MSRE-qPCR for analysis of gene specific methylation can be accurately used for detection and validation of colorectal cancer-specific patterns

4BIO Summit: Europe, November 28th, 2018

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Aim of UDX

- **Make cancer a curable disease by detecting its pre- or early stages and recurrence**
  - Develop **blood-based cancer screening tests** and **monitoring tests** using and combining various **OMICS** (epigenomics, transcriptomics, metabolomics) and **Bioinformatics** approaches
- We aim to develop a **high accuracy combinatory platform** that could be used for wide range of **cancers**
- The first cancer we tackle is **colorectal cancer** and its pre-cancer stage (advanced adenoma) detection

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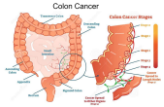
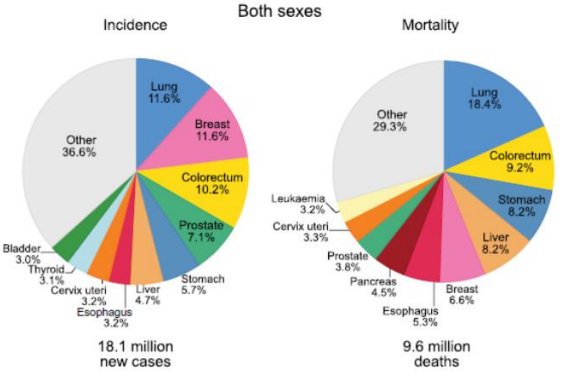
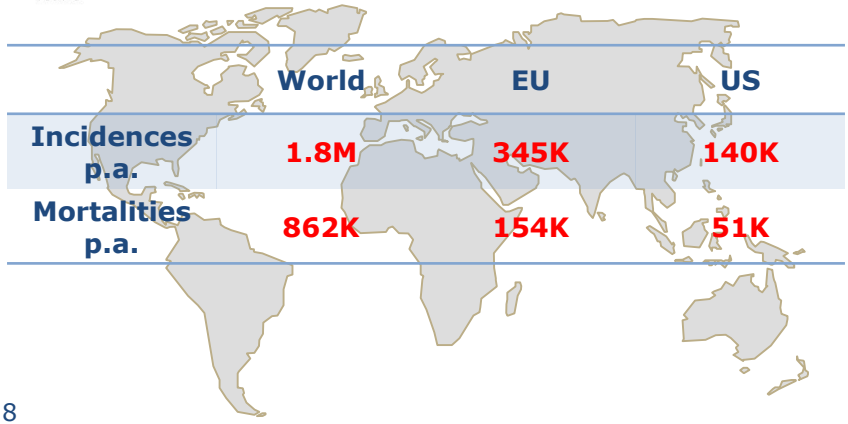


Colorectal cancer statistics





Source:Global Cancer Statistics 2018

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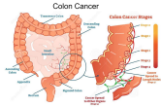
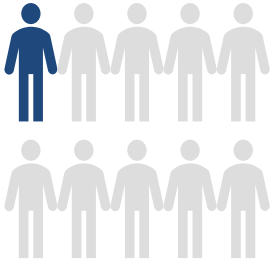
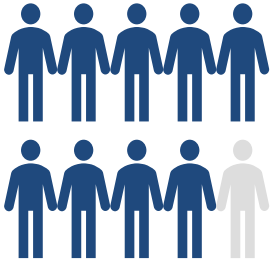


Early detection of colorectal cancer saves lives



CRC stage IV: 1 out of 10
survive 5 years



CRC stages I: 9 out of 10
survive 5 years

Detecting CRC early → **saves lives**

Current screening tests struggle with low compliance- patients prefer blood-based assays to stool
Current blood-based assays have low accuracy

Source: SEER 18 2004-2010

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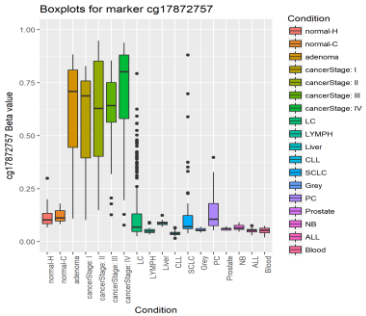
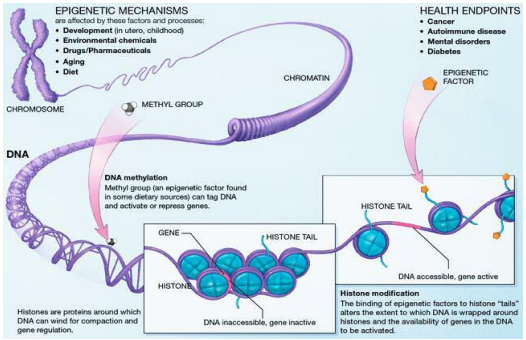
DNA- Methylation Biomarkers

- **DNA methylation = „ideal" biomarker**
  - **mC- changes = early event in cancerogenesis /complex disease development**
  - influence course of illness, response to drugs
  - **tissue specific (cancer specific)**
  - *heritable* pattern
  - chemically as stable as DNA
  - **detectable and stable in blood plasma** (e.g. plasma cfDNA (ctDNA))





http://commonfund.nih.gov/epigenomics/figure

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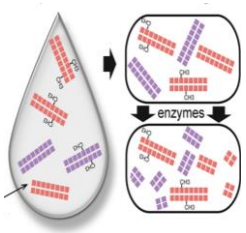
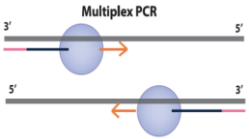
Methylation specific restriction enzyme (MSRE)-qPCR

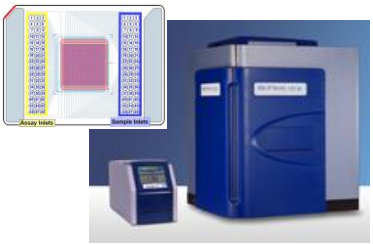
Methylation sensitive restriction digest



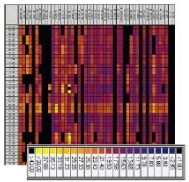
Pre-amplification



96x96 high-throughput µ-fluidic qPCR



Array of cp values



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Method suitable for blood based screening				
	high multiplexing capacity	pre-amplification	<10 copy detection	plasma validation
MSRE (< 0.5ug DNA)	+	+	+	+

Sample set

- 4 ml of plasma prospectively collected from 133 patients attending colorectal cancer screening and oncology units in Spain and USA
  - All patients were colonoscopy checked and classified as:
    - CRC- patients with confirmed colorectal cancer stage 0-IV
    - Control- patients with no finding, patients with hyperplastic polyps and non-advanced adenomas
- Setup
  - 70 samples were used as training set- case vs control
  - 63 used as validation set

	Training (n = 70)			
	CRC	Healthy + Non-advanced adenoma	Healthy	Non-advanced adenoma
Female (%)	15 (21.4)	20 (28.6)	15 (21.4)	5 (7.1)
Male (%)	15 (21.4)	20 (28.6)	15 (21.4)	5 (7.1)
Age (range)	64 (41-80)	60 (46-84)	60 (46-77)	60 (48-84)
BMI (sd)	25.0 (± 4.4)	26.7 (± 4.3)	26.7 (± 4.7)	27.2 (± 3.0)
	CRC all	Proximal	Distal	
Localized	17	6	11	
Advanced	13	9	4	

	Validation (n = 63)			
	CRC	Healthy + Non-advanced adenoma	Healthy	Non-advanced adenoma
Female (%)	11 (17.5)	25 (39.7)	20 (31.7)	5 (7.9)
Male (%)	9 (14.3)	18 (28.6)	11 (17.5)	7 (11.1)
Age (range)	61 (45-84)	57 (47-80)	55 (47-80)	63 (55-70)
BMI (sd)	25.4 (± 3.8)	26.3 (± 4.0)	26.3 (± 4.0)	26.7 (± 4.0)
	CRC all	Proximal	Distal	
Localized	13	5	8	
Advanced	7	4	3	

