

# RNA-Seq Now What?

BIS180L  
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# We have differentially expressed genes, what do we want to know about them?

- What biological processes are affected by:
  - treatment
  - genotype
  - genotype x treatment interaction
- What transcription factors might control expression of these genes?
- We have hundreds of differentially expressed genes, how do we figure this out?

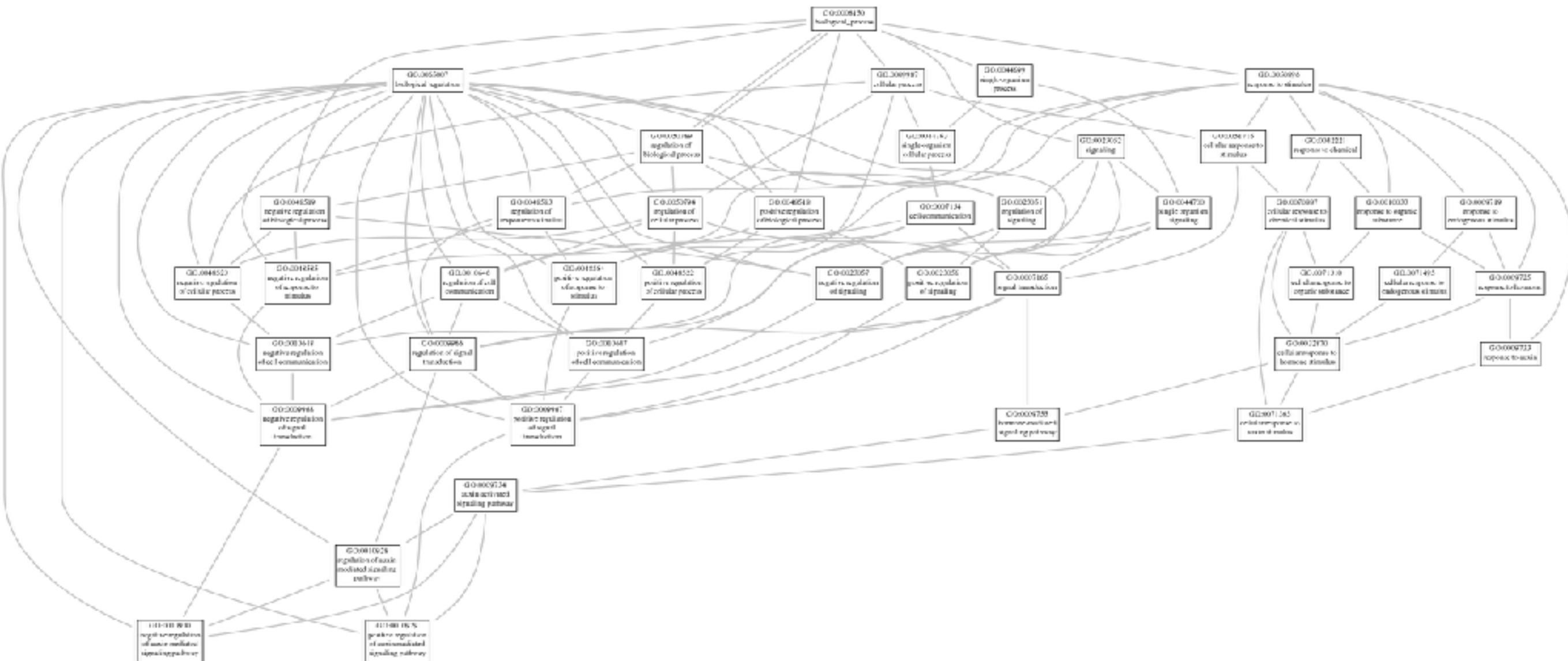
