



microRNA

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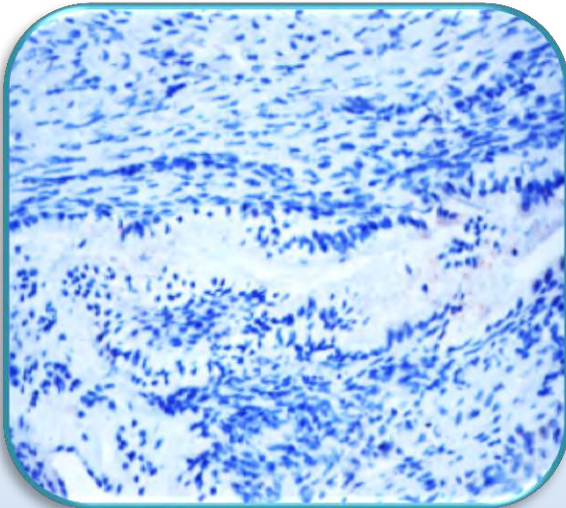
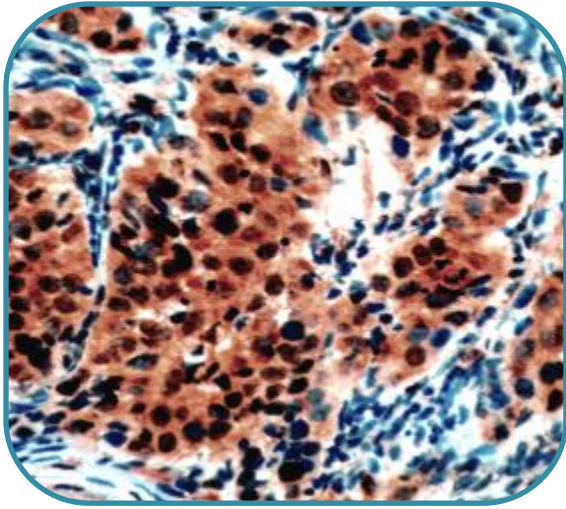
Date:



microRNA

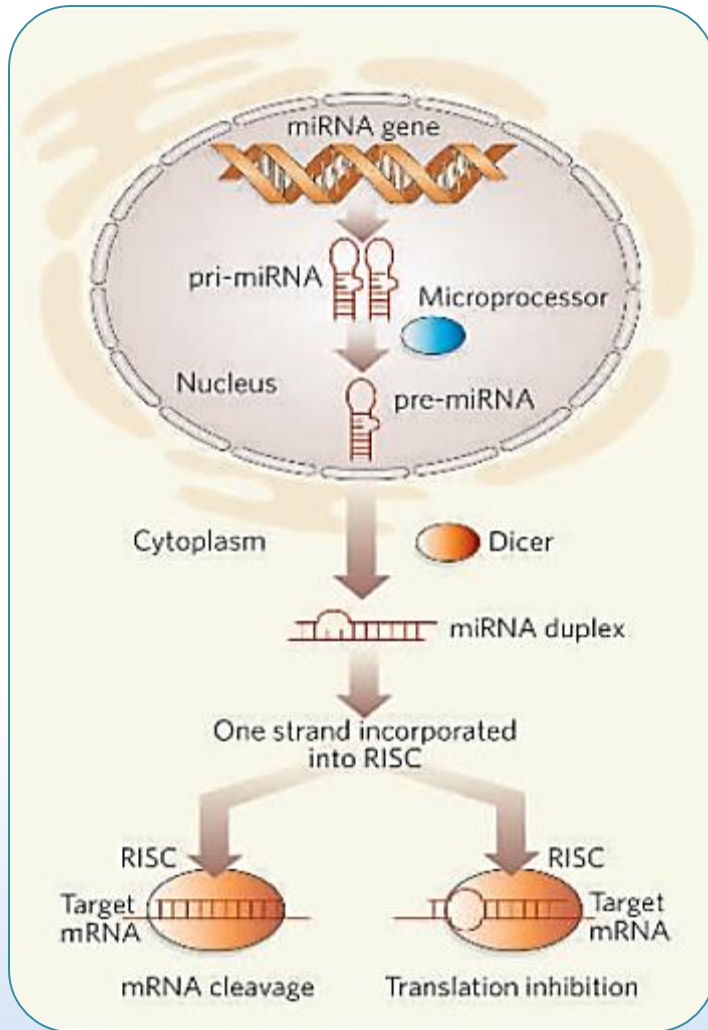


microRNAs



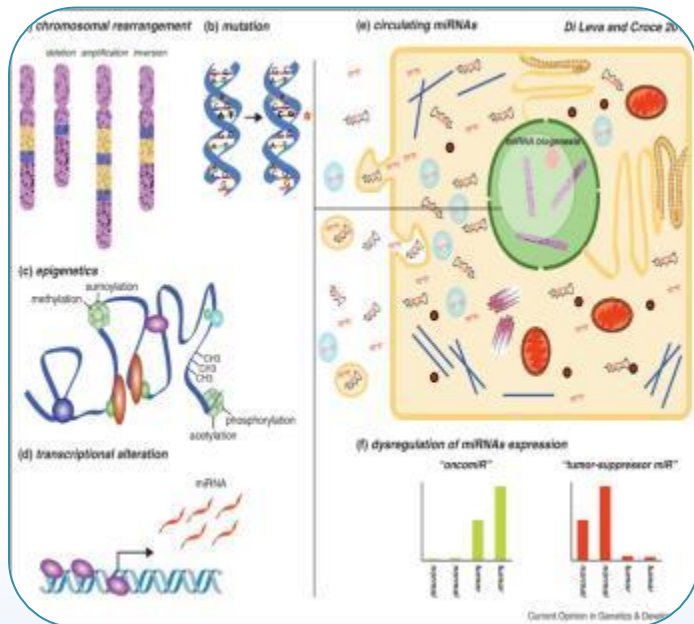
- Non protein coding, endogenous RNAs of 21-22nt length
- Evolutionarily conserved
- Regulate gene expression by binding complementary regions at 3' regions of target mRNAs
- Act as negative regulators of gene expression
- miRNA expression profile is tissue specific

miRNA functions



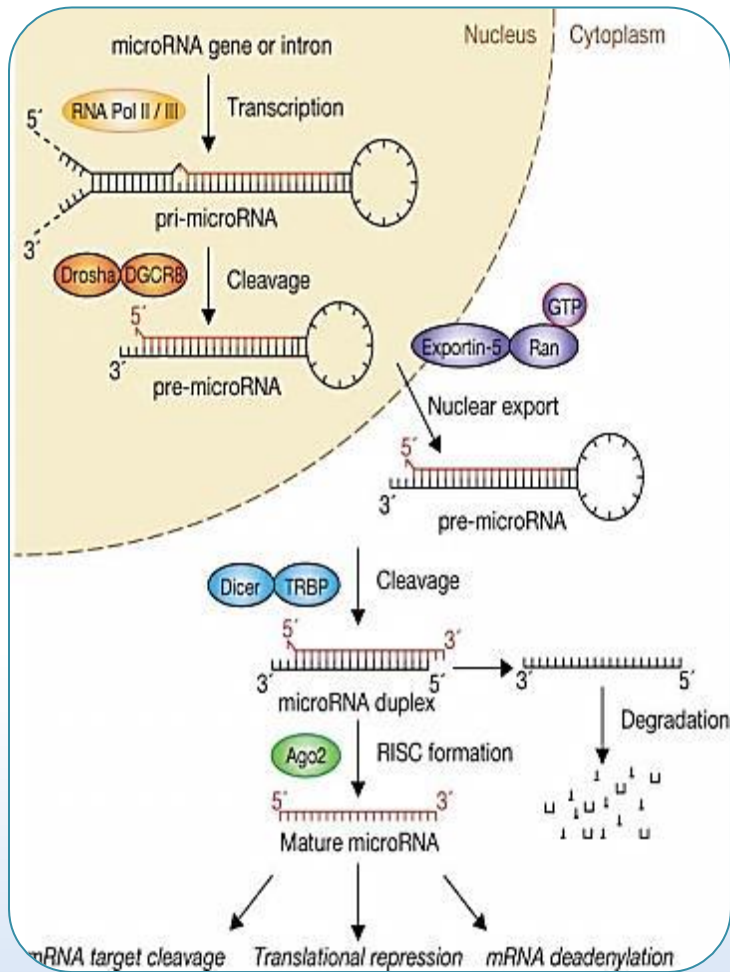
- Regulate 30% of coded genes
- Bring about translation inhibition and mRNA cleavage
- A part of the miRNA matches the target mRNA
- The greater the degree of complementarity with the target mRNA, greater is the probability of mRNA degradation

Diversity of miRNA functions



- Development
 - Timing
 - Cell proliferation
 - Stem cell
- Cell death
- DNA methylation and chromatin formation
- Diseases
 - Cancer
 - Diabetes
 - Cholesterol biosynthesis
 - Nervous system disorders
 - Autoimmune diseases
 - Metabolic diseases

