

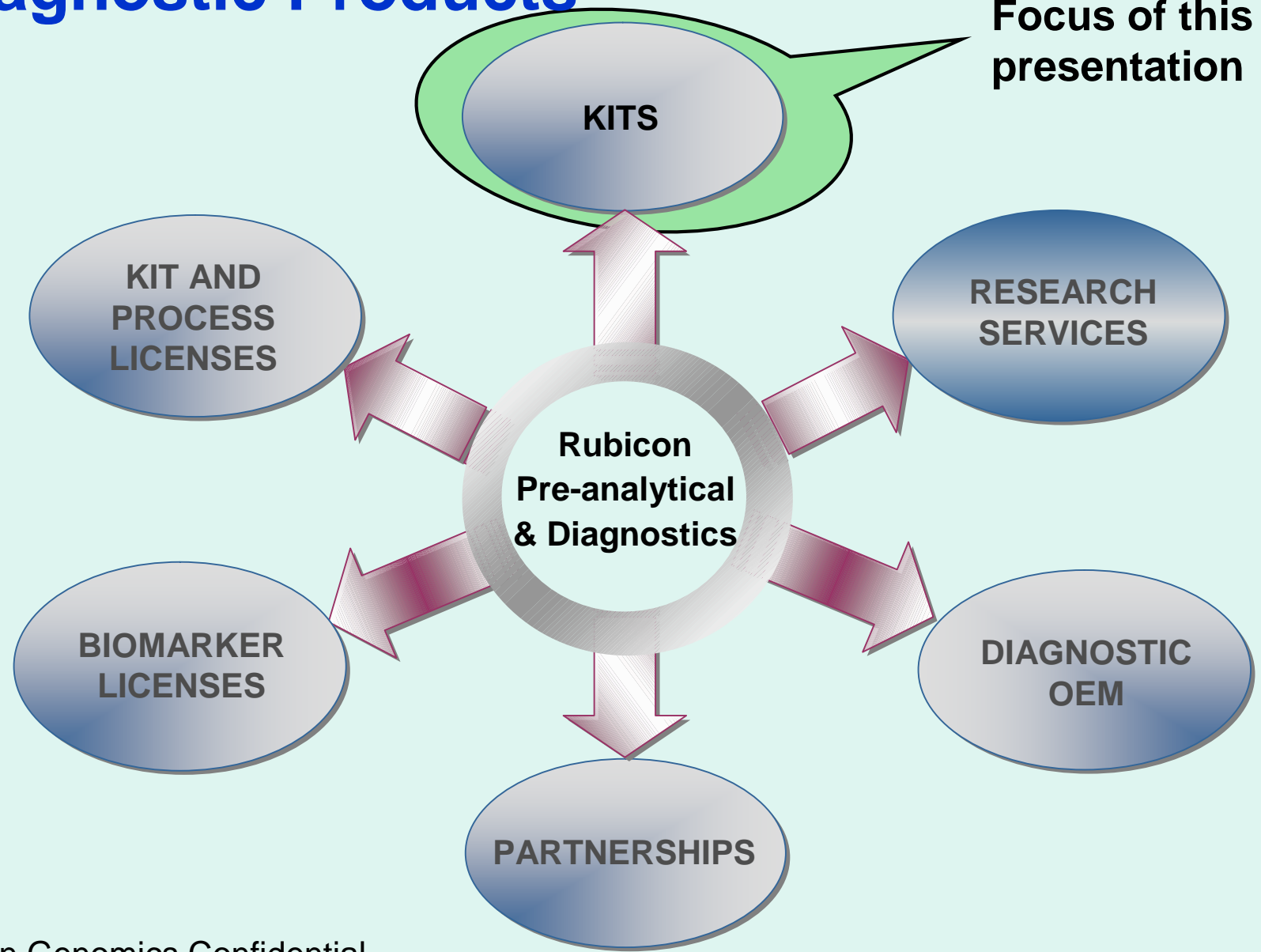
## WGA Product Summary

Focus on Pre-Analytical Platforms for Improved Analytical Performance

March 3, 2009

# Rubicon Commercial Pre-Analytical and Diagnostic Products

Focus of this presentation



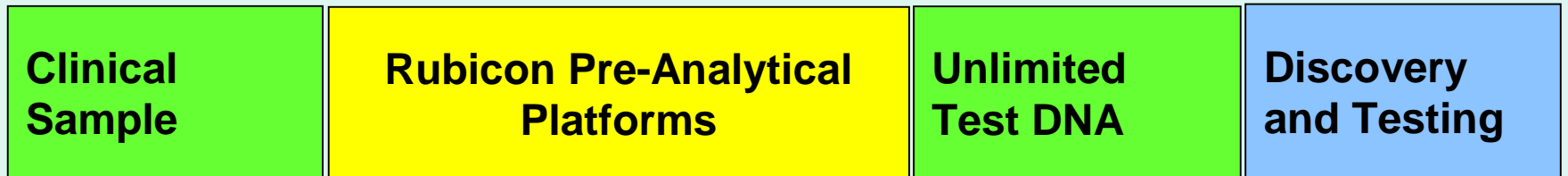
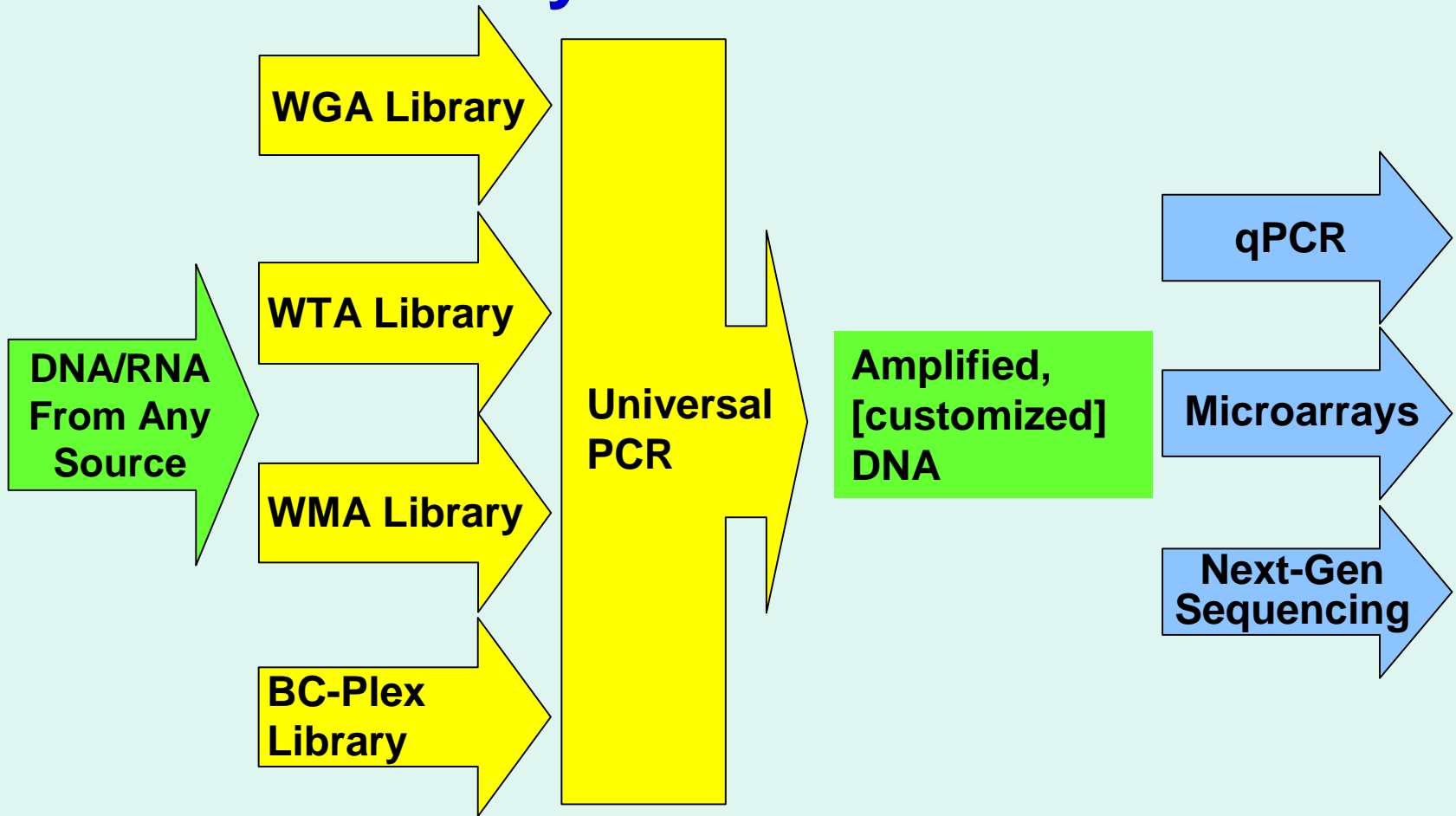
# Important Limitations of Nucleic Acid Analysis