# Gene Ontology (GO) terms

- GO terms are a defined hierarchical vocabulary for describing gene functions

# GO term example: response to auxin



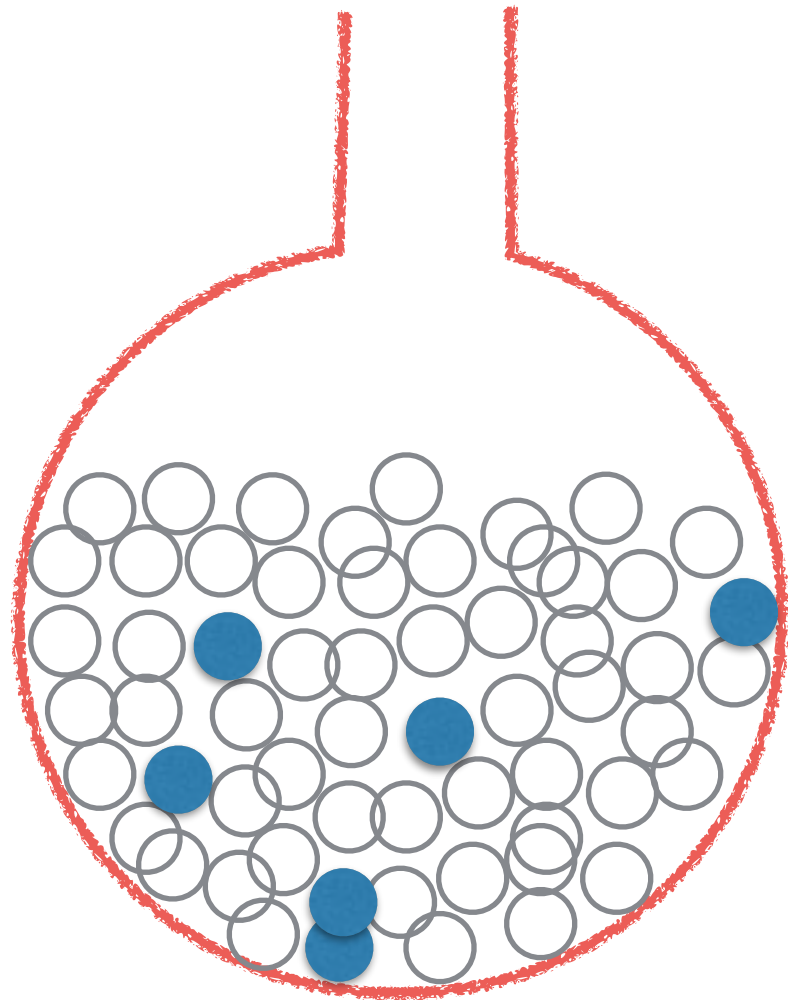
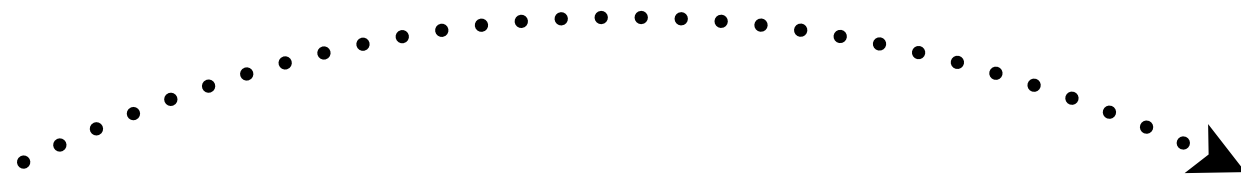
# More of the auxin GO hierarchy



# GO and differential expression analysis

- Alternative to analyzing individual genes
- Determine which GO terms are over-represented among the differentially expressed genes

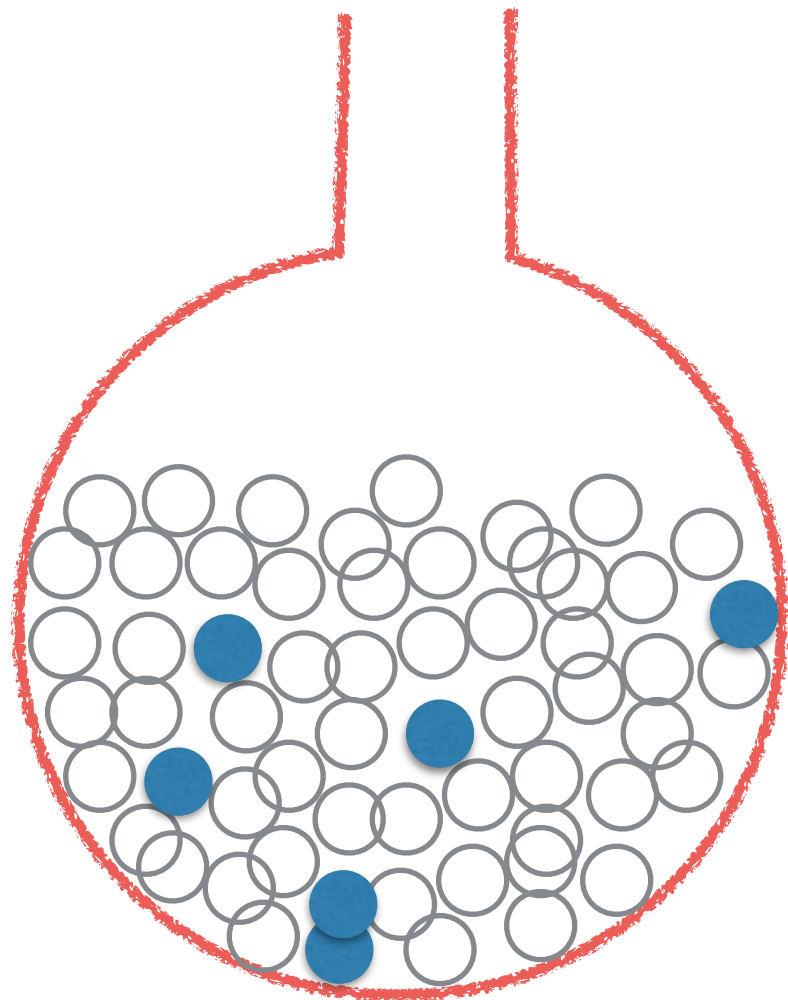
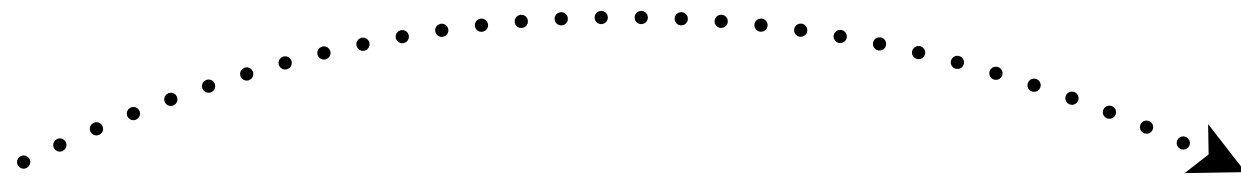
# Over-representation analysis



Remove 20  
How many white and blue  
do you expect?

Jar with 1000 marbles  
800 white, 200 blue

# Over-representation analysis



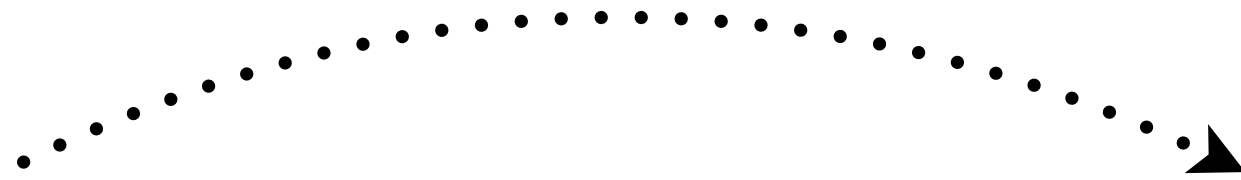
What if you find  
10 white and 10 blue?

Blues are **over-represented**  
in your sample

Jar with 1000 marbles  
800 white, 200 blue

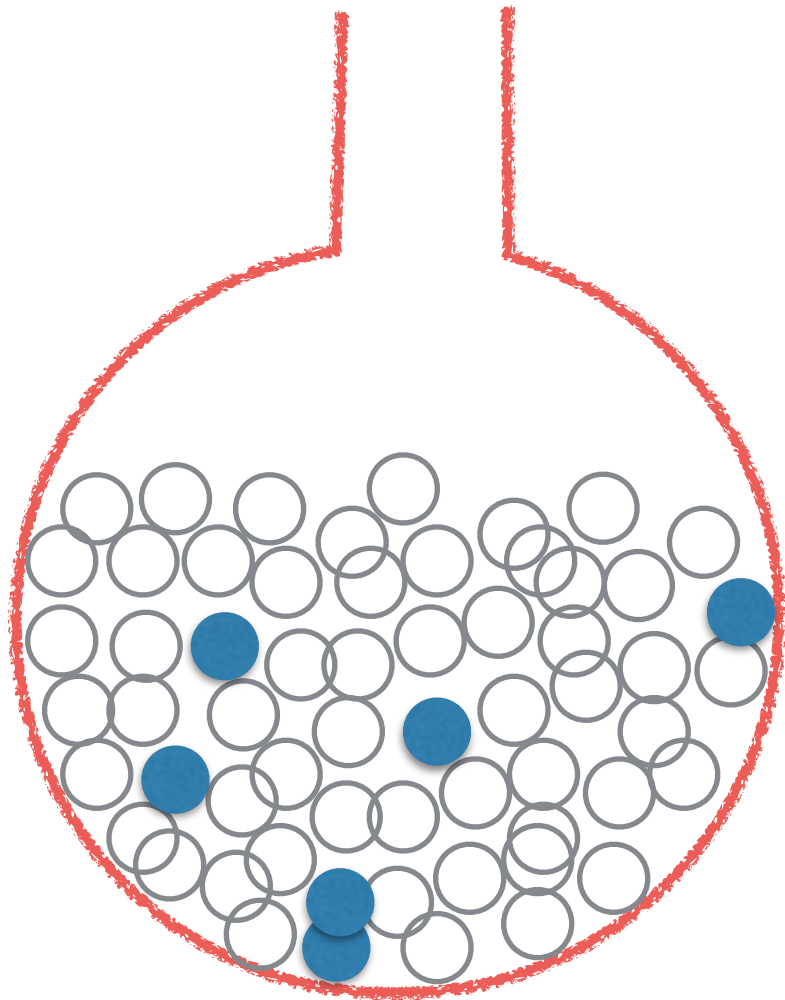


# Over-representation analysis



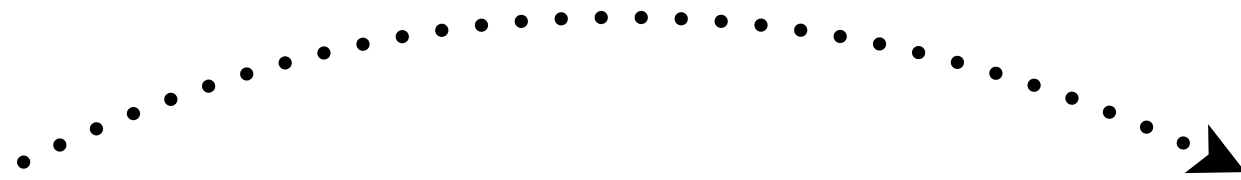
Find 1,000 genes differentially expressed in response to shade.

How many do you expect to have the GO term “cell wall modification”?



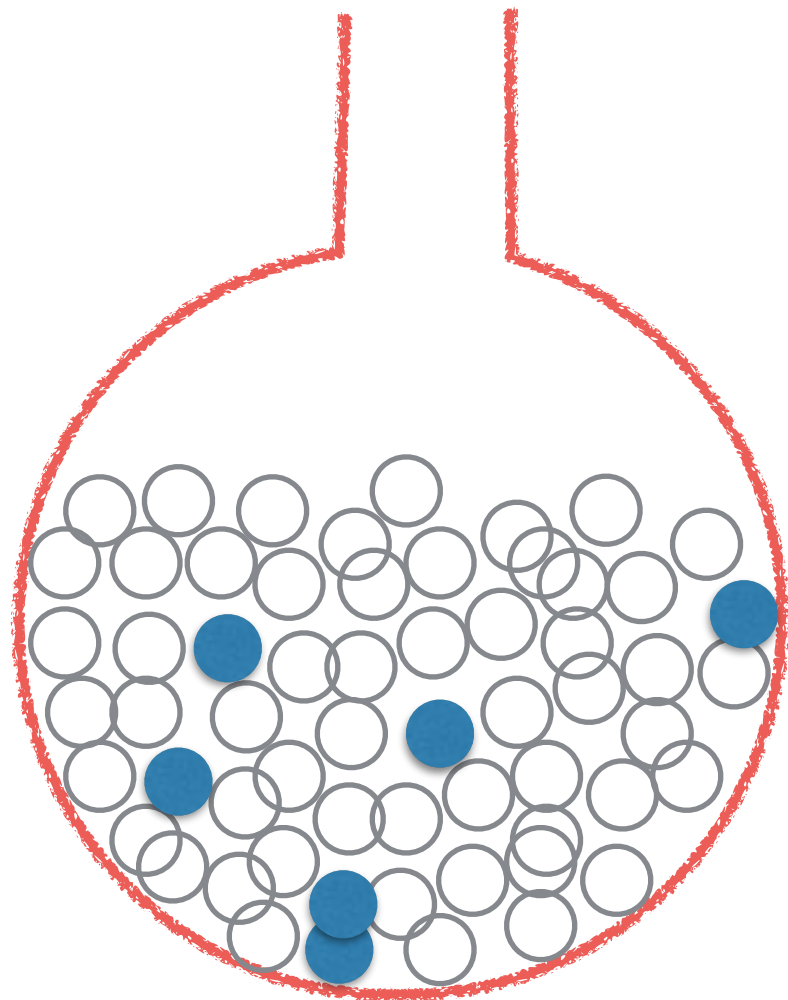
Genome with 34,000 genes  
340 have GO term:  
“Cell wall modification”

