolated warts furnished us our original material for investigation. A little later, in a shipment of a dozen wild cottontail rabbits from southern Kansas, three were found to be affected with the same wart-like disease. To date, out of 75 wild cottontail rabbits received from Kansas eleven have been found to carry one or more warts. These eleven animals serve as the basis for our description of the naturally occurring disease.

### Description of the Naturally Occurring Disease

In wild cottontail rabbits the presence of warts has caused no apparent discomfort in our experience and induced no demonstrable evidence of generalized illness. Most of the animals were sacrificed, shortly after their arrival, for pathological material, but four, kept under observation for 7 weeks or longer, at no time appeared ill and were in good physical condition when finally killed. The number

<sup>1</sup> We are grateful to Mr. T. A. McKichan of Cherokee, Iowa, who first told us of the disease, and to Mr. Clifford Peck of Cherokee and Mr. Earl Johnson of Rago, Kansas, who furnished us with naturally occurring cases of the disease.

[CANCER RESEARCH 36, 794, February 1976]

## Condylomata Acuminata and Human Genital Cancer

Harald zur Hausen

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The subsequent review by Goldberg and Gravel (8) stresses the possible role of HSV-2<sup>1</sup> infections in human cervical cancer. Although seroepidemiological studies do suggest an association of HSV-2 with this type of cancer (12, 14), repeated attempts, with 1 exception (6), have failed to demonstrate viral DNA by nucleic acid hybridizations in cervical cancer cells (Refs. 13 and 21; H. Schulte-Holthausen and H. zur Hausen, unpublished data), even when assays were performed under very sensitive conditions. Although these negative results are not yet conclusive, additional arguments have been raised that do not support the HSV-2 etiology of cervical cancer (19).

It is the intention of this note to draw attention to a virus-induced and virus particle-containing tumor revealing a similar epidemiological pattern, as does cervical cancer: the genital warts or condylomata acuminata. Condylomata acuminata have been shown to contain a papilloma virus (5, 13), which appears to differ from human wart virus when tested biochemically (20) or immunologically (1). These particles can be detected by electron microscopy in about 50% of all condylomas examined (13). Attempts to grow this virus in tissue culture or to isolate it directly from condylomata have not been very successful in the past (unpublished data).

Genital warts seem to be transmitted primarily by sexual contact and are predominant in populations of high sexual promiscuity. In contrast to common warts, condylomata may become malignant (although rarely), and there exist a number of clinical observations on malignant transition of this tumor (3, 4, 7, 9-11, 15, 17). In particular, penile carcinomas have been observed to develop within or at a site of condylomata acuminata (2, 16, 18).

The condyloma agent has been entirely neglected thus far in all epidemiological and serological studies relating not only to cervical and penile, but also to vulvar and perianal, carcinomas. This is particularly unusual in view of the localization of genital warts, their mode of venereal transmission, the number of reports on malignant transition, and the presence of an agent belonging to a well-characterized group of oncogenic DNA viruses.

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**Harald zur Hausen, 1976**  
HPV παίζουν σημαντικό ρόλο  
στην ανάπτυξη καρκίνου του  
τραχήλου της μήτρας

**Harald zur Hausen**  
**1983 και 1984**  
απομόνωσε τα πρώτα στελέχη  
HPV16 και HPV18

Gissmann et al., 1984,  
Zur Hausen, 1996

Minireview

## Classification of papillomaviruses

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### Abstract

One hundred eighteen papillomavirus (PV) types have been completely described, and a yet higher number of presumed new types have been detected by preliminary data such as subgenomic amplicons. The classification of this diverse group of viruses, which include important human pathogens, has been debated for three decades. This article describes the higher-order PV taxonomy following the general criteria established by the International Committee on the Taxonomy of Viruses (ICTV), reviews the literature of the lower order taxa, lists all known “PV types”, and interprets their phylogenetic relationship. PVs are a taxonomic family of their own, *Papillomaviridae*, unrelated to the polyomaviruses. Higher-order phylogenetic assemblages of PV types, such as the “genital human PVs”, are considered a genus, the latter group, for example, the genus “Alpha-Papillomavirus”. Lower-order assemblages of PV types within each genus are treated as species because they are phylogenetically closely related, but while they have distinct genomic sequences, they have identical or very similar biological or pathological properties. The taxonomic status of PV types, subtypes, and variants remains unchanged and is based on the traditional criteria that the sequence of their L1 genes should be at least 10%, 2–10%, and maximally 2% dissimilar from one another.

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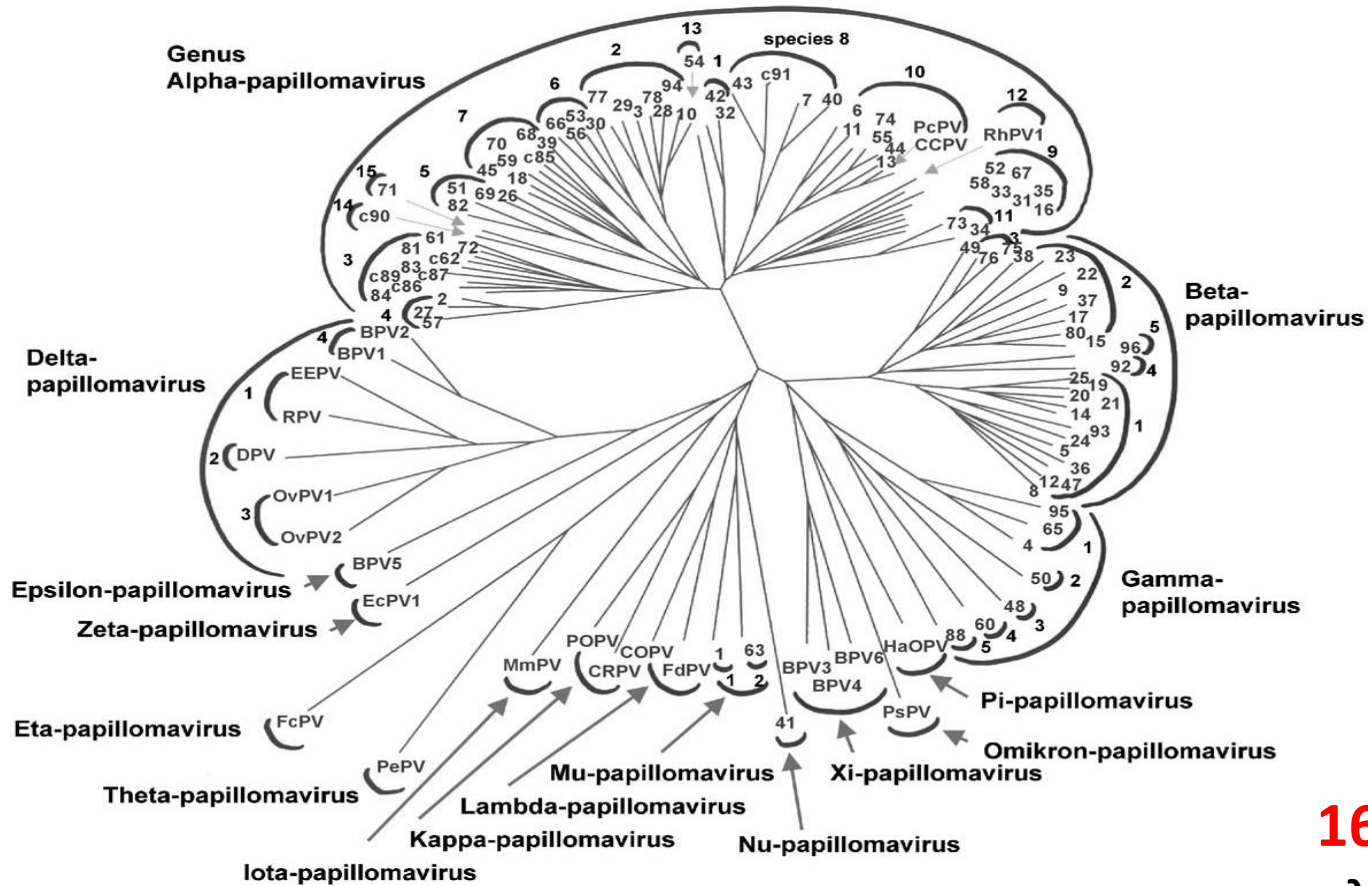
**Keywords:** Papillomaviruses; HPV type; Pathogen

Διαμόρφωση των φυλογενετικών δέντρων των PVs με βάση:

- τη νουκλεοτιδική αλληλουχία,
- τα βιολογικά χαρακτηριστικά (είδη ξενιστών, ιστός, παθογένεια, οργάνωση γενώματος)

**Θέσπισαν την ταξινόμηση των PVs σε γένη**





# Φυλογενετικό δένδρο με αλληλουχίες του L1 γονιδίου

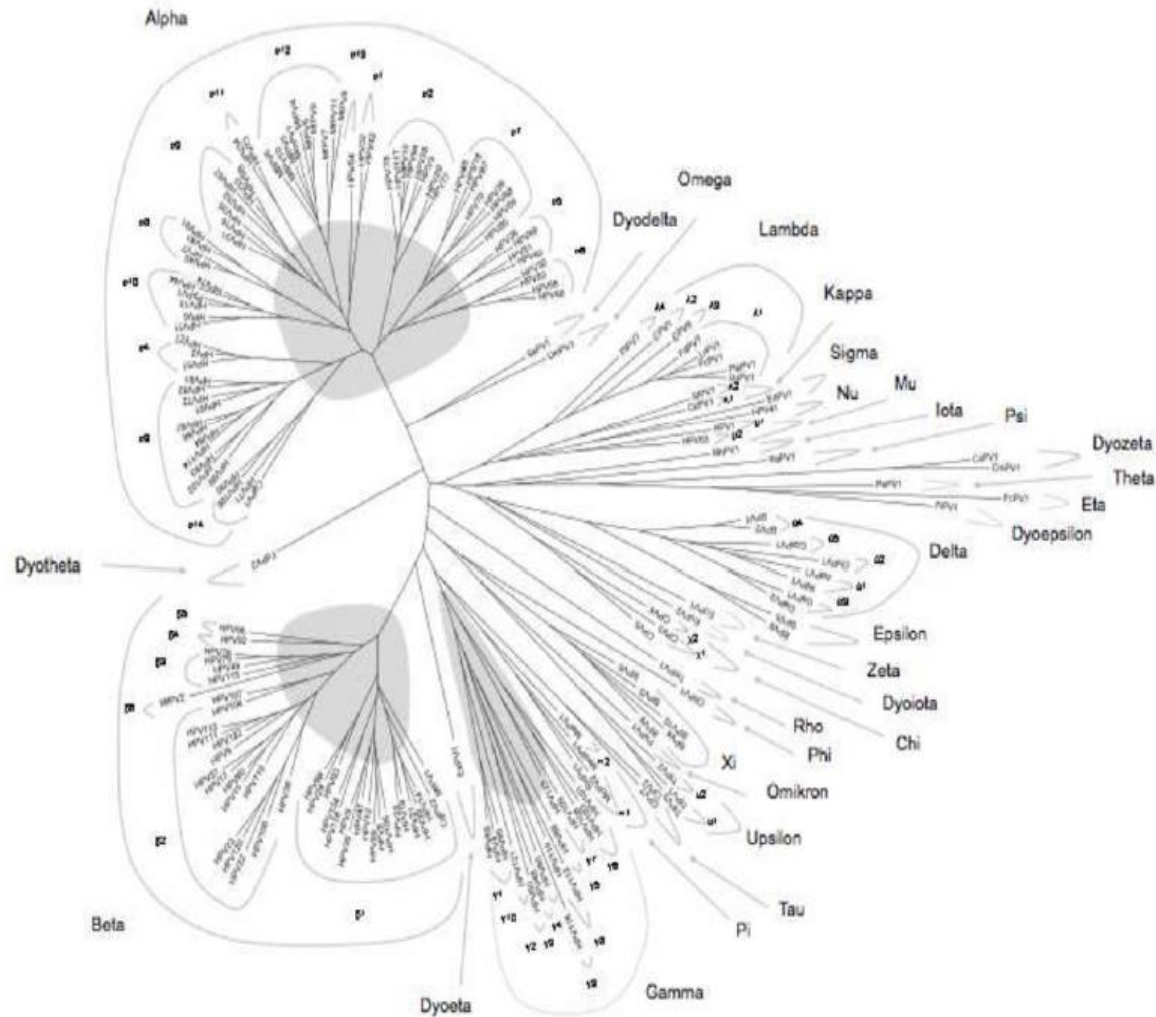
de Villiers et al., Virology 2004

Νέος τύπος HPV: αλληλουχία L1  
 ORF > 10% κοντινότερο γνωστό τύπο-  
 Υπότυπος: 2-10%-Variant: < 2%

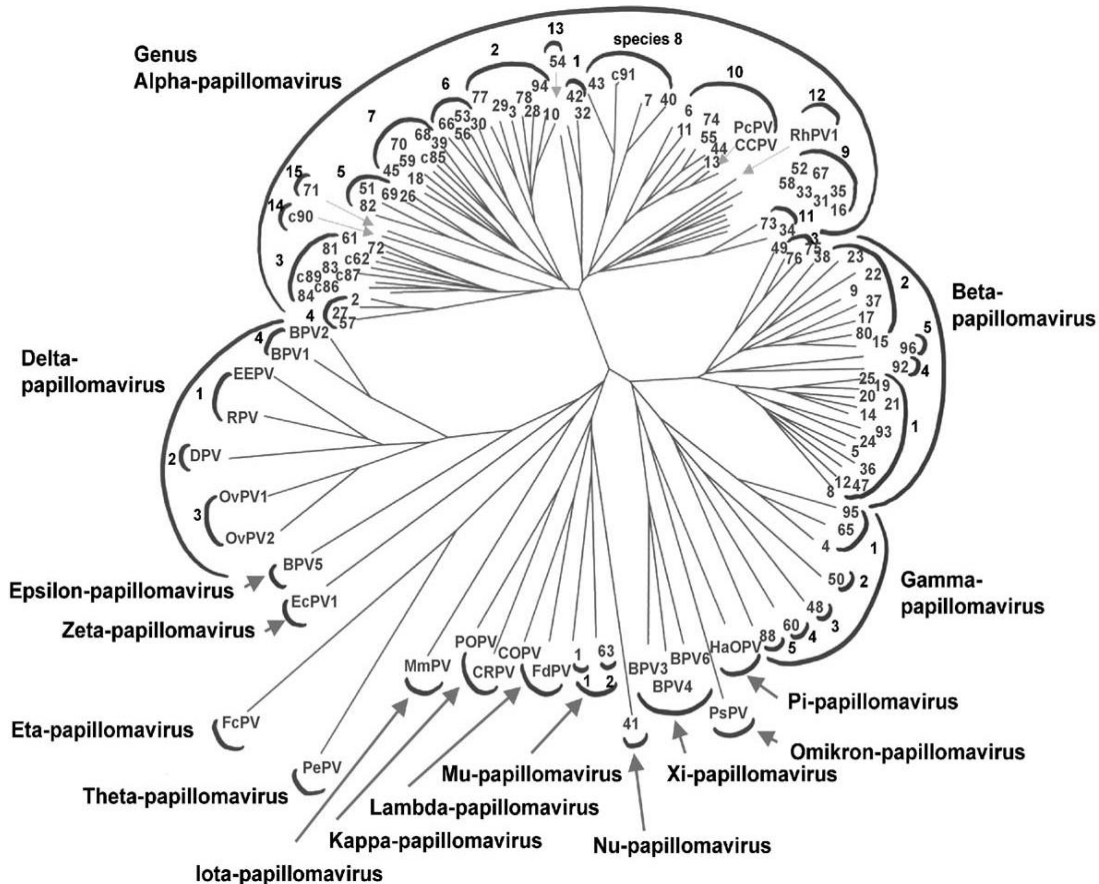
**16 γένη** PVs-  
 ελληνική αλφάβητο από το γράμμα Άλφα μέχρι το γράμμα Πι με βάση τα κριτήρια που θεσπίστηκαν

Σήμερα 29 γένη PV

πολλαπλή ομοπαράθεση L1  
αλληλουχιών 189 PV τύπων



# Human Papillomavirus (HPV)



[IARC Monogr Eval Carcinog Risks Hum. 2007;90:1-636.](#)

## Human papillomaviruses.

[IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.](#)

IARC MONOGRAPHS - 100B

Table 2.1 HPV types in the high-risk clade

| Alpha HPV species | Types classified as Group 1 carcinogens in Volume 90 | Other Types in Species |
|-------------------|--|------------------------|
| 5                 | 51   | 26, 69, 82             |
| 6                 | 56, 66   | 30, 53                 |
| 7                 | 18, 45, 39, 59                                       | 68, 70, 85, 97         |
| 9                 | 16, 31, 33, 35, 52, 58                               | 67                     |
| 11                |  | 34, 73                 |

✓ γένη στον άνθρωπο  
Άλφα-, Βήτα-, Γάμμα-, Μι-  
και Νι-ΡVs

Τρίτη, 9 Μαρτίου 2021

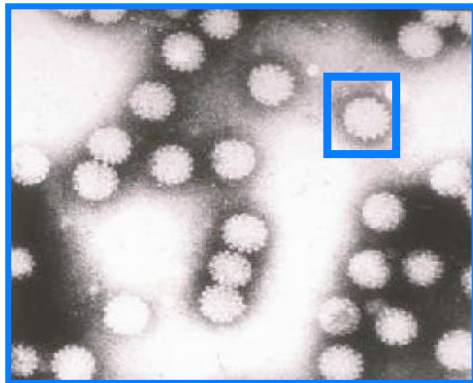
39/45/59/51/56  
= 18 related  
("A7 species")

31/33/35/52/58  
= 16 related  
(A9 species)

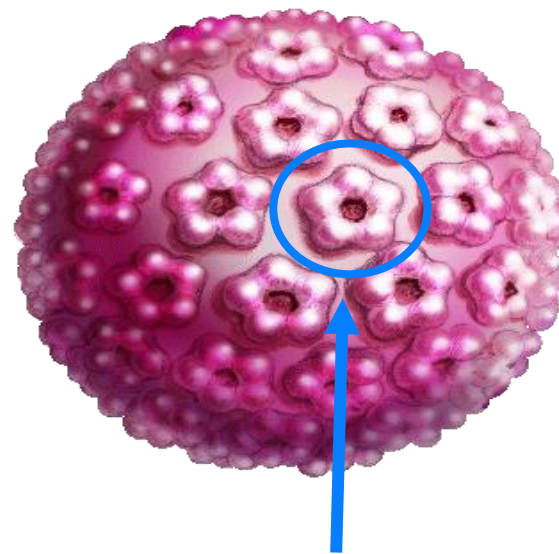


# Η δομή του ιού

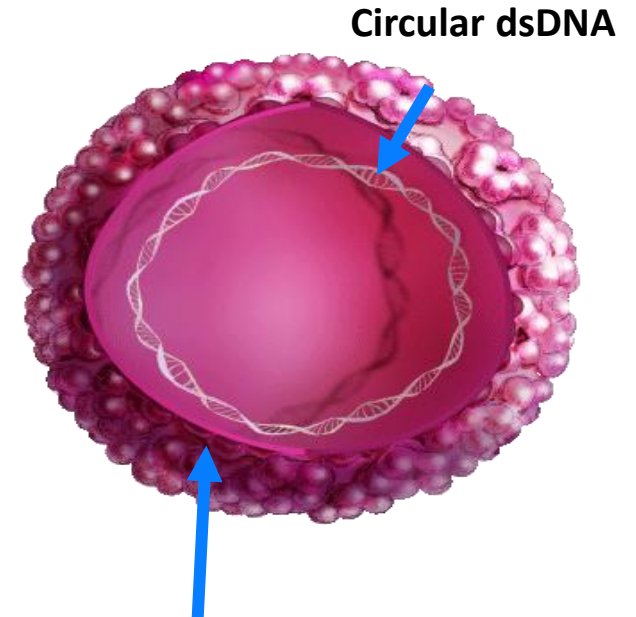
- Ο HPV είναι σχετικά μικρός ιός και περιέχει διπλή έλικα DNA μέσα σε σφαιρικό καψίδιο το οποίο αποτελείται από 2 πρωτεΐνες, L1 και L2



HPV capsids,  
approximately 55 nm in diameter



L1 protein pentamer

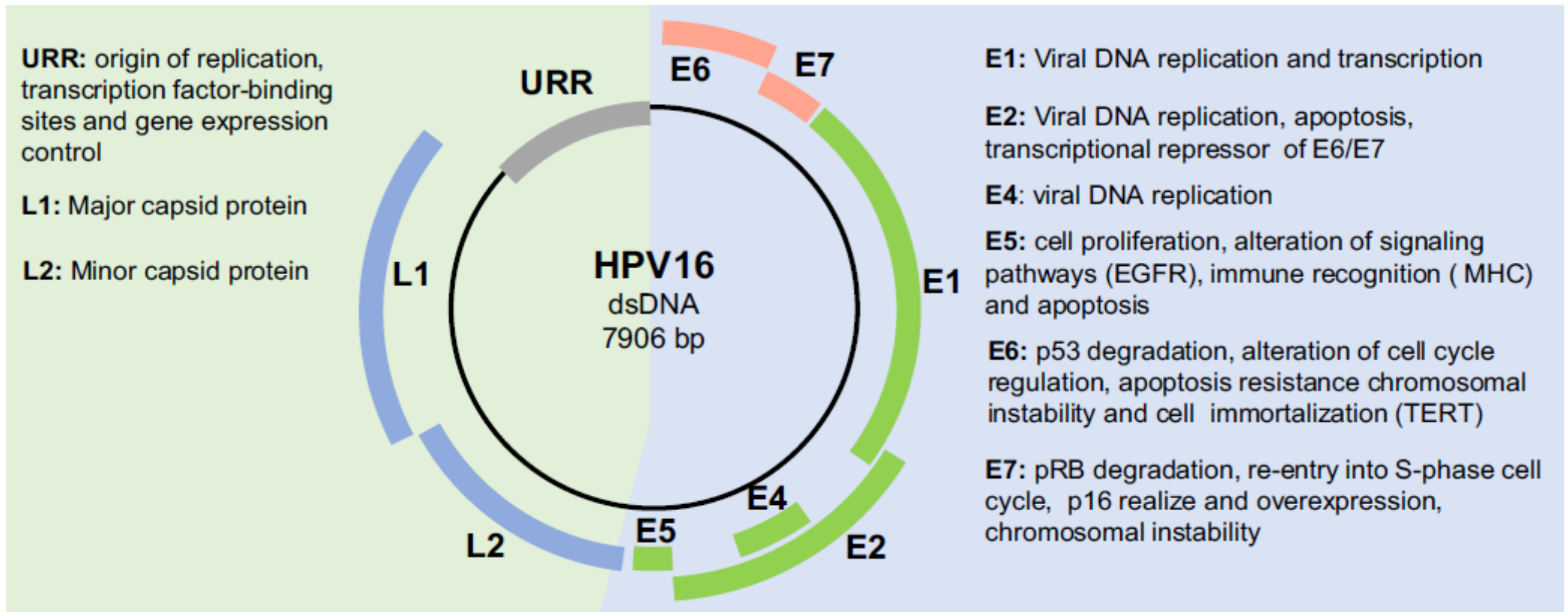


L2 supporting protein

Οι τύποι HPV 16 και 18 είναι υπεύθυνοι για > 70% των περιστατικών ΚΤΜ

Οι τύποι HPV 16,18,45 και 31 είναι υπεύθυνοι για >90 των περιστατικών αδενοκαρκινώματος

# Η οργάνωση του γονιδιώματος του HPV



S. de Sanjose et al. Best Practice & Research Clinical Obstetrics and Gynaecology 47 (2018) 2-13

# Η ιική Ε6 πρωτεΐνη

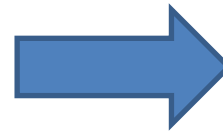
16-18 kDa , 150 αα

Μετασχηματισμός,  
αθανатоποίηση

Σύνδεση με κυτταρικές  
πρωτεΐνες



- Αλληλεπίδραση της με την ογκοκατασταλτική πρωτεΐνη p53  
E6+p53+E6 ουβικουτινική λιγάση E6AP



Ουβικιτινίωση της p53 και αποδόμησή της στο 26S πρωτεάσωμα

✓ μόνο οι τύποι υψηλού κινδύνου διεγείρουν το μονοπάτι της ουβικουτινίωσης και την αποδόμησης της πρωτεΐνης

- Ενεργοποίηση της καταλυτικής υπομονάδας της τελομεράσης hTERT



Διατήρηση μεγάλων τελομερών κατά την κυτταρική διαίρεση αναστέλλοντας την κυτταρική γήρανση



Αθανατοποίηση των κυττάρων

# Η ιική E7 πρωτεΐνη

CR1, CR2, CR3 συντηρημένες περιοχές-100 αα

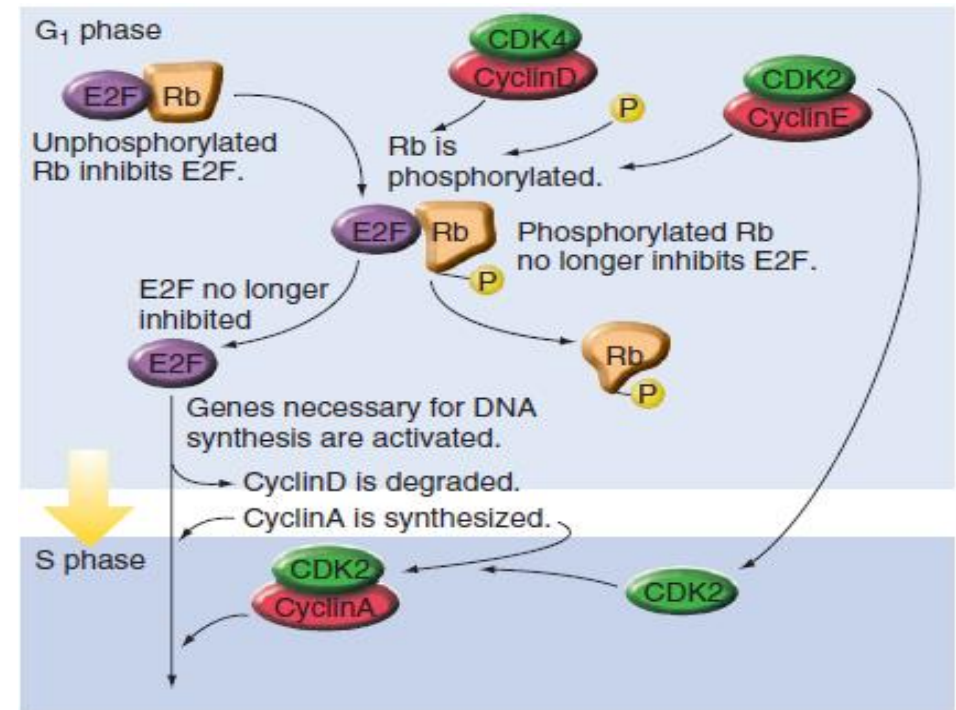
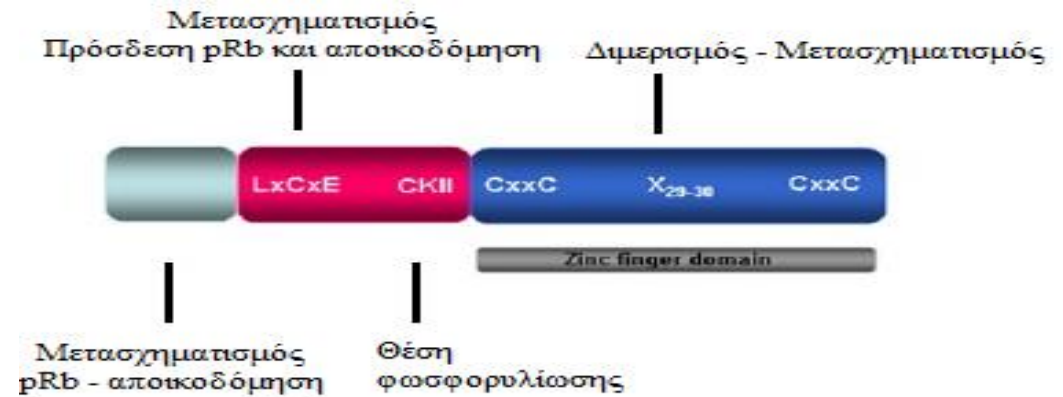
- Αλληλεπίδραση της με την οικογένεια των πρωτεϊνών του ρετινοβλαστώματος (pRb, p107 και p103)



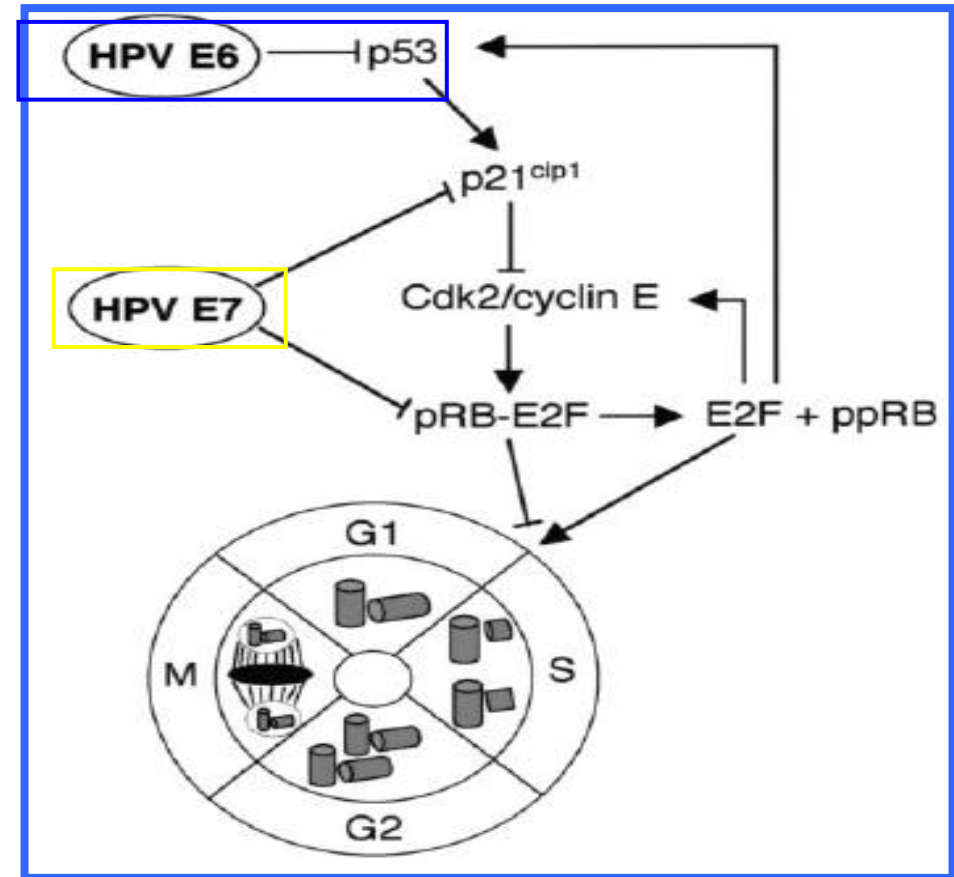
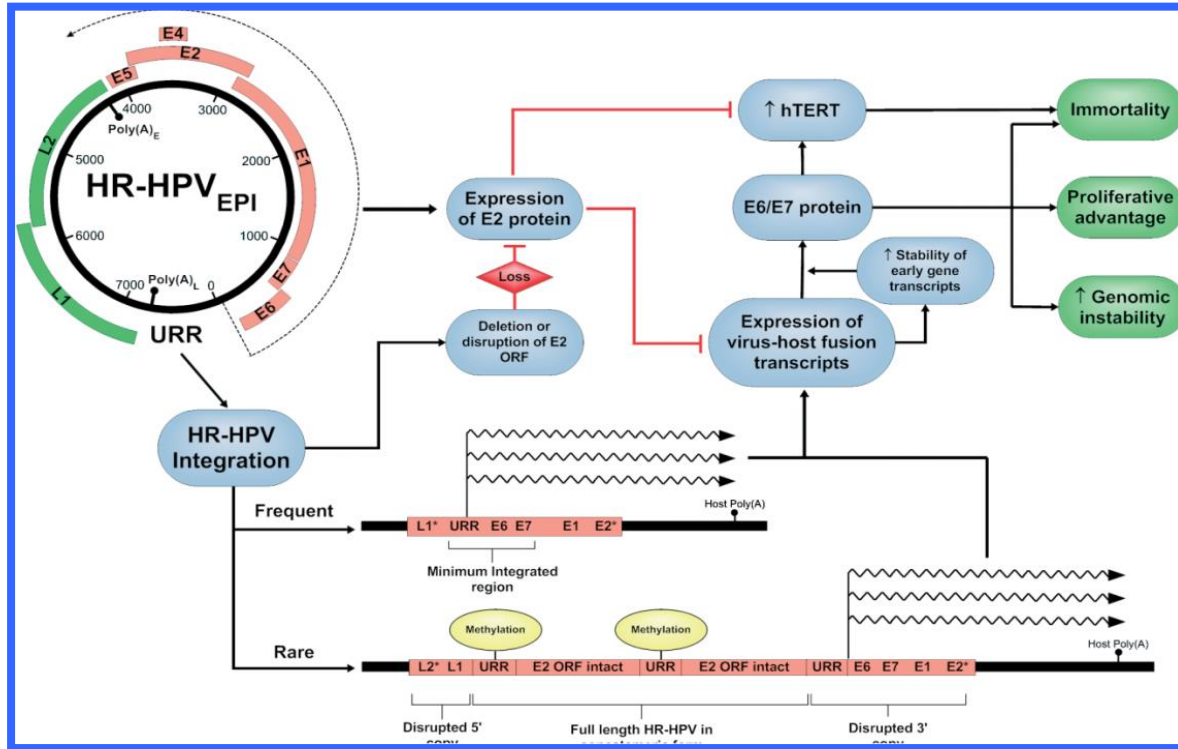
- ✓ επάγει την αποικοδόμηση της pRb μέσω του μονοπατιού του ουβικουτινικού πρωτεοσώματος
- ✓ συνδέεται με τις κυκλίνες A και E και με τους αναστολείς των κύκλινο-εξαρτώμενων κινασών (cdk) p21 και p27 → επάγεται η φωσφορυλίωση της pRb



**Απορρύθμιση του κυτταρικού κύκλου με συνέπεια τον ανεξέλεγκτο κυτταρικό πολλαπλασιασμό**



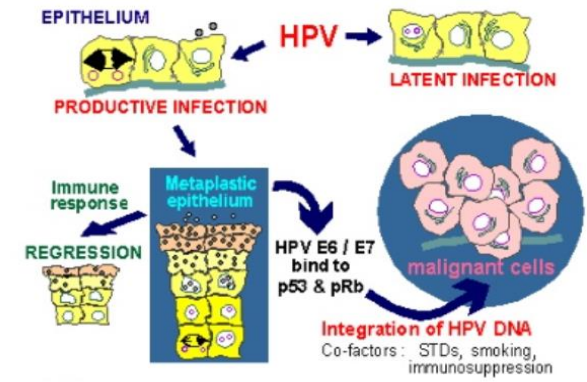
# E6 & E7-επίδραση στη ρύθμιση του κυτταρικού κύκλου



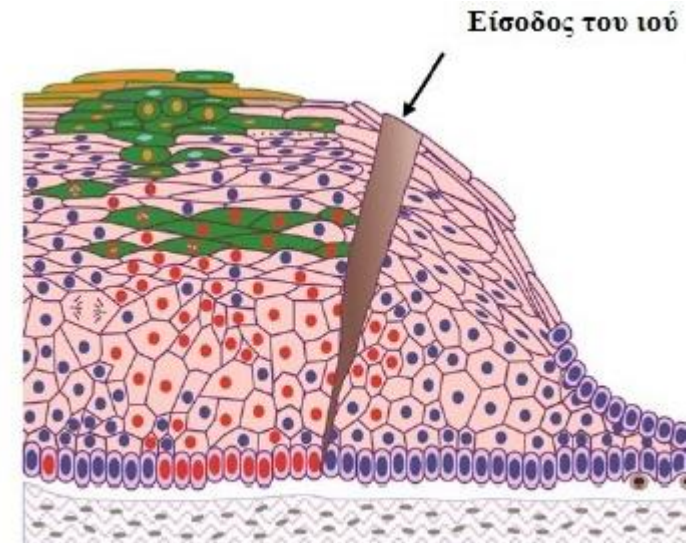
- Αναστολή των ογκοκατασταλτικών γονιδίων p53, pRb
- Κυτταρική αθανατοποίηση
- Κακοήθης μετασχηματισμός
- Γενωμική αστάθεια



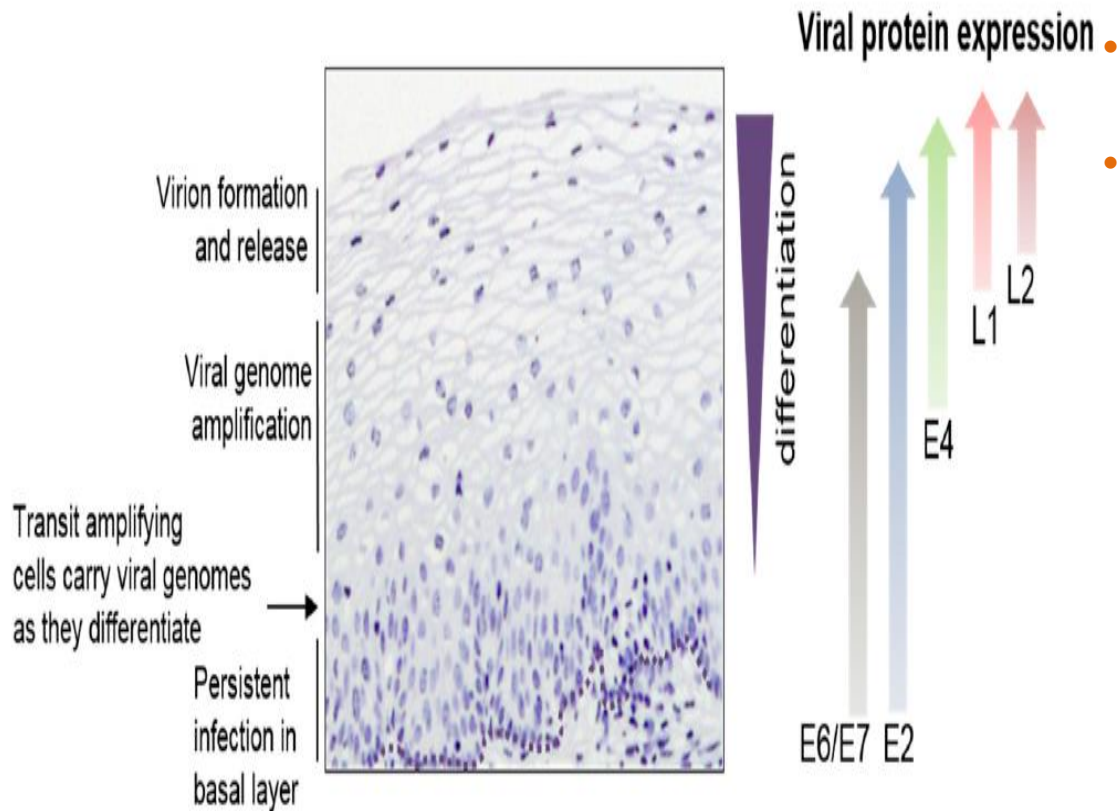
# Κύκλος ζωής του ιού



- Ακολουθεί το πρόγραμμα διαφοροποίησης των κερατινοκυττάρων του ξενιστή
- Τα ιικά σωματίδια προσβάλλουν τους επιθηλιακούς ιστούς μέσω μικροτραυματισμών
- Ο ιός εισβάλλει στα κύτταρα της βασικής στοιβάδας



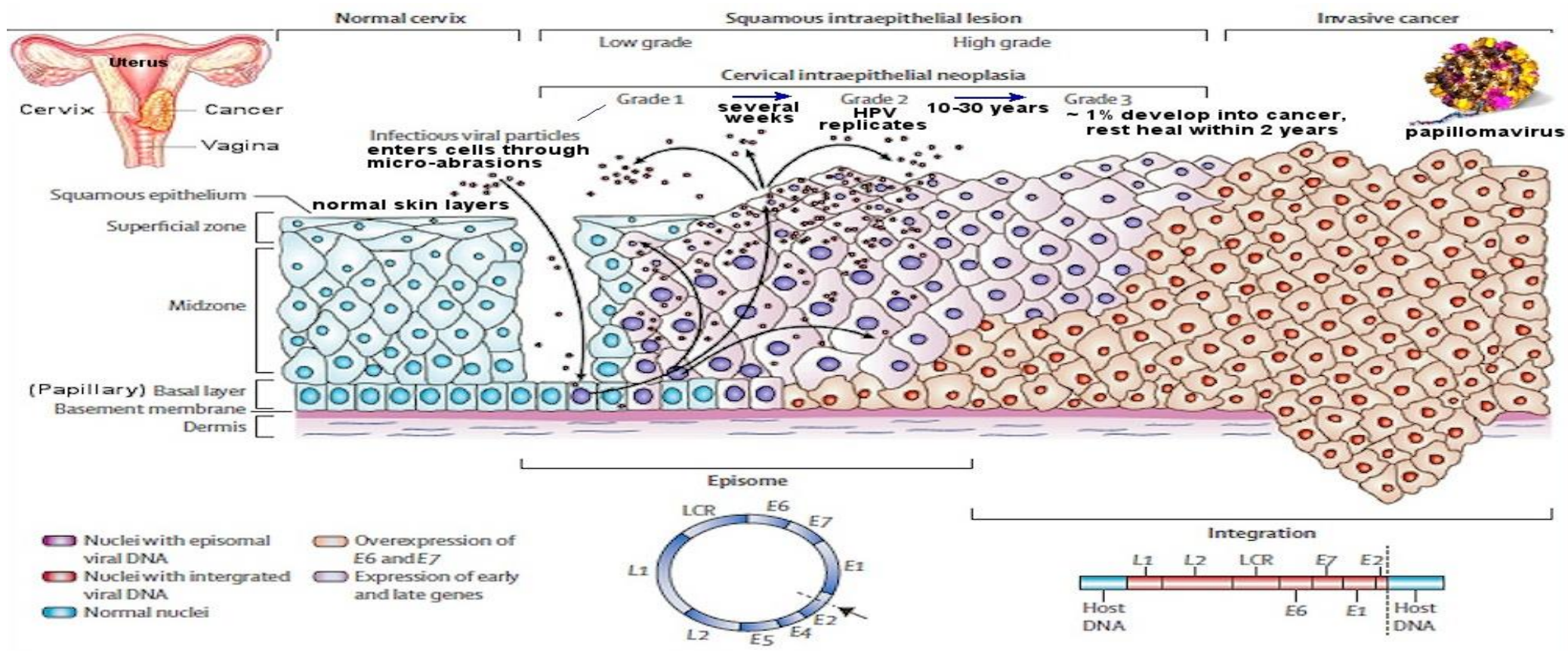
# Μεταγραφή του ιικού γονιδιώματος



- Ο όψιμος υποκινητής ενεργοποιείται ειδικά σε διαφοροποιημένες στοιβάδες του επιθηλίου
- Έκφραση των καψιδιακών πρωτεϊνών L1 και L2, οι οποίες είναι απαραίτητες για τον σχηματισμό του ιικού καψιδίου και το πακετάρισμα του ιικού γονιδιώματος στα νεοσχηματιζόμενα ιικά σωμάτια

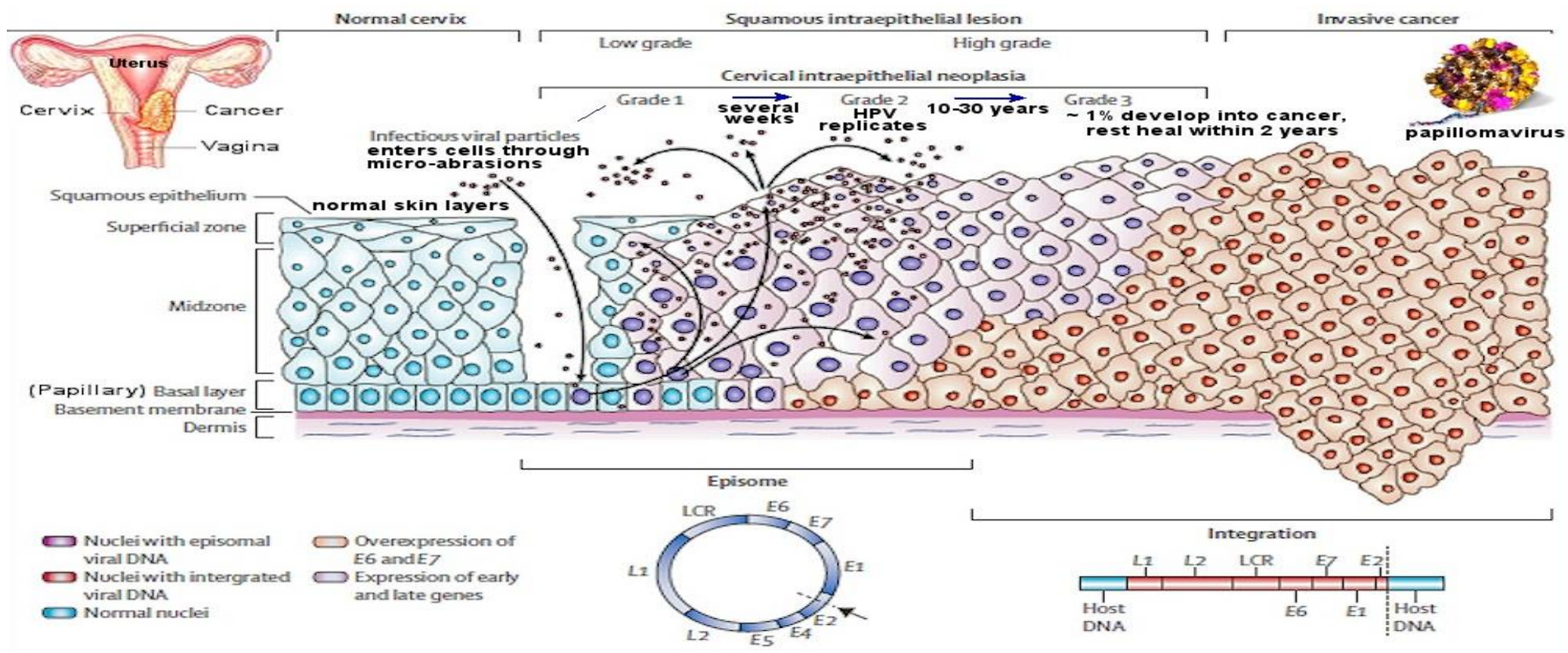
- Στα αδιαφοροποίητα κερατινοκύτταρα ή τα ενδιάμεσα διαφοροποιημένα κερατινοκύτταρα εκφράζονται έξι μη δομικές ρυθμιστικές πρωτεΐνες (E1, E2, E4, E5, E6, και E7) από την πρώτη περιοχή του ιικού γονιδιώματος
- Τα E1, E2, E4, E5, E6, E7 βρίσκονται υπό τον άμεσο έλεγχο του πρώιμου μεταγραφικού υποκινητή (p97)

**Η έκφραση των γονιδίων του ιού εξαρτάται από το πρόγραμμα διαφοροποίησης των κερατινοκυττάρων**



- Ο ιός HPV εισέρχεται στα κύτταρα της βασικής μεμβράνης μέσω μικροτραυματισμών στο επιθήλιο του τραχήλου.
- Μετά τη μόλυνση τα πρώιμα (Early) γονίδια E1, E2, E4, E5, E6 και E7 εκφράζονται και το ιικό γονιδίωμα αντιγράφεται.



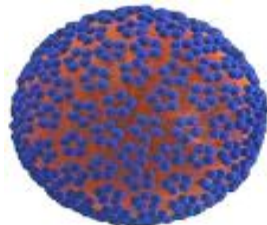


Στα ανώτερα στρώματα του επιθηλίου, το ιικό γονιδίωμα αντιγράφεται με γρηγορότερους ρυθμούς και εκφράζονται τα γονίδια L1, L2 και E4, τα οποία συμμετέχουν στο σχηματισμό του καψιδίου και στην απελευθέρωση των ισωματίων.

Η εξέλιξη των κυτταρικών αλλοιώσεων σε διηθητικό καρκίνο σχετίζεται με την ενσωμάτωση του ιικού γονιδιώματος στο ανθρώπινο χρωμόσωμα, με την απώλεια έκφρασης της E2 πρωτεΐνης και την επακόλουθη υπερέκφραση των γονιδίων E6 και E7

# HPV detection: Basic principles

- Isolation of HPV nucleic acids
  - DNA is present in cells (episomal, integrated)
  - mRNA
- Denaturation of nucleic acids
  - DNA is denatured in PCR (usually 94-95°C) to create single strands
  - mRNA is denatured in lower temperature (40-60°C) only to unfold any 2ndary structures
- Amplification
  - DNA via PCR (DNA pol, cycling T from 55-95°C)
  - mRNA using RT-PCR or isothermic reaction (RNA pol, RT, RNase-H)
- Signal detection (fluorescence, chemiluminescence)
- Most DNA techniques target L1 (conserved region). Most mRNA techniques target the transcripts of the E6/E7 oncogenes





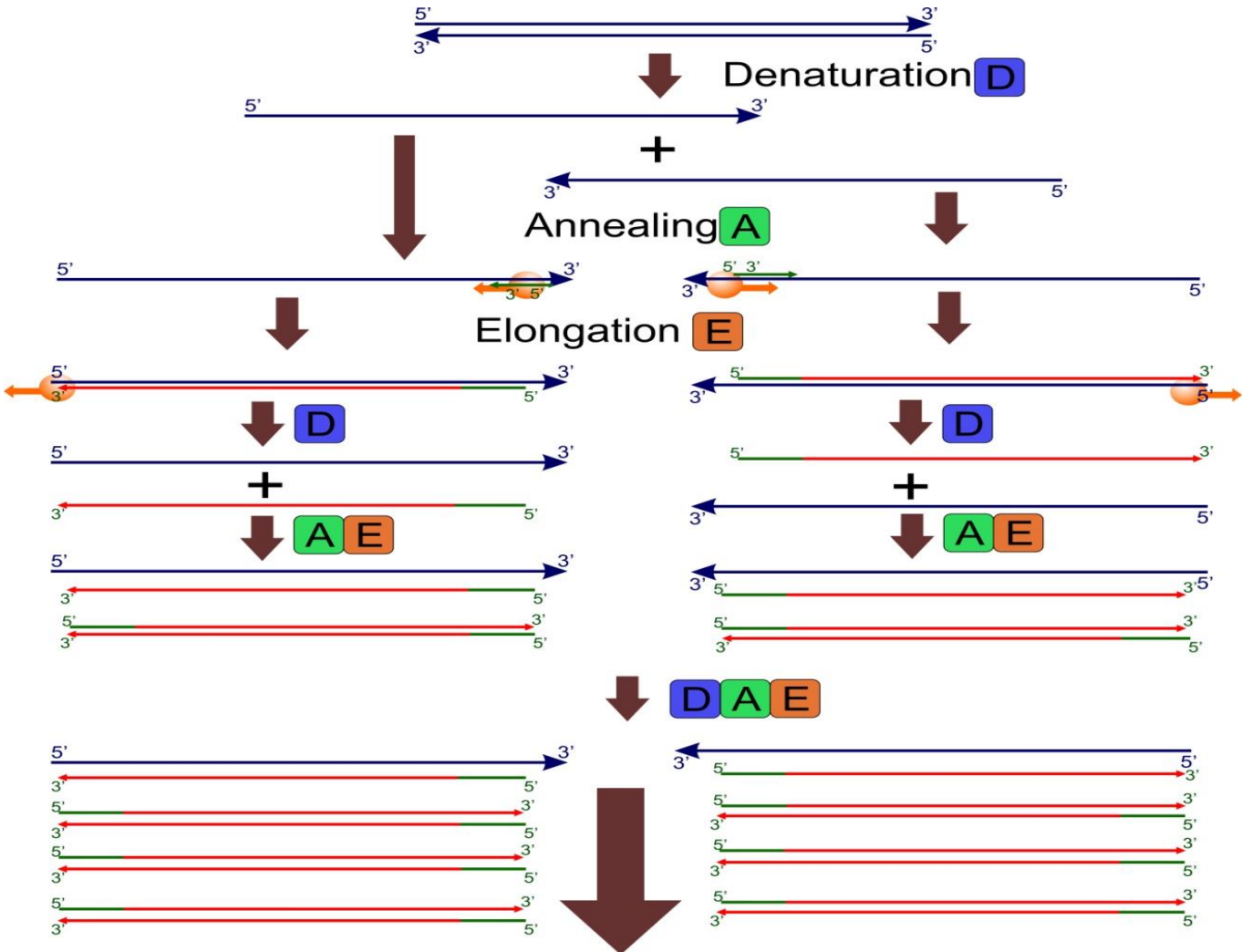
# DNA

- Μέθοδοι που βασίζονται στο PCR
- Μέθοδοι που βασίζονται στο real-time PCR
- Μέθοδοι που δε βασίζονται στο PCR

# RNA

- Μέθοδοι που ανιχνεύουν το mRNA E6 και E7 ογκογονιδίων του ιού

# PCR

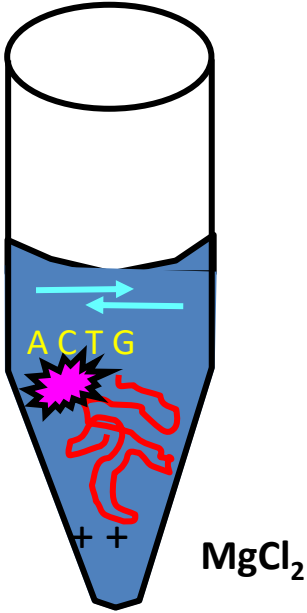


Exponential growth of short product

$$n^{36} = 68,719,476,736 \text{ copies in } \sim 2 \text{ hrs}$$

Buffer  
Primers

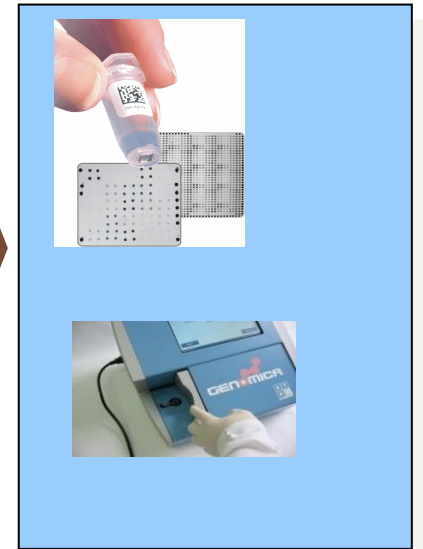
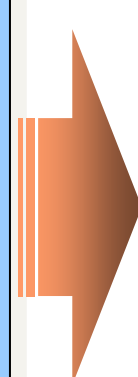
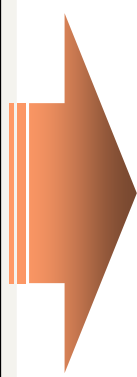
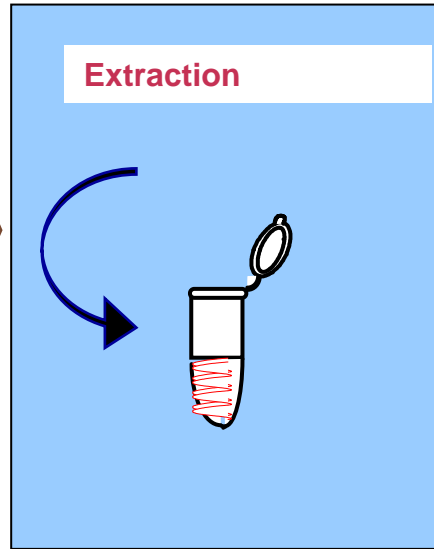
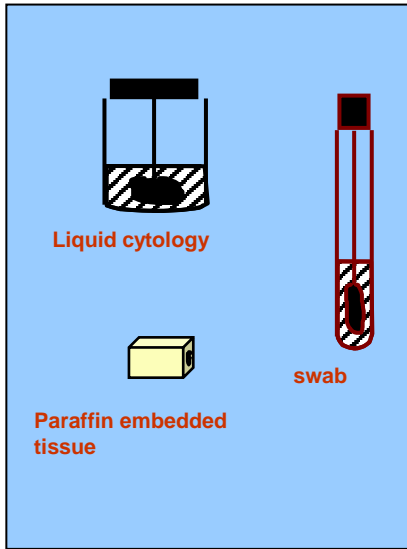
DNA template



# Μέθοδοι που βασίζονται στο PCR-ενίσχυση στόχου

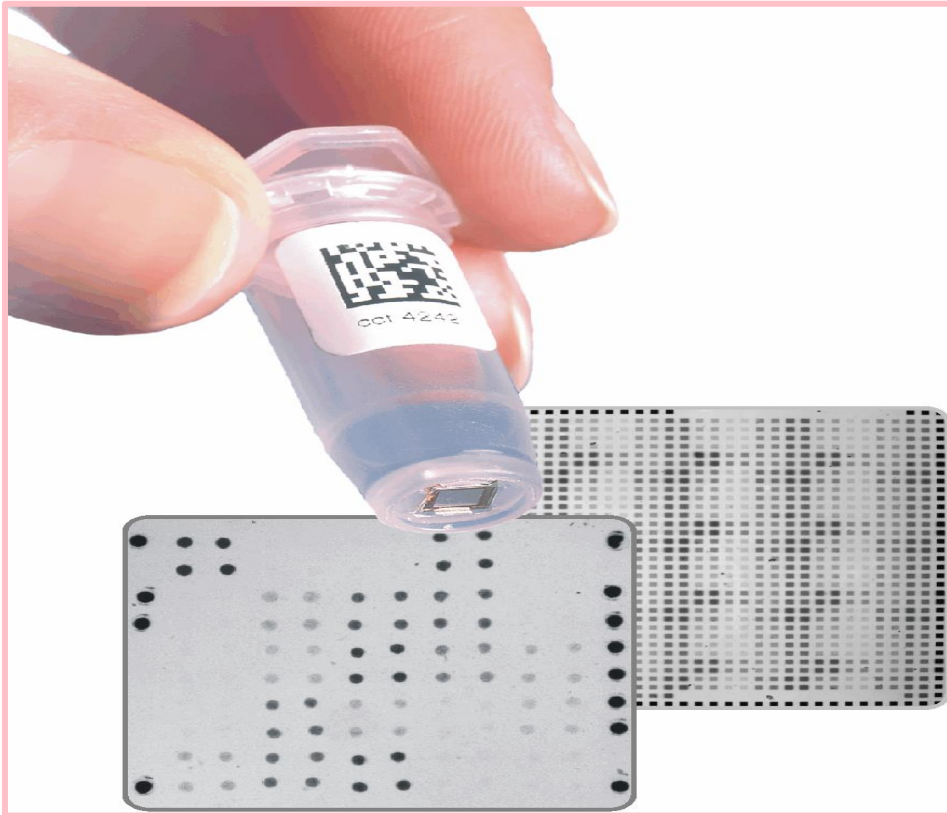
- AMPLICOR® - LINEAR ARRAY HPV GENOTYPING Tests
- CLART® HPV2
- INNO-LiPa HPV-SPF10LiPA25
- HPV Direct Flow-chip
- PapilloCheck® HPV-Screening
- HybriBio 21 HPV GenoArray
- GenoFlow HPV array (DiagCor)





# HPV CLINICAL MICROARRAYS

# Microarrays Technology

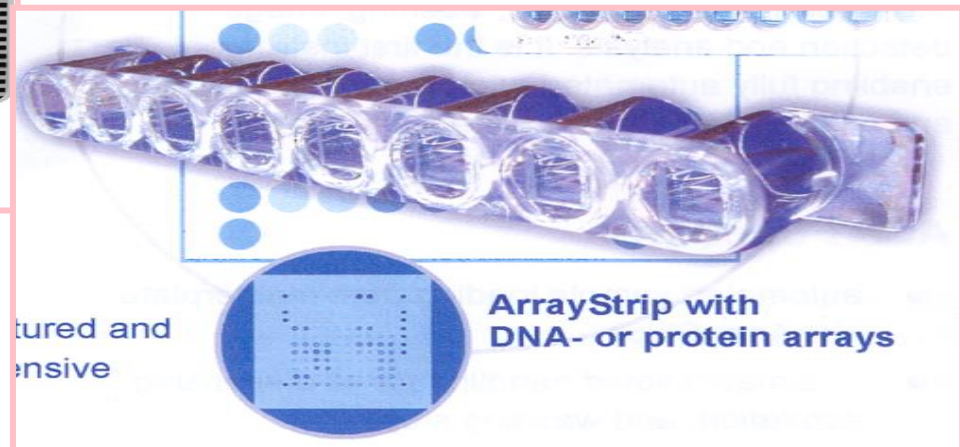


**Biochip (3x3mm):**

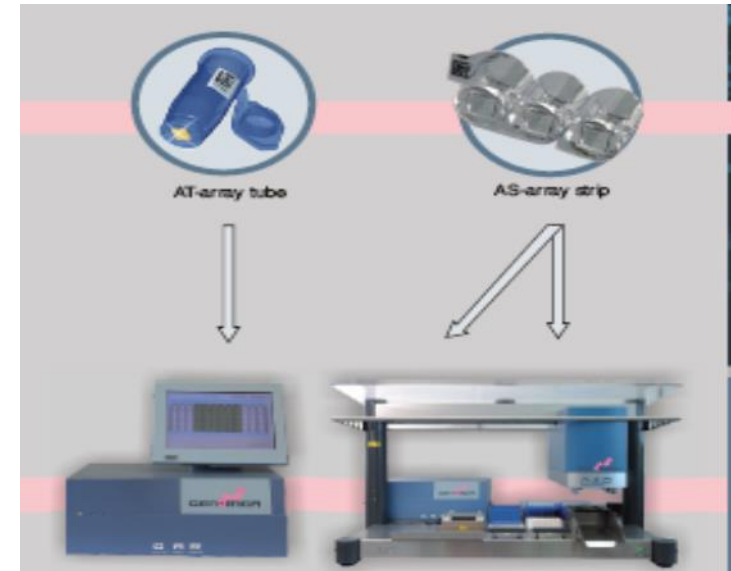
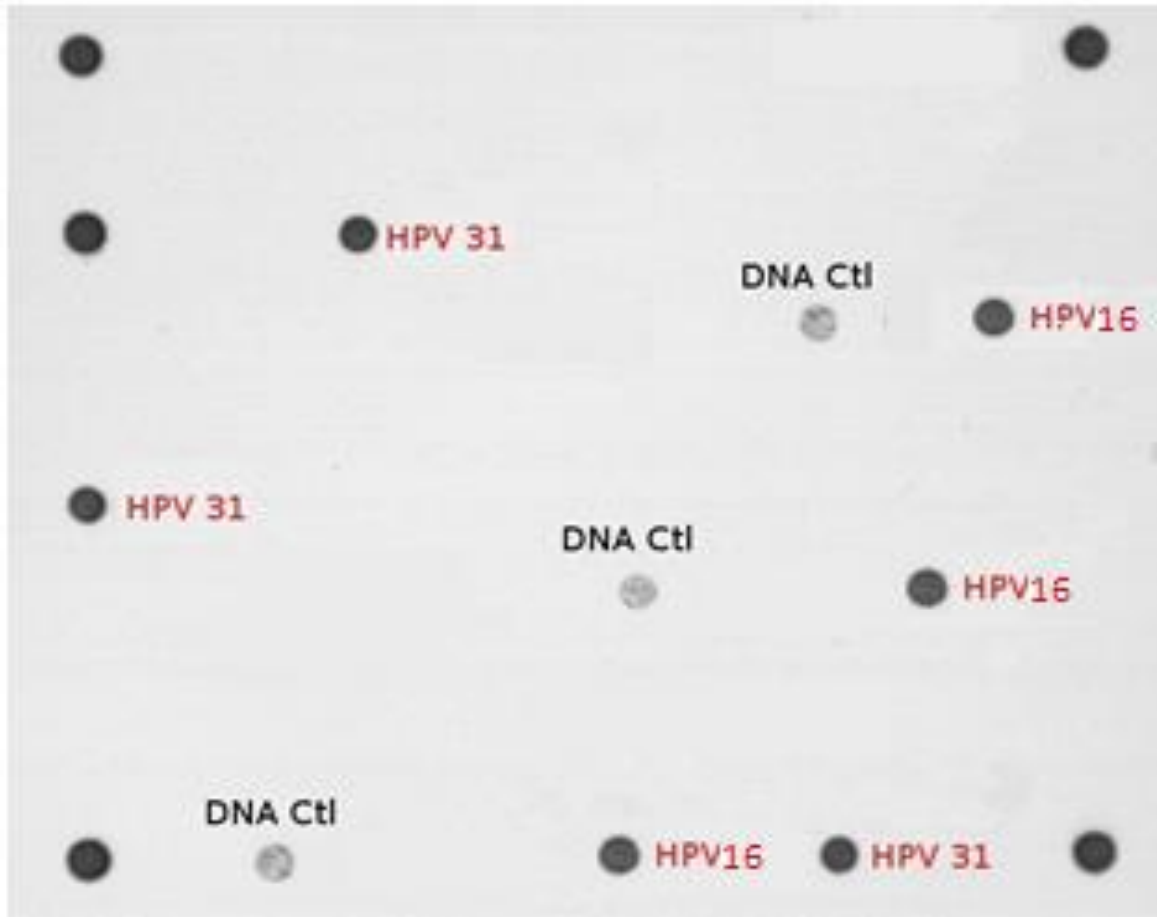
probes in triplicate

