

RNA-Seq Now What?

BIS180L
Professor Maloof
May 23, 2019

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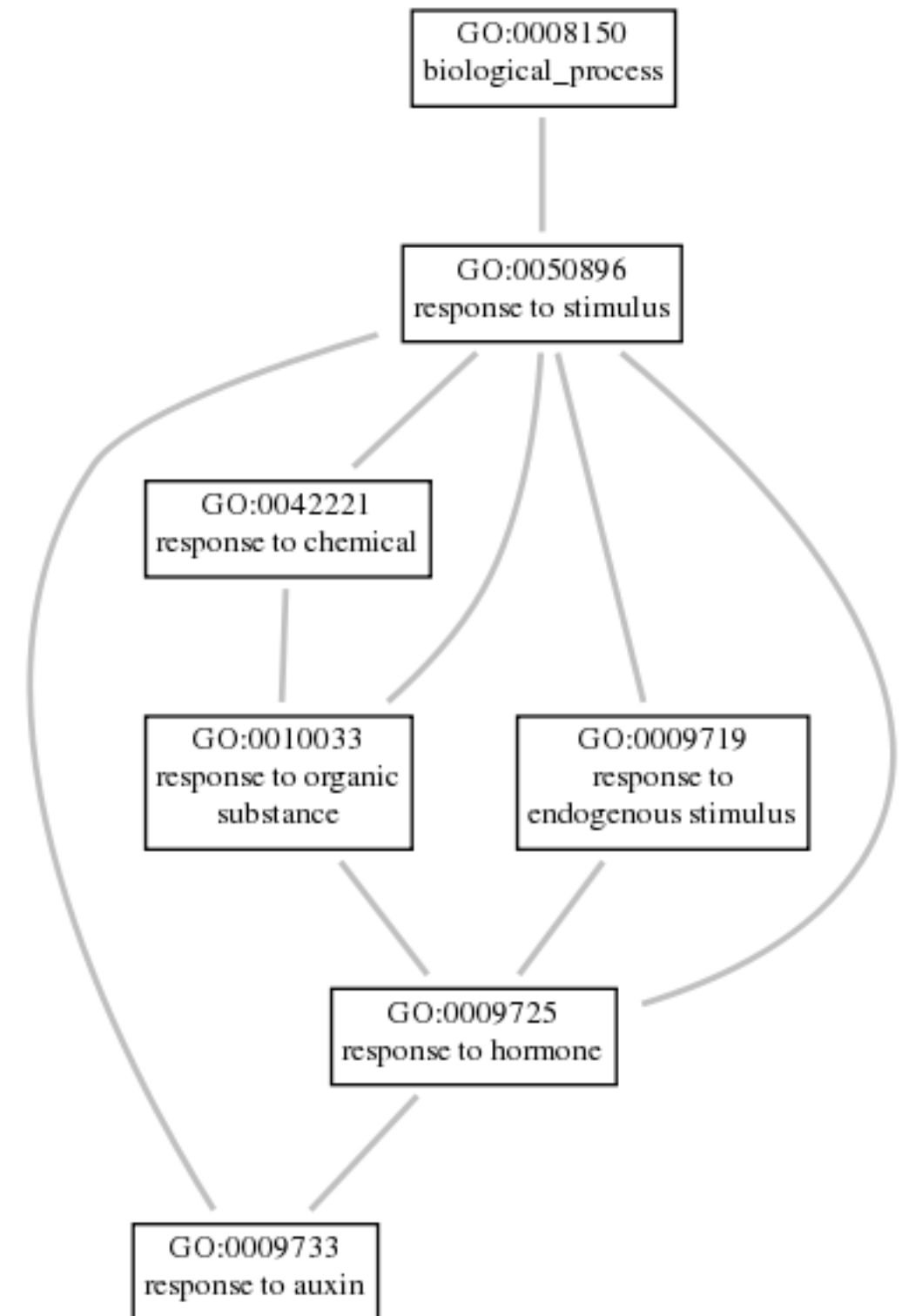
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- What biological processes are affected by:
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- We have hundreds of differentially expressed genes, how do we figure this out?

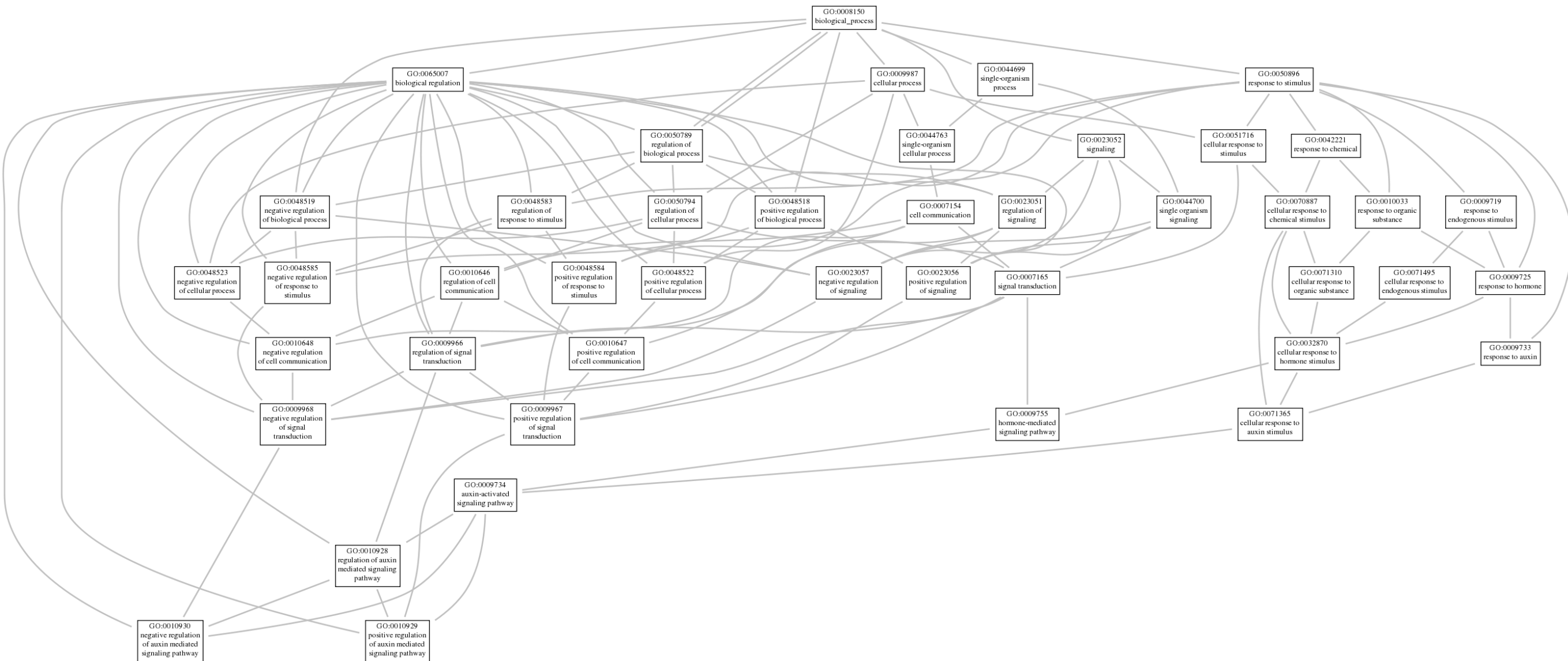
Gene Ontology (GO) terms

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

GO term example: response to auxin



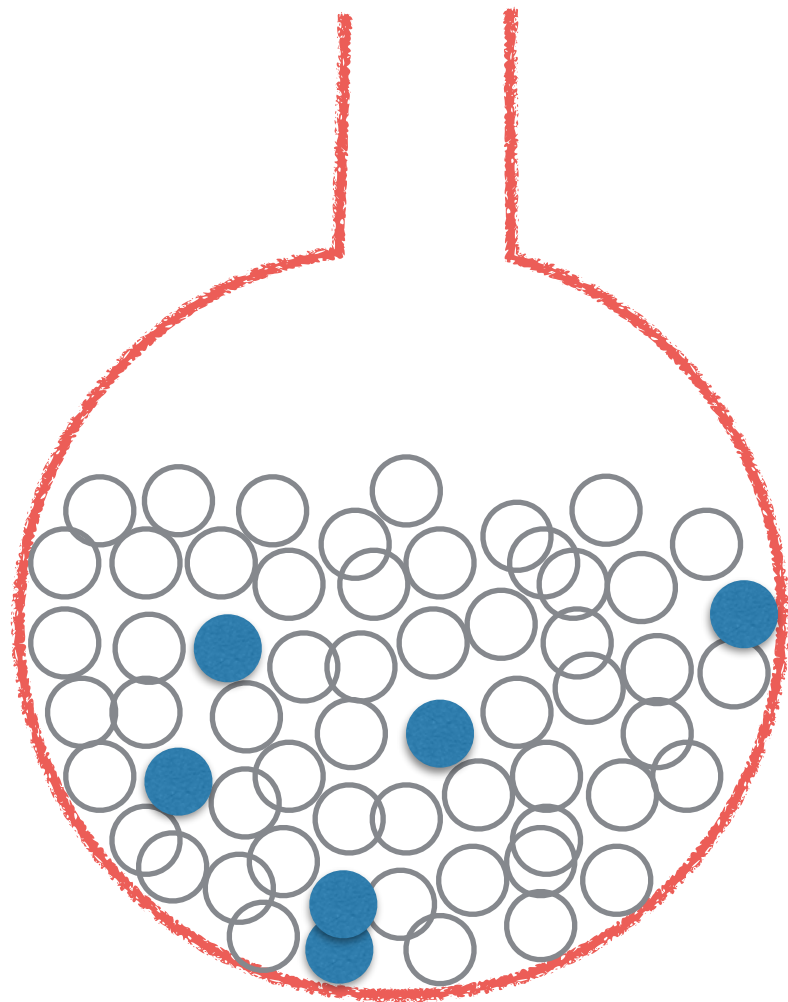
More of the auxin GO hierarchy



GO and differential expression analysis

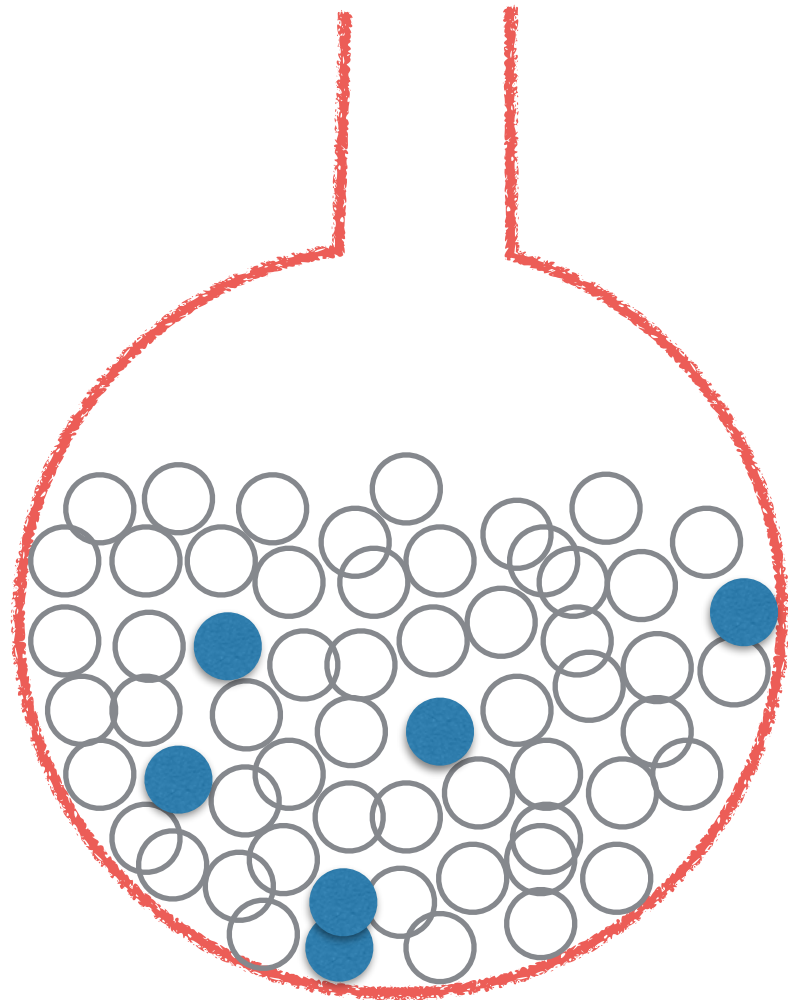
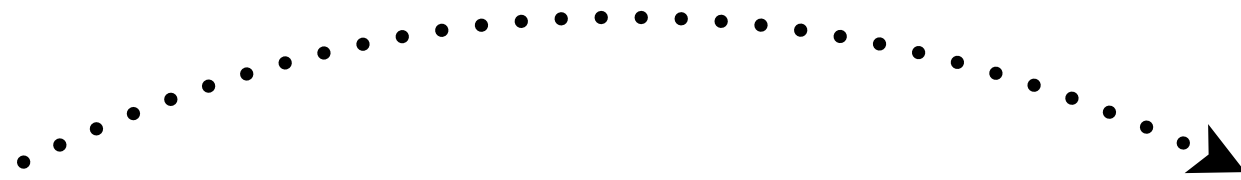
- Alternative to analyzing individual genes
- Determine which GO terms are over-represented among the differentially expressed genes

Over-representation analysis



Jar with 1000 marbles
800 white, 200 blue

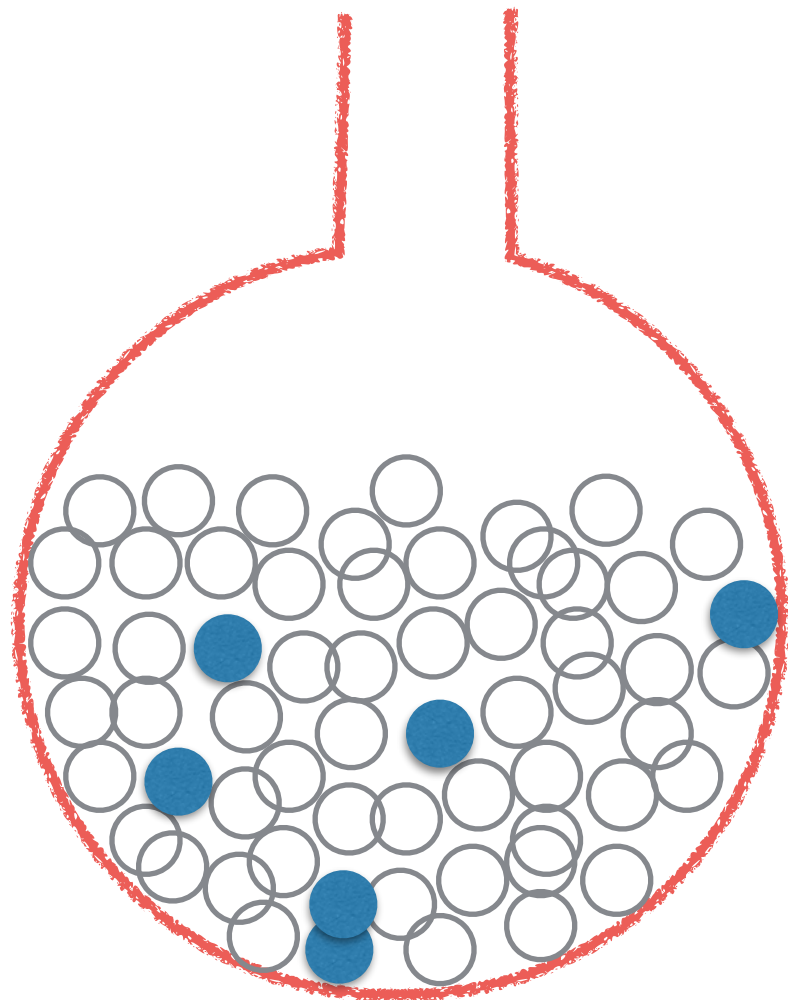
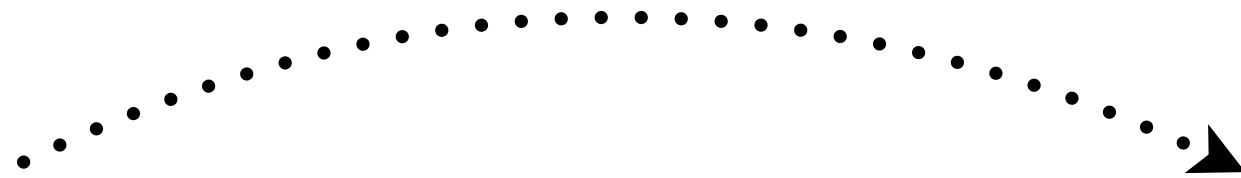
Over-representation analysis



