
# Recap of Lecture 8

- Introduction to machine learning
  - Unsupervised, supervised and reinforcement learning
- Clustering
  - K-means clustering
  - Hierarchical clustering
- Dimensionality reduction, visualization and 'structure' analysis
  - Principal Component Analysis (PCA)

[Muddy Point Feedback Link]

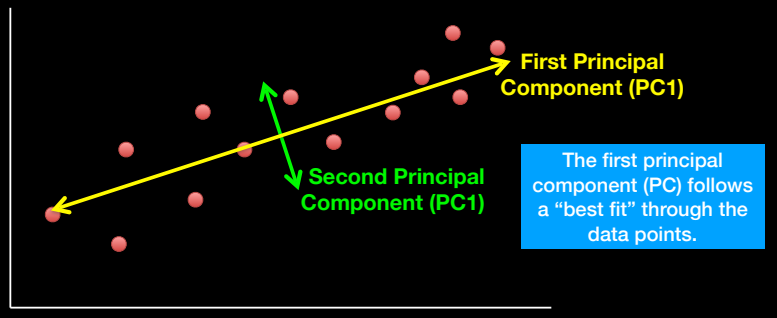
# Recap: PCA objectives

- To reduce dimensionality
- To visualize multidimensional data
- To choose the most useful variables (features)
- To identify groupings of objects (e.g. genes/samples)
- To identify outliers

# PCA: Principal Component Analysis

PCA projects the features onto the principal components.

The motivation is to reduce the features dimensionality while only losing a small amount of information.



# Practical PCA issue: Scaling

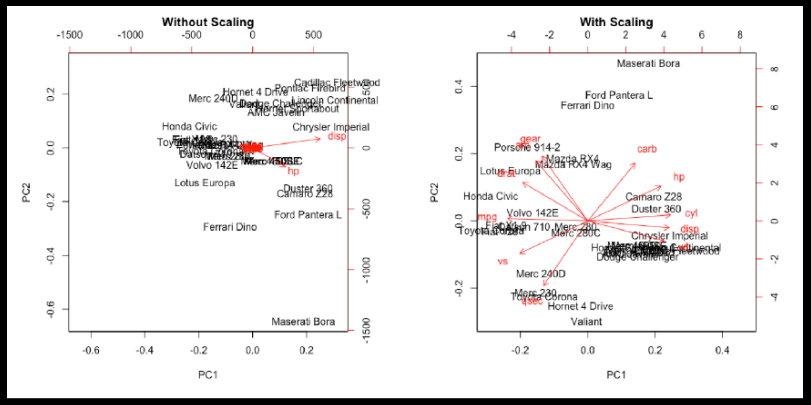
```
> data(mtcars)
> head(mtcars)
                   mpg cyl disp  hp drat    wt  qsec vs am gear carb
Mazda RX4         21.0   6  160 110 3.90 2.620 16.46  0  1    4    4
Mazda RX4 Wag     21.0   6  160 110 3.90 2.875 17.02  0  1    4    4
Datsun 710        22.8   4  108  93 3.85 2.320 18.61  1  1    4    1
Hornet 4 Drive    21.4   6  258 110 3.08 3.215 19.44  1  0    3    1
Hornet Sportabout 18.7   8  360 175 3.15 3.440 17.02  0  0    3    2
Valiant           18.1   6  225 105 2.76 3.460 20.22  1  0    3    1

# Means and standard deviations vary a lot
> round(colMeans(mtcars), 2)
   mpg    cyl   disp     hp   drat     wt   qsec     vs     am   gear   carb
 20.09   6.19 230.72 146.69   3.60   3.22  17.85   0.44   0.41   3.69   2.81
> round(apply(mtcars, 2, sd), 2)
   mpg    cyl   disp     hp   drat     wt   qsec     vs     am   gear   carb
  6.03   1.79 123.94  68.56   0.53   0.98   1.79   0.50   0.50   0.74   1.62
```

# Practical PCA issue: Scaling

`prcomp(x, scale=FALSE)`

`prcomp(x, scale=TRUE)`




Do it Yourself!

# Your turn!

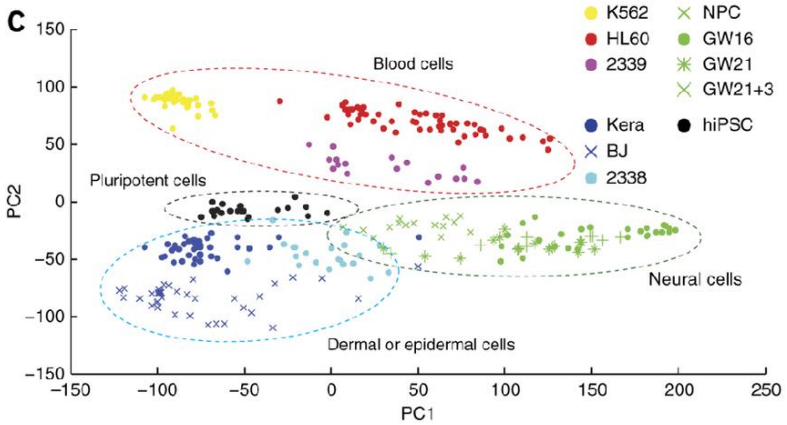
Unsupervised Learning Mini-Project

**Input:** read, View/head,
**PCA:** prcomp,
**Cluster:** kmeans, hclust
**Compare:** plot, table, etc.

# Reference Slides

# This PCA plot shows clusters of cell types.

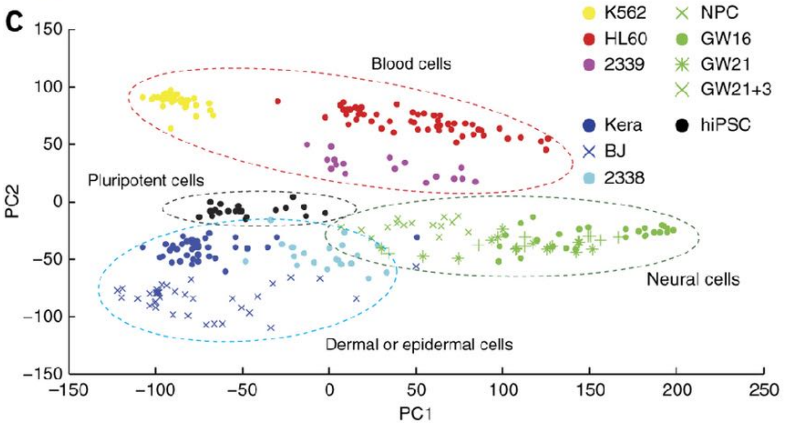
This graph was drawn from single-cell RNA-seq.
There were about 10,000 transcribed genes in each cell.




Pollen et al. Nature Biotechnology 2014

# This PCA plot shows clusters of cell types.

Each dot represents a single-cell and its transcription profile
The general idea is that cells with similar transcription should cluster.

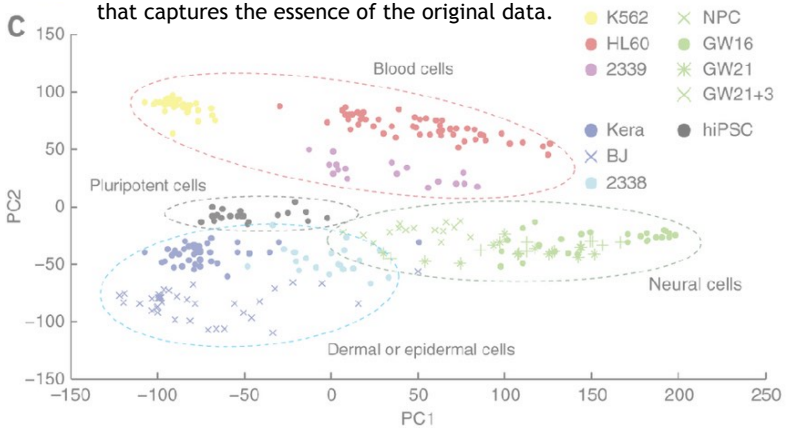



Pollen et al. Nature Biotechnology 2014

# This PCA plot shows clusters of cell types.

How does transcription from 10,000 genes get compressed to a single dot on a graph?

PCA is a method for compressing a lot of data into something that captures the essence of the original data.




# What does PCA aim to do?

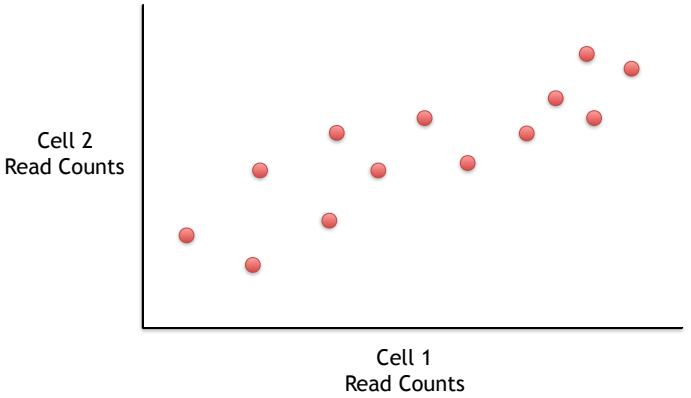
- PCA takes a dataset with a lot of dimensions (i.e. lots of cells) and flattens it to 2 or 3 dimensions so we can look at it.
  - It tries to find a meaningful way to flatten the data by focusing on the things that are different between cells. (much, much more on this later)

