Translating Nanotechnology and Microfluidics for Analysis of DNA Methylation

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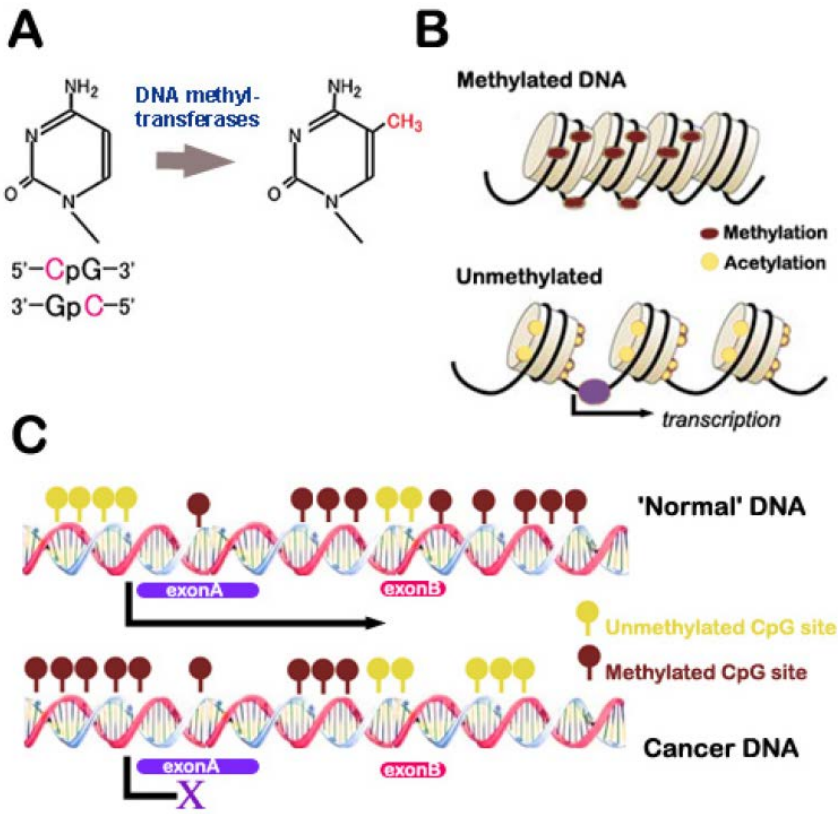
Methylation As a Cancer Biomarker

DNA Methylation contributes to the progression of carcinogenesis by silencing of tumor suppressor genes



(J.G. Herman & S. Baylin, *New England Journal of Medicine*, 2003)

Abnormal epigenetic changes appear to be an early event before detection of genetic mutations. Thus, detection of promoter methylation is a promising approach for early diagnosis of cancer.

Current Method for Promoter Methylation Detection

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 9821–9826, September 1996
Medical Sciences

Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands

(DNA methylation/tumor suppressor genes/*p16*/*p15*)

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DNA Extraction

- Phenol-chloroform
- Ethanol precipitation

Bisulfite Conversion

Non-Methyl C → U

Methyl C → C

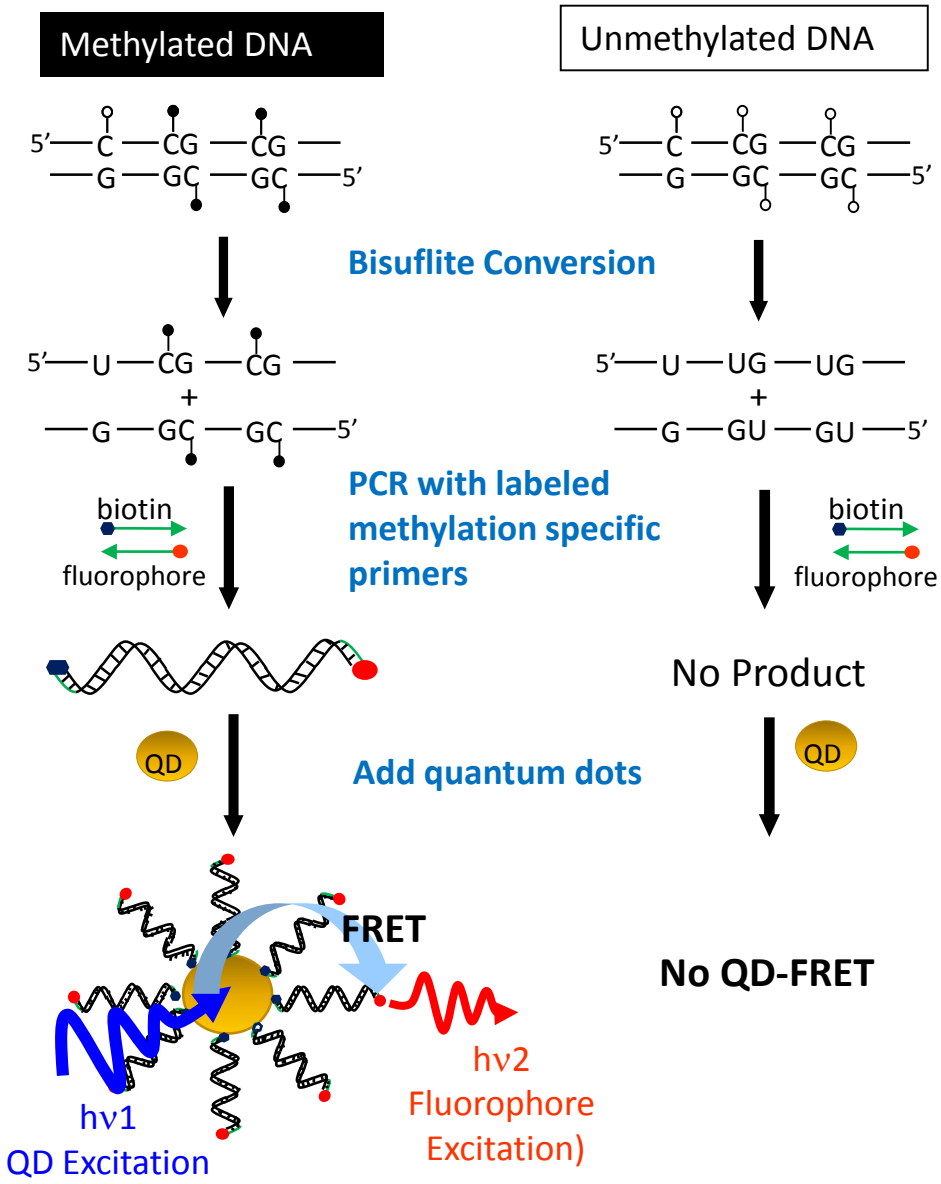
PCR Detection

Advantages: High sensitivity and specificity

Limitations:

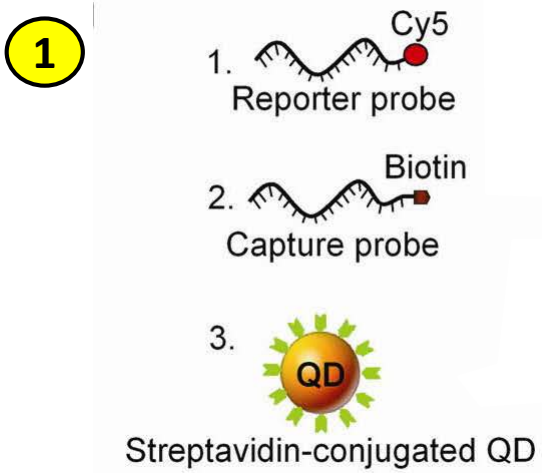
- Still not sensitive enough to reliably detect methylated DNA in body fluids such as serum, sputum , stool and urine (requiring nested PCR, digital PCR).
- Tedious and labor-intensive process which is not amenable for routine clincal utilizations.
- Sub-optimal effieiecny in recovery of circualting DNA

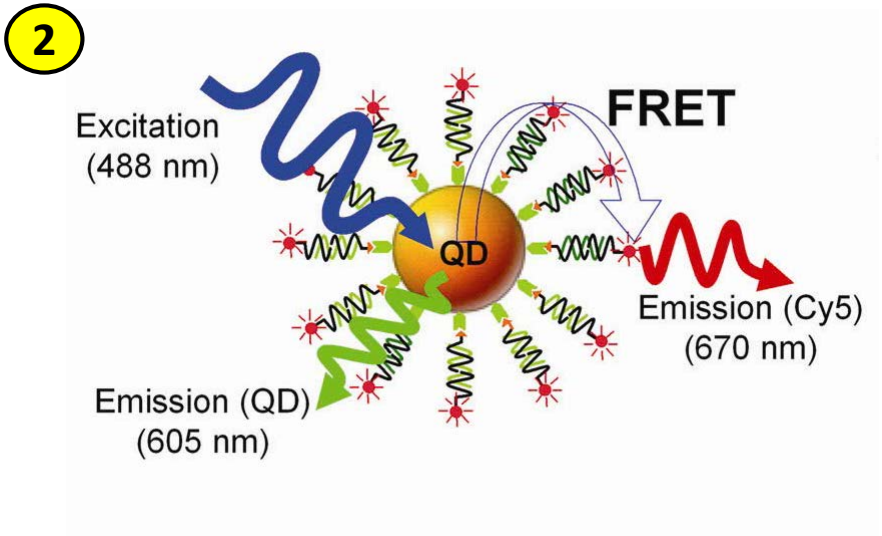
MS-qFRET: Methylation-Specific QD-FRET for DNA Methylation Detection



(Bailey *et al*., *Genome Research*, 2009; Bailey, *Methods*, 2010; Zhang, *Theranostics*, 2012)

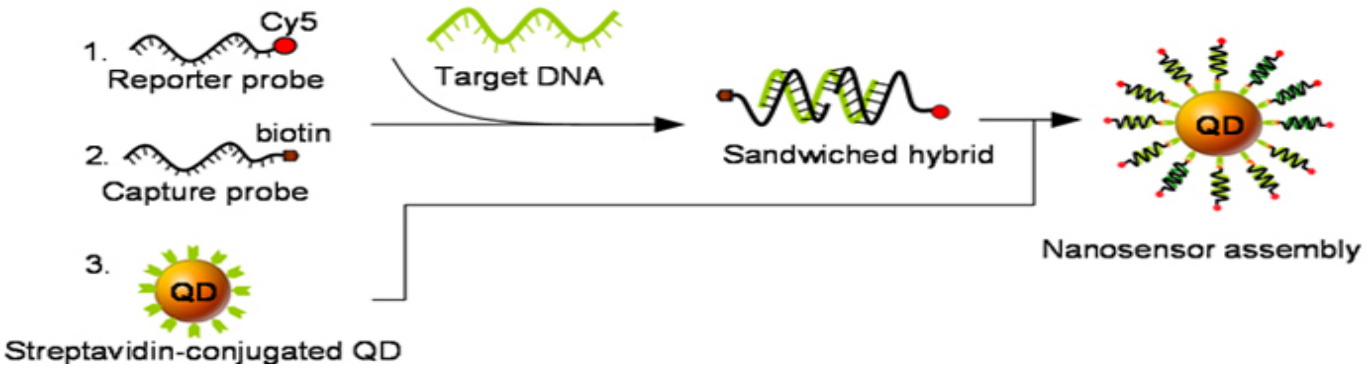
Quantum Dot-Fluorescence Resonance Energy Transfer (QD-FRET) DNA Nanosensor

