NCS Maternal and Cord Blood Samples: Whole-Genome Arrays and DNA Methylation

Charlotte A. Hobbs, M.D., Ph.D
Arkansas Children's Hospital Research Institute
Department of Pediatrics, College of Medicine, University of Arkansas for Medical Sciences

NCS Biologics

- NCS biologics (DNA, RNA, protein) for ….
  - Genomics
  - Epigenomics
  - Proteomics
- Longitudinal/epidemiologic studies – robust quality biologics depend on
  - Collection
  - field processing
  - shipment
  - storage
  - retrieval

Study Objective

- Phase 1
  - Compare DNA and protein concentration of NCS samples with samples from Arkansas DNA Bank for Congenital Malformations.
- Phase 2
  - Evaluate suitability of NCS blood samples to generate genotypes and gene-specific DNA methylation profiles.

Experience

- National Birth Defects Prevention Study
  - Collection of biologics
  - Blood vs buccal derived DNA compared on highly multiplexed genotyping platform

Cheek cells by buccal cytobrush for DNA collection

- After maternal interview , buccal (cheek cell) collection kit is mailed
- Sample requested for biological mother, father and baby
- DNA extracted, quantified, whole genome amplified, SNP genotyped, data analyzed

Image: courtesy of cdc.gov

96 DNA samples: from buccal brush and blood

DNA quantity	Brush_GQ	Brush_WGA_Q	Blood_GQ
Mean: ng/µl	3.54	129.33	260.13

SNP Genotyping platform: Illumina™ Golden Gate

Number of SNPs genotyped/sample: 1536

Blood Vs Buccal SNPs genotyping concordance rate: 98.2%

Why analyze DNA?

- Changes in DNA sequence (SNPs, copy number variation) have been associated with disease risk
- Epigenetic changes (DNA methylation) have also been associated with human development as well as disease risk

DNA Extraction Methods

- ≈ 100 mothers and cord blood samples from NCS repository
- DNA extracted from fractionated blood using Qiagen® PureGene® Blood Kit.
- DNA quantified by spectrophotometry ($A_{260/280}$)

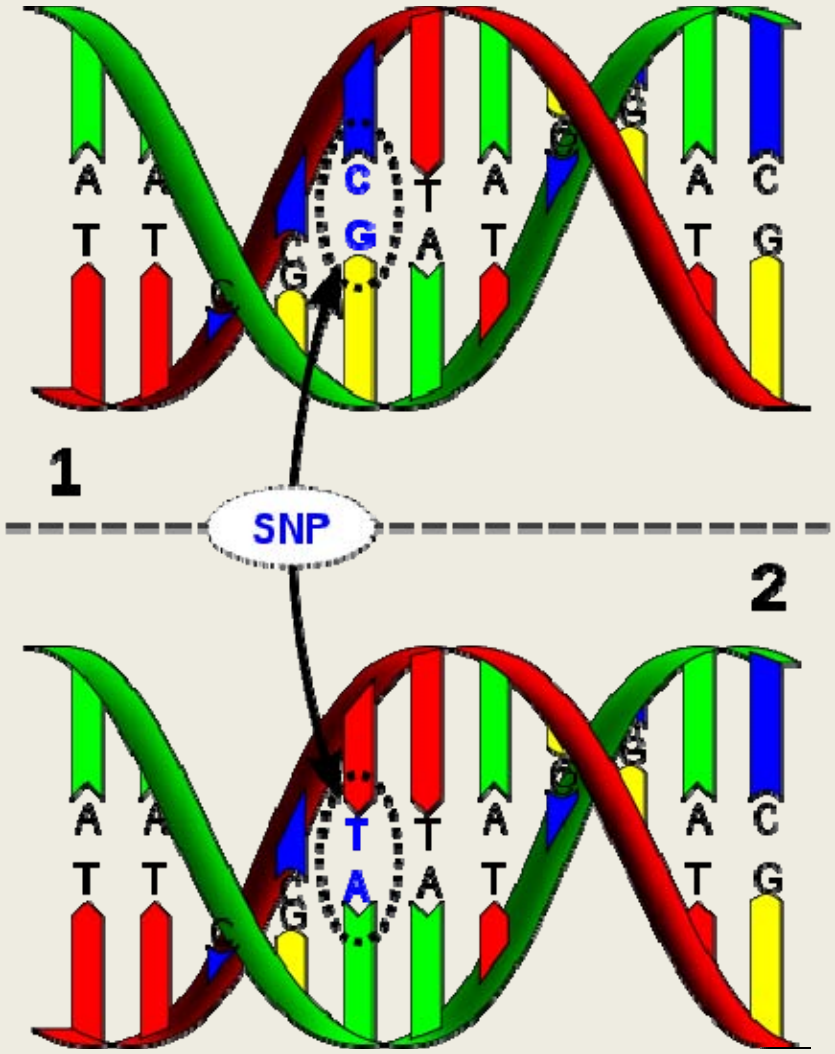
- ≈ 14 samples A_{260}:A_{280} < 1.4 repurified with PureGene® reagents.
- ≈ 23 samples with inadequate yields, DNA was extracted from plasma

Analysis of DNA

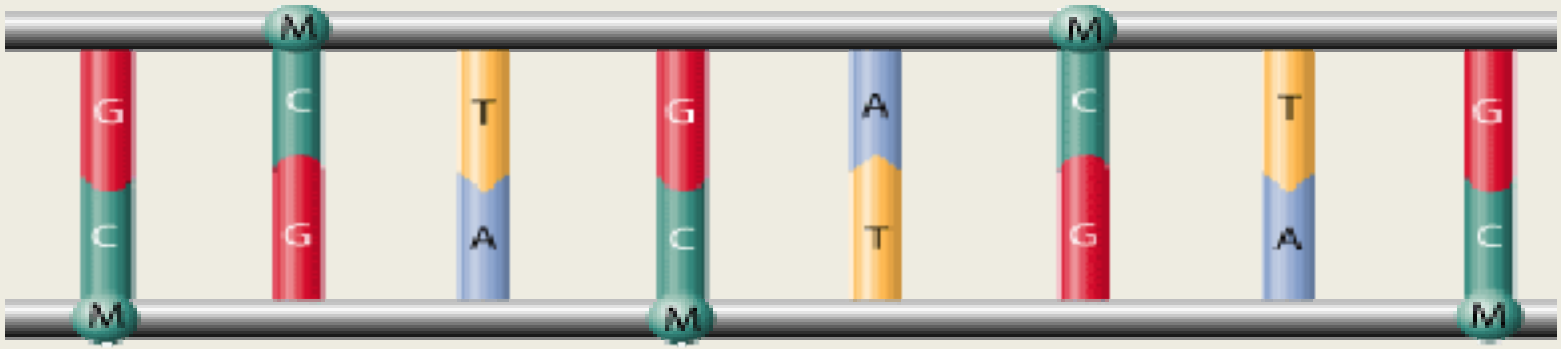
- HumanOmni 2.5-8 BeadChip

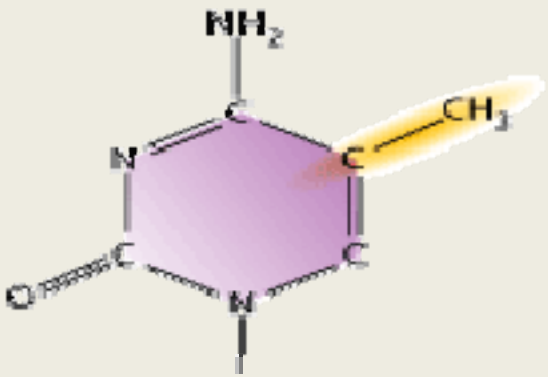
  - Coverage: 2.5 million SNPs across the human genome
- SNPs identified by:
  - International HapMap Project
  - 1000 Genomes Project
- HumanMethylation450 BeadChip
  - Measures methylation at 450,000 CpG sites across the human genome
  - Methylation = addition of methyl group(CH_3) to cytosine in DNA
  - CpG Methylation of C in CpG in humans
- DNA adducts: chemicals bound to DNA as a result of exposure through the environment or diet

