

**Discovery of a miRNA-based RT-qPCR signature able to detect early stage colorectal cancer in blood plasma**

**AACR 102<sup>nd</sup> Annual Meeting – April 4, 2011**

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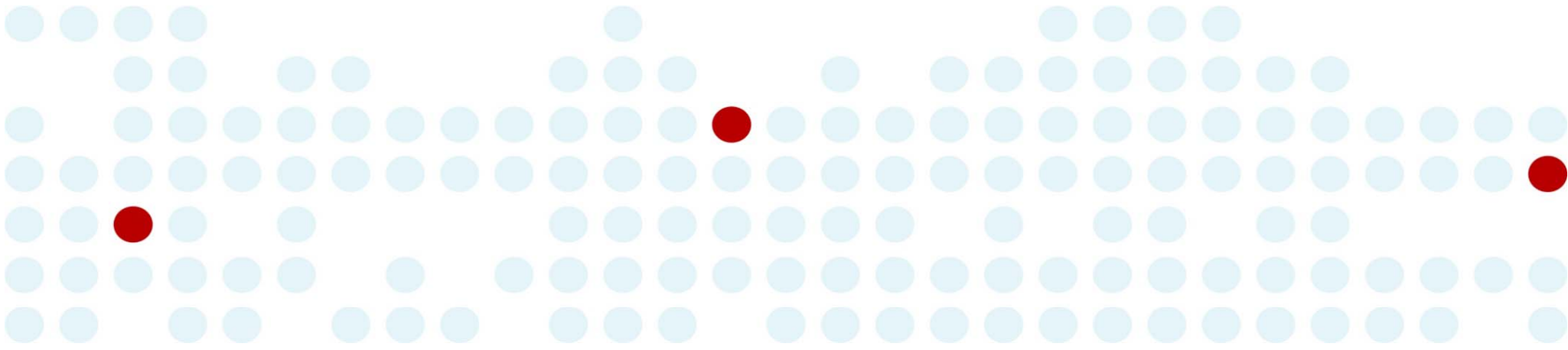
## AACR 102nd Annual Meeting – Adam Baker

I have the following financial relationships to disclose:

I am an Employee of Exiqon A/S, Denmark

*- and -*

I will not discuss off label use and/or investigational use in my presentation.



- Introduction – Why early detection of colorectal cancer and why miRNAs as biomarker
- Development of a sensitive RT-qPCR platform for quantification of microRNAs in clinical samples
- miRNAs in plasma

## Unmet need for early detection test of colorectal cancer

### Colorectal cancer

- Estimated new cases in 2010 in the USA:
  - Colon: 102,900
  - Rectum: 39,670
- Estimated deaths in 2010 in the USA:
  - Combined: 51,370
- **Early diagnosis results in resectable cancer with much improved prognosis**



Stage	5 yr relative survival	Treatment
0-I	93%	Surgery
II	80%	Surgery/discretionary adjuvant chemotherapy
III	58%	Surgery/adjuvant chemotherapy
IV	6.9%	Chemotherapy

## Rationale for early detection test of CRC in blood plasma

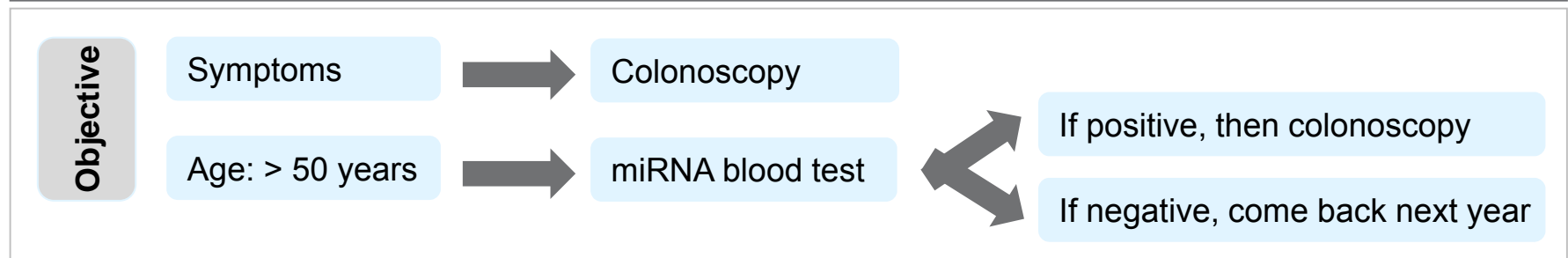
### Colorectal cancer (CRC) screening guidelines

