

Step 1: Significant Differential Expression Filtering

Experiment 1 Experiment 2
45,101 45,101

A

1a
Change p-value must be $\leq .002$ OR $\geq .998$

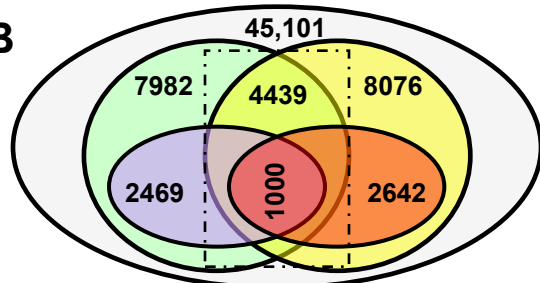
↓ ↓
Is expression above detection threshold? → **No**

1b
Differential expression must be > 4 -fold OR > 2 -fold change

↓ **Yes** ↓
Is the transcript differentially expressed? → **No**

↓ **Yes** ↓
4-fold: 2,469 4-fold: 2,642
2-fold: 7,982 2-fold: 8,076

B



Step 2: Consistency Filtering

2a
Each probe set must have passed Step 1 in both expts

↓ ↓
Significant in both experiments? → **No**
↓ **Yes** ↓

2b
Euclidean distance between replicate profiles is ≤ 1.4

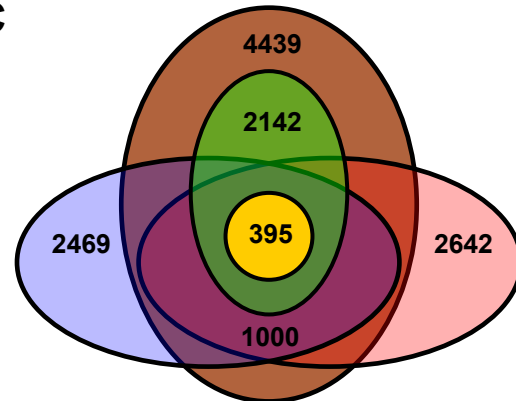
4-fold: 1,000
2-fold: 4,439
↓
Consistent expression levels? → **No**
Yes ↓

2c
Pearson's Correlation between replicate profiles is $\geq .76$

Consistent profile patterns? → **No**
Yes ↓

4-fold: 395
2-fold: 2,142

C



Step 3: Cluster Analysis

3a
Figure of Merit Analysis

↓
Which clustering algorithm and how many clusters are optimal?

3b
Consensus Clustering

↓
Do the expression profiles cluster consistently in both experiments? → **No**
↓ **Yes** ↓

4-fold: 361; 47 clusters
2-fold: 2,025; 140 clusters