# Over-representation analysis



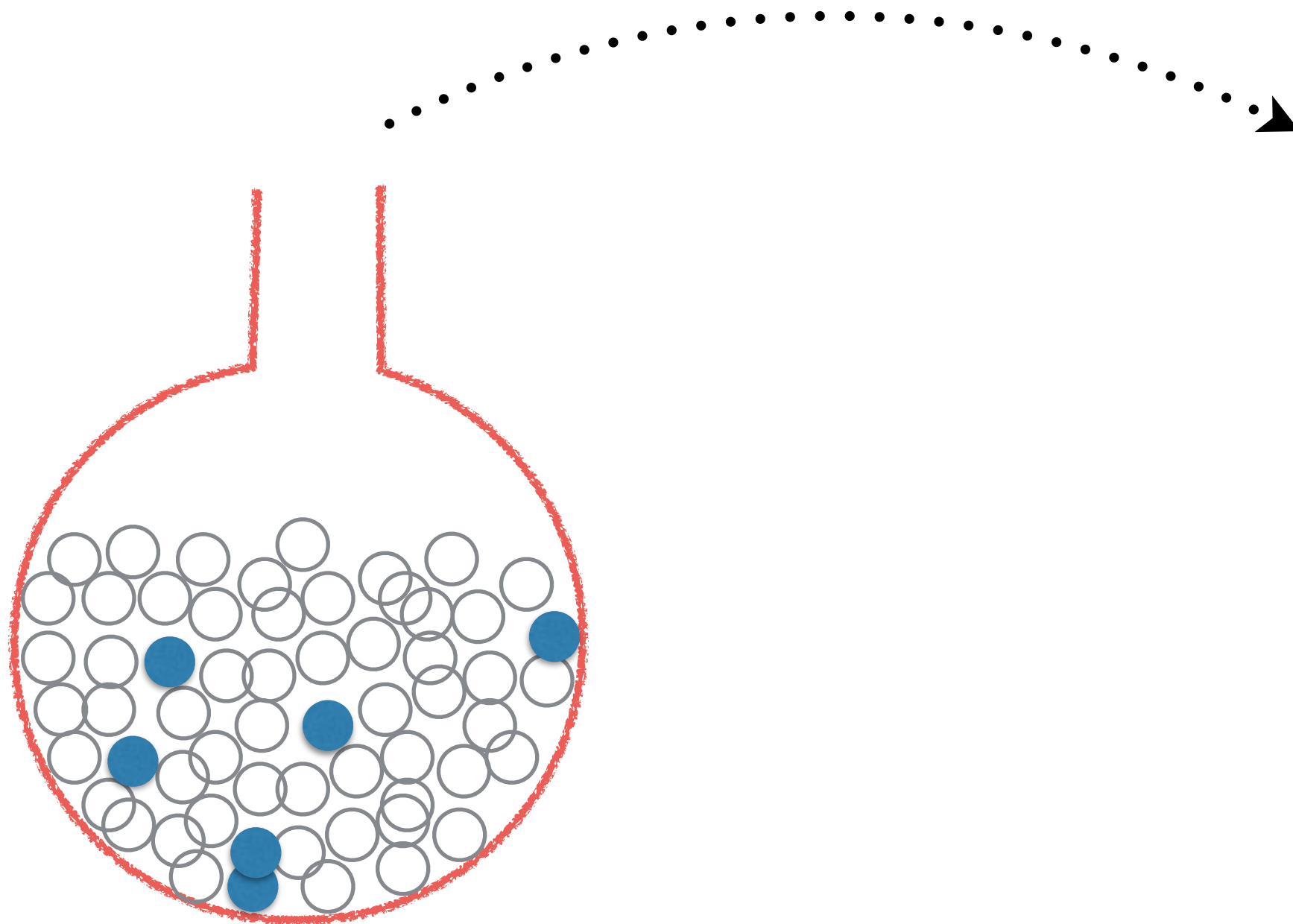
Find 1,000 genes differentially expressed in response to shade.

50 of these 1,000 have the GO term “Cell wall modification”



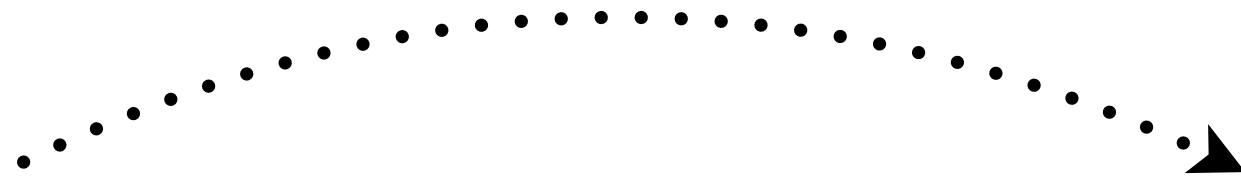
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# Over-representation analysis: additional considerations

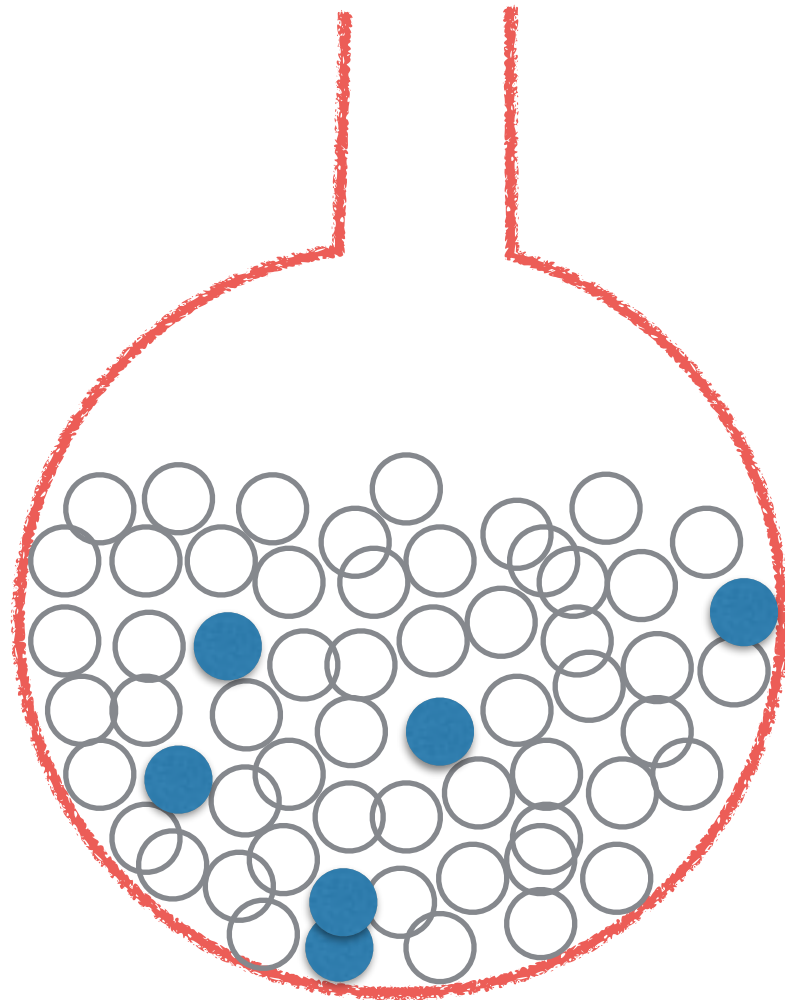


The Jar (aka Universe) should only contain **expressed genes**. (Why?)

# Over-representation analysis: additional considerations



Biases in detecting differentially expressed genes should be considered



The Jar (aka Universe) should only contain **expressed genes**. (Why?)

# Statistical tests for over (or under) representation:

- Build a contingency table categorizing genes by D.E. and GO

		Differentially Expressed	
		NO	YES
GO term “cell wall modification” present?	NO	32,710	950
	YES	290	50

- Use a (modified) Fisher’s Exact Test to look for unequal ratios (is 32710:950 different from 290:50)

# Promoter Motif Analysis

- As you know transcription factors bind to specific DNA sequences or “motifs”.
- Similar to looking for enrichment of GO terms among our DE genes we can also look for enrichment of transcription factor binding motifs in the promoters of DE genes.
- Why would we want to do this? (What hypotheses might be generated by such an analysis?)