microRNAs



microRNAs



Tumourigenesis

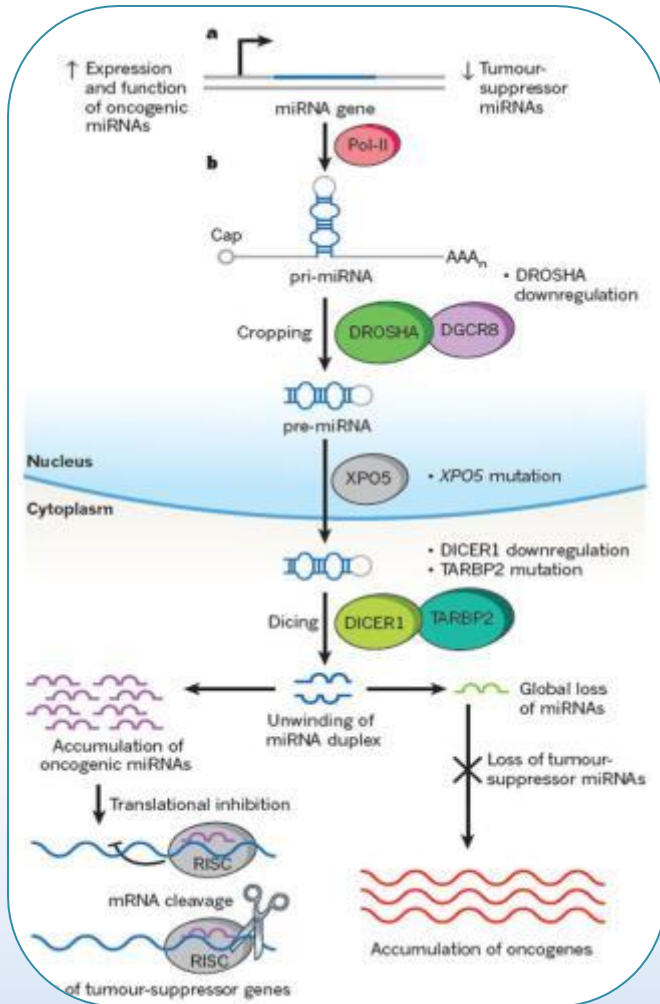
Metabolism

Viral Infection

Aging and Apoptosis

Development / Differentiation

miRNA and Cancer

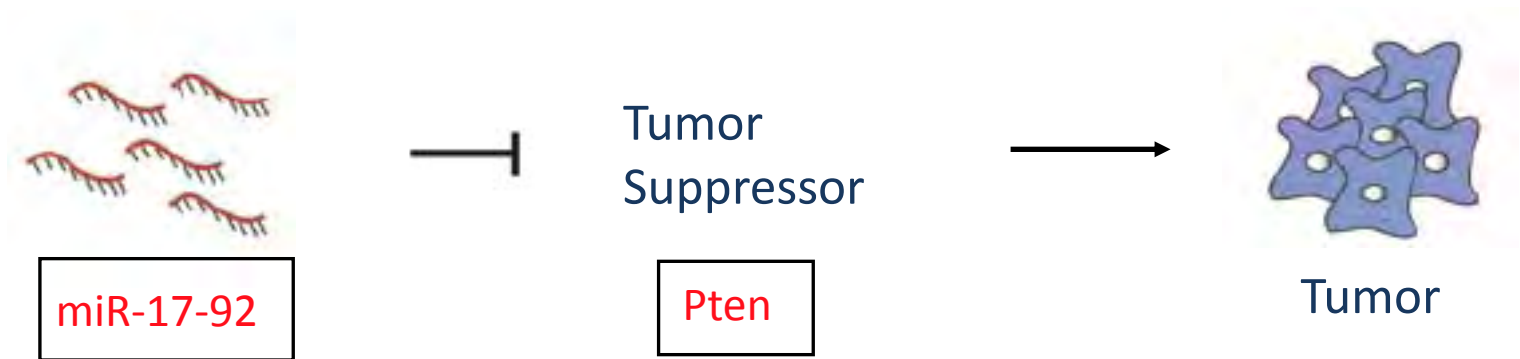


- Majority of miRNA genes are located in cancer associated regions and chromosome fragile sites
- Indicate a differential expression pattern in normal and tumor tissues
- miRNA display tissue and cell lineage specificity
- Altered miRNA expression pattern can serve as markers for
 - Tumor diagnosis
 - Disease prognosis
 - Therapeutic response prediction
 - Cancer of unknown primary

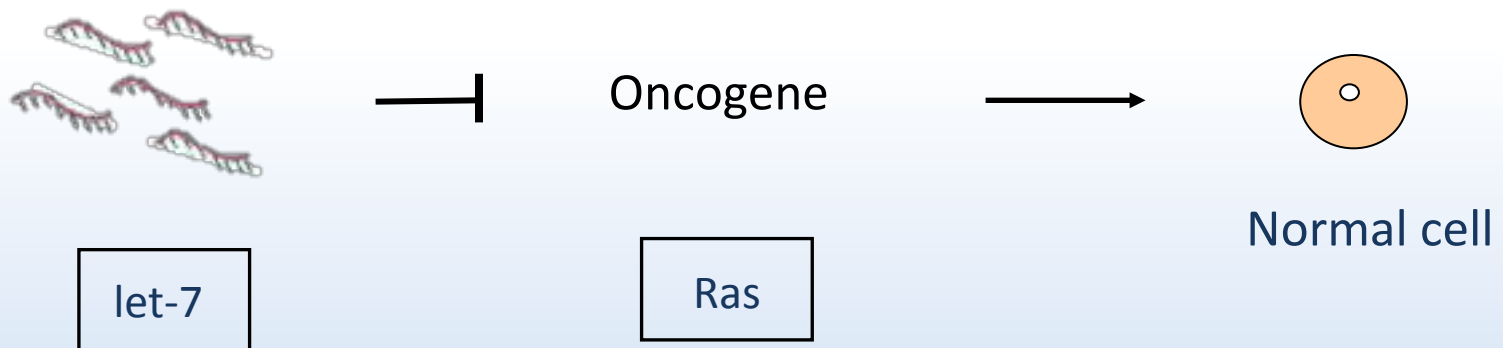
<http://dx.doi.org/10.1038/nature10888>

microRNAs: Oncogene / Tumor Suppressor

Oncogenic miRNAs:



Tumor suppressor miRNAs:



miRNAs: Oncogene / Tumor Suppressor

| Cancer Type | Human microRNA | Potential Function |
|--------------------------|---|---------------------------------------|
| Breast Cancer | miR-21, miR-125b; miR126, miR-206, miR-335 | OncomiR; Metastasis Suppressor |
| Colon Cancer | miR-21; miR-34a | OncomiR; Tumor Suppressor |
| Lung Cancer | miR-21, miR17-92 cluster, miR-106b/93/25 cluster; Let-7a, miR-143, miR-145 | OncomiR; Tumor Suppressor |
| Pancreatic Cancer | miR-196a, miR196b | OncomiR |
| Prostate Cancer | miR-21, miR-15/16; miR145, miR-146, miR-330, miR205 | OncomiR; Tumor Suppressor |
| Ovarian Cancer | miR-141, miR-200a/b/c; miR-199a/b, miR-140, miR-145, miR-204, miR125a/b, | OncomiR; Tumor Suppressor |
| Hepatocellular Carcinoma | miR-21, miR-224, miR34a, miR221, miR-222, miR-106, miR-303; miR26a/b, let-7g, miR-122, miR-422b, miR-145, miR-199 | OncomiR; Tumor Suppressor; Metastasis |
| Thyroid Cancer | miR-146, miR-221, miR222, miR-181b, miR-155, miR224 | OncomiR |

miRNA & Cancer of Unknown Primary (CUP)

- Malignant cancers whose origins cannot be identified based on
 - Clinical history
 - Complete physical examination
 - Routine laboratory tests
 - Imaging and radio metabolic techniques
 - Histological analysis
- Accounts for 3-5% of all cancers
- The prognosis of patients with CUP is poor with a median survival of 4-12 months

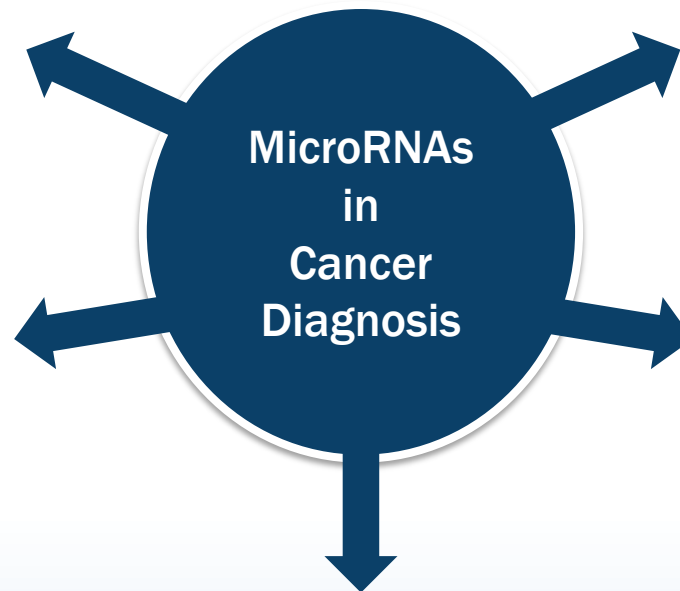
miRNA & Cancer of Unknown Primary (CUP)