Remove 20
How many white and blue
do you expect?

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Over-representation analysis

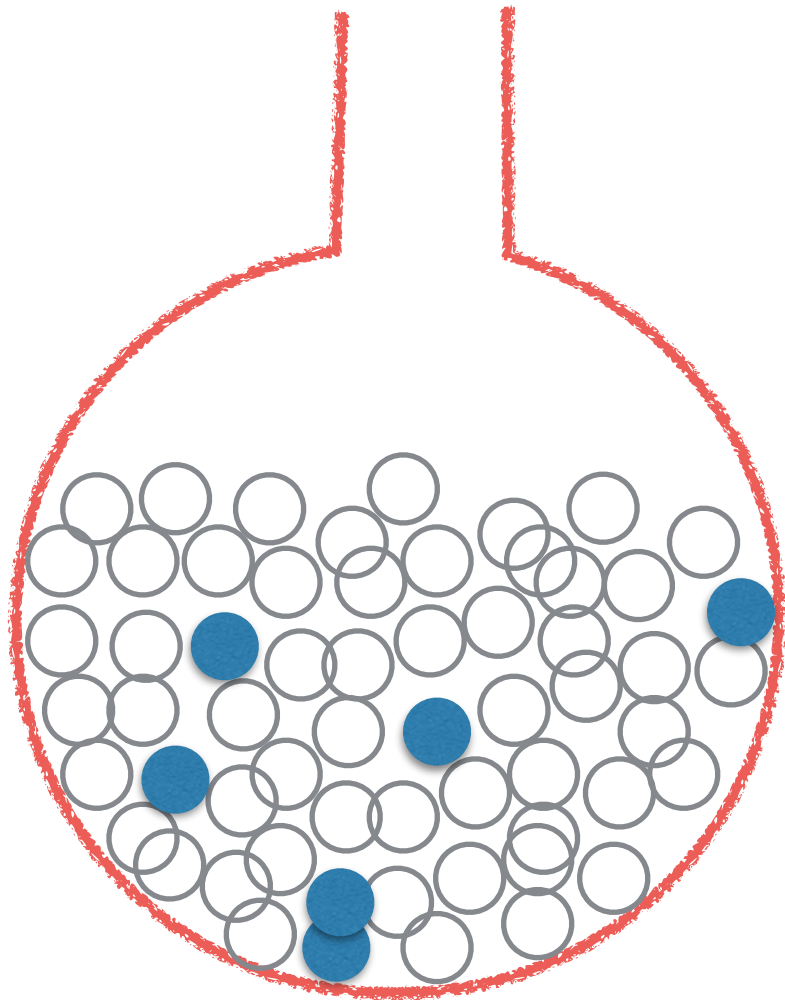


What if you find
10 white and 10 blue?

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

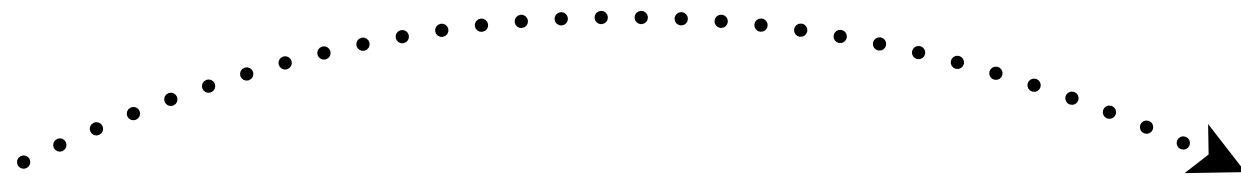
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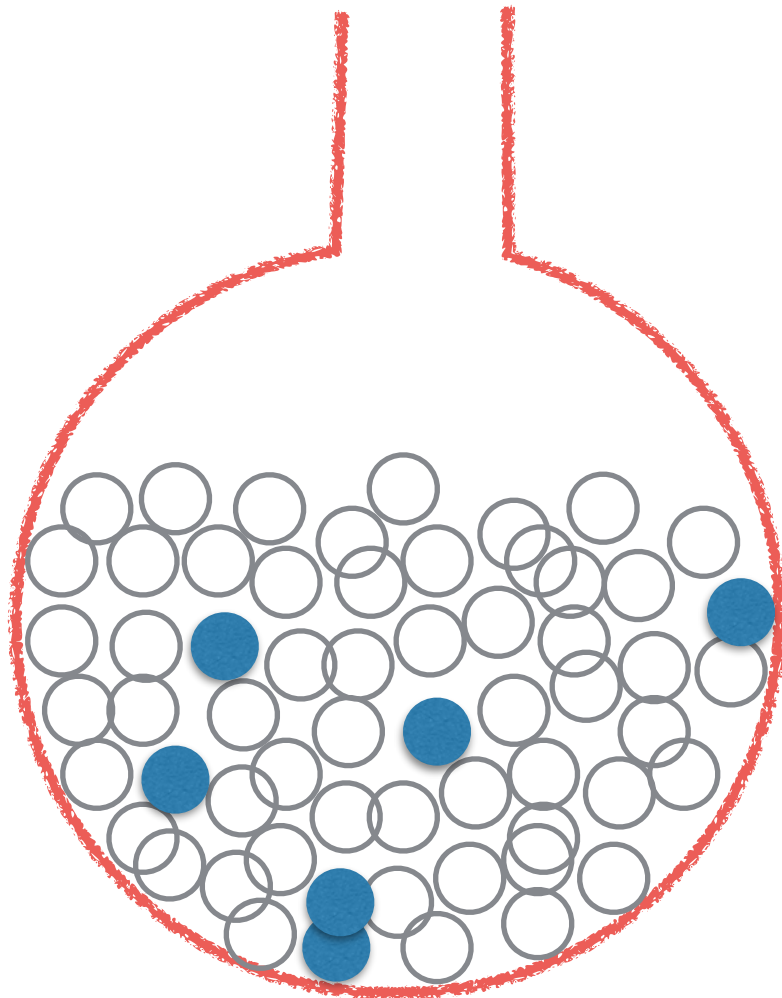
Genome with 34,000 genes
340 have GO term:
“Cell wall modification”

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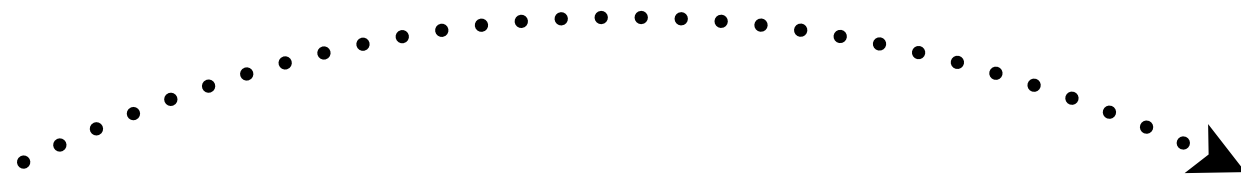
Find 1,000 genes differentially expressed in response to shade.

