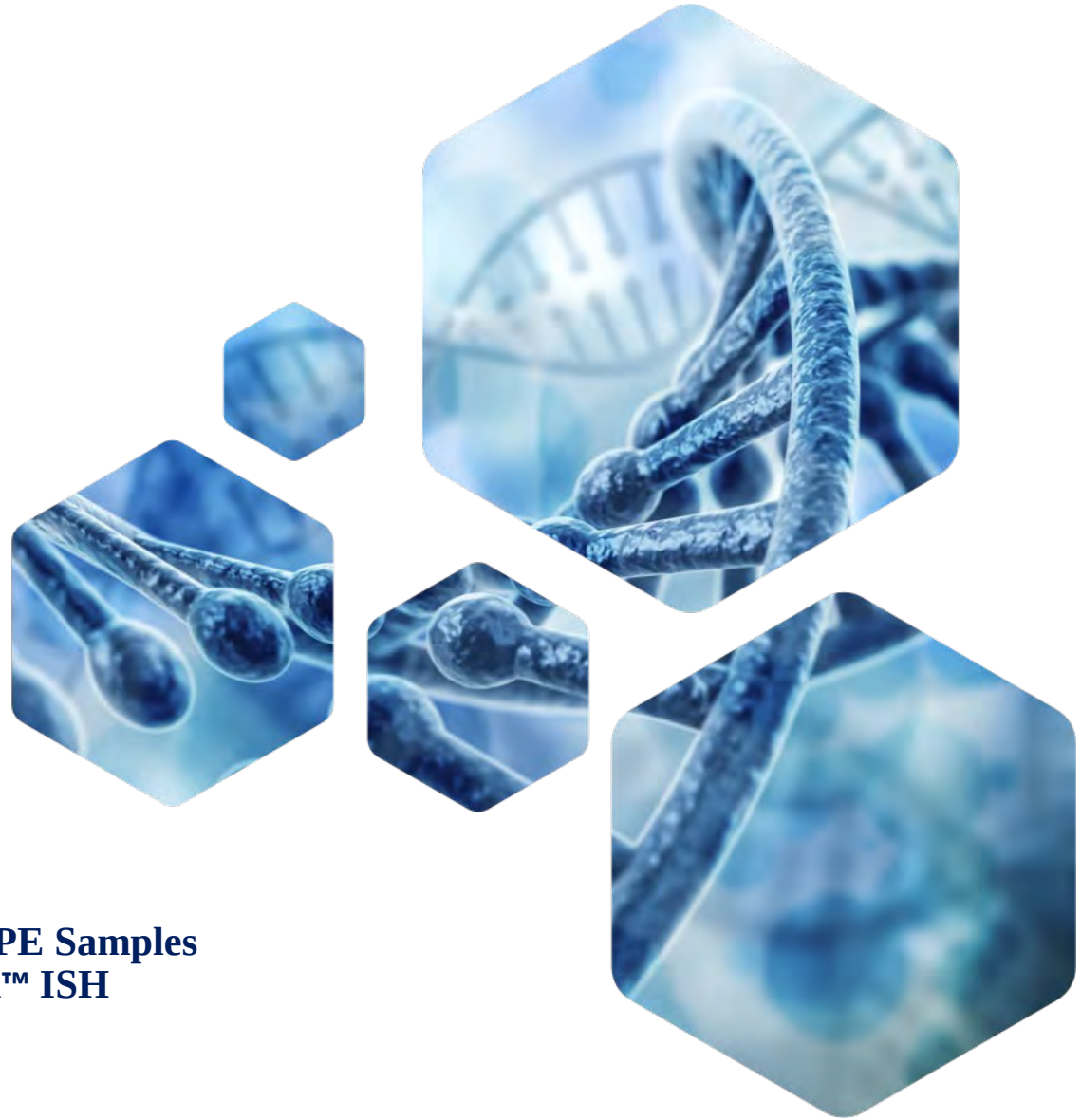


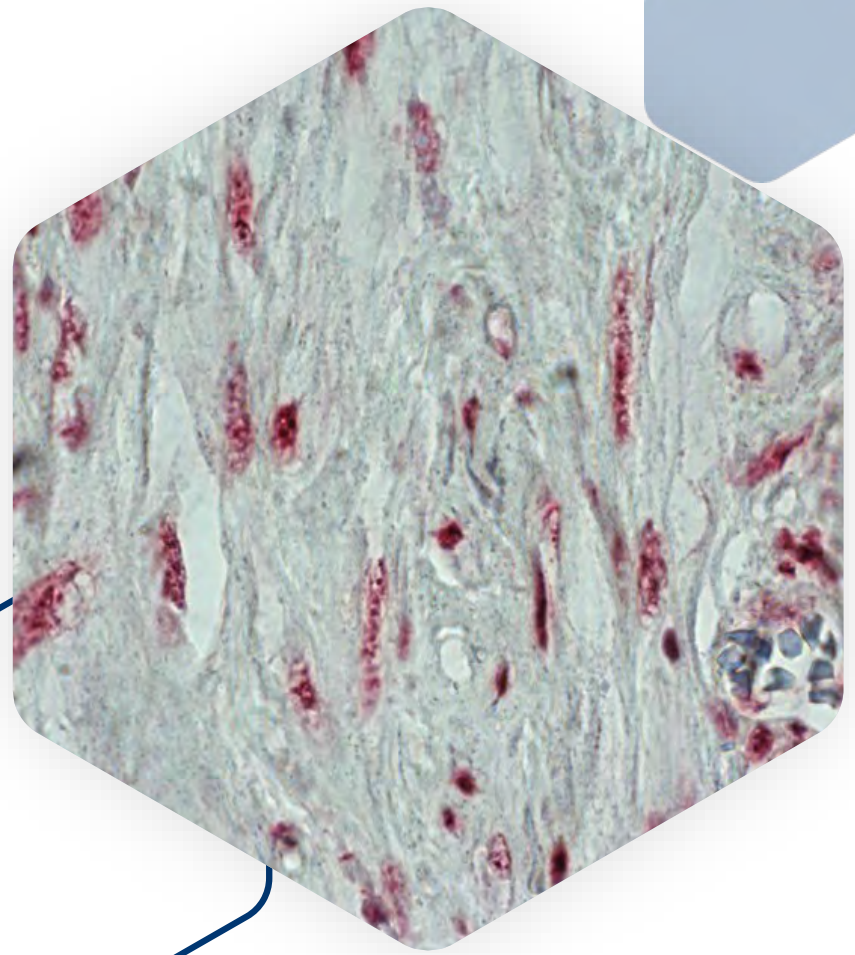
Detection of Human Papillomavirus (HPV) Nucleic Acid in FFPE Samples with AMPIVIEW® RNA Probes, Powered by Enzo's LoopRNA™ ISH Technology

Deborah Holzapfel, Ph.D. – **VIRCAN 2024**

AMPIVIEW® RNA Probes

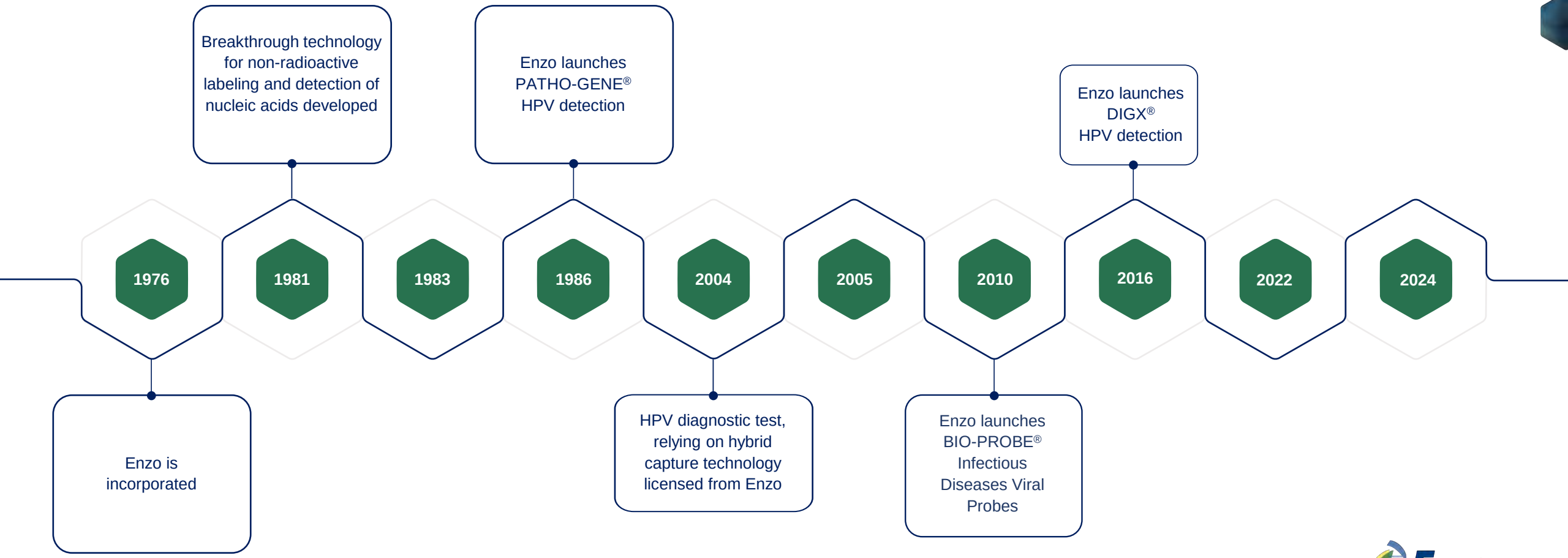
Outline

- Enzo Technologies
- Introduction to AMPIVIEW® RNA Probes
- HPV Detection
- Other Viral Detection
- Type of Detection
- Probes in the Pipeline
- Conclusion



Over 45 years of Innovation

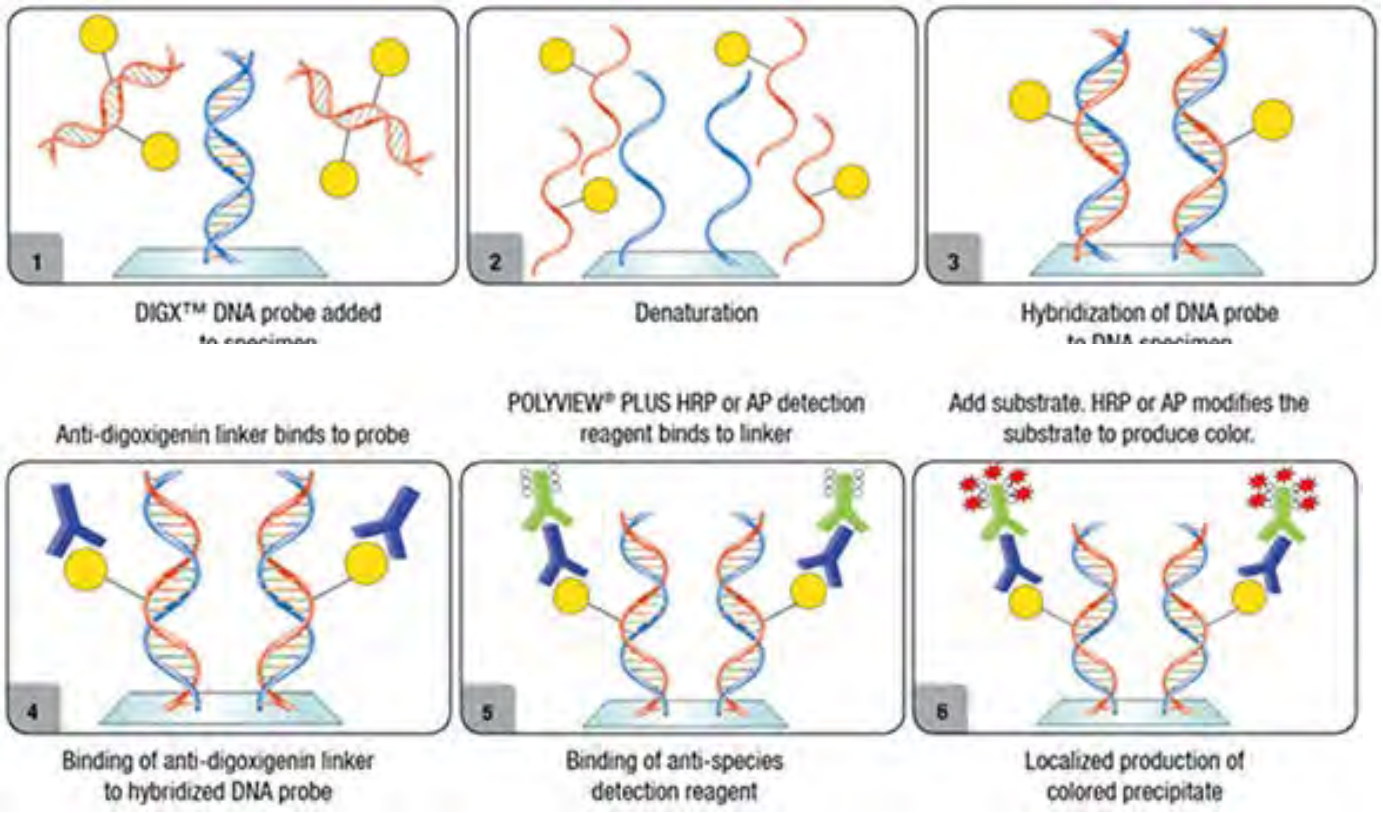
Enabling Transformative Science for Decades



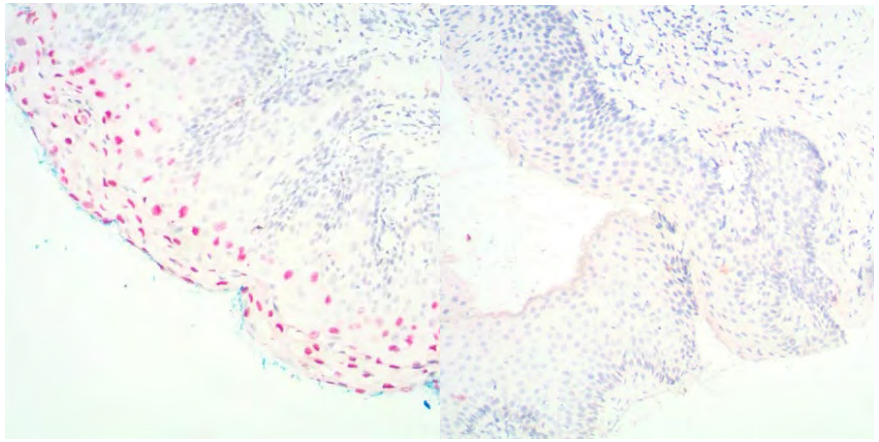
In situ Hybridization

Chromogenic *in situ* Hybridization (ISH)

in situ hybridization (ISH) is a laboratory technique that allows for precise localization of specific segments of nucleic acid within a histologic section.