- Present treatment is based on consideration for the cell type
- This leads to reduced antineoplastic efficacy and poor outcome.
- miRNA based classification gave 90% accuracy in classifying cancers of unknown primary
- Classification based on differential expression of miRNA that correlated with cancer type, stage and clinicopathological variables
- Provides superior classification of CUP when compared with classification based on mRNA

miRNAs as diagnostic markers

Utilizing a signature of altered miRNA expression to differentiate cancer tissue from normal tissue

Profiling circulating blood or tumor derived exosomal miRNAs, surpassing the invasive procedures to aid in early detection of cancers



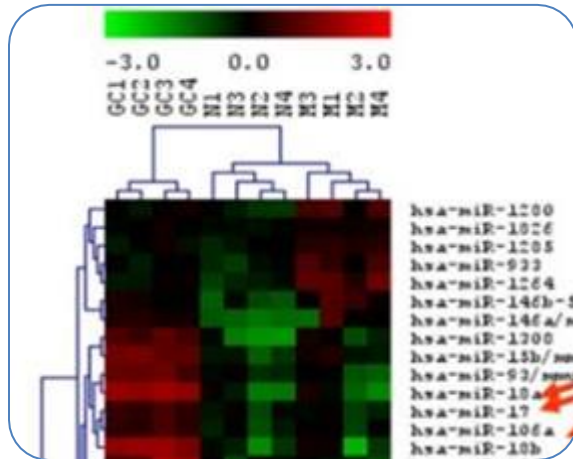
Use of miRNA based classifier to identify tissue of origin for cancers of unknown primaries

Distinguishing tumor subtypes using a panel of miRNAs that show differential expression within one cancer type

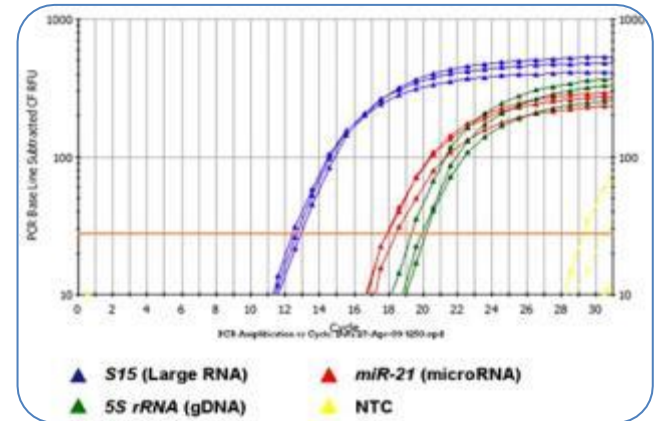
Study SNPs in the miRNA genes, miRNA binding sites in the target mRNA genes or in the miRNA processing/ machinery pathway genes to predict cancer predisposition

miRNA Analysis

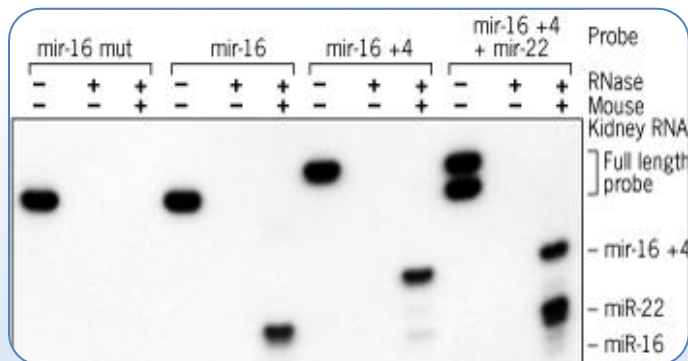
Microarray



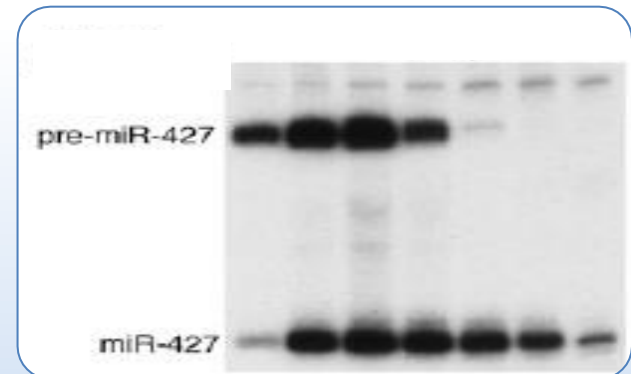
qRT-PCR



RNase protection assay



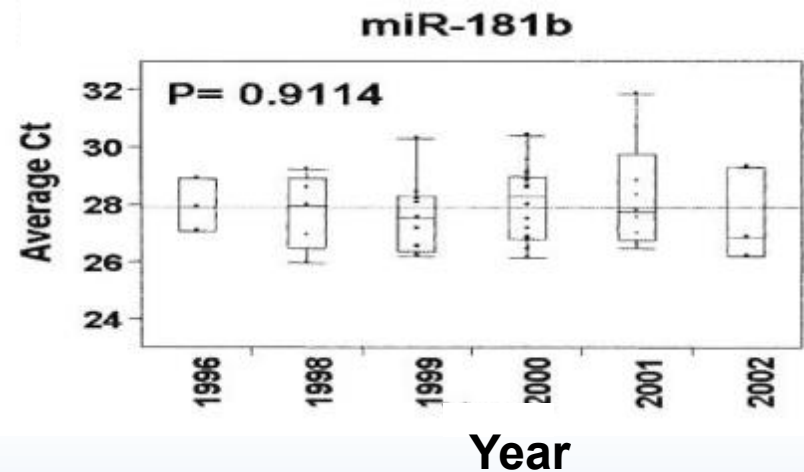
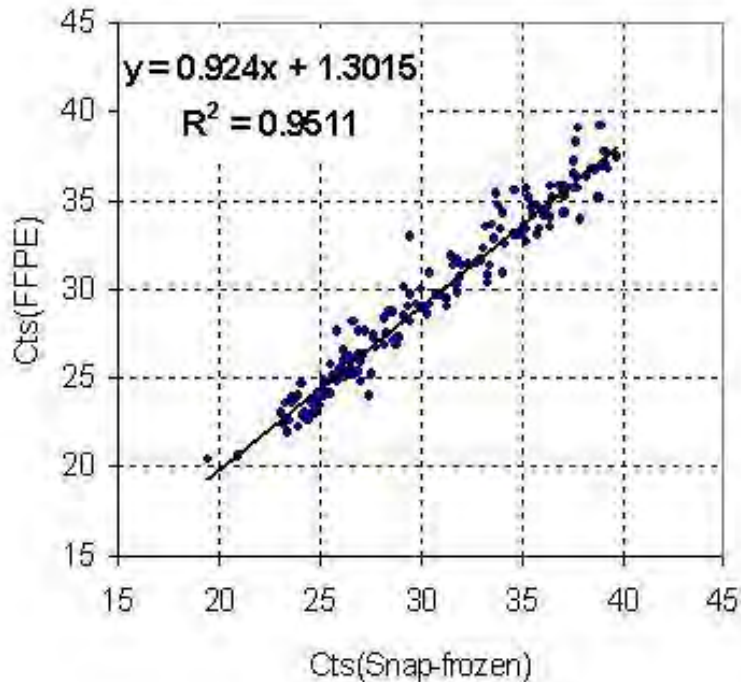
Northern blot