# A PCA example

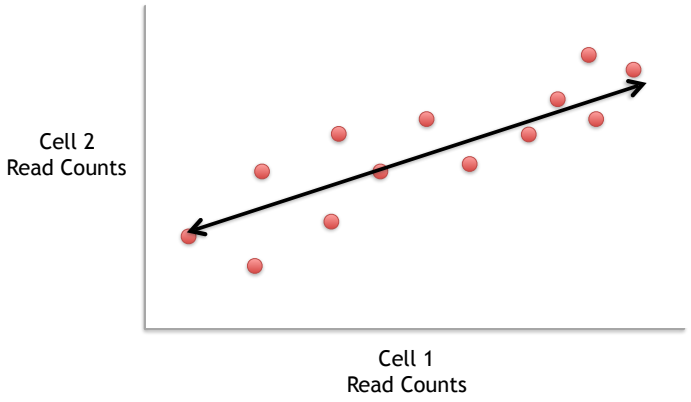
Again, we'll start with just two cells
Here's the data:

| Gene | Cell1 reads | Cell2 reads |
|---|---|---|
| a | 10 | 8 |
| b | 0 | 2 |
| c | 14 | 10 |
| d | 33 | 45 |
| e | 50 | 42 |
| f | 80 | 72 |
| g | 95 | 90 |
| h | 44 | 50 |
| i | 60 | 50 |
| … (etc) | … (etc) | … (etc) |

Here is a 2-D plot of the data from 2 cells.

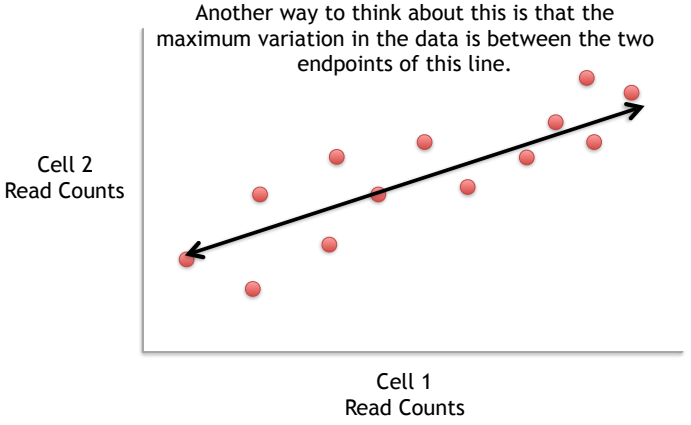



Generally speaking, the dots are spread out along a diagonal line.

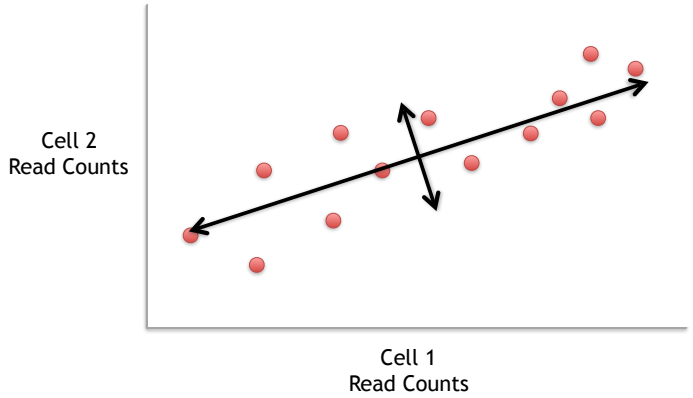



Generally speaking, the dots are spread out along a diagonal line.

Another way to think about this is that the maximum variation in the data is between the two endpoints of this line.

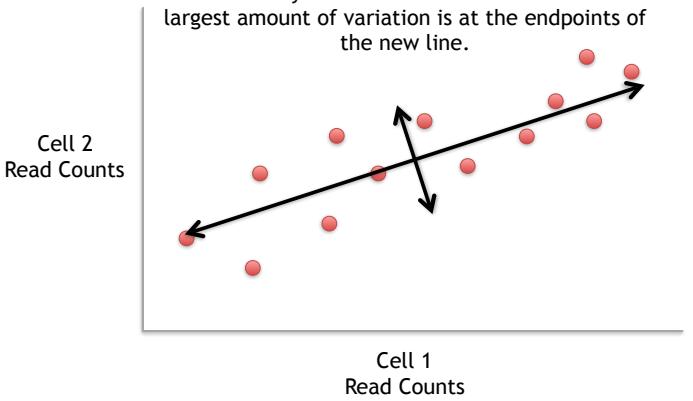


Generally speaking, the dots are also spread out a little above and below the first line.

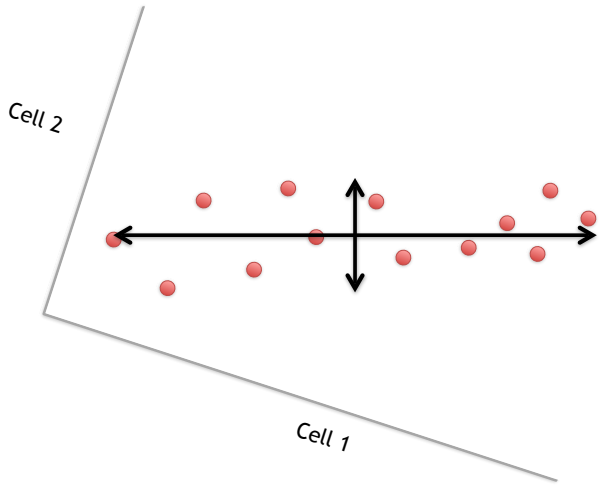



Generally speaking, the dots are also spread out a little above and below the first line.

Another way to think about this is that the 2nd largest amount of variation is at the endpoints of the new line.

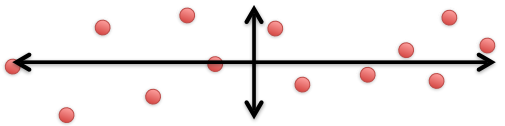



If we rotate the whole graph, the two lines that we drew make new X and Y axes.




If we rotate the whole graph, the two lines that we drew make new X and Y axes.

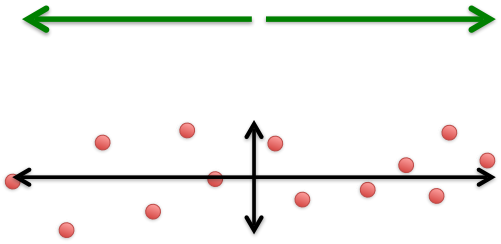
This makes the left/right, above/below variation easier to see.



If we rotate the whole graph, the two lines that we drew make new X and Y axes.

This makes the left/right, above/below variation easier to see.

1) The data varies **a lot** left and right



If we rotate the whole graph, the two lines that we drew make new X and Y axes.

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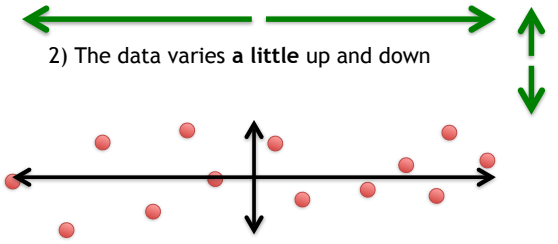
1) The data varies **a lot** left and right

2) The data varies **a little** up and down



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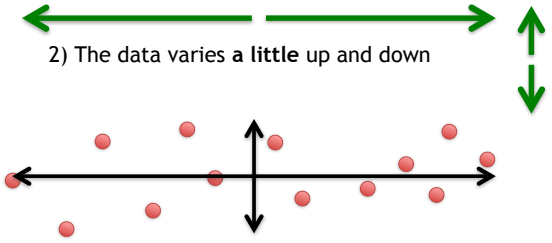
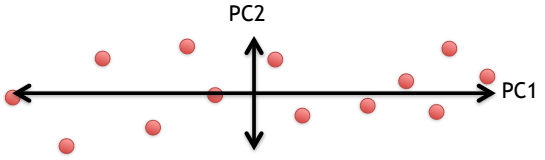


Note: All of the points can be drawn in terms of left/right + up/down, just like any other 2-D graph.

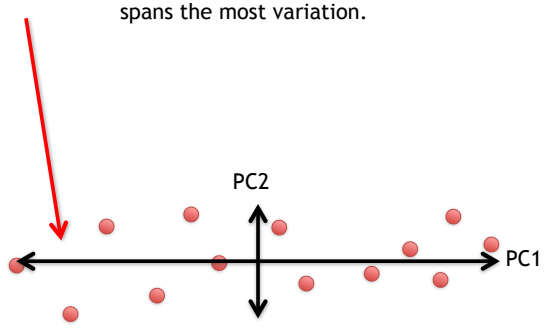
That is to say, we do not need another line to describe "diagonal" variation – we've already captured the two directions that can have variation.

These two "new" (or "rotated") axes that describe the variation in the data are "Principal Components" (PCs)



These two “new” axes that describe the variation in the data are “Principal Components” (PCs)

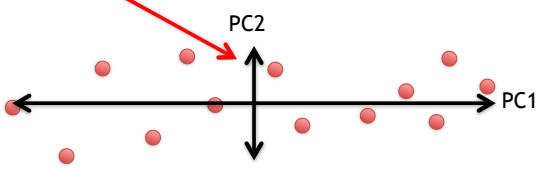
PC1 (the first principal component) is the axis that spans the most variation.