For individuals between 50 and 75:

- Colonoscopy every 5 years (False-negative rate ~5%)  
*or*
- Annual FOBT (False-negative rate 20-75%)
- Poor compliance: <50% of population are screened

**Large unmet need for minimally invasive screening assay for detection of CRC**

### Proposed application of early detection blood test in Colorectal cancer (CRC) screening



## miRNAs fulfill the requirement of being a "Great Biomarker"

### Facts about miRNAs:

- Involved in pathway regulation & tissue differentiation
- miRNA expression profiles are associated with diseases
- Stable in body fluids including plasma/serum
- Can be sampled under standard clinical SOPs

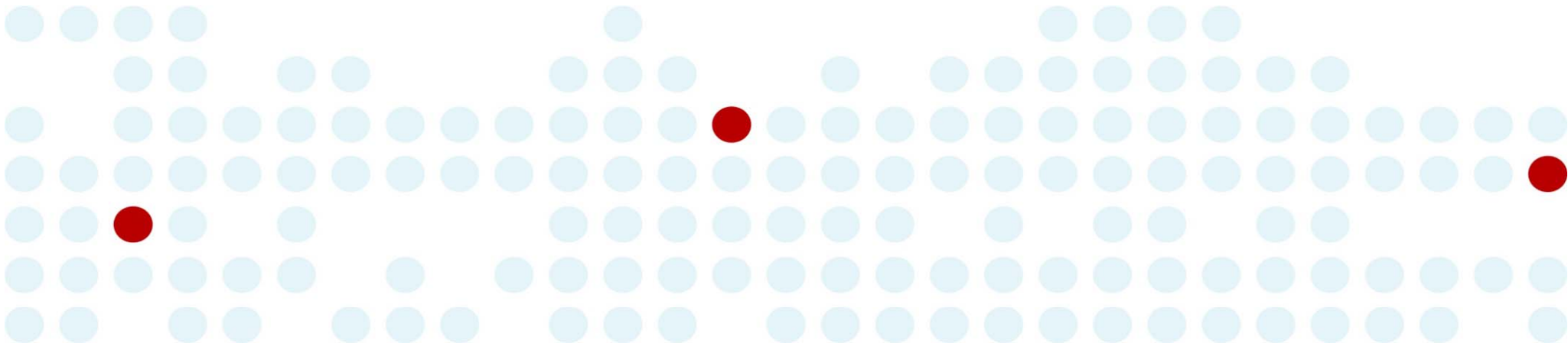
### Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma

Jason D. Arroyo<sup>a</sup>, John R. Chevillet<sup>a</sup>, Evan M. Kroh<sup>a</sup>, Ingrid K. Ruf<sup>a</sup>, Colin C. Pritchard<sup>a</sup>, Donald F. Gibson<sup>b</sup>, Patrick S. Mitchell<sup>a,1</sup>, Christopher F. Bennett<sup>a,c</sup>, Era L. Pogosova-Agadjanian<sup>d</sup>, Derek L. Stirewalt<sup>d</sup>, Jonathan F. Tait<sup>b</sup>, and Muneesh Tewari<sup>a,d,e,2</sup>

nature  
cell biology

MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins

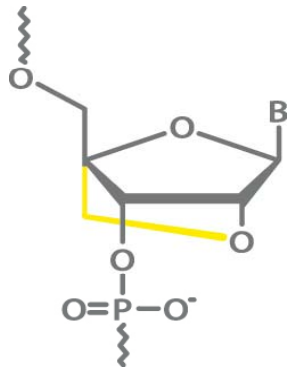
Kasey C. Vickers<sup>1,2</sup>, Brian T. Palmisano<sup>1</sup>, Bassem M. Shoucri<sup>1</sup>, Robert D. Shamburek<sup>1</sup> and Alan T. Remaley<sup>1</sup>



- Introduction – Why early detection of colorectal cancer and why miRNAs as biomarker
- **Development of a sensitive RT-qPCR platform for quantification of microRNAs in clinical samples**
- miRNAs in plasma

# LNA™ Universal RT microRNA PCR System enables robust plasma miRNA profiling

## LNA™



## Technology allows for improved binding

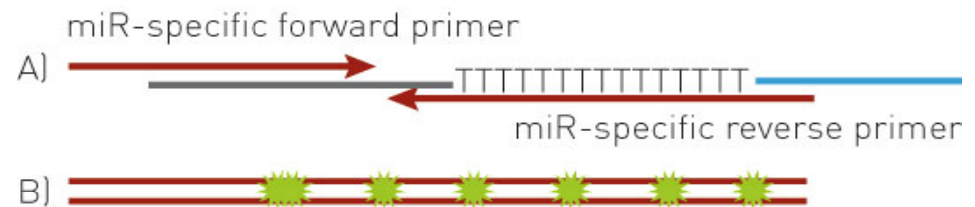
- LNA™ is a bicyclic RNA mimic
- Increased  $T_m$  ( $T_m$  increases by 2 - 8°C per base)
- Improved mismatch discrimination
- Unprecedented sensitivity and specificity

## A unique system for miRNA detection

### Step 1: First-strand synthesis (RT)



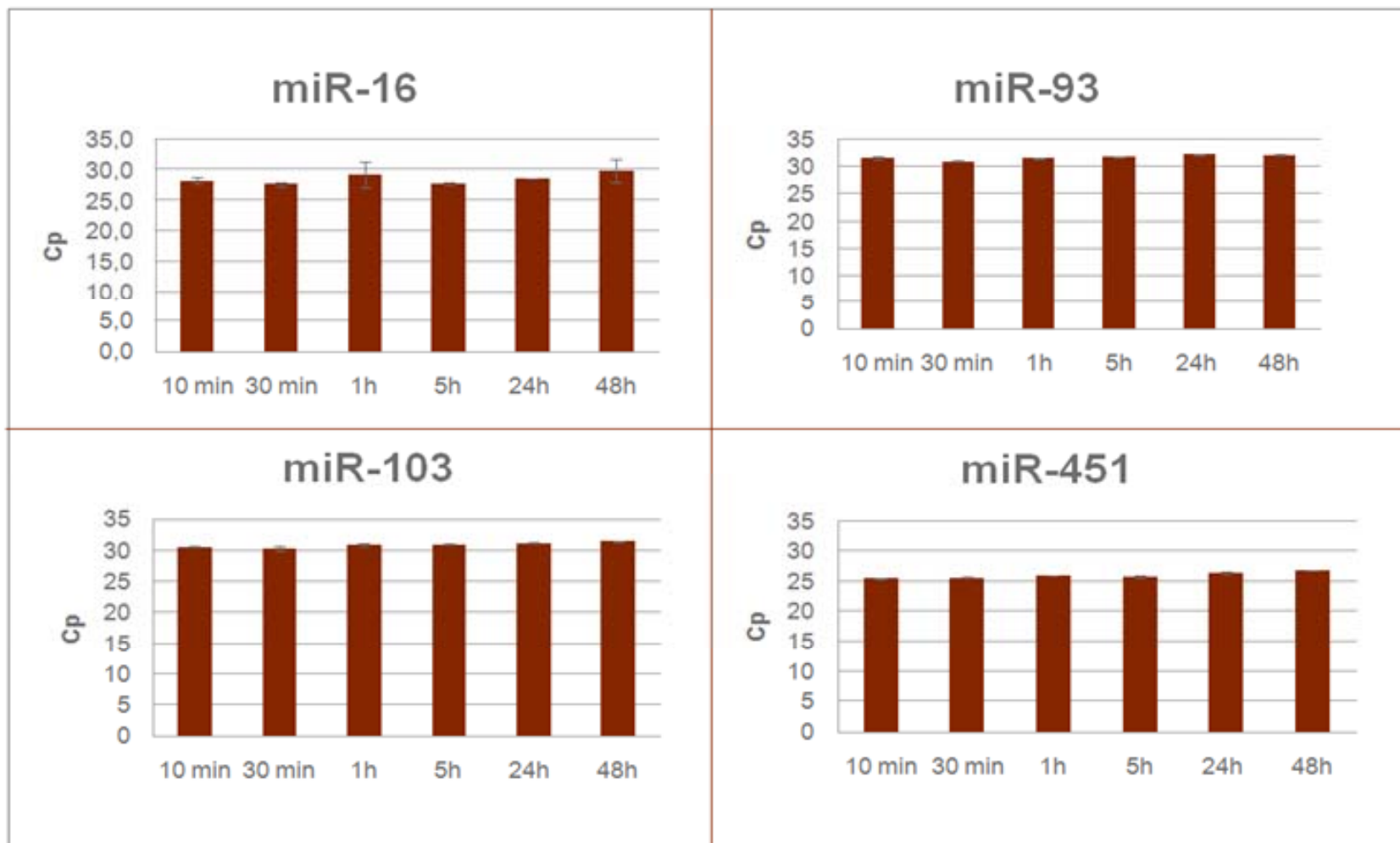
### Step 2: Real-time PCR amplification



### Advantages:

- Universal RT → No bias, no pre-amplification
- LNA™ in two specific primers → sensitivity and specificity

## miRNAs are very stable in plasma at room temperature



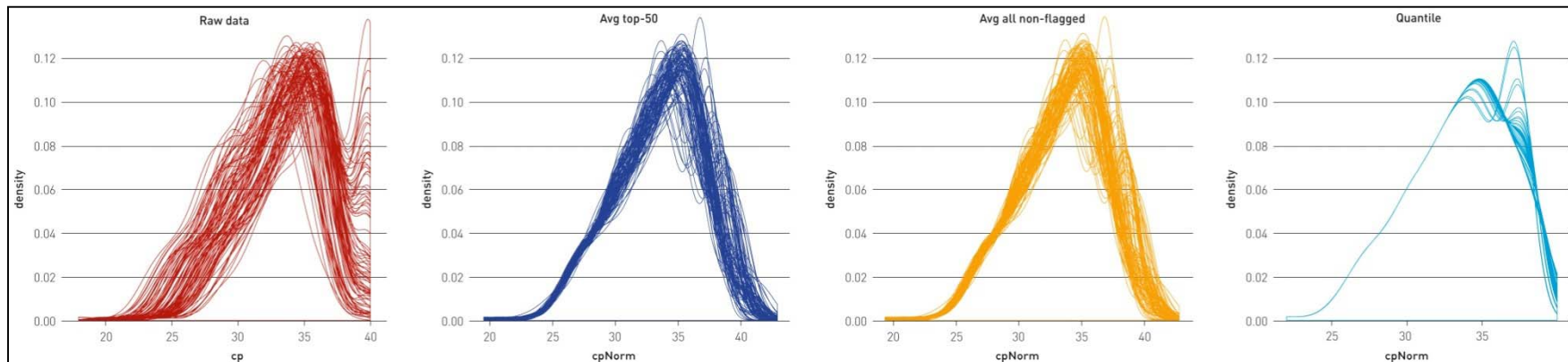
## Normalization of qPCR data is straight forward