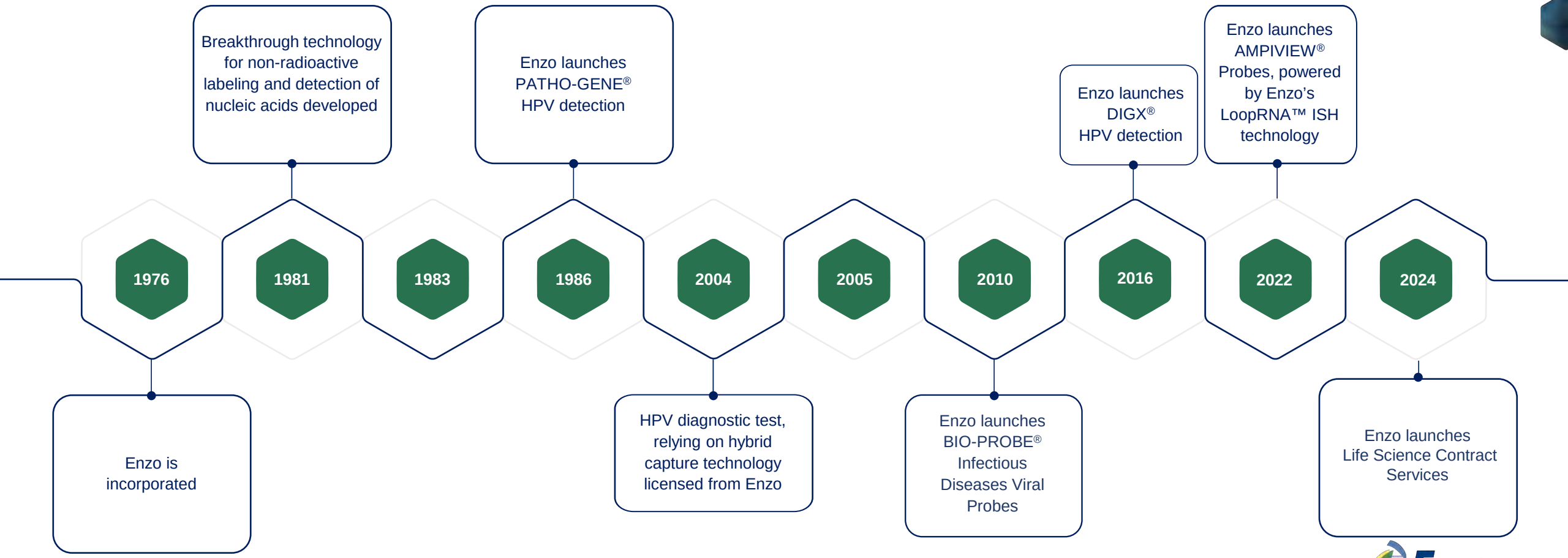
A. B.



Positive detection of HPV 16, 18, 31, 33, 51 with PATHO-GENE® HPV 16/18/31/33/51 probes in A. HPV infected cervical FFPE tissue and B. vulva FFPE tissue

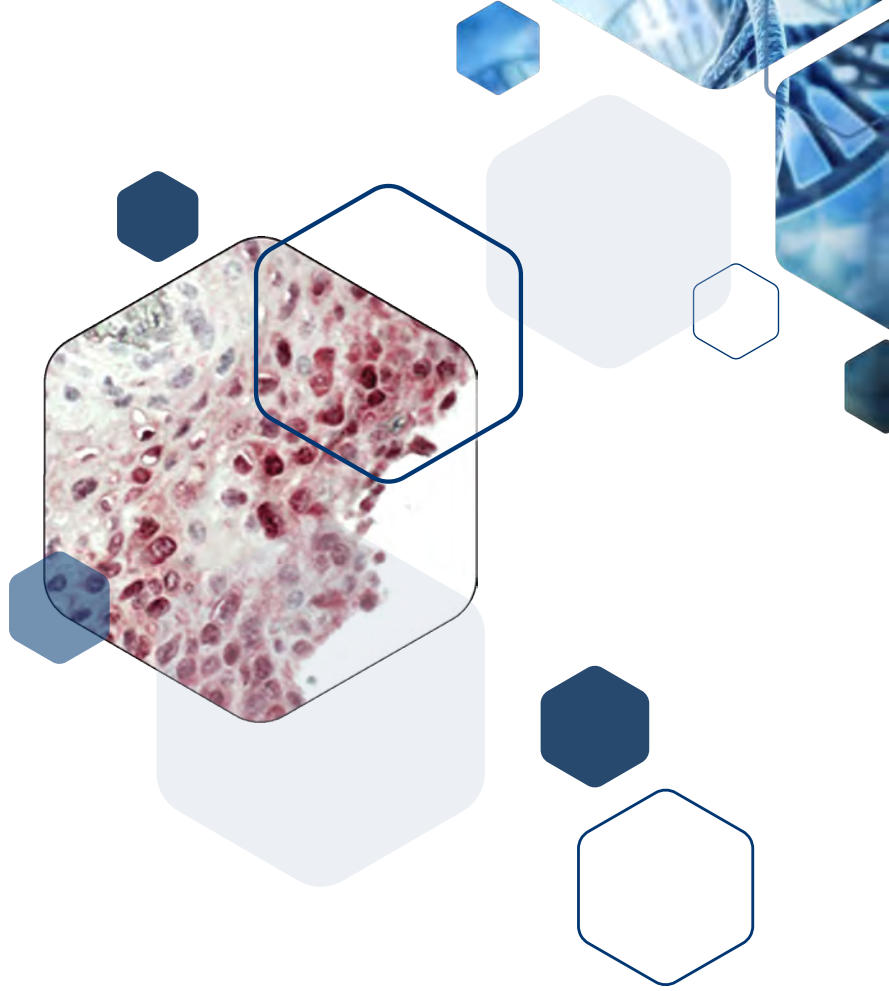
Over 45 years of Innovation

Enabling Transformative Science for Decades



AMPIVIEW® RNA Probes

Powered vy Enzo’s LoopRNA™ ISH Technology



- Visualize nucleic acid at the single cell level
- High sensitivity - as sensitive as PCR
- Flexible protocols
- Cost-effective
- Adaptable workflow: manual or automated
- No specialized equipment needed
- No specialized reagents needed – compatible with IHC reagents

How it works?

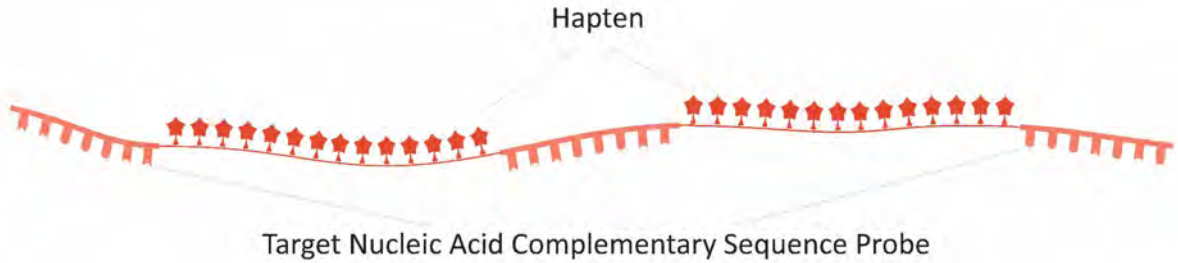
AMPIVIEW® RNA Probes

How it works?

AMPIVIEW® RNA Probes

DNA or RNA target

AMPIVIEW® RNA Probe

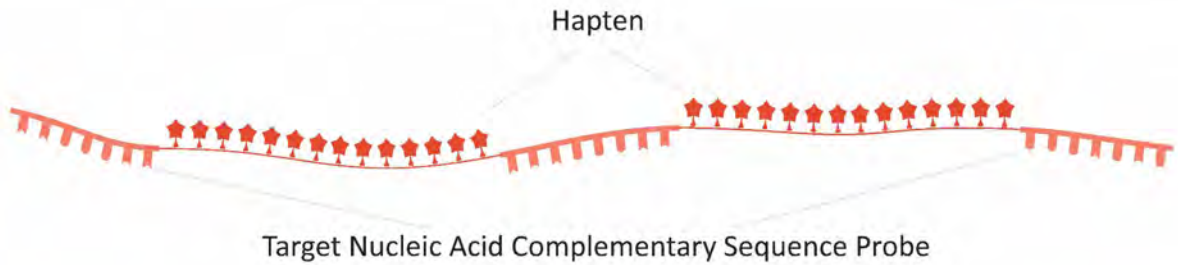


How it works?

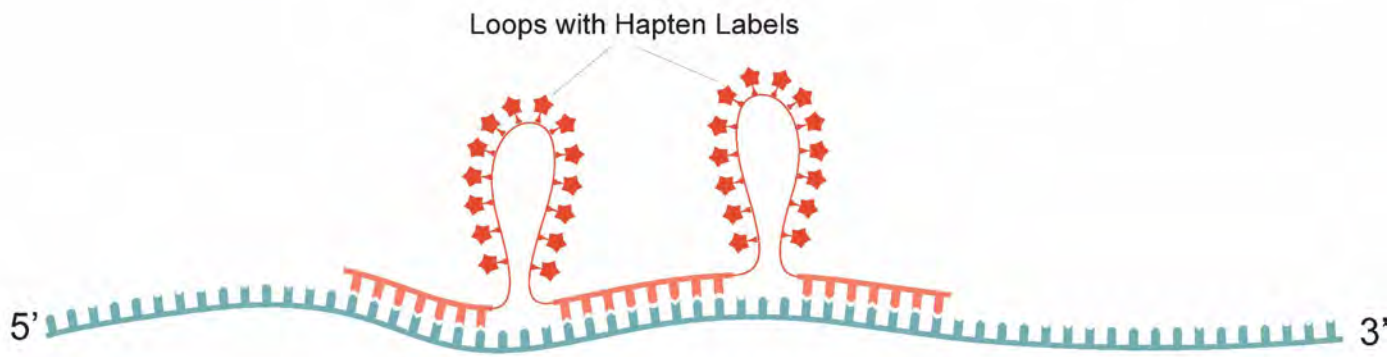
AMPIVIEW® RNA Probes

DNA or RNA target

AMPIVIEW® RNA Probe



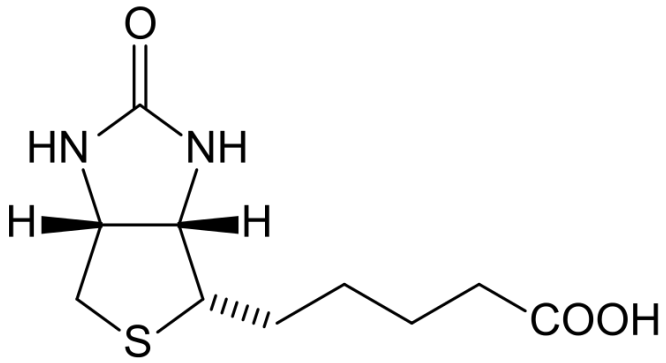
Hybridization



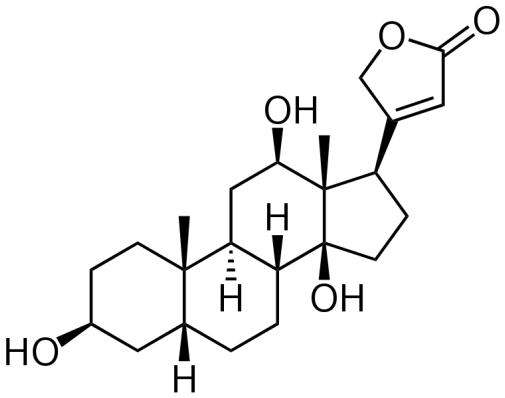
How it works?