These two “new” axes that describe the variation in the data are “Principal Components” (PCs)

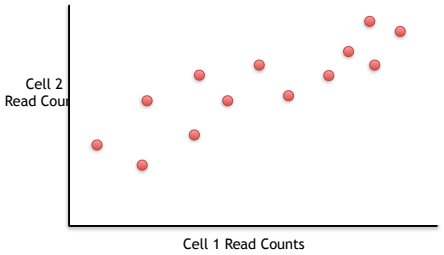
PC1 (the first principal component) is the axis that spans the most variation.

PC2 is the axis that spans the second most variation.




# General ideas so far…

- For each gene, we plotted a point based on how many reads were from each cell.

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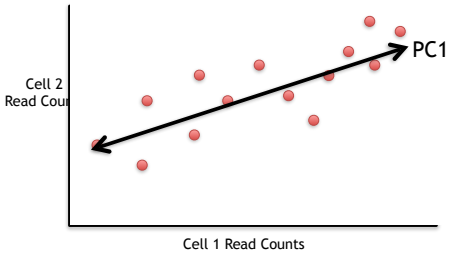


- PC1 captures the direction where most of the variation is.

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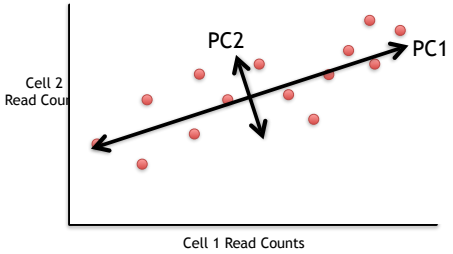
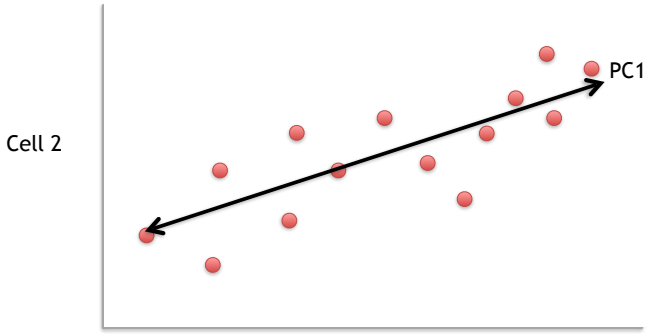
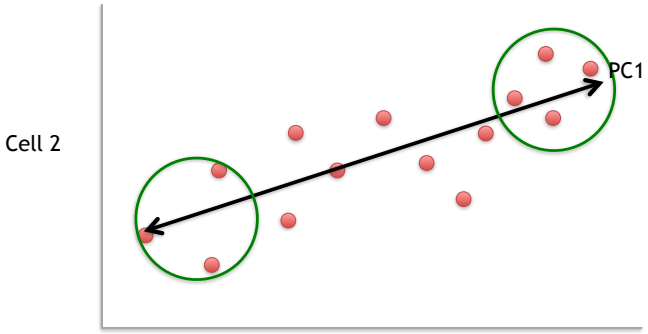



- PC1 captures the direction where most of the variation is.
- PC2 captures the direction with the 2nd most variation.

For now, let's focus on PC1




The length and direction of PC1 is mostly determined by the circled genes.

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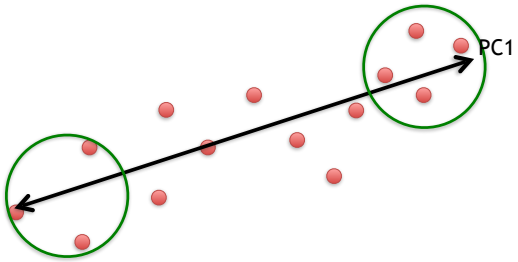

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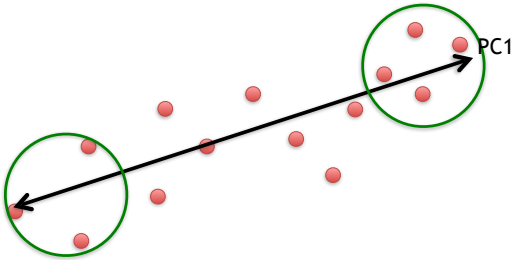
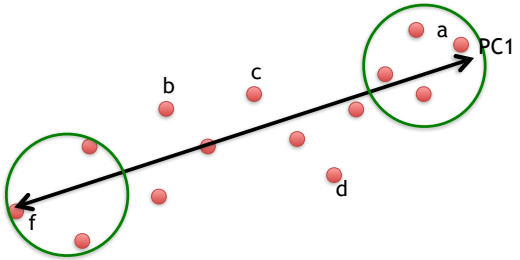



We can score genes based on how much they influence PC1.

The length and direction of PC1 is mostly determined by the circled genes.




