1. **Data exploration**
   1. Visualization of all TPM values for all genes as **BOXPLOT**
   2. **HEATMAP (not correlation)** all genes & CERES scores with three colours (red <0, orange =0, green >0)
   3. Maybe general distribution of CERES scores of all genes – three bars (one for negative, second for 0 and third for positive scores)- **BARPLOT**
2. **PCA Analysis** of [TPM Matrix]- dataset reduced to all genes important in given cell lines for breast cancer
   1. Visualize impact of viability of genes via Boxplot of CERES important genes
      1. Direct identification of important genes with no impact on cell viability
   2. Heat map of PCA genes (y-axis), cell samples (x-axis) and colours representing CERES scores
      1. Could help identify important genes reducing viability when knocked out
   3. Compare PCA genes to driver mutations found in literature research and visualize on Venn diagram
3. Visualize CERES and TPM values of 5 chosen driver mutations **BOXPLOT** 
   1. Should have negative CERES values and high TPM values

**Start with 1 driver mutation (Gene A):**

1. Generate heatmap with all genes (y-axis) & all selected cell samples (x-axis) with colours representing CERES values
   1. Identification of genes with negative values (in general without differentiating between mutated and not mutated genes)
2. **K-means clustering** of genes into k groups (based on silhouette/elbow factor) based on average CERES values in all cell lines
   1. Identify cluster of driver mutation
   2. Check for presence of second site targets in cluster around driver mutation
3. Generate filter with information on the presence of gene mutation in cell line or not (for all genes) – matrix/filter with 1 for mutation and N/A for not mutated
4. Apply filter to [CERES Matrix] with all genes
5. **Paired Wilcoxon signed rank Test** (non-parametric test) calculating t-values for Gene A with all other genes in
   1. Perhaps apply correction to p-value (Bonferroni correction etc.)
   2. Identify X number of second site targets (genes) based on p-value (range needs to be specified)
   3. Should correlation value coincide with t-value?
6. Check TPM values for X second site targets
   1. Overexpressed, underexpressed?
7. Could analyze type mutation in [Mutation matrix] or [Copy number matrix] if gene amplified or deleted
8. Compare CERES scores of: a) cell lines with driver mutation and second site target mutation and b) cell lines with driver mutation but no second site target mutation (regarded as wildtype)

**Repeat for other driver mutations**

**Obtain 5 sets of driver mutations and interactions with second site targets (maybe second site targets overlap?)**

1. **Regression analysis**- check if CERES scores of second site targets can be used to predict CERES scores of driver mutations
   1. Regression model
   2. F-test to verify regression model in comparison to null model of no correlation
      1. If good regression model- evidence for genetic interaction between driver mutations and second site targets