- **Real DNA/RNA samples are often imperfect**
  - Inadequate quantity
  - Variable quantity (e.g., plasma, urine, biopsy)
  - Variable quality (e.g., fixed, apoptotic or necrotic tissue, plasma)
- **Analytical platforms perform sub-optimally with imperfect samples**
  - Reduced analytical sensitivity, specificity, reproducibility
  - Sample, locus, allele drop-outs
  - Microarray and multi-analyte testing limited or problematic
  - Reduced clinical sensitivity, specificity, robustness
- **Examples of NAT limitations**
  - Single-cell molecular assays impossible (except FISH)
  - IVD-MIA of plasma, needle aspirate, CTC impossible
  - Valuable biospecimens quickly exhausted
- **Strong market need for pre-analytical processing to improve analytical and clinical performance of NAT**

# Rubicon Solutions

- **Best-in-field total DNA, meDNA, RNA amplification**
  - PCR-amplified molecular libraries with custom adaptors on both ends
  - Output validated on all analytical instruments and applications
  - Template can be degraded or intact
  - Simple, rapid, reproducible
  - High representation
  - High fidelity
  - No background
- **Standardization of output**
  - Constant molecular weight and purity
  - Constant concentration
- **Customized for analytical platforms**
  - Custom work flow to reduce labor and increase throughput
  - Custom molecular weight
  - Custom base composition
- **Benefits**
  - Improved analytical and clinical performance for genomic, epigenomic, and expression research and clinical testing

# Rubicon Pre-Analytical Platforms



# WGA Technical and Commercial Landscape

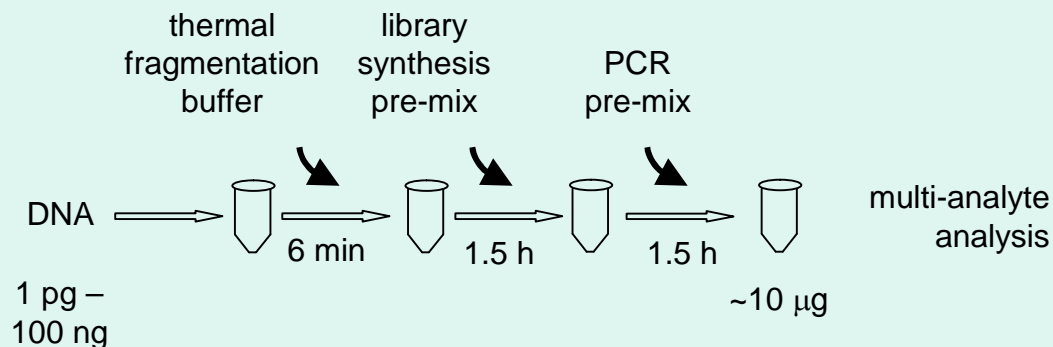
Samples	Pre-Analytical Platforms	Analytical Platform	Analytical Result	Market
<b>Degraded DNA(ng)</b> Fixed biopsy Fixed surgical Plasma/serum ChIP Blood card Swabs/smears Urine CSF Sputum Feces/hair Environment	<b>MDA</b> GE Qiagen  <b>WGA-I</b> Sigma Rubicon  <b>WGA-II</b> Rubicon	<b>Microarray</b> Affymetrix Agilent Roche Life  <b>NGS</b> Illumina Roche Life	<b>Genotype</b> SNP CNV STR Microsatellite  <b>Sequence</b> De novo Point mutation Rearrangement	<b>Univ and Inst</b> Core facility PI  <b>Pharma</b> Discovery PGx  <b>MDx</b> IVD ASR/home-brew
<b>Single cells (pg)</b> FACS CTC LCM Blastomeres Amniocytes Environment	<b>NGS-WGA-II</b> Rubicon  <b>WGA-III</b> Rubicon	<b>Q-PCR</b> Life Roche Promega Qiagen, etc	<b>Copy number</b> Deletion Amplification	<b>Agbio</b>  <b>Forensic</b>
<b>Intact DNA (ng)</b> Blood Saliva Urine Environment	<b>BC-WGA/MDA</b> Qiagen Rubicon	<b>Capillaries</b> Life Promega		

