**Basic Data Access & Validation**

**Positive testing:**

* "Show me the raw counts for GAPDH across all samples" 🡪 OK
* "What's the correlation between sample\_A\_1 and sample\_A\_2?" 🡪 KO – need to modify prompt to query diagonal matrix
* "Display the metadata for differentiated vs NS samples" 🡪 OK
* "How many genes are in the counts matrix?" 🡪 OK

**Negative testing:**

* "Display the metadata for control group samples" 🡪 OK (no control group)
* "What samples have the highest library sizes?" 🡪 KO – should say we don’t have that information

**Statistical Analysis Queries**

**Positive testing:**

* “With what comparisons has deseq2 been run?” 🡪 OK
* "Which genes are significantly upregulated in E-GSC vs L-GSC with padj < 0.01?" 🡪 OK
* "Show me the log2 fold changes for the top 20 differentially expressed genes" 🡪 OK (takes all samples subset)

**Negative testing:**

* "Run DESeq2 comