Experience with the Human 1M and HumanMethylation27 BeadChips in Human and Non-human Primates

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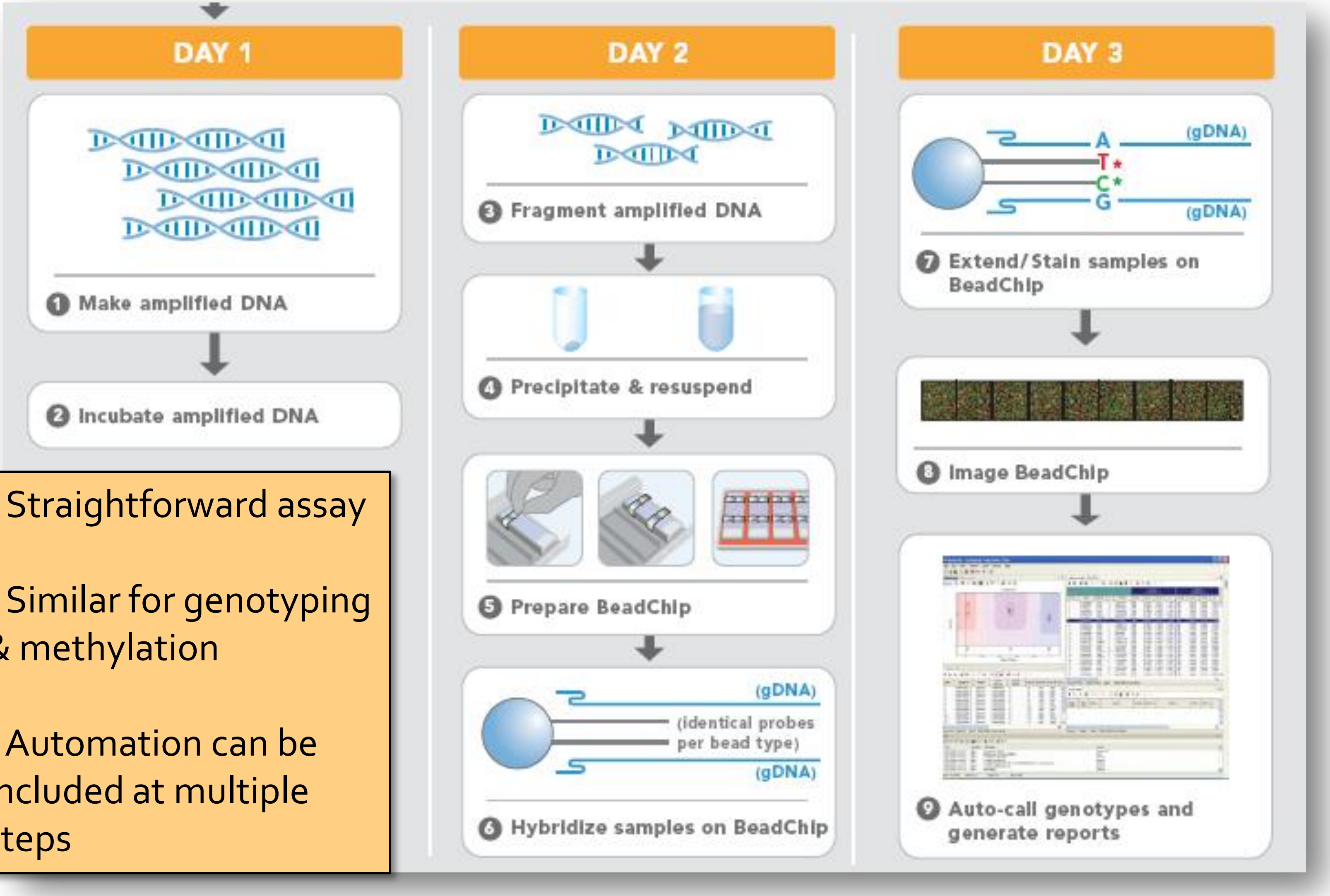
Infinium Assay



- Straightforward assay
- Similar for genotyping & methylation
- Automation can be included at multiple steps

Infinium HD - Human1M-Duo v2.0

Human1M-Duo

- 2.4 million genetic variations
- Over 1 million SNPs per Sample
- 2 Samples per Chip
- Doubles sample capacity per run
- Lower DNA input per sample (400ng)
- Additional high value content
  - Disease SNPs
  - Additional gene centric SNPs (Seattle SNPs)
  - 5000 additional CNV probes
  - SNPs associated with diseases (found in GWAS)
  - Reduced number of gaps
  - Additional TagSNPs

Our setup

- ***Laboratory***
  - Mostly manual method
  - Staining and washes automated with TECAN Freedom Evo washstation
  - One BeadArrayGX500

- ***Genotype Calling/preliminary analysis***
  - BeadStudio (no concerns with calling algorithm)
  - Run on a desktop PC
    - Dell Optiplex GX620, 3 GHz, 4Gb RAM
    - /3Gb Startup Switch in the boot.ini file (allows for more memory to be used for applications, e.g., BeadStudio)

The STAMPEED experience with the Illumina 1M BeadChip

- **STAMPEED**: *SNP Typing for Association with Multiple Phenotypes from Existing Epidemiologic Data*
- 13 GWAS studies funded by NHLBI to investigate a variety of cardiovascular, lung and blood disorders
- Genome Wide Association for Asthma and Lung Function (PI: Deborah Meyers, PhD)
  - Over 3,000 asthmatic and control samples (a combination of samples from the Collaborative Study of the Genetics of Asthma (CSGA) and the Severe Asthma Research Program (SARP))

The STAMPEED experience with the Illumina 1M BeadChip

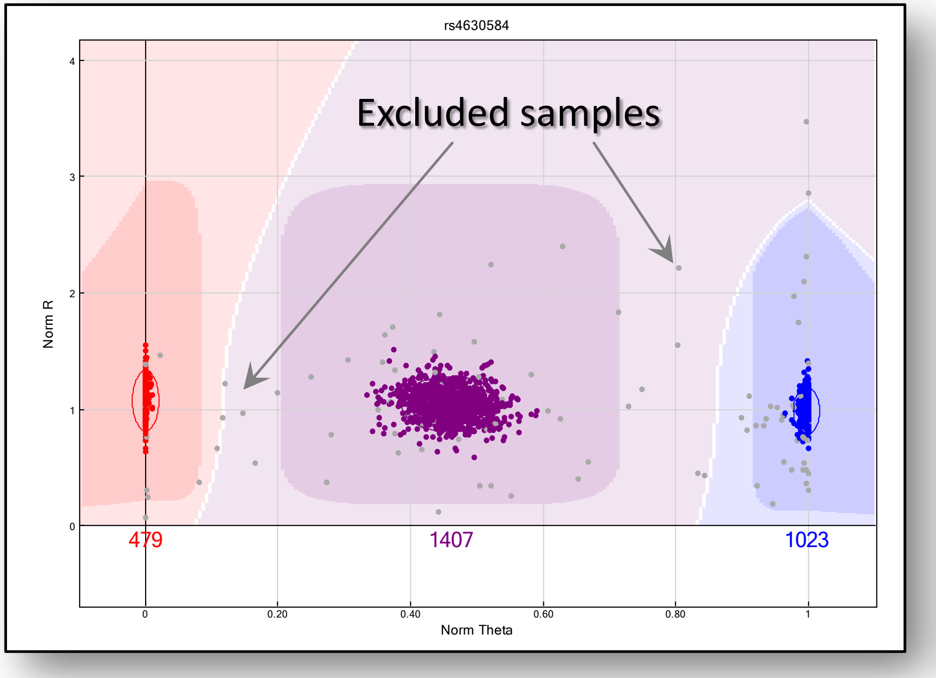
- Allele calling/genotype QC
  1. Cluster with Illumina 1M cluster definitions
  2. Remove ***samples*** with call rates < 90%
  3. Re-cluster with remaining samples
  4. Remove ***SNPs*** with call rates < 90%
  5. Re-calculate and remove ***samples*** with call rates < 95%
  6. Re-cluster and re-calculate calling statistics
  7. Fine-tune remaining SNPs using the GenTrain and ClusterSep scores

- 2911 ***samples*** (95.51%) passed QC
- 1,029,883 ***SNPs*** (97.58%) passed QC

The STAMPEED experience with the Illumina 1M BeadChip

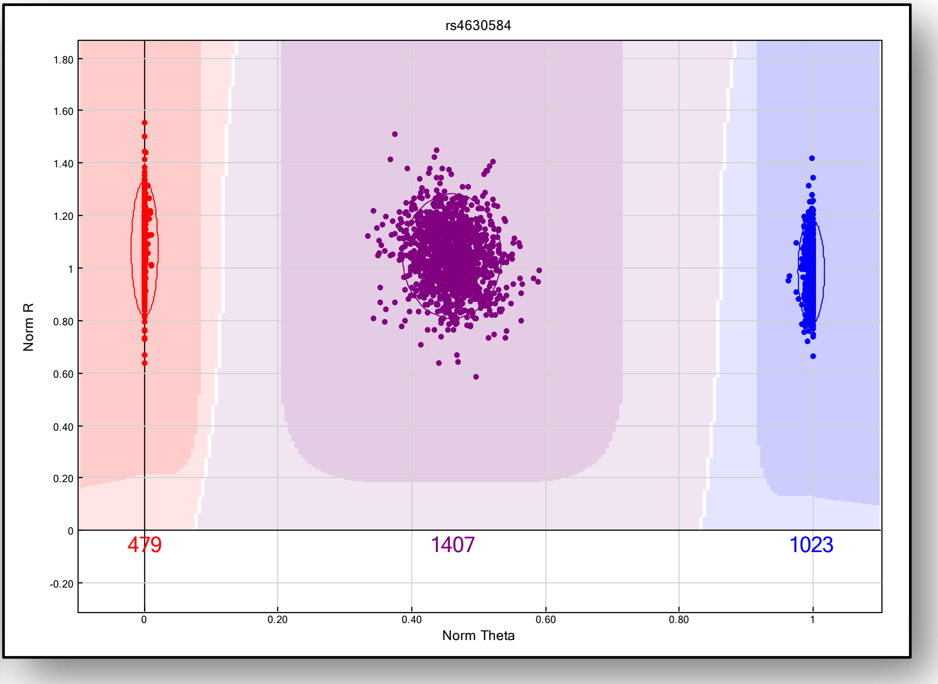
- The advantage of re-clustering

What goes in…



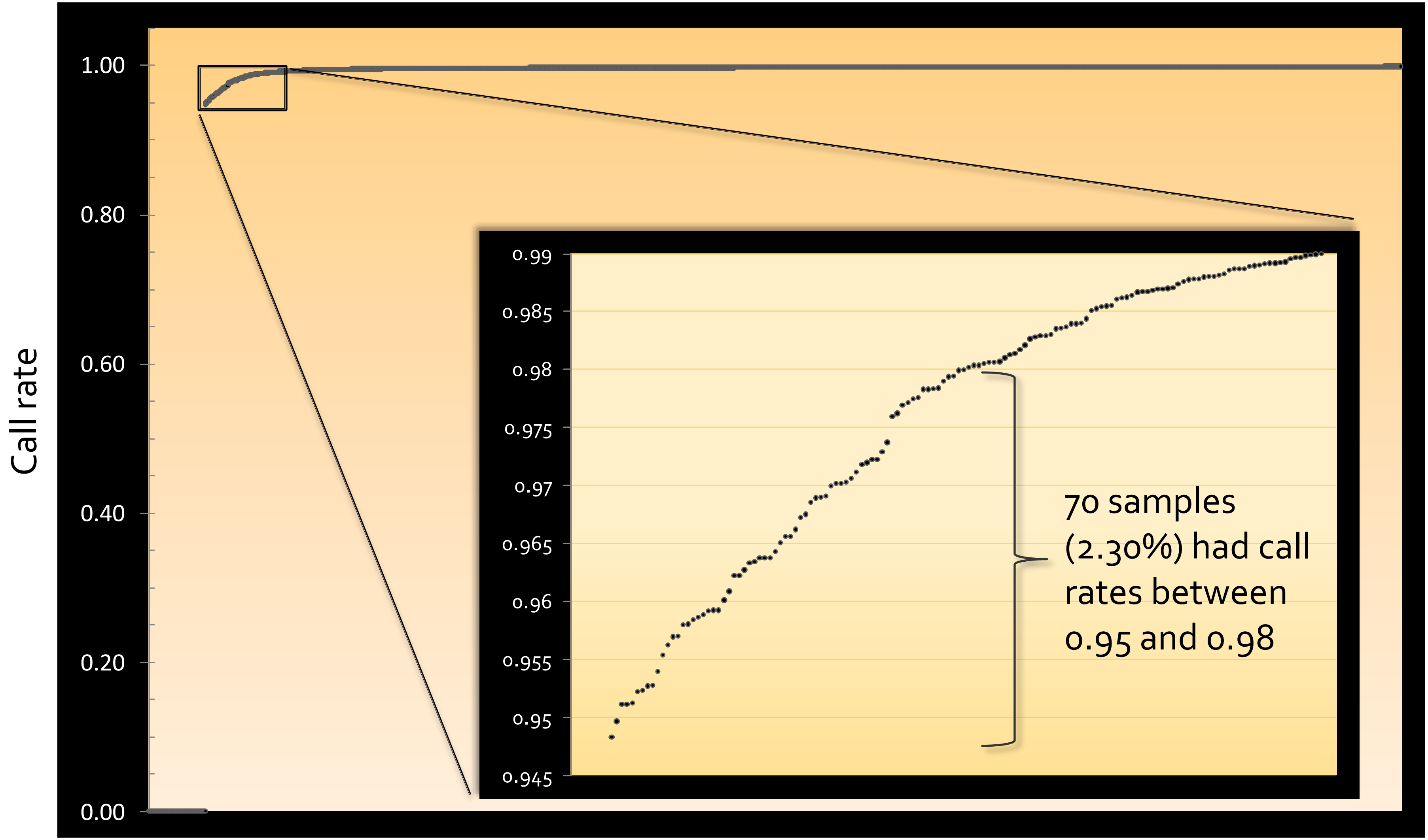
Excluded samples

What comes out…



STAMPEED

Sample Call rates (95.51% of samples ≥ 95%)



70 samples (2.30%) had call rates between 0.95 and 0.98

STAMPEED

Analysis of replicate samples

No. of replicate pairs	Average Number of differences	Range of differences	Concordance Rate
106	889	21 – 48,566	0.9996
104*	135	21 - 1234	0.9999

*Two samples were considered outliers and removed from analysis

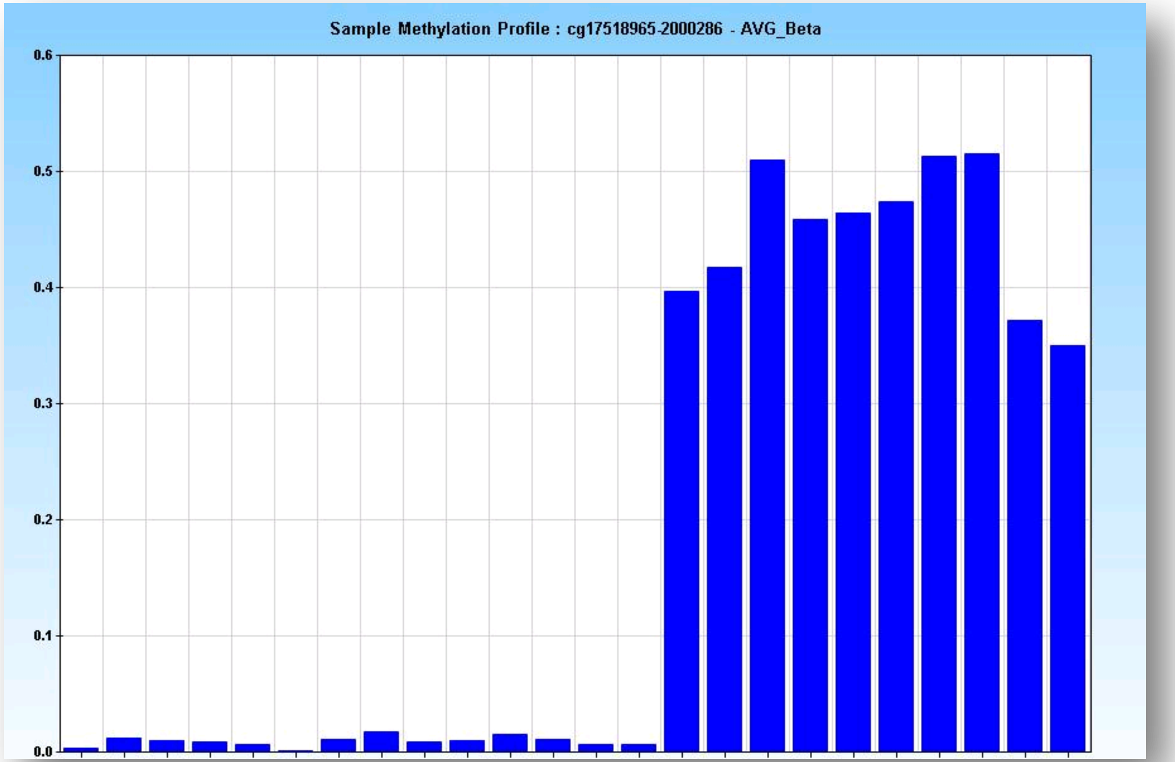
HumanMethylation27 BeadChip in Humans and NHPs

Description	Markers	Avg coverage (# of sites)
Total RefSeq Genes	14,495	1.9
Well-annotated genes described in the NCBI CCDS database (build36)	12,833	1.9
Methylation hotspots in cancer genes	144	7.6
Cancer related targets	982	1.9
MicroRNA promoters	110	2.3

Human Methylation27 with Human Samples

- 6 DNA samples from purchased primary monocyte cultures
- 5 DNA samples from buccal cells
- All samples were replicated on two different chips
- One monocyte sample was also replicated on the same chip

S1PR4 (sphingosine-1-phosphate receptor 4 precursor)

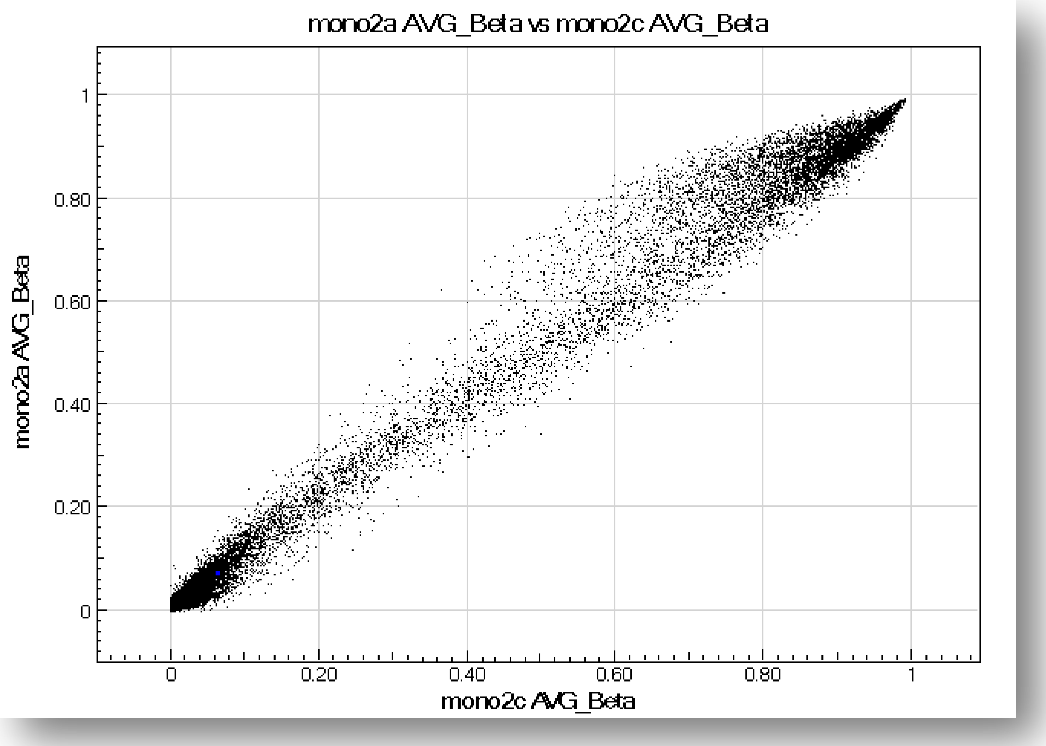


Monocytes

Buccal DNA

QC Experiments with Human Monocytes

- ***Intra-chip variability***
  - Sample run in duplicate on same BeadChip



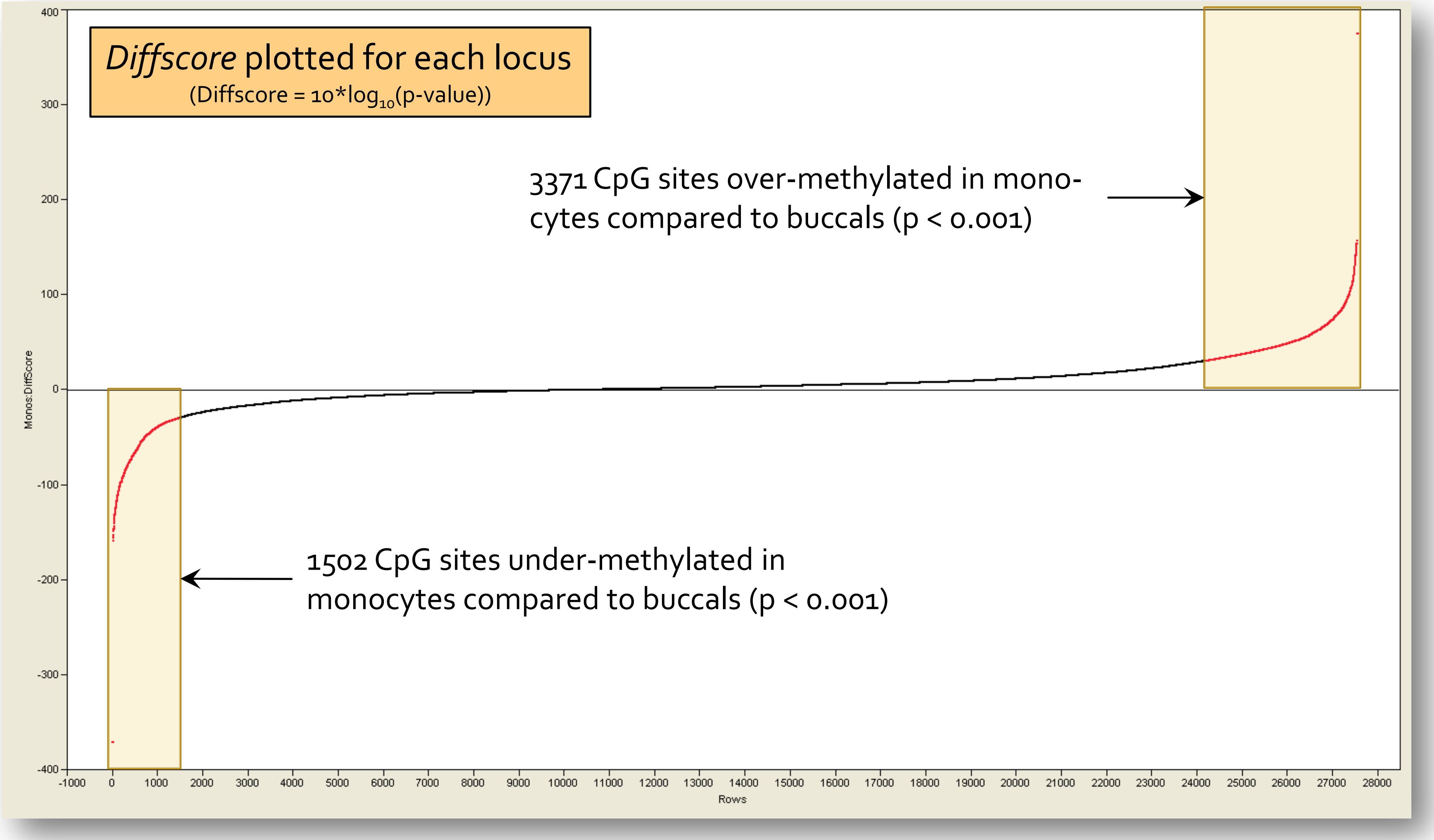
$r^2 = 0.991$

QC Experiments with Human Monocytes

- ***Inter-chip variability***
  - Sample run in duplicate on two different BeadChips

$r^2 = 0.998$

Differential Methylation using the "Diffscore" values in BeadStudio



Diffscore plotted for each locus
(Diffscore = $10*\log_{10}(\text{p-value})$)

3371 CpG sites over-methylated in monocytes compared to buccals ($p < 0.001$)

1502 CpG sites under-methylated in monocytes compared to buccals ($p < 0.001$)

HumanMethylation27 BeadChip with Non-Human Primate samples

- Evaluated
  - Vervet liver
  - Vervet visceral fat
  - Vervet pancreas
  - Cyno liver
  - Cyno visceral fat
  - Cyno subcutaneous fat
  - Cyno pancreas
  - Human control (buccal)

Cynomolgus macaque
"Cynos"

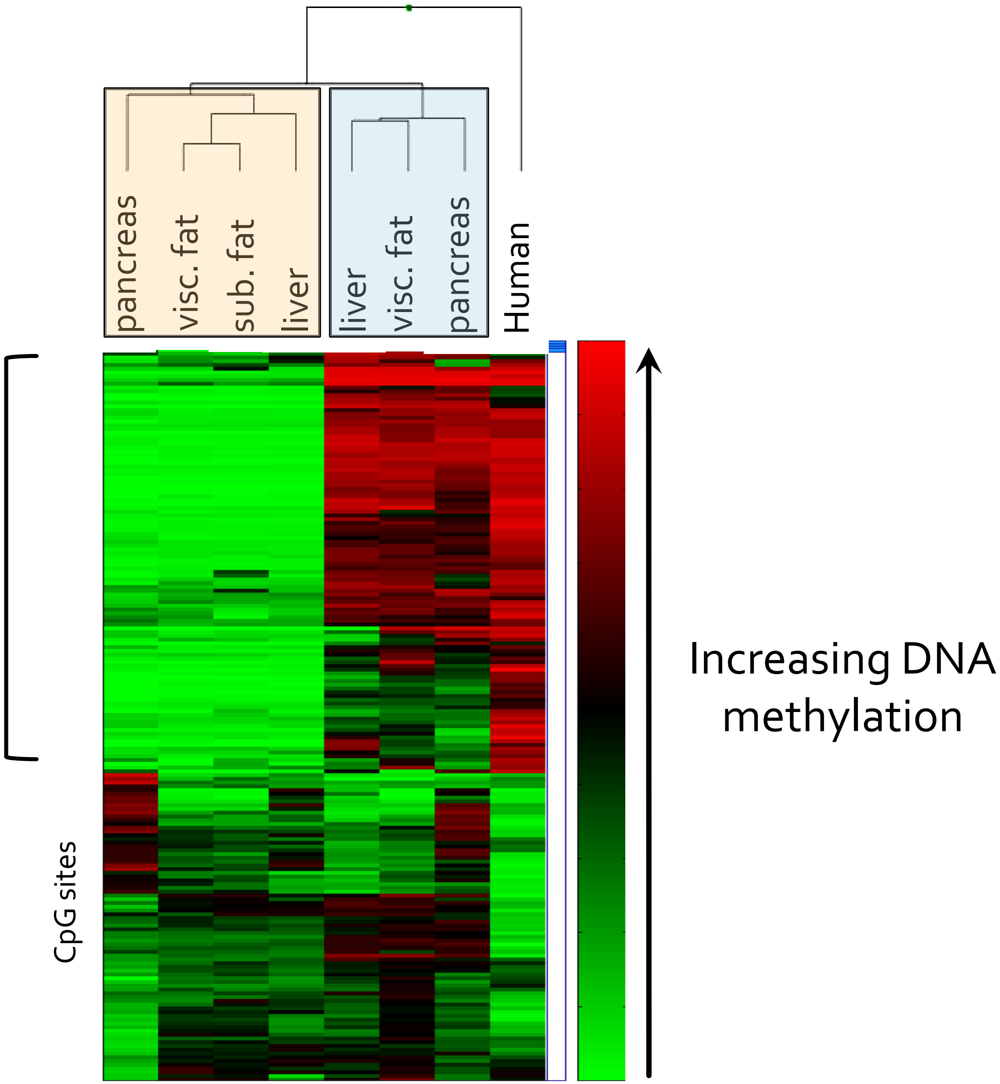
80.6% success rate
(22,214 sites)

Chlorocebus aethiops sabaeus
"Vervets"

74.4% success rate
(20,524 sites)

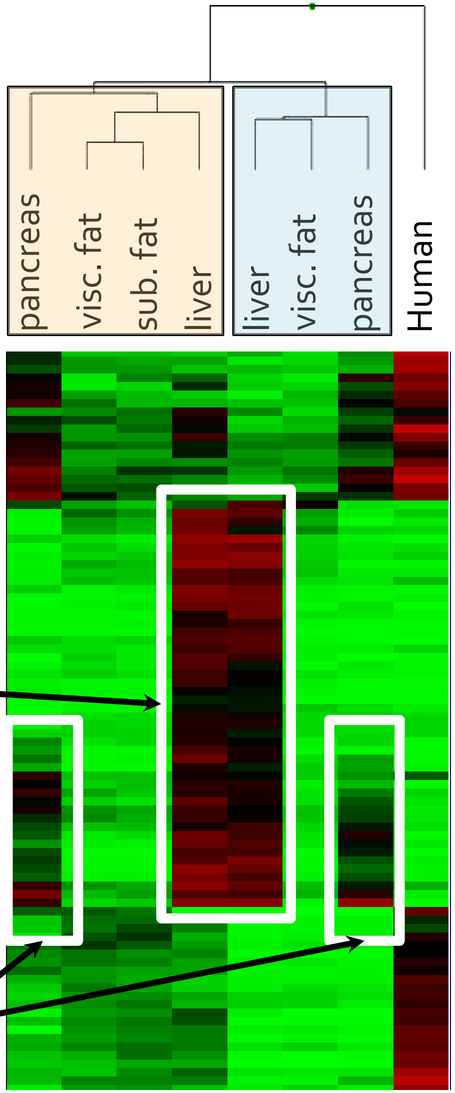
Preliminary methylation data in non-human primates

- BeadStudio clustering algorithm clustered cynos and vervets together based on methylation patterns.
- Differences could be due to variability of
  - Genomes
  - Diets
  - Environments (vervets were at UCLA, cynos at WFU)
  - Social factors (stress, dominance, etc.)
  - All of the above



Preliminary methylation data in non-human primates

- Some genes from the same tissues from cynos and vervets clustered together based on methylation patterns

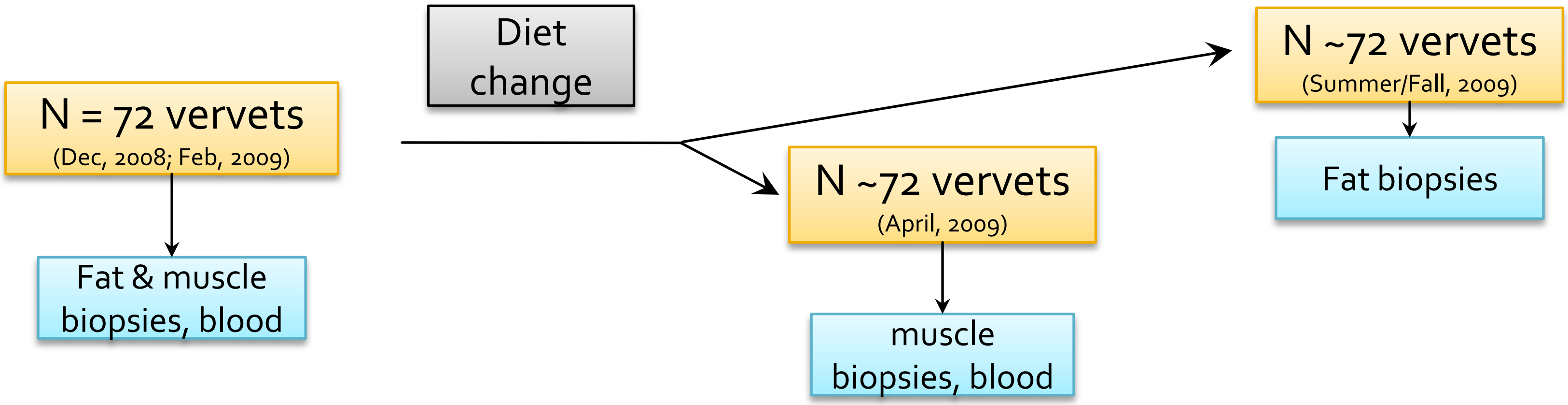


Cyno/vervet liver

Cyno/vervet pancreas

Advantage of the HumanMethylation27 BeadChip in NHP studies

1) **Evaluate the change in methylation status of CpG sites before and after the diet change in a sub-sample of the VRC.** Fat and muscle biopsies will be obtained from approximately 72 animals. The Illumina HumanMethylation27 BeadChip will be used to evaluate fat and muscle tissue, before and after the diet change.



Diet change

N = 72 vervets
(Dec, 2008; Feb, 2009)

Fat & muscle biopsies, blood

N ~72 vervets
(April, 2009)

muscle biopsies, blood

N ~72 vervets
(Summer/Fall, 2009)

Fat biopsies

Conclusions

- The 1M BeadChip worked very well with the STAMPEED GWAS project
  - Call rates and accuracy were excellent
  - Genotyping with BeadStudio was simple, but time-consuming on a desktop computer

- HumanMethylation27 BeadChip
  - Works very well with human DNA samples
  - Produces acceptable data for NHP samples
  - BeadStudio is an excellent tool for QC and preliminary statistical analysis

Acknowledgments

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  - Jim Parina, Geoff Krummell, Noreen Contreras, Andrea Misiner
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- Methylation studies
  - Yongmei Liu, Jan Wagner, Stanton Gray, Li Zhang, Wei Cui