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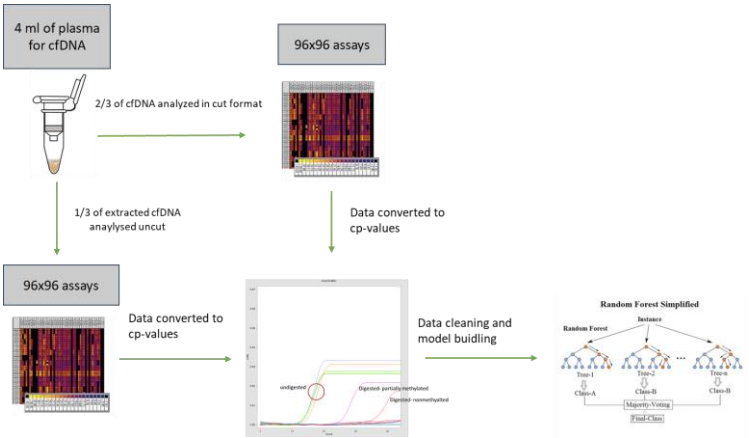
Experimental set-up

- 250 initial CpG-s selected by analysing publically available data (TCGA, Luo *et al*.)
- 180 targets + 6 controls assayed to 2 x 96 marker assays
- 1/3 of cfDNA analysed uncut- QC
- 2/3 of extracted cfDNA cut with AciI, Hin6I or HpyCH4IV (1-15 cut-sites per target)
- 45-cp values used for down-stream statistical analysis with random forest



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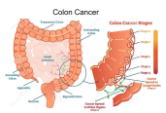
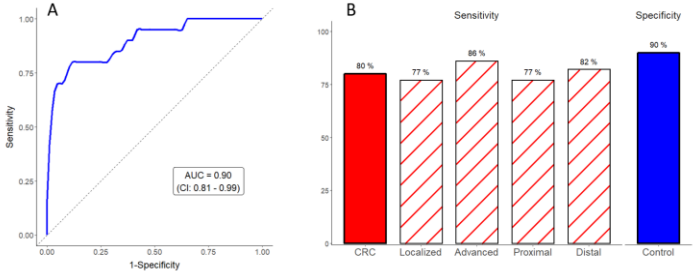
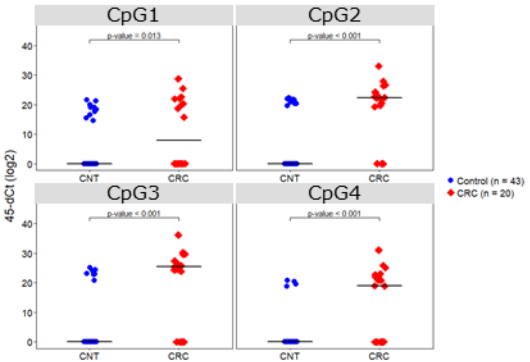


Results for CRC vs CNT+NAA from 9-marker model





- Validation set:
  - **AUC 90%**
  - Overall **sensitivity 80%** at **90% specificity**
  - **Early-localized stage sensitivity of 77%**
  - Good sensitivity both for left and right side cancer

- **Single markers** showing good discriminative power with **high specificity**
- Biological relevance of the top 9 markers verified and mapped

Methylation markers give a clear and strong CRC signal with high specificity

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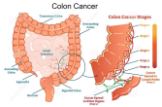

Conclusion

- **MSRE-qPCR** technology could be successfully used for **accurate multiplexed analysis** of **methylation** markers from **plasma** samples
- **Methylation markers** can successfully be used for plasma-based detection of **colorectal cancer** patients
- **80% sensitivity** at **90% specificity** could be achieved for colorectal cancer with **77% sensitivity** for **early-localized cancer** detection

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UDX team!

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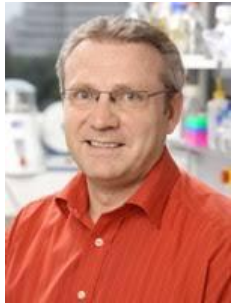

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Special Thanks!

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Thank you for your attention!

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