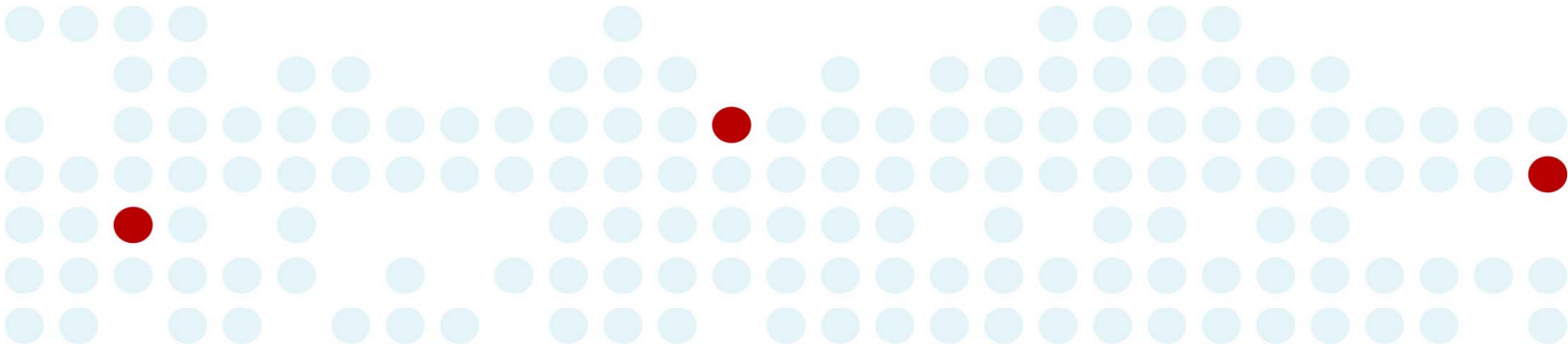
Normalization of qPCR data is necessary to make expression values comparable across samples

Normalization adjusts for technical biases:

- variable amount of sample input RNA
- variable sample input RNA quality
- variable assay efficiencies due to e.g. sample specific assay inhibition

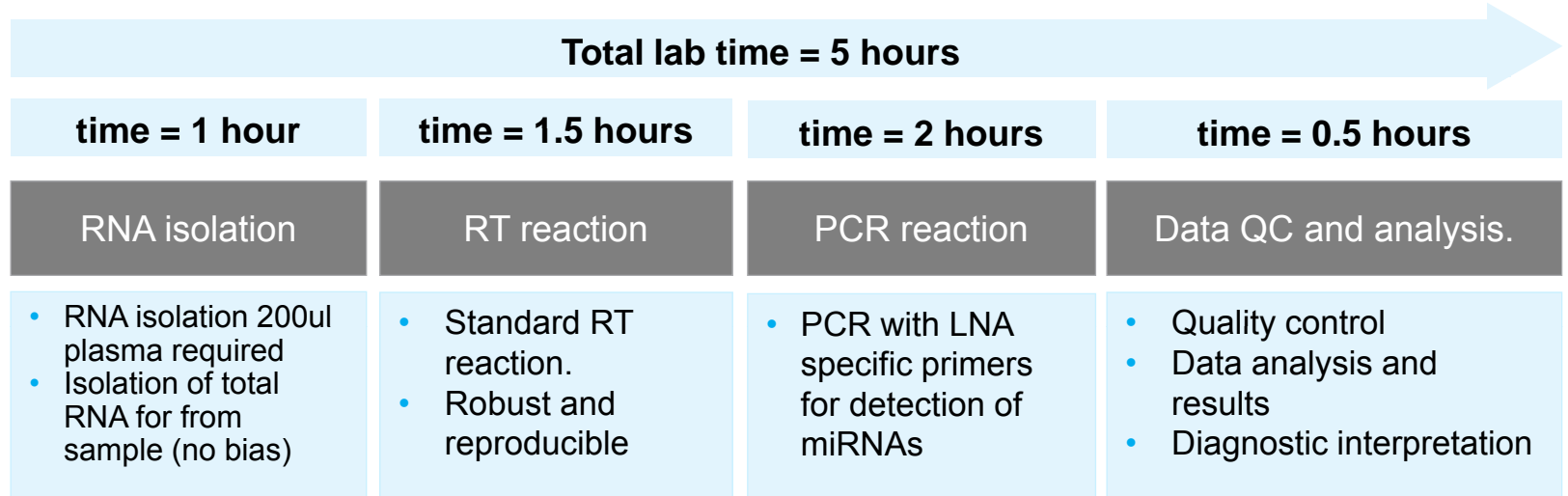
**Prior assumptions about housekeeping genes across the samples of interest is not needed**





