

Measuring the Potency of Dendritic Cell Preparations Using Transcriptional Analysis

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Presenter Disclosure Information

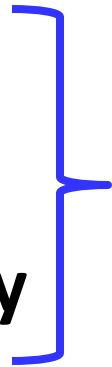
David Stroncek

The following relationships exist related to this presentation:

No Relationships to disclose

Quality Assessment of Cellular Therapies

- Identity
- Purity
- Sterility
- Potency
- Stability
- Comparability



Require biomarker(s)
reflecting critical biological
function(s)

Potency biomarkers are the cornerstone of quality assessment

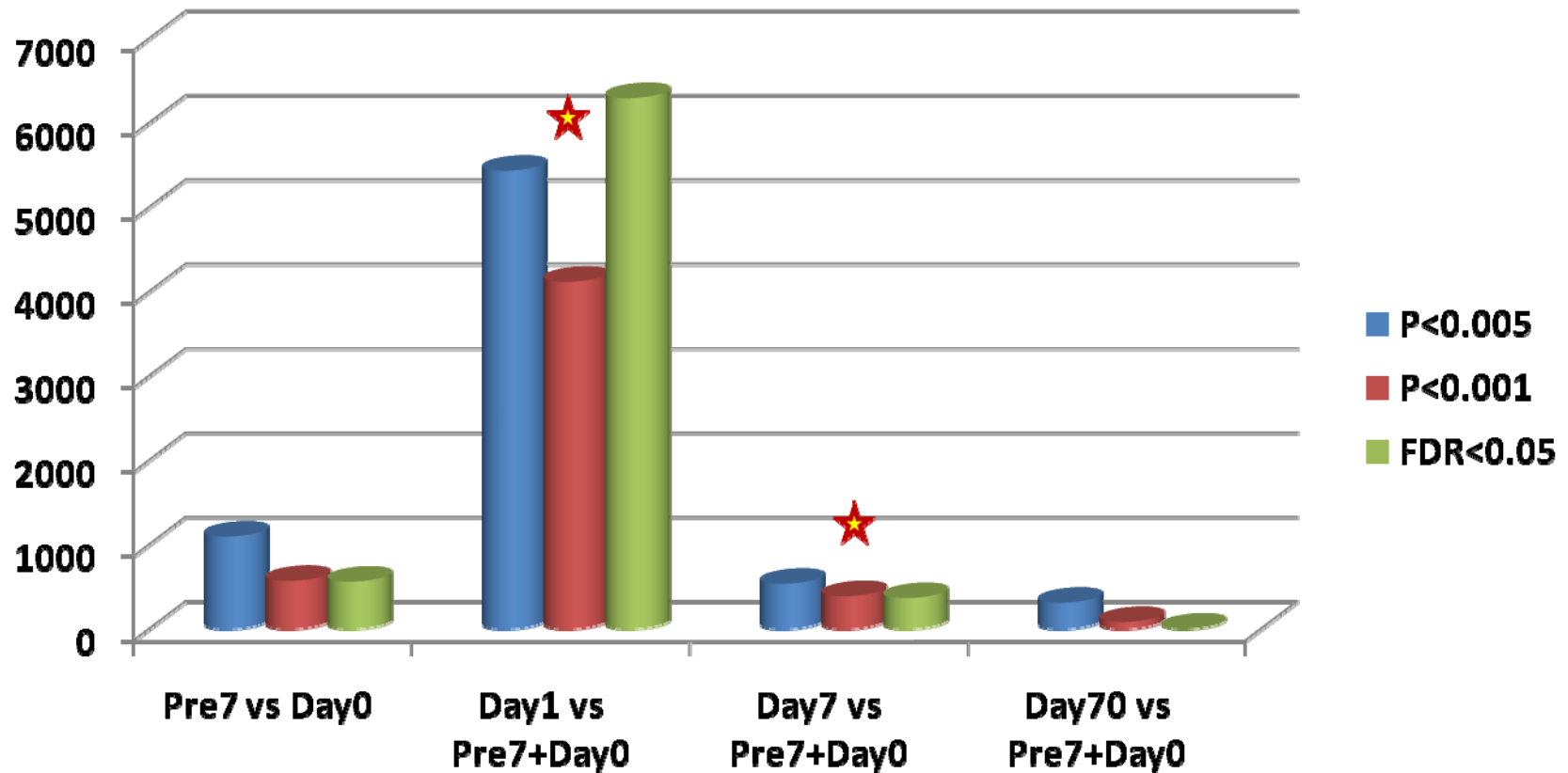
Biomarker Assessment: Sources and Degree of Variability

Sources of Variability

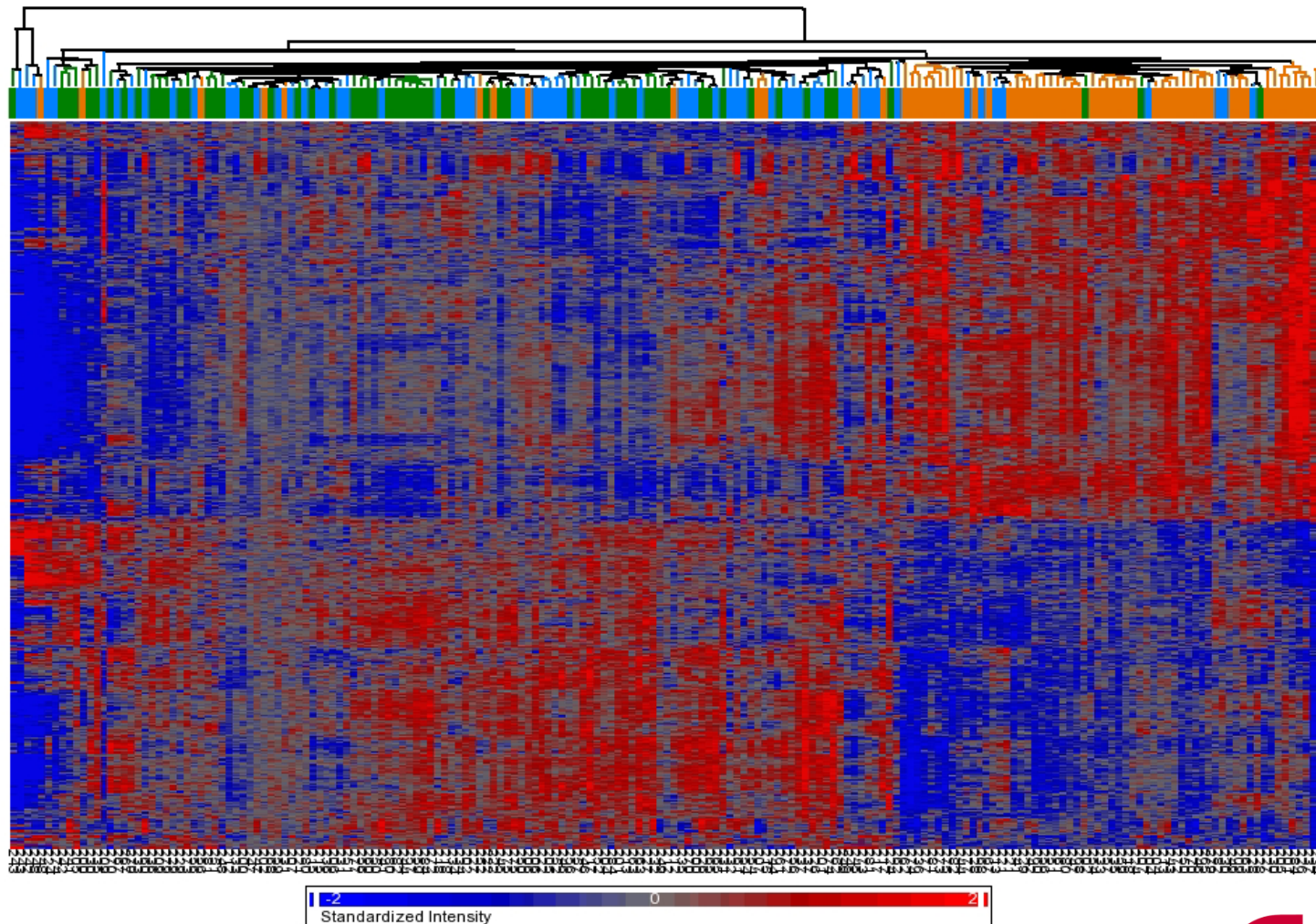
- **Assay variability**
- **Changes in donors from day to day**
- **Inherent differences among donors**
- **Variability in manufacturing**

Seasonal and H1N1 Vaccine Induced Gene Expression Changes

CHI protocol 09-H-0239 design (*Olnes*)



Visualize differentially expressed genes on **day1** by Hierarchical cluster
(Day1/Pre7+day0, $p < 0.001$. 4114 genes)



Day1

Pre 7

Day0

Biomarkers for New Cellular Therapies:

Identification of Biomarkers

Consistency (technical validation)

- **Method validation (Assays)**
- **Process validation (Manufacturing)**

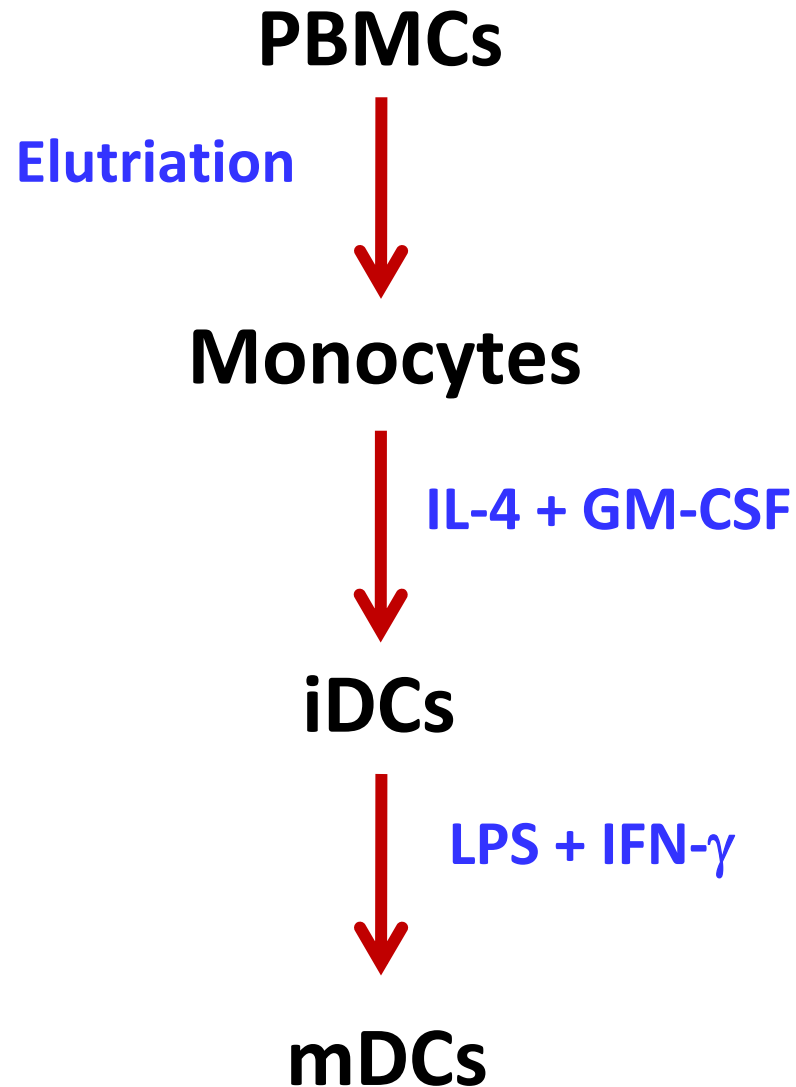
Biological Variability

- **Inter-individual – genetic, epigenetic, clinical condition**
- **Intra-Individual – changes over time, state of health**

Potency Biomarkers

- **Ability to discriminate between a biologically active and inactive product**
- **Minimal assay variability**
- **Reflect manufacturing and individual variability**

Production of iDCs and mDCs



Goal: Identify Biomarkers for Assessing mDCs

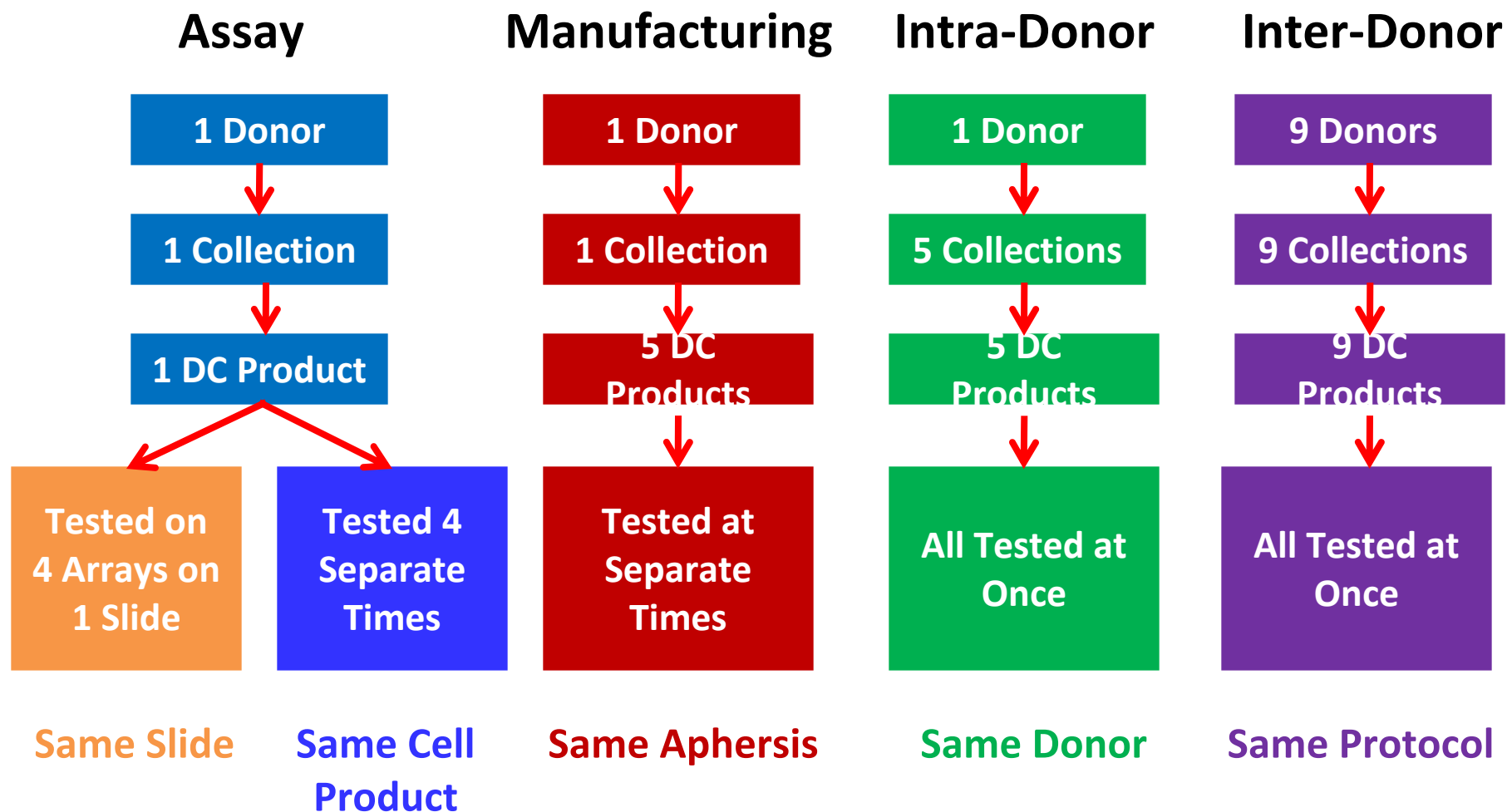
Flow Cytometry Analysis of DCs from 9 Healthy Subjects: Traditional biomarkers are useful for purity but not functional analysis

Donor	Viability (%)	CD86+ (%)	CD83+ (%)	CD80+ (%)	HLA-DR+ (%)	CD123+ (%)	CD11c+ (%)	CD209+ (%)	CCR7+ (%)	CD40+ (%)	CD54+ (%)
340	78	99.6	99.4	96.8	89	99.1	85.5	68.1	83.3	97.5	98.3
882	84	99.5	99.9	99.1	97.6	100	81.3	87.7	92	99.3	99.2
172	79	98.5	99.5	83.8	89.4	99.8	64.8	60.5	90.5	92.9	94.7
892	67	99.8	99.7	92.2	99.7	99.7	98.1	85.8	58.3	99.9	99.9
852	91	99.9	98.7	97.8	99.1	100	98.4	90	61	99.9	99.9
249	66	99.5	99.6	94.8	99.5	99.9	95.9	68.3	37.3	99.4	99.9
717	89	99.8	99.9	99.2	99.6	100	98.2	76.3	54.8	99.6	99.9
910	74	99.2	*	*	99.5	*	98.9	93	*	99.1	83.8
085	79	98.8	*	97	98.3	*	98.7	93.8	*	98.8	99.3

All the marker are consistent, only CD11c CD209 and CCR7 show inter-donor differences

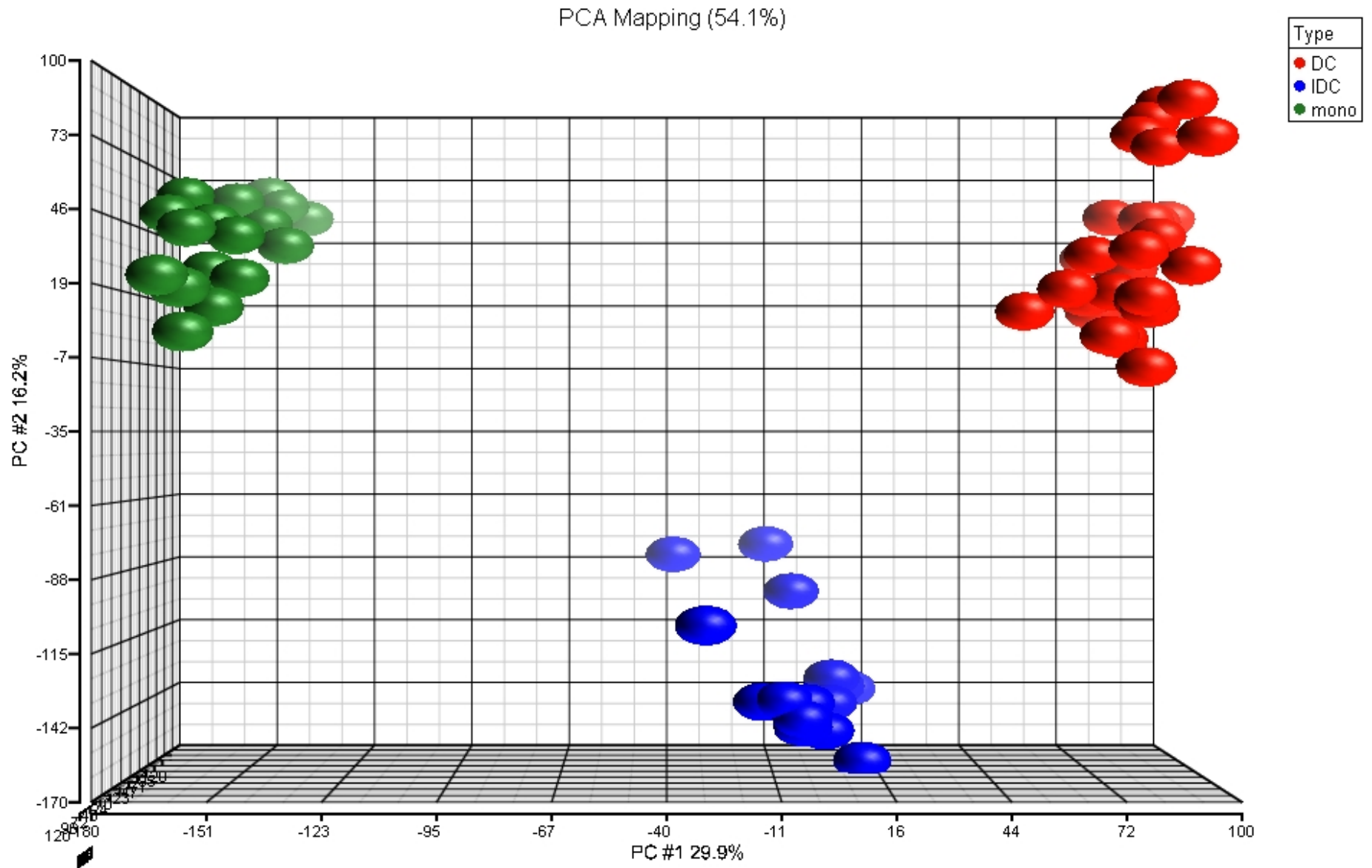
* Denotes technical problem

Assessing DC Biomarker Measurement Consistency

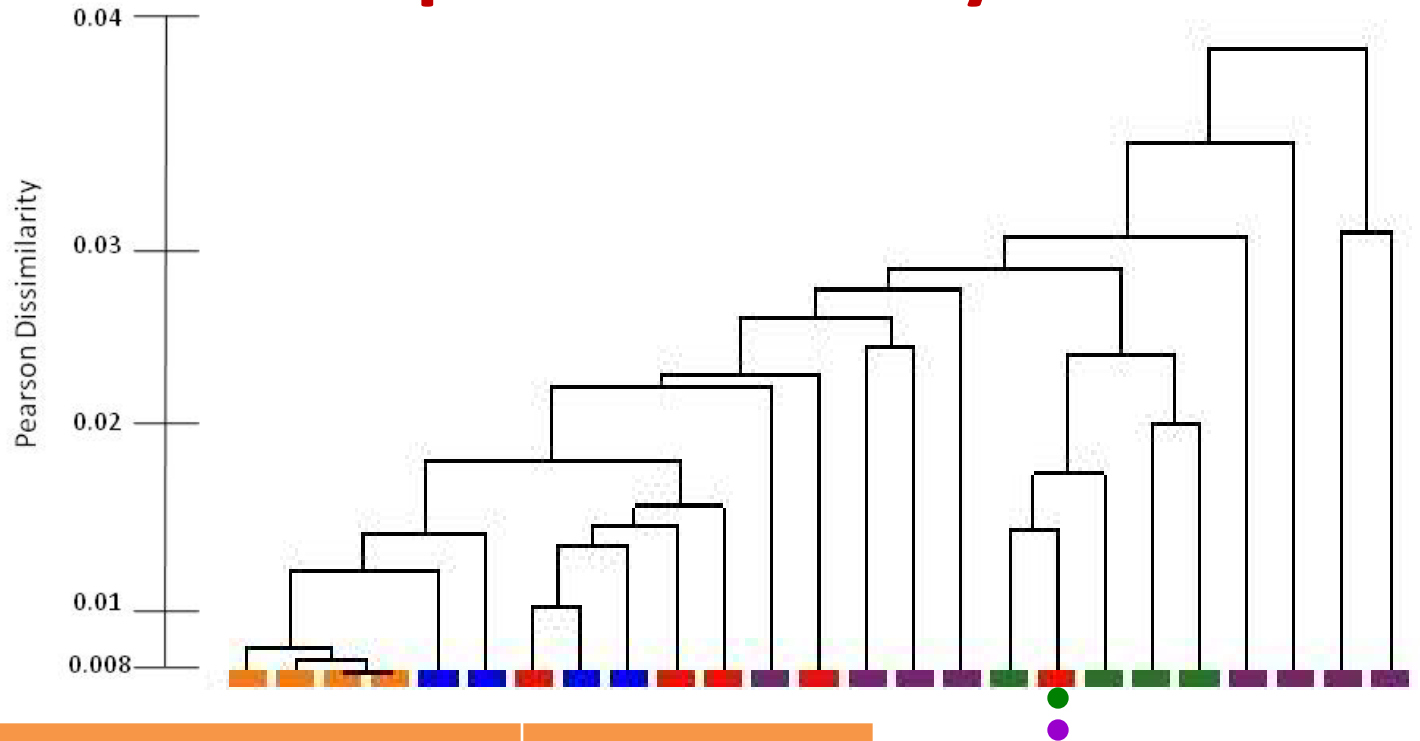


Samples assessed with Agilent 44k oligonucleotide microarrays

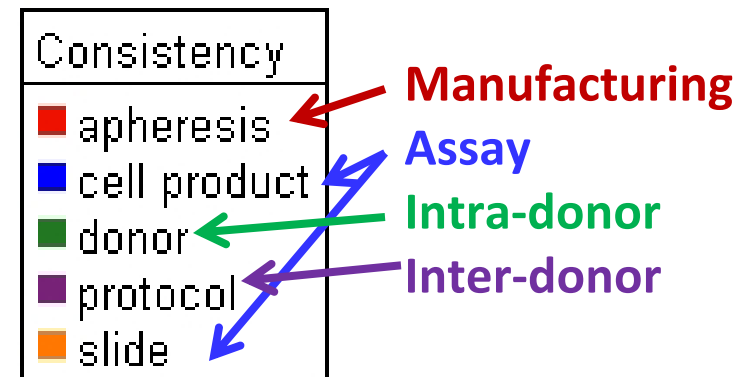
Global Transcriptome Analysis of Monocytes ●, iDCs● and mDCs●



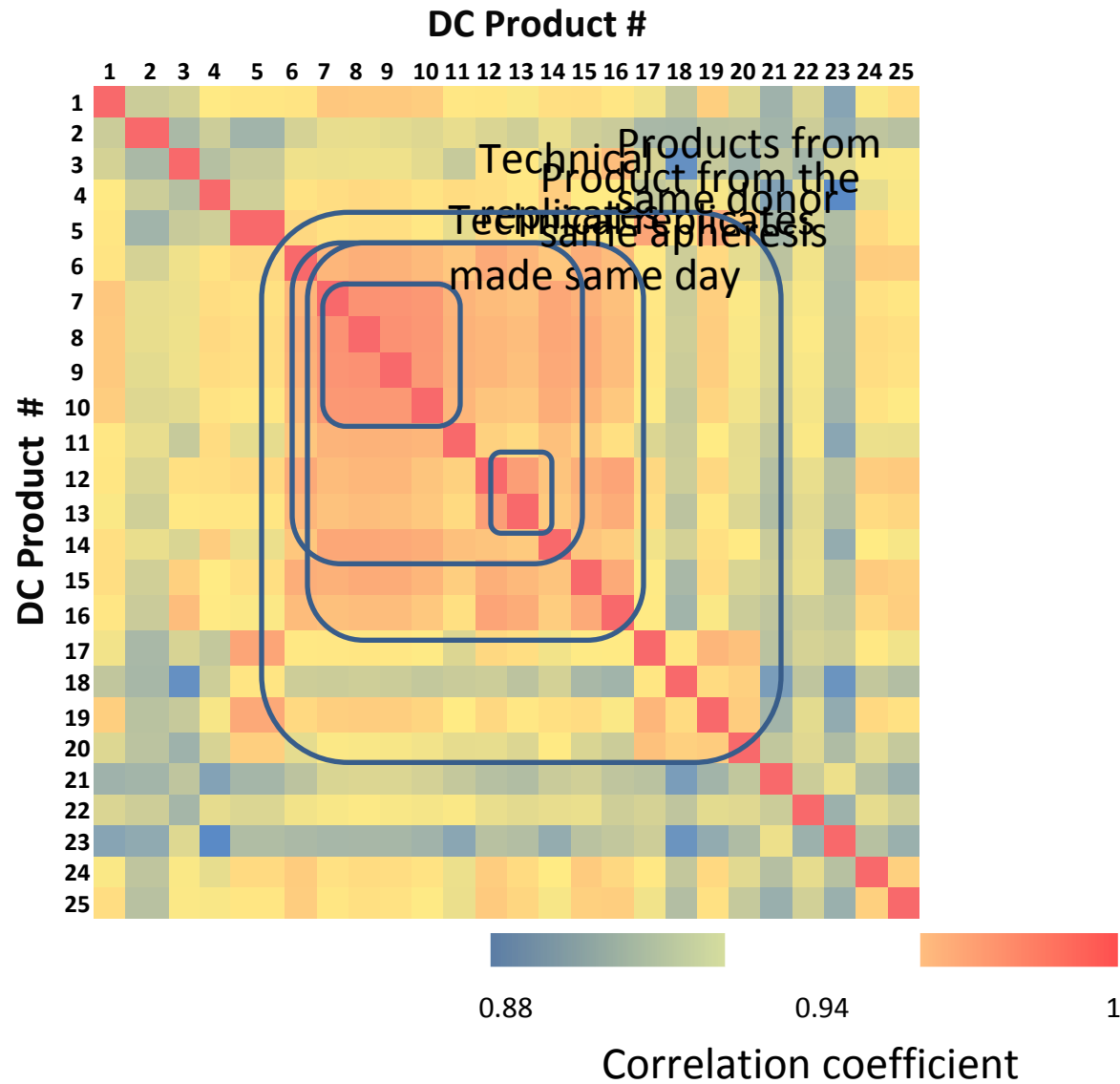
Gene Expression Analysis of mDCs



	Intra-class Correlation
Slide (Within Assay)	0.9821
Cell product (Between Assay)	0.9626
Apheresis (Manufacturing)	0.9477
Donor (Intra-donor)	0.9466
Protocol (Inter-donor)	0.9245



Similarity Matrix: Comparison of 25 DCs



DC Genes Differently Expressed Compared to Monocytes and iDCs

Genes specifically up-regulated in mature DCs versus monocytes and iDCs

Class comparison DC vs monocytes (0.001)

N=9

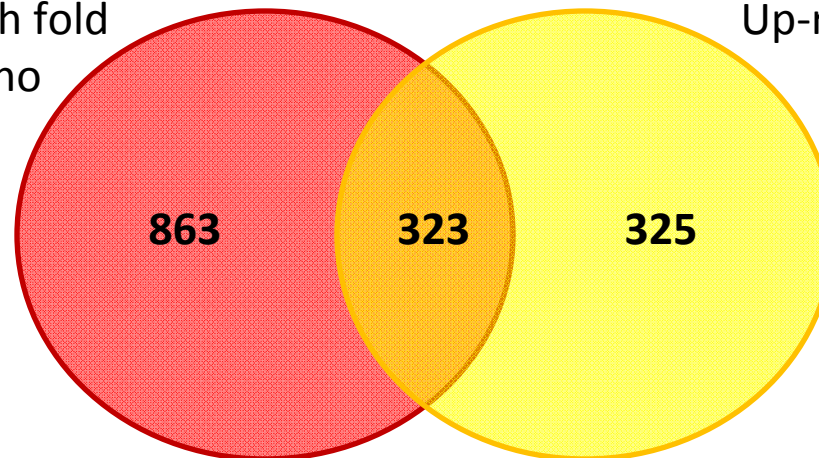
- 14082 genes
- 6902 with greater expression in DCs
- 1186 change more than 5-fold

Class comparison DC vs iDC (0.001)

N=5

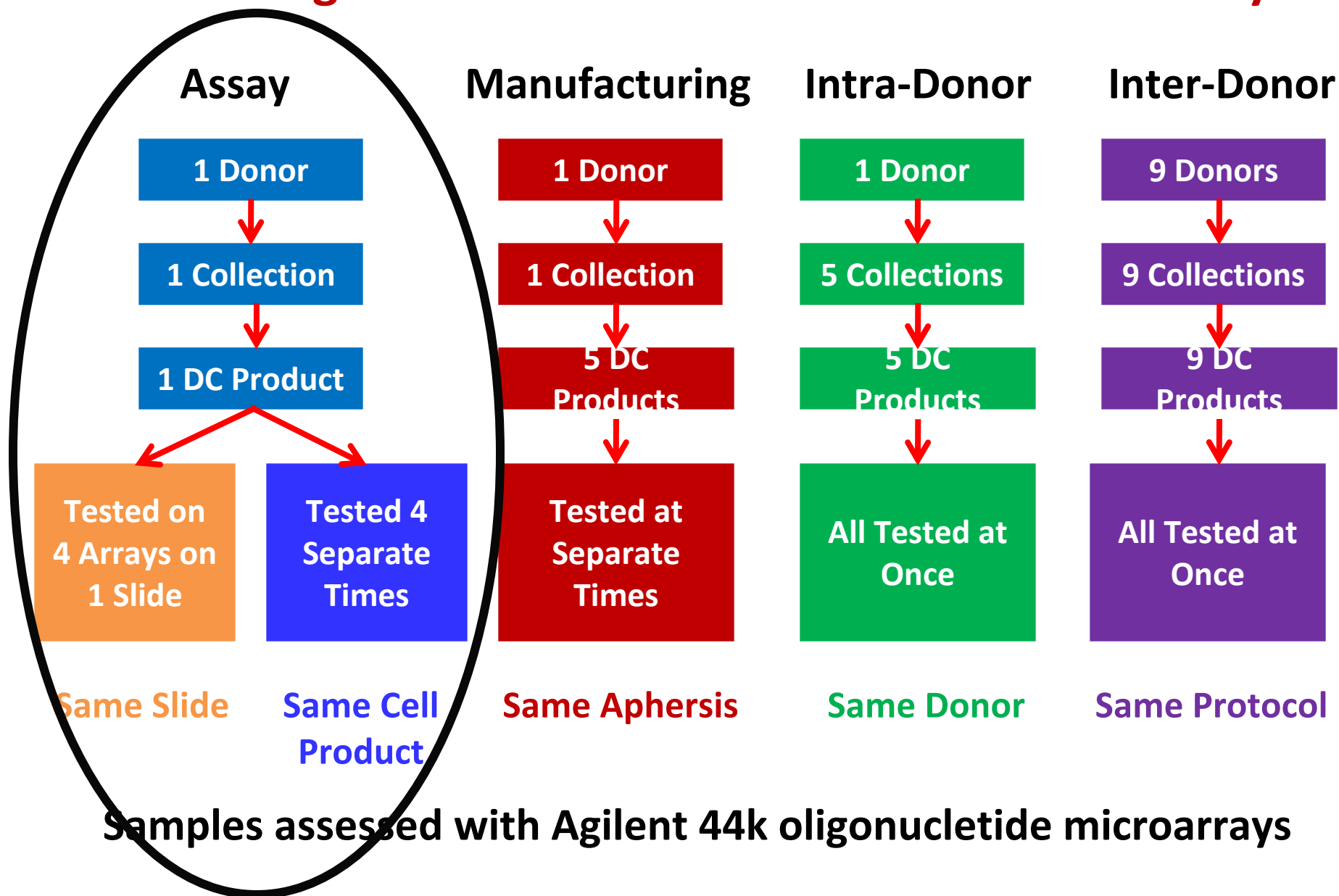
- 7403 genes
- 3410 with greater expression DC
- 648 changed more than 5-fold

Up-regulated genes with fold change > 5 in DC vs mono



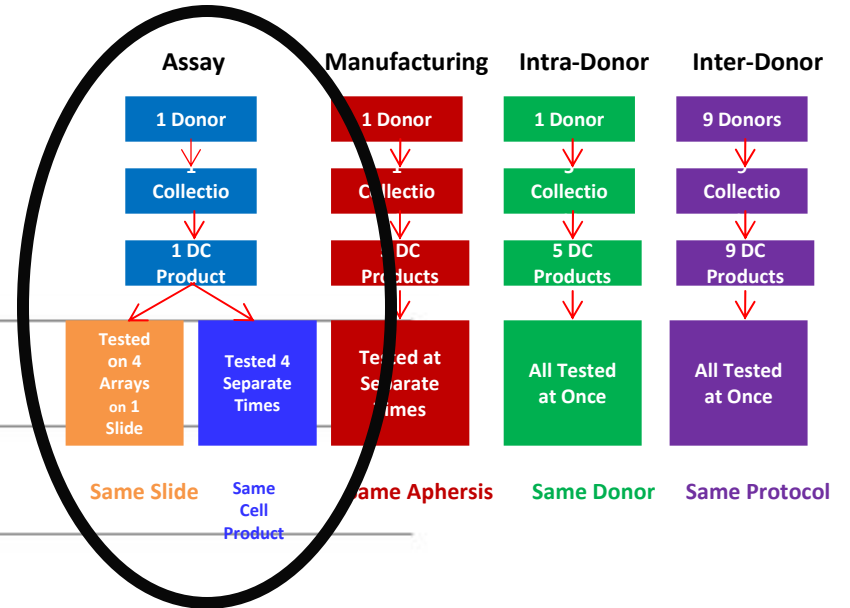
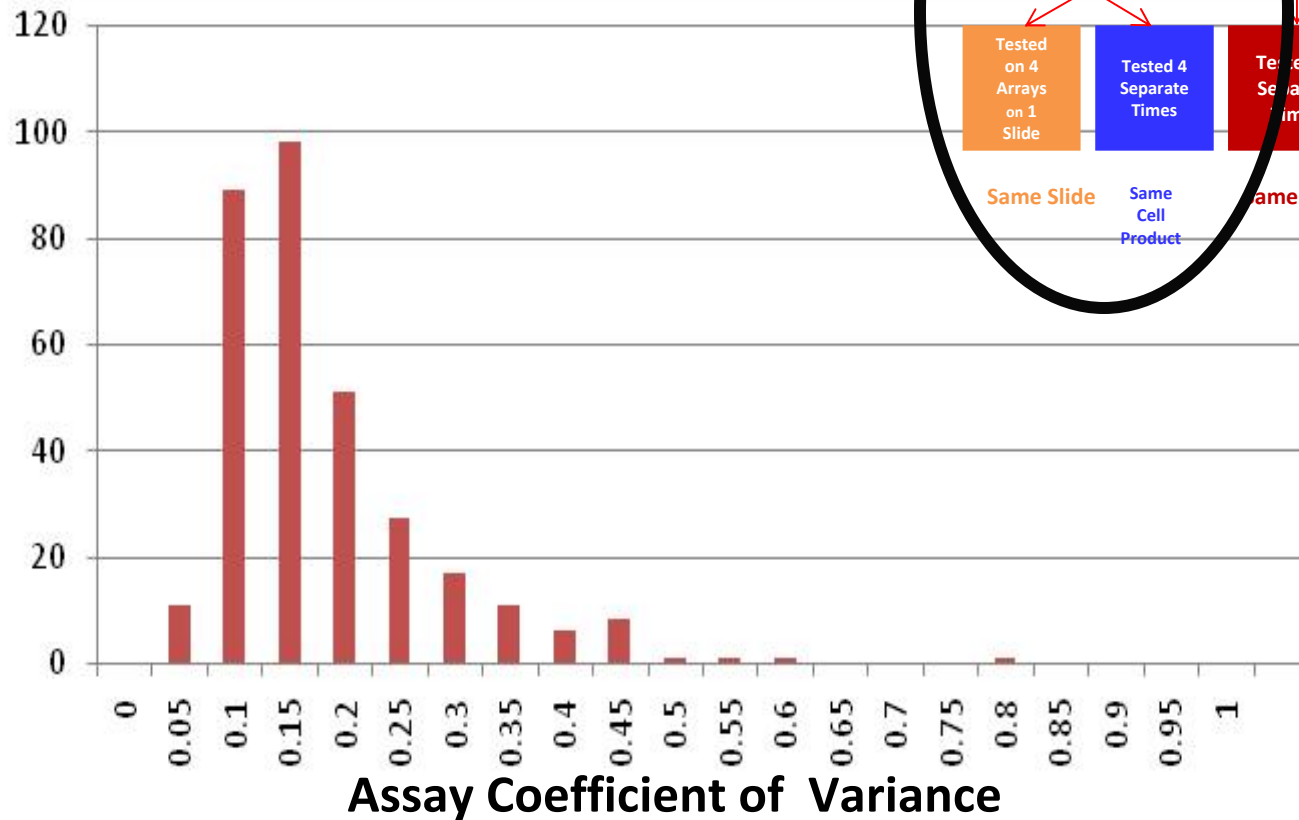
Up-regulated genes with fold change > 5 in DC vs iDC

Assessing DC Biomarker Measurement Consistency



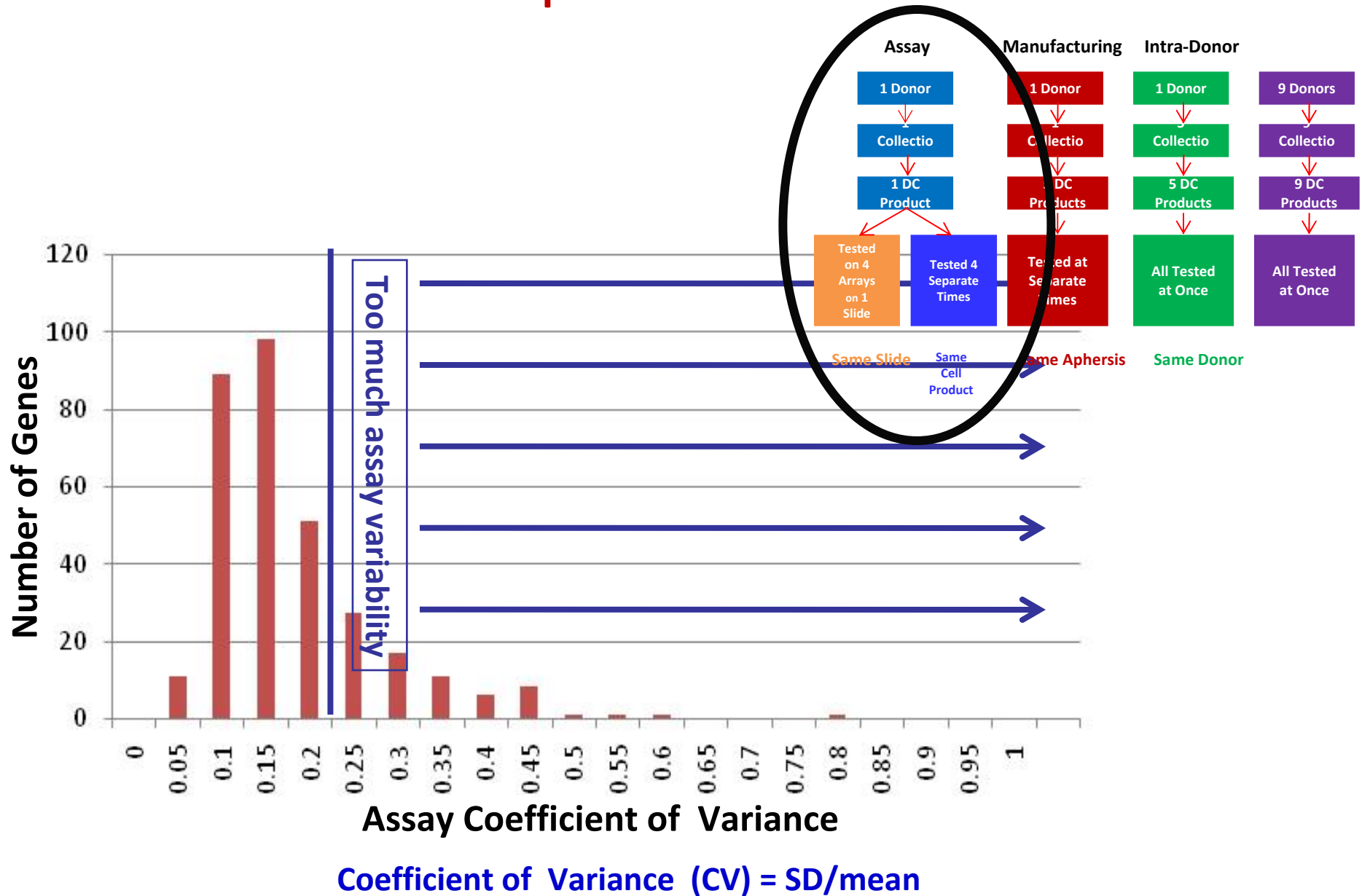
Assay Coefficient of Variance (CV) for 323 Differentially Expressed DC Genes

Number of Genes

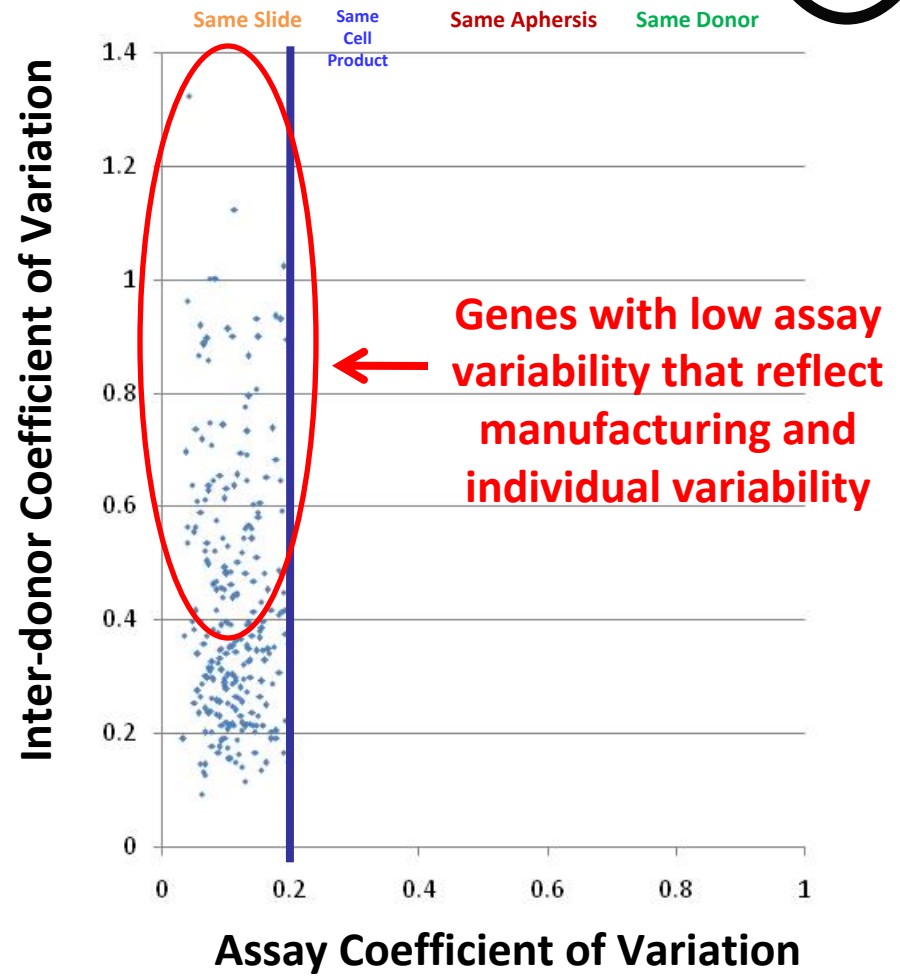
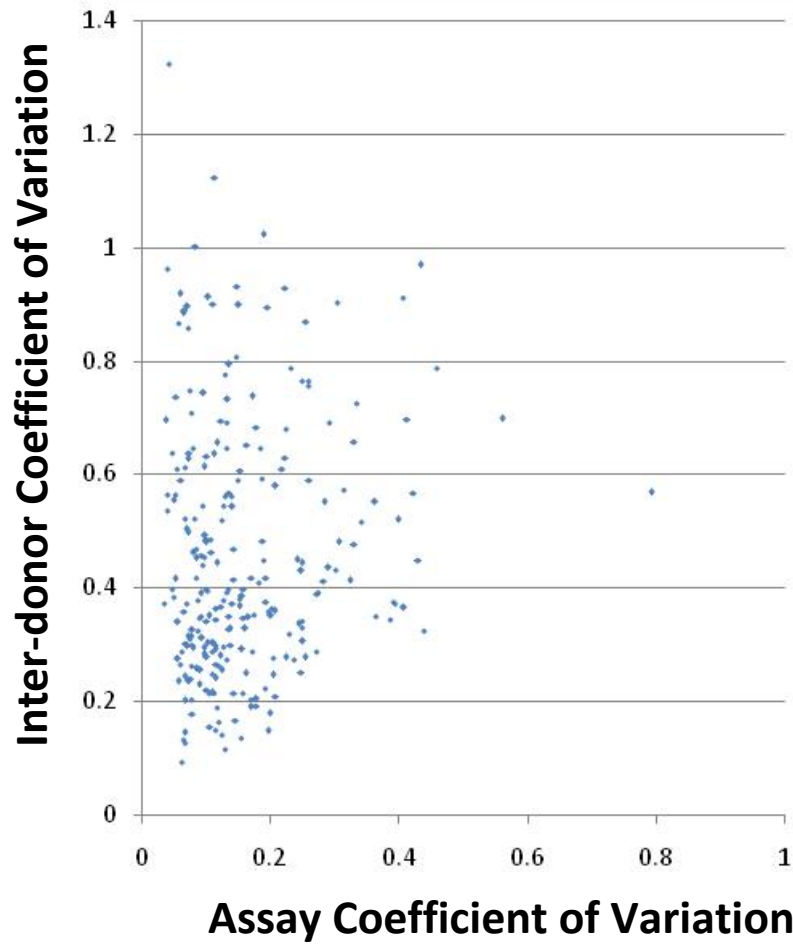
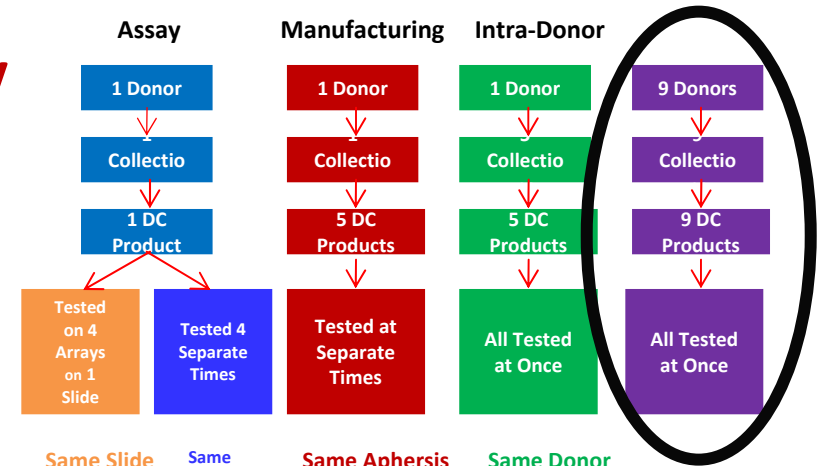


Coefficient of Variance (CV) = SD/mean

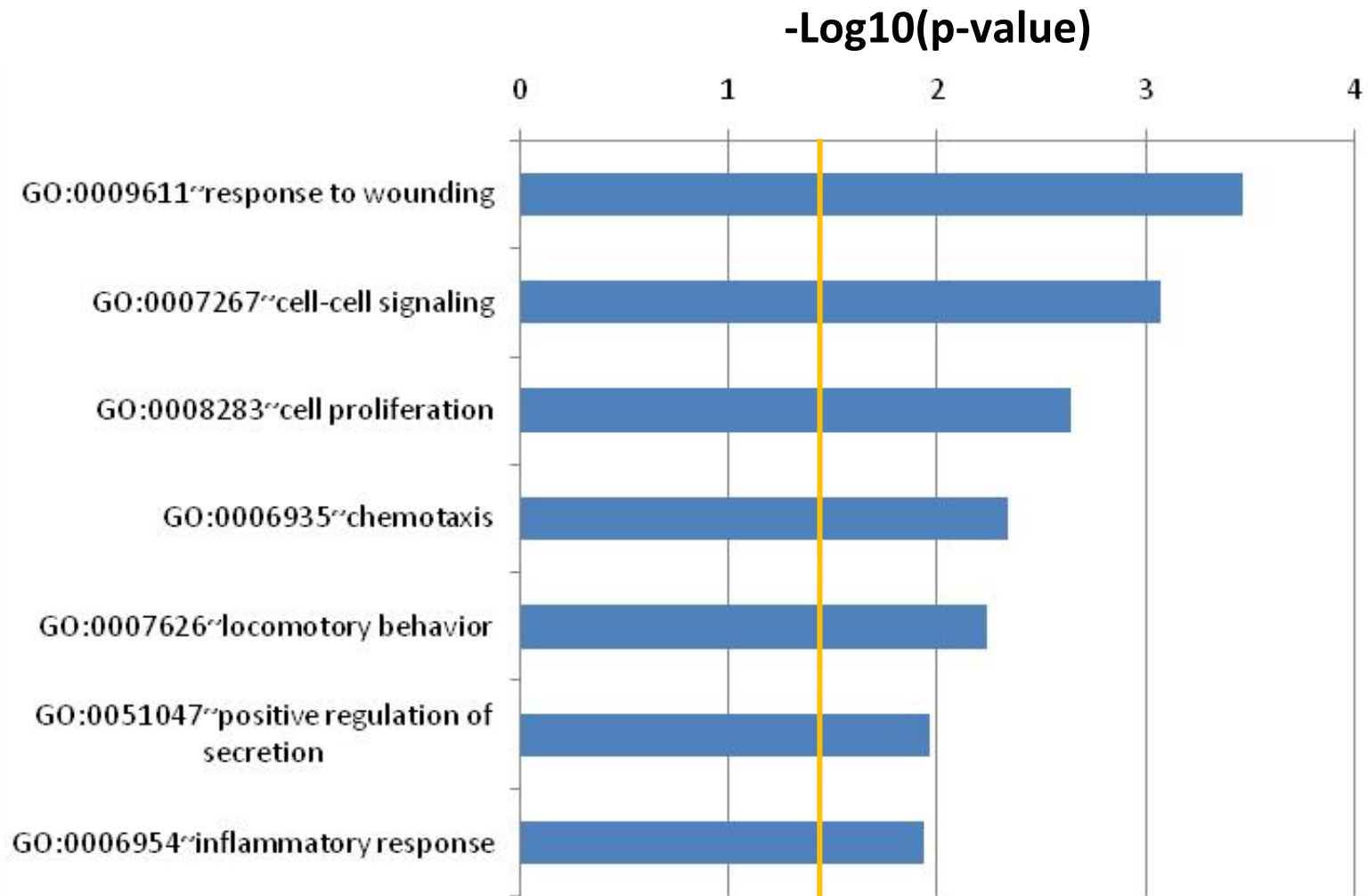
Assay Coefficient of Variance (CV) for 323 Differentially Expressed DC Genes



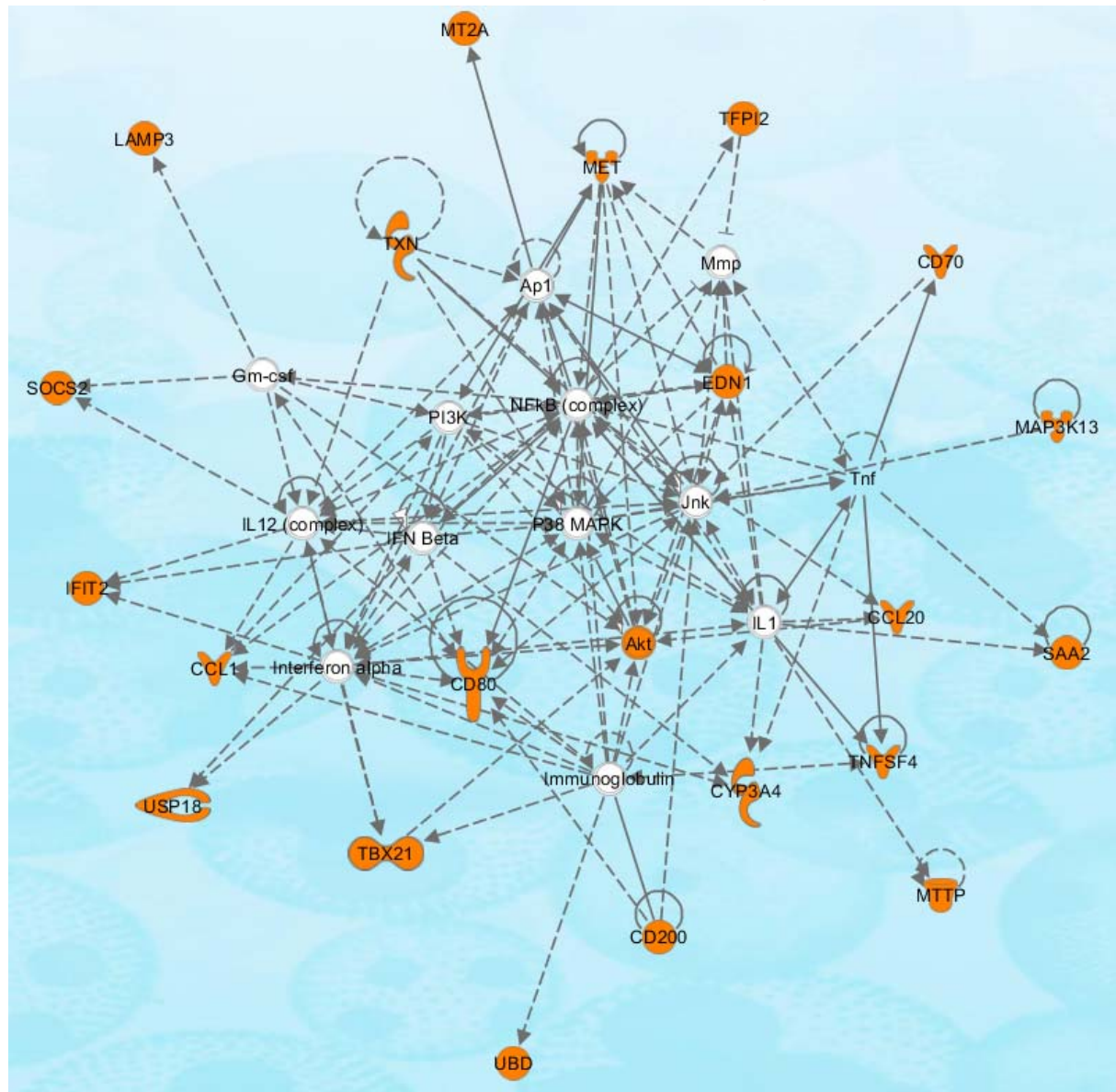
Inter-Donor Gene Expression Variability Among 323 Differentially Expressed DC Genes : Comparison with Assay Variability



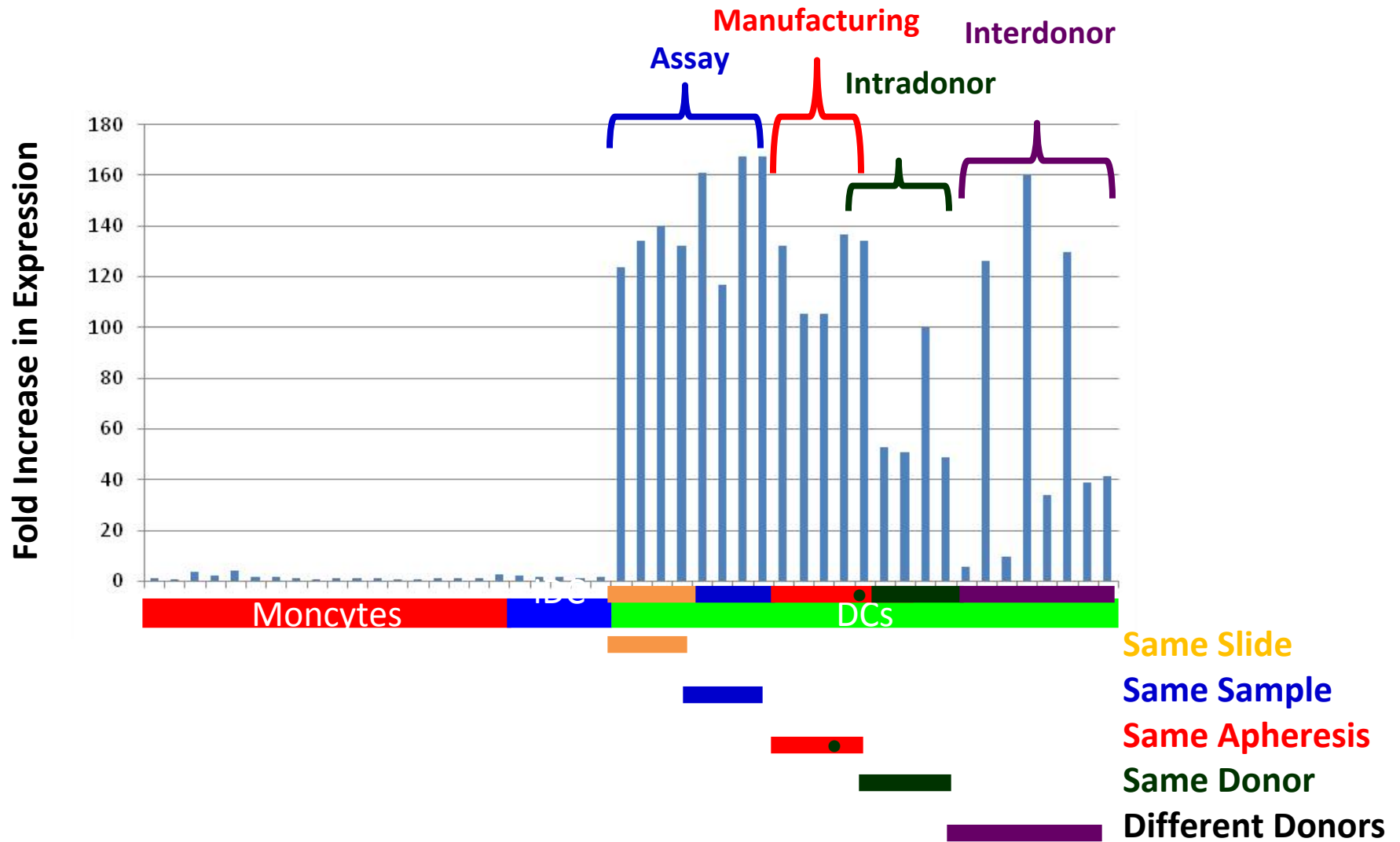
Differentially Expressed DC Genes with High Inter-Donor but Low Assay Variability (n=106): Gene Ontology Analysis



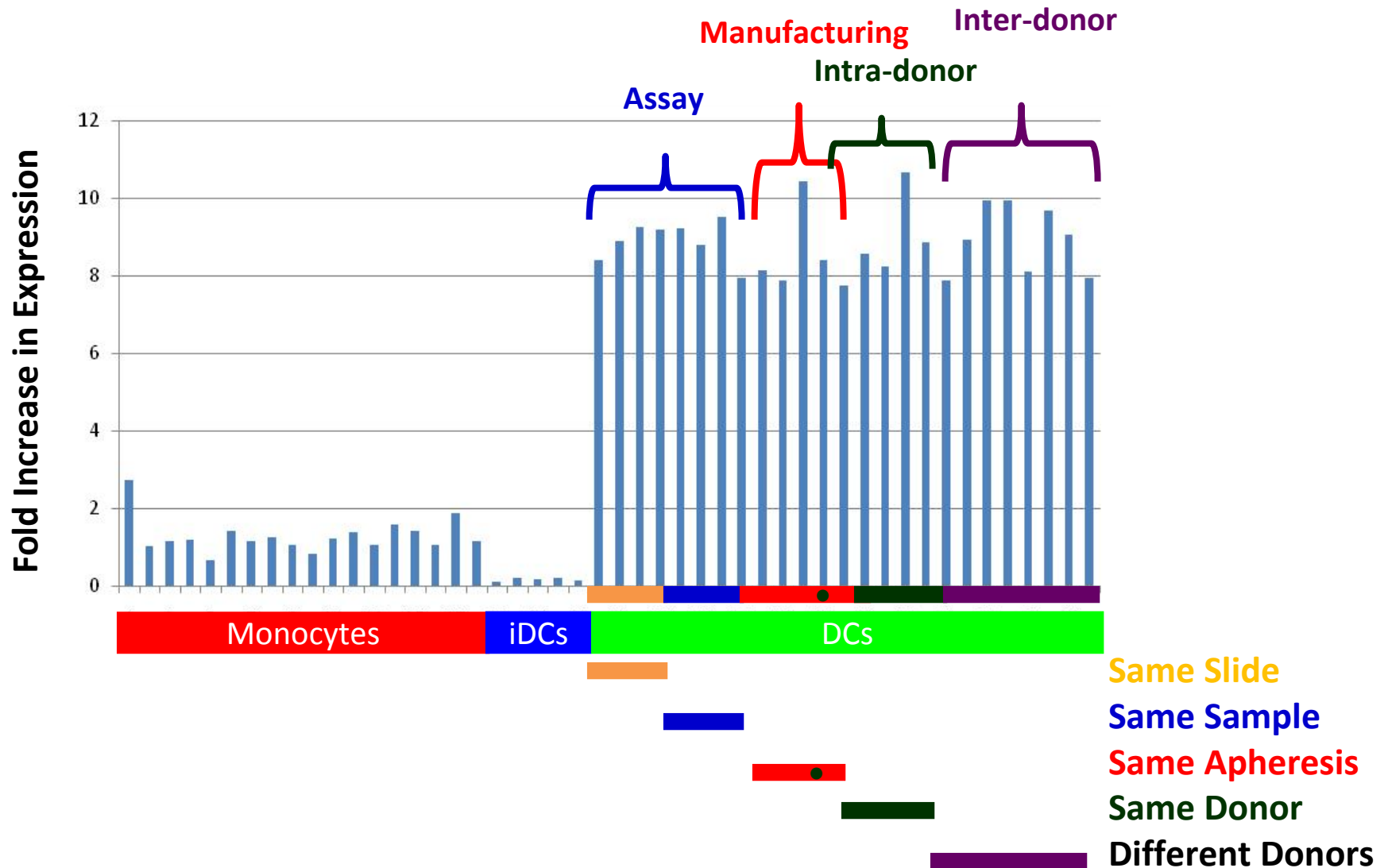
Differentially Expressed DC Genes with High Inter-Donor but Low Assay Variability (n=106): IPA Network Analysis



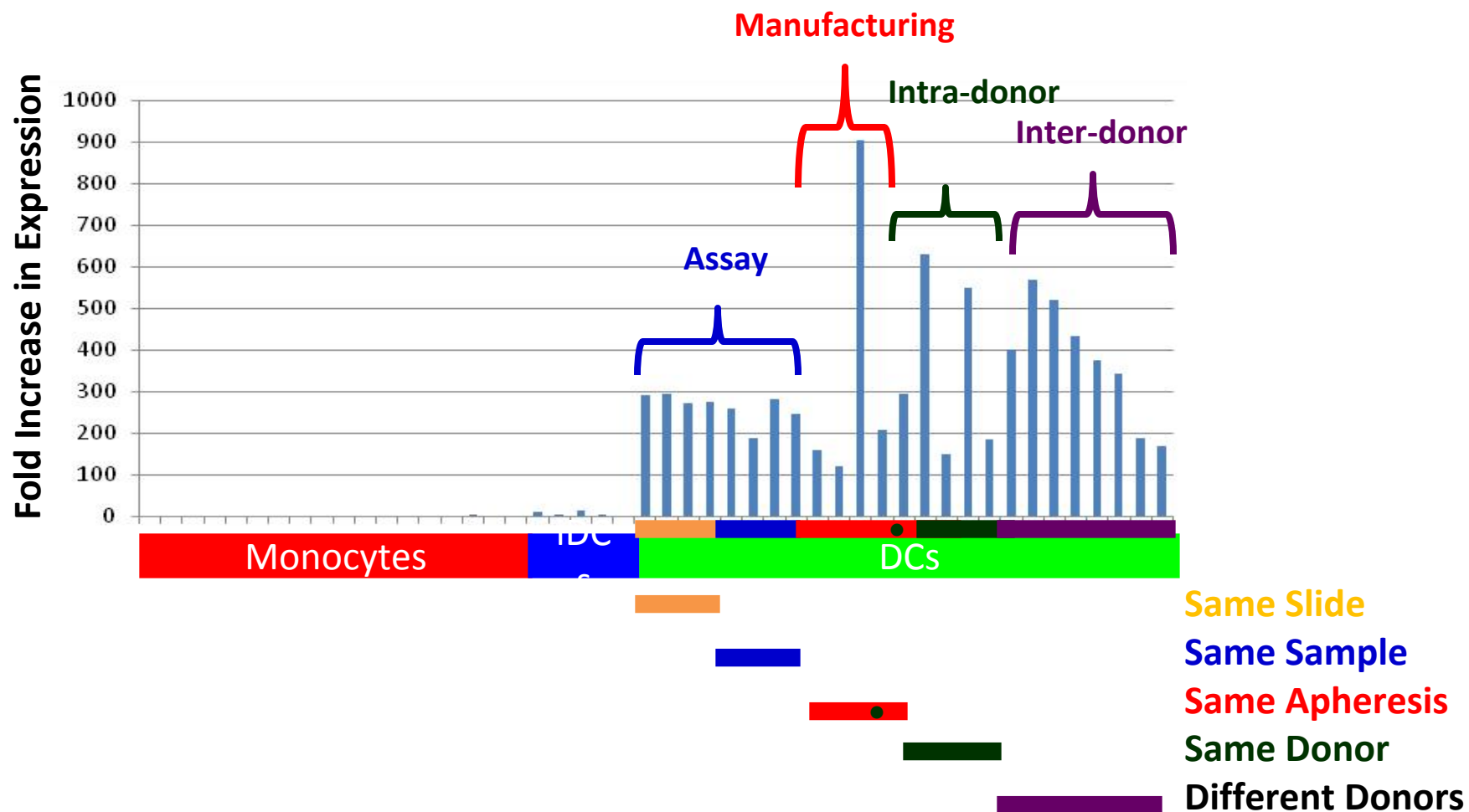
CCL1 – High Inter-Donor Variability



AIM2- Low Inter-Donor Variability



CD80 - A Standard DC Biomarker



Conclusions

We are developing a strategy to systemically characterize cell therapy biomarkers

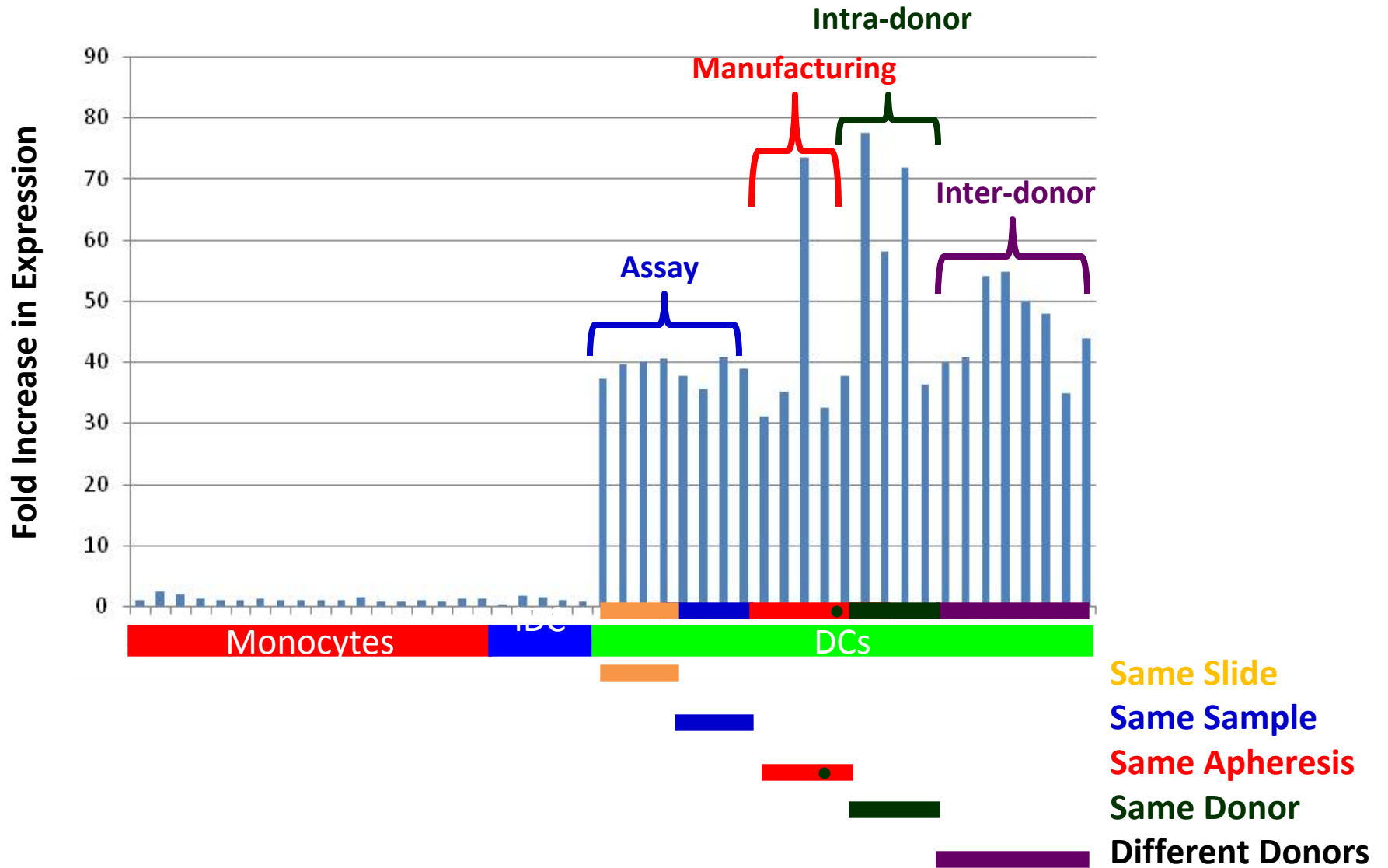
- **Reflect product consistency**
- **Reflect individual and manufacturing variability**

The concepts of this board approach will be used to design validation studies during clinical trials

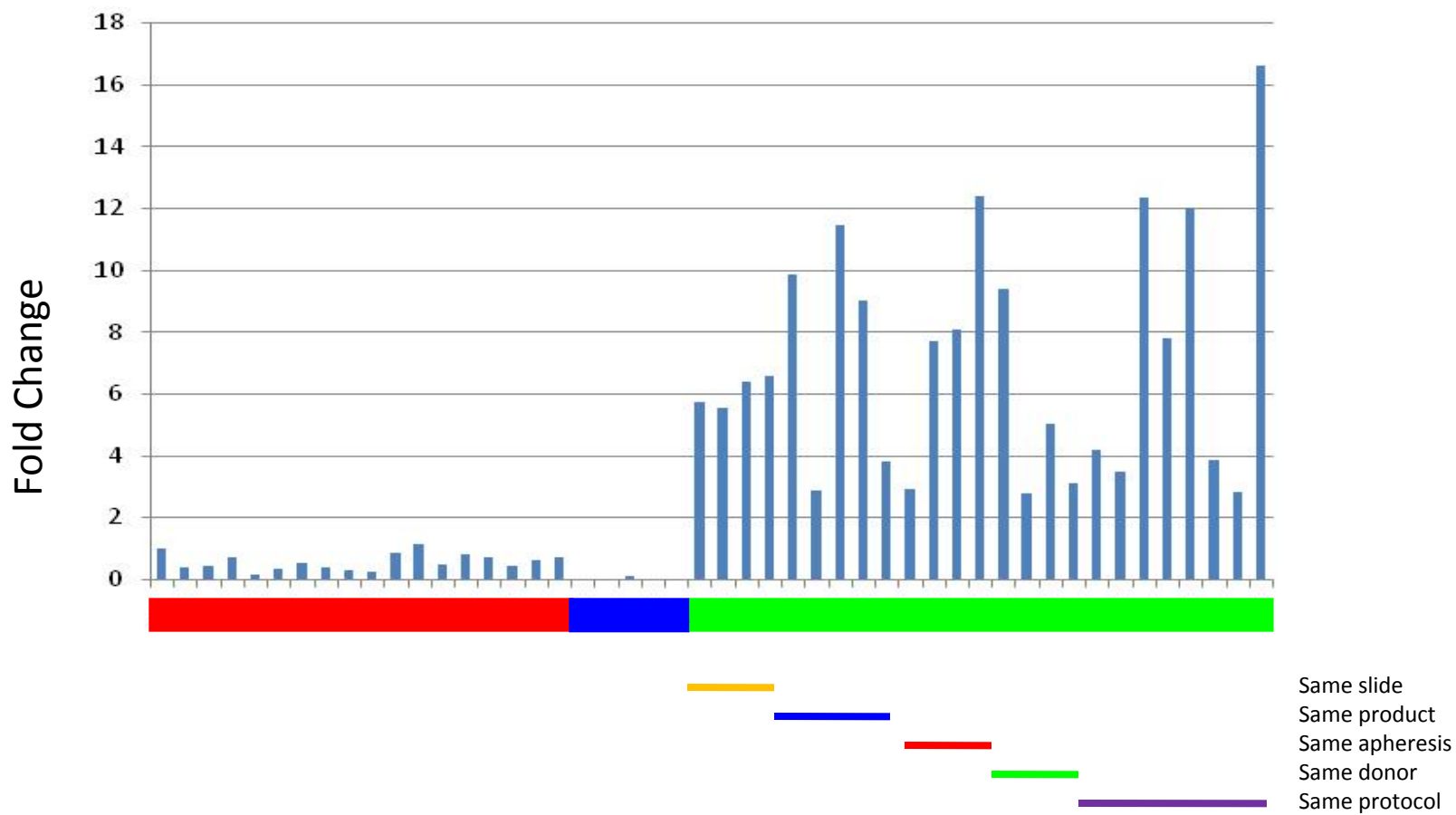
Mark O. Hatfield
Clinical Research Center



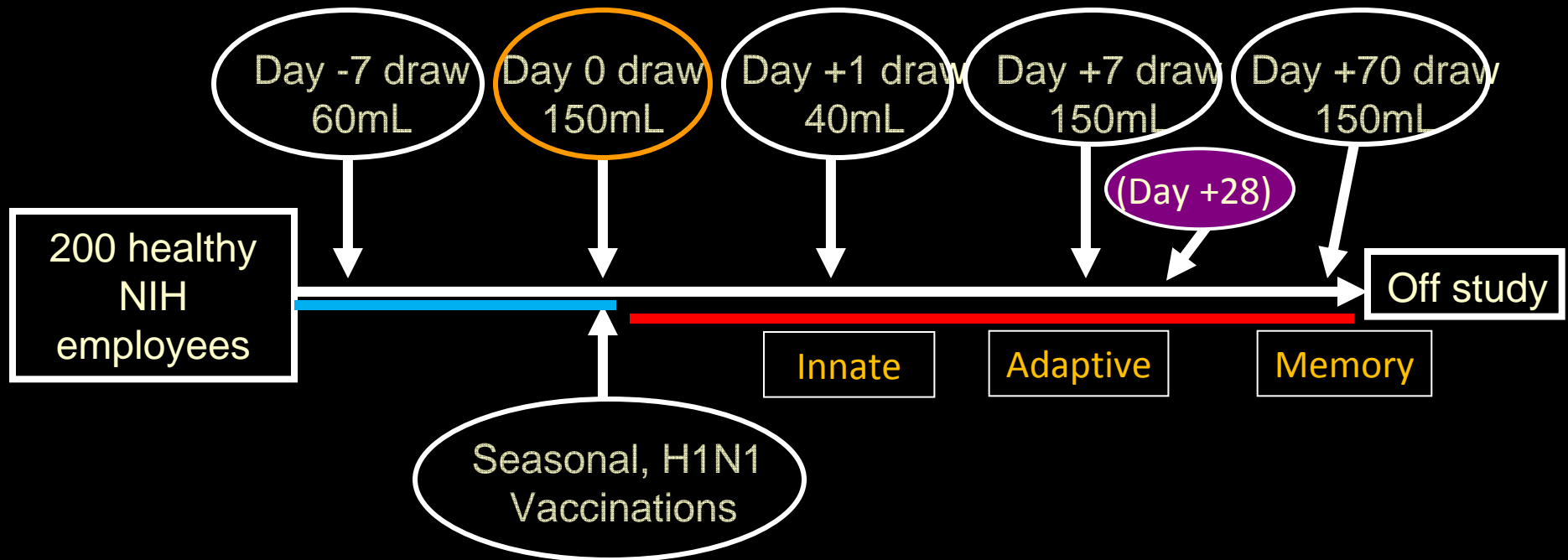
CD38



CXCL9



CHI protocol 09-H-0239 design (*Olnes*)



Fasting Blood Draws to minimize metabolic changes

Day 0 critical since vaccination coincided with H1N1 peak in the community

Parallel study to examine innate responses with multiple collections in first 24h (*Perl*)

Identification of Potential Biomarkers

Technical

- Expressed by cell of interest
- Good assay consistency (**low assay CV**)
- Biologically relevant

Relevant biological functions are not always known (Differentially expressed by DCs, iDCs and monocytes)

Bio variability

- Does the biomarker reflect manufacturing variability
- Does the biomarker reflect inter-donor and intra-donor variability

Determination of reference intervals

- Single biomarkers
- Multiple biomarkers

What are “normal” products? (Products in separate clusters)

Validation

- Do the biomarkers and reference intervals separate potent and impotent products

What do we use as a “gold standard”? (Correlation with clinical outcome)

Ideal Biomarker

Measures a critical biological function

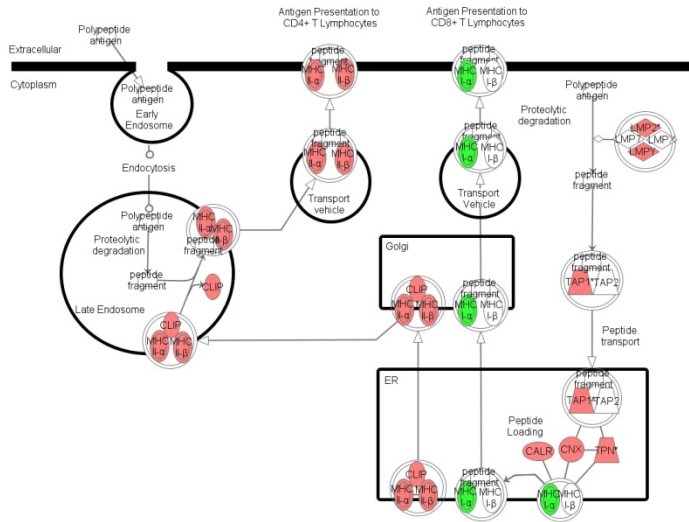
Highly sensitive to changes in manufacturing

- **Effected by variations in manufacturing**
- **Reflects important differences in starting materials**

Reflects differences in donors

Multiple biomarkers many be required

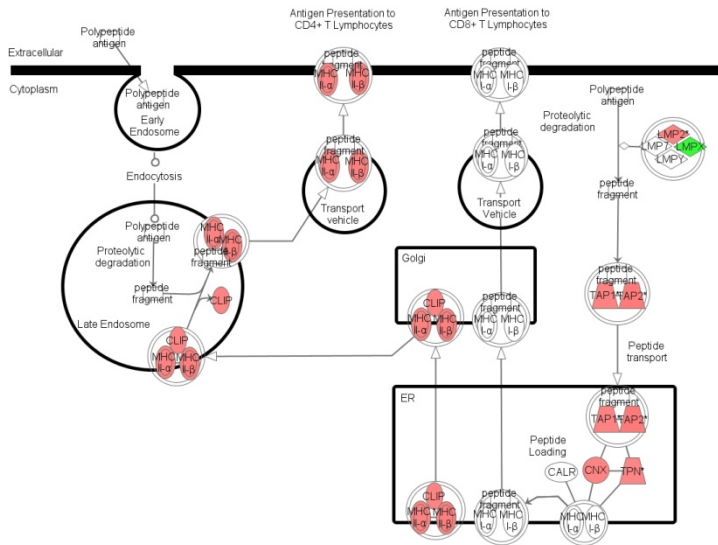
24 subjects



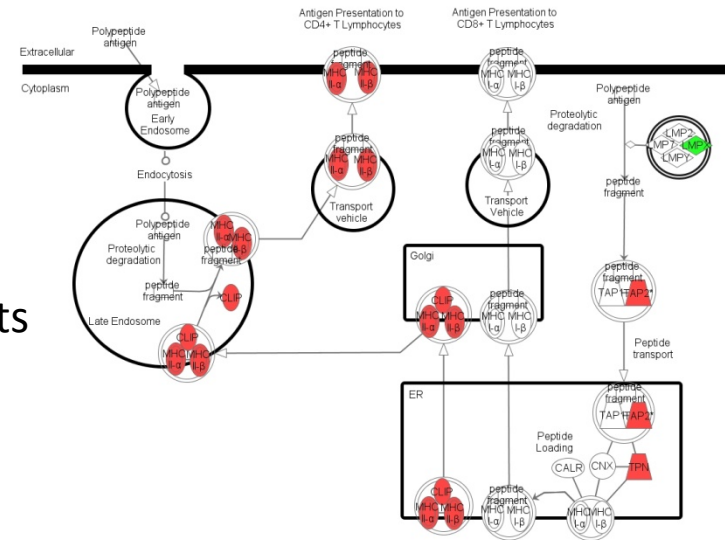
Antigen presentation pathway

Day 1 overlapping genes baseline pre7 and day0

Day 1 vs mean of pre7 and day0

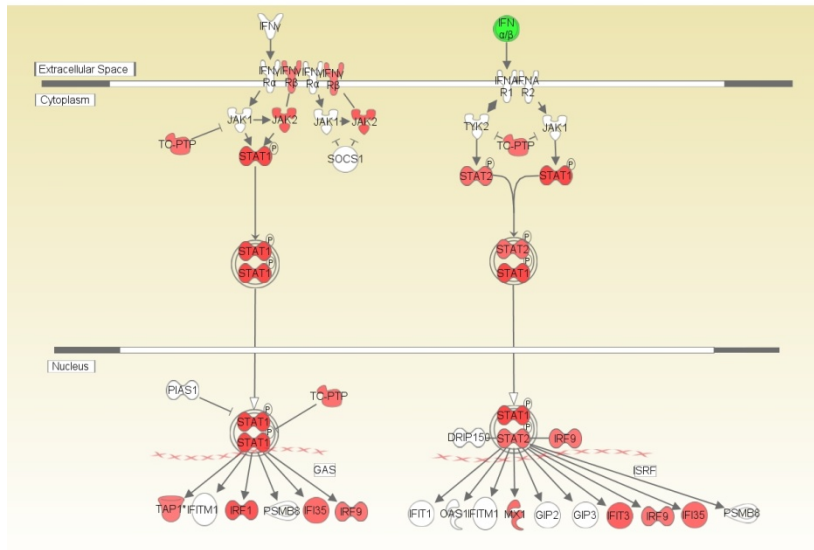


63 subjects



24 subjects

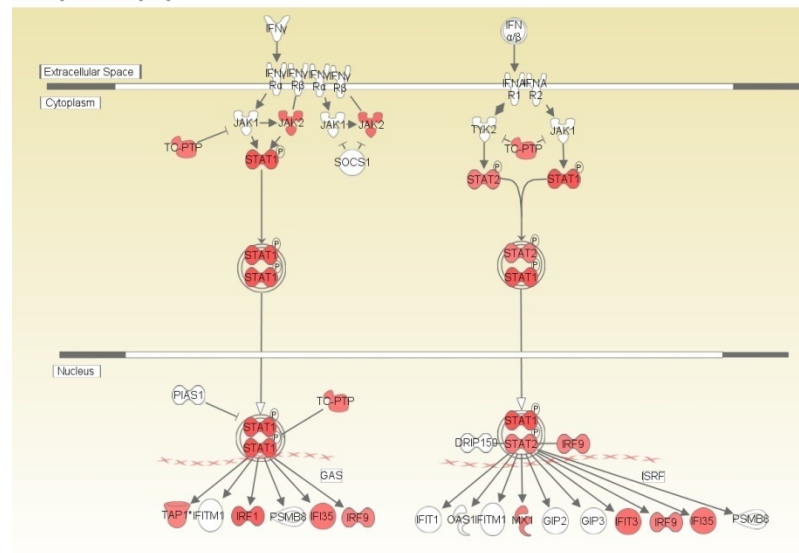
Path Designer Interferon Signaling



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63 subjects Day1 paired with Pre7Day0

Path Designer Interferon Signaling

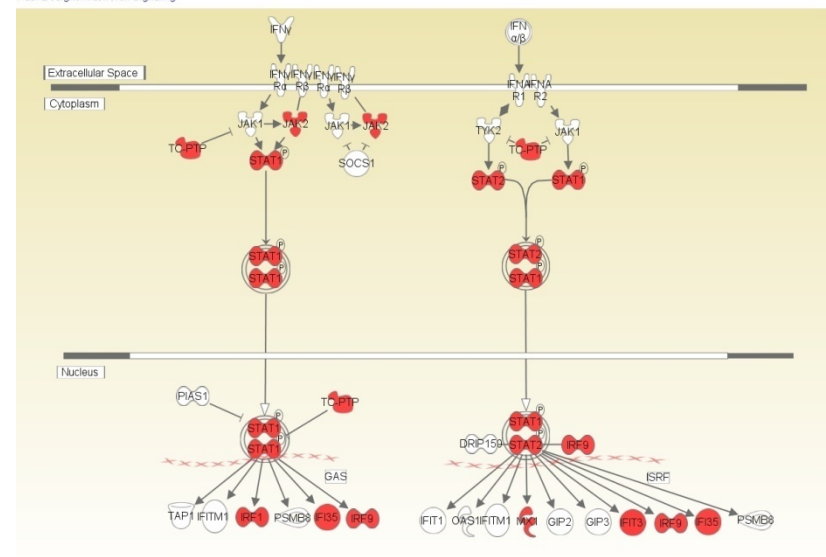


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Interferon signaling pathway

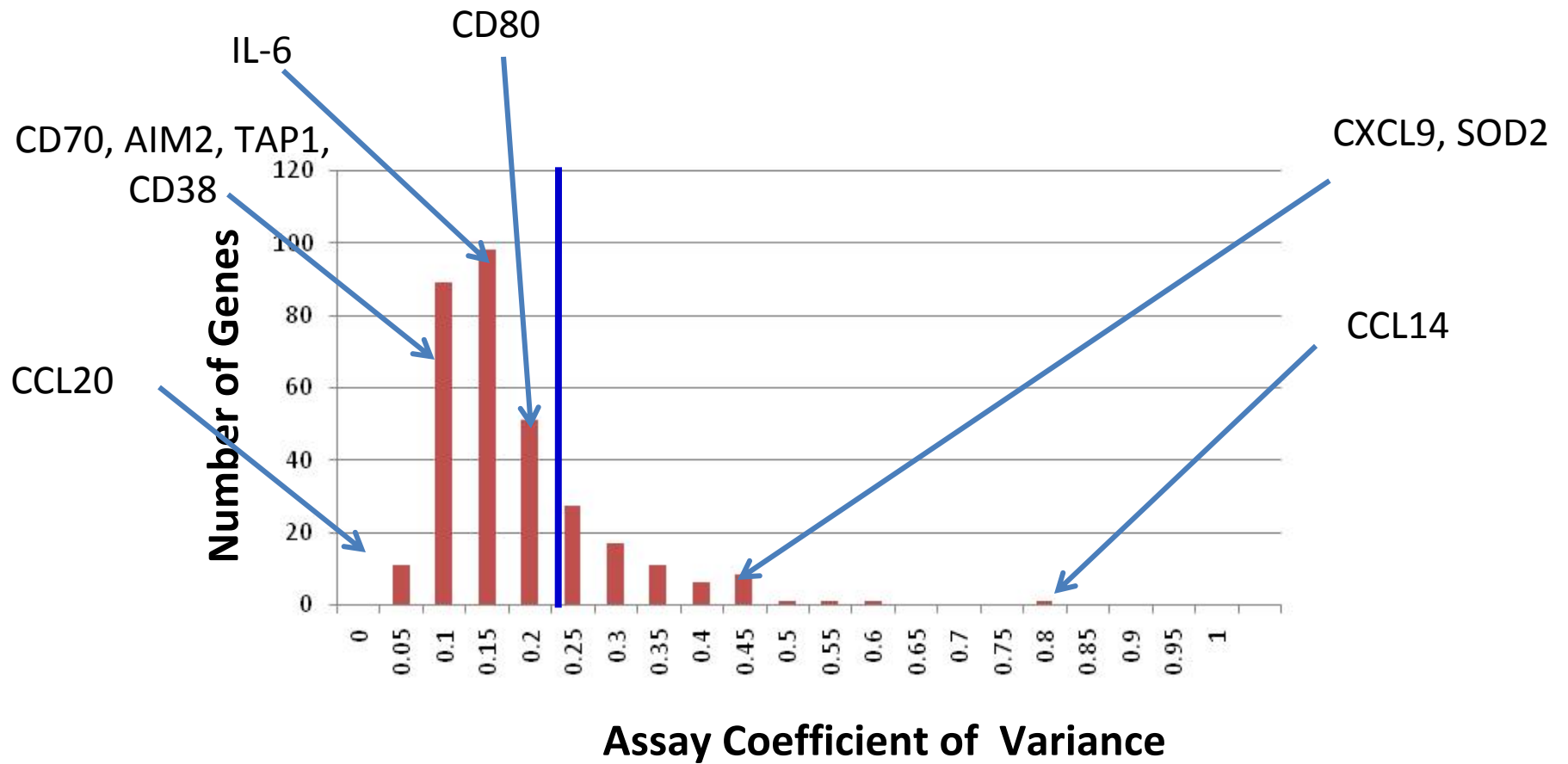
63 subjects Day1 overlapping genes
baseline Pre7 and Day0

Path Designer Interferon Signaling

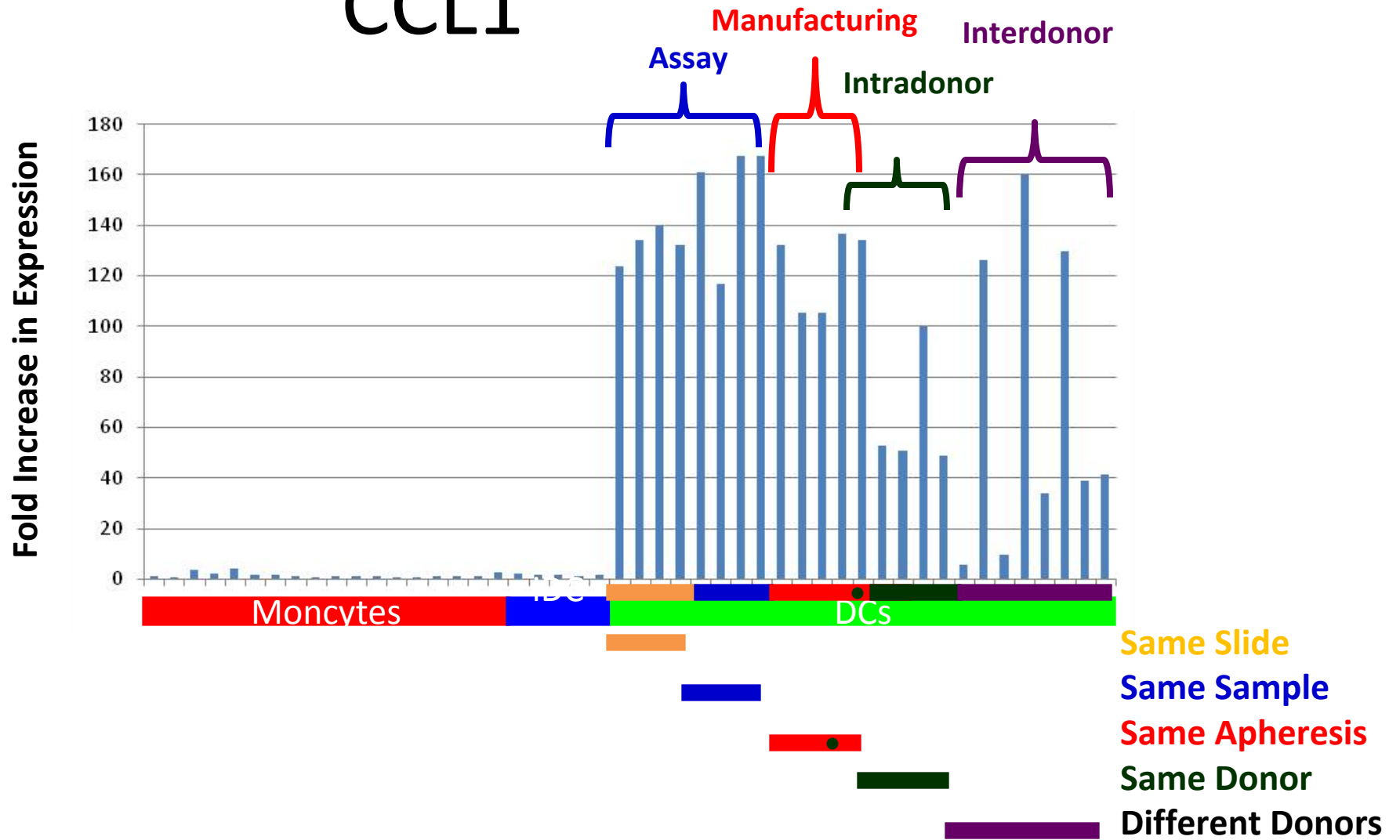


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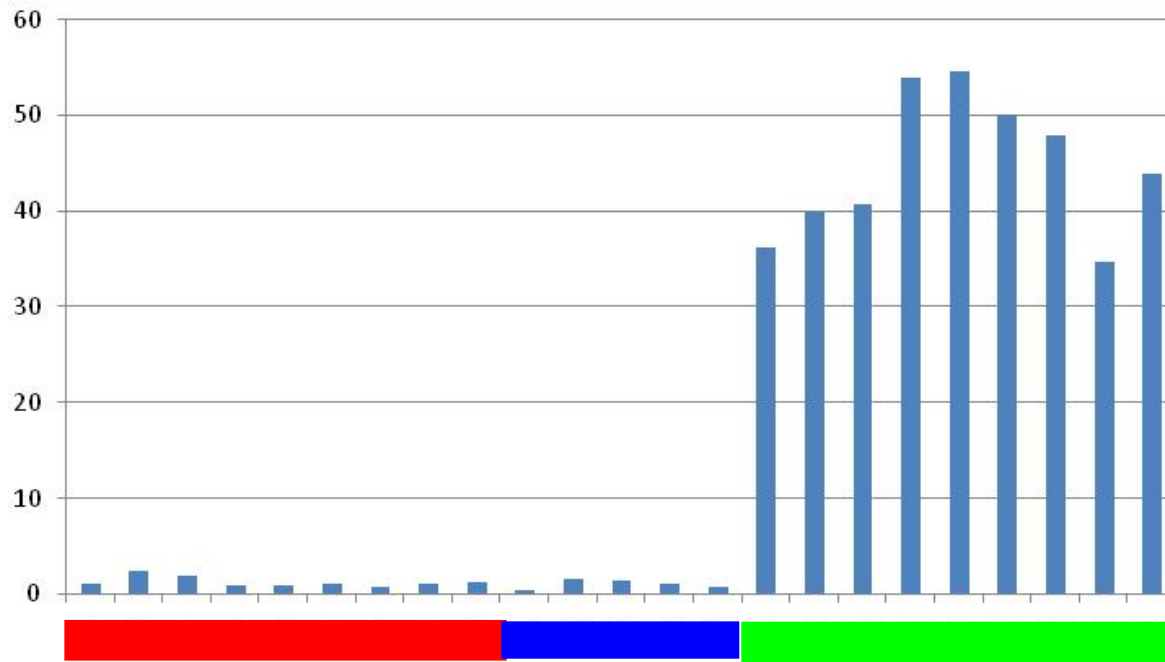
Assay CV of Selected Differentially Expressed DC Genes

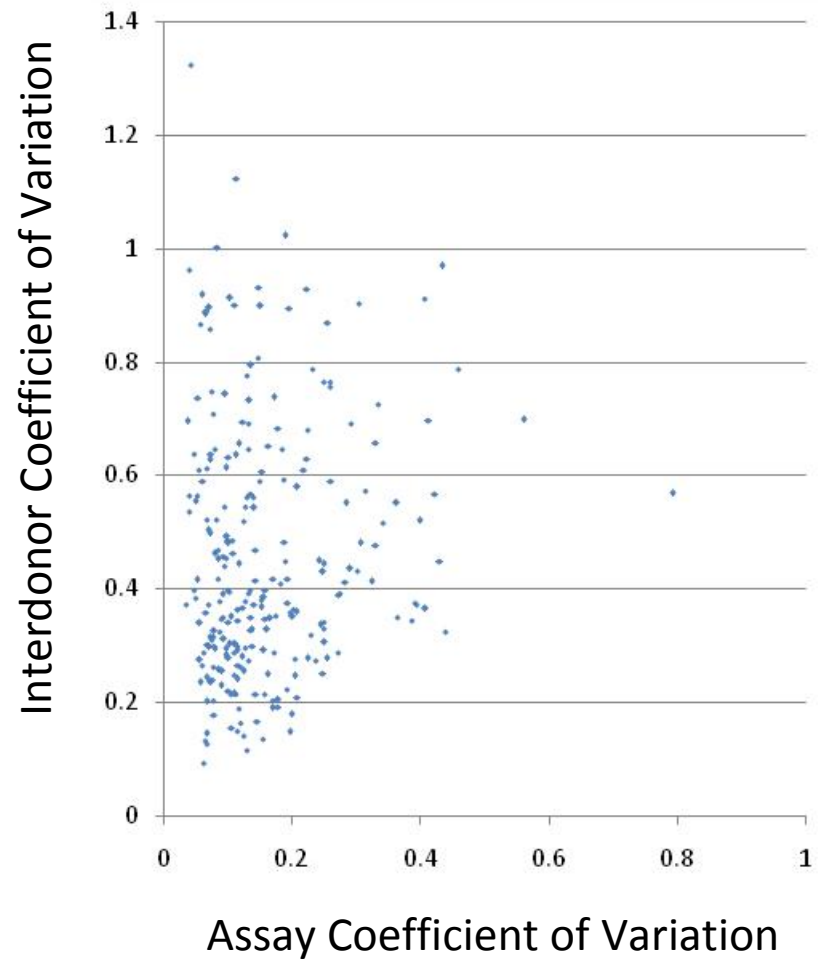


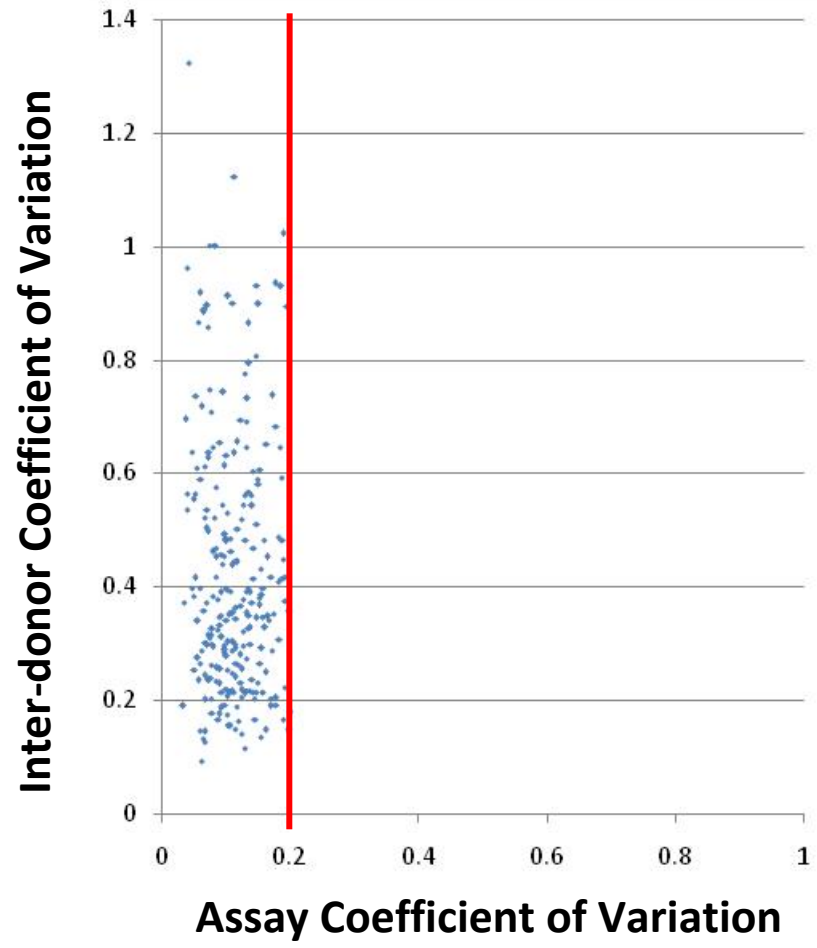
CCL1



CD38 (correct)

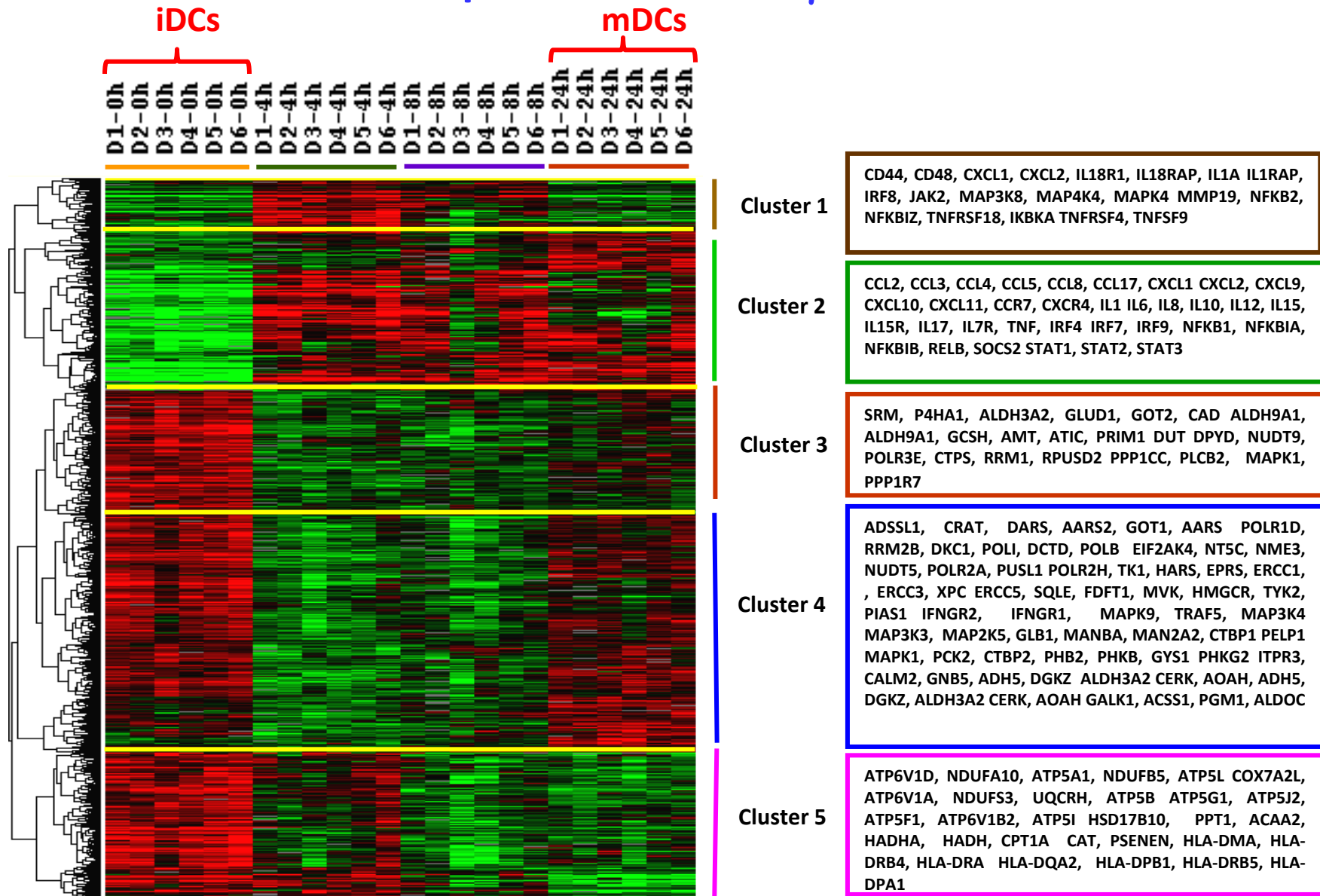






Changes associated with DC maturation

iDC plus LPS and IFN- γ \rightarrow mDCs



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