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

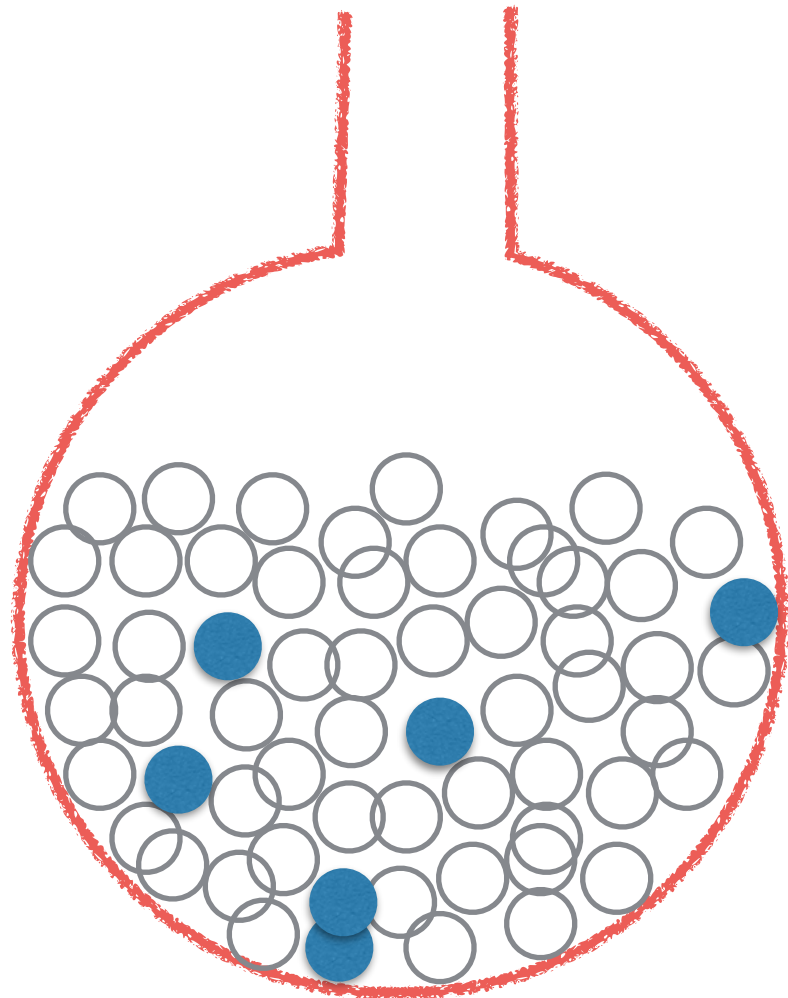


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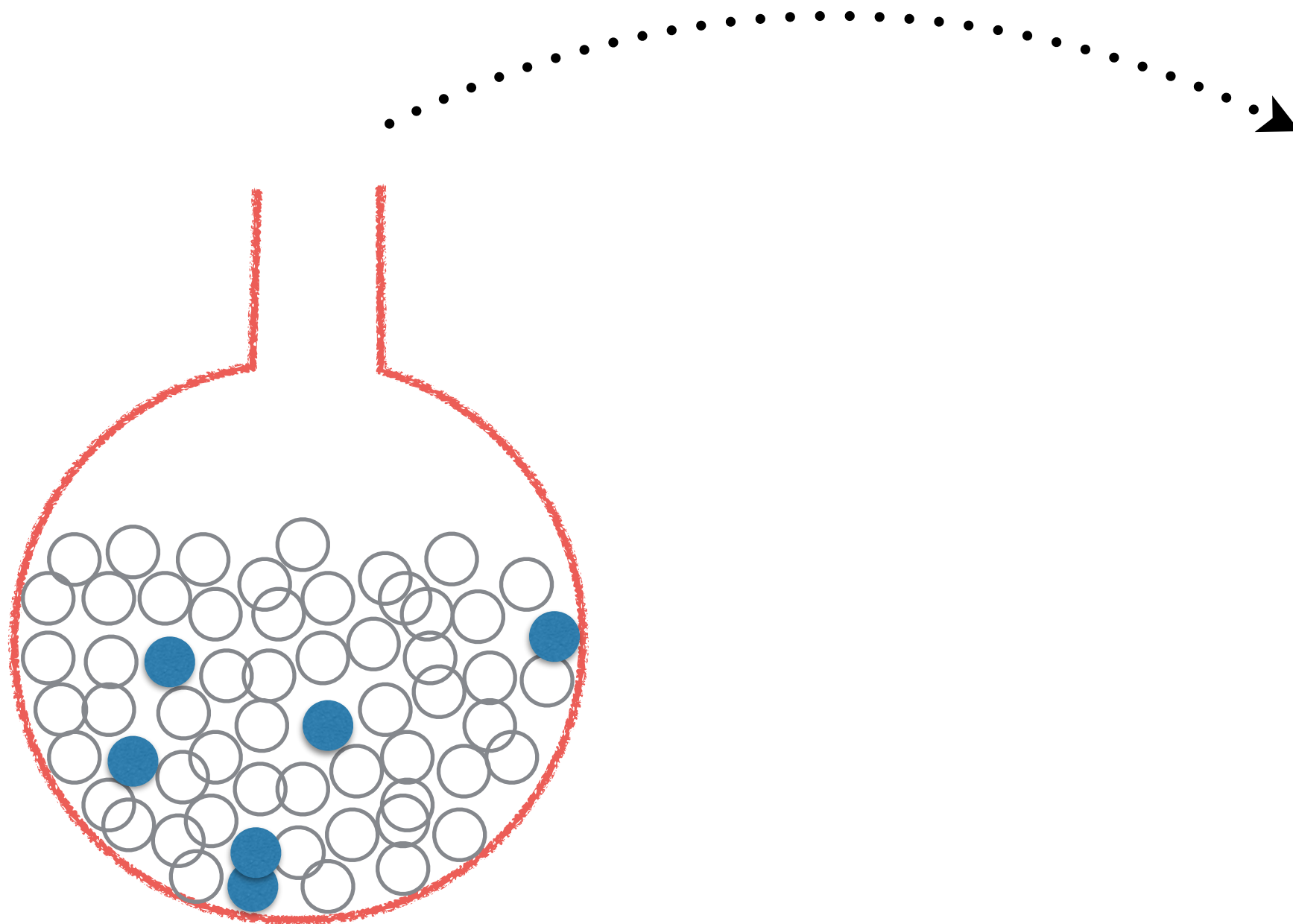
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If instead of expectation, you find that 50 of these 1,000 have the GO term “Cell wall modification”, then what do you conclude?

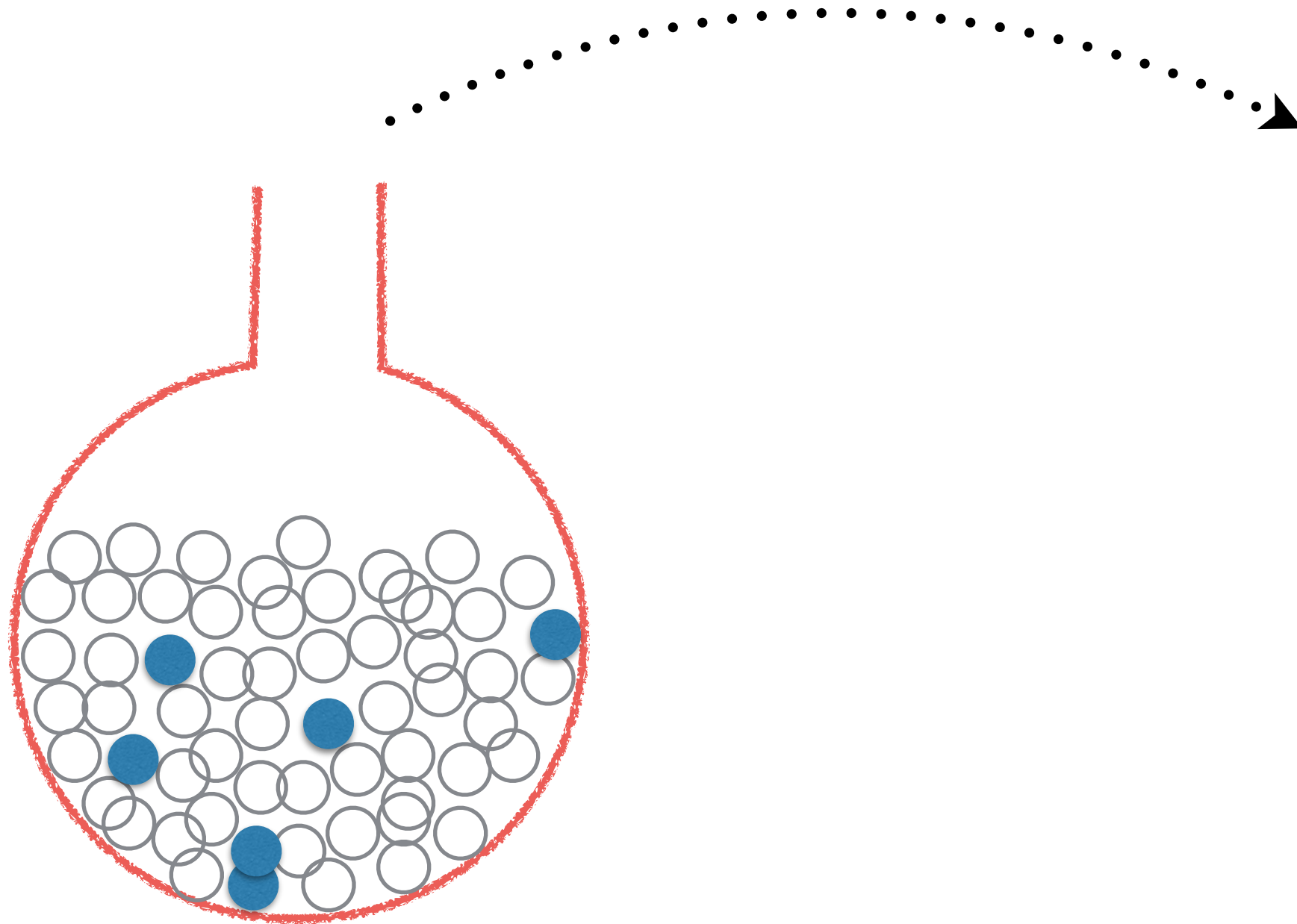
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Over-representation analysis: additional considerations

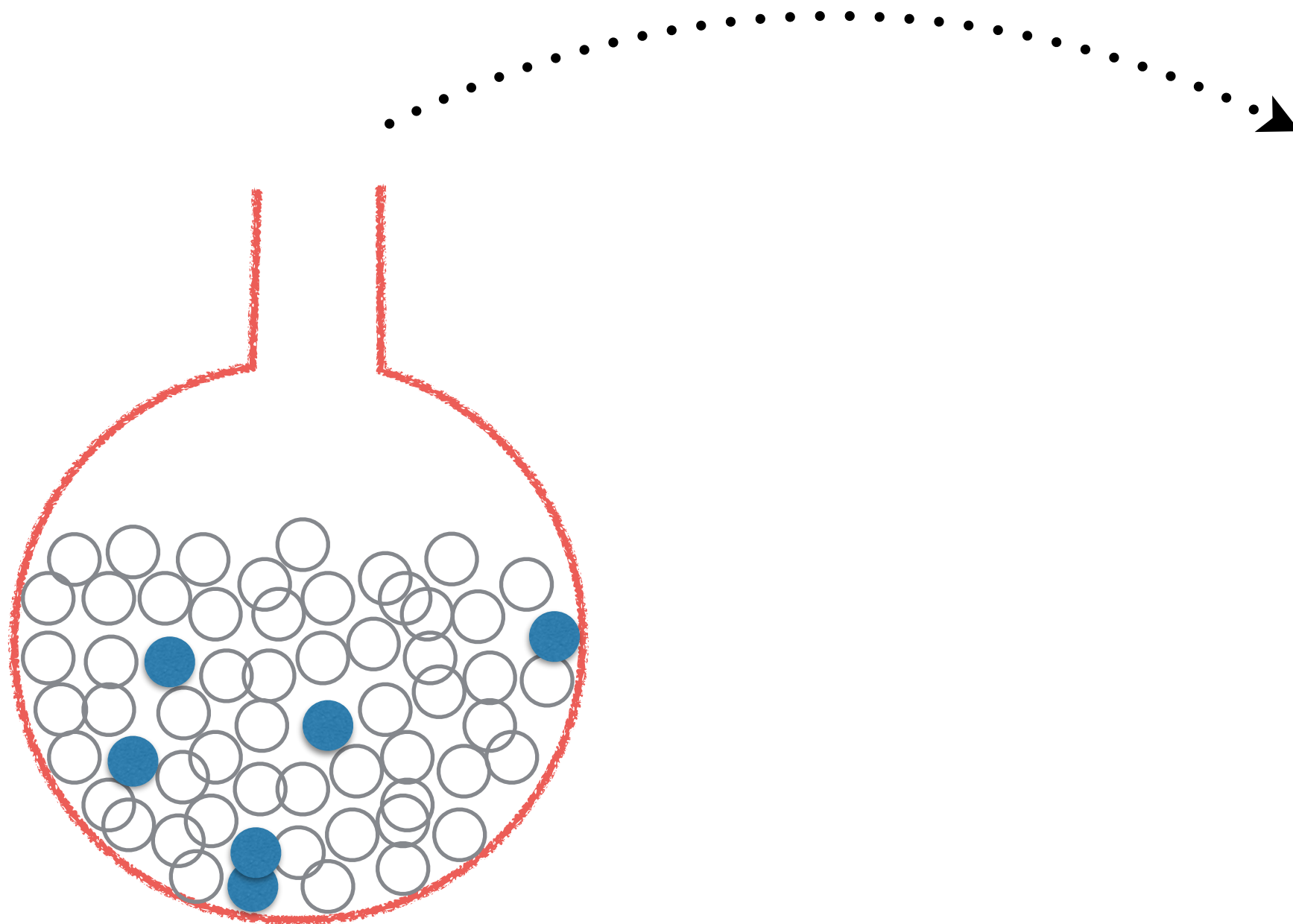


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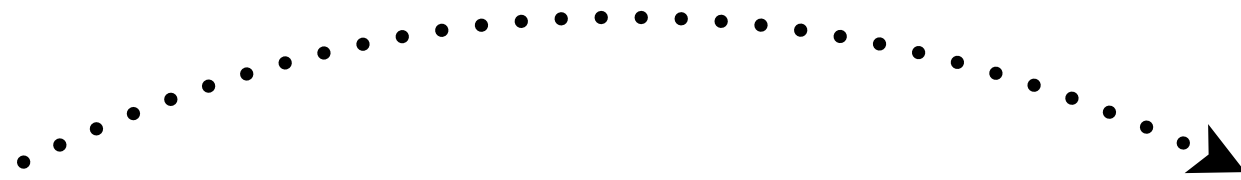


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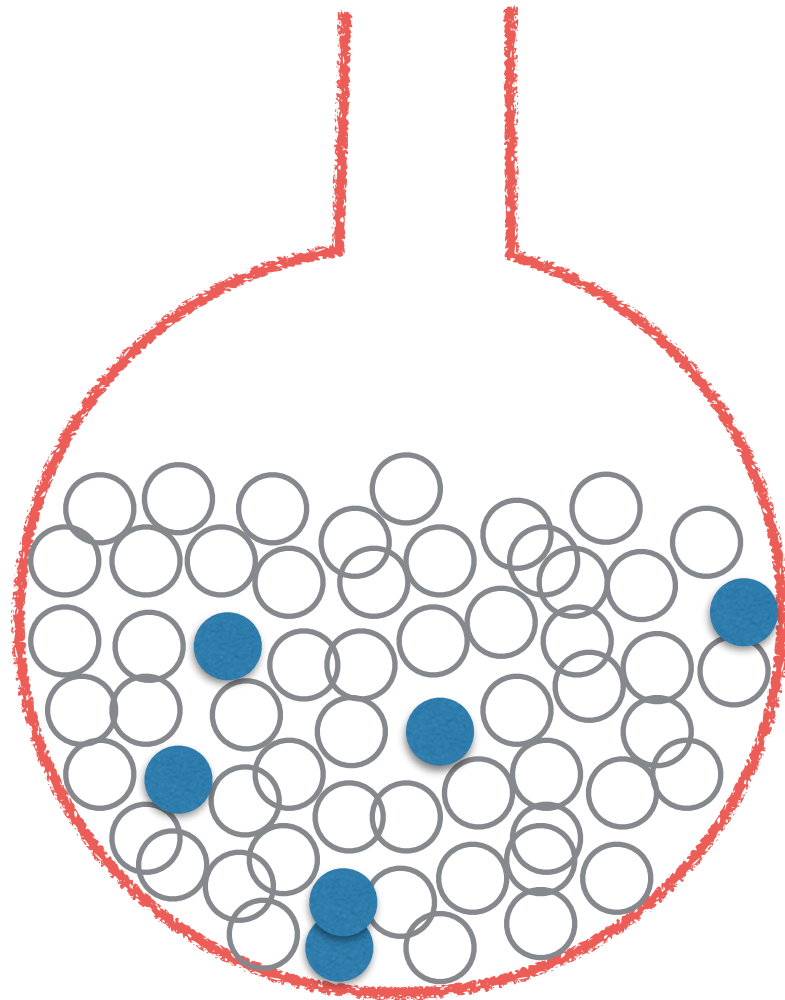


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2) Sequence length bases in detecting differentially expressed genes should be considered



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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 32710:290 different from 950:50)?

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- 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?)