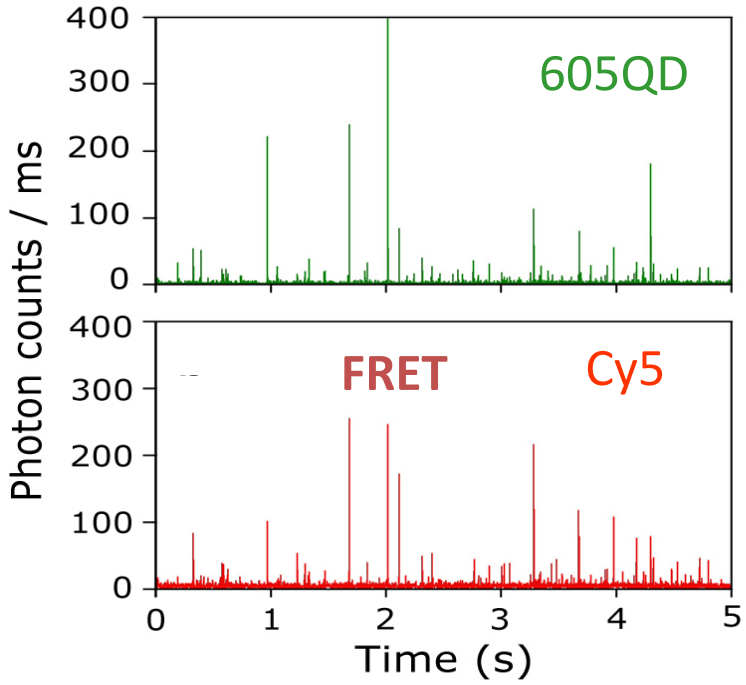




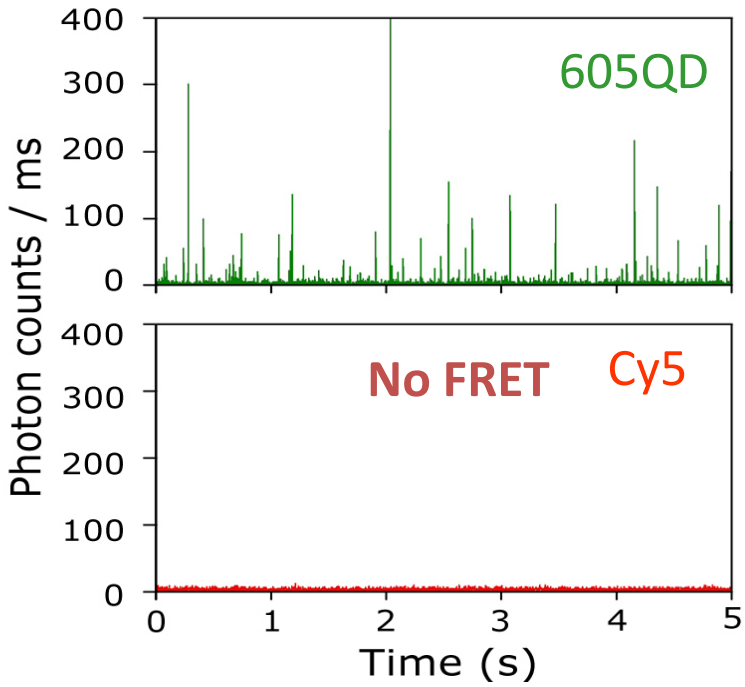
(Zhang *et al.*, *Nat Mater*, 2005)

Single-molecule/nanoassembly Detection



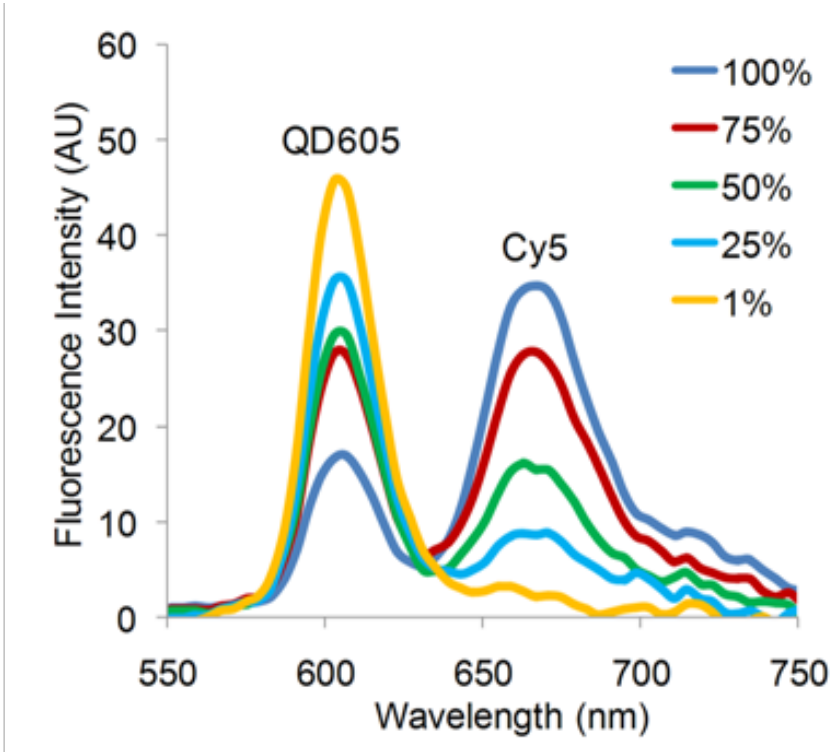


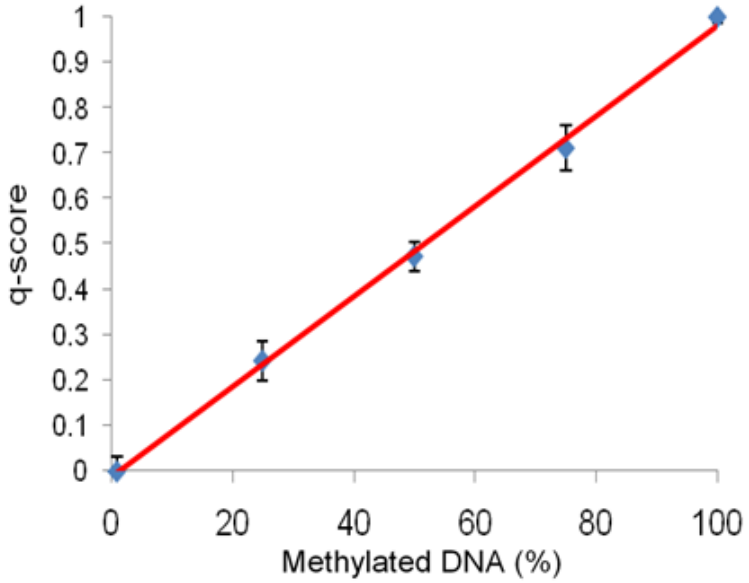
In the presence of targets



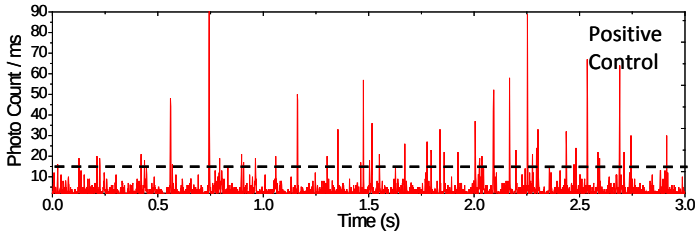
In the absence of targets

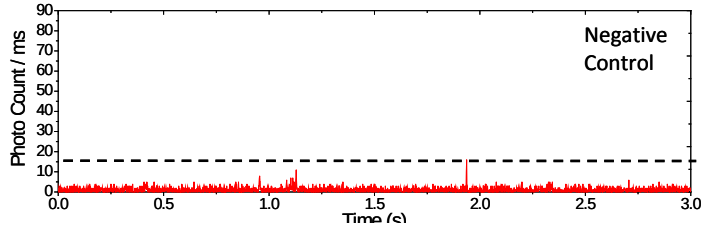
Quantitative Aanalyis of DNA Methylation Detection with QD-FRET Nanosensor

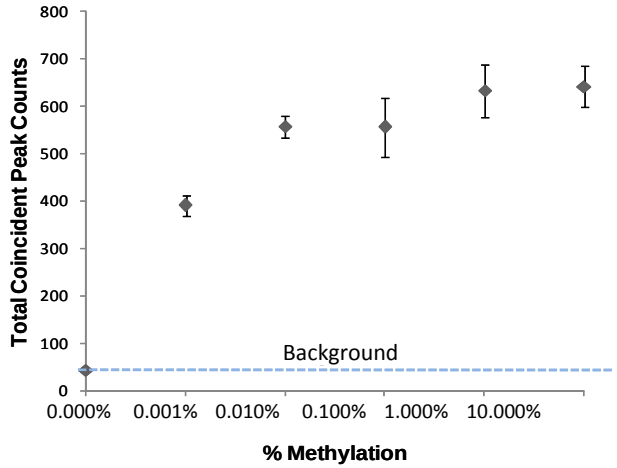




DNA Methylation Detection with QD-FRET Nanosensor



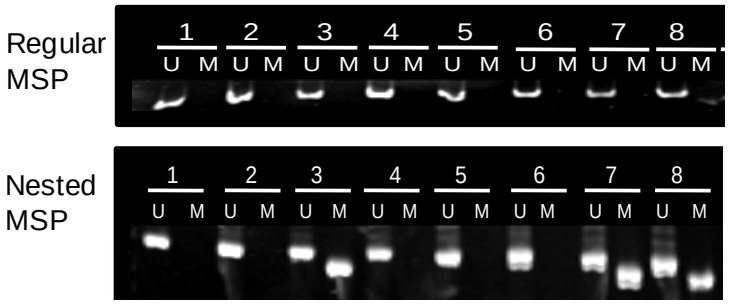




Detection limit:

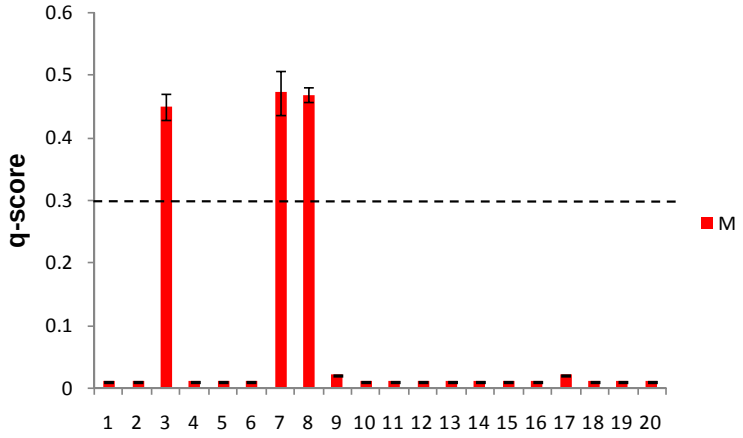
- Detect methylated DNA in the presence of 10,000 excess of unmethylated alleles
- 15 pg DNA (5 genomic equivalents)

a



PYCARD

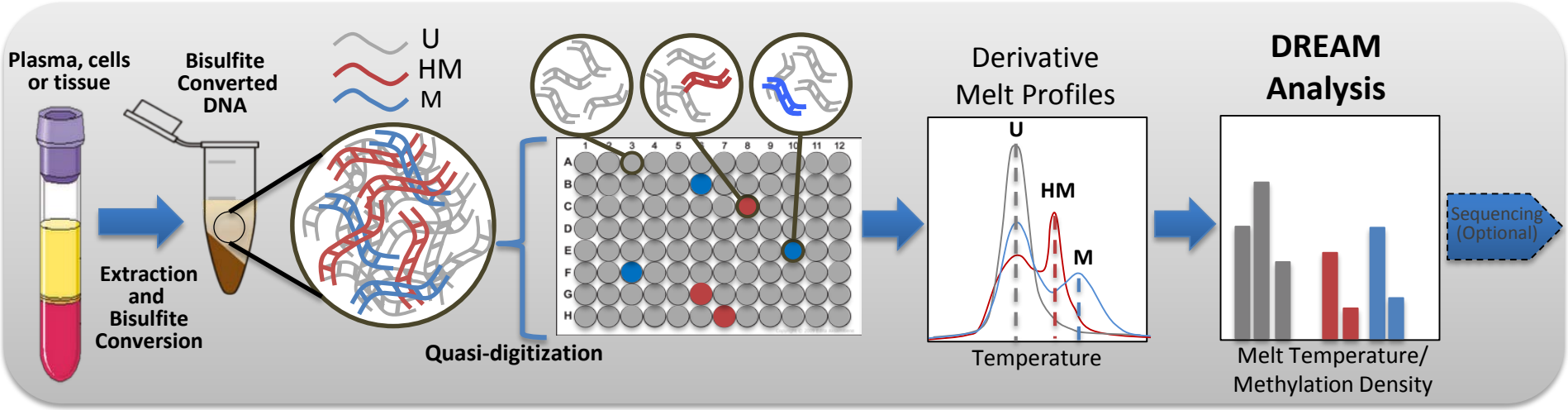
c



Analysis with clinical (sputum) samples

DREAMing
Discrimination of Rare EpiAlleles by Melt

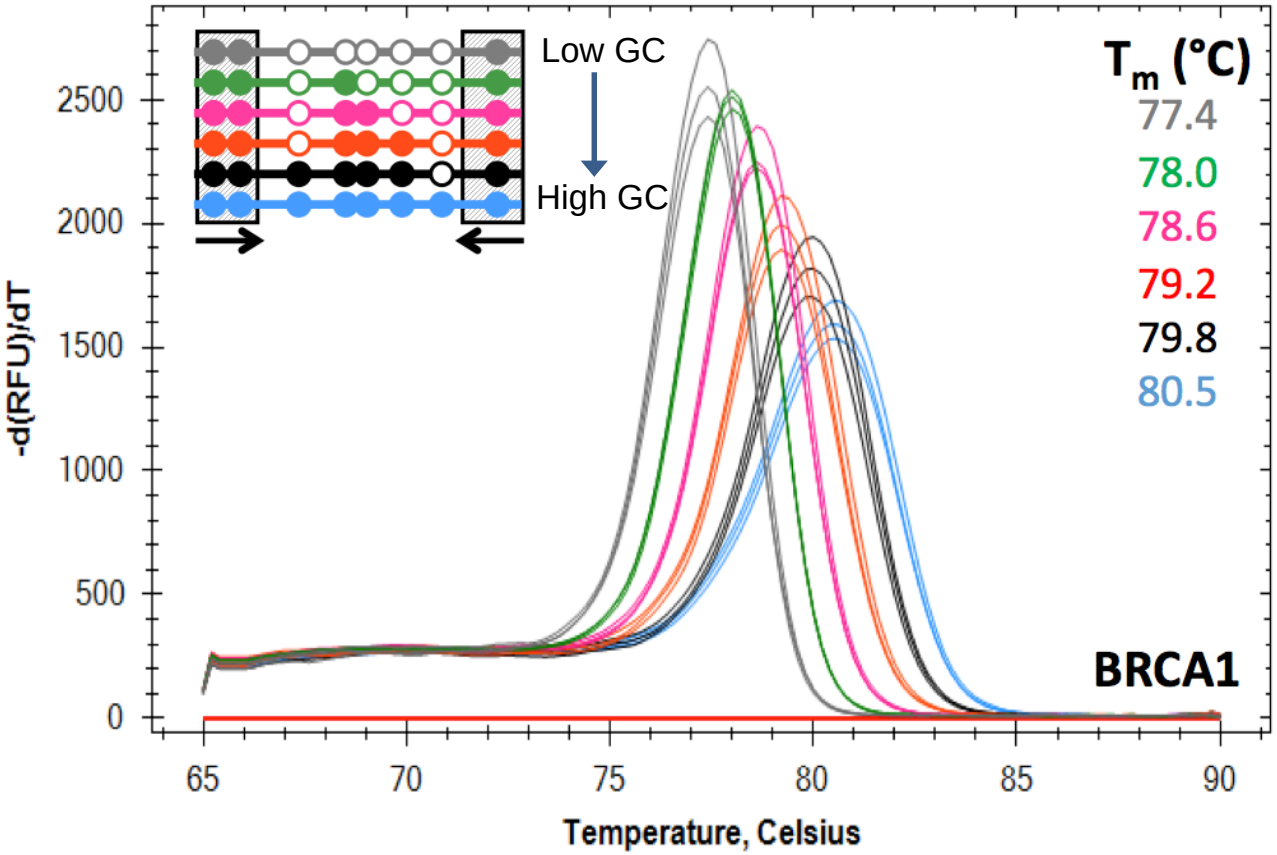
- Cancers comprise heterogeneous populations of cells at primary and metastatic sites.
- DREAMing uses quasi-digital detection and precise melt curve analysis to distinguish *individual copies* of epiallelic species at *single-CpG-site resolution*



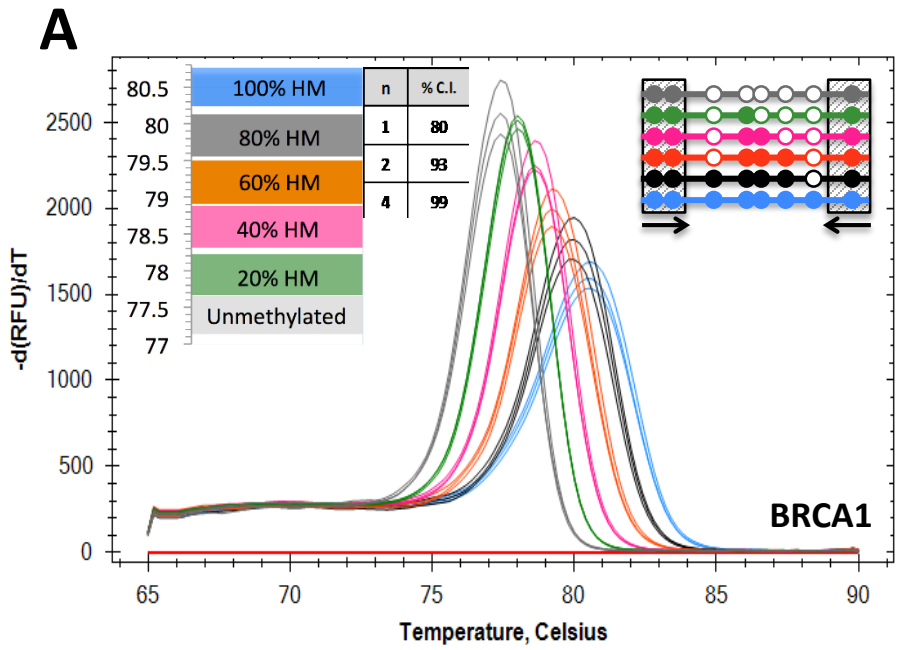
(Pisanic et al, *Nucleic Acids Res*, 2015)

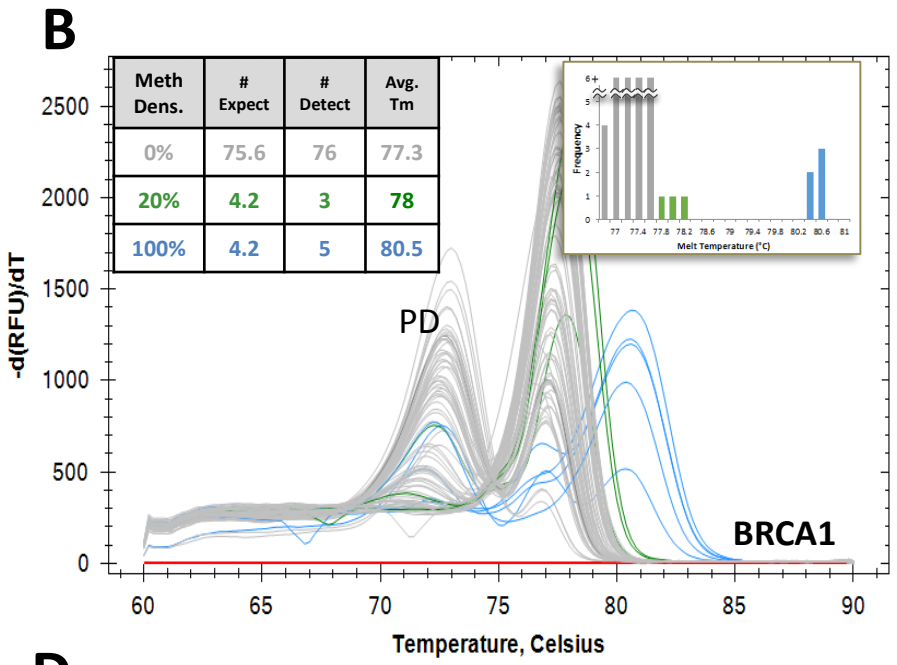
Discrimination of Epiallelic Variants Based on Melt Temperature