# Rubicon Pre-Analytical Kits

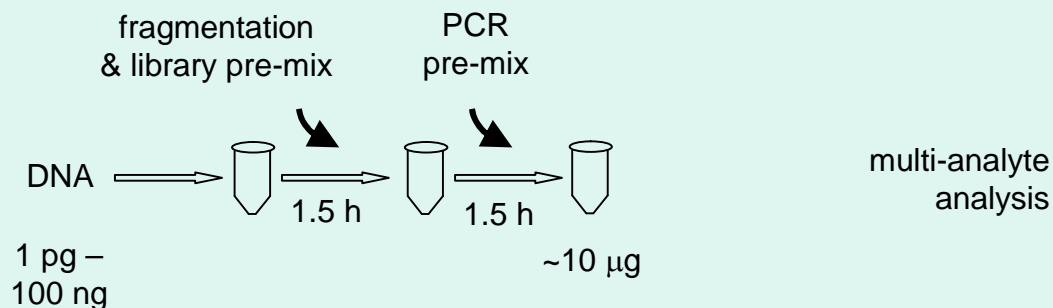
- **First-generation amplification kits (WGA I, WTA I, WMA I)**
  - Based on quasi-random primed library made from ss or ds DNA
  - WGA I and WTA I licensed and sold by Sigma-Aldrich for research market only
  - Clinical, C-WGA I, C-WTA I, and cWMA I sold by Rubicon for clinical applications
  - Adopted by diagnostics industry for manufacturing and patient testing
- **Second-generation amplification kits (WGA II, WMA II)**
  - Based on efficient, single-step ligation-based library made from dsDNA
  - Most useful for plasma/serum, ChIP, and other fragmented templates
  - Highest representation and sequence fidelity
  - Used >4 y in-house and by MDx partners for Q-PCR and microarray assays
- **Third-generation amplification kits (WGA III, WMA III)**
  - Based on most novel method of making library from ss or ds DNA
  - Best representation and reproducibility for single-cell genetic and epigenetic applications.
  - Used for Pre-Implantation Genetic Diagnostics and Screening applications.
- **Customized kits**
  - Complexity reduction for more sensitive profiling using microarrays and sequencing
  - BC-Plex bisulfite-converted DNA WGA for diagnostic testing and sequencing
  - Single-tube next-gen sequencing template prep (in development)