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## Work flow compatible with standard clinical procedures



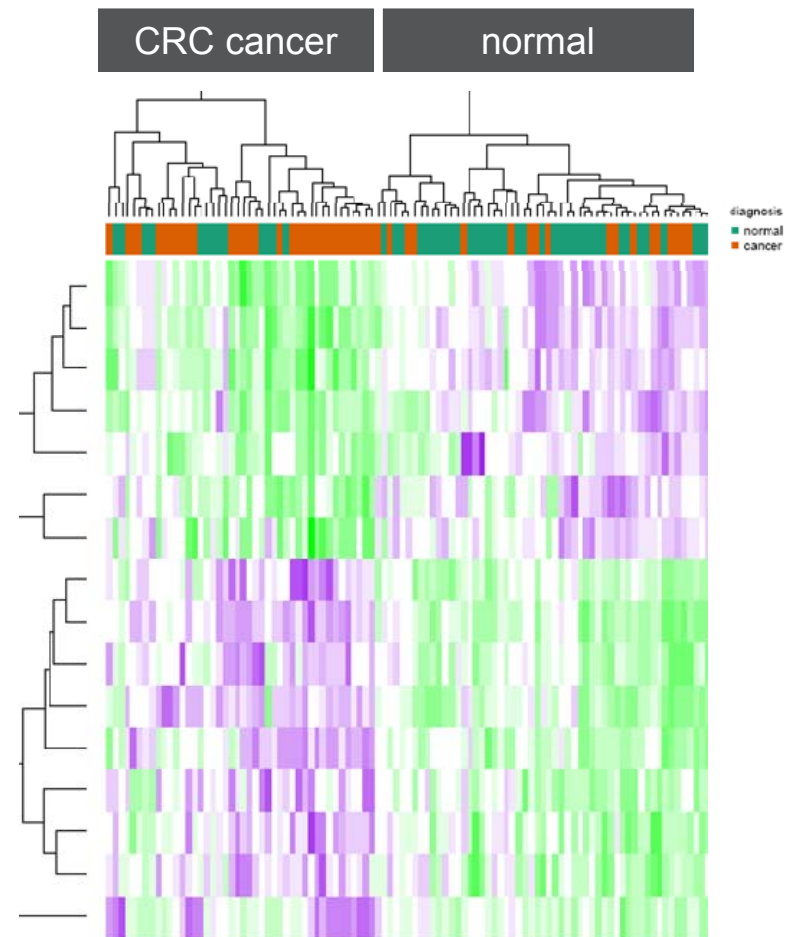
- Assessible to standard hospital protocols
- No special handling/ storage requirements
- Low volume requirement