AMPIVIEW® RNA Probes Haptens

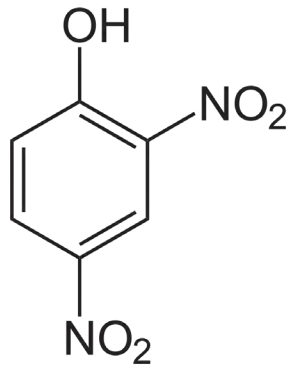
Biotin



Digoxigenin

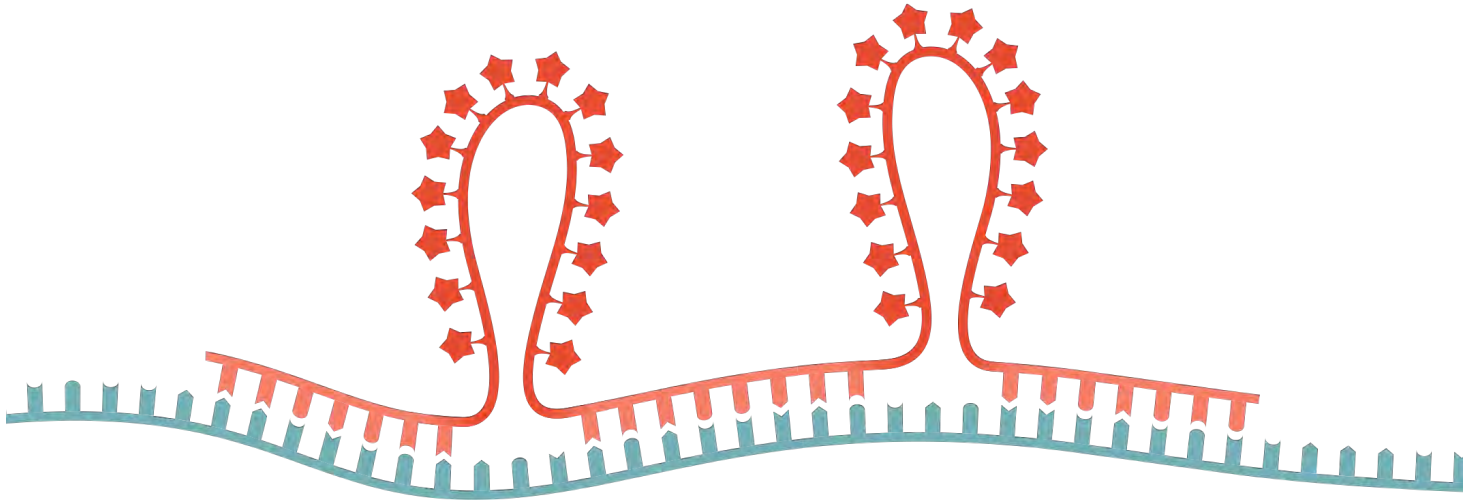


Dinitrophenol (DNP)



How it works?

One-Step Detection: Biotin-labeled AMPIVIEW® RNA probes with SAVIEW® PLUS detection system



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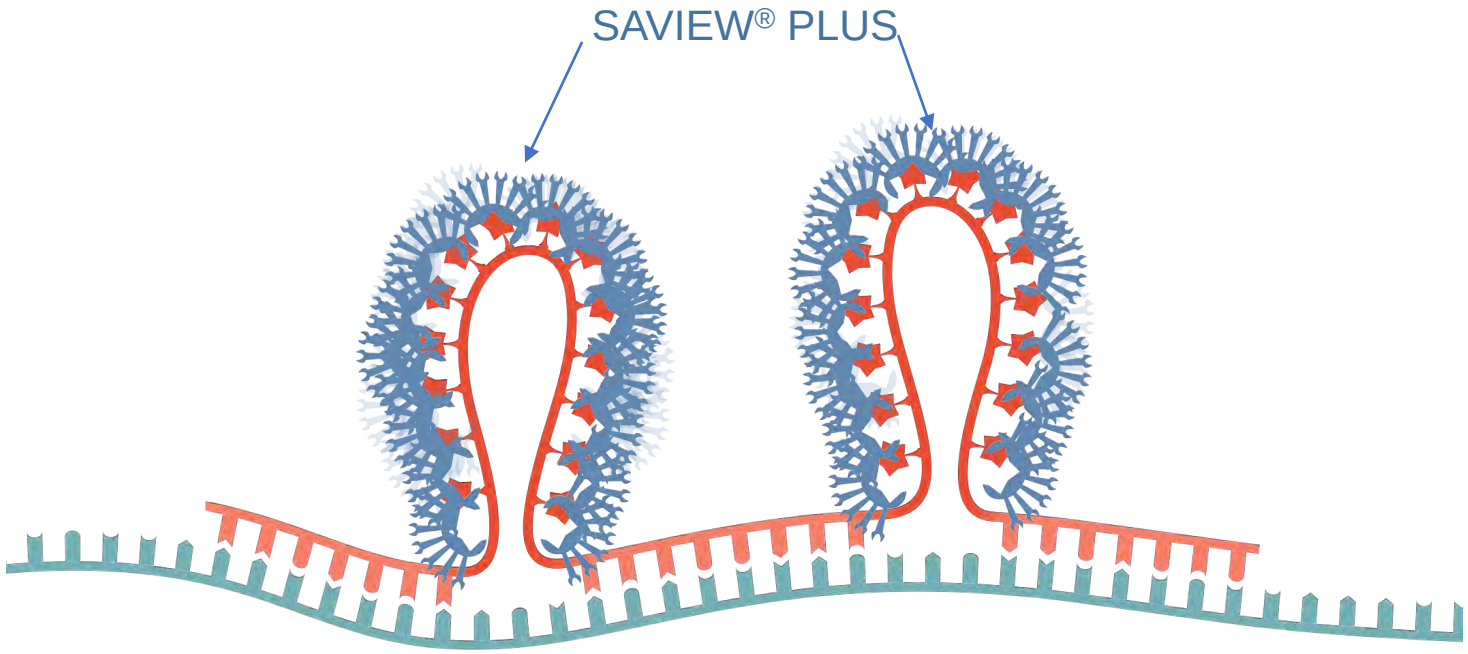

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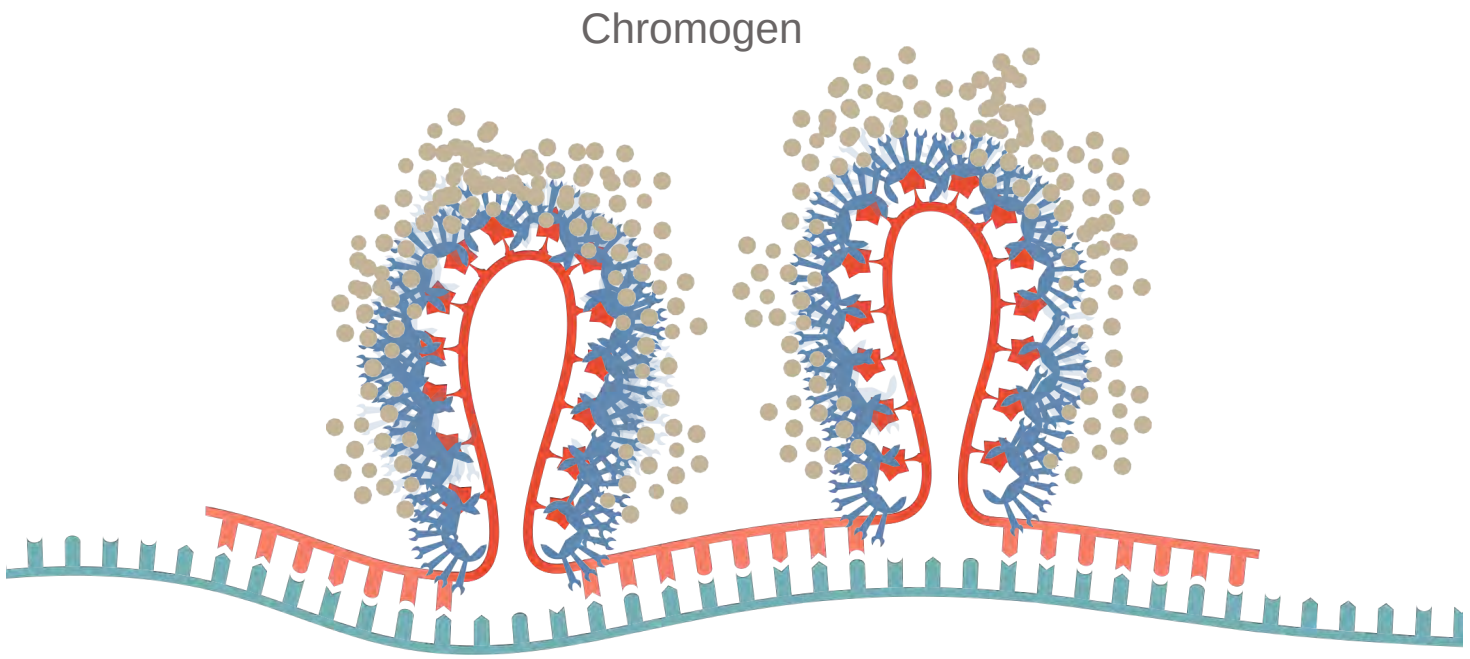

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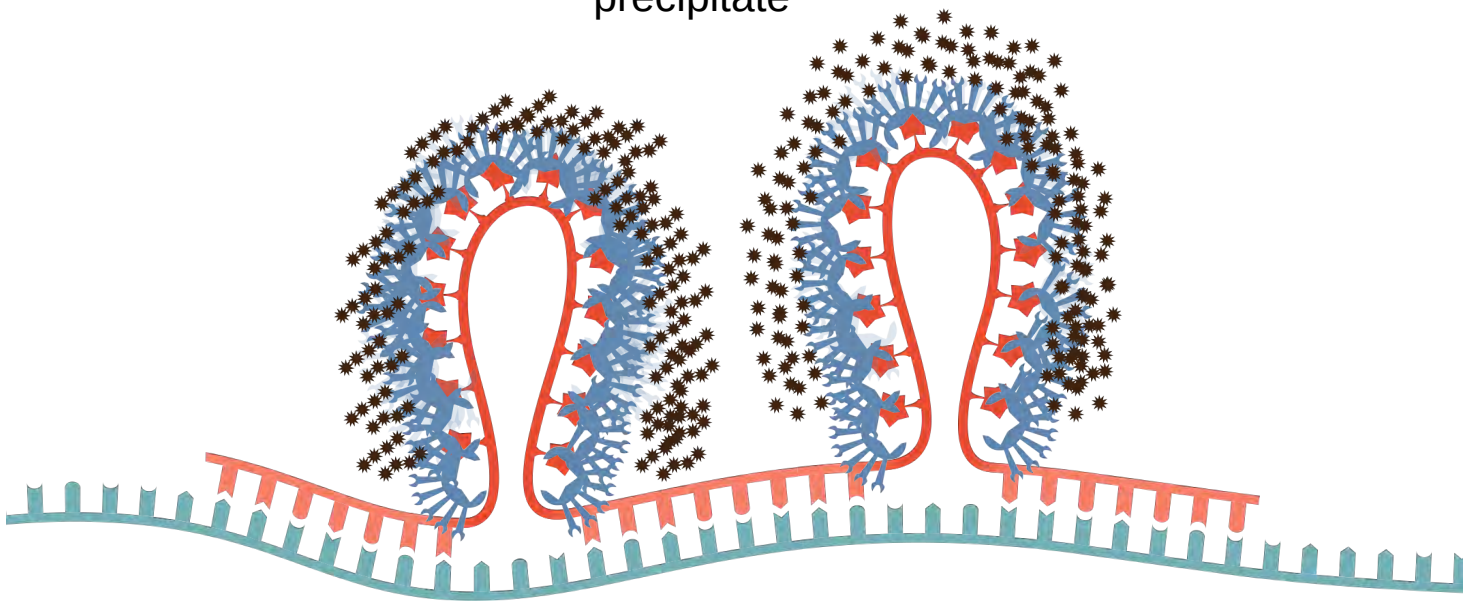
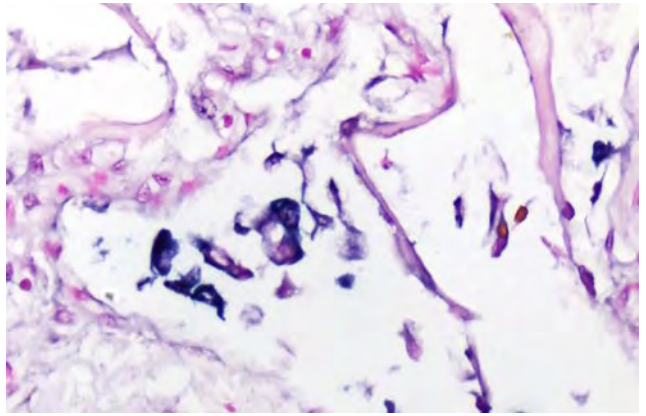
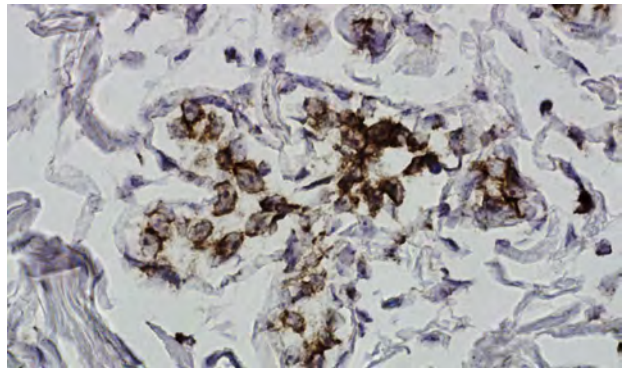




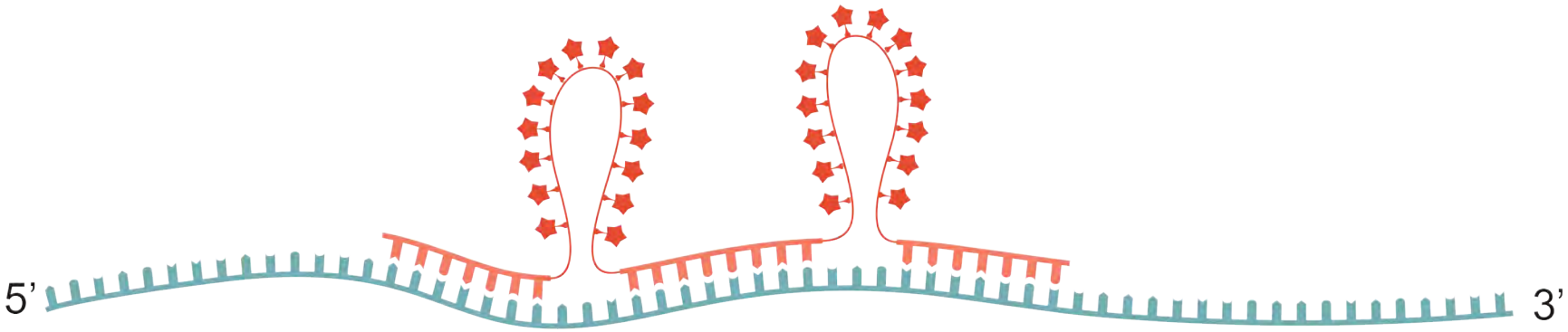
SARS-CoV-2 detection (blue) in lung tissue developed with SAVIEW® PLUS AP/Blue Chromogen



HER-2 detection (brown) in breast tissue developed with SAVIEW® PLUS HRP/DAB Chromogen

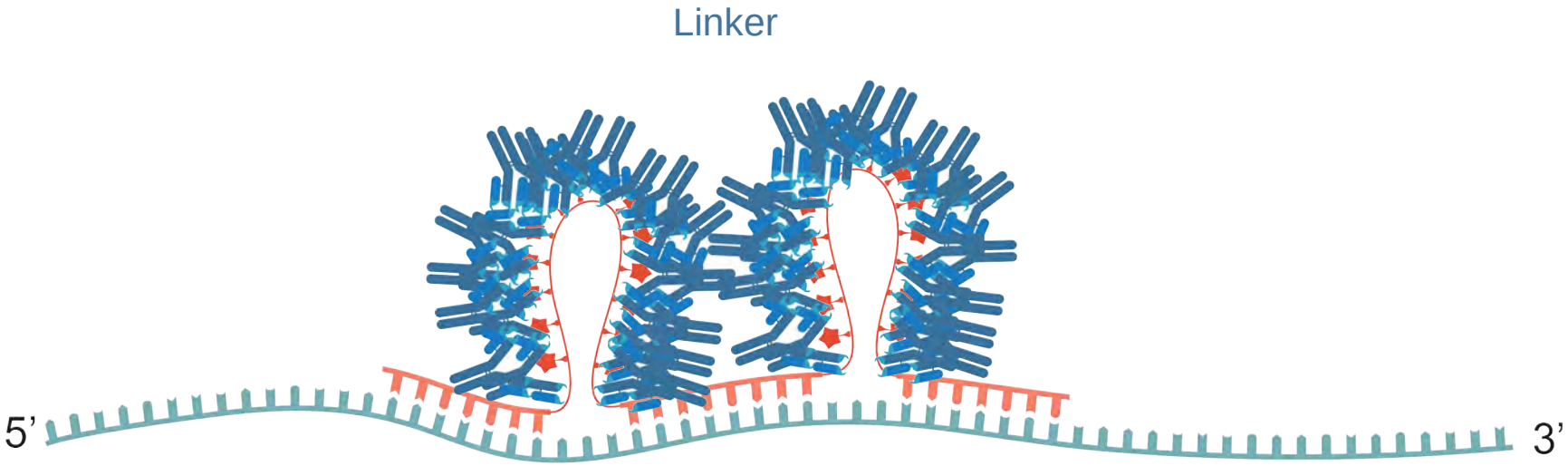
How it works?

Two-Step Detection: AMPIVIEW® RNA Probes with POLYVIEW® PLUS detection system



How it works?

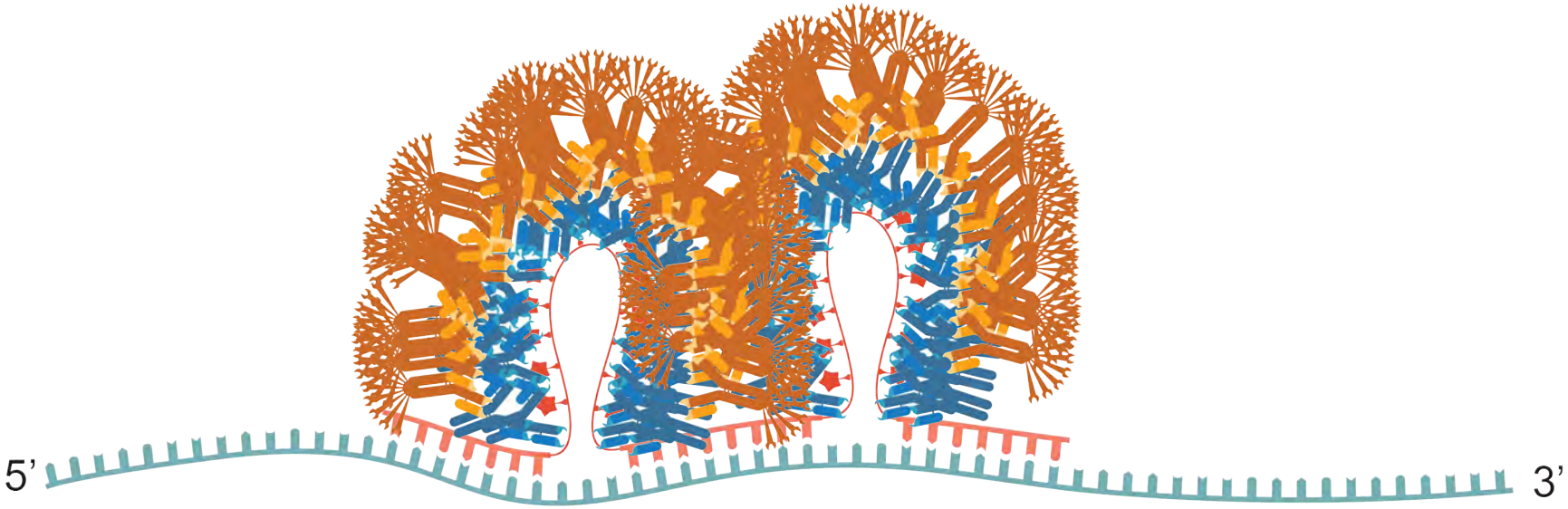
Two-Step Detection: AMPIVIEW® RNA Probes with POLYVIEW® PLUS detection system



How it works?

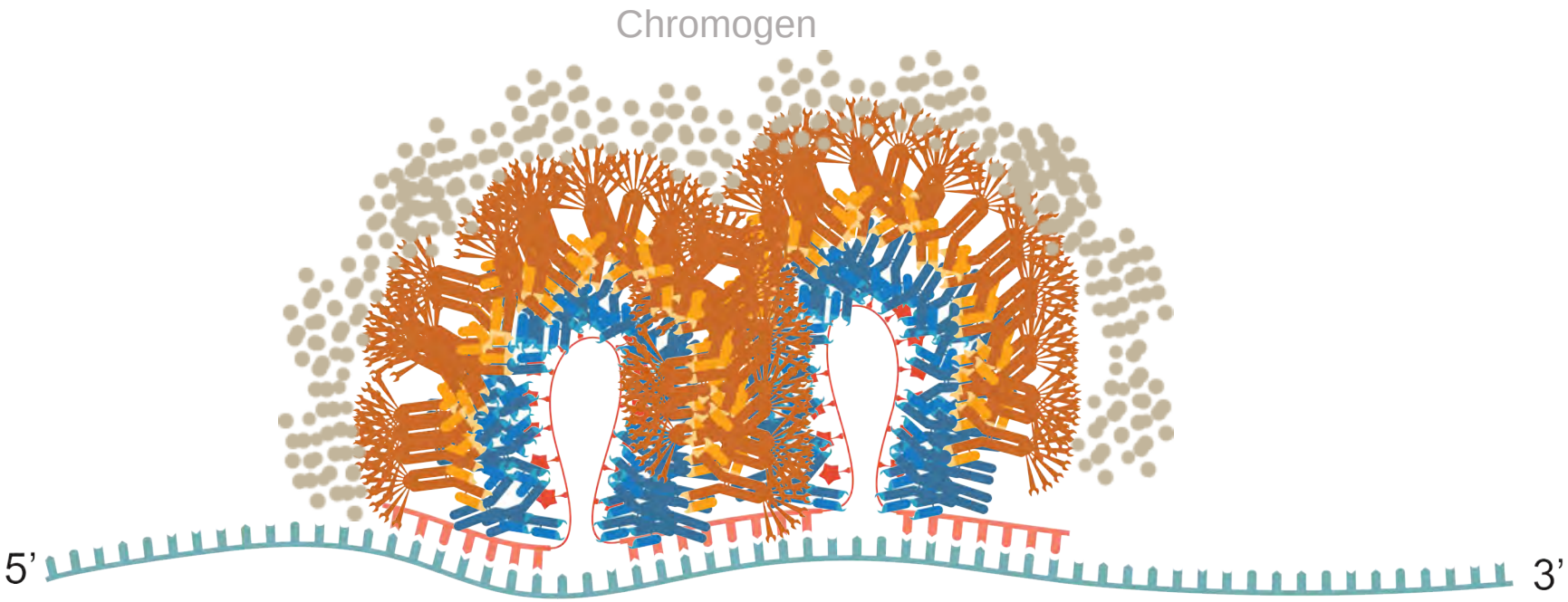
Two-Step Detection: AMPIVIEW® RNA Probes with POLYVIEW® PLUS detection system

POLYVIEW® PLUS



How it works?

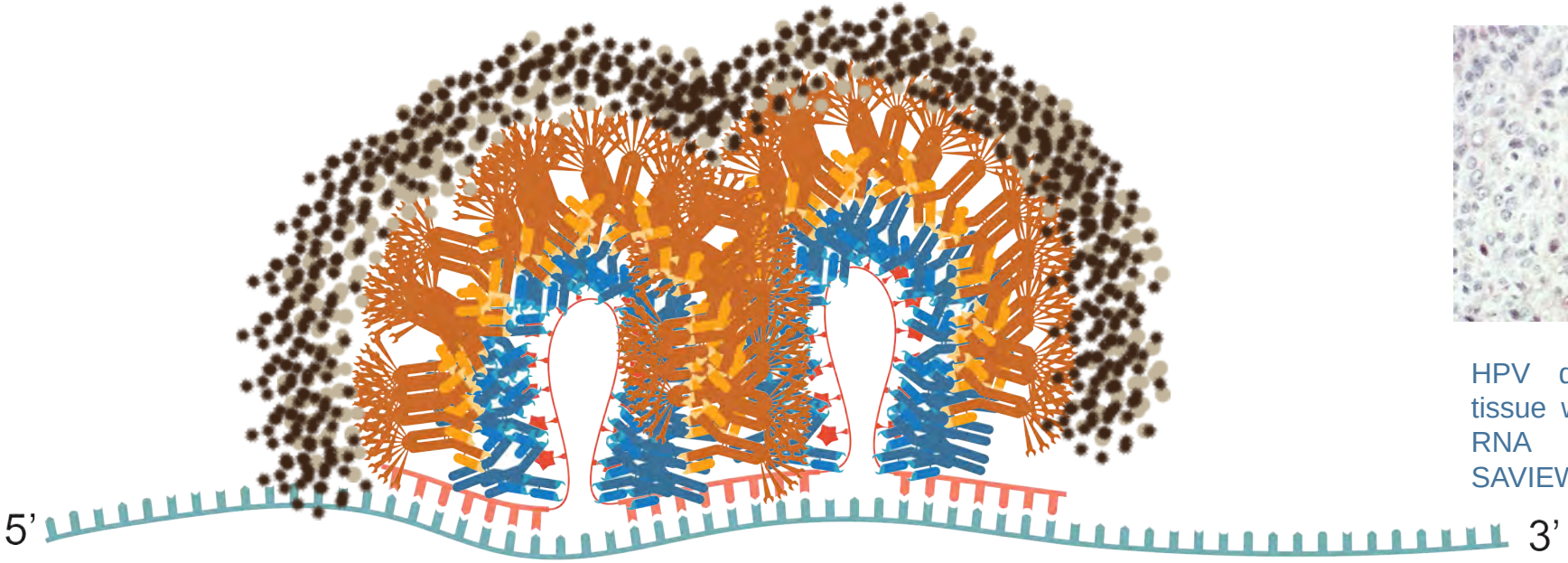
Two-Step Detection: AMPIVIEW® RNA Probes with POLYVIEW® PLUS detection system

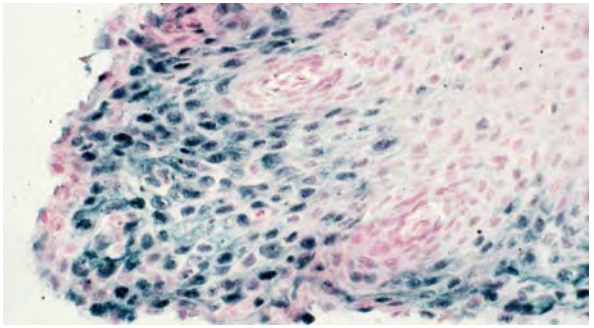


How it works?