# Rubicon WGA Flowcharts

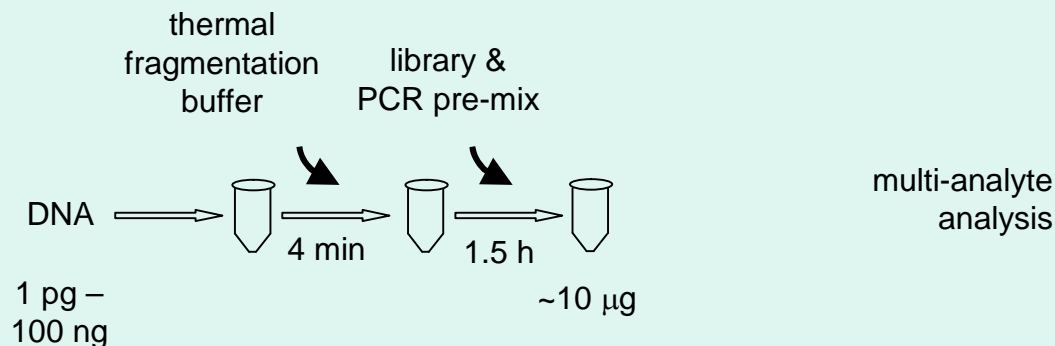
## C-WGA I



## WGA II

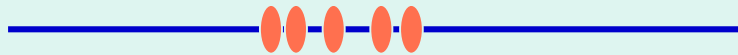


## WGA III



# MethylPlex WMA is “Methylation-Specific Genomic DNA Amplification”

DNA from cancer cells  
Methylated CpG sites

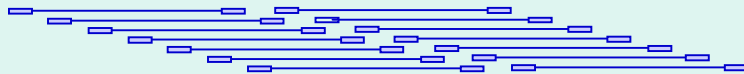


DNA from normal cells  
Non-methylated CpG sites



MethylPlex WMA library synthesis and amplification

Amplified MethylPlex library from cancer cells



No gap

Amplified MethylPlex library from normal cells

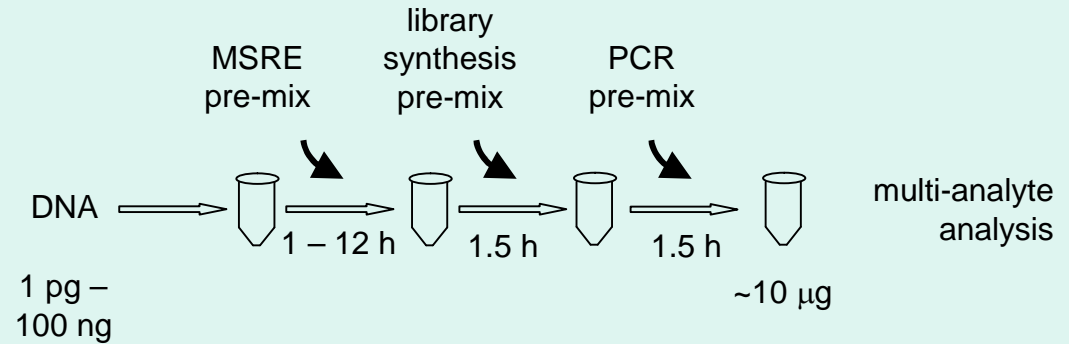


gap

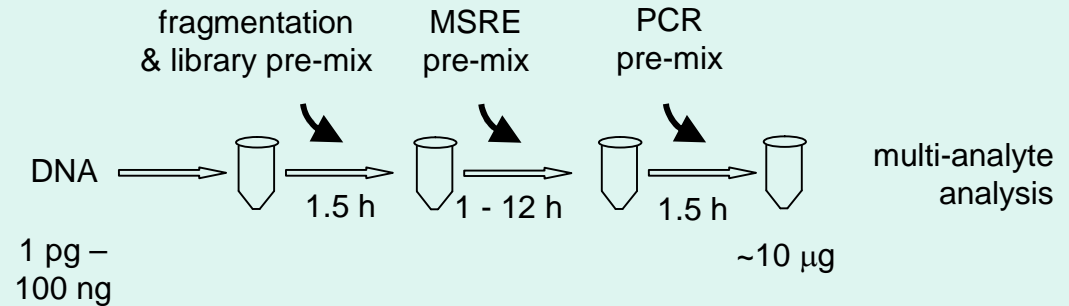
Locus-specific copy number assays using qPCR,  
microarrays, or tag-sequencing

# MethylPlex Flowcharts

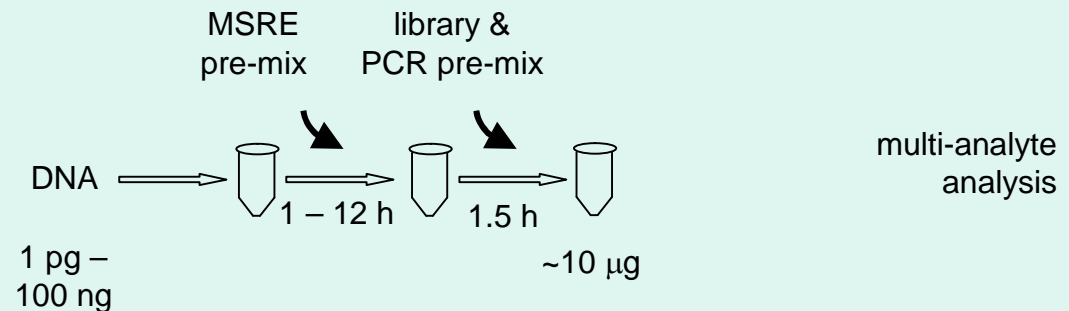
## C-WMA I



## WMA II



## WMA III



# Rubicon DNA Pre-Analytical Platforms Most Applicable for Q-PCR and Array Applications

<b>tissue</b> <b>application</b>	<b>frozen tissue</b>	<b>fixed tissue</b>	<b>IP (plasma)</b>	<b>single cell</b>
<b>CGH</b>	<b>C-WGA I</b> <b>WGA II</b>	<b>C-WGA I</b> <b>WGA II</b>		<b>WGA III</b>
<b>ChIP</b>			<b>WGA II</b>	
<b>methylation</b>	<b>C-WMA I</b>	<b>C-WMA I</b>	<b>WMA II</b>	<b>WMA III</b>
<b>genotyping</b>	<b>C-WGA I</b>	<b>C-WGA I</b>	<b>WGA II</b>	<b>WGA III</b>

# Sigma & Rubicon WGA CGH Performance Expectations, Based on Q-PCR Trends

method feature	C-WGA I	WGA II	WGA III
representation bias (10 ng frozen tissue)	+/- 2 cycles	+/- 1 cycle	+/- 5 cycles
locus DO (>10 cycles delay with 10 ng frozen tissue)	<0.1% DO	<0.1% DO	~5% DO
reproducibility (10 ng frozen tissue)	+/- 0.5 cycles	+/- 0.5 cycles	+/- 0.5 cycles
reproducibility for 1 cell	+/- 5 cycles	unknown	+/- 1 cycle
strengths	better representation for all sequences including GC-rich loci	best representation for 10 ng	excellent reproducibility for single cells
weaknesses	poor reproducibility for single cells	Requires dsDNA	some loci systematically under-represented

# Rubicon WGA ChIP Performance Expectations, Based on Q-PCR Results and ChIP Publications

<b>method</b> <b>feature</b>	<b>C-WGA I</b>	<b>WGA II</b>
<b>amplification efficiency for 1000 b fragments</b>	~90%	~100%
<b>amplification efficiency for 200 b fragments</b>	~10%	~100%
<b>ability to detect methylation in ChIP</b>	yes, at low-resolution	yes, at high and low resolution
<b>strengths</b>	better than conventional LM-PCR for low resolution ChIP; works for CpG islands; works for methylation analysis	best representation high or low resolution ChIP, including CpG islands; works for methylation analysis
<b>weaknesses</b>	worse than conventional LM-PCR for high resolution ChIP	

# Sigma & Rubicon WMA Performance Expectations, Based on Q-PCR Results

method feature	C-WMA I	WMA II
efficiency for detection of methylated loci	excellent amplification of GC-rich sequences and ability to detect methylation	best amplification of GC-rich sequences and ability to detect methylation
detection of methylation in frozen or fixed tissue	yes	yes
detection of methylation in plasma	no, fragments too small	yes
detection of methylation in protein-bound ChIP	yes, at low resolution	yes, at high or low resolution
sensitivity to low levels of methylation	~3% methylation detectable, using enrichment	~3% methylation detectable using enrichment

# Major Points

- Rubicon has developed, patented, and commercialized effective pre-analytical platforms for amplification and standardization of DNA for all array applications.
- C-WGA I kits are superior for WGA of formalin-fixed paraffin-embedded samples
- WGA II kits are superior to all other WGA methods for ChIP, plasma, or other samples with short dsDNA.
- WGA III kits have superior sensitivity and reproducibility for applications with very small amounts of DNA such as single cells.