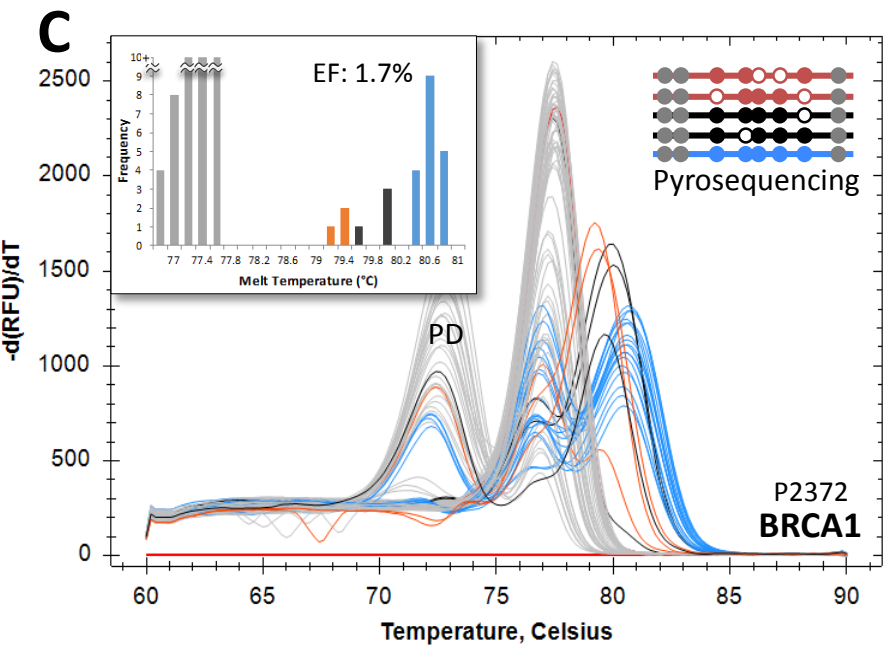
Bisulfite DNA conversion

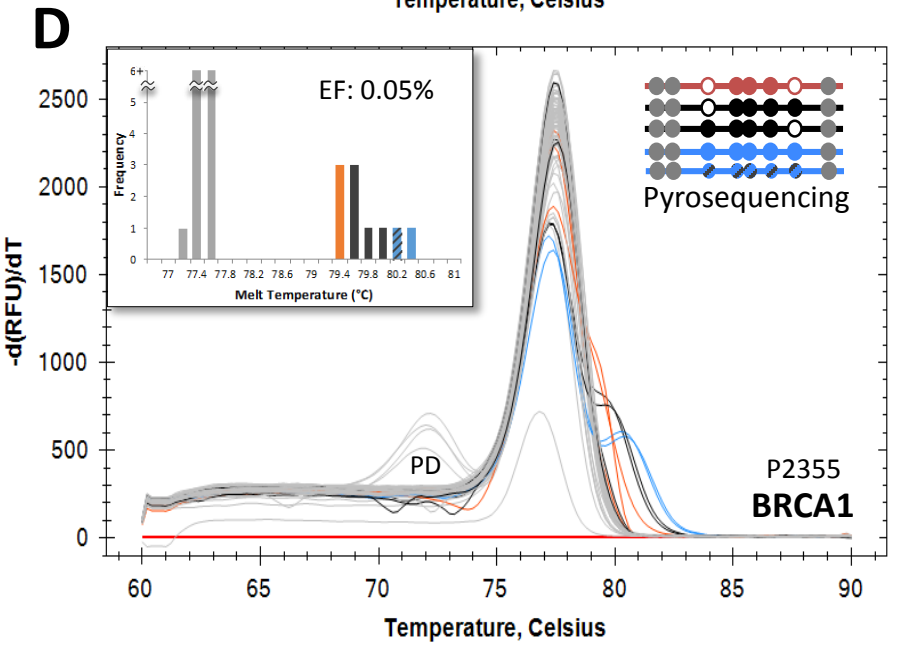


Assessment of *BRAC1* Epigenetic Heterogeneity MDS/MPN Patient Samples

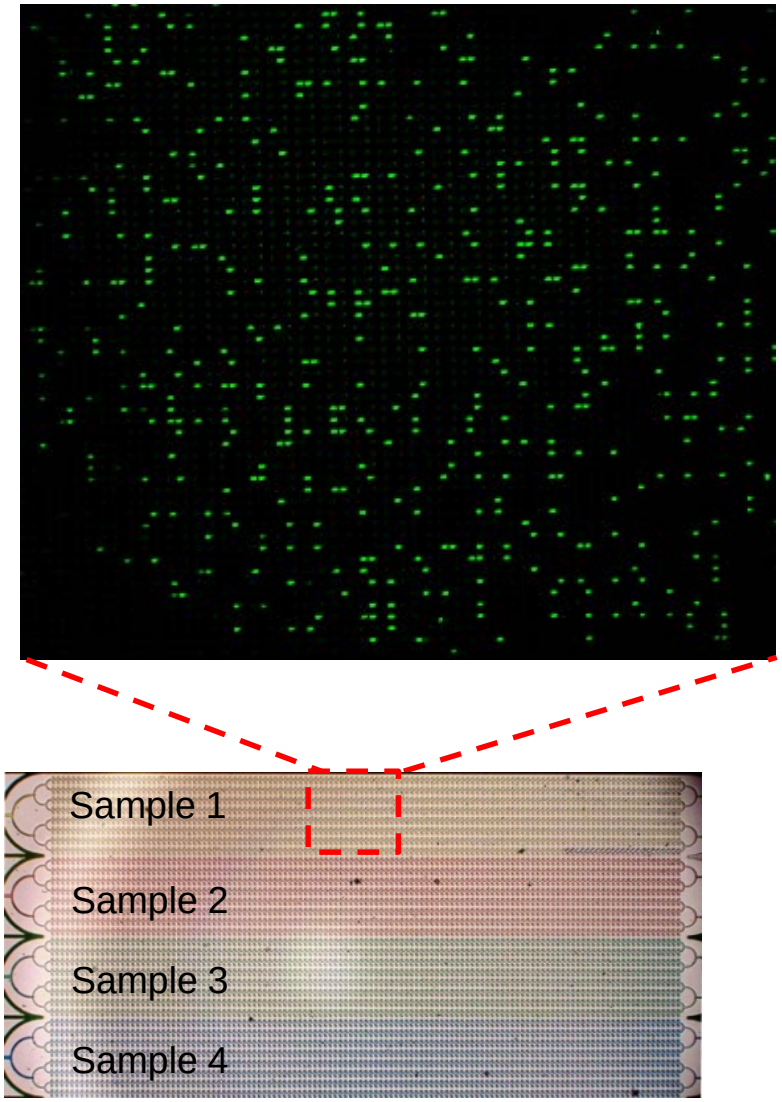




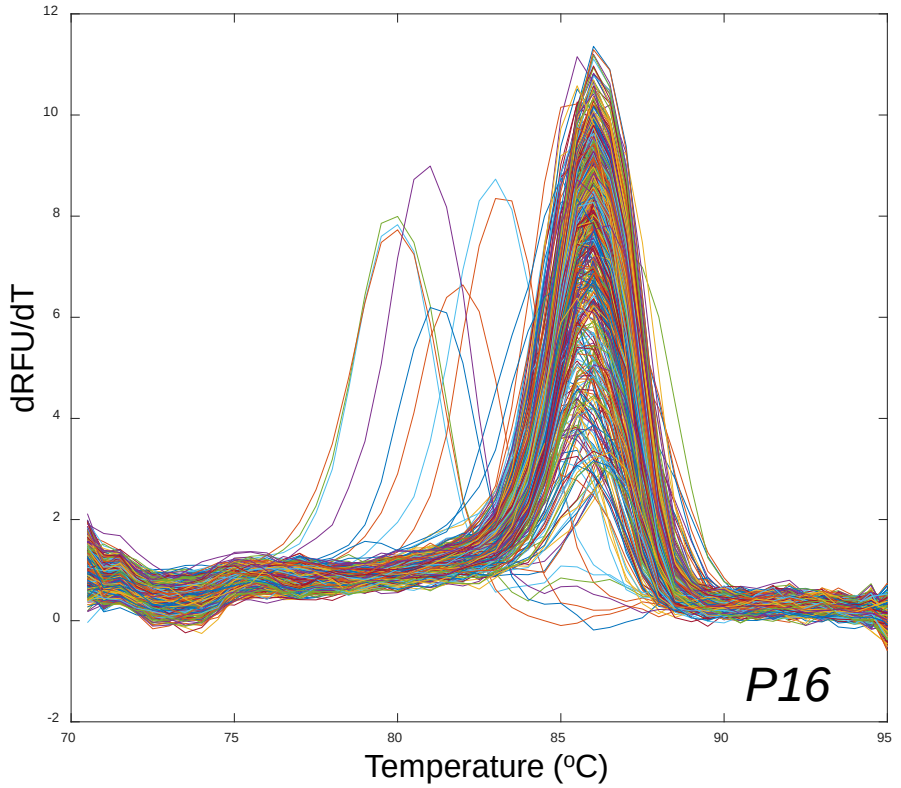




Microfluidic Array Chip for Digital Melt Analysis of Methylation Heterogeneity

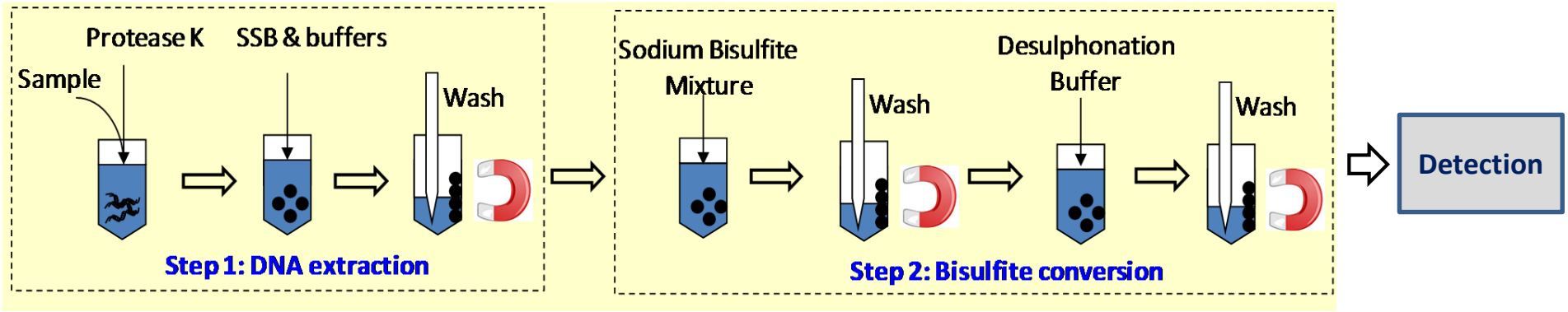


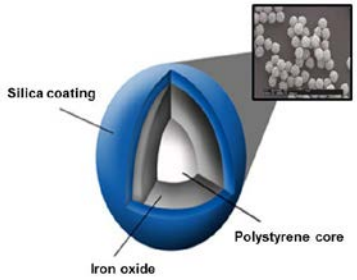
4 x 4,096 wells

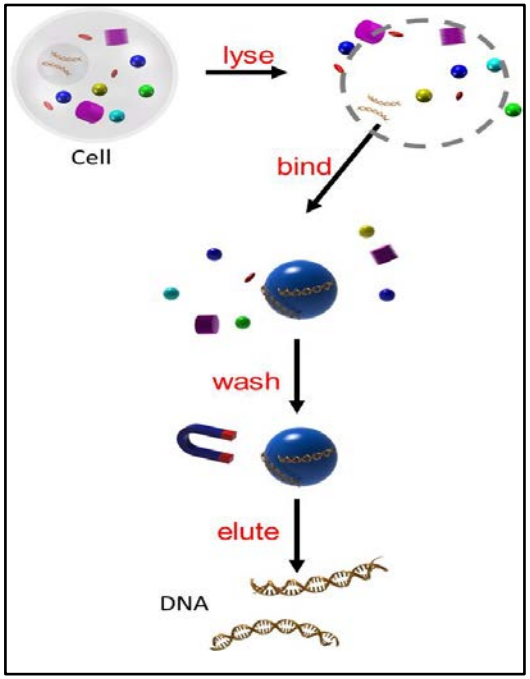


Integrate DNA Isolation and Bisulfite Conversion Using Silica Superparamagnetic Particles