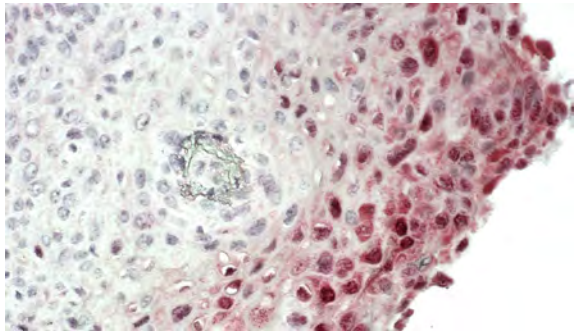
Two-Step Detection: AMPIVIEW® RNA Probes with POLYVIEW® PLUS detection system

Chromogen reacts with reporter enzyme to form color precipitate





HPV detection (blue) in cervical tissue with AMPIVIEW® HPV 16/18 RNA probes developed with POLYVIEW® PLUS HRP/DAB

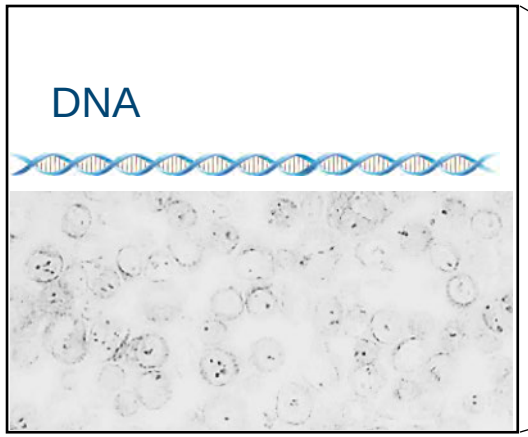


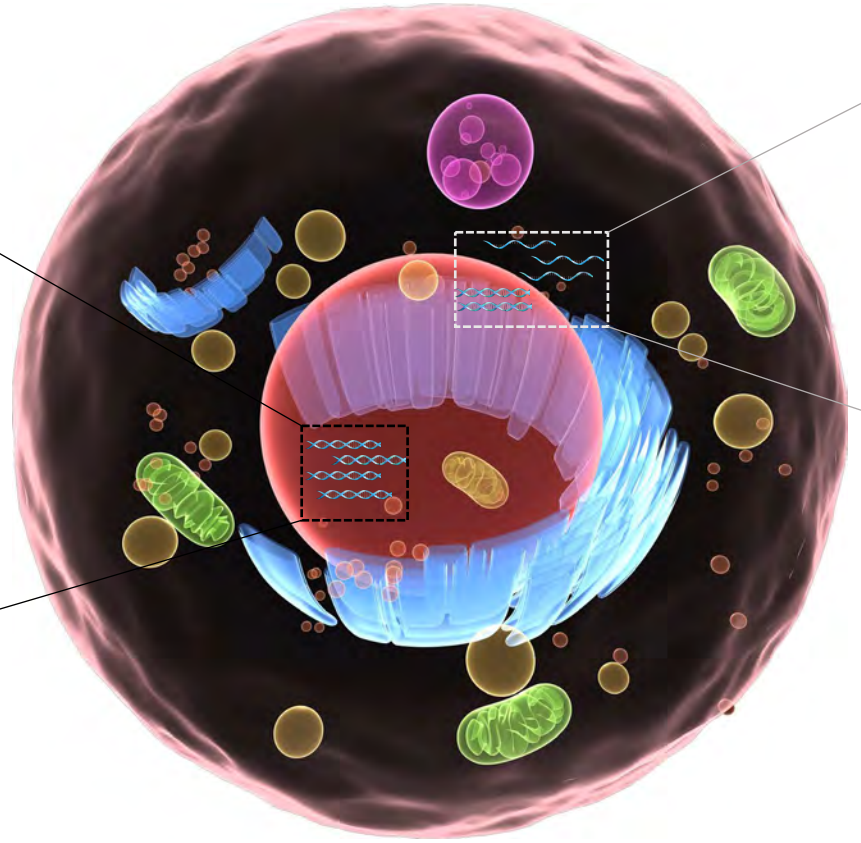
HPV detection (blue) in cervical tissue with AMPIVIEW® HPV 16/18 RNA probes developed with SAVIEW® PLUS HRP/DAB

AMPIVIEW® RNA Probes

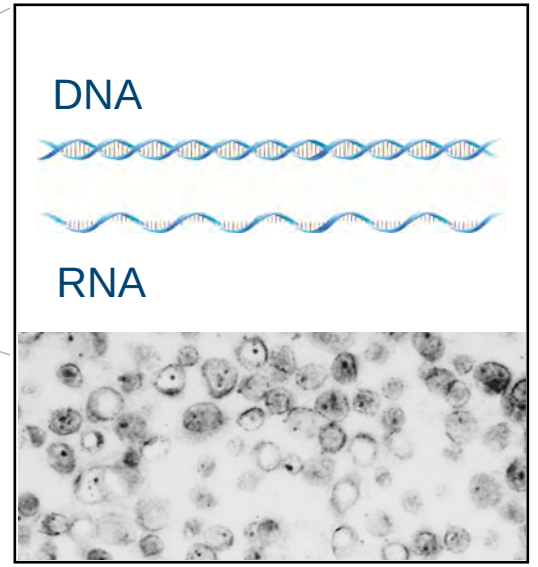
Powered by Enzo's LoopRNA™ ISH Technology

Sense probes





Anti-Sense probes

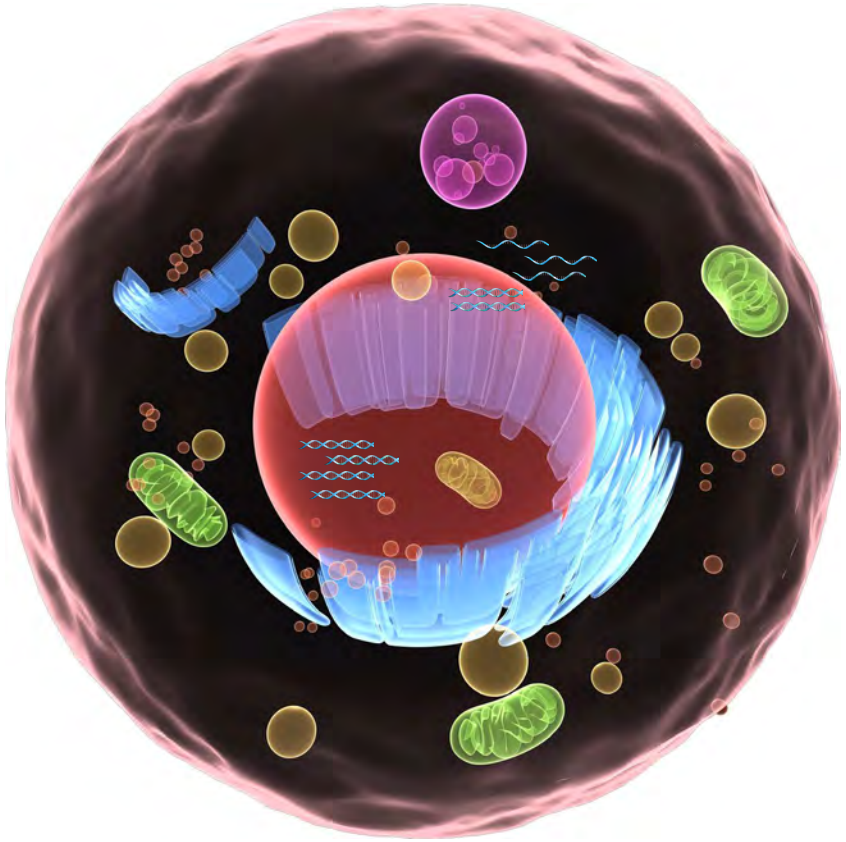


FOR MORE INFORMATION VISIT ENZO.COM/AMPIVIEW

AMPIVIEW® RNA Probes

Powered by Enzo's LoopRNA™ ISH Technology

- Superior **sensitivity**
- High **flexibility**
- **Simple** protocol
- Cost-effective

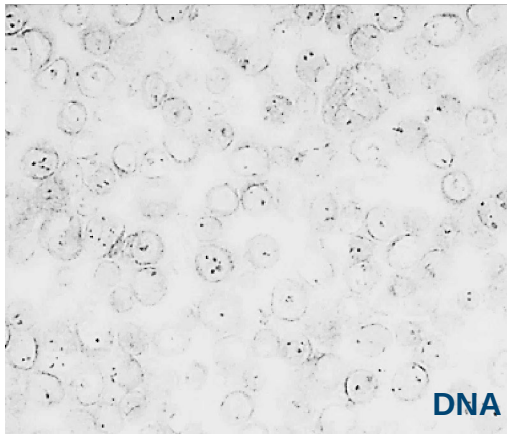


FOR MORE INFORMATION VISIT ENZO.COM/AMPIVIEW

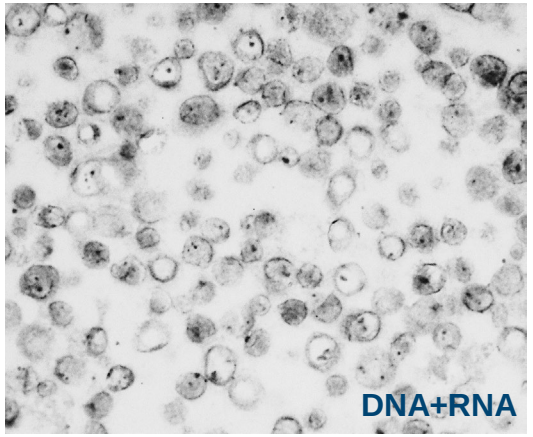
AMPIVIEW® RNA Probes

Detection of DNA vs DNA/RNA

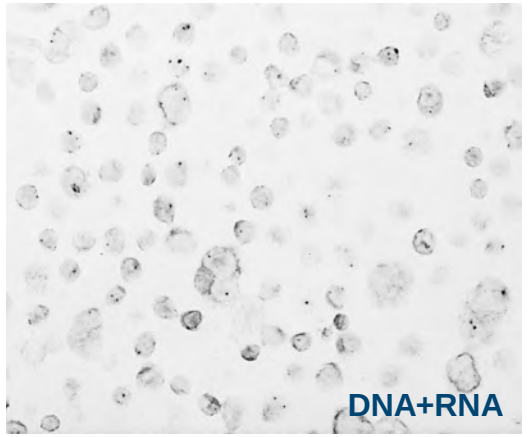
SiHa Cells
1-3 HPV integrated copies (sense probe)



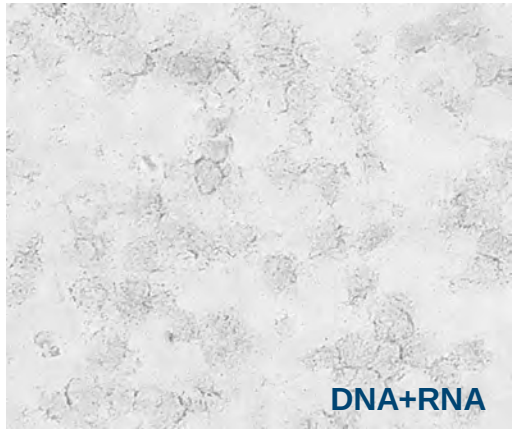
SiHa cells
1-3 HPV integrated copies (antisense probe)



SiHa cells + RNase
1-3 HPV-integrated copies (antisense probe)



C33A cells
No HPV copies (antisense probe)



AMPIVIEW® HPV type 16/18 RNA probe | Detection with AP/NBT/BCIP | Images in B/W | Black spots = positive signal

Highly sensitive probes can detect DNA or DNA and RNA in cells and tissue samples

Human Papillomavirus and Cancer

Key Facts

- Papillomaviruses are small, non-enveloped icosahedral viruses, possessing a circular double-stranded DNA (dsDNA) genome of about 8 kb in length[1]. More than 200 types of HPV have been identified. HPVs can be grouped as high-risk (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) and low-risk (HPV types 6, 11, 42, 43 and 44)[2]. The two most common HPV high-risk genotypes are HPV 16 and HPV 18, which cause approximately 70% of all cervical cancers[3].

- Furthermore, HPV high-risk infection is associated with cancers at a variety of other anogenital sites: around 50% of penile, 25% of vulvar, 80% vaginal, and close to 90% of anal cancers[4].

[1]Doorbar J, et al. 2015; [2]Munoz N, et al. 2003; [3]Graham, SV 2017; [4]de Martel C, et al. 2017

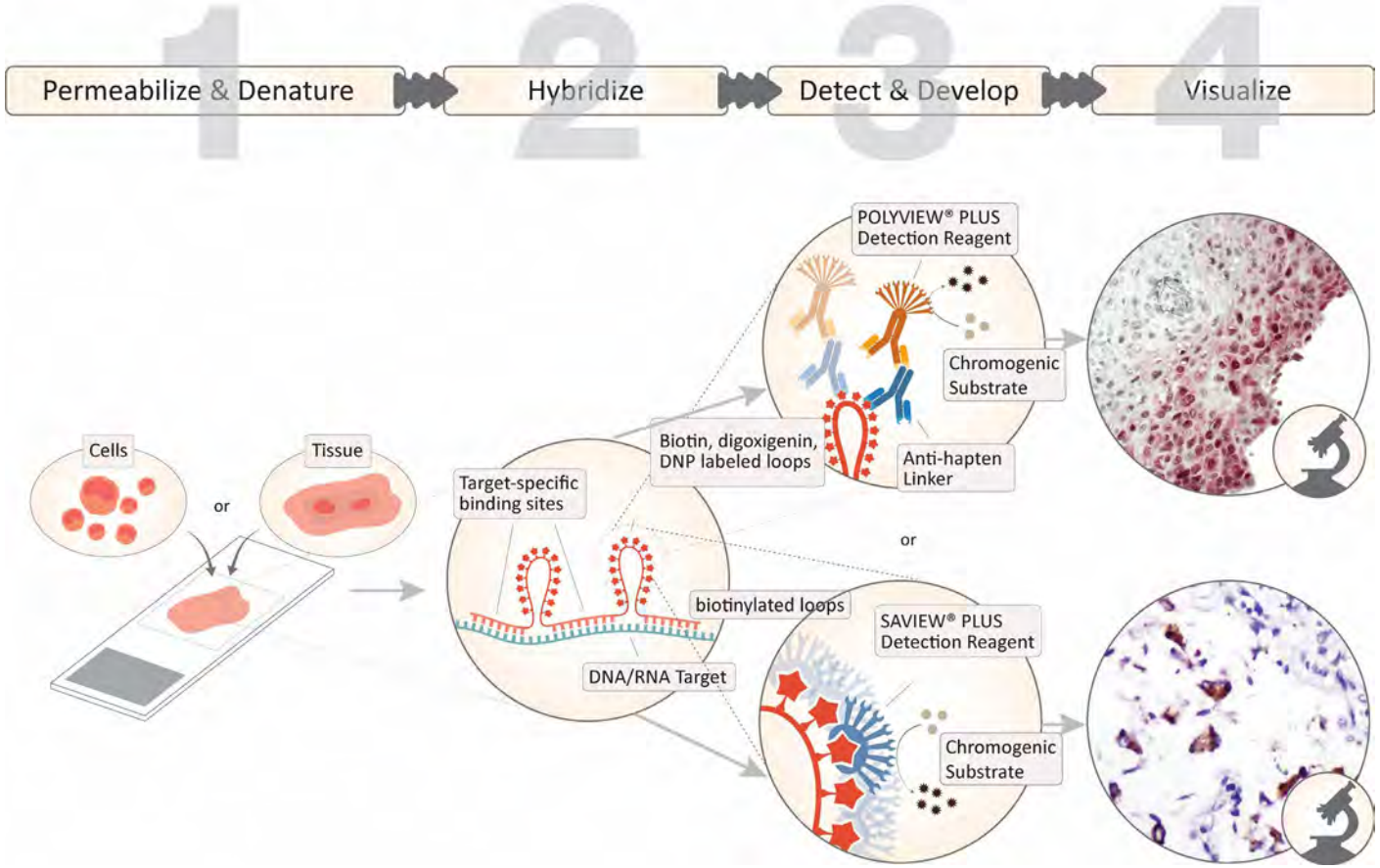
Challenge

HPV Detection

- *in situ* hybridization (ISH) is a powerful tool used in clinical and research labs for the detection of HPV infection in formalin-fixed paraffin-embedded (FFPE) tissue samples and cells.
- One of the main challenges for ISH applications is sensitivity, especially for those involving the detection of low-copy targets. In clinical settings, insufficient sensitivity can lead to misdiagnosis. To overcome this limitation, Enzo developed AMPIVIEW® RNA probes, powered by Enzo's LoopRNA™ ISH technology to deliver high sensitivity to visualize the spatial biology of nucleic acids in the sample.
- While PCR assays require the homogenization of the samples, ISH results can be observed under a light microscope without disrupting the morphology of the sample.

AMPIVIEW® RNA Probes

Workflow

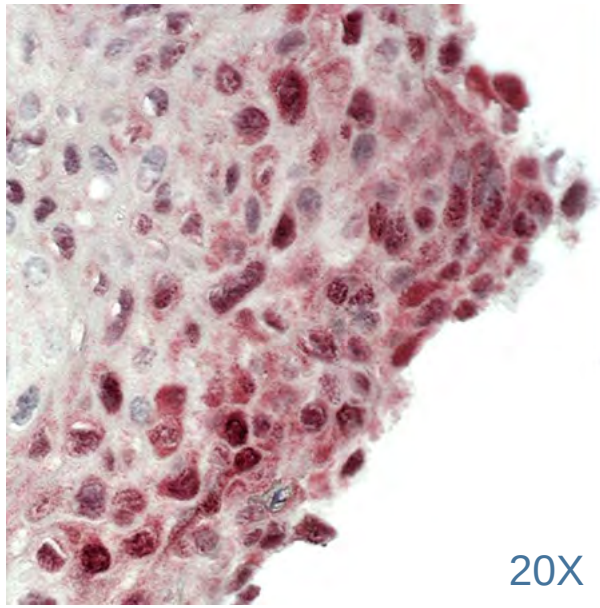


AMPIVIEW® HPV High-Risk RNA Probes

Detection of HPV 16, 18, 31, 33, 51 E2 and E6/E7

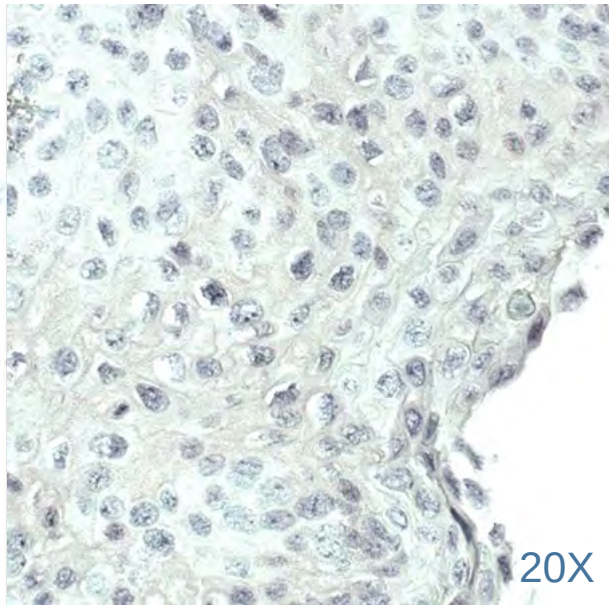
Cervical cancer tissue infected with HPV

AMPIVIEW® HPV High Risk RNA Probes (ENZ-GEN148)



20X

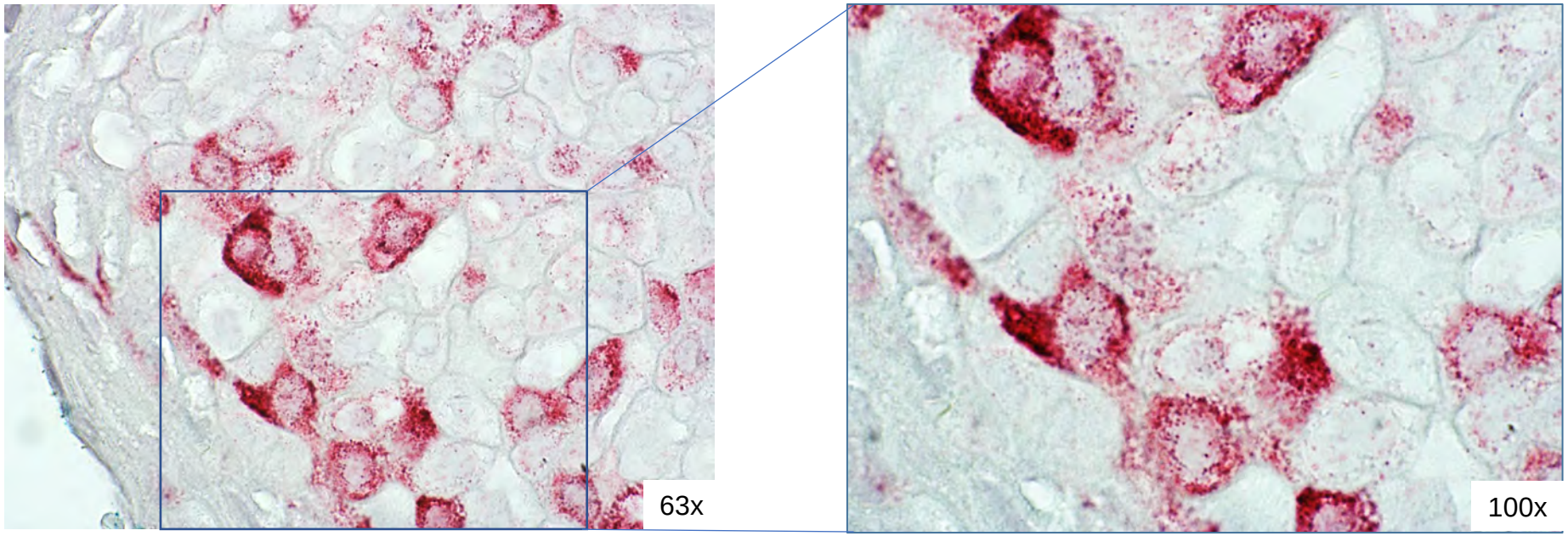
AMPIVIEW® NSP Dig RNA Probes (ENZ-GEN162)



20X

AMPIVIEW® HPV High-Risk RNA Probes

63X and 100X Magnification

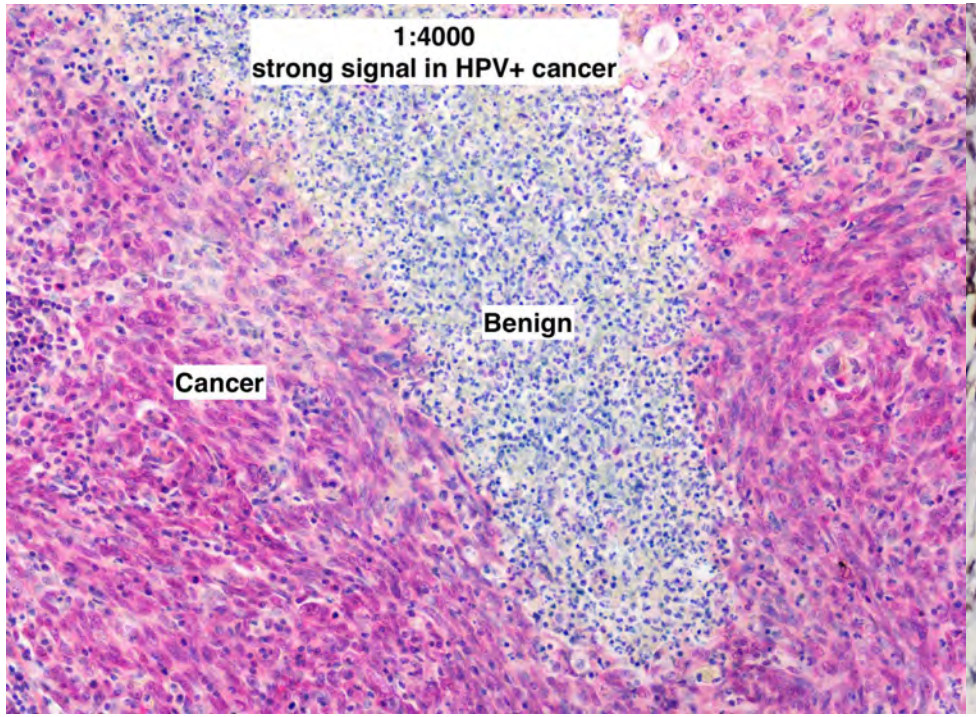


HPV High-Risk detected in HPV infected cervical tissue samples developed with POLYVIEW® PLUS Universal AP and HIGHDEF® Red AP Chromogen/Substrate

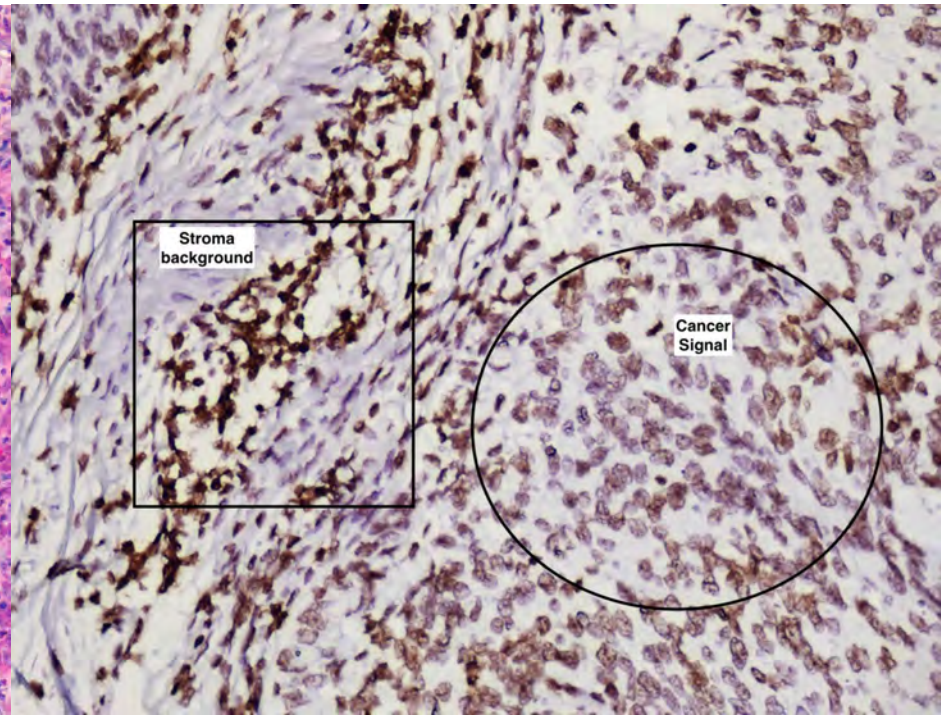
AMPIVIEW® HPV High-Risk RNA Probes

Higher specificity compared to similar products in the market

AMPIVIEW® HPV High-Risk RNA probes

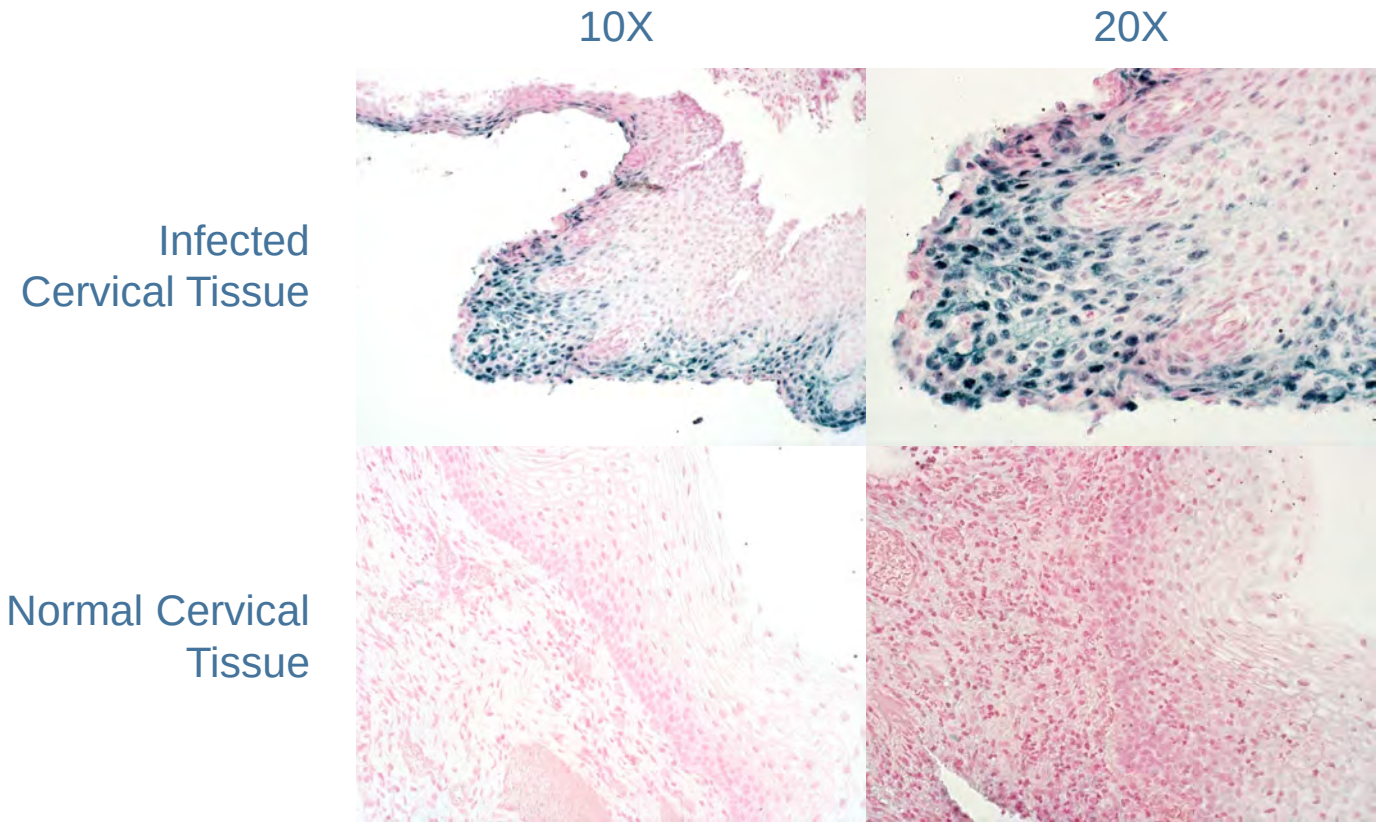


Leading Competitor



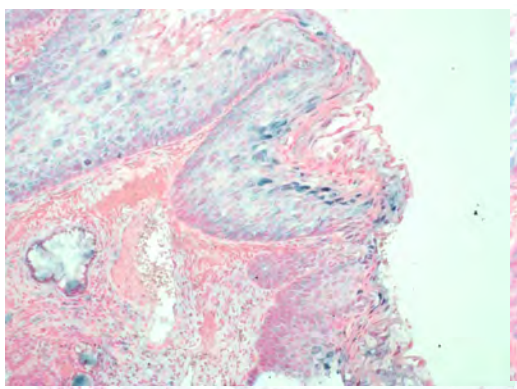
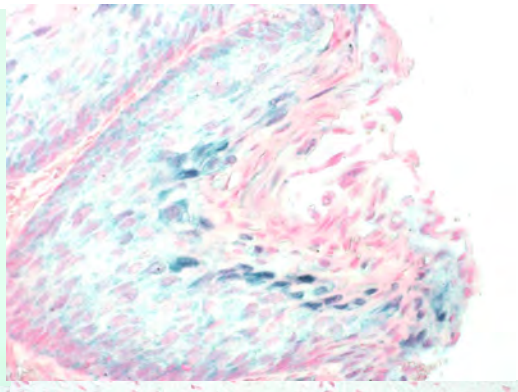
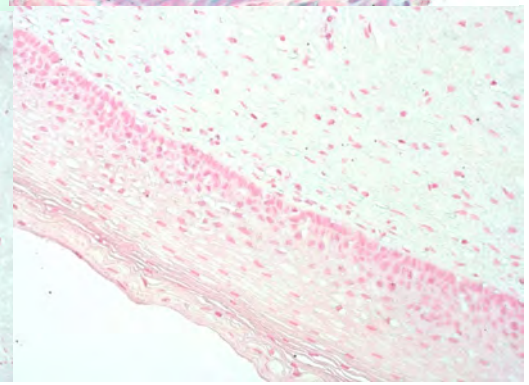
AMPIVIEW® HPV 16/18 RNA Probes

Detection of HPV 16, 18 E2 and E6/E7



AMPIVIEW® HPV 31/33/51 RNA Probes

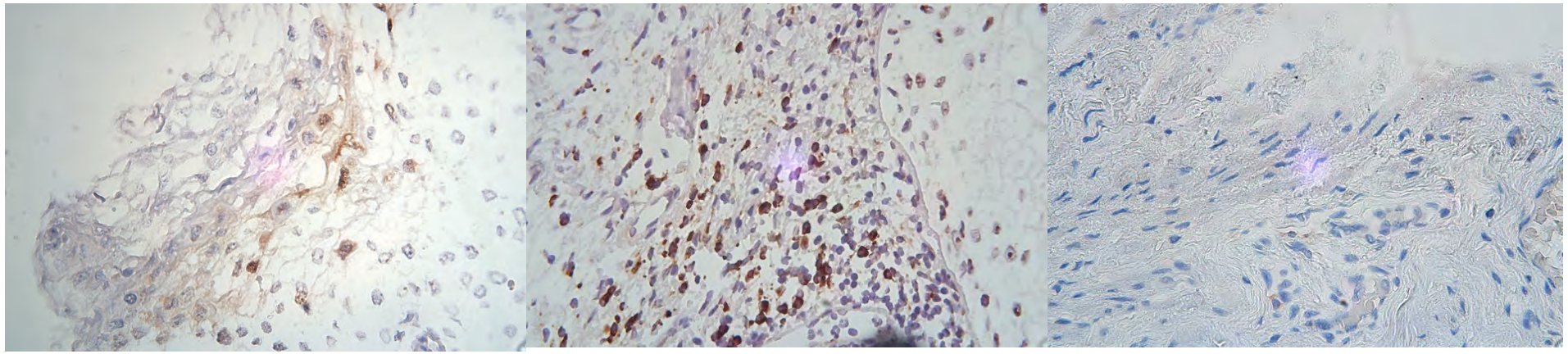
Detection of HPV 31, 33, 51 E2 and E6/E7

	10X	20X
Infected Cervical Tissue		
Normal Cervical Tissue		

AMPIVIEW® HPV HPV-15 (AS) RNA Probes

Detection of HPV 16/18/31/33/35/39/45/51/52/56/58/59/66/68/82 E6/E7 target

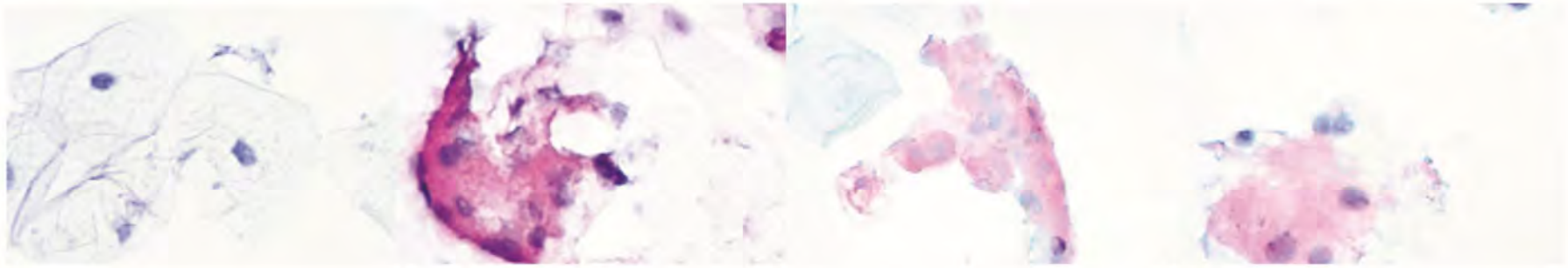
A. B. C.



A. HPV 16/18 infected tissue. B. HPV 31/33/51 infected issue. C. Non-infected tissue

AMPIVIEW® RNA Probes vs PCR

As sensitive as PCR



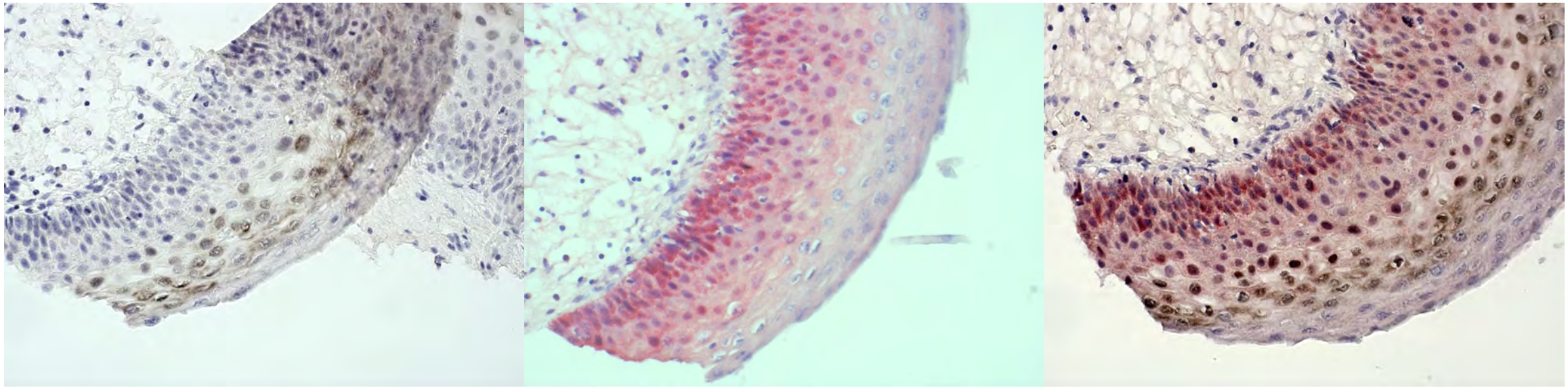
ISH Results	Negative	Positive	Positive	Positive
PCR Results	Negative	Positive	Positive	Positive
Ct Value*	ND	27.8	36.8	38.6

*Real-time PCR cycle threshold. Ct levels are inversely proportional to the amount of target nucleic acid in the sample.

Multiplexing

ISH and IHC

A. B. C.

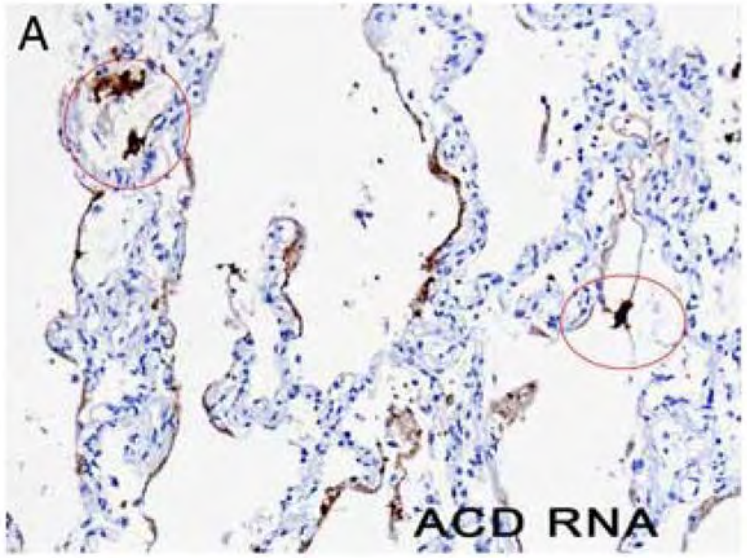


A. Detection of HPV 16, 18, 31, 33, 51 infection in cervical cancer tissue with AMPIVIEW® HPV High-Risk RNA Probes, B. Detection of p16 with anti-p16 antibody. C. Multiplex with AMPIVIEW® HPV High-Risk RNA Probes and p16 antibody in cervical cancer tissue.