miRNAs in Situ Hybridization

- Larger scale analysis of miRNAs is required for fully understanding their biological functions within disease setting. FFPE tissue samples are invaluable source for this study.
- Study the macroscopic distribution and cellular localization of miRNAs in a heterogeneous cell population.
- Several technique problems in miRNAs in situ hybridization: RNA integrity, lower concentration, very short RNA nucleotides, protection from associated proteins and cellular structure.

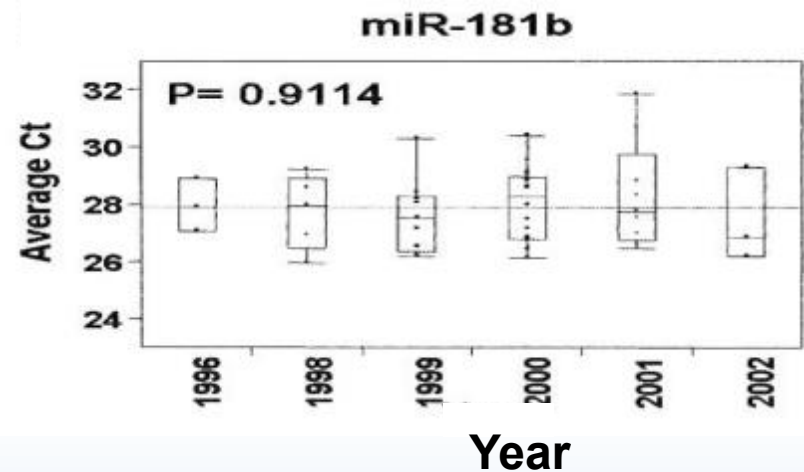
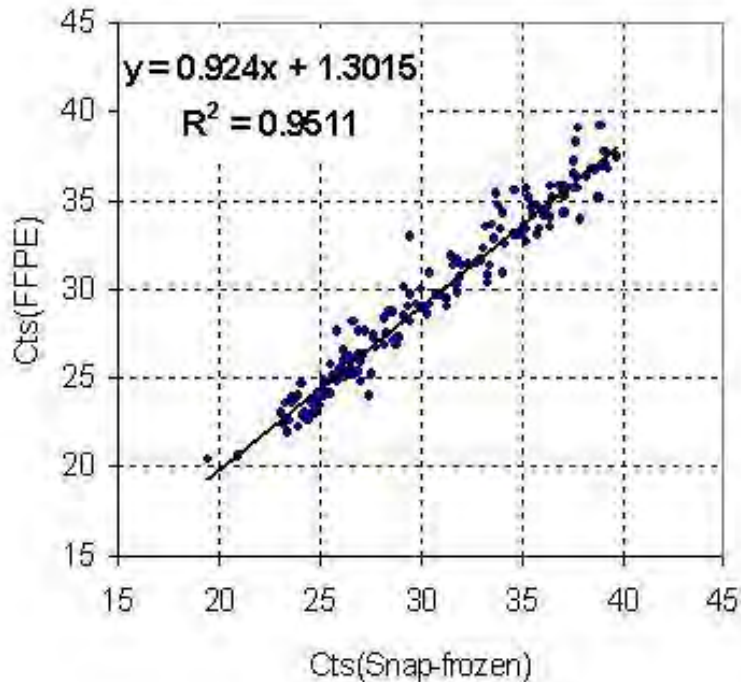
microRNAs are Stable in FFPE Tissues



Xi et al. 2007 *RNA* 13:1-7

Li et al. *BMC Biotechnol.* 2007; 7: 36. 2

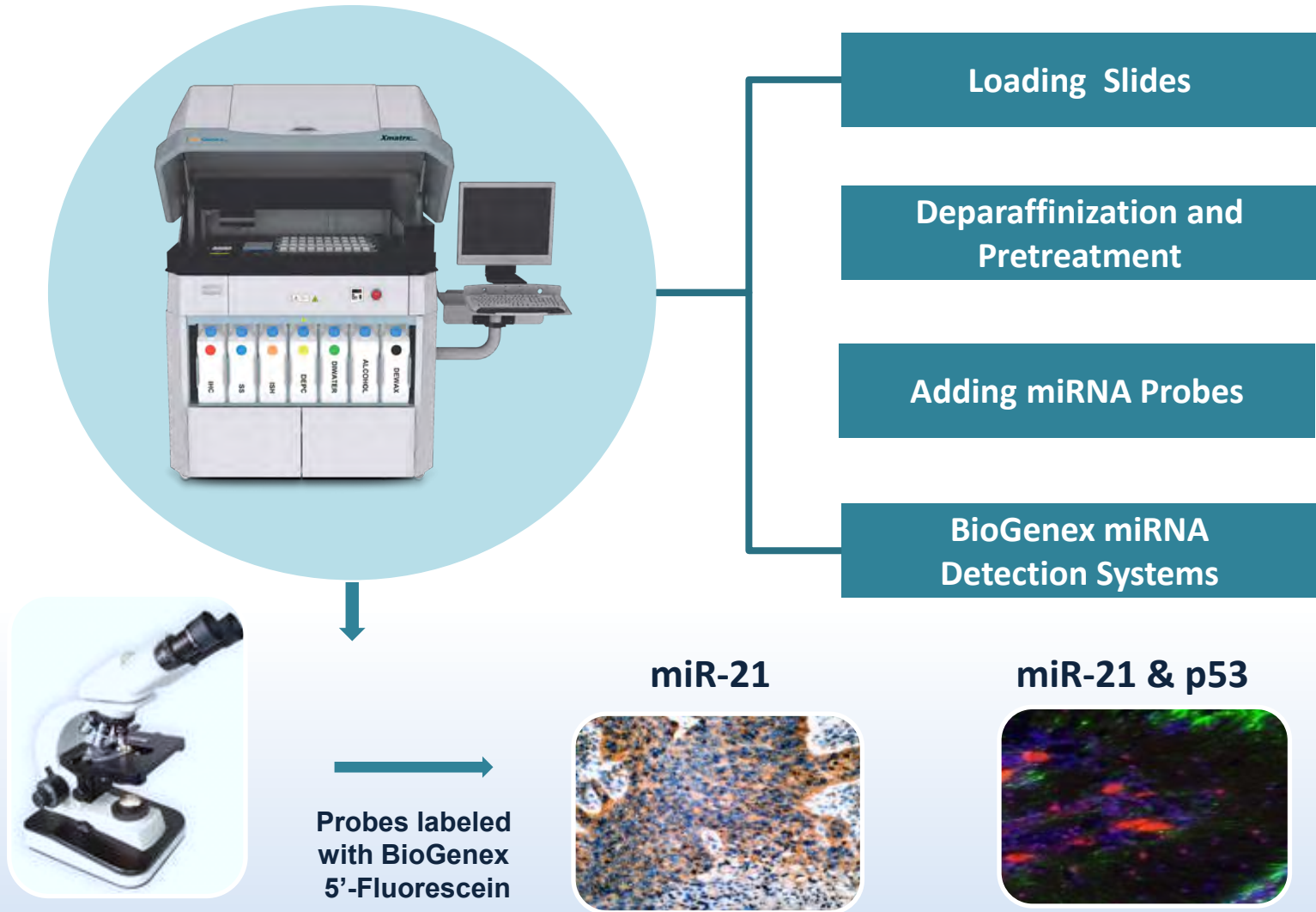
microRNAs are Stable in FFPE Tissues



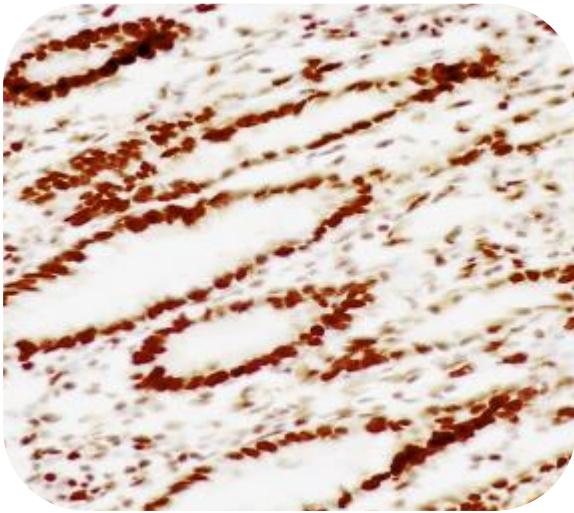
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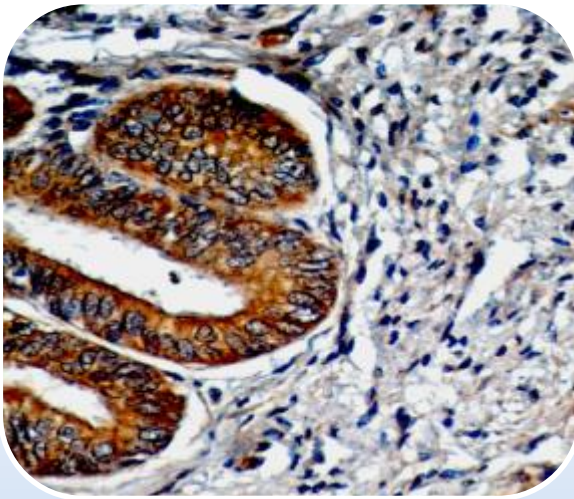
Workflow for MicroRNA Detection