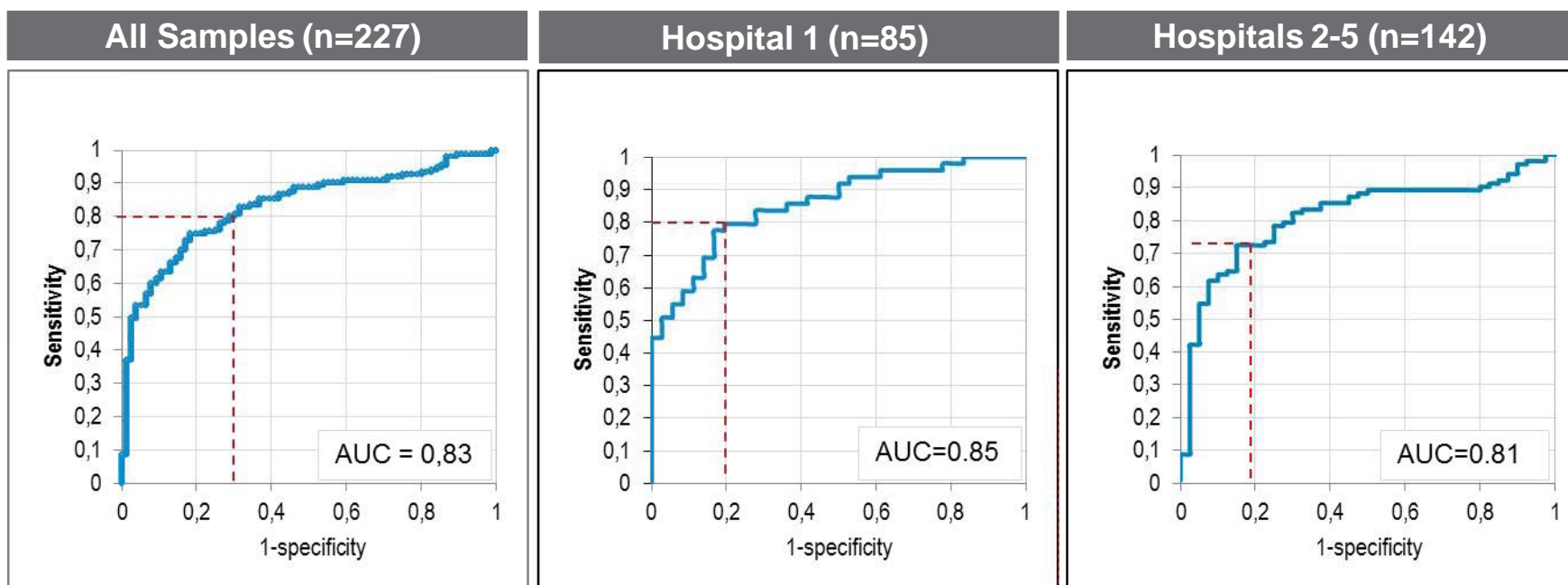
- FDA approved technology
- Conventional qPCR
- No black box algorithms
- Automatable

## Pre-screen: Profile differs in plasma from CRC patients and healthy controls

- 50 stage II colorectal cancer patients and 50 age- and sex-matched colonoscopy negative controls
- Plasma samples (pre-endoscopy)
- Supervised approach on 730 miRNAs. Lasso-based feature selection and simple classifier. 10-fold X-validation
- Screening defined 378 candidate miRNAs present in plasma



## Focused panel of miRNAs in plasma may be used as biomarker for CRC

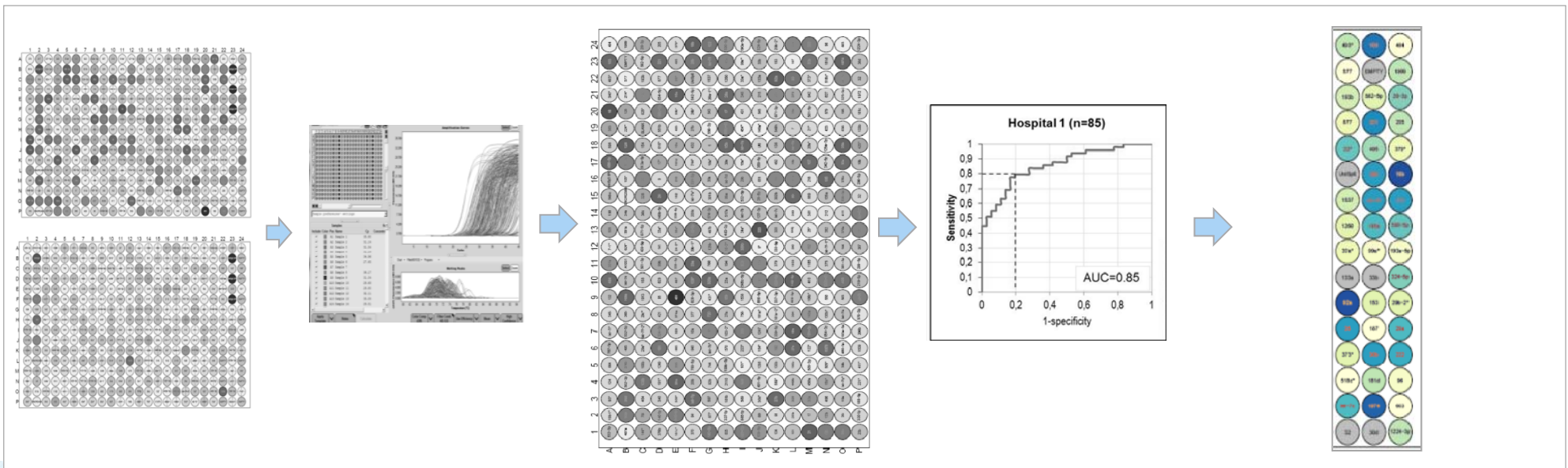


	All Samples	Hospital 1	Hospitals 2-5
Sensitivity *	75%	80%	73%
Specificity *	80%	78%	82%
(n) Cancer	151	49	102
(n) Control	76	36	40