Internal control for nucleic acid extraction

Internal control for PCR inhibition







## PCR πραγματικού χρόνου Real time PCR/Q PCR

- Βασίζεται στην ανίχνευση και ποσοτικοποίηση ενός φθορίζοντος μορίου αναφοράς (ειδικού για συγκεκριμένη αλληλουχία) σε κάθε κύκλο της αντίδρασης PCR

Reporter and  
quencher-marked  
probe

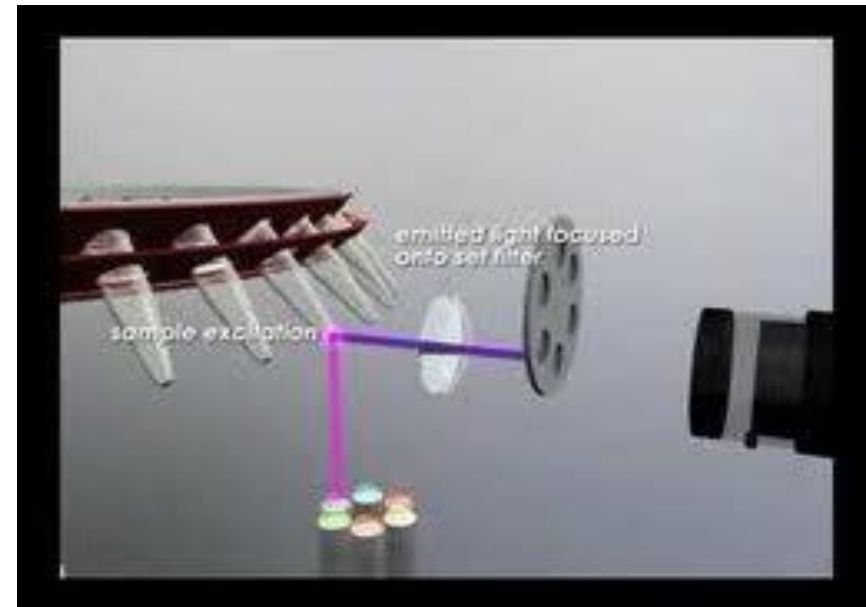
Target DNA strand  
hybridized with probe

*Δεν απαιτείται ανάλυση με ηλεκτροφόρηση*

# PCR πραγματικού χρόνου (qPCR)

## ➤ Επιτρέπει

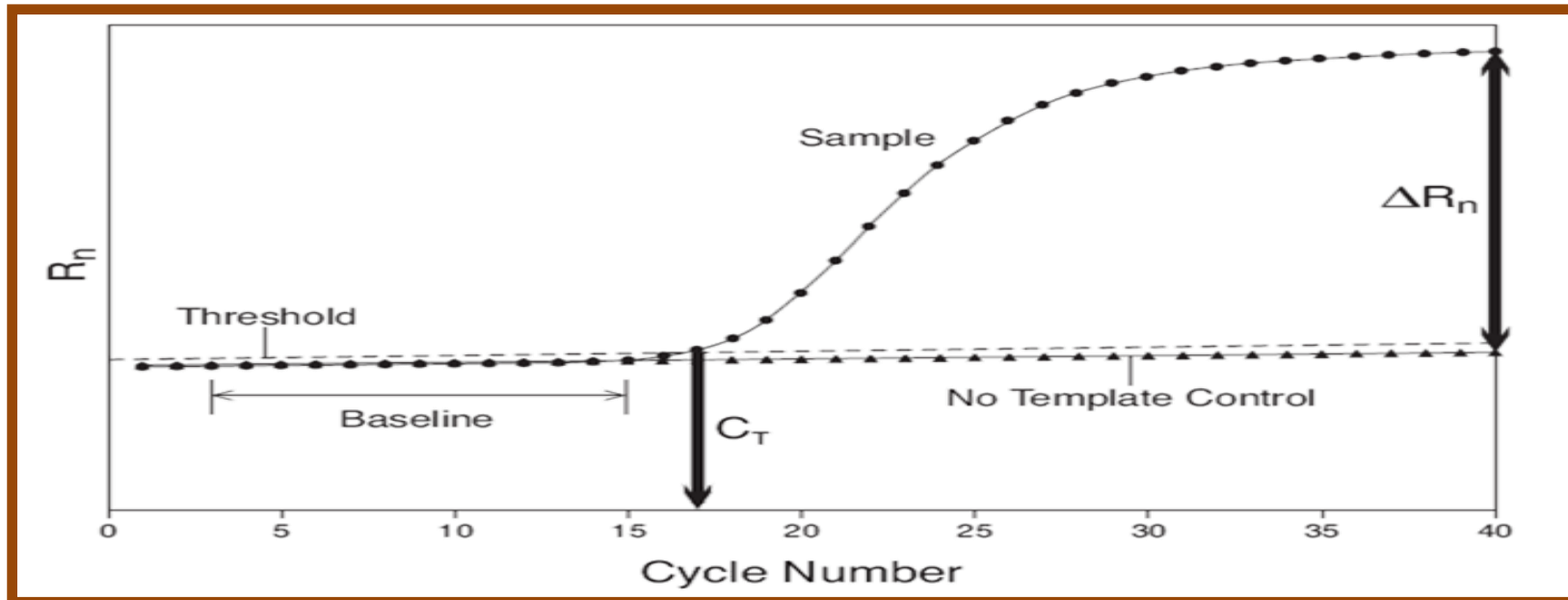
✓ την αδιάλειπτη παρακολούθηση των προϊόντων της PCR κατά την ενίσχυση μέσω της μέτρησης του εκπεμπόμενου φθορισμού των προϊόντων σε κάθε κύκλο



# PCR πραγματικού χρόνου (qPCR)

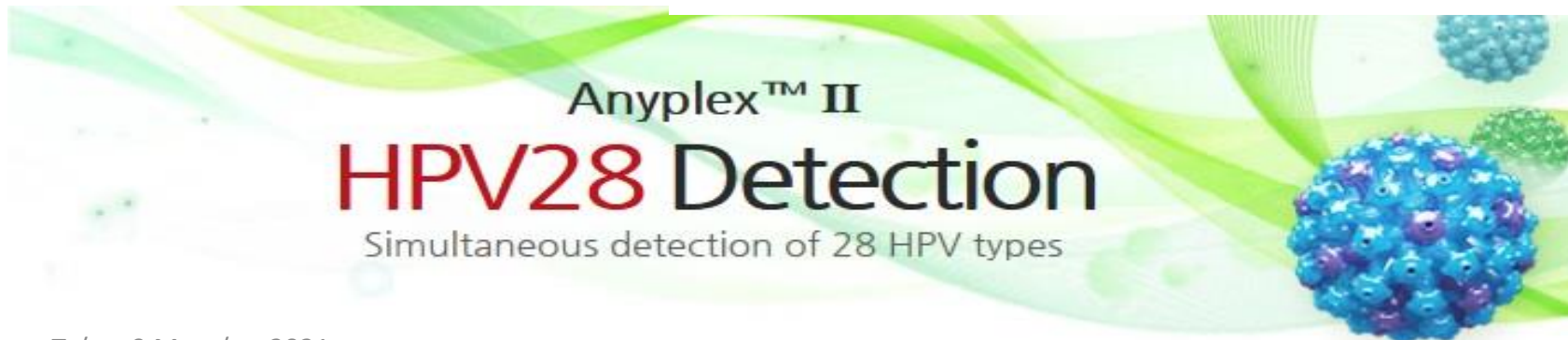
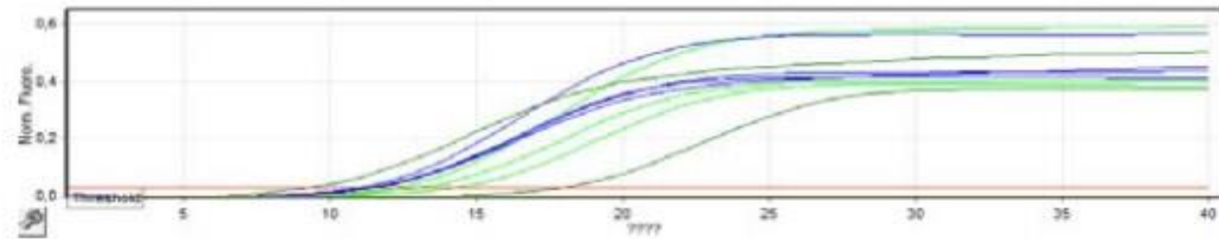
## ➤ Επιτρέπει

- ✓ Ταυτοποίηση του κύκλου εισόδου στην εκθετική φάση (κύκλος-κατώφλι) και, της σχετικής ποσοτικοποίησης της μήτρας DNA στην αρχή της αντίδρασης.
- ✓ Τα προϊόντα του πολλαπλασιασμού αναλύονται σε πραγματικό χρόνο όπως συντίθενται σε κάθε κύκλο.



# Μέθοδοι που βασίζονται σε PCR πραγματικού χρόνου

- cobas<sup>®</sup> HPV Test
- Anyplex<sup>™</sup> II HPV28 Detection
- Abbott RealTime High HPV
- Sacace 14 HR



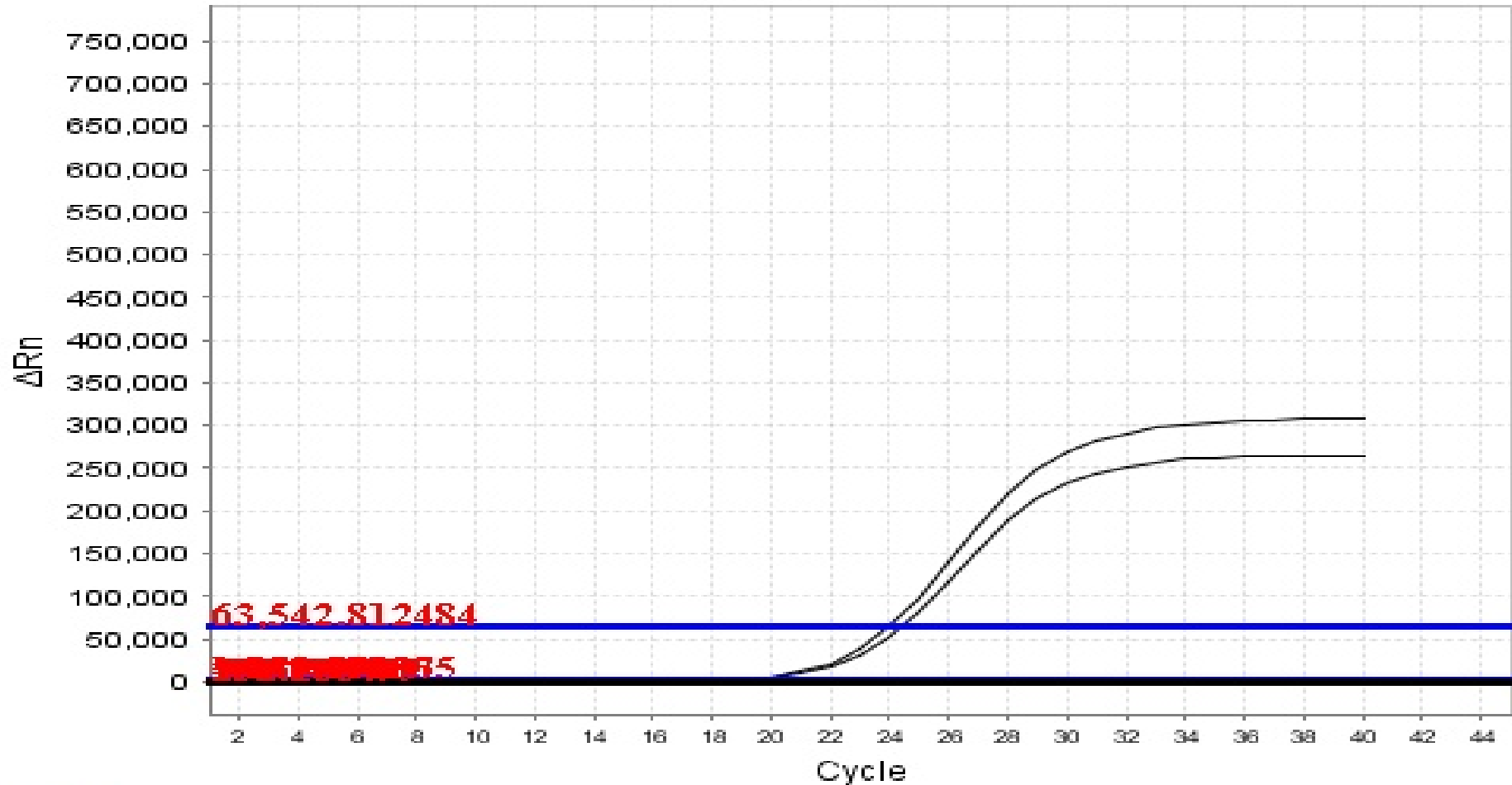


# 14 High-risk HPV Genotyping Real-time PCR Kit

- 14 high-risk HPV types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- Cellular IC for the monitoring of the entire process
- ✓ **Demonstrate no cross-reactivity with non-high risk HPV genotypes, ensuring that positive results are clinically meaningful**

# Negative clinical sample

Amplification Plot

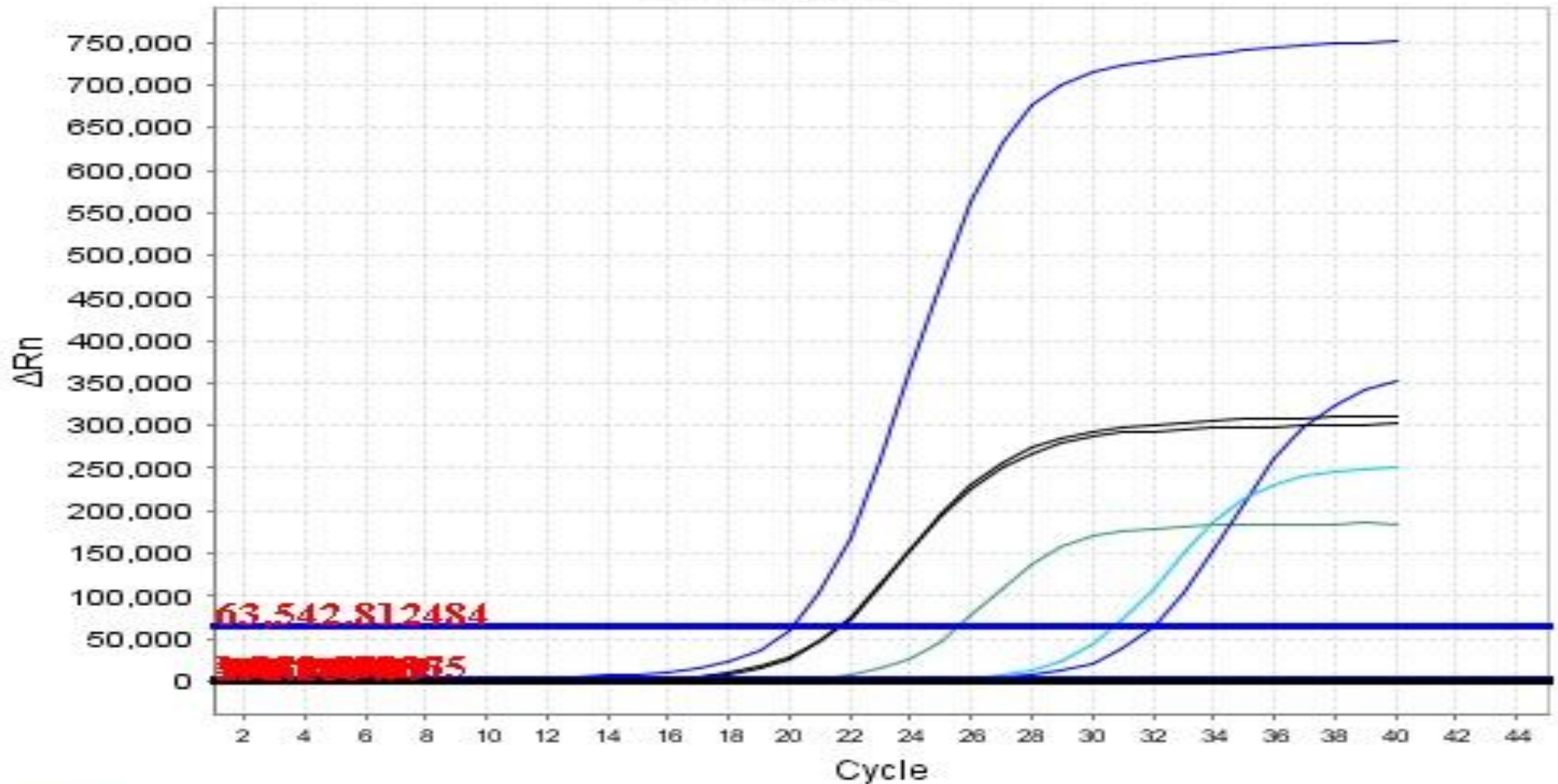


Legend



# Positive clinical sample

Amplification Plot





- 12 HR: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- Γονοτύπιση: 16, 18

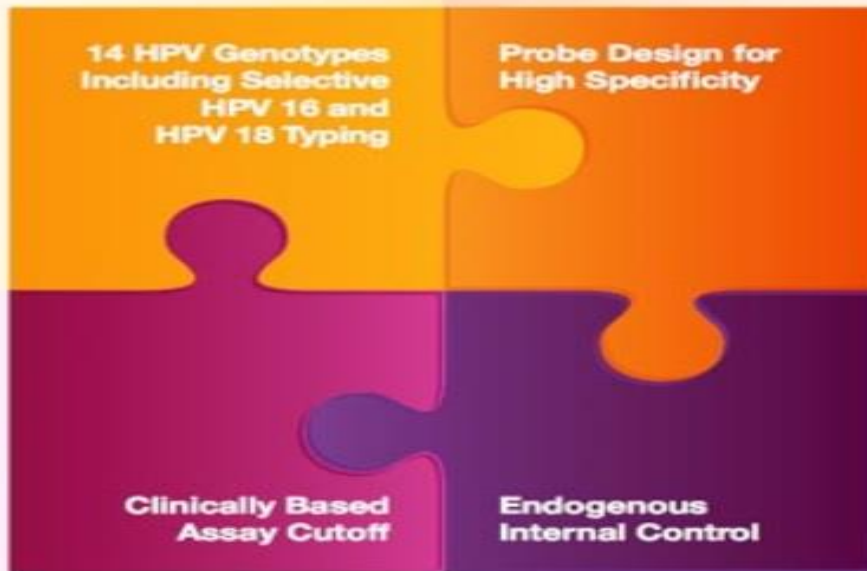


# Abbott Real Time High Risk (HR) HPV

Multiplex real-time PCR for the separate detection of HPV 16, HPV 18, and 12 other high-risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) to identify women who would benefit from immediate follow-up.

Genotype specific single-stranded lin probes in conjunction with an optimized thermal cycling profile for highly specific detection of targeted high-risk HPV genotypes to avoid cross-reactivity with non targeted HPV types.

**Clinically Meaningful Results**



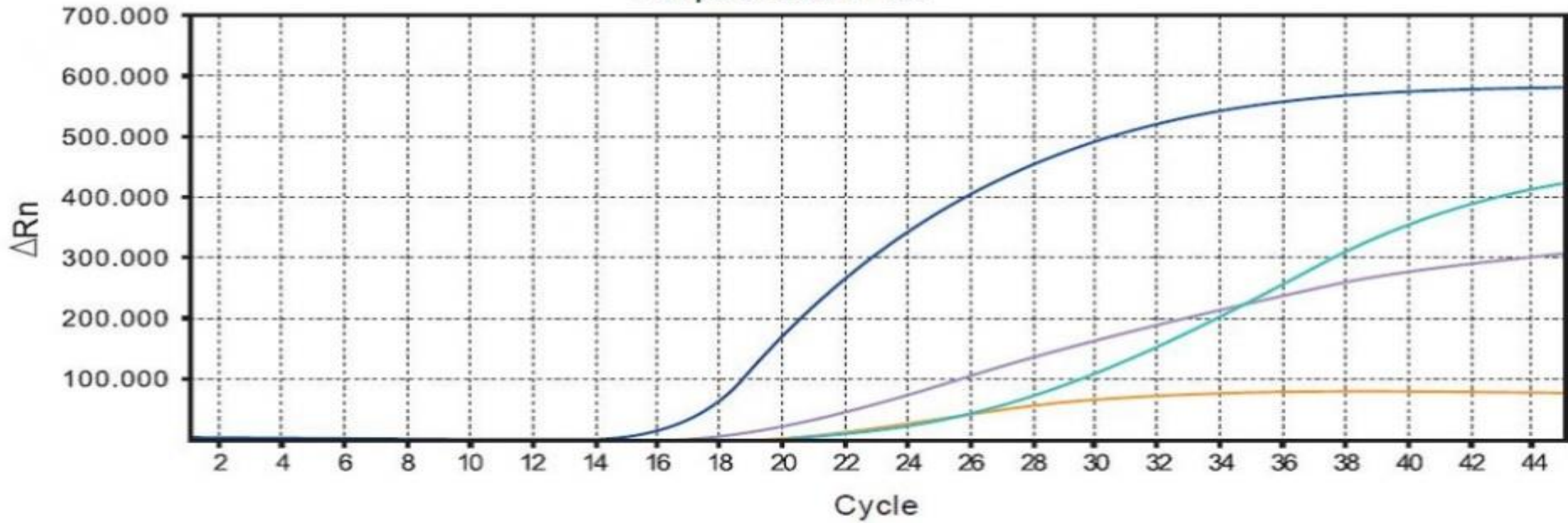
Assay cut-off established based on correlation to disease status in order to differentiate women at risk of cervical cancer or precancer from those at no or low risk.

True cellular internal control (human beta-globin) for high reliability and confidence in HPV-negative test results.

- 12 HR: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- Γονοτύπιση: 16, 18



Amplification Plot



# Μέθοδοι που δε βασίζονται στο PCR-μέθοδοι ενίσχυσης σήματος

- *digene* Hybrid Capture 2
- Cervista®

*Cervista* HPV HR  
Be Certain. Be in Control.



# *digene* Hybrid Capture 2

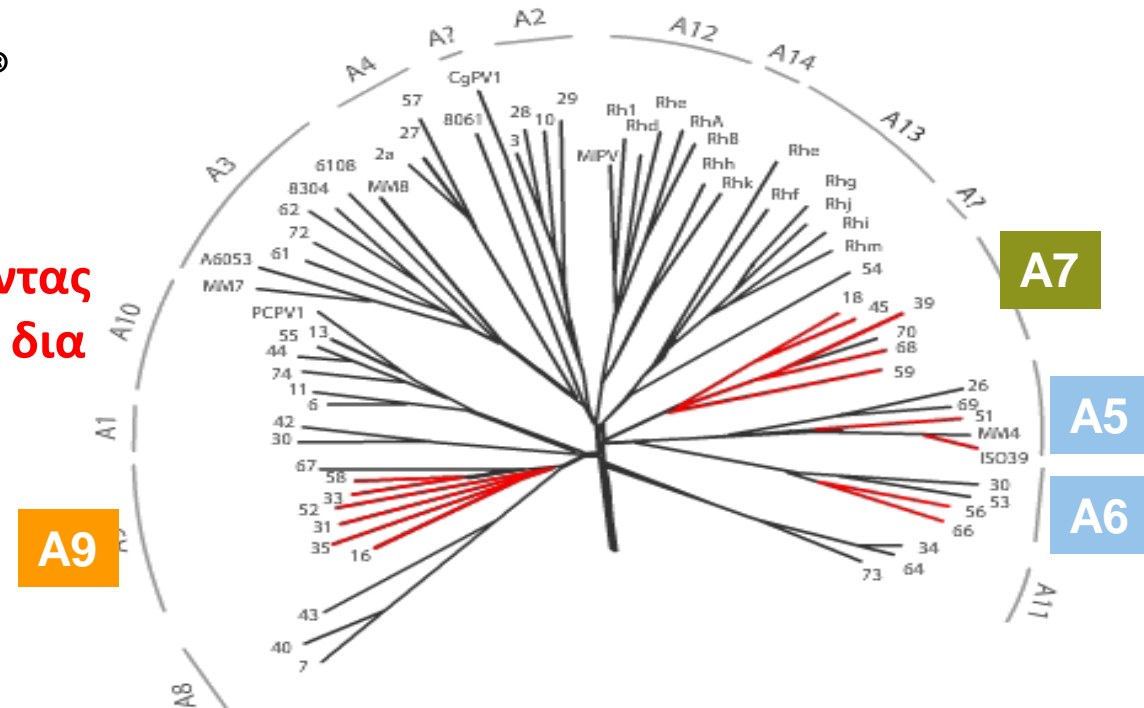
- Χρησιμοποιεί μίγμα από ανιχνευτές RNA που αντιστοιχούν στους τύπους του ιού που ανιχνεύει
- Διαδικασία ανίχνευσης DNA:RNA υβριδίων με χρήση ειδικών μονοκλωνικών αντισωμάτων και χημειοφωταύγειας για την ποιοτική ανίχνευση 18 τύπων χαμηλού και υψηλού κινδύνου HPV
- Διαφοροποίηση σε δύο ομάδες HPV DNA
- Χαμηλού κινδύνου (6, 11, 42, 43, 44)
- Υψηλού κινδύνου (16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68)
- Εύκολη στη χρήση για κλινικές μελέτες-προσφέρεται για αυτοματοποίηση-ισχυρή και αναπαραγώγιμη μέθοδος
- **Δεν επιτρέπει την ακριβή γονοτύπωση του ιού**
- **Λιγότερο ευαίσθητη από την PCR**

# Cervista™ HPV HR Test Design

- Ενζυματική δράση της νουκλεάσης Cleavase® (Hologic™, Inc)
- Η χημεία Invader χρησιμοποιεί ενίσχυση σήματος και ανίχνευση φθορισμού, παράγοντας εκατομμύρια ειδικά σήματα προς ανίχνευση διαμέσου ταυτόχρονων διπλών ισοθερμικών αντιδράσεων:

1. Επιβεβαιώνουν την ύπαρξη επαρκούς ποσότητας DNA στο υπό εξέταση δείγμα
2. Ανιχνεύουν την ύπαρξη των 14 HPV χαρακτηρισμένων ως HR από την IARC (International Agency for Research on Cancer)

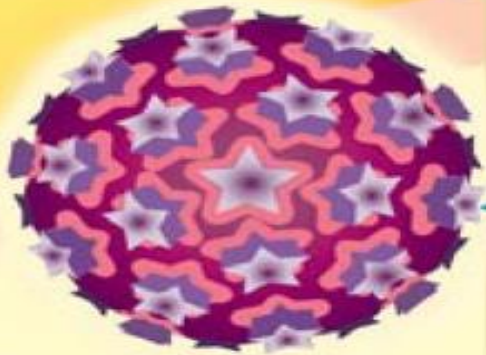
|       |                        |
|-------|------------------------|
| A5/A6 | 51, 56, 66             |
| A7    | 18, 39, 45, 59, 68     |
| A9    | 16, 31, 33, 35, 52, 58 |



- Φυλογενετικό δέντρο της A υπεριοικογένειας HPV, βασισμένο στην L1 περιοχή του ιού
- Cervista HPV είναι ειδικό για τους HR γονότυπους που έχουν επιλεγθεί από τις A5/A6, A7 & A9 ιικές ομάδες:

de Villiers et al., Virology, 2004

HPV DNA tests



HPV virus



DNA indicates presence of HPV



E6/E7 mRNA indicates activity of HPV oncogenes



E6/E7 oncoproteins induce cell transformation



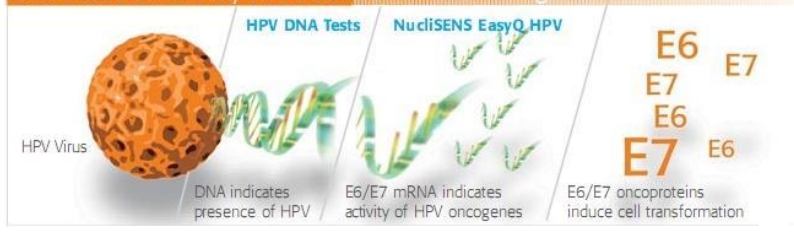
Clear focus on the key risk factor:  
**Active HPV oncogenes**



# Μέθοδοι ανίχνευσης HPV E6/E7 mRNA

1. Nuclisens HPV (Biomerieux)-ισοθερμική NASBA τεχνολογία-πλήρη μετάγραφα από 5 υψηλού κινδύνου HPV τύπους
2. APTIMA (GenProbe)-ισοθερμική TMA τεχνολογία-πλήρη μετάγραφα από 14 υψηλού κινδύνου HPV τύπους
3. HPV Oncotect (inCellDx)-κυτταρομετρία ροής/FISH-E6/E7 mRNA κύτταρα από όλους τους υψηλού κινδύνου HPV τύπους
4. In-house nested-RT PCR/ηλεκτροφόρηση σε πήκτωμα αγαρόζης-πλήρη και διακεκομένα μετάγραφα

1. Molden et al., *J Virol Methods* 2007;142:204-12
2. Szarewski et al., *Epidemiol Biomarkers Prev* 2008; 17:3033-42
3. Narimatsu and Patterson, *Am J Clin Pathol* 2005; 123:716-23
4. Sotlar et al., *J Med Virol* 2004; 74:107-16

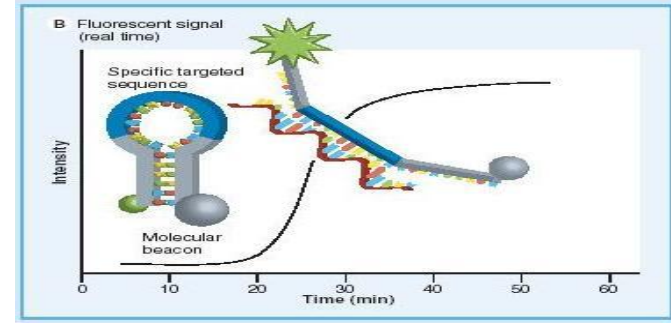
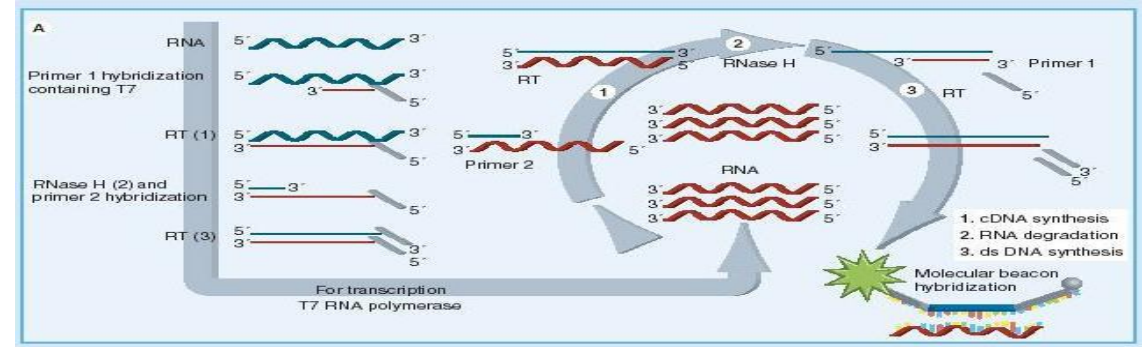
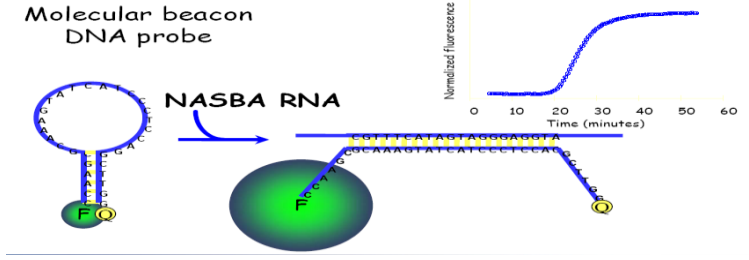


# NucliSens EasyQ® HPV (Biomerieux, France)

- ✓ Βασίζεται στην ποιοτική ανίχνευση παραγόμενου E6/E7 mRNA από τους υψηλού κινδύνου τύπους HPV 16, 18, 31, 33 και 45
- ✓ Χρησιμοποιεί μοριακούς ιχνηθέτες (molecular beacons) για την ανίχνευση του παραγόμενου προϊόντος
- ✓ Διαθέτει εσωτερικό μάρτυρα

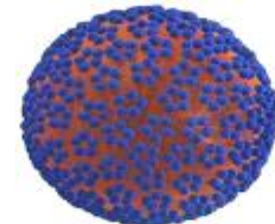
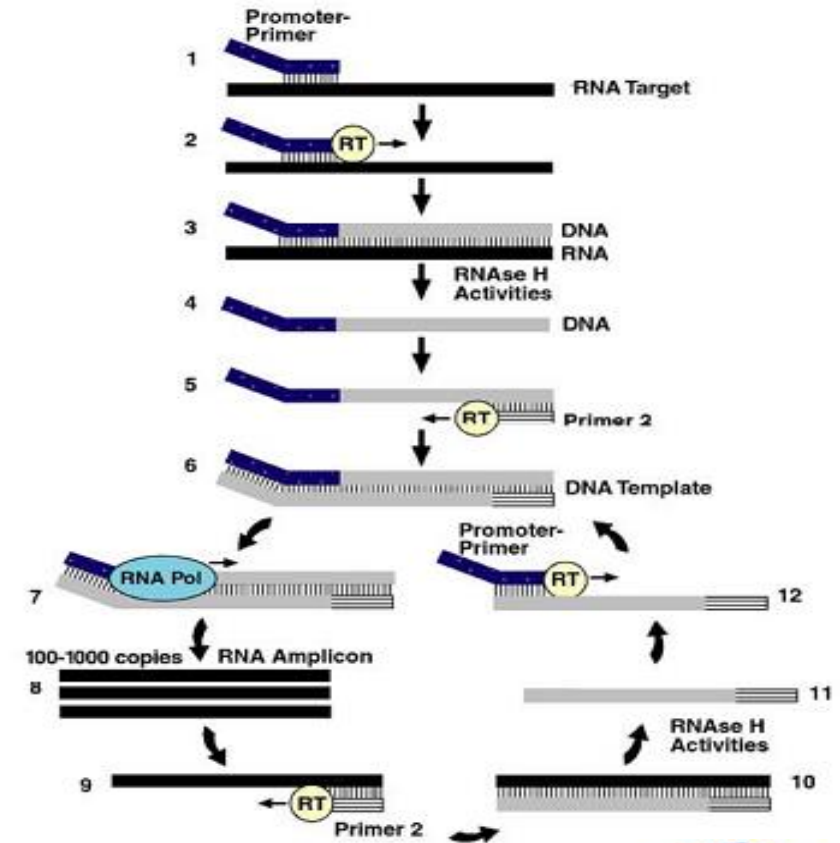


Real-time detection by NASBA



# Aptima HPV (Hologic)

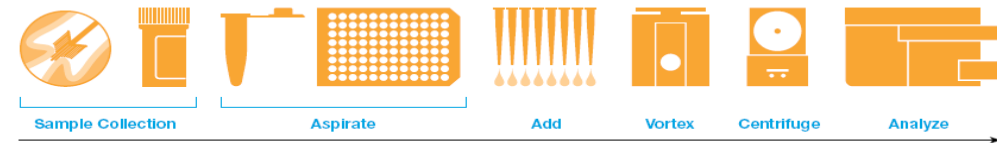
- Transcription mediated amplification (TMA) of HPV mRNA with **chemiluminescent**
- Qualitative assay for detection of E6/E7 mRNA of **14 HR HPVs** (16/18/31/33/35/39/45/51/52/56/58/59/66/68) with optional **typing for 16 and 18/45**
- Internal controls and calibrators
- Sample stability in ThinPrep for up to 30 days, Aptima Specimen Transfer 105 days (2°C to 20°C) or 24 months at <-20°C
- 1 ml of LBC, swabs, urine...



# OncoTect E6/E7 (Incell Dx)

✓ Κυτταρομετρία ροής-μέσο ανίχνευσης της υπερ-έκφρασης του ενδοκυττάριου mRNA των γονιδίων E6 και E7 όλων των ογκογόνων τύπων του HPV ως συνέπεια της ενσωμάτωσης του ιού στο γενωμικό DNA

## Simple Workflow



## 24 & 96 Test Procedure

|                                  | Aspirate   | Add        | Vortex           | Centrifuge | Waterbath      |          |
|----------------------------------|--|------------|------------------|------------|----------------|----------|
| Start To Finish: Approx. 3 Hours | 1  | ●          | 1 mL PBS         | ●          | ○              |          |
|                                  | 2  | ●          | 300 uL R1        | ○          | ○              |          |
|                                  |  | ○          | 1 mL R2          | ●          | ○ (1 hr. wait) |          |
|                                  | 3  | ●          | 1 mL R3          | ●          | ○              |          |
|                                  | Make the master mix by combining 100 uL R4 and 3 uL R5 per sample, vortex. |            |                  |            |                |          |
|                                  | 4  | ○          | 100 uL R4/R5 mix | ●          | ○              | ● 30 min |
|                                  | ○  | 1 mL R6    | ●                | ●          | ○              |          |
|                                  | ●  | 1 mL R7    | ○                | ○          | ● 15 min       |          |
|                                  | ○  | ○          | ●                | ●          | ○              |          |
|                                  | ●  | 400 uL PBS | ●                | ○          | ○              |          |

● Action ○ No Action Required

The sample is ready for analysis on the flow cytometer.

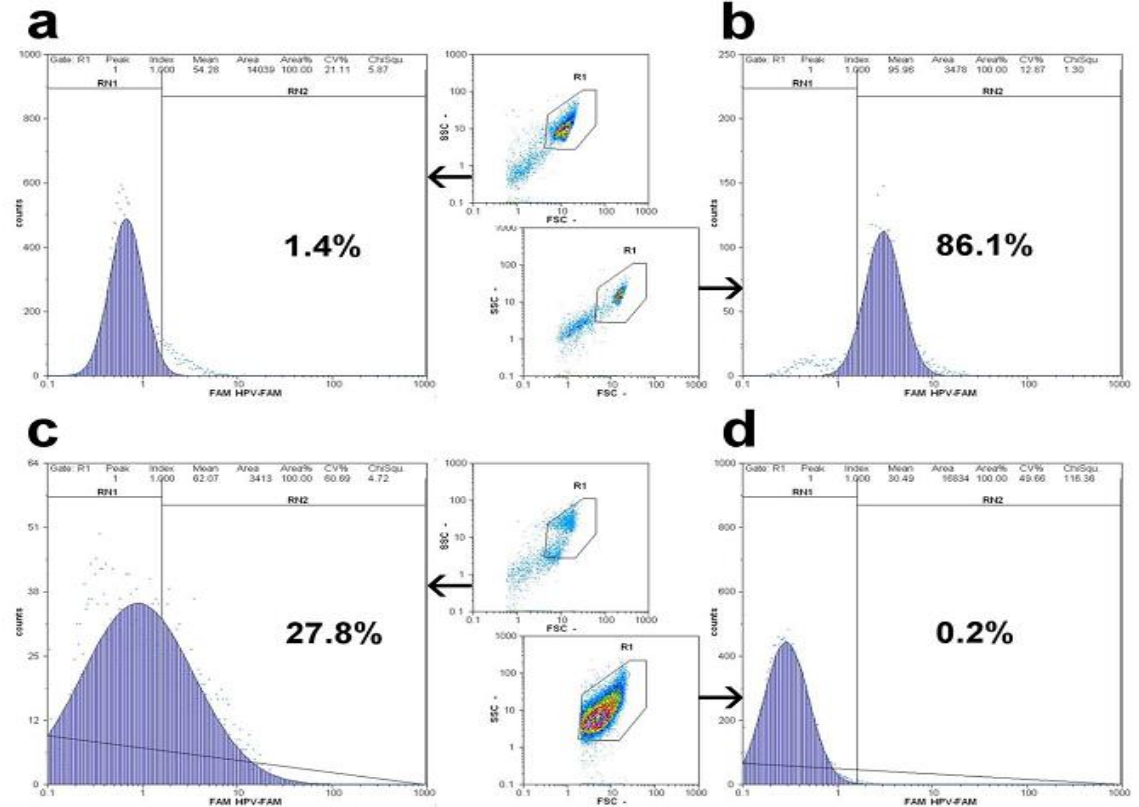
✓ Βασίζεται στον υβριδισμό ενός FAM σημασμένου μίγματος HPV ιχνηθετών σε κύτταρα εξωτραχήλου προερχόμενα από εναιώρημα σε φιαλίδιο κυτταρολογίας υγρής φάσης (ThinPrep®)

Narimatsu R, et all (2005) High-Throughput Cervical Cancer Screening Using Intracellular Human Papillomavirus E6 and E7 mRNA Quantification by Flow Cytometry. American Journal of Clinical Pathology 123: 716–723.

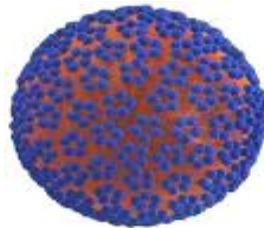


# Analysis

- Gating of cells of interest using a FSC/SSC dot plot
- Percentage of green fluorescent cells is calculated on gated cells
- Cut-off for cells set by using clinically negative samples (1.5% for our lab)



Spathis A et al. (2012) mRNA and DNA Detection of Human Papillomaviruses in Women of All Ages Attending Two Colposcopy Clinics. PLoS ONE 7(11): e49205. doi:10.1371/journal.pone.0049205

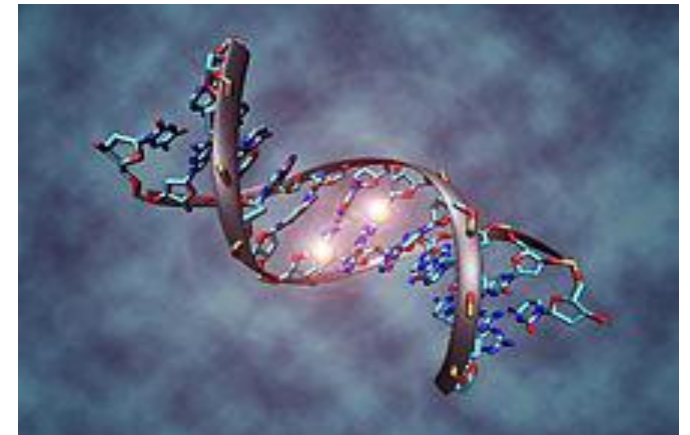




|                 | Aptima HPV  | HPV Oncotect                                       | NASBA NucliSens HPV   |
|-----------------|---|--|---|
| Detection       | 14 HR   | All HR   | 5 HR  |
| Typing          | 16, 18/45   | None   | 16, 18, 31, 33, 45  |
| Isolation       | mRNA  | None   | DNA & RNA   |
| Detection       | Chemiluminescence   | Fluorescence                                       | Fluorescence  |
| Automation      | Full  | Semi   | Semi  |
| Result analysis | Automated   | Lab cut-off  | Automated   |
| QA              | Calibrators / IC  | Fixed cell lines                                   | PC, IC  |
| Throughput      | <b>275 / 8h</b>   | 24 / 4h  | 28 / 8h   |
| Expandability   | None  | mRNA or proteins                                   | None  |
| References      | (Szarewski, 2008; Castle, 2011; Ratnam, 2011; Castle, 2015; Reid, 2016) | (Kottaridi, 2010; Coquillard, 2011; Spathis, 2012) | (Molden, 2006; Szarewski, 2008; Keegan, 2009; Sørbye, 2014) |

# Επιγενετικές αλλαγές και HPV

- Η εμμένουσα λοίμωξη και ενσωμάτωση του ιού-επιγενετικές αλλαγές
- Μεθυλίωση του ιικού DNA και του DNA του ξενιστή, τροποποιήσεις της ιστόνης και διαφοροποίηση της έκφρασης μη κωδικών RNA μορίων (miRNAs, lncRNAs)
- Η ρύθμιση της μεταγραφής των γονιδίων του ξενιστή μέσω επιγενετικών μηχανισμών, συμπληρώνει τη δράση των ογκοπρωτεϊνών προς τον κυτταρικό μετασχηματισμό (αύξηση κυτταρικού πολλαπλασιασμού, διαφυγή ανοσιακής επιτήρησης, αναστολή απόπτωσης)



# Μεθυλίωση του ιικού DNA

- Μηχανισμός που χρησιμοποιεί ο ιός για να παραμείνει στον ξενιστή
- Η μεθυλίωση στα *L1* και *L2* γονίδια παρουσιάζει αυξημένα ποσοστά στα καρκινικά κύτταρα
- Η μεθυλίωση στην *LCR* αντικρουόμενα ευρήματα
- Η μεθυλίωση στο 3' *LCR* μπορεί να σχετίζεται με την επισωματική κατάσταση του ιού

## Molecular progression to cervical precancer, epigenetic switch or sequential model?

Belinda Nedjai<sup>1,2</sup>, Caroline Reuter<sup>1,3</sup>, Amar Ahmad<sup>1,3</sup>, Rawinder Banwait<sup>1</sup>, Rhian Warman<sup>1</sup>, James Carton<sup>2</sup>, Sabrina Boer<sup>3</sup>, Jack Czick<sup>1</sup> and Attila T. Lorincz<sup>1</sup>

<sup>1</sup>Barts and the London School of Medicine, Charterhouse Square, Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, London, EC1M 6BQ, United Kingdom

<sup>2</sup>Department of Histopathology, Charing Cross Hospital, Fulham Palace Road, London, W6 8RF, United Kingdom

<sup>3</sup>Department of Urology, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands

The evolution of precancerous cervical lesions is poorly understood. A widely held model of cervical intraepithelial neoplasia grade 3 (CIN3) development is sequential progression from normal through CIN1 and CIN2 to CIN3. Another hypothesis, the “molecular switch” model, postulates that CIN3 can evolve directly from human papillomavirus (HPV)-infected normal epithelium without progressing through CIN1 and CIN2. To shed light on this process, we compared DNA methylation of selected human biomarkers and HPV types in two groups of CIN1: CIN1 that were near or adjacent to CIN3 (adjacent-CIN1) and CIN1 that were the principal lesions with no CIN3 detected (principal-CIN1). 354 CIN (CIN1 and CIN3) and normal tissue areas were dissected and typed for HPV from 127 women who underwent loop electrosurgical excision procedures (LEEP). Methylation of genes *EPB41L3* and the viral regions of HPV16-L1/L2, HPV18-L2, HPV31-L1, and HPV33-L2 were determined by a highly accurate quantitative pyrosequencing of bisulfite converted DNA. There was a significant trend of increased methylation with disease grade comparing normal to CIN1 and CIN3 ( $p < 0.0001$ ). Adjacent-CIN1 predominantly shared the same HPV types as the CIN3, however, methylation differed substantially between adjacent-CIN1 and CIN3 ( $p = 0.008$ ). In contrast diagnostically principal-CIN1 had an indistinguishable methylation distribution compared to adjacent-CIN1 (*EPB41L3*:  $p = 0.49$ ; HPVme-All:  $p = 0.11$ ). Our results suggest that progression from normal epithelium to CIN1 or CIN3 is usually promoted by the same HPV type but occurs via distinct DNA epigenotypes, thus favoring the “molecular switch” model.

## Methylation of the L1 gene and integration of human papillomavirus 16 and 18 in cervical carcinoma and premalignant lesions

FRANCISCO ISRAEL TORRES-ROJAS<sup>1</sup>, LUZ DEL CARMEN ALARCÓN-ROMERO<sup>2</sup>, MARCO ANTONIO LEYVA-VÁZQUEZ<sup>1</sup>, JULIO ORTIZ-ORTIZ<sup>1</sup>, MIGUEL ÁNGEL MENDOZA-CATALÁN<sup>1</sup>, DANIEL HERNÁNDEZ-SOTELO<sup>1</sup>, OSCAR DEL MORAL-HERNÁNDEZ<sup>1</sup>, HUGO ALBERTO RODRÍGUEZ-RUIZ<sup>1</sup>, DINORAH LEYVA-ILLADES<sup>1</sup>, EUGENIA FLORES-ALFARO<sup>1</sup> and BERENICE ILLADES-AGUIAR<sup>1</sup>

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**Abstract.** High-risk human papillomavirus (HPV) is the primary cause of cervical carcinoma (CC). Viral integration into the host chromosomes is associated with neoplastic progression, and epigenetic changes may occur as a result. The objective of the present study was to analyze HPV L1 gene methylation and to compare the use of quantitative polymerase chain reaction (qPCR), *in situ* hybridization (ISH) and L1 methylation analysis as methods for detecting HPV integration. Cervical scrapes or biopsy samples positive for HPV 16 or 18, from 187 female patients with CC, squamous intraepithelial lesions (SILs) or no intraepithelial lesion (non-IL) were analyzed. Methylation of the L1 gene was determined using bisulfite modification followed by PCR, and HPV integration was subsequently analyzed. HPV 16 L1 gene methylation was revealed to increase with histological grade, with statistically significant differences observed as follows: Low-grade SIL vs. CC,  $P < 0.0001$  and non-IL vs. CC,  $P < 0.0001$ . HPV 18 L1 gene methylation also increased according to histological grade, however, no statistically significant differences were observed. Methylation at CpG site 5608 of the HPV 16 L1 gene was associated with all grades of cervical lesions, whereas methylation at CpG site 5617 demonstrated the strongest association with CC (odds ratio, 42.5; 95% confidence interval, 4.7-1861;  $P < 0.0001$ ). The concordance rates between the various methods for the detection of the physical status of

HPV 16 and HPV 18 were 96.1% for qPCR and ISH, 76.7% for qPCR and L1 gene methylation, and 84.8% for ISH and L1 gene methylation. In conclusion, methylation of the HPV 16 L1 gene increases significantly according to the grade of the cervical lesion, and methylation at CpG sites 5608 and 5617 of this gene may be used as prognostic biomarkers. ISH and L1 gene methylation have good concordance with qPCR with regards to the detection of HPV integration. Therefore, these are useful methods in determining the physical state of HPV.

