Improving the Utility of Microarray Technology

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Overview

- Identifying the Problem
- Solving the Problem
  - Protocol Optimisations, Modifications and Improvements
  - Manufacturing Improvements
  - Informatics
  - New Technologies

Identifying the Problem

- Despite the tremendous potential and promise of genomics technologies, and microarrays in particular, there has been disappointingly limited impact on drug discovery, diagnostics and general improvements to health care
- The root of this problem lies in both the technology and it's implementation

Lack of Standards

- Protocol, platform, design standards
  - Difficult to impose
  - Imposing standards that are too strict leads to suppression of novel approaches
  - However, lack of standards leads to poor inter-platform and inter-study comparability
- Data Standards
  - MGED (www.mged.org)

Requirements for Diagnostics and Drug Discovery

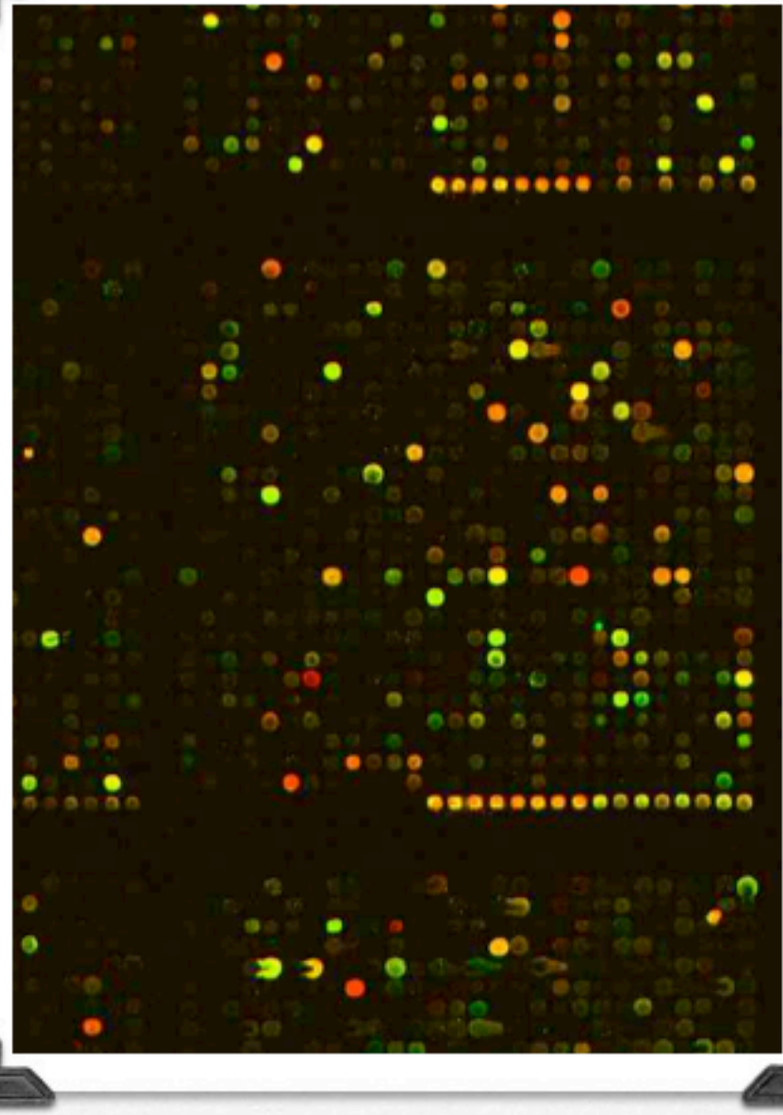
- ☐ High Sensitivity
- ☐ High Reproducibility
- ☐ Low Cost
- ☐ Ease of Use
- ☐ High Throughput

High Sensitivity & Specificity

- The ultimate goal would be to avoid amplification if possible
- Sensitivity can be increased with more "detectable" signal molecules, better hybridisation and capture efficiencies and, where necessary, signal amplification

Improving Hybridisation (Oligo Arrays)

- Traditional hybridisation:
  - DIG Easy Hyb
  - 37 °C
  - Overnight
  - Humid Chamber