We can score genes based on how much they influence PC1.

| Gene | Influence on PC1 |
|---|---|
| a | high |
| b | low |
| c | low |
| d | low |
| e | high |
| f | high |
| … | … |

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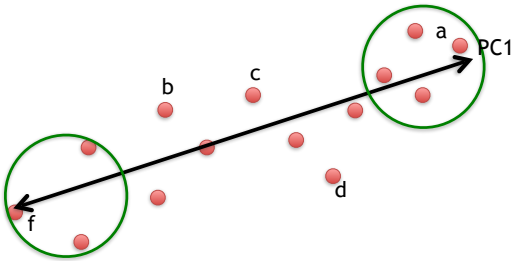


Some genes have more influence on PC1 than others.

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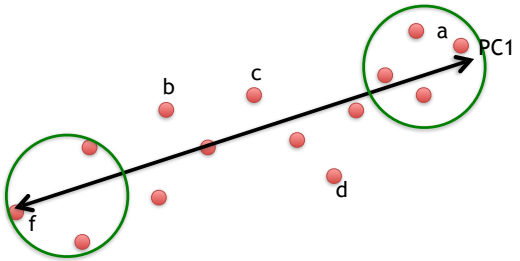
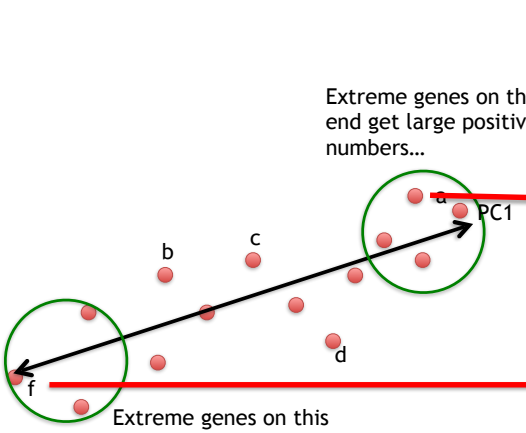



Some genes have more influence on PC1 than others.

| Gene | Influence on PC1 | In numbers |
|---|---|---|
| a | high | 10 |
| b | low | 0.5 |
| c | low | 0.2 |
| d | low | -0.2 |
| e | high | 13 |
| f | high | -14 |
| … | … | |

Genes with little influence on PC1 get values close to zero, and genes with more influence get numbers further from zero.

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Extreme genes on this end get large positive numbers…

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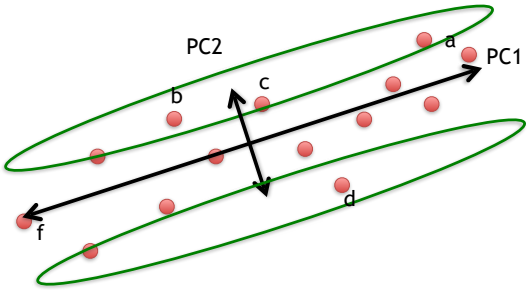
# Genes that influence PC2




| Gene | Influence on PC2 | In numbers |
|---|---|---|
| a | medium | 3 |
| b | high | 10 |
| c | high | 8 |
| d | high | -12 |
| e | low | 0.2 |
| f | low | -0.1 |
| … | … | |

# Our two PCs

PC1

| Gene | Influence on PC1 | In numbers |
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# Using the two Principal Components to plot cells

Combining the read counts for all genes in a cell to get a single value.

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The original read counts

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| … | … | |

Cell1 PC1 score = (read count * influence) + … for all genes

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| d | high | -12 |
| e | low | 0.2 |
| f | low | -0.1 |
| … | … | |

Cell1 PC1 score = (10 * 10) + …

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| c | high | 8 |
| d | high | -12 |
| e | low | 0.2 |
| f | low | -0.1 |
| … | … | |

Cell1 PC1 score = (10 * 10) + (0 * 0.5) + …

# Using the two Principal Components to plot cells

Combining the read counts for all genes in a cell to get a single value.

The original read counts

| Gene | Cell1 | Cell2 |
|---|---|---|
| a | 10 | 8 |
| b | 0 | 2 |
| c | 14 | 10 |
| d | 33 | 45 |
| e | 50 | 42 |
| f | 80 | 72 |
| g | 95 | 90 |
| h | 44 | 50 |
| i | 60 | 50 |
| etc | etc | etc |

PC1

| Gene | Influence on PC1 | In numbers |
|---|---|---|
| a | high | 10 |
| b | low | 0.5 |
| c | low | 0.2 |
| d | low | -0.2 |
| e | high | 13 |
| f | high | -14 |
| … | … | |