## Increased methylation of human papillomavirus type 16 DNA is associated with the severity of cervical lesions in infected females from northeast China

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**Abstract.** Hypermethylation of the cytosine-phosphate-guanine (CpG) sites located at the 3'-major capsid protein L1 (3'L1) and the long control region (LCR) of the human papillomavirus (HPV) genome may be associated with the progression of cervical cancer (CC). However, the methylation status of the LCR of HPV type 16 DNA remains to be elucidated in an infected Chinese population. The aim of the present study was to investigate the association between methylation of the HPV 16 L1 gene and LCR, and the severity of cervical lesions in infected female patients. Therefore, bisulfite modification, polymerase chain reaction amplification and sequencing were used to analyze 122 HPV 16-positive clinical cervical swabs obtained from patients in northeastern China. The proportion of methylated samples at each of the 7 CpG sites within the 3'-L1/5'-LCR and 5 CpG sites within the promoter region was significantly increased in patients with CC, compared with that observed in high-grade squamous intraepithelial lesions (HSIL) and normal tissue/low-grade intraepithelial lesions (LSIL) ( $\chi^2$  test,  $P < 0.01$ ). The mean methylation frequencies of the CpG sites 7089 and 7143 exhibited an area under the curve value of 0.822 [95% confidence interval (CI)=0.733-0.911] for distinguishing CC from other lesions; 0.787 (95% CI=0.700-0.874) for distinguishing normal/LSIL from HSIL and CC, and 0.763 (95% CI=0.652-0.874) for distinguishing CC from HSIL. These results suggest that the

methylation of CpG sites within the HPV 16 3'-L1 and LCR region is correlated with the severity of cervical lesions. Quantification of HPV DNA methylation in the L1 gene and promoter region appears to provide a promising novel marker for distinguishing between normal tissue/LSIL, HSIL and CC in a Chinese population.

## Quantitative Measurement of L1 Human Papillomavirus Type 16 Methylation for the Prediction of Preinvasive and Invasive Cervical Disease

Christine Kottaridi<sup>1</sup>, Maria Kyrgiou<sup>2,3</sup>, Abraham Poulidakis<sup>1</sup>, Maria Magkana<sup>1</sup>, Evangelia Aga<sup>1</sup>, Aris Spathis<sup>1</sup>, Anito Mitre<sup>4,5</sup>, George Makris<sup>1</sup>, Charalampos Chrelias<sup>1</sup>, Vassiliki Mpakou<sup>6</sup>, Evangelos Parsakevaldis<sup>1</sup>, John G. Panayiotides<sup>7</sup> and Petros Karakitsos<sup>1</sup>

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**Background.** Methylation of the human papillomavirus (HPV) DNA has been proposed as a novel biomarker. Here, we correlated the mean methylation level of 12 CpG sites within the L1 gene, to the histological grade of cervical precancer and cancer. We assessed whether HPV L1 gene methylation can predict the presence of high-grade disease at histology in women testing positive for HPV16 genotype.

**Methods.** Pyrosequencing was used for DNA methylation quantification and 145 women were recruited.

**Results.** We found that the L1 HPV16 mean methylation ( $\pm$ SD) significantly increased with disease severity (cervical intraepithelial neoplasia [CIN] 3, 17.9% [ $\pm$ 7.2] vs CIN2, 11.6% [ $\pm$ 6.5],  $P < .001$  or vs CIN1, 9.0% [ $\pm$ 3.5],  $P < .001$ ). Mean methylation was a good predictor of CIN3+ cases; the area under the curve was higher for sites 5611 in the prediction of CIN2+ and higher for position 7145 for CIN3+. The evaluation of different methylation thresholds for the prediction of CIN3+ showed that the optimal balance of sensitivity and specificity (75.7% and 77.5%, respectively) and positive and negative predictive values (74.7% and 78.5%, respectively) was achieved for a methylation of 14.0% with overall accuracy of 76.7%.

**Conclusions.** Elevated methylation level is associated with increased disease severity and has good ability to discriminate HPV16-positive women that have high-grade disease or worse.

**Keywords.** cervical intraepithelial neoplasia; CIN; HPV L1 gene methylation; pyrosequencing; Human Papillomavirus

## Searching HPV genome for methylation sites involved in molecular progression to cervical precancer

Christine Kottaridi<sup>1,2,3</sup>, Danaï Leventakou<sup>1</sup>, Abraham Poulidakis<sup>1</sup>, Vasileios Pergaliotis<sup>2</sup>, George Chrelias<sup>2</sup>, Eugenia Patsouri<sup>1</sup>, Andriani Zacharotou<sup>1</sup>, Eleni Panopoulou<sup>1</sup>, Vasileia Damaskou<sup>1</sup>, Vasileios Sioulas<sup>2</sup>, Charalampos Chrelias<sup>2</sup>, Sofia Kalantaridou<sup>2</sup>, Ioannis G. Panayiotides<sup>1</sup>

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7270

ATACGTGT

7429, 7455, 7461

7535, 7553

7862, 31, 37, 43, 52, 58

494, 502, 506

765, 780, 790

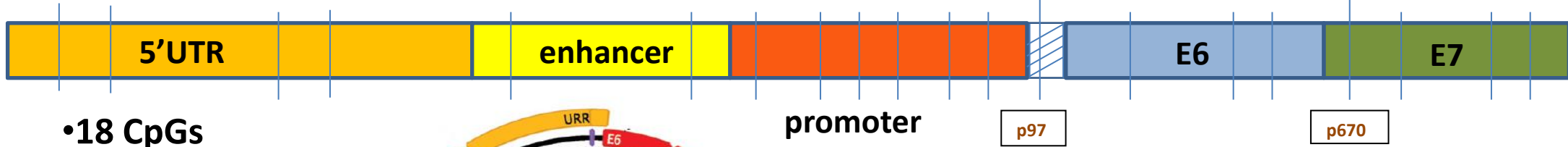
CG TTATAAA...GG GTTGAAG CGAATTCG

ACGTATGG...GTAGGAAACG

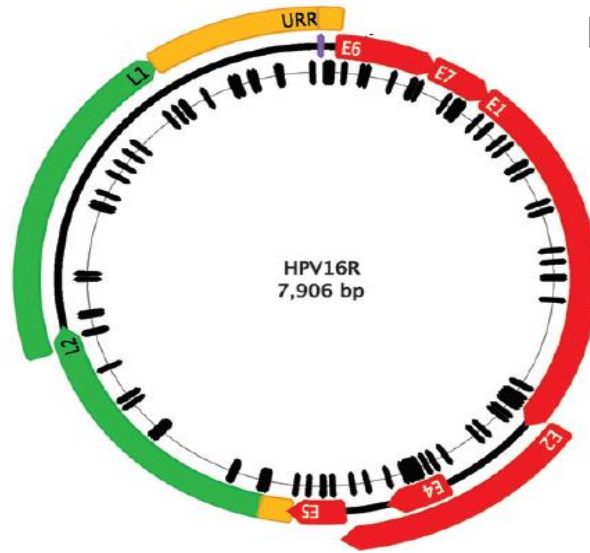
CGAT..CGT..CG..TCG..CGA..CG

CGGTGGA TCGGTTCG

CG.. CGTA..TTCG



- 18 CpGs
- ✓ 12 UTR CpGs
- ✓ 3 E6 CpGs
- ✓ 3 E7 CpGs



PCR1 primers VL5602F-R, 241bp

Blue: seq primers

Yellow: CpGs 5602, 5608, 5611, 5617

Green: CpGs: 5709, 5726

PCR 2 VL5927F-R, 248bp

purple: seq primers

grey: CpGs 5927, 5963

PCR 3 VL6367F-R, 279bp

CpGs: 6367, 6389/ 6457/ 6581

PCR 4 VL6650F-R, 245bp

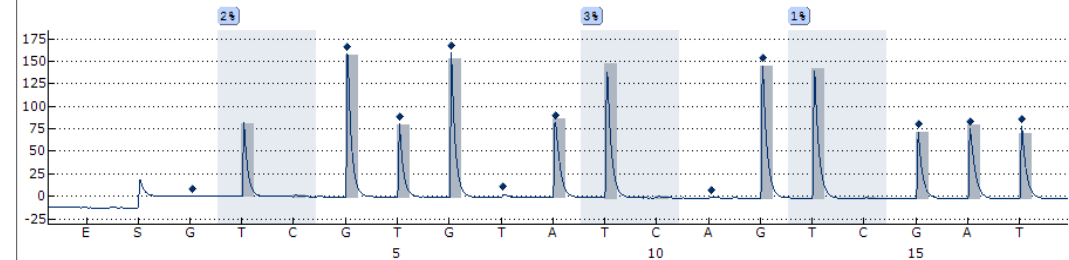
CpGs 6650, / 6731, / 6796

PCR 5 VL7034F-R, 190 bp

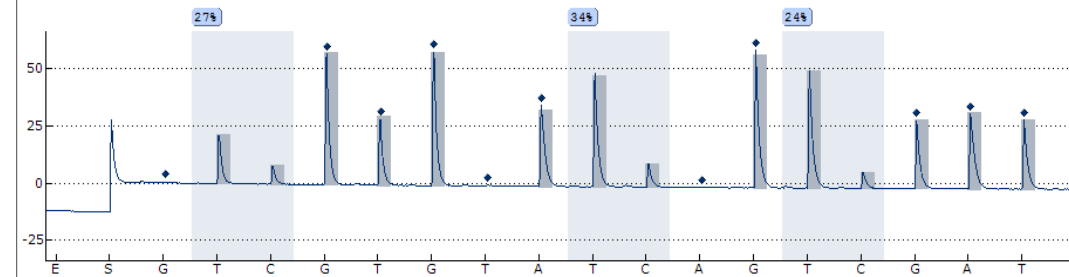
CpGs: 7034, / 7091, / 7136, 7145

L1 gene:12 CpGs

CB C/TGGTGGATC/TGGTC/TGATGTATGTTTTGT



BB C/TGGTGGATC/TGGTC/TGATGTATGTTTTGT





# L1 γονίδιο

•Αύξηση του ποσοστού μεθυλίωσης σε υψηλού βαθμού αλλοιώσεις

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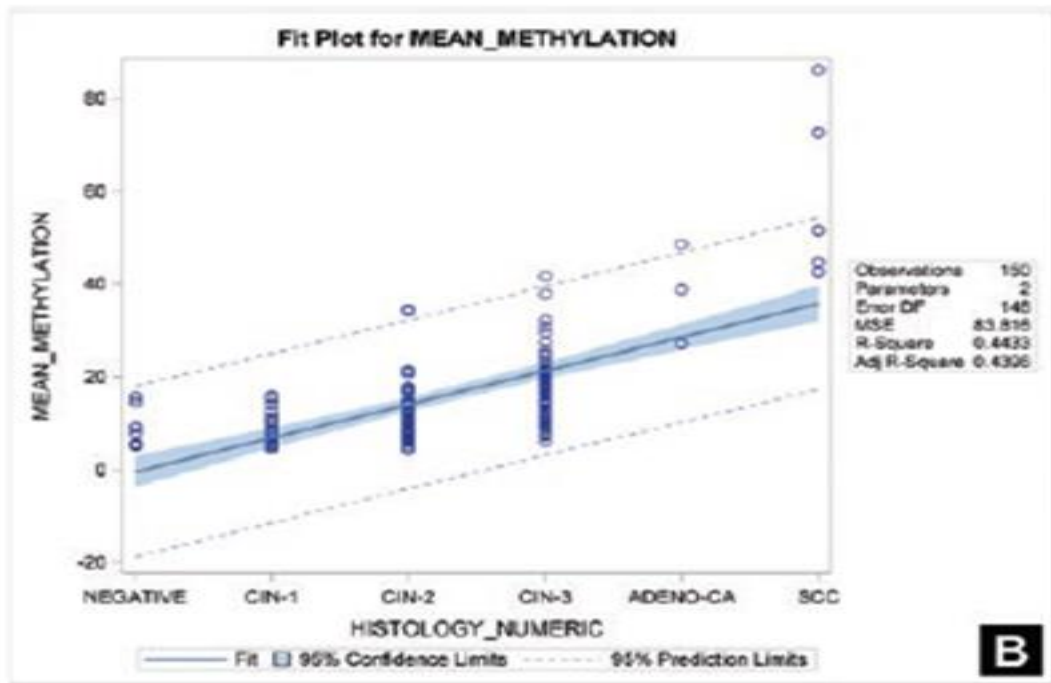
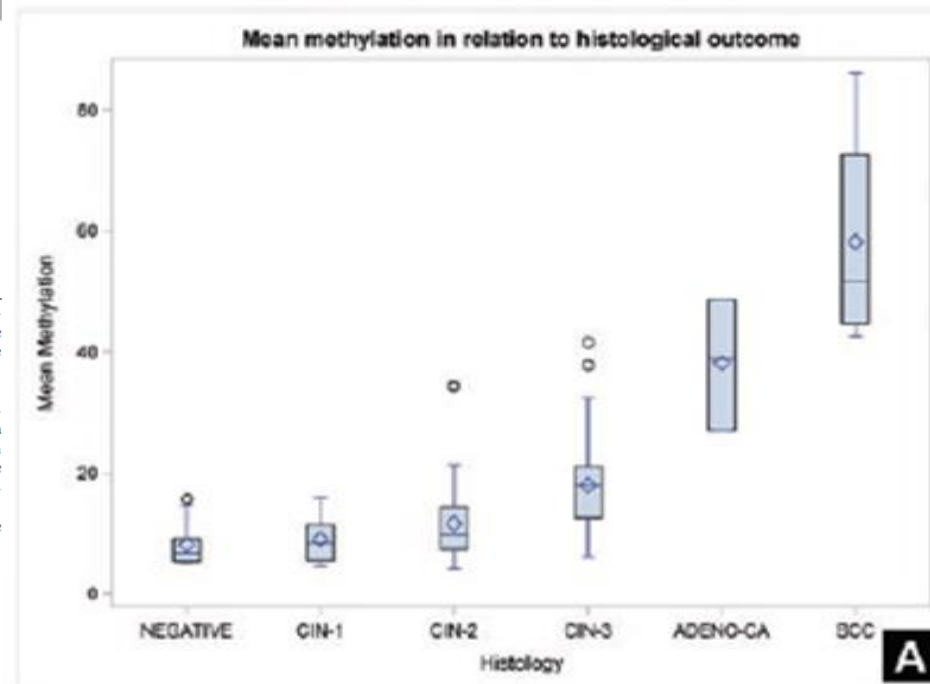
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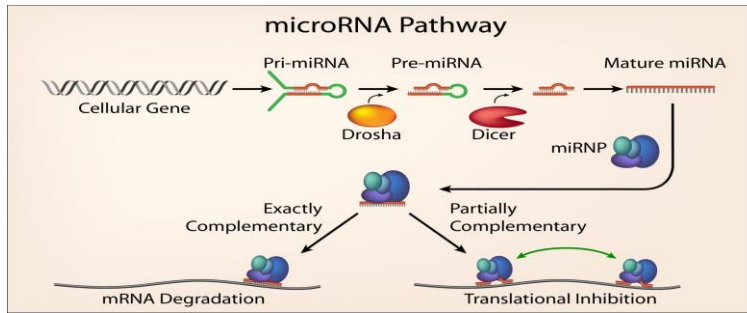


## Searching HPV genome for methylation sites involved in molecular progression to cervical precancer

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## Αποσιώπηση της γονιδιακής έκφρασης

- Η διερεύνηση του προφίλ έκφρασης διαφόρων microRNAs που συμμετέχουν στη ρύθμιση του μονοπατιού σηματοδότησης PD-1/PD-L1, προκειμένου να κατανοηθεί πληρέστερα ο ρόλος τους στον καρκίνο του τραχήλου της μήτρας και να συσχετιστούν με την παρουσία του HPV.
- Η έκφραση των miRNAs απορρυθμίζεται στον καρκίνο, επομένως θα εκτιμηθεί η πιθανή χρήση των μελετηθέντων μορίων ως προγνωστικών δεικτών για την εξέλιξη της νόσου.

A rectangular yellow sticky note is pinned to a brown corkboard. A single orange pushpin is attached to the top center of the note. The words "Thank you" are written in a red, cursive-style font across the middle of the note.

Thank you