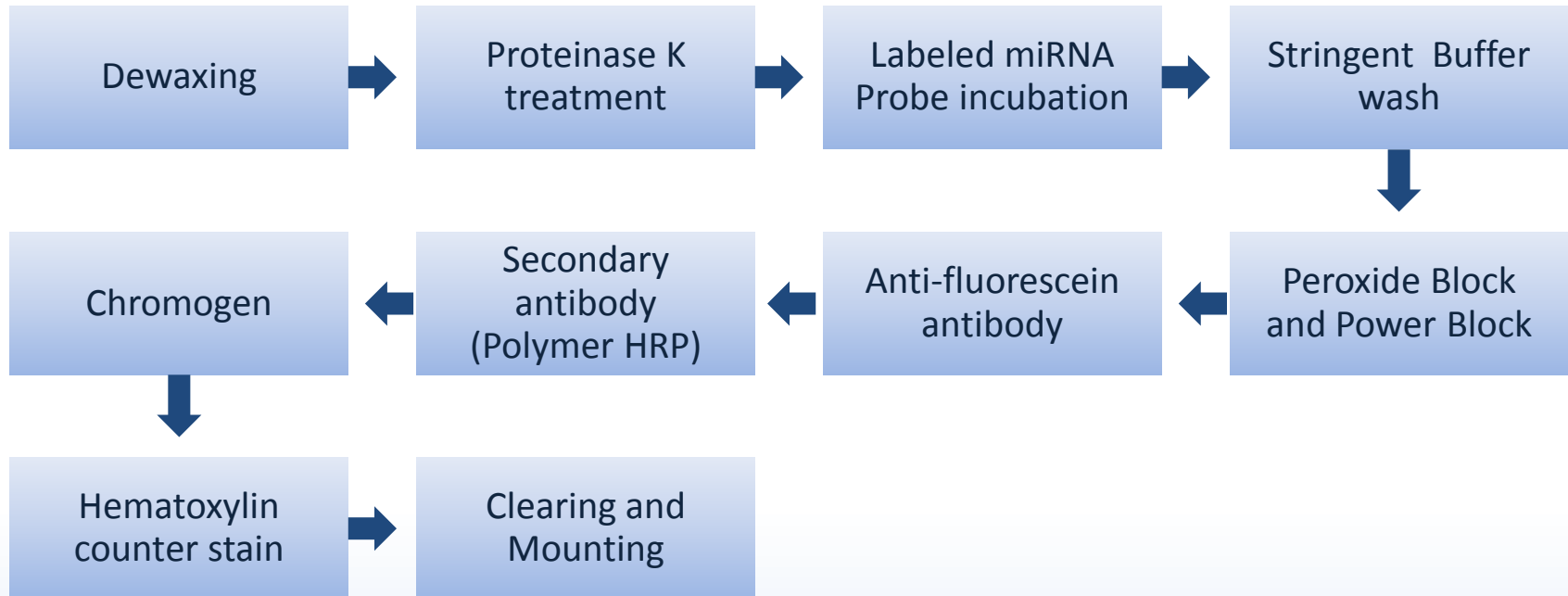
miRNA Analysis - ISH



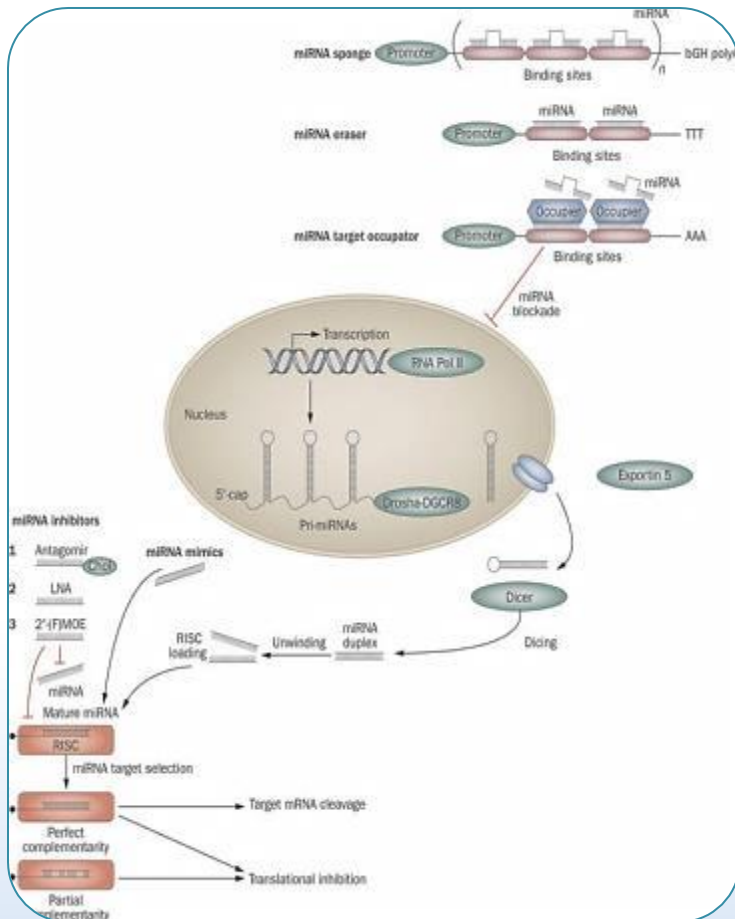
- miRNA's are well preserved in FFPE and frozen samples
- Can be exploited to understand their role in diseases
- Allows to study macroscopic distribution and cellular localization of miRNA in a heterogeneous population



miRNA in-situ hybridization work flow

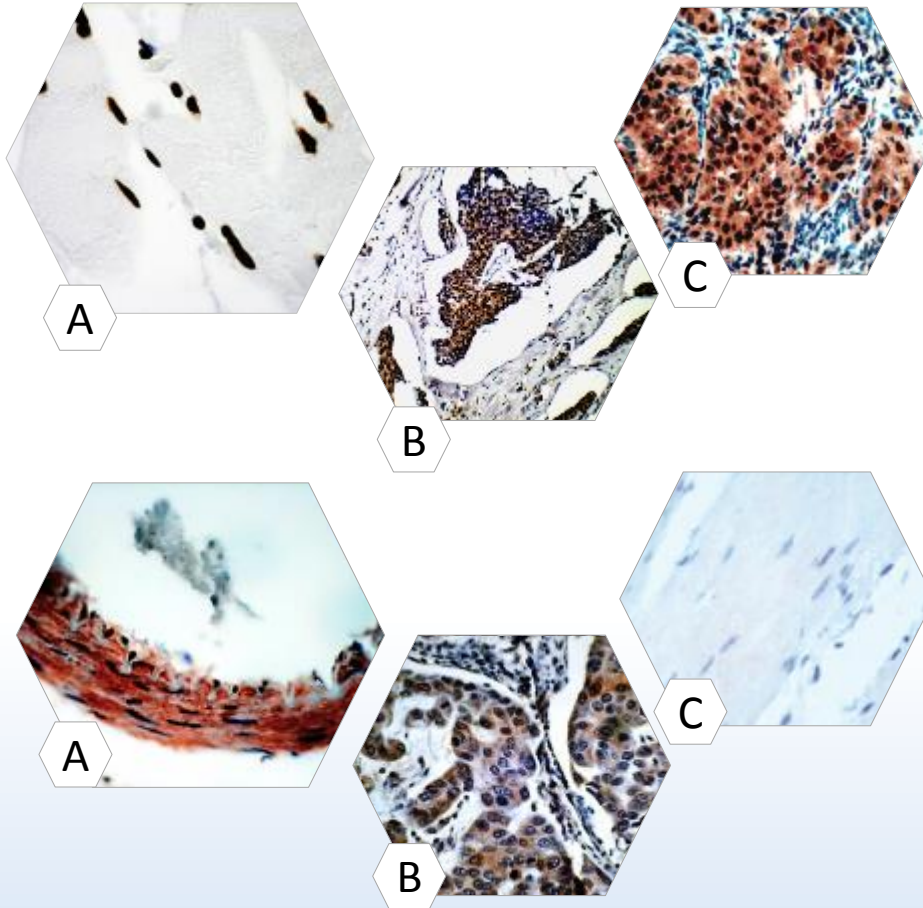


miRNA as Therapeutic Targets



- OncomiRs can act as targets for down regulation
- Can be targeted by anti-sense nucleotides to bring about anti-cancer effect
- Tumor suppressive miRNAs can be upregulated to inhibit tumor progression

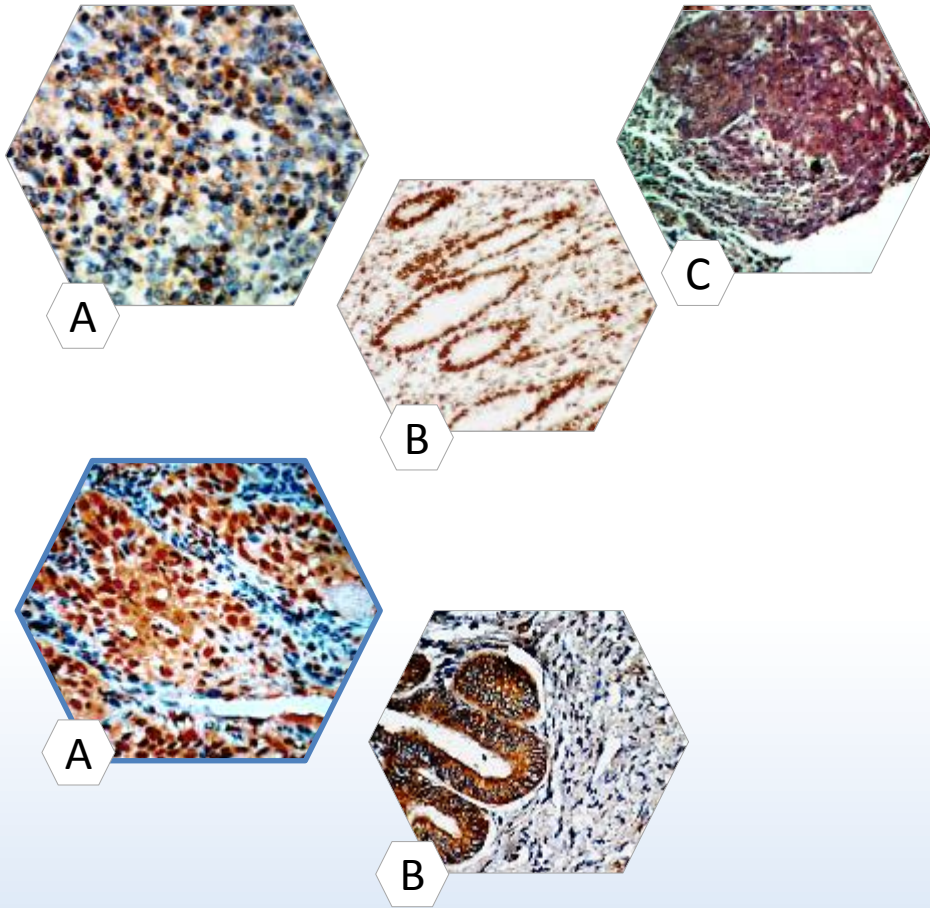
Biogenex miRNA probes I



- A. Has-miR-1
- B. Has-miR-21
- C. Has-miR-146a

- A. Has-miR-145
- B. Has-miR-205
- C. miR-145 mutant

Biogenex miRNA probes II



- A. Has-miR-328
- B. SnRNA U6
- C. Has-miR-221

- A. Has-miR-146 +anti-p53
- B. Has-miR-222

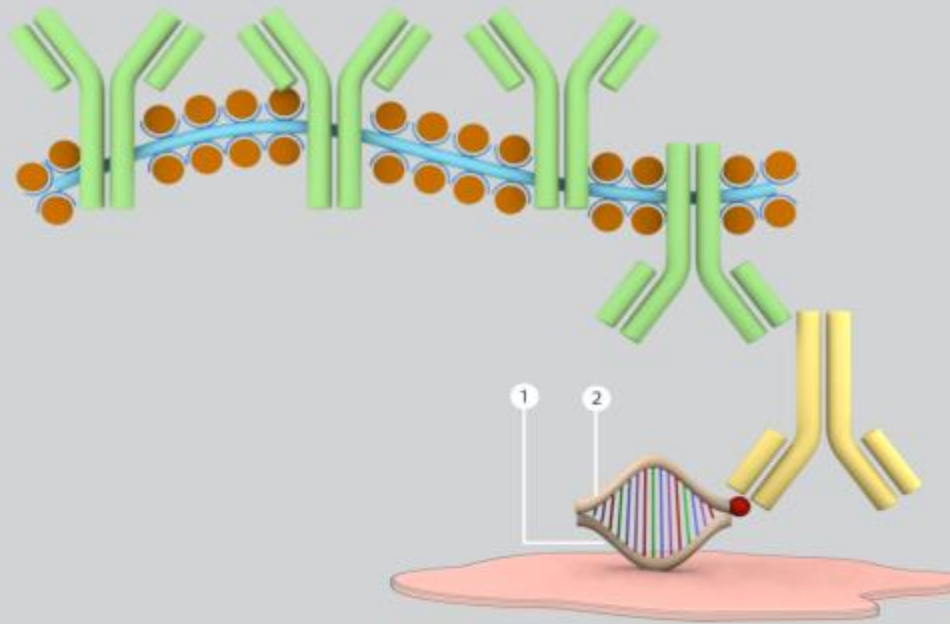
One Step ISH detection Kit










- BioGenex has launched in-situ detection of microRNAs in FFPE tissues on fully automated Xmatrix system.
- It offers the following advantages:
 - Complete kit with all reagents needed, we also offer positive (snRNA U6 probe) and negative (Scramble sequence) probes
 - Biotin-free system to avoid endogenous biotin activity.
 - Poly-HRP detection system provides higher sensitivity.
 - Can be combined with IHC to co-detect miRNA and protein biomarkers.

One Step ISH detection Kit

miRNA Detection System



- Substrate 
- Polymer - HRP 
- Secondary Antibody 
- Anti-Fluorescein Antibody 
- miRNA Probe (Conjugated with Fluorescein) 
- Mature miRNA in cell 
- Tissue 



Molecular Pathology Workflow Solution

Please visit www.biogenex.com for more details on our product portfolio

