High throughput MSREqPCR for colon-cancer DNA-methylation biomarker testing

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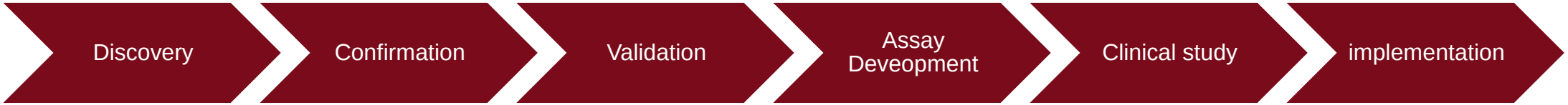
DIAGNOSTIC NEED & TECHNICAL OPTIONS

- **High need** for improving DX
  - Early diagnostic markers, predictive / stratification & monitoring markers
  - **DNA methylation** – is an epigenetic key player and a dysregulation an early event in cancerogenesis
- **DNA methylation biomarker** tools emerged over past >10 years ….
  - 2 major principles:
    - **Bisulfite** (SO_3) deamination based distinction (ME: **^{m}C>C;** UM: C>T,)
    - **MSRE** - methylation sensitive restriction enzymes (AciI, HpaII, …)

      UM MSRE-digested (not amplified), ME undigested & amplified
  - Technical approach from omics discovery to single “minimal invasive” assays
    - **Microarray** based discovery (from tissue DNA) very powerful… Illuminas EPIC array (SO_3 based)
    - **qPCR** based multiplexed confirmation & validation using cfDNA ➔ MSRE best choice



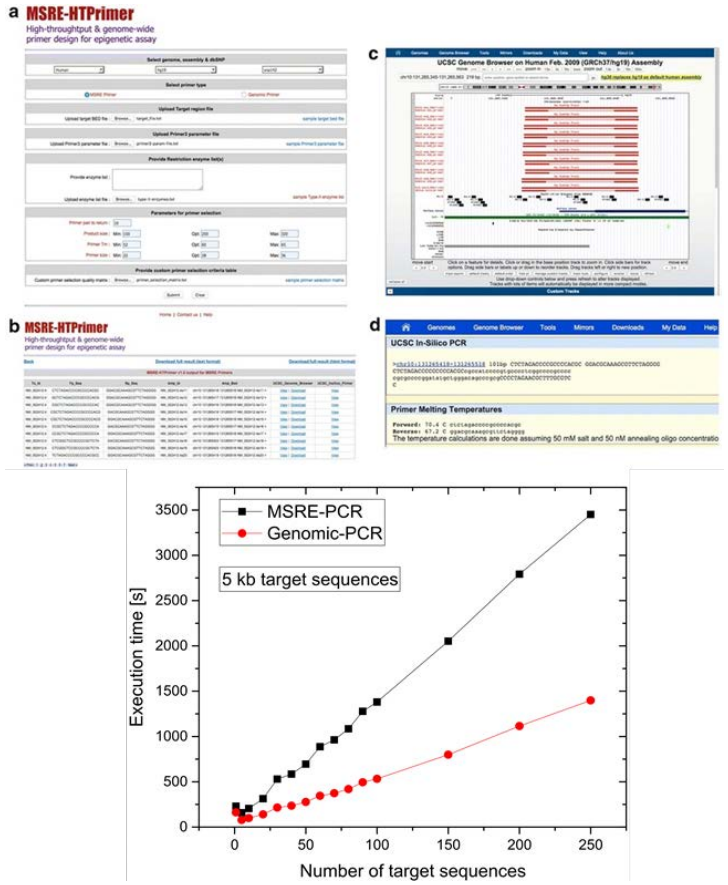
Tissue DNA ➔ microarray ➔ PCR

use PCR confirmed tissue derived candidates ➔ SELECT best performing markers using **Plasma cfDNA** - **PCR based**