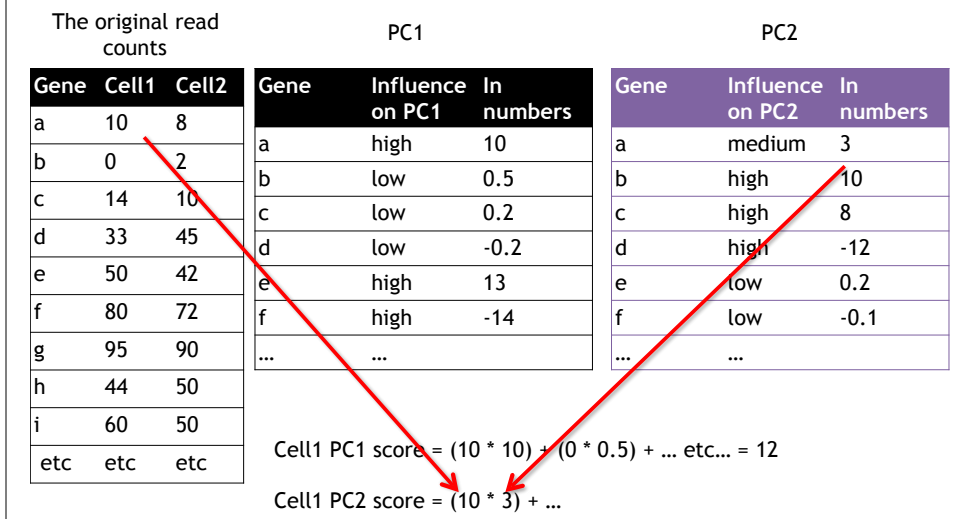
PC2

| Gene | Influence on PC2 | In numbers |
|---|---|---|
| a | medium | 3 |
| b | high | 10 |
| c | high | 8 |
| d | high | -12 |
| e | low | 0.2 |
| f | low | -0.1 |
| … | … | |

Cell1 PC1 score = (10 * 10) + (0 * 0.5) + … etc… = 12

# Using the two Principal Components to plot cells

Combining the read counts for all genes in a cell to get a single value.



The original read counts

PC1

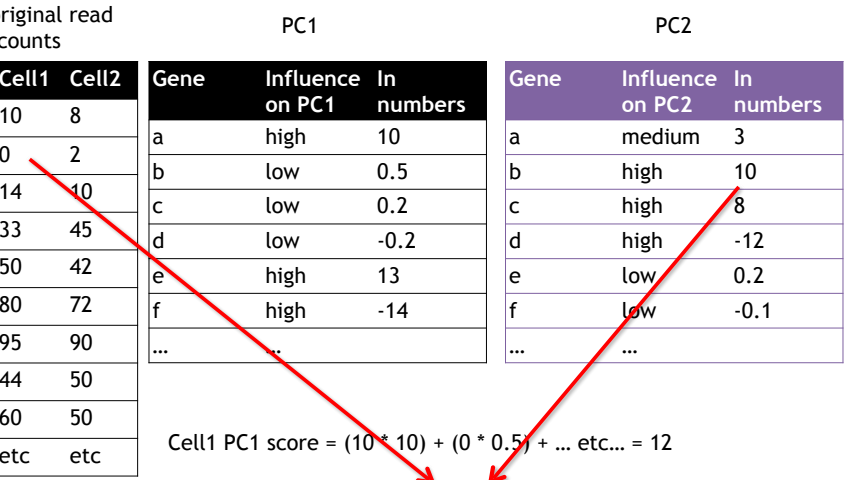
PC2

Cell1 PC1 score = (10 * 10) + (0 * 0.5) + … etc… = 12

Cell1 PC2 score = (10 * 3) + …

# Using the two Principal Components to plot cells

Combining the read counts for all genes in a cell to get a single value.



The original read counts

PC1

PC2

Cell1 PC1 score = (10 * 10) + (0 * 0.5) + … etc… = 12

Cell1 PC2 score = (10 * 3) + (0 * 10) + …

# Using the two Principal Components to plot cells

Combining the read counts for all genes in a cell to get a single value.

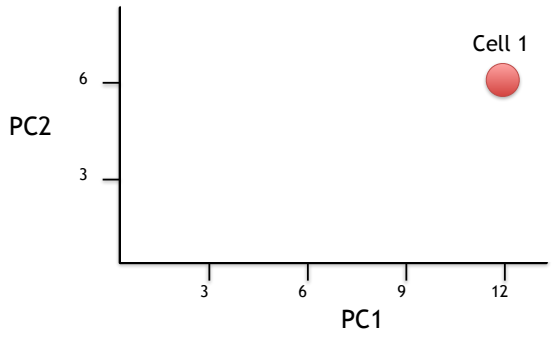
The original read counts

PC1

PC2

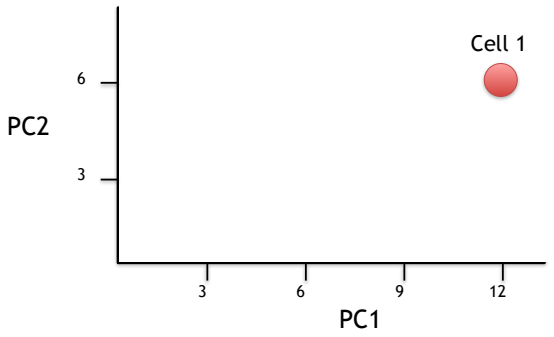
Cell1 PC1 score = (10 * 10) + (0 * 0.5) + … etc… = 12