AMPIVIEW® COVID-19 RNA probes vs ACD

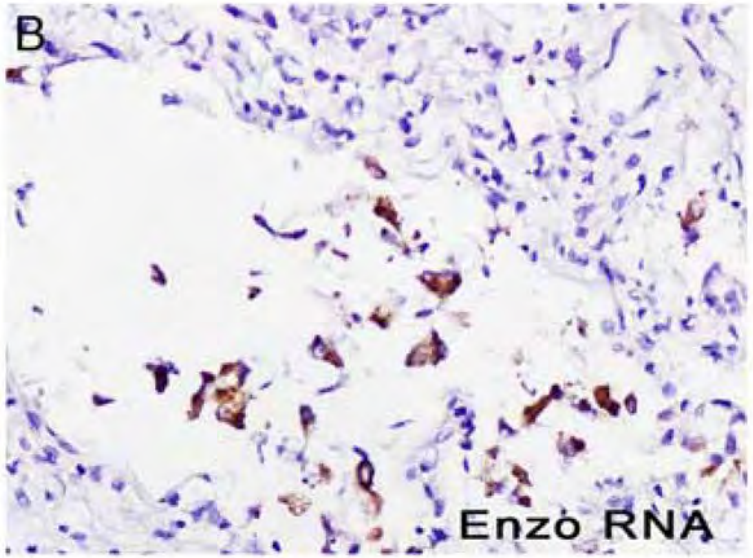
Comparing ISH on lung tissue section

ACD COVID-19 RNA probe



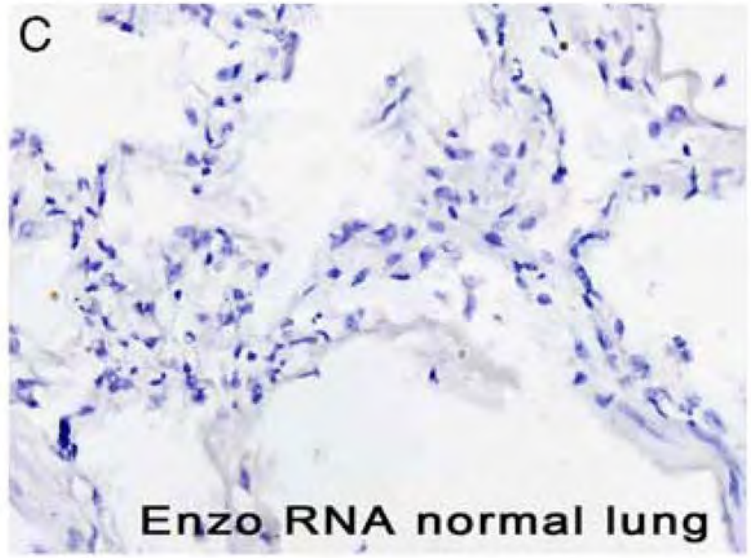
| **A**: SARS-CoV2 RNA detection in fatal COVID-19 pneumonia. ACD probes also stained macrophages (circles)

AMPIVIEW® COVID-19 RNA probe



| **B**: SARS-CoV2 RNA detection in fatal COVID-19 pneumonia.

AMPIVIEW® COVID-19 RNA probe

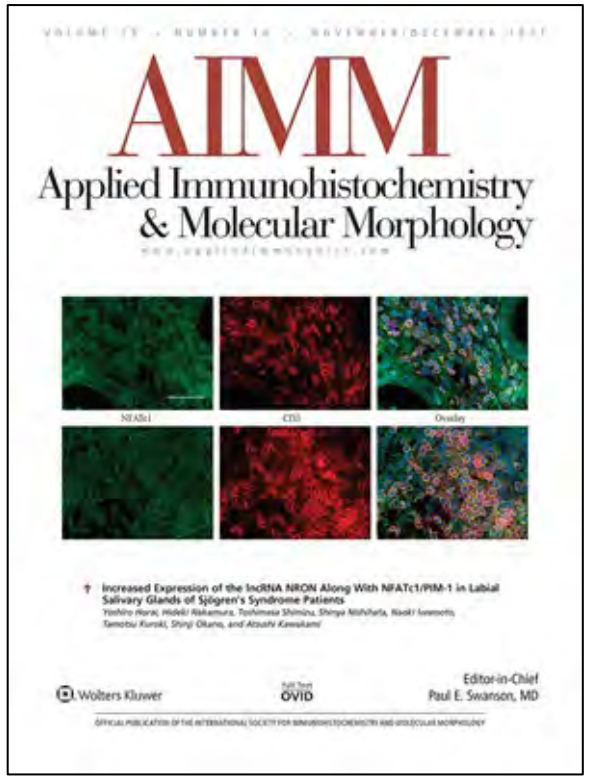


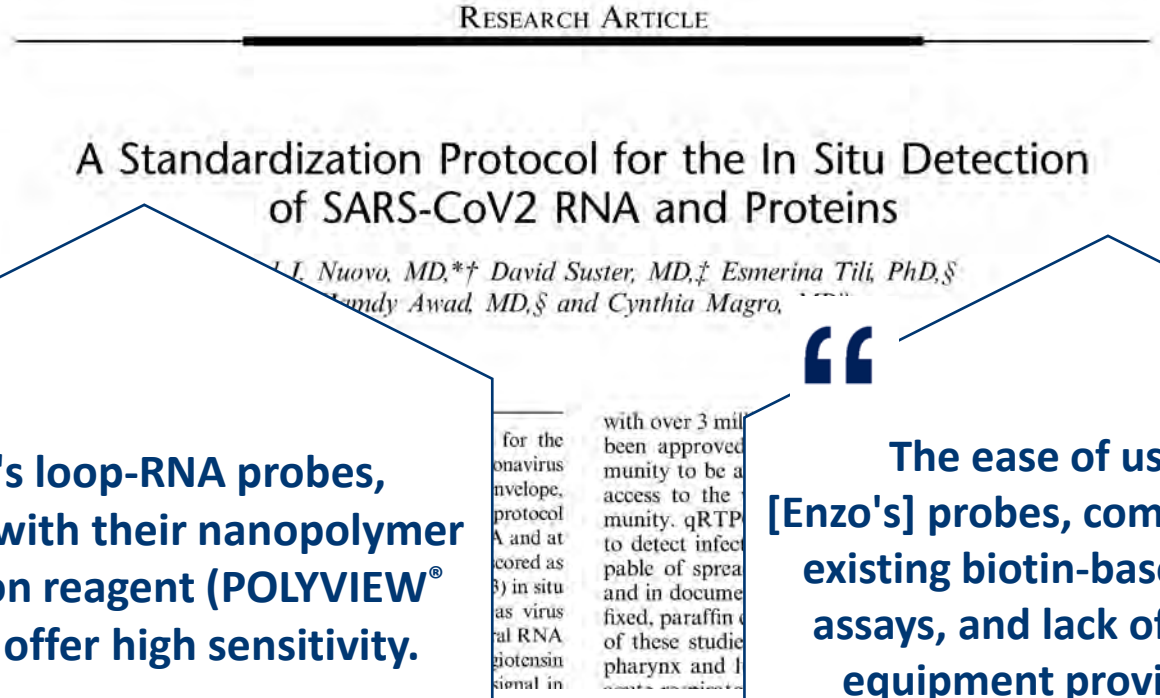
| **C**: SARS-CoV2 RNA detection in normal lung tissue.

Nuovo, GJ et al. 2022 *App Immunohistochem Mo Morphol*

High specificity confirmed with Enzo's SARS-CoV-2 RNA probes

AMPIVIEW® SARS-CoV-2 RNA Probes




RESEARCH ARTICLE

A Standardization Protocol for the In Situ Detection of SARS-CoV2 RNA and Proteins

J. Nuovo, MD,† David Suster, MD,‡ Esmerina Tili, PhD,§*

"Enzo's loop-RNA probes, coupled with their nanopolymer detection reagent (POLYVIEW® PLUS), offer high sensitivity."

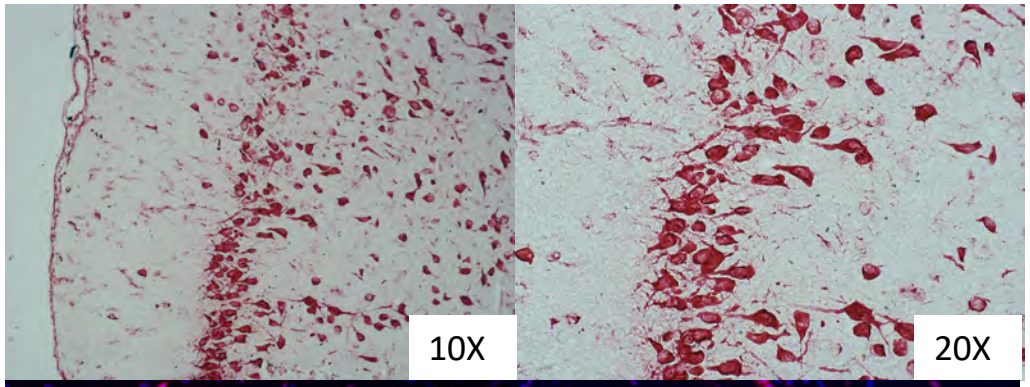
"The ease of use of the [Enzo's] probes, compatibility with existing biotin-based detection assays, and lack of specialized equipment provide several advantages over existing technologies."

AMPIVIEW® NORAD (AS) Dig RNA Probes (Mouse)

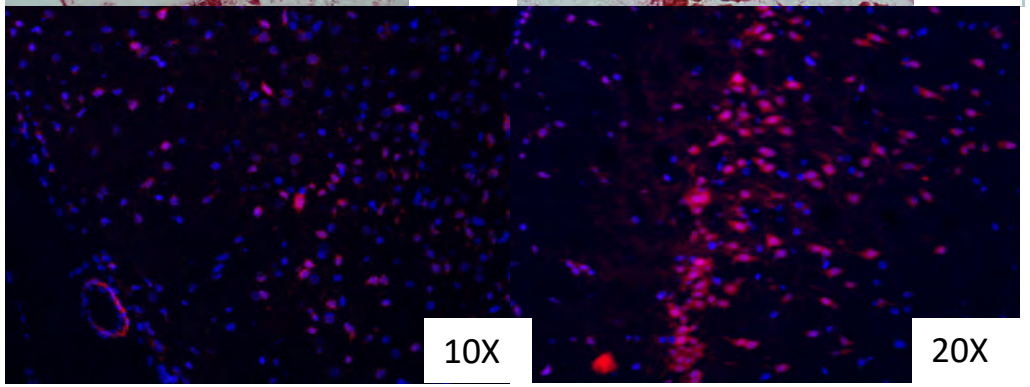
ENZ-GEN178-2000

Non-coding RNA activated by DNA damage (NORAD) is a long non-coding RNA and critical regulator in cerebral damage, in addition to playing a critical role in aging. AMPIVIEW® NORAD (AS) Dig RNA probes, powered by Enzo's LoopRNA ISH™ technology, are designed to detect NORAD in mouse and rat specimens.

AMPIVIEW® Norad (AS) Dig RNA Probes



AMPIVIEW® Norad (AS) Dig RNA Probes



AMPIVIEW® RNA Probes

Pipeline

- ENZ-GEN131 AMPIVIEW® PTEN (AS) DNP RNA Probe
- ENZ-GEN133 AMPIVIEW® GATA3 (AS) Dig RNA Probe
- ENZ-GEN139 AMPIVIEW® HER-2 (AS) Dig RNA Probe
- ENZ-GEN269 AMPIVIEW® HER-2 (AS) DNP AS Dig RNA Probes
- ENZ-GEN166 AMPIVIEW® CD19 (AS) Dig RNA Probes
- ENZ-GEN198 AMPIVIEW® Bcl2 (AS) Dig RNA Probes
- ENZ-GEN267 AMPIVIEW® EGFR (AS) DNP AS Dig RNA Probes
- ENZ-GEN271 AMPIVIEW® Ki67 (AS) Dig AS Dig RNA Probes
- ENZ-GEN279 AMPIVIEW® p53 (AS) Dig RNA Probes
- ENZ-GEN281 AMPIVIEW® PD-1 (AS) Dig RNA Probes
- ENZ-GEN283 AMPIVIEW® PD-L1 (AS) Dig RNA Probes
- ENZ-GEN291 AMPIVIEW® PD-L1 (AS) DNP RNA Probes
- ENZ-GEN285 AMPIVIEW® NFkB (AS) Dig RNA Probes
- ENZ-GEN287 AMPIVIEW® Yap1 (AS) Dig RNA Probes
- ENZ-GEN295 AMPIVIEW® HPV HR-15 Dig RNA Probes
- ENZ-GEN297 AMPIVIEW® HPV 45 (AS) DNP RNA Probes
- ENZ-GEN304 AMPIVIEW® Ki6 (AS) DNP RNA Probes
- ENZ-GEN306 AMPIVIEW® Wnt5a (AS) DNP RNA Probes (Human)
- ENZ-GEN308 AMPIVIEW® miR200 (AS) DNP RNA Probes
- ENZ-GEN310 AMPIVIEW® miR-let-7 (AS) DNP RNA Probes
- ENZ-GEN312 AMPIVIEW® HPV 6/11 DNP RNA Probes
- ENZ-GEN314 AMPIVIEW® HPV 16/18 DNP RNA Probes
- ENZ-GEN316 AMPIVIEW® HPV 31/33/51 DNP RNA Probes
- ENZ-GEN318 AMPIVIEW® HPV High-Risk DNP RNA Probes
- ENZ-GEN308 AMPIVIEW® miR200 (AS) DNP RNA Probes
- ENZ-GEN110 AMPIVIEW® miR-Let7 (AS) DNP RNA Probes

Conclusion

AMPIVIEW® RNA Probes

- AMPIVIEW® RNA probes are uniquely designed with the precision of targeted, sequence-specific RNA probes, powered by Enzo's LoopRNA™ ISH technology to deliver superior sensitivity and specificity.
- AMPIVIEW® RNA probes sensitivity proved to be comparable to RT-PCR results, while preserving the morphology of the sample.
- The design of the probes makes them adaptable to any workflow (manual or automated) and compatible with immunohistochemistry detection systems.
- AMPIVIEW® RNA probes are easy-to-use and flexible with existing ISH and IHC setups and probes can be designed with virtually unlimited potential to detect any gene and transcript of interest.

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All of Enzo family

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Thank you!

Questions, comments?