Single Nucleotide Polymorphism



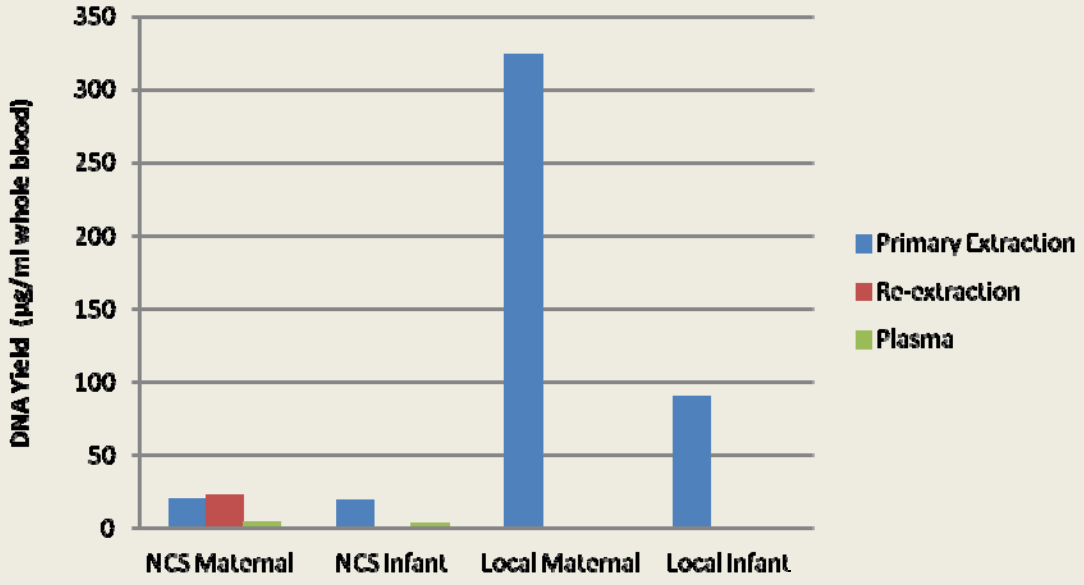
DNA Methylation



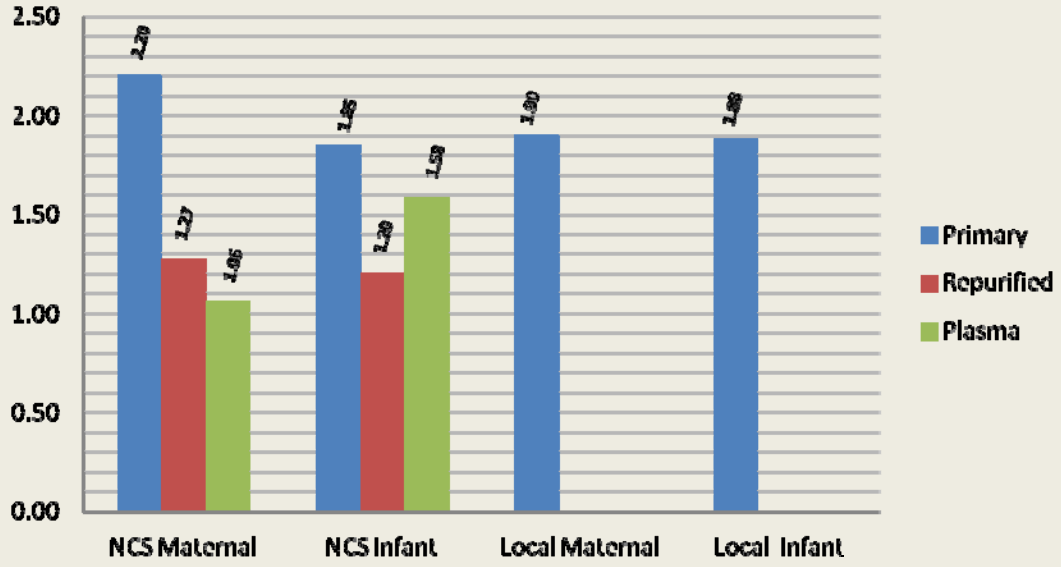


DNA methylation is the addition of a methyl group (M) to the DNA base cytosine (C).

DNA Yields from NCS Whole and Cord Blood

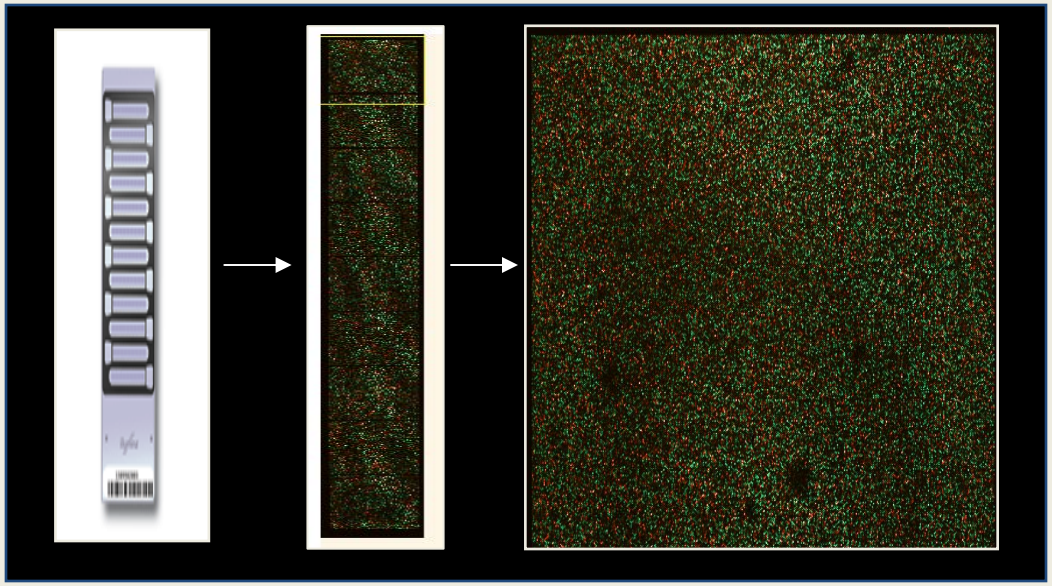


Median DNA 260/280 ratio

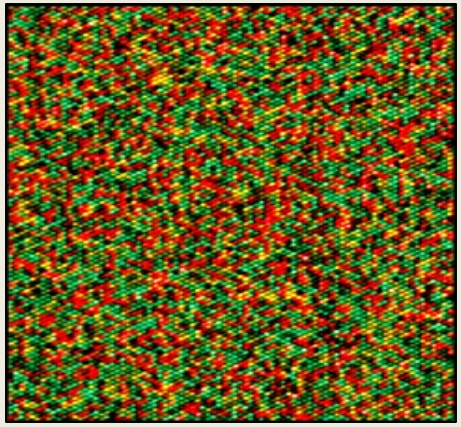


BeadChip Array Processing

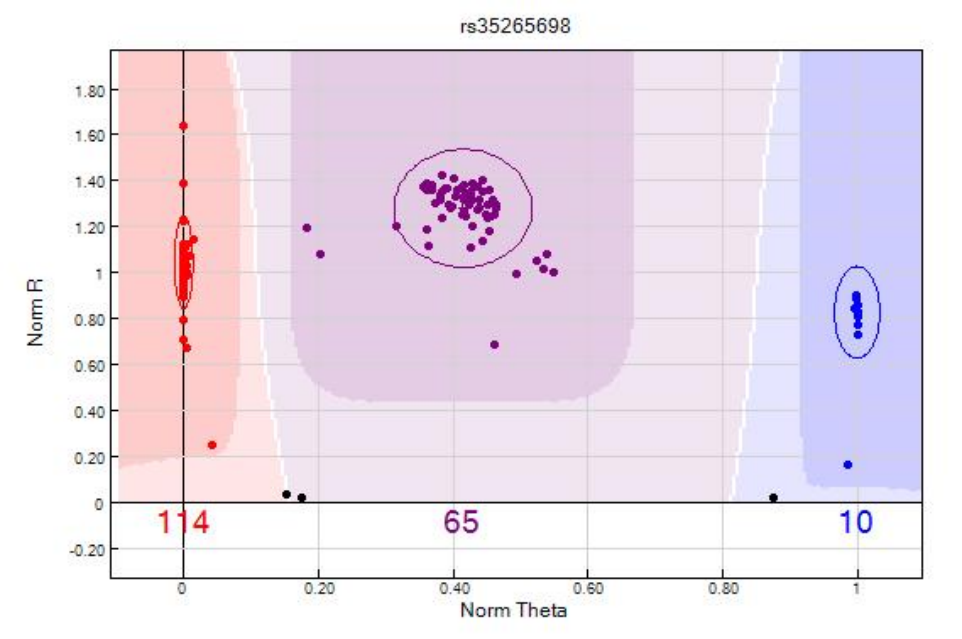
- Red or green flourescent signal coresponding to SNP identity
- Example: red=CC, green=TT, red+green=yellow=CT



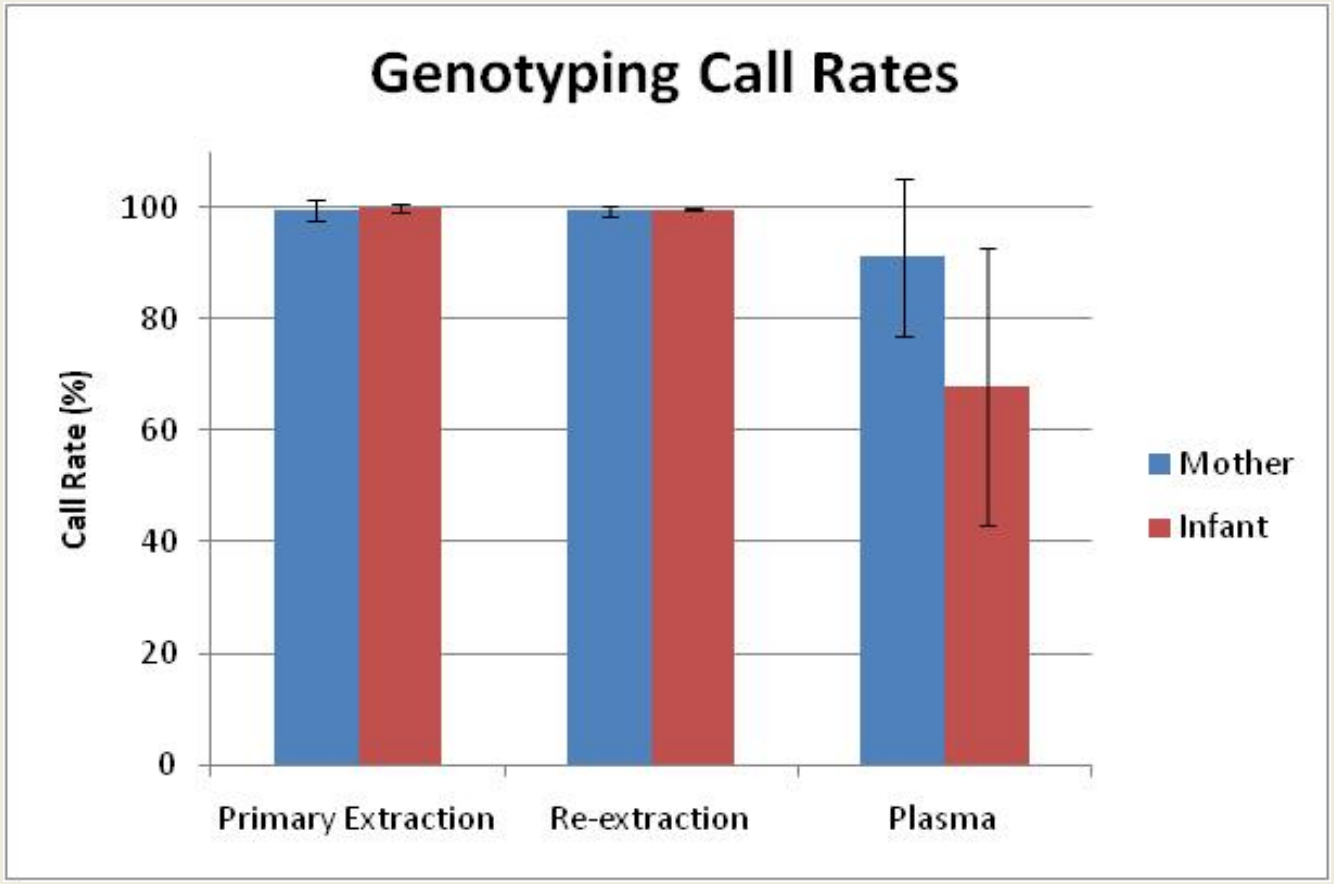
Genotype Analysis

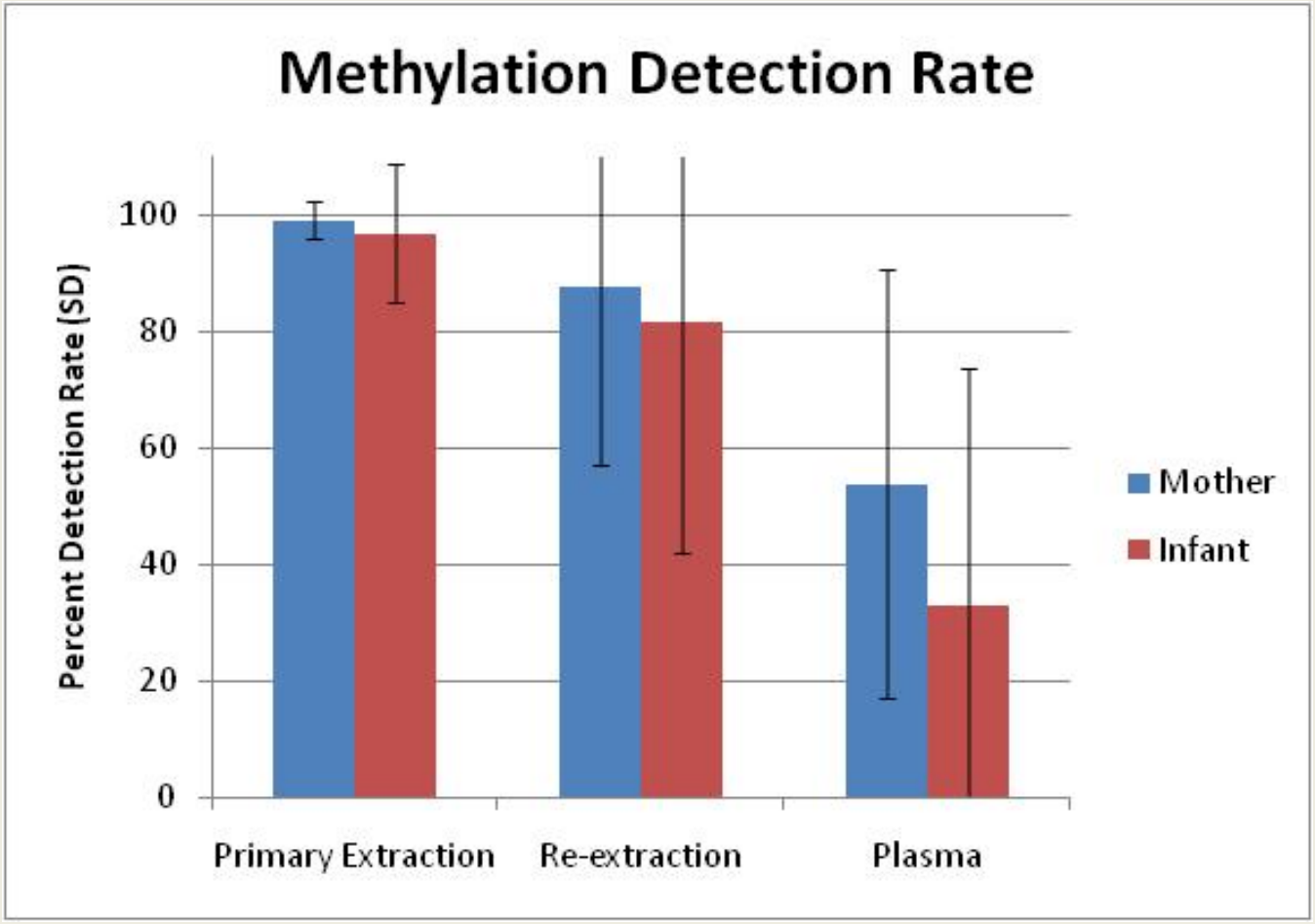


Scanned Illumina Beadchip displays genome wide SNPs.



SNP clusters show homo and heterozygosity including poor quality samples receiving no call.


Genotyping Call Rates
Call Rate (%)
100
80
60
40
20
0
Primary Extraction
Re-extraction
Plasma
Mother
Infant


Methylation Detection Rate
Percent Detection Rate (SD)
100
80
60
40
20
0
Primary Extraction
Re-extraction
Plasma
Mother
Infant

Summary

- Genotyping and methylation analysis was successful for 89.6% and 75.5% of samples, respectively
- Significant variability in NCS DNA yield and quality, when compared with local collected samples, suggests deviations in sample collection, handling and storage.
- Suggests further optimization of protocol for blood collection is needed prior to beginning the NCS Main Study
- DNA adducts
    - integration of genomic, epigenomic and DNA adduct data.

Acknowledgements

- National Children's Study Initial Vanguard Study
- Birth Defects Genomics Laboratory staff and faculty