Cell1 PC2 score = (10 * 3) + (0 * 10) + … etc… = 6

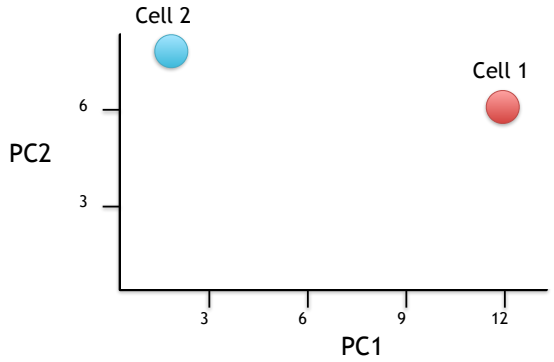



Cell1 PC1 score = (10 * 10) + (0 * 0.5) + … etc… = 12

Cell1 PC2 score = (10 * 3) + (0 * 10) + … etc… = 6



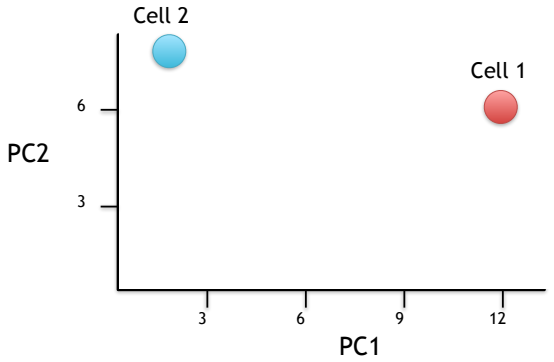

Now calculate scores for Cell2



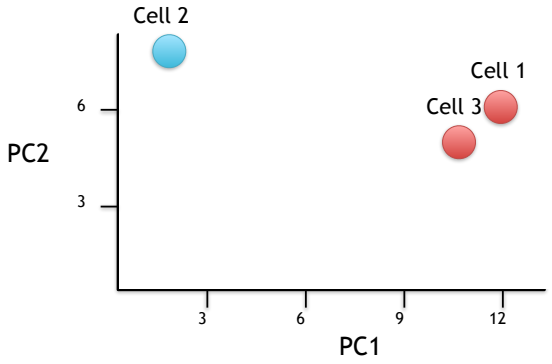

Now calculate scores for Cell2

Cell2 PC1 score = (8 * 10) + (2 * 0.5) + … etc… = 2

Cell2 PC2 score = (8 * 3) + (2 * 10) + … etc… = 8

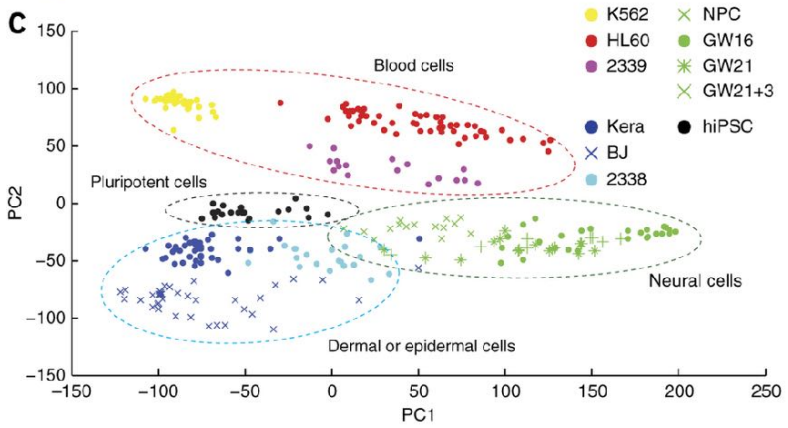



If we sequenced a third cell, and its transcription was similar to cell 1, it would get scores similar to cell 1's.




If we sequenced a third cell, and its transcription was similar to cell 1, it would get scores similar to cell 1's.

## Hooray! We know how they plotted all of the cells!!!




Do it Yourself!

# Back to lab Section 3 to 6…

Unsupervised Learning Mini-Project

**Input:** read, View/head,
**PCA:** prcomp,
**Cluster:** kmeans, hclust
**Compare:** plot, table, etc.

[ Muddy Point Assessment ]