Methylation on Beads (MOB)



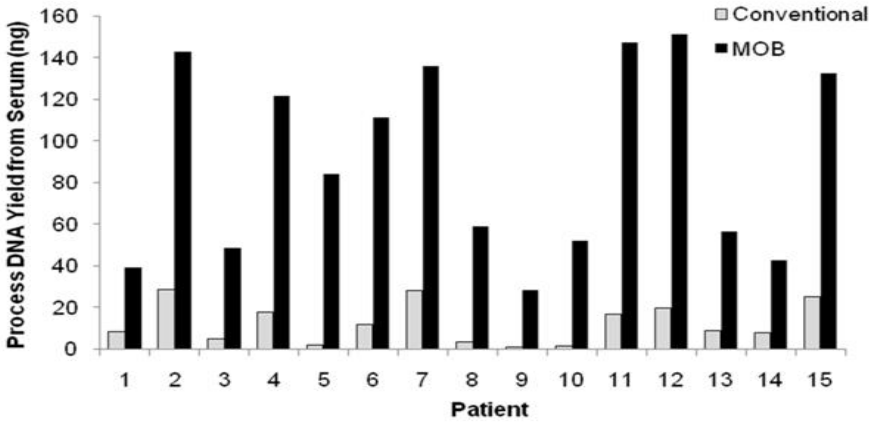


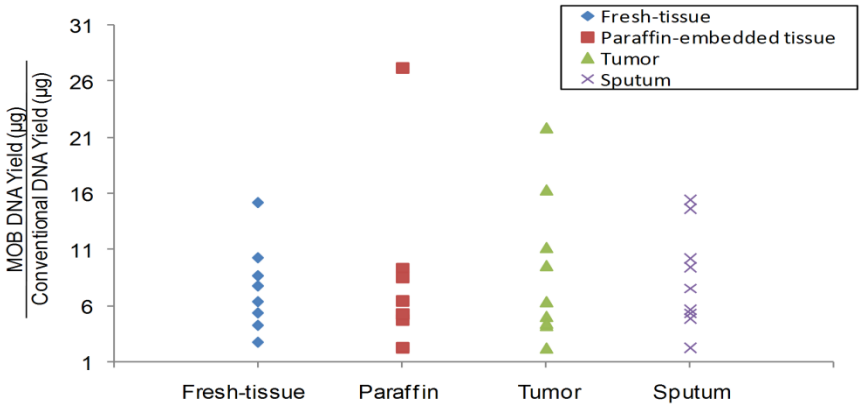


- Induced magnetic property in a magnetic field, easy for manipulation
- Reversible DNA binding and desorption by tuning the buffer condition
- Small size provides large surface area for DNA binding
- Size varies from 10 nm to 1000 nm

(Bailey et al, *Clinical Chem*, 2010; B. Keeley, *Clinica Chimica Acta*, 2013)

Comparison of MOB and Conventional Method





Methylation Detection in Primary Tissue

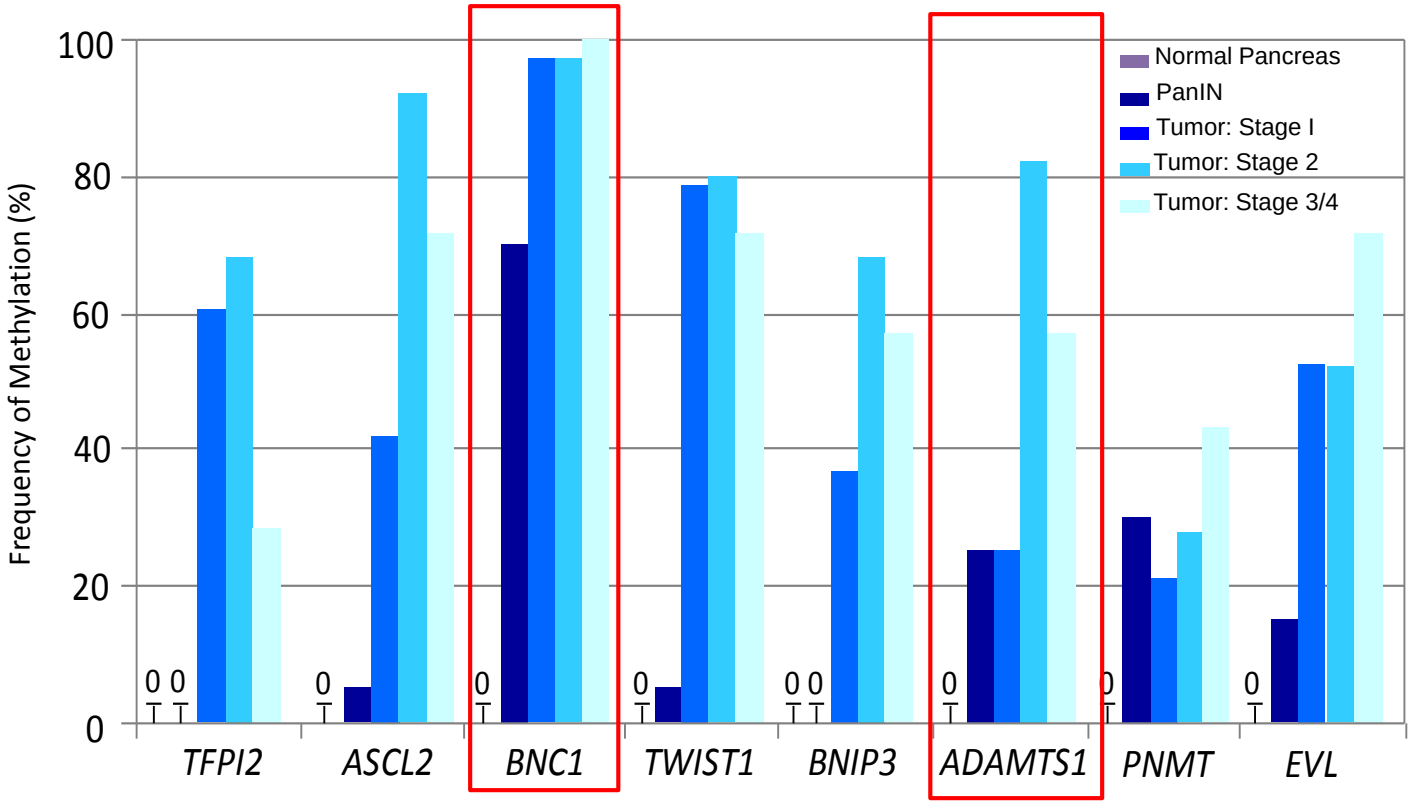


Table 1. Clinical information for primary pancreatic samples

			Pancreas Primary Tumors (N= 123)			
	Normal (N=4)	PanINs (N=20)	Stage I (N=38)	Stage II (N=78)	Stage III (N=5)	Stage IV (N=2)
Median Survival (months)		137.3	42.4	17.8	13.9	19.6

(*Yi et al., Clinical Cancer Research*, 2013)

Methylation Detection in Sera

Table 2. Sensitivity and specificity of *BNC1* and *ADAMTS1* in pancreatic cancer patient serum samples

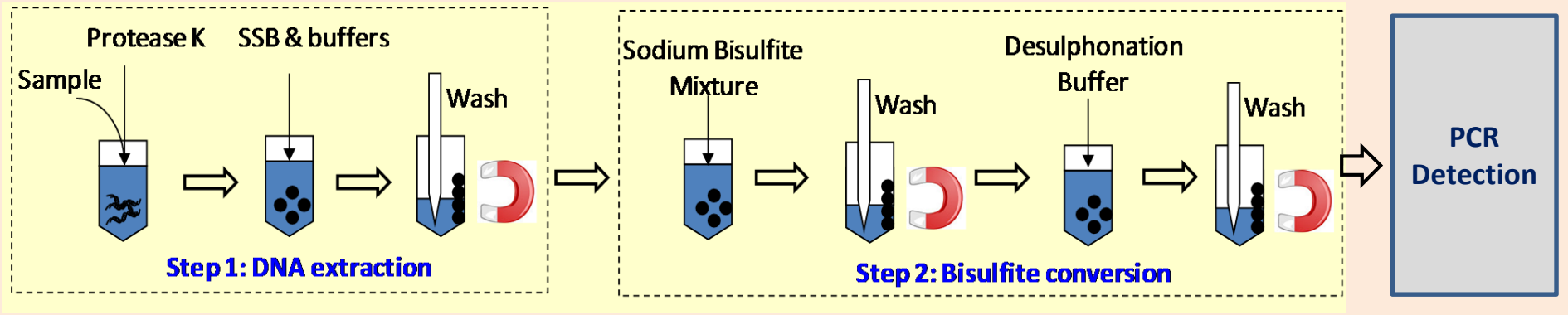
		BNC1		*ADAMTS1*		
Pancreatic Cancer		***Sensitivity(%)***				
Stage	n	Estimated Value	95% CI	Estimated Value	95% CI	
I	10	90% (9/10)		90% (9/10)		
II-IV	32	75% (24/32)		34% (11/32)		
Total	42	79% (33/42)	66-91%	48% (20/42)	33-63%	(84%) Overall
		Specificity(%)				
		Estimated Value	95% CI	Estimated Value	95% CI	
Normal	26	89%	76-100%	92%	82-100%	(85%) Overall

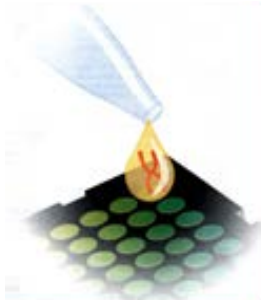
CI- confidence interval

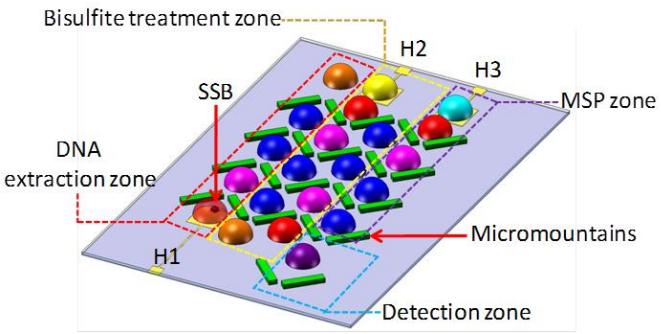
(*Yi et al., Clinical Cancer Research*, 2013)

Fully Integrated Device for Robust Methylation Detection

Methylation on Beads (MOB)



