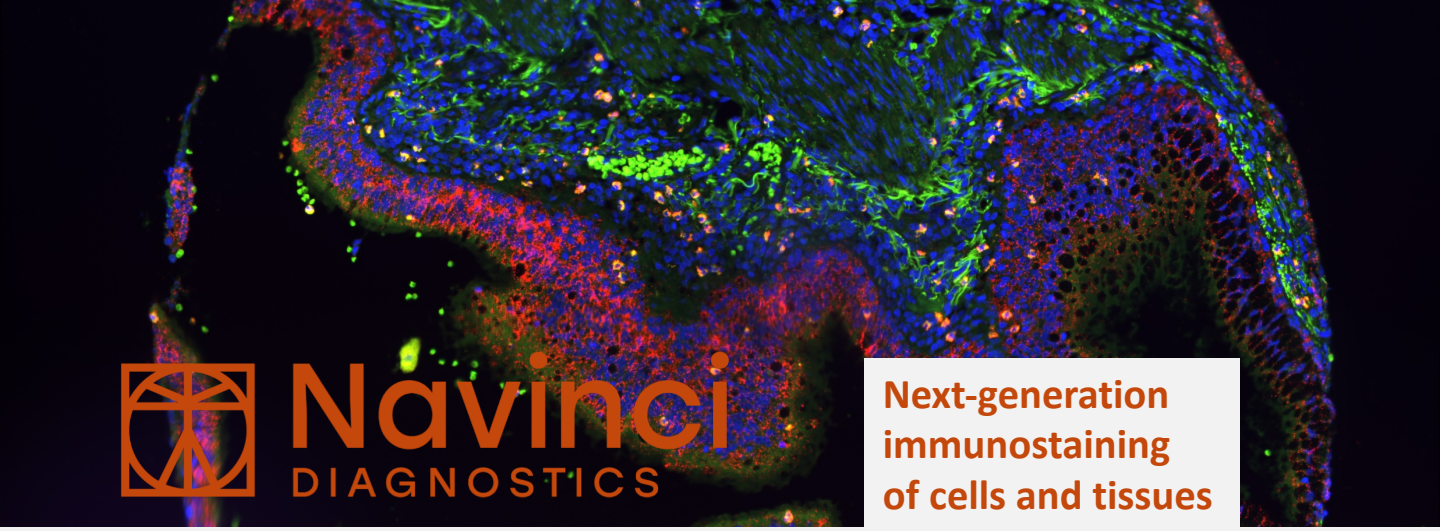


Navinci DIAGNOSTICS

Next-generation immunostaining of cells and tissues

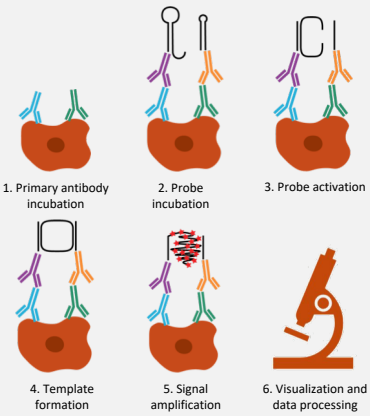
Navigating chromatin organization with NaveniFlex™

Navinci Diagnostics develops protein detection immunoassays based on *in situ* proximity ligation. The NaveniFlex™ kits (MM, RR or MR) enable the detection of proteins, protein interactions, and protein modifications in cultured cells, fresh-frozen tissue, and FFPE samples. Proteins or interactions are represented as quantifiable fluorescent dots and visualized by conventional microscopy. NaveniFlex™ kits are a valuable tool to study epigenetic mechanisms behind genome regulation.

How NaveniFlex™ MR works:

Navinci Diagnostic's kits are based on our proprietary Unfolding Proximity Probes technology. They combine antibody-based proteomic technologies with fluorescence readout to create next-generation immunostaining. **NaveniFlex™ MR** detects pairs of antibodies specific for your targets of interest offering high sensitivity and specificity in one assay, enabling high-quality protein detection and analysis in cells and tissues.

Primary antibodies directed to the desired targets are recognized by oligo-conjugated secondary probes that, after activation and ligation, give rise to DNA circles that serve as a template for rolling circle amplification (RCA) and specific fluorescent detection.



Chromatin and 3D nuclear organization impacts genome regulation:

The eukaryotic genome is packed inside the cell nucleus and functionally organized through a complex of DNA and histone proteins called chromatin. Different degrees of compaction makes chromatin more accessible and permissive for transcription (euchromatin), or more condensed and unreachable for the transcriptional machinery (heterochromatin). Several epigenetic mechanisms, including methylation of the DNA sequence, the action of non-coding RNAs, or histone post-translational modifications (PTMs), modulate chromatin structure, and its accessibility. These changes in chromatin organization play a crucial role in transcriptional regulation, DNA damage response, DNA replication, or cellular division.

Chromatin is organized in the 3D space of the nucleus. DNA loci localization and its association with certain nuclear compartments have a direct impact on chromatin function. For instance, chromatin in close contact with the nuclear envelope or lamina is highly condensed and is considered a repressive environment for transcription. These lamina-associated domains (LADs) are enriched in repressive histone PTMs such as H3K9me2/3 or H3K27me3 and contain transcriptionally inactive genes and genomic regions with low gene density.

Alterations of the epigenetic mechanisms controlling chromatin function and disruption of nuclear compartments are directly associated with the outcome and progression of many diseases such as cancer. Therefore, epigenetic regulators have been proposed as promising therapeutic targets for tumor progression

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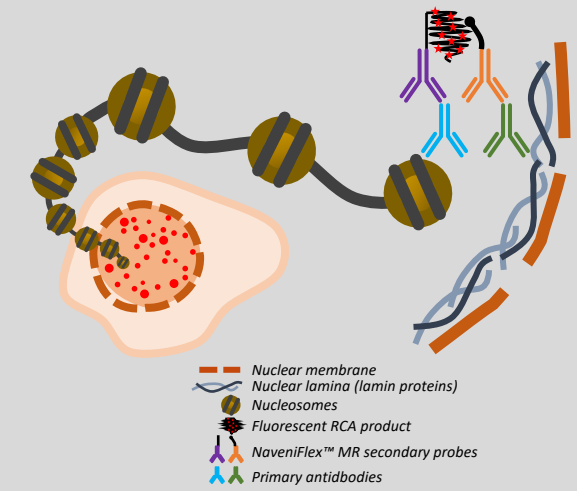
APPLICATION NOTE

Visualizing chromatin contacts with the nuclear periphery with NaveniFlex™ MR:

The precise molecular mechanisms mediating chromatin contacts with the nuclear lamina are still largely unresolved. Although chromatin immunoprecipitation (ChIP) and DNA adenine methyltransferase identification (DAM-ID) are typically the methods of choice to study such interactions, novel complementary methodologies are still in need to score protein or protein modifications associated with LADs, and the proteins responsible for such interactions.

The **NaveniFlex™** *in situ* immunoassays provide a fast, reproducible, and quantifiable solution to investigate proximity events, keeping subcellular spatial information and allowing the study of complex tissues and heterogeneous cell populations at single-cell resolution. By using the proper set up of primary antibodies, interactions of candidate proteins or histone PMTs with nuclear lamina components can be visualized using standard fluorescent microscopy.

As an example, Figure 1 illustrates how chromatin interaction with the nuclear lamina is visualized with the **NaveniFlex™ MR** kit by scoring the physical proximity between the ubiquitous histone H3 with lamin-B1, a constituent of the nuclear lamina, in BT474 breast cancer cells (A) and human skin tissue (B).



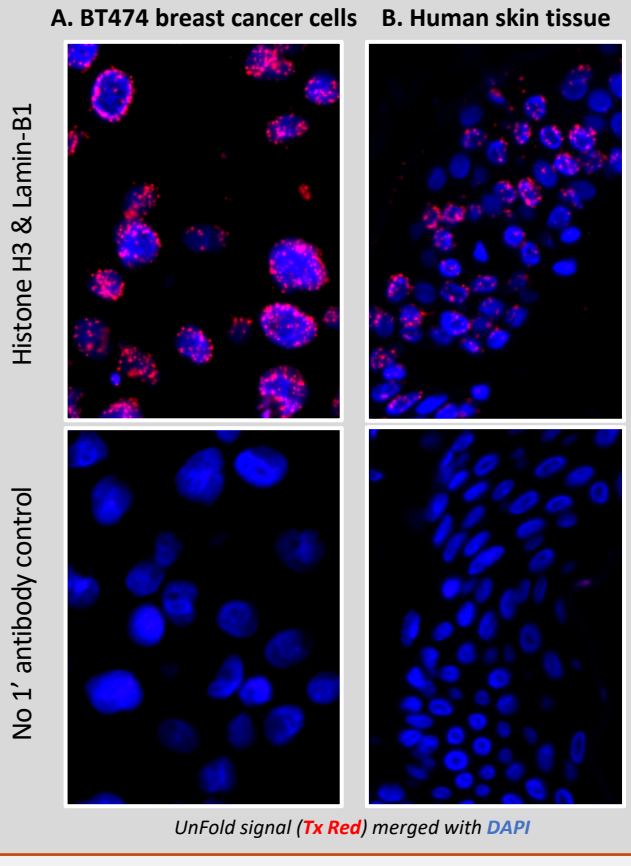


Figure 1.
*Application of the **NaveniFlex™ MR** UnFold technology to detect interaction between components of the nuclear lamina and chromatin proteins. Physical proximity between the histone H3 protein and lamin-B1 is scored by dual recognition of specific primary antibodies (anti-mouse and anti-rabbit, respectively) that generate a rolling circle amplification (RCA) reaction that can be fluorescently detected when secondary probes bind in close proximity. The assay has been performed in BT474 breast cancer cells **(A)** and human skin tissue **(B)**. A technical control without using primary antibodies shows the background of the method. Images taken with Leica epifluorescent microscope.*

Conclusions:

In situ **technologies with single-cell resolution might need to be implemented to unravel how the eukaryotic genome is regulated and how epigenetic mechanisms influence chromatin dynamics and function in the context of the 3D space of the nucleus. Navinci Diagnostics's NaveniFlex™ kits can easily be applied to study nuclear targets, protein interactions in the nucleus, and interactions between chromatin proteins or modifications with different nuclear compartments.**

Reference publication:

Klaesson A, Grannas K, Ebai T, Heldin J, Koos B, Leino M, Raykova D, Oelrich J, Arngården L, Söderberg O, Landegren U. Improved efficiency of in situ protein analysis by proximity ligation using UnFold probes. Sci Rep. 2018 Mar 29;8(1):5400. PMID: 29599435

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