\* The same cutoff score was applied on all samples in the study

# Development of miRNA early detection test of CRC in blood plasma

DISCOVERY PHASE				VALIDATION PHASE
Genome wide screening	Normalization, QC, processing	Candidate miRNA discovery screen.	Bioinformatics, data analysis,	Validation Set miRNA signature .
<ul style="list-style-type: none"> <li>50 controls</li> <li>50 CRC patients</li> <li><b>730 miRNA screen</b></li> </ul>	<ul style="list-style-type: none"> <li>Multiple QC check</li> <li>Data flagging</li> <li>Normalization</li> </ul>	<ul style="list-style-type: none"> <li>76 controls</li> <li>151 CRC patients</li> <li><b>378 custom miRNAs screened</b></li> <li>Multiple controls</li> </ul>	<ul style="list-style-type: none"> <li>Data analysis</li> <li>Quality control</li> <li>ROC curve</li> <li>miRNA selection</li> </ul>	<ul style="list-style-type: none"> <li>3000 patients (2011)</li> <li><b>Defined miRNA signature</b></li> <li>Multiple controls</li> </ul>



## Major validation study to be completed late 2011

### **Conclusions:**

- Robust and reliable platform for detection of miRNA in plasma/serum
- miRNAs fulfill the requirements for being a clinical applicable biomarker
- miRNA profile in plasma/serum may be applied for early detection of CRC
- miRNA profile obtained from 200  $\mu$ l blood in 5 hours
- Major validation study (3,000 individuals) to be completed late 2011

## Acknowledgement

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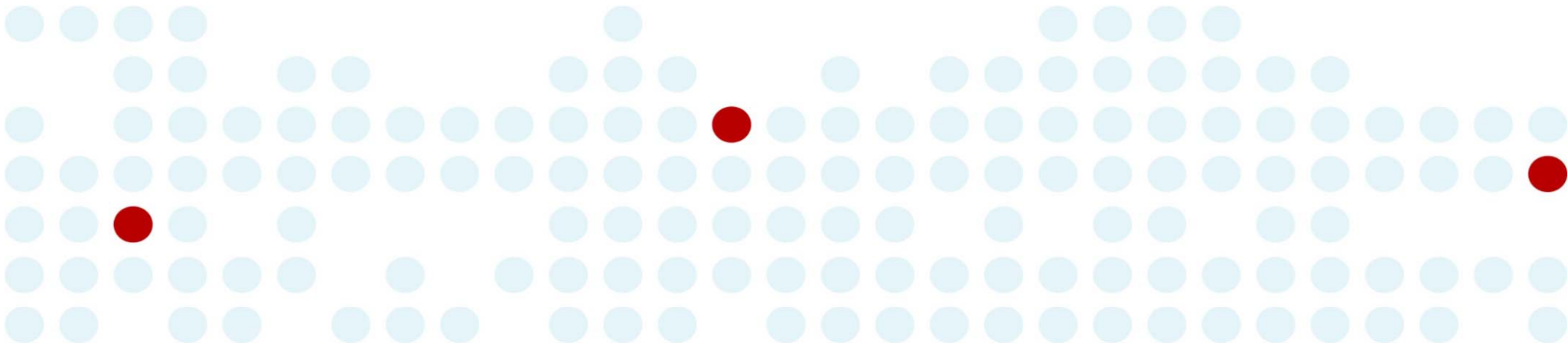
Platform grant 007-2009-2

Concerning the miRCURY LNA™ Universal RT microRNA PCR system:

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