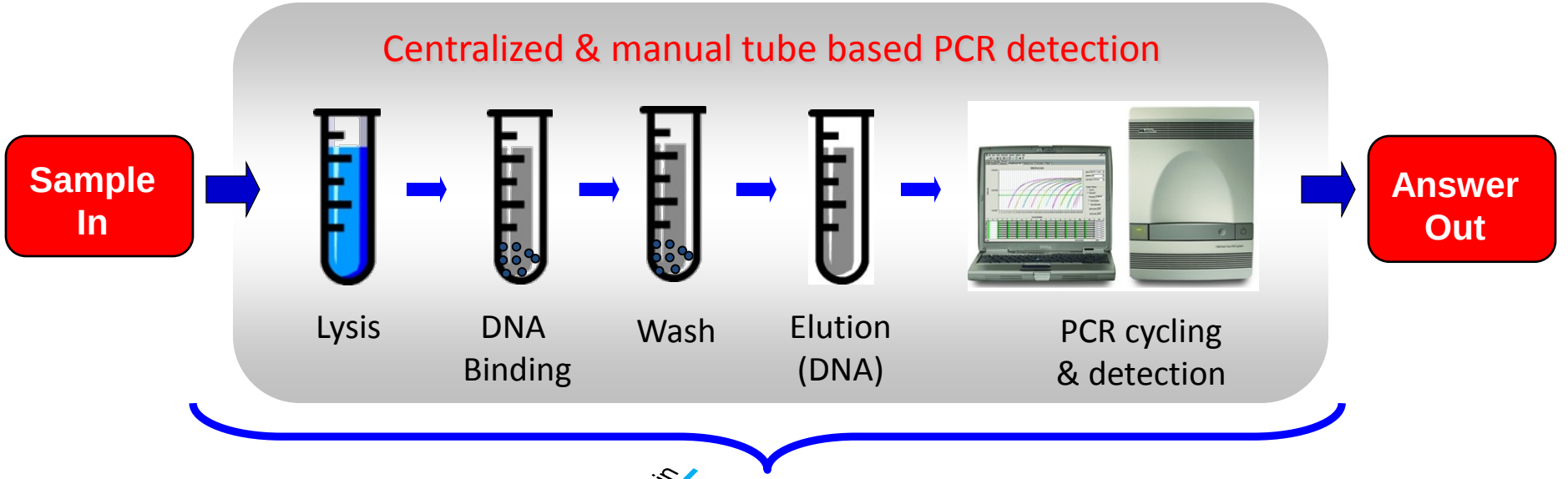


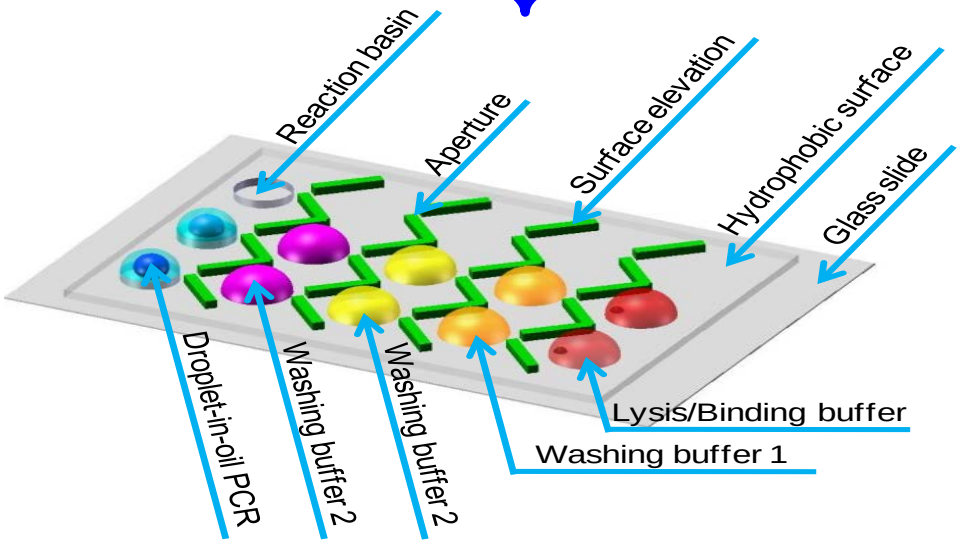


Methylation on a Chip

Droplet Magnetofluidics for Integrated DNA Preparation and PCR

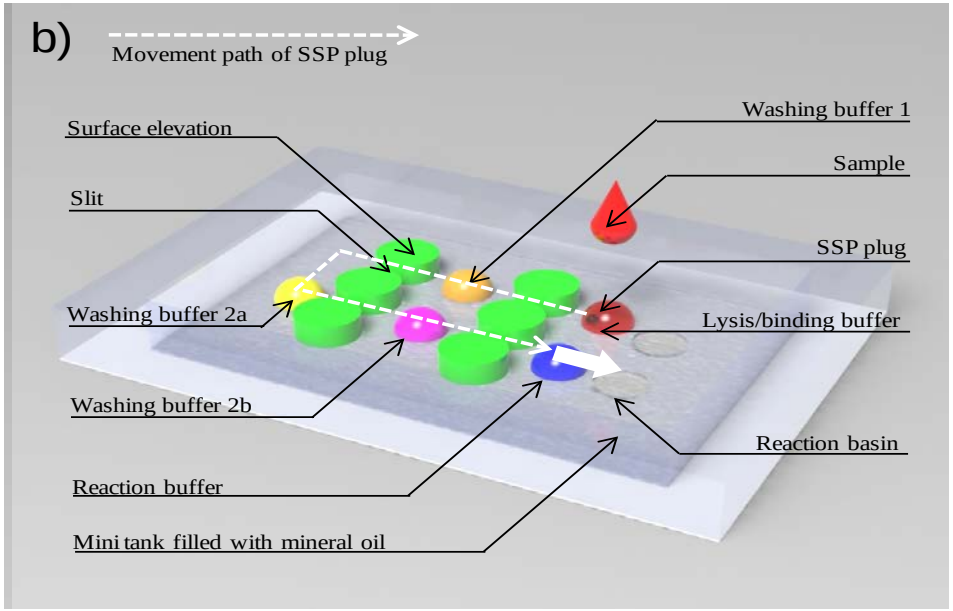
Using ***Silica Superparamagnetic Particles (SSP)*** as a solid phase within droplets



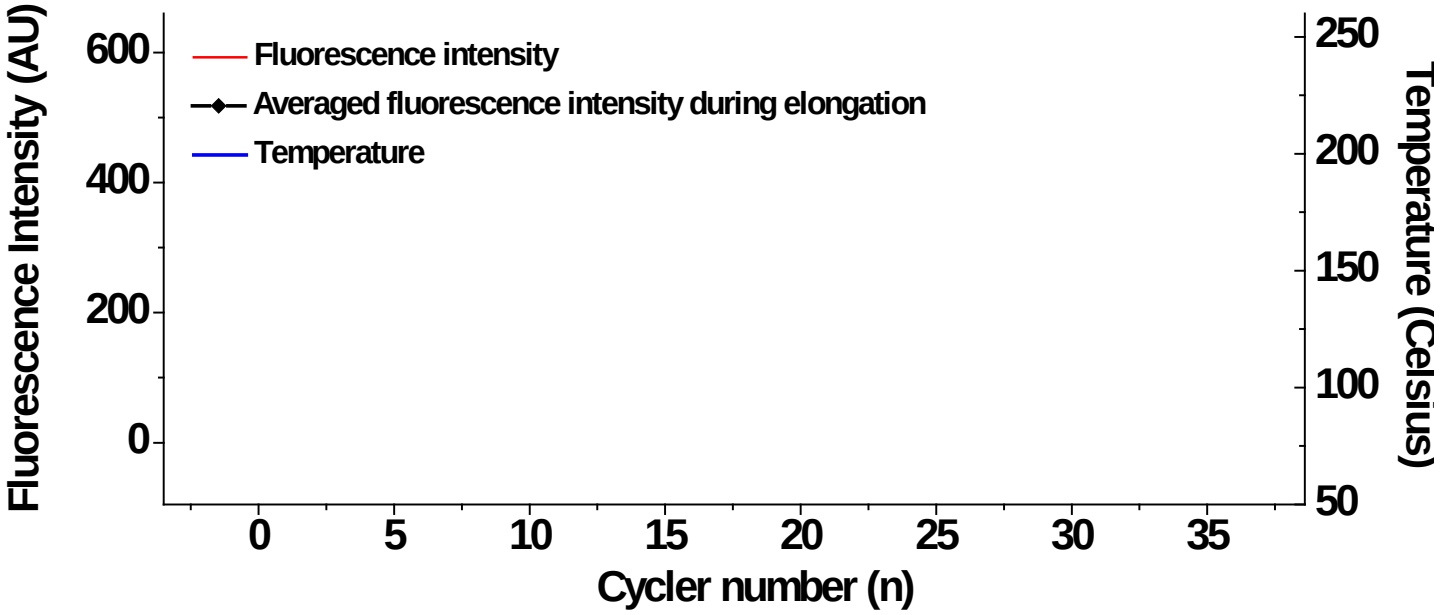


(Zhang, *et al* . *Lab Chip* 2011; Zhang *et al*. *Advanced Materials* 2013)

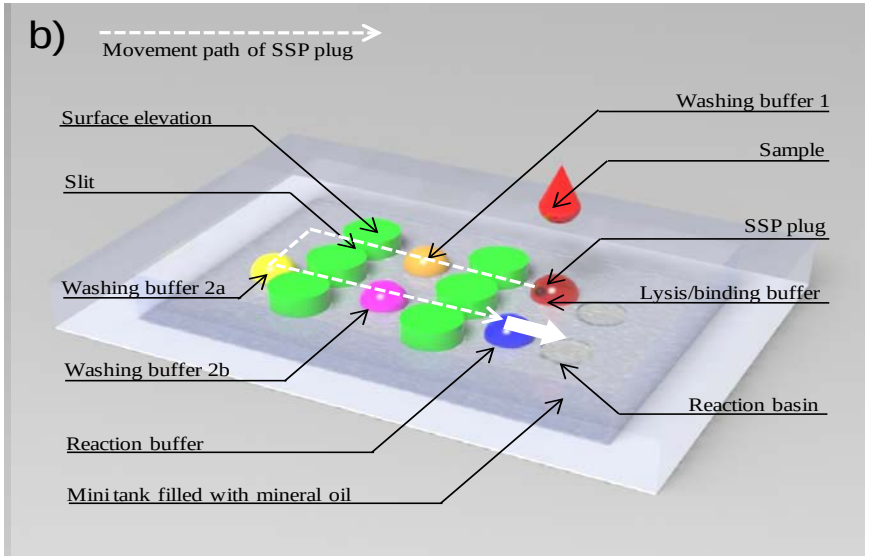
Fully Integrated Sample Processing and PCR



