Load required functions and modules:

```
In [1]: from urllib.parse import quote
        from pandas import read_csv, MultiIndex
        from re import sub
        from numpy import log10
        from rpy2 import robjects
        from rpy2.robjects.packages import importr
        from rpy2.robjects.pandas2ri import rpy2py_dataframe
        from matplotlib.pyplot import subplots, style, rc
        from seaborn import scatterplot, clustermap
        from warnings import catch_warnings, simplefilter

        deseq2 = importr("DESeq2")
        style.use(["seaborn-poster", "seaborn-whitegrid"])
        rc("axes", linewidth=1, edgecolor="black")
        %matplotlib inline
```

Construct base query:
RNA-Seq counts of all Mus musculus liver samples with the factor of "spaceflight" in the database; additionally, request that these samples have unnormalized counts files associated with them, which will also include the names of these files in the returned table.

```
In [2]: API_ROOT = "https://visualization.genelab.nasa.gov/GLOpenAPI"

        query_components = {
            "study.factor value.spaceflight": "",
            "investigation.study assays.study assay technology type": "RNA Sequencing (RNA-Seq)",
            "study.characteristics.organism": "Mus musculus",
            "study.characteristics.material type": "Liver",
            "file.datatype": "unnormalized counts",
            "format": "csv",
        }

        query = "&".join(f"{quote(k)}={quote(v)}" for k, v in query_components.items())
        query
```

```
Out[2]: 'study.factor%20value.spaceflight=&investigation.study%20assays.study%20assay%20technolog
        y%20type=RNA%20Sequencing%20%28RNA-Seq%29&study.characteristics.organism=Mus%20musculus&st
        udy.characteristics.material%20type=Liver&file.datatype=unnormalized%20counts&format=csv'
```

Retrieve metadata:
the first two rows of the CSV are the header; the first three columns are the index.

```
In [3]: metadata = read_csv(f"{API_ROOT}/samples/?{query}", header=[0, 1], escapechar="#")
        metadata.index = MultiIndex.from_frame(metadata[["id"]])
        del metadata["id"]
        metadata.head()
```

Out[3]:

			investigation.study assays	study.characteristics		stu val
			study assay technology type	material type	organism	spa
(id, accession)	(id, assay name)	(id, sample name)				
GLDS-168	nist-liver_transcription_profiling_RNA_Sequencing_(RNA-Seq)	Mmus_BAL-TAL_LVR_RR3_BSL_wERCC_Rep1_B2	RNA Sequencing (RNA-Seq)	Liver	Mus musculus	
		Mmus_BAL-TAL_LVR_RR3_BSL_wERCC_Rep2_B4	RNA Sequencing (RNA-Seq)	Liver	Mus musculus	
		Mmus_BAL-TAL_LVR_RR3_BSL_wERCC_Rep3_B6	RNA Sequencing (RNA-Seq)	Liver	Mus musculus	
		Mmus_BAL-TAL_LVR_RR3_BSL_wERCC_Rep4_B7	RNA Sequencing (RNA-Seq)	Liver	Mus musculus	
		Mmus_BAL-TAL_LVR_RR3_FLT_wERCC_Rep1_F1	RNA Sequencing (RNA-Seq)	Liver	Mus musculus	Sp

Note that this allowed us to retrieve multiple samples across *multiple datasets:*

```
In [4]: metadata.index.to_frame()["id"][["accession", "sample name"]].groupby("accession").count()
```

Out[4]:

	sample name
accession	
GLDS-164	6
GLDS-168	52
GLDS-47	9
GLDS-48	14

Retrieve unnormalized counts:
the first *three* rows are the header (corresponding to the index of `metadata`), the first column is the index (gene ID). Note that while we requested the names of unnormalized counts files, we do not need to retrieve these files directly; GLOpenAPI processes them remotely and delivers a merged counts table to the user.

```
In [5]: counts = read_csv(f"{API_ROOT}/data/?{query}", header=[0, 1, 2], index_col=0, escapechar="#")
        counts.head()
```

Out[5]:

*	GLDS-47			
*	rr1-casis_transcription_profiling_RNA_Sequencing_(RNA-Seq)			
index	**Mmus_C57-6T_LVR_BSL_Rep1_B1**	**Mmus_C57-6T_LVR_BSL_Rep2_B2**	**Mmus_C57-6T_LVR_BSL_Rep3_B3**	**Mmus_C57-6T_LVR_**
ENSMUSG00000000001	1983.0	2708.0	2532.0	
ENSMUSG00000000003	0.0	0.0	0.0	
ENSMUSG00000000028	31.0	38.0	42.0	
ENSMUSG00000000031	35.0	37.0	48.0	
ENSMUSG00000000037	0.0	0.0	1.0	

5 rows × 81 columns

Construct R dataframes for use in DESeq2:
note that we collapse multilevel indices and columns to single-level rownames and colnames for simplicity, and replace spaces with dots in factor values ("Ground Control" becomes "Ground.Control") simply to avoid R/DESeq2 throwing warnings.

Since there are samples present from multiple studies, we will also account for it by introducing a factor "batch"

```
In [6]: metadata = metadata.reindex(counts.columns)

        colData = robjects.DataFrame({
            "batch": robjects.FactorVector(
                metadata.index.get_level_values(0).map(lambda s: s.replace("-", "_")),
            ),
            "spaceflight": robjects.FactorVector(
                metadata[("study.factor value", "spaceflight")].map(lambda s: s.replace(" ", ".")),
            ),
        })
        colData.rownames = metadata.index.map("/".join).tolist()

        counts_fillna = counts.fillna(0)
        countData = robjects.DataFrame({"/".join(col): robjects.IntVector(counts_fillna[col]) for col in counts_fill
        countData.rownames = counts.index.tolist()
```

Run DESeq2 to infer differentially expressed genes between the conditions "Space Flight" and "Ground Control", convert the results back to pandas DataFrames, add custom columns:

```
In [7]: analysis = deseq2.DESeq(
            deseq2.DESeqDataSetFromMatrix(
                countData=countData, colData=colData,
                design=robjects.Formula("~spaceflight+batch"),
            ),
            test="LRT", reduced=robjects.Formula("~batch"),
        )

        r_results = deseq2.results(
            analysis, contrast=robjects.StrVector([
                "spaceflight", "Space.Flight", "Ground.Control",
            ]),
        )

        results = rpy2py_dataframe(robjects.r("function(x) data.frame(x)")(r_results))
        results["-log10(padj)"] = -log10(results["padj"].fillna(1))
        results["significant"] = (results["padj"]<.05) & (results["log2FoldChange"].abs()>2)
```

```
R[write to console]: estimating size factors

R[write to console]: estimating dispersions

R[write to console]: gene-wise dispersion estimates

R[write to console]: mean-dispersion relationship

R[write to console]: final dispersion estimates

R[write to console]: fitting model and testing

R[write to console]: -- replacing outliers and refitting for 560 genes
-- DESeq argument 'minReplicatesForReplace' = 7
-- original counts are preserved in counts(dds)
```

Display the table of genes identified as significantly differentially expressed:

```
In [8]: results[results["significant"]].drop(columns=["-log10(padj)", "significant"]).sort_values(by="log2FoldChange
```