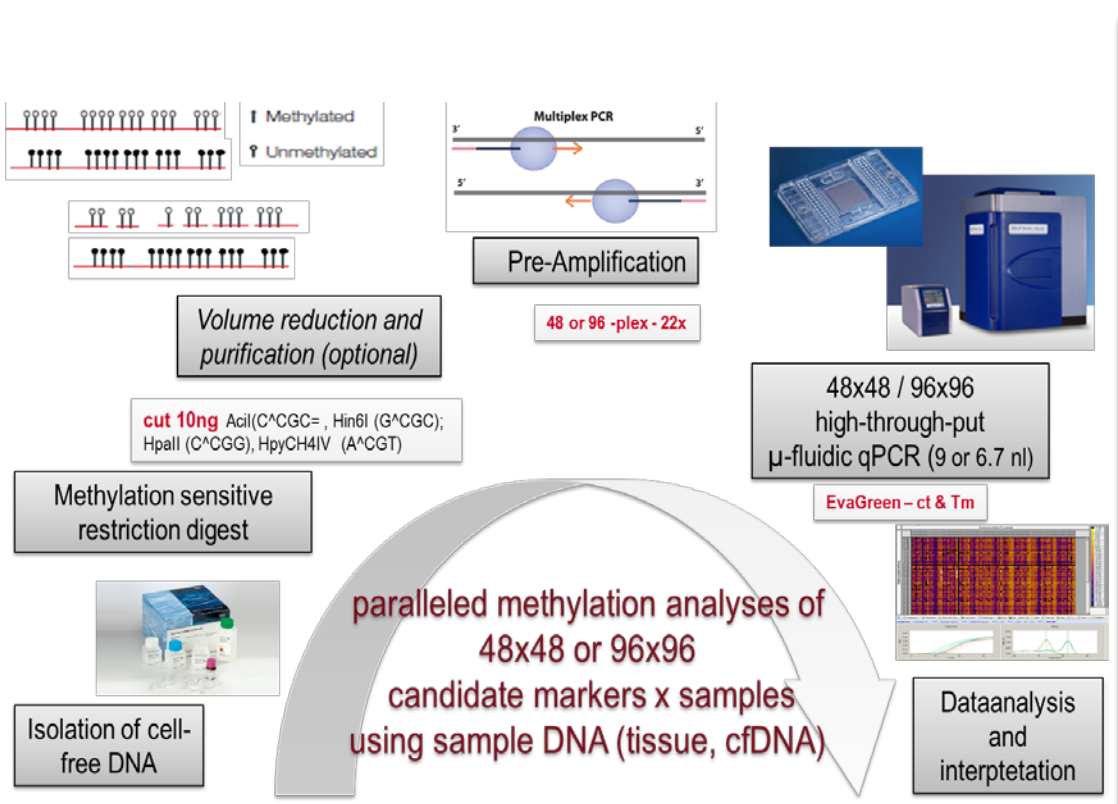
METHODS: high throughput design & qPCR testing

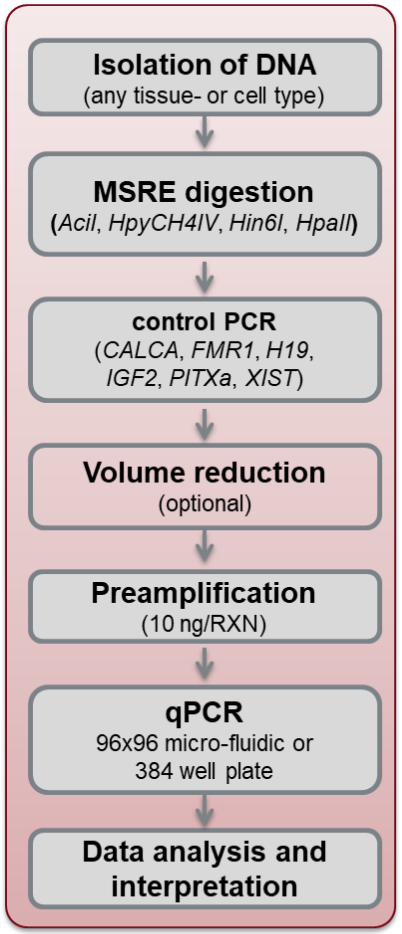
AUSTRIAN INSTITUTE OF TECHNOLOGY

In silico **assay design** pipeline:



laboratory workflow:





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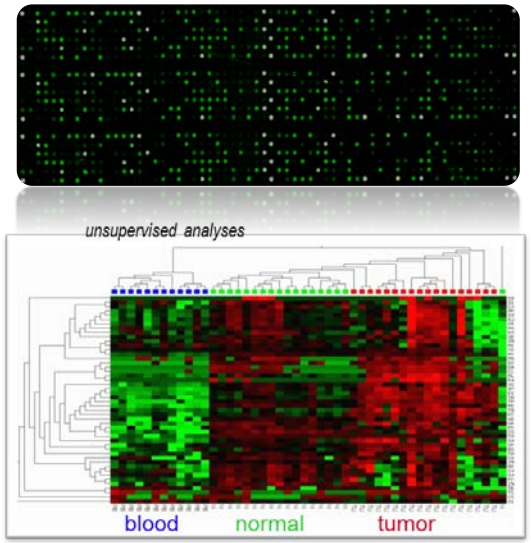
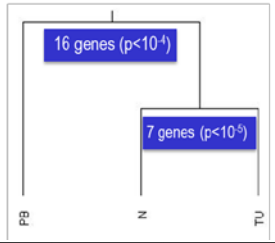
RESULTS: Colon markers from AIT's 360plex panel

confirmation of markers derived from 360plex panel run on **Colon-tissue**:

AITs „CpG 360 Methylation-Test (320 genes) *



- **48.48 upon preamp:**
  - 100% correct classification of „sex of samples"
  - 100% PB vs TU; 98% PB vs Colon; 100% TU vs N



No de	Group 1 Classes	Group 2 Classes	Mis-classification rate (%)
1	N, TU	PB (n=12)	0
2	N (n=18)	TU (n=18)	0

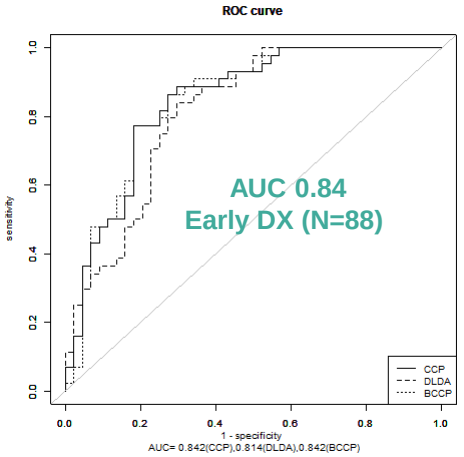
➔ Successful transfer from arrays to MSRE-HTqPCR & confirmed by SO_3 - deamination based MSP

48 selected markers on plasma cfDNA:

A) early DX - plasma of treatment naïve patients (n=44) & controls (n=44) – 2ml plasma used

➔ **33 of the 48 markers remained signif (p<0.05)**

➔ **AUC = 0.84 (potential for further optimisation**

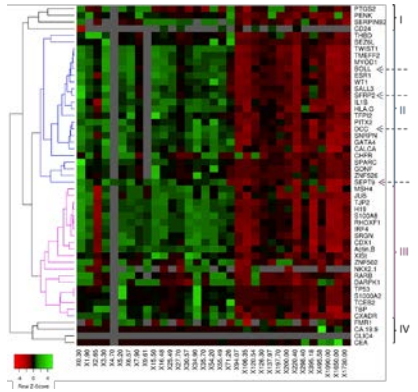


B) patient stratification & therapy monitoring:

in patients (n=34) with liver metastasis receiving chemotherapy

➔ Almost all markers remained significant for distinguishing "responders and non responders" (**heatmap**)

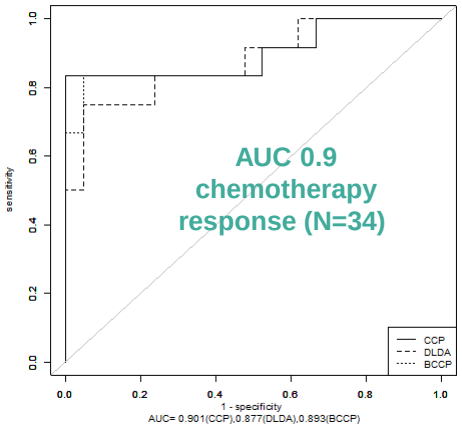
➔ AUC 0.9 before and upon first CTX cycle



➔ 19 markers have a high **correl ≥ 0.7** with tumor burden (volume)

➔ Top-3 genes based on *AUC* upon 1st CTX:

BOLL *0.87*
DCC *0.92*
SFRP2 *0.91*
SEPT9 - 0.86 (EPIGENOMICS)



* Methylation Assay. EP 09450020.4. (2009); WO2010086389 A1 (2010)

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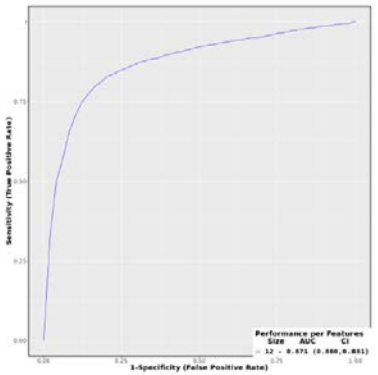
Application of MSREqPCR to qualify markers derived from genomic screen

- **180 candidate marker**s selected from colon cancer Human Methylation 450K data from The Cancer Genome Atlas (TCGA) consortium
- 2x 96plex MSREqPCR assay qualified
  - Run in 96.96 HTqPCR (6nl PCR vol)
- **53 markers significantly hypermethylated** in plasma samples
- 12-marker SVM prediction algorithm was built on Study 1 samples using reproducible markers leading to initial **Training Set** (**N=215**) prediction performance accuracy of **AUC 0.87** (*Figure A*)
- **12-marker classifier validated** on large independent Study 2 sample set (**N=774**) that included samples from colorectal cancer, NAA, control and also lung and breast cancer patients to test the marker-panel tissue-specificity towards colorectal cancer
- Model performance on Study 2 validation set can be seen on Figure 1 B with **AUC 0.88** (CI: 85.5-91%) and overall sensitivity of 77% and specificity of 88% (*Figure B*)

	CRC	Control + NAA	Control	NAA
Female	46	59	46	13
Male	47	63	45	18
Total	**93**	**122**	**91**	**31**
Total sample	**215**			

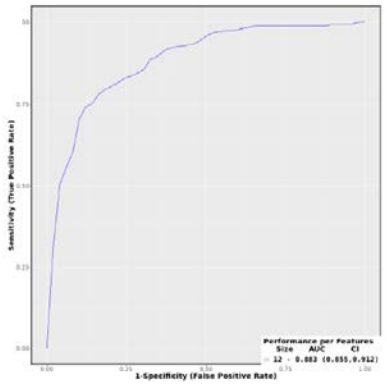
A



	CRC	Control + NAA	Control	NAA	Breast C.	Lung C.
Female	71	296	223	73	26	6
Male	81	274	199	75	0	20
Age (range)	65 (41-84)		61 (41-82)	66 (50-82)	55 (41-77)	61 (48-75)
Total	**152**	**570**	**422**	**148**	**26**	**26**
Total samples	**774**					

	CRC All	Breast C.	Lung C.
Stage 0-I	35	16	15
Stage II	49	8	4
Stage III	48	2	6
Stage IV	13		
Unknown	7		1
Total	**152**	**26**	**26**

B



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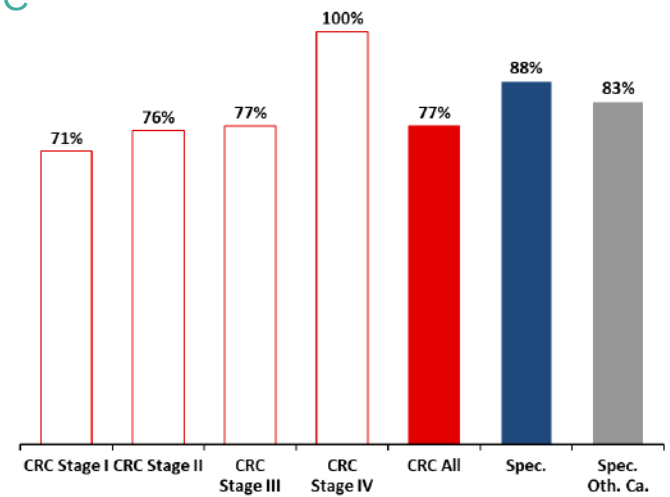

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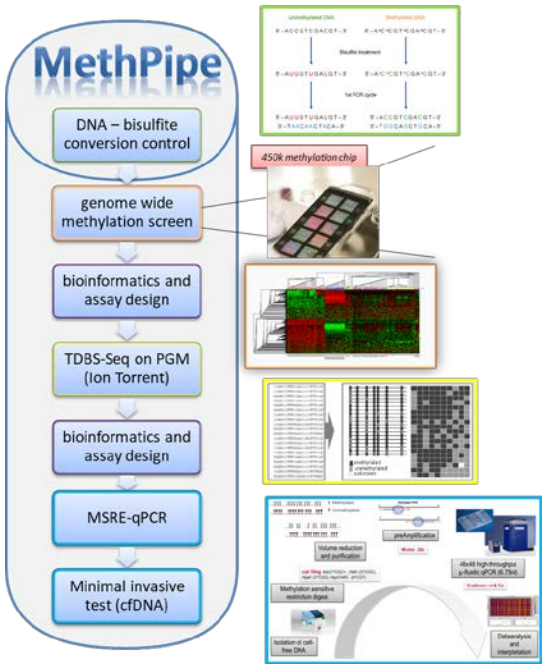
SUMMARY & CONCLUSIONS:

DNA methylation testing for **liquid biopsy** based diagnostics is a very powerful promising tool and is well suited for different a variety of applications.

- We have a complete „methylation marker“ pipeline from genome-wide discovery to cfDNA methylation analysis (applying EPIC arrays & NGS and PCR based SO_3 and MSRE-assays)
- we have confirmed a 48plex panel for a broad range of CRC-diagnostics applications
- early diagnostics, stratification & monitoring

MSREqPCR is a very **efficient approach** for marker confirmation and validation

- Best suited for bringing markers from tissue to „Liquid biospsy“ application
- targeted investigation of 96 DNA methylation sites using the cfDNA content of only 2 ml of plasma
  - Multiplexing, low input needed, can be run on **standard qPCR** cylcers
  - Works with low DNA amounts in almost all kind of clinical samples (also FFPE)



- ***We are open for cooperation and contract research services***

- Acknowledgements: