

Application Note

Wild Type and Mutated *BRCA* - Differentiation of Breast Cancer using New miRNA Biomarker Panel

Ready-to-Use fully optimized **SSNA** miRNA *in situ* hybridization (ISH) Kit

Application Highlights:

- Abnormal expression of miRNAs has been reported in various types of cancer, including breast cancer
- *BRCA* mutated breast carcinomas are commonly seen in younger patients and have a more aggressive clinical course with limited diagnostic assays
- BioGenex Xmatrx® automated systems and BioGenex SSNA *BRCA* Breast panel miRNA probes were used to successfully differentiate the miRNA expression pattern between patients with wild type and *BRCA* mutated breast cancer
- The *in situ* experimental conditions for hybridization were optimized for both BioGenex manual and automated systems
- BioGenex miRNA ISH Panel Probes are currently one of the best products for complex diagnostic assays

BioGenex Products Used:

- #HM021-100: miR-21
- #HM017-100: miR-17
- #DF400-YADE: XISH™ One-Step Polymer-HRP ISH Detection Kit (Automation)
- #DF400-50KE: Super Sensitive One-Step Polymer-HRP ISH Detection Kit (Manual)

Keywords:

Breast carcinoma, *BRCA* mutation, *In situ* hybridization, miRNA, Xmatrx®

Introduction:

Breast cancer is the leading cause of cancer-related mortality among women, globally. Annually, an estimated 266,120 new cases of breast cancer are expected to be diagnosed in women in the United States and over 40,920 cases are expected to be fatal. Breast cancer constitutes around 30% of newly diagnosed cancer incidences among women in the United States. Specifically, *BRCA* mutated breast carcinomas are commonly seen in younger patients and have a more aggressive clinical course. Identifying genomic biomarkers for breast cancer prognosis will lead to a better understanding of breast cancer genetics and a more accurate understanding of tumor behavior. Utilizing microRNAs (miRNAs) as a potential biomarker to differentiate *BRCA* will allow for specialized treatment regimens and increased clinical care.

miRNAs are small, endogenous, functional non-coding RNAs that are involved in mammary gland development, proliferation and, by association, breast cancer. miRNAs have been recognized as an important regulator of both normal and cancerous cells, allowing for great potential as diagnostic and prognostic biomarkers in various types and subtypes of breast cancer. The detection of miRNA in clinical samples has been difficult, requiring total RNA extracts which lack critical spatial differentiation. *In situ* hybridization (ISH), however, has enabled the direct assessment of miRNAs expression level in formalin-fixed paraffin-embedded (FFPE) tissues, malignant cells, stromal cells and invading lymphocytes. Performing miRNA ISH using BioGenex Super Sensitive Nucleic Acid (SSNA) probes is promising for improving the understanding of pathogenesis and therapeutic outcome in patients with *BRCA* mutated breast cancer.



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Super Sensitive Nucleic Acid (SSNA) miRNA probes:

BioGenex has developed proprietary SSNA miRNA probes that are specially designed to enhance signals from the intrinsically low-copy-number miRNAs. These probes have high melting temperatures enabling stringent washes to remove non-specific binding. BioGenex miRNA probes are dual-end labeled with an anti-fluorescein reporter to amplify the signal, yielding intense stains. Overall, the BioGenex SSNA probes aid in studying the regulatory functions of miRNA.

This Application Note highlights how BioGenex SSNA miRNA ISH probes can differentiate distinct miRNA expression profiles between wild type and *BRCA* mutated cancer cases. The original study and the results were presented at the Annual Meeting of the United States and Canadian Academy of Pathology (USCAP) (1).

Study samples and detection methods:

The miRNA expression profile was evaluated in 13 invasive ductal carcinoma cases containing 6 *BRCA* mutated and 7 wild type breast carcinomas (1). Differential expression of miRNAs was documented using the BioGenex Xmatrx® automated system and miRNA ISH *BRCA* Breast panel probes.

Experimental- *In situ* hybridization:

ISH miRNA probes were used for evaluating the expression pattern of miR-17 and miR-21. The *in situ* experimental conditions for hybridization were optimized for both manual and automated systems. The hybridized probes were visualized using the BioGenex Super Sensitive Polymer-HRP IHC detection system. Nuclear staining was evaluated semi-quantitatively by intensity; as weak, moderate, or strong. Scramble probes were used as the negative control.

Results and conclusion:

As compared with wild type breast cancer, expression of miR-17 and miR-21 was downregulated in *BRCA* mutated breast cancer (Figure 1), suggesting that the miRNA expression pattern may serve as a potential biomarker in predicting *BRCA* mutation status in invasive breast cancer.

miR-17 has been linked to tumorigenesis in a broad range of cancers, including gastric cancer (2), and colon cancer (3). In a recent study, miR-17 has been shown to play a significant role in breast cancer progression (4). Several studies have also reported miR-21 as a potential novel diagnostic biomarker for breast cancer (5, 6). A study by Wang et al. suggests that circulating miR-21 could serve as a potential serum-based biomarker for breast cancer detection in the Chinese population, with 80.0% sensitivity and 87.7% specificity (6). In table 1, *BRCA* mutated carcinomas demonstrated minimal nuclear positivity in benign epithelium and tumor nuclei, with 17% of cases staining for miR-17 and miR-21, respectively.

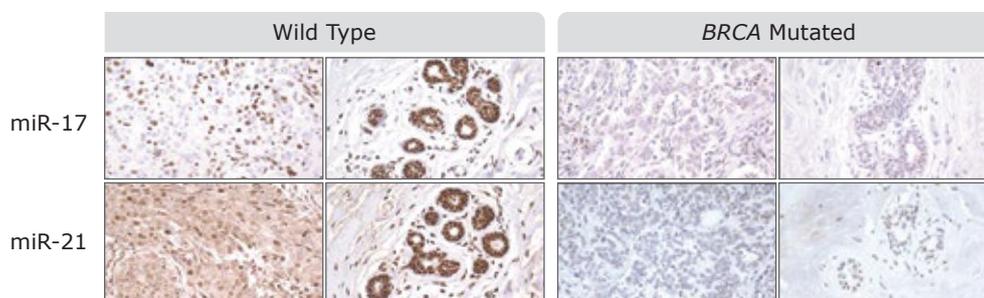


Figure 1. Differential miRNA expression between wild type and *BRCA* mutated tumors tissues.

Table 1: Expression levels of miR-17 and miR-21.

Tumor type	Total cases	miR-17 +	miR-21 +	Molecular subtype
Wild	7	7 (100%)	7 (100%)	Basal: 2, Luminal: 5
<i>BRCA</i> mutated	6	1 (17%)	1 (17%)	Basal: 1, Luminal: 5

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To conclude, *BRCA* mutated breast cancer currently has limited diagnostic assays. However, BioGenex SSNA ISH probes can be successfully used to differentiate the distinct miRNA expression profile of wild type and *BRCA* mutated breast carcinoma. The high melting temperatures and dual-end labeled SSNA probes help in studying the differential expression pattern of the low abundant miRNAs. Understanding the relationship of the miRNA expression level to the *BRCA* mutation status can help develop effective targeted interventions and specialized treatment options. BioGenex SSNA probes combined with the automated processing using Xmatrx® greatly increases the reliability of the test results through the elimination of error-prone ISH procedure.

Datasheets:

The BioGenex miRNA probe datasheets provide additional information on the recommended usage guidelines and storage. Refer to the datasheets below before use:

- #HM021-100 • #HM017-100

Refer to the user manual for the automated detection kit and manual kit

1. **DF400-YADE:** XISH™ One-Step Polymer-HRP ISH Detection Kit (Automation)
2. **DF400-50KE:** Super Sensitive One-Step Polymer-HRP ISH Detection Kit (Manual)

Disclaimer:

The research group and authors have expressed no conflict of interest. BioGenex has optimized the protocols for optimal staining results, using positive tissue controls. Due to complex ISH procedures care should be taken in each step. Variations in tissue embedding and fixation and tissue nature should be taken into account for variation in results. Reagents and probes must be prepared and handled according to the manufacturer's instructions.

References:

1. Mohanty SK et al. miRNA Expression Pattern in Predicting *BRCA* Mutation Status in Invasive Breast Carcinoma. Presented as Poster in Annual Meeting of the United States & Canadian Academy of Pathology (USCAP) 2014.
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3. Knudsen KN et al. microRNA-17 is the most up-regulated member of the mir-17-92 cluster during early colon cancer evolution. *PLoS One*. 2015;10:e0140503.
4. Yang F et al. miR-17 as a diagnostic biomarker regulates cell proliferation in breast cancer. *OncoTargets Ther*. 2017 ;10:543-50.
5. Gao Y et al. MicroRNA-21 as a potential diagnostic biomarker for breast cancer patients: a pooled analysis of individual studies. *Oncotarget*. 2016;7:34498-506.
6. Wang B, Zhang Q. The expression and clinical significance of circulating microRNA-21 in serum of five solid tumors. *J Cancer Res Clin Oncol*. 2012;138:1659-66.

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