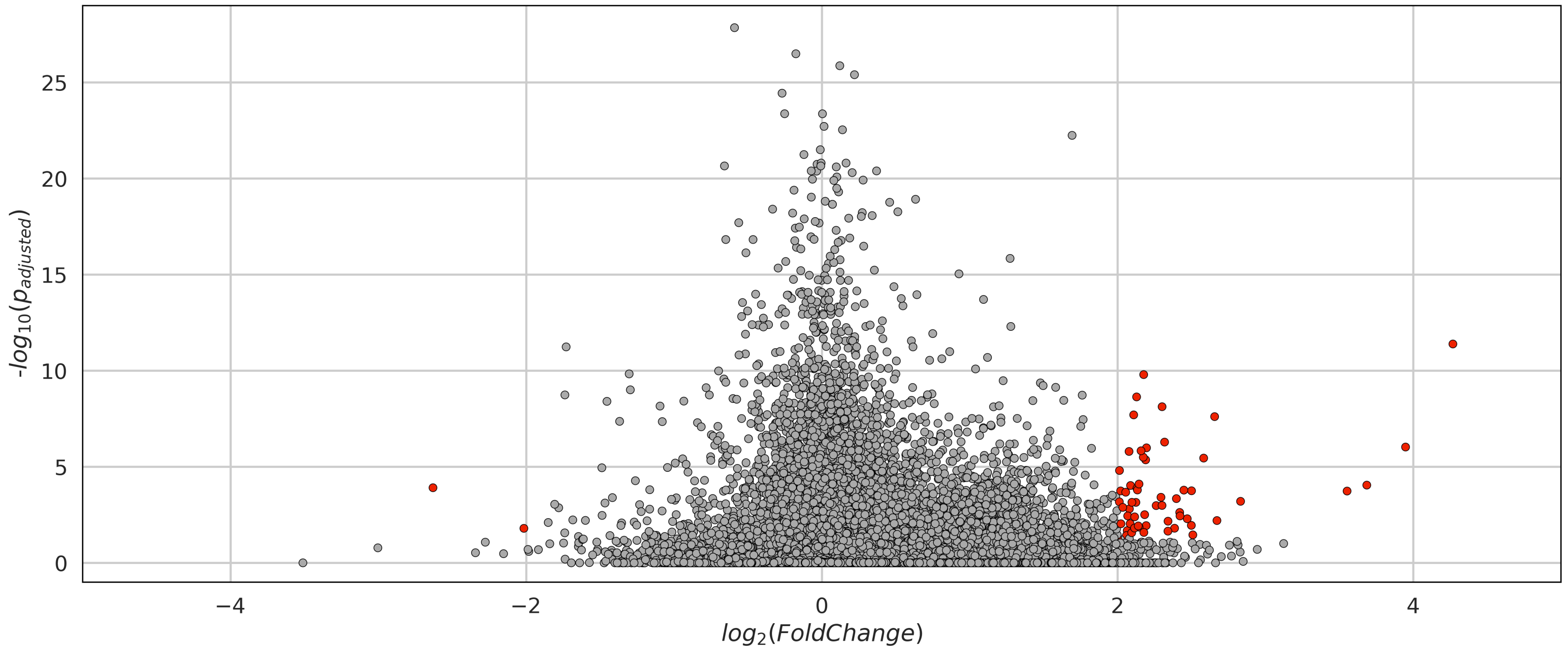
```
Out[8]:
```

	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
ENSMUSG00000096490	4.253197	-2.629335	0.615761	26.085283	9.153208e-06	1.235335e-04
ENSMUSG00000095351	42.389562	-2.014465	0.565094	13.551140	3.584393e-03	1.603816e-02
ENSMUSG00000049252	1.207579	2.000743	1.302016	10.484232	1.486824e-02	4.849641e-02
ENSMUSG00000083070	1.145412	2.013473	0.517538	21.902540	6.834892e-05	6.700135e-04
ENSMUSG00000118203	10.702841	2.014427	0.444351	31.111709	8.052326e-07	1.565135e-05
...	...	...	...	...	...	...
ENSMUSG00000028332	5.951008	2.833538	0.754895	22.057580	6.345610e-05	6.297004e-04
ENSMUSG00000020490	4.308256	3.554023	0.846128	25.111848	1.463099e-05	1.842217e-04
ENSMUSG00000029866	3.493003	3.687375	0.773622	26.851885	6.323662e-06	9.045238e-05
ENSMUSG00000092563	3.437059	3.949610	0.734759	37.733661	3.218307e-08	9.434801e-07
ENSMUSG00000110384	30.618018	4.269812	0.481242	65.989478	3.080932e-14	4.095827e-12

61 rows × 6 columns

Example: render a volcano plot

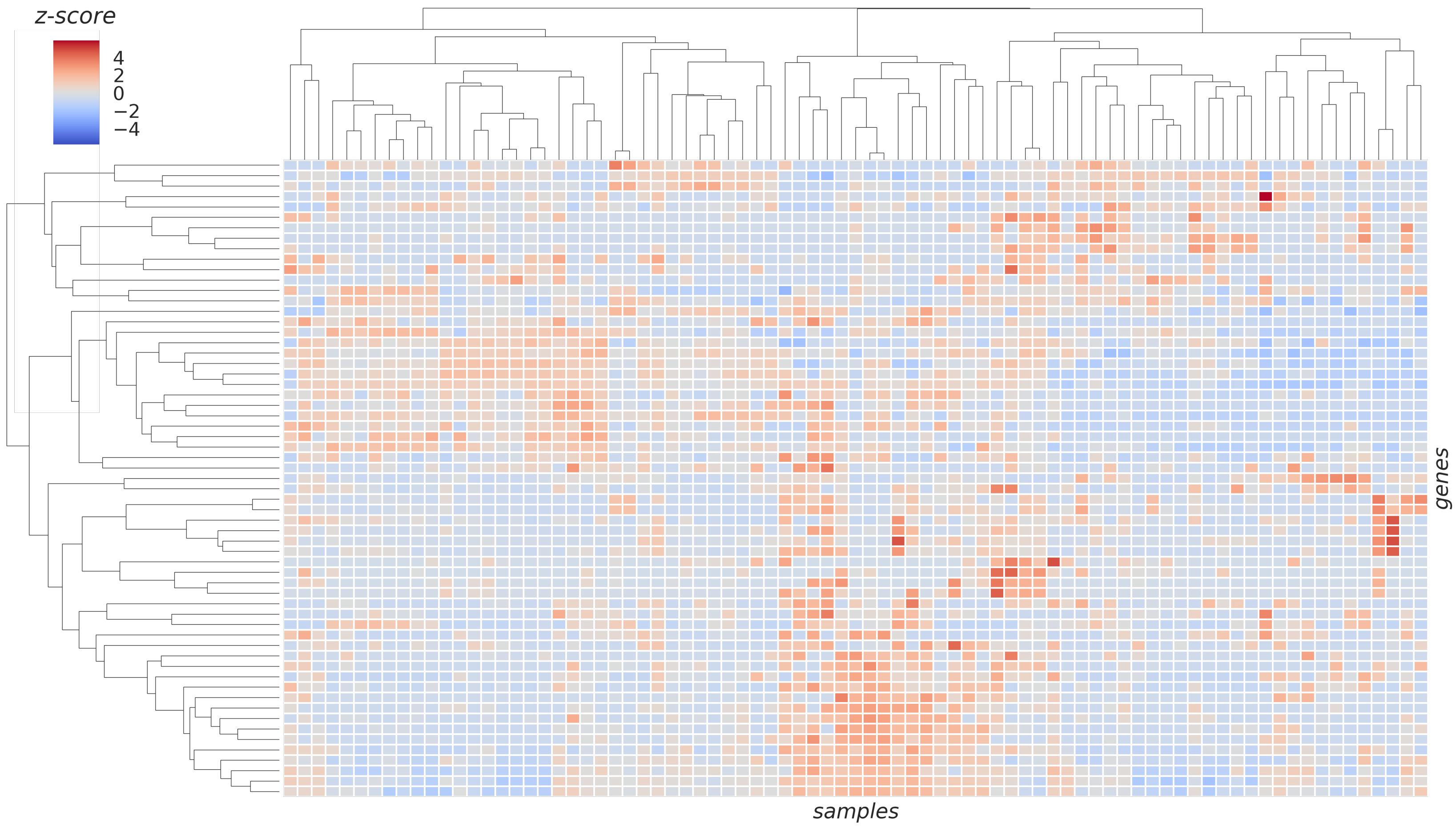
```
In [9]: figure, ax = subplots(figsize=(20, 8), dpi=300)
        scatterplot(
            ax=ax, data=results, x="log2FoldChange", y="-log10(padj)",
            hue="significant", palette=["#AAA", "#E20"], ec="#000", s=36,
        )
        ax.set(xlim=(-5, 5), ylim=(-1, 29), xlabel="$log_{2}(FoldChange)$", ylabel="-$log_{10}(p_{adjusted})$")

        with catch_warnings():
            simplefilter("ignore")
            ax.legend().remove()
```



Example: render a clustermap of z-scores of log-transformed normalized counts of differentially expressed genes

```
In [10]: sigcounts = counts.reindex(results[results["significant"]].index)
         lognorm_sc = log10(.1 + sigcounts / sigcounts.mean())
         zlognorm_sc = lognorm_sc.sub(lognorm_sc.mean(axis=1), axis=0).div(lognorm_sc.std(axis=1), axis=0)
         vmax = zlognorm_sc.abs().max().max()
```

```python
g = clustermap(data=zlognorm_sc, metric="correlation", vmin=-vmax, vmax=vmax, cmap="coolwarm", lw=1, figsize
g.ax_heatmap.set(xticks=[], yticks=[], xlabel="$samples$", ylabel="$genes$")
g.ax_cbar.set(xlim=(-15, 5), ylim=(-36, 7), zorder=-1, title=f"{' '*5}$z$-$score$")
g.fig.dpi = 300
```


z-score
genes
samples