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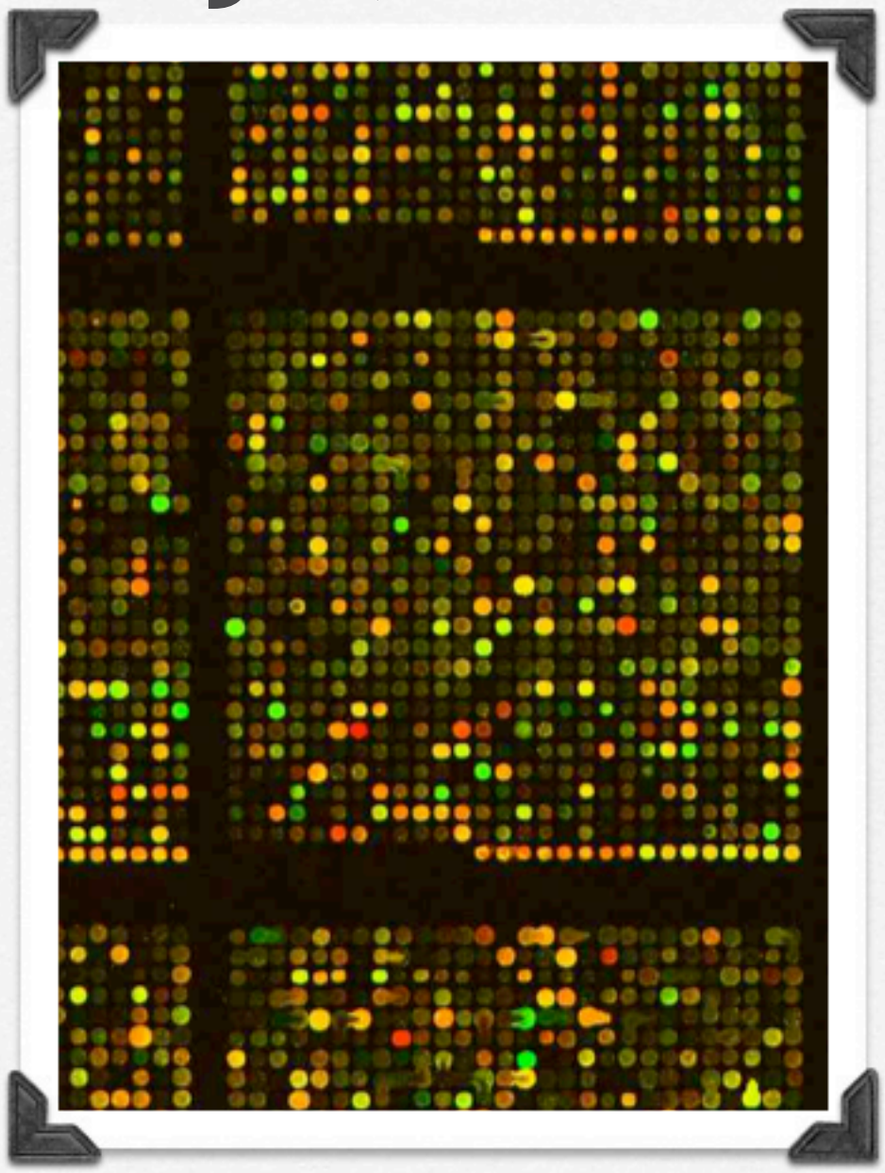
Improving Hybridisation (Oligo Arrays)

- Advalytix Slide Booster
- DIG Easy Hyb
- 37 °C
- Overnight
- Constant Agitation



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Advalytix Slide Booster

Improving Hybridisation (cDNA Arrays)

- Traditional hybridisation:
  - DIG Easy Hyb
  - 37 °C
  - Overnight
  - Humid Chamber

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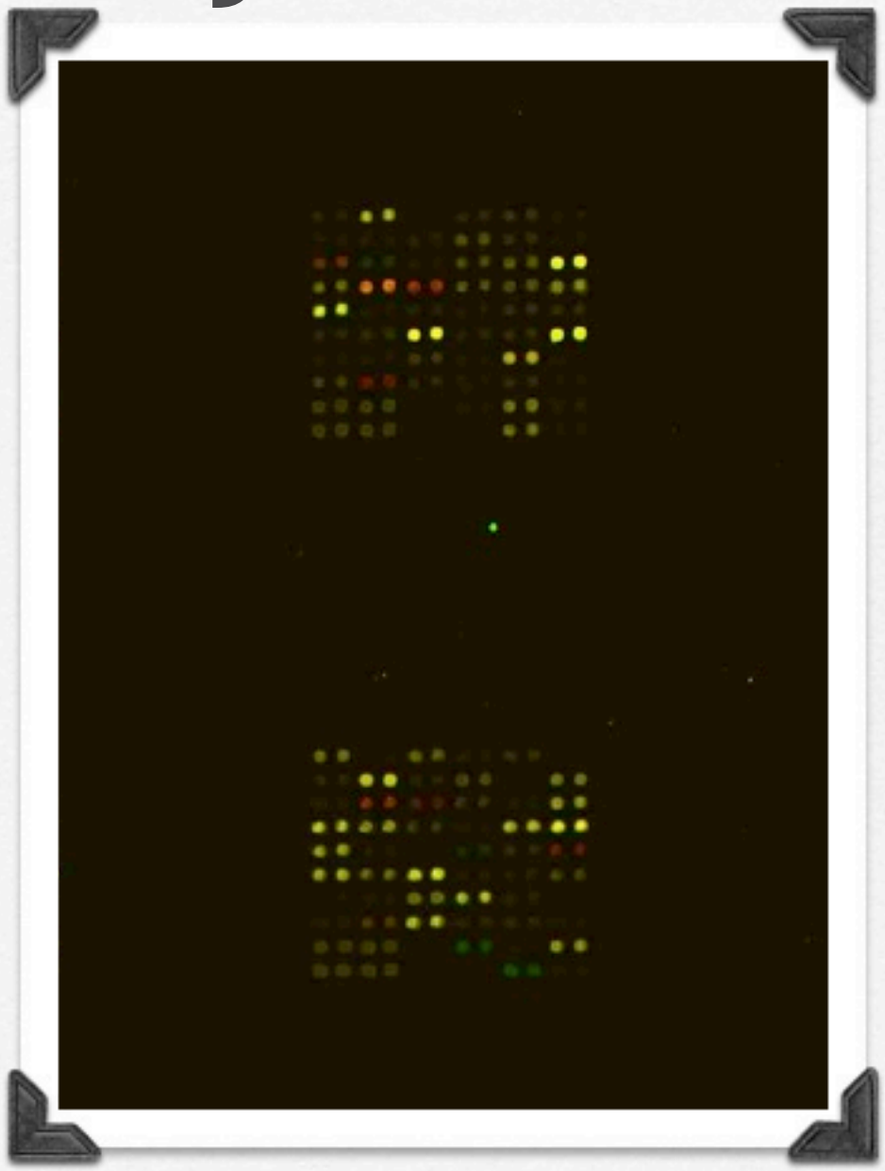
Improving Hybridisation (cDNA Arrays)

- Advalytix Slide Booster
  - Advalytix cDNA Hyb Buffer
  - 70 °C for 10 minutes
  - 42 °C Overnight
  - Constant Agitation



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If you still can't see it, you must amplify....

- Improved hybridisation, flurophores etc...have gotten us to the point where 1-2 µg of total RNA is possible for a microarray experiment.
- However, 1-2 µg represents more than 100,000 cells. For diagnostics for example, this is not possible.

Not all amplification methods are created equal

Amplification Method	Amount of Total RNA Required	Amount Used in Present Study	Time to Prepare aa- Labelled Product
Isothermal Linear Amplification	5-100 ng	20 and 100 ng	6 hours
T7-based Linear Amplification	100-2000 ng	100 and 500 ng	11-19 hours
PCR Based Amplification	20-1000 ng	100 ng	8 hours
Global RT-PCR	As low as 10 pg	0.2 and 20 ng	10 hours

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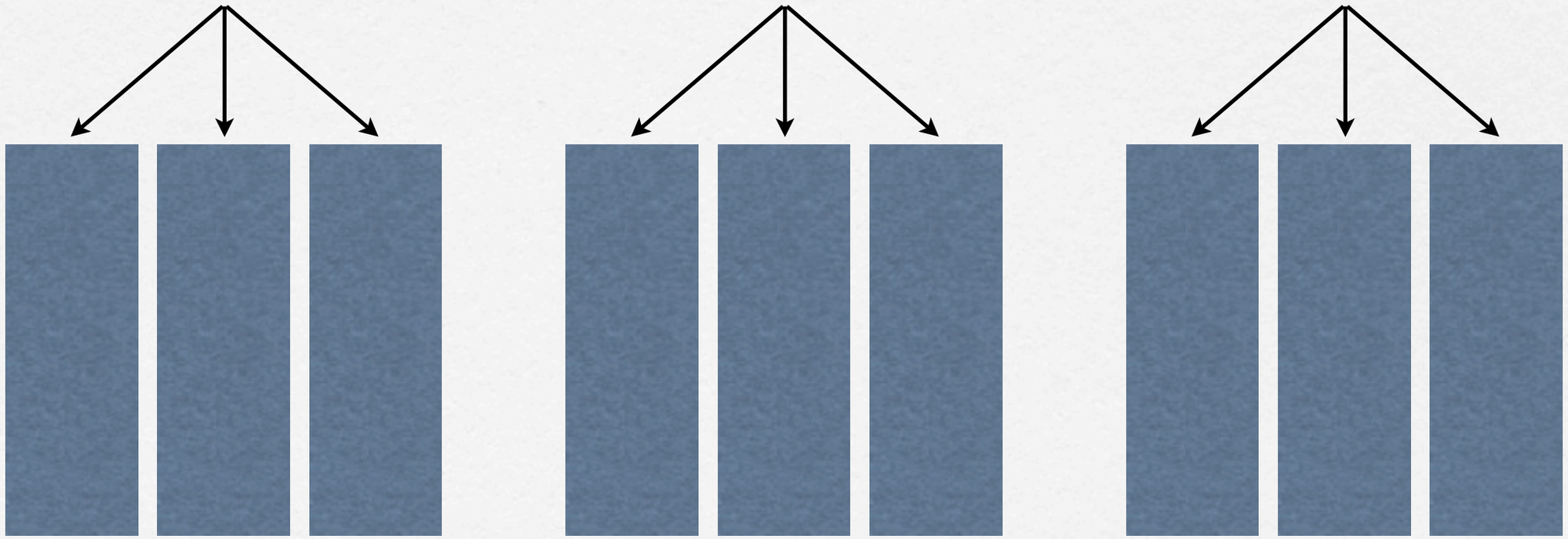
Experimental Design - Approach 1

x ng of total RNA
amplified UHRR
v.
amplified HeLa RNA

y ng of total RNA
amplified UHRR
v.
amplified HeLa RNA

10 µg of total RNA
non-amplified UHRR
v.
non-amplified HeLa RNA



Human 19k cDNA Arrays

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Analysis Schema

One Class SAM Analysis on all non-amplyfied hybridisations
(15 replicates, 3 per day, 5 days)

↓

8933 Significantly different genes

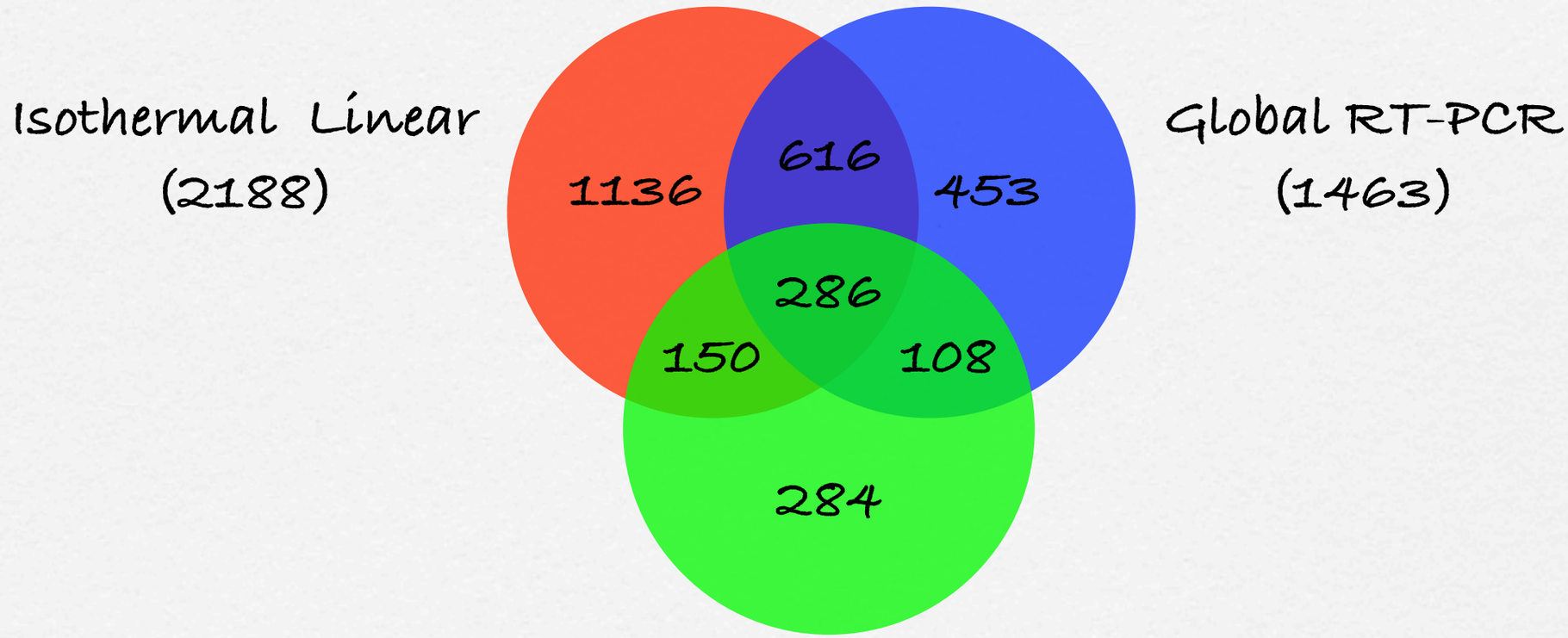
↓

Filter each amplified data set for the 8933 genes

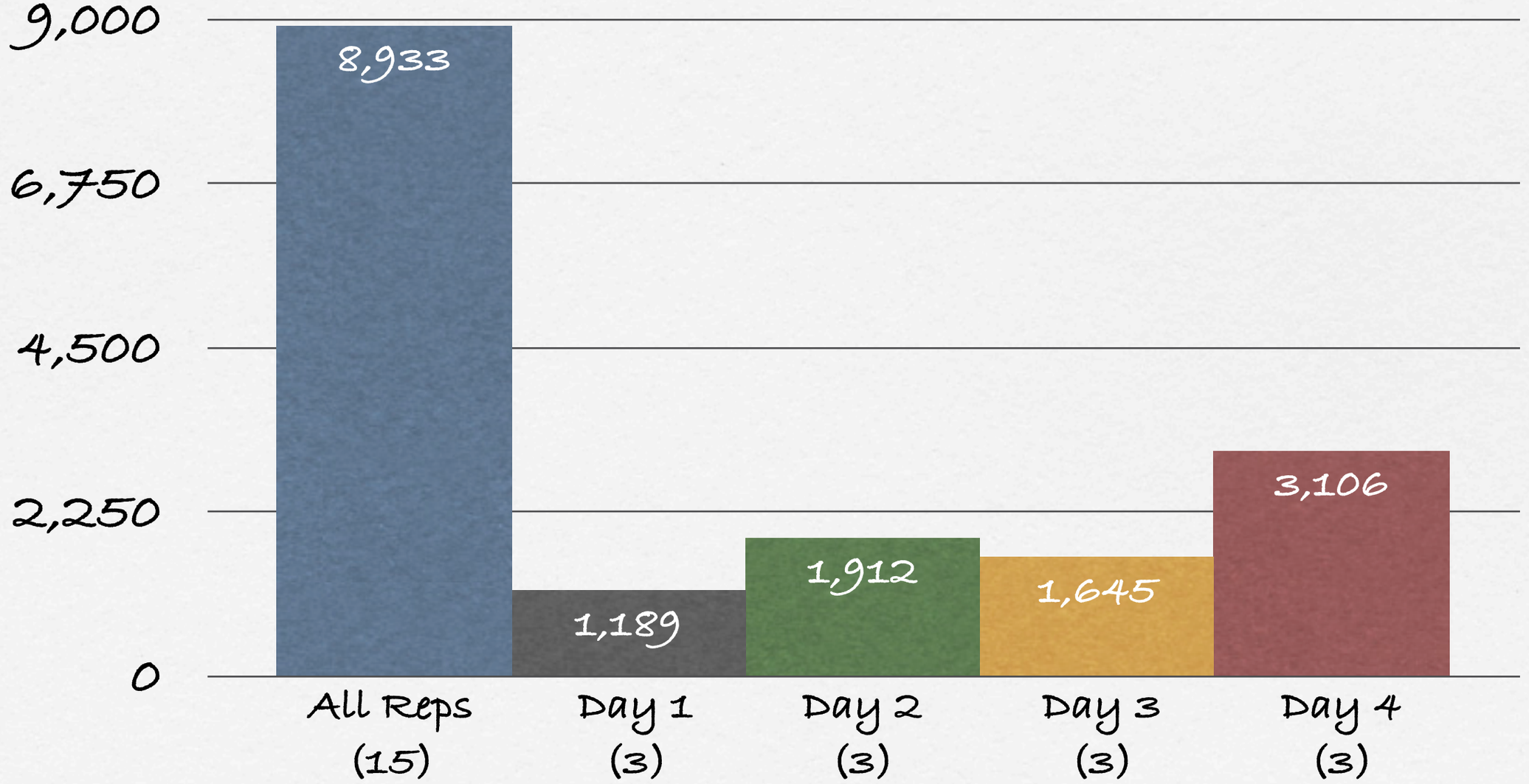
↓

Perform one class SAM for each amplification
(3 replicates each)

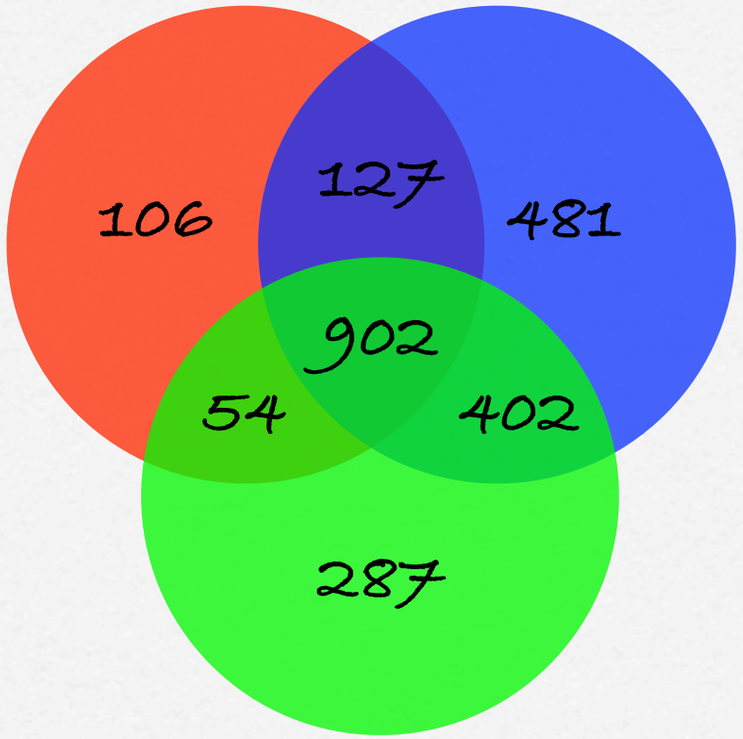
Comparison of Significant Genes By Amp Method



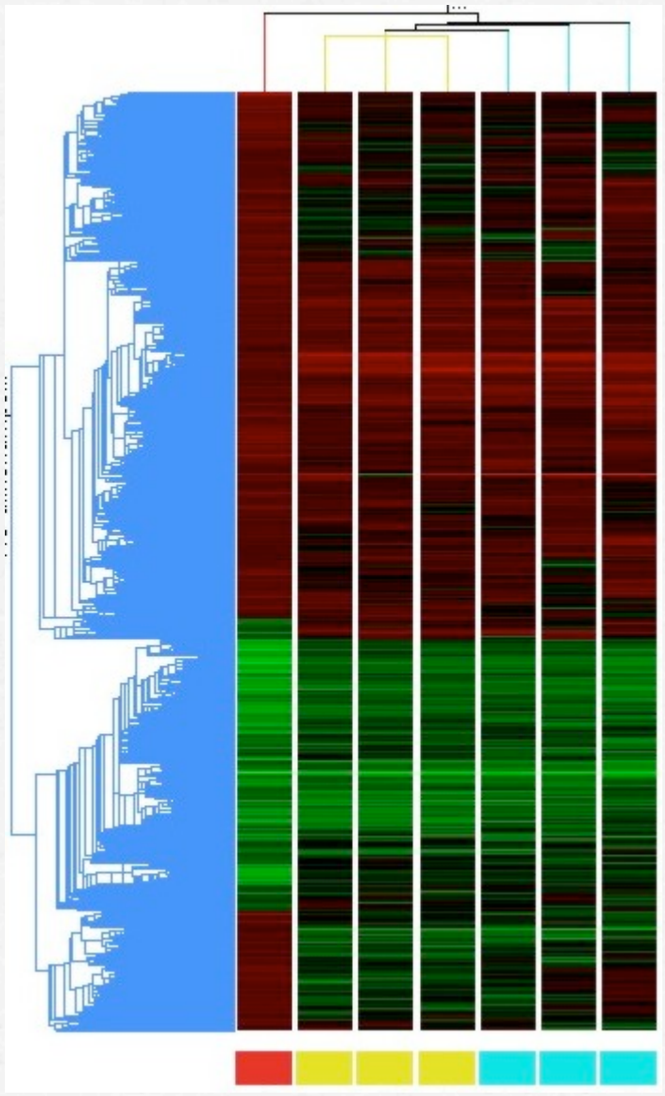
Affect of Sample Size on SAM Algorithm



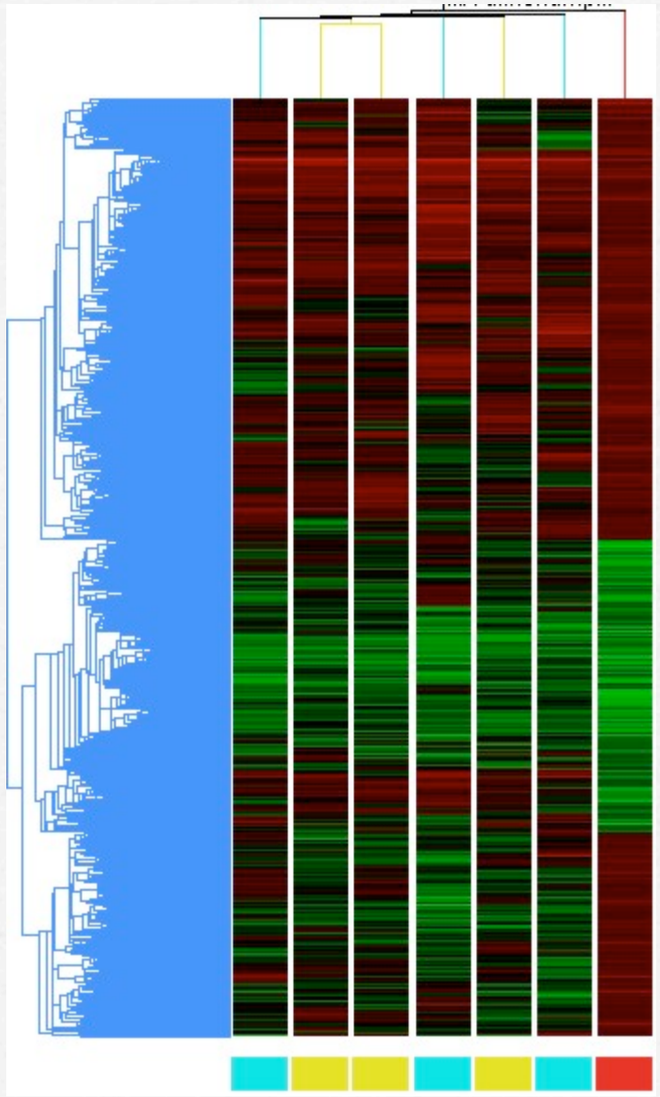
Significant Genes For Non-Amplification By Day



Assessing Reproducibility



Isothermal



T7-based

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Comparing Gene Expression to Protein Expression

- Often times - a change on a gene expression microarray does not indicate an actual change in protein expression
- A lack of a change on a gene expression microarray does not necessarily indicate a lack of change in protein activity
- Assaying protein expression globally is non-trivial

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Causes of Gene and Protein Expression Discordance

- Protein function does not need to be regulated at the level of expression - much of protein activity is controlled post-translationally
- Microarrays measure steady state RNA levels - and thus a change in "expression" can be either an actual change in the amount of new transcription OR a change in RNA stability

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Assaying *de novo* transcription with microarrays

- Ideal would be for a microarray based nuclear run-on
  - has been demonstrated using radioactivity but not fluorescence
- Alternative is transient labelling of nascent transcripts

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Measuring *de novo* Transcription

- Cells are "doped" with modified nucleotides
- Post doping, cells are allowed to recover for a short period of time
- Cells are treated, and then harvested
- Labelled transcripts are then hybridised to an array

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Arrays to measure protein expression

- Antibody arrays are the protein "analogue" of a cDNA/oligo array
  - The largest issues are obtaining enough high quality validated antibodies to print and keeping the antibodies functional
- Reverse phase arrays measure a few (1-10) analytes at a time in many lysates
  - Typical multiplexing by fluors allows 2 colours

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Arrays to Measure DNA-Protein Interaction

- CpG and Regulatory Region Arrays
- ChIP on Chip and Protein Binding Microarrays
- Measures differential control of gene expression by specific transcription factors

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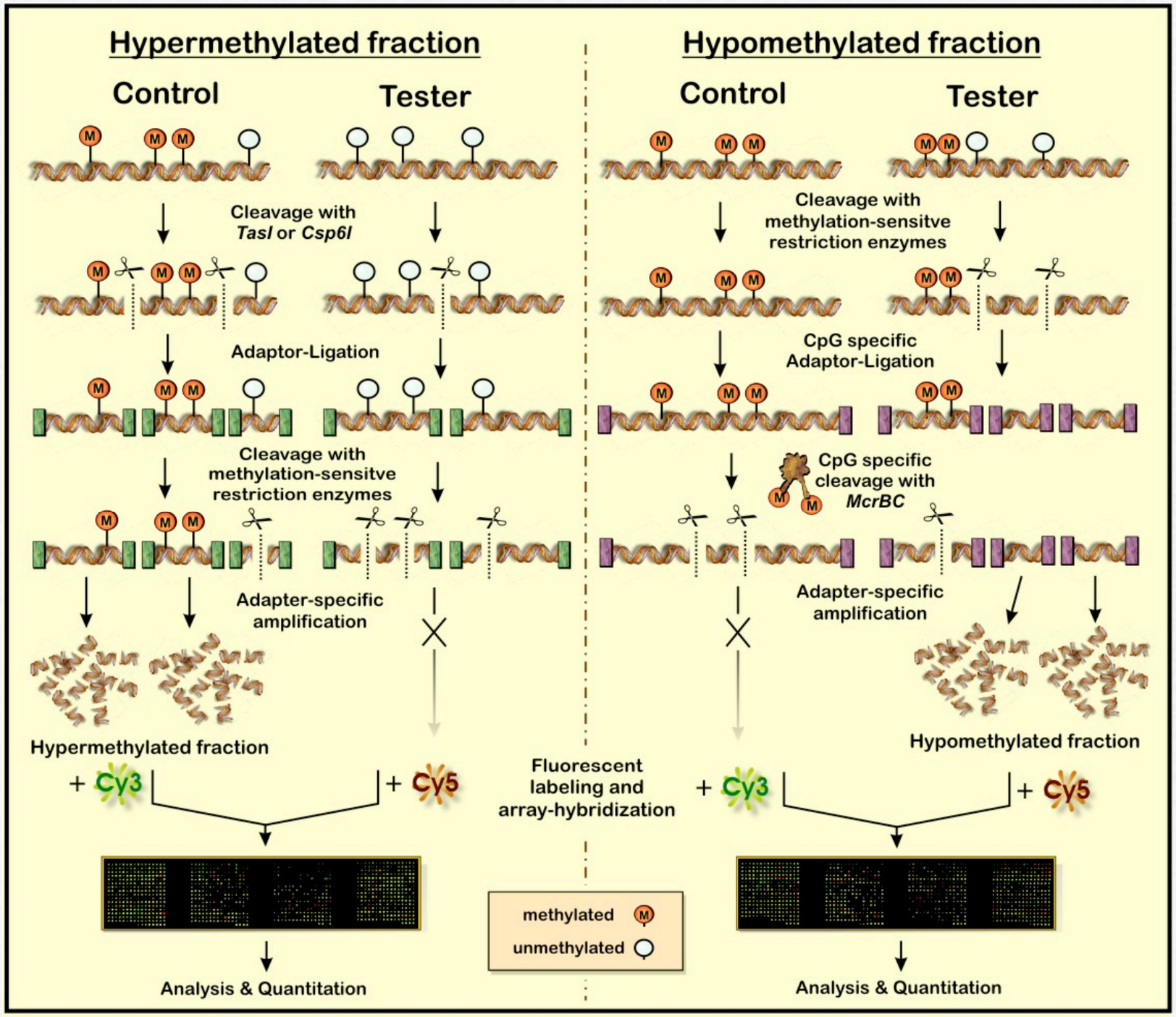
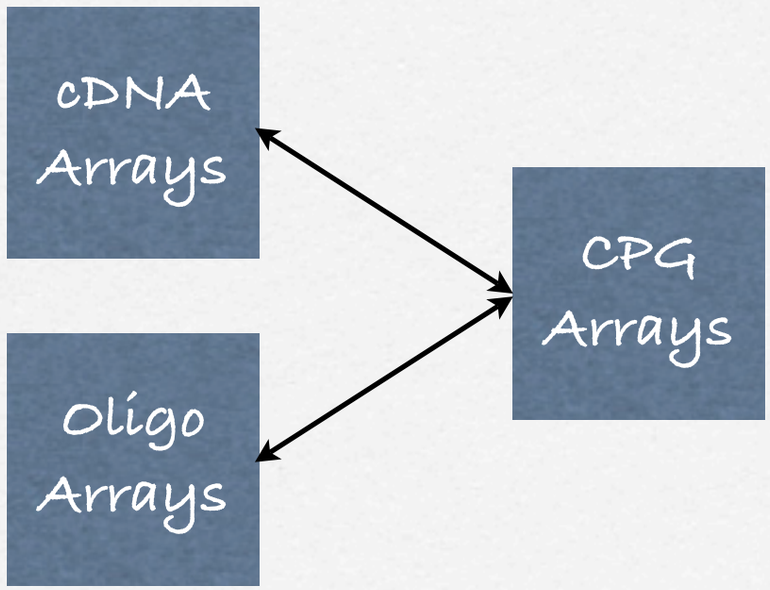
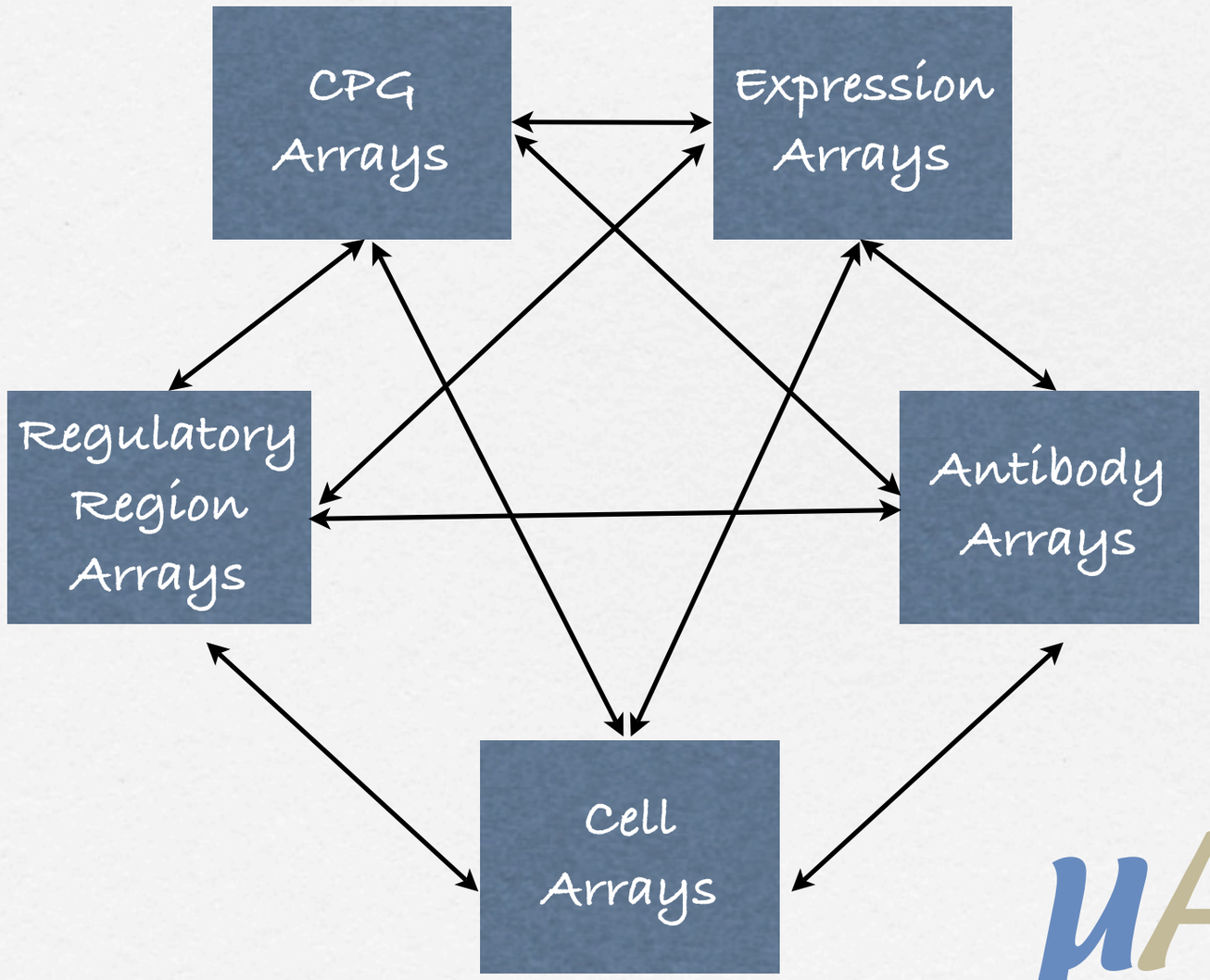
Epigenetics - Methylation



Integrated Array Design





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