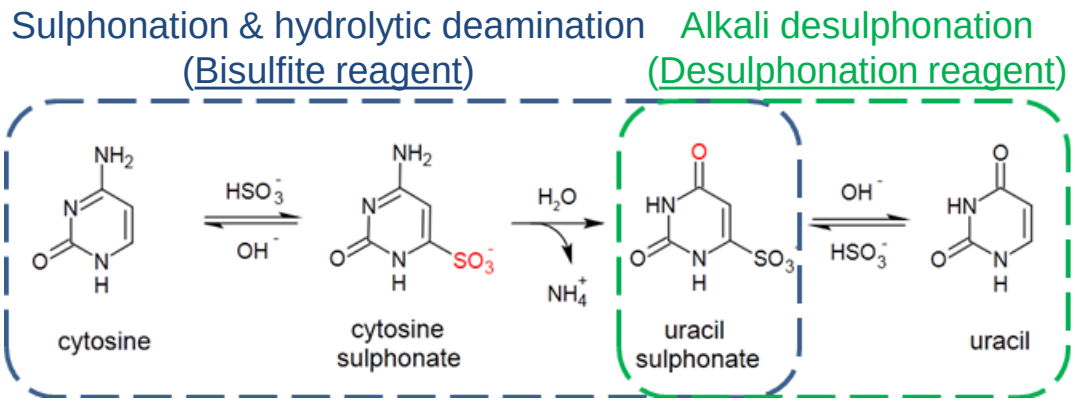
“Sample-To-Answer “ Genetic Detection in Droplets

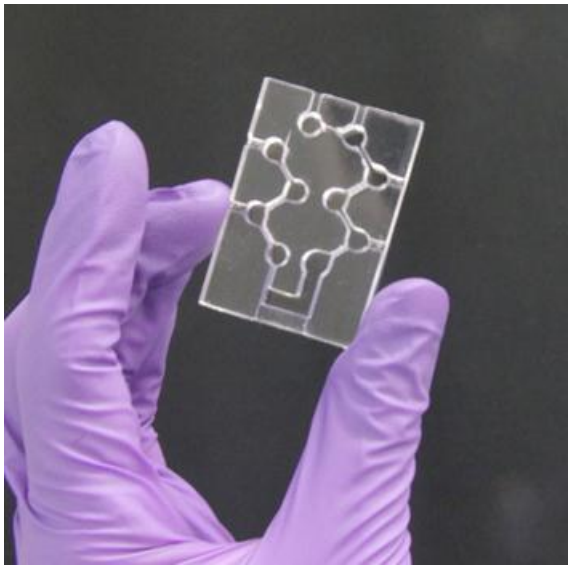


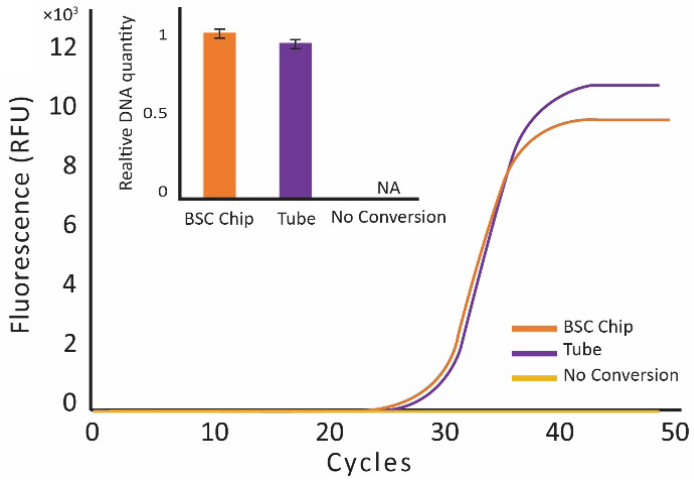
Quantitative Detection of Rsf1 marker in droplets

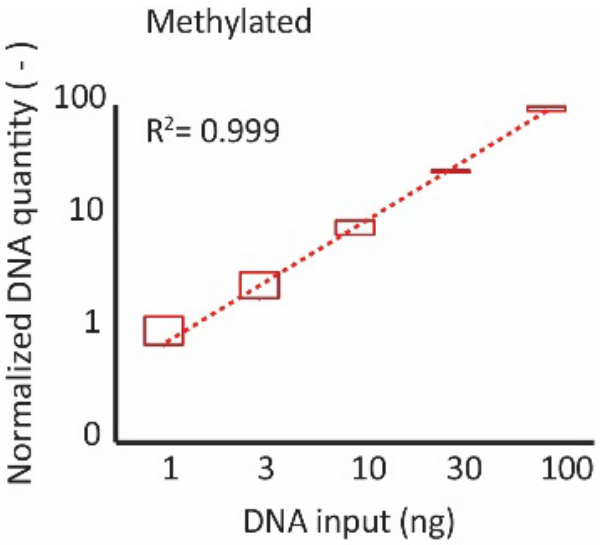


DNA bisulfite Conversion Chip for Methylation Analysis



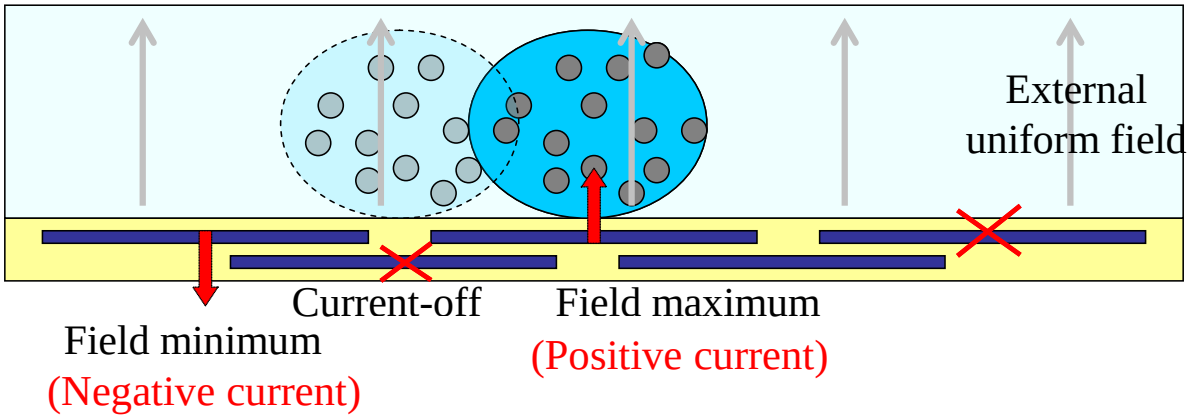


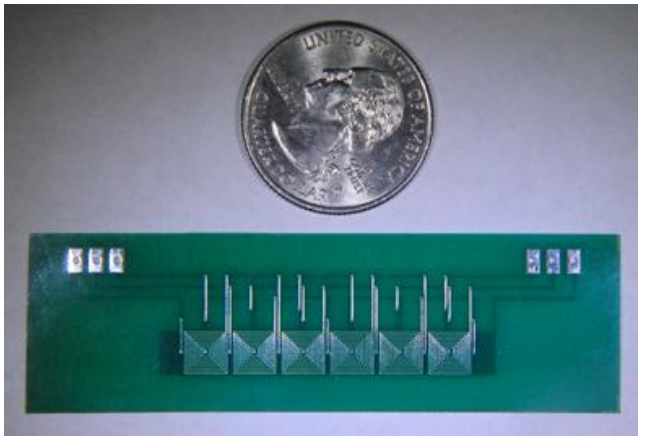


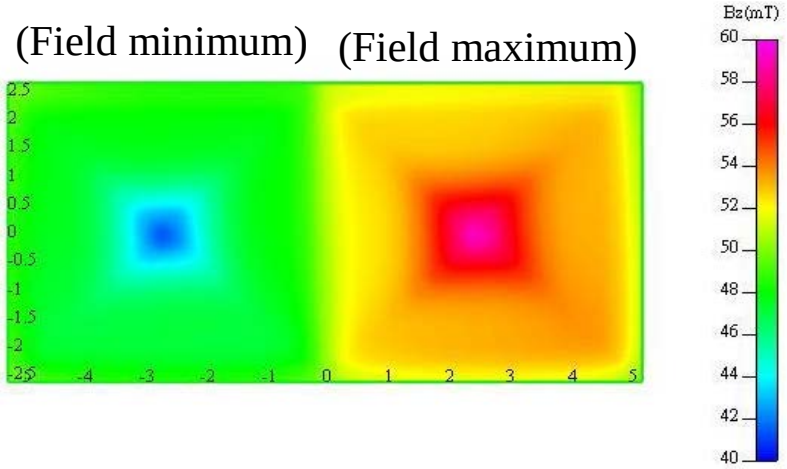


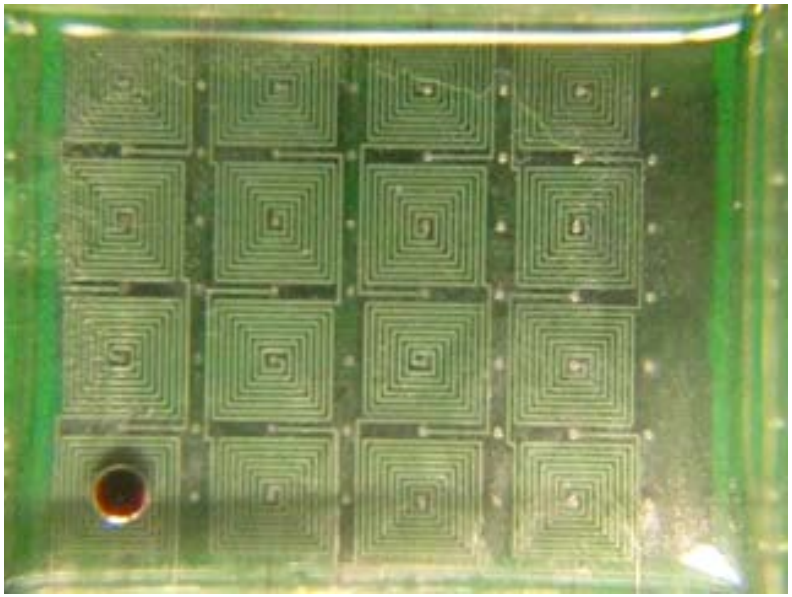
A. Stark et al , *Biomedical Microdevices*, 2016

Electromagnetic Droplet Manipulation





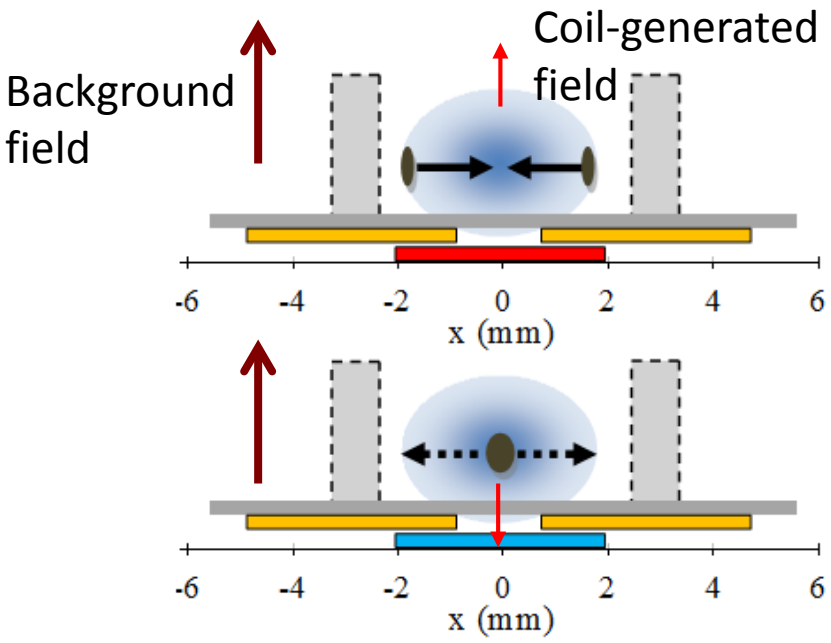


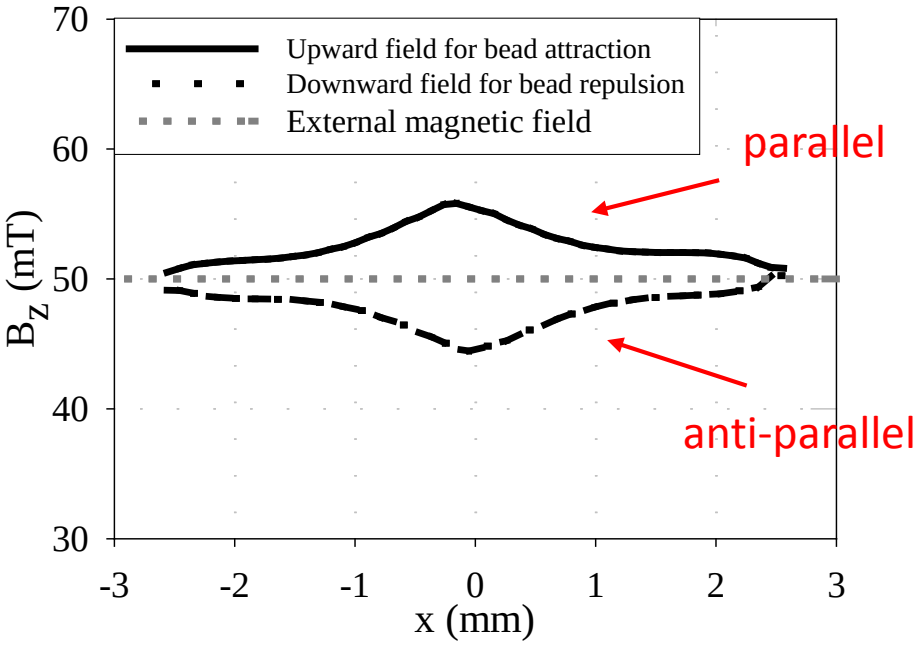


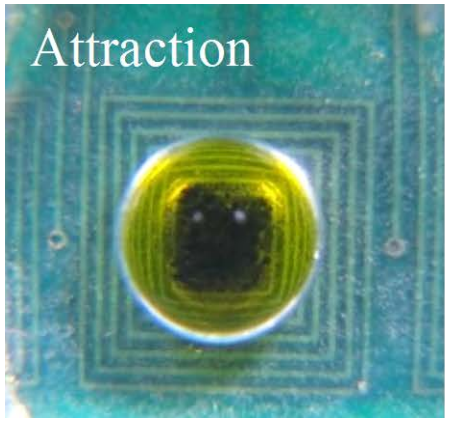
Magnetic force： $F_m = N_{mp} \frac{V_{mp}\chi_{mp}}{\mu_0} B_0 \partial B_z / \partial x$

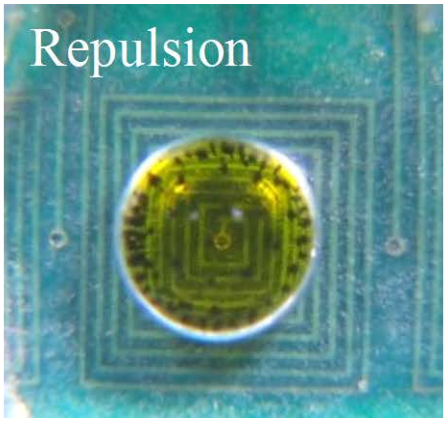
C.H. Chiou et al , *Biosensors and Bioelectronics*, 2013

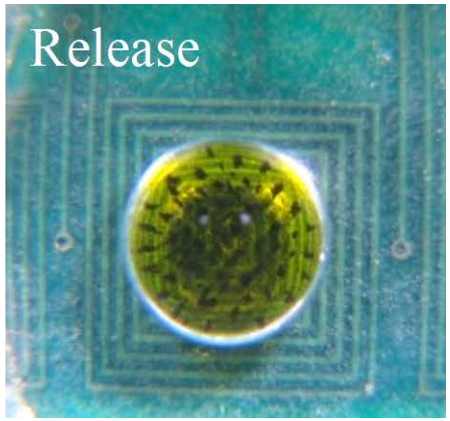
Mechanism of Mixing

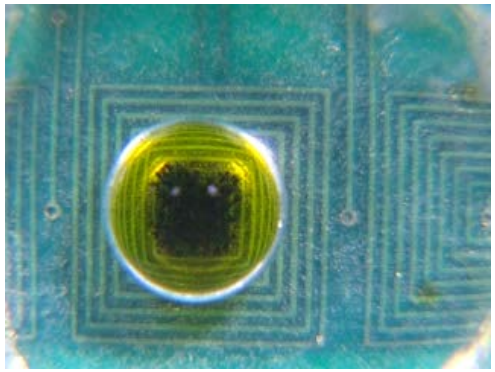




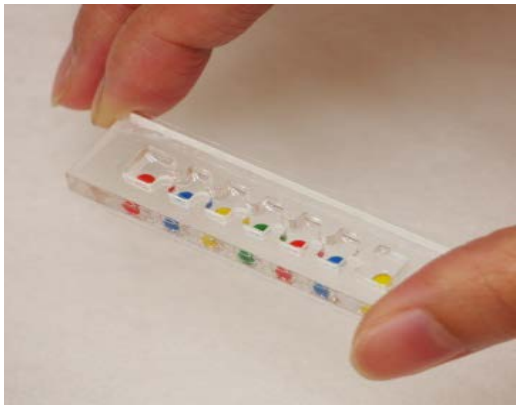


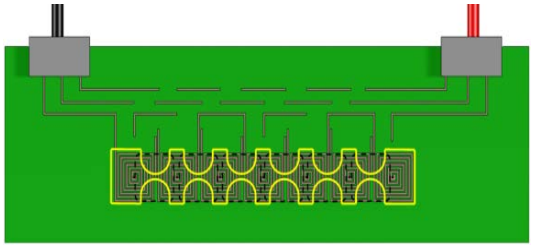


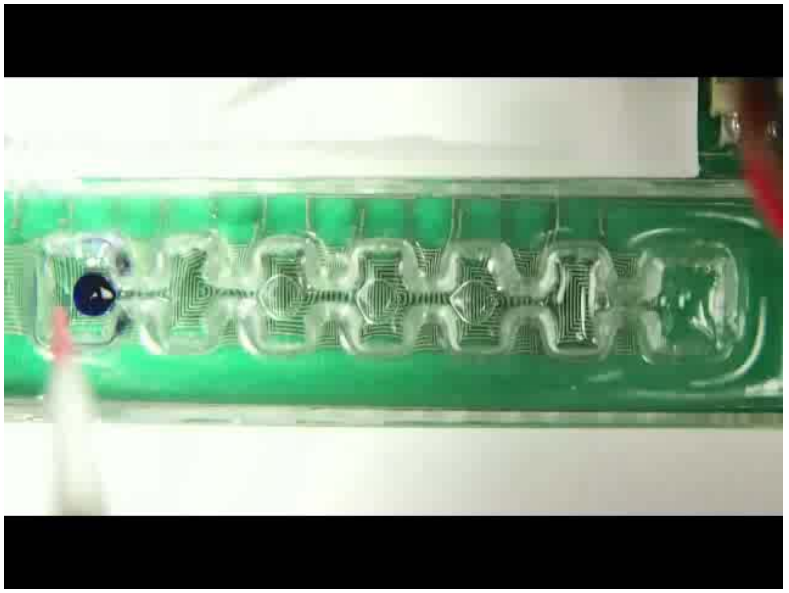


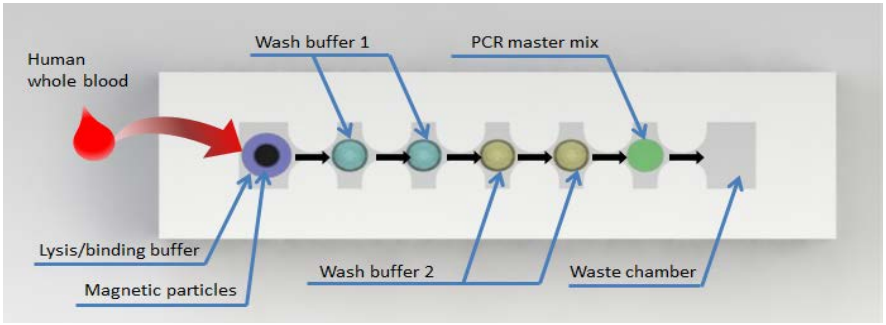


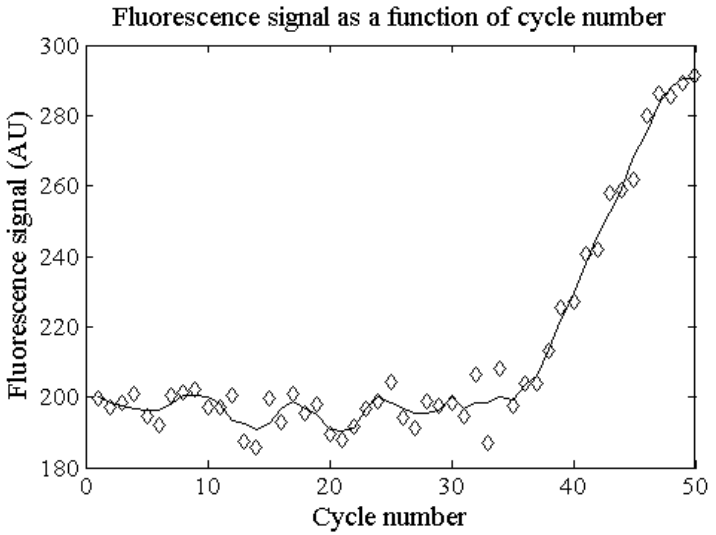
On-Chip DNA Extraction and Real-time PCR



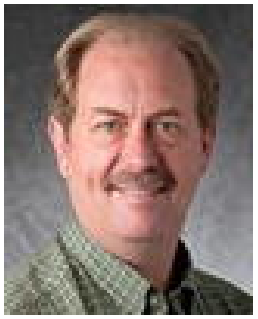




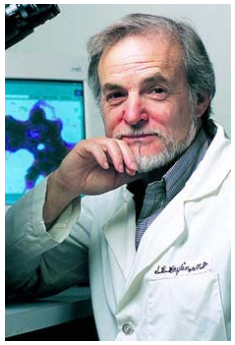




Acknowledgements



James Herman



Steve Baylin

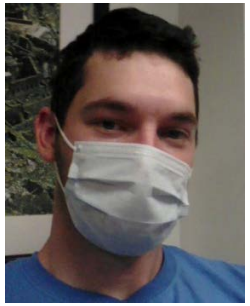
Malcolm Brock

Nita Ahuja

Tom Pisanic

Alex Stark

Weijie Poh



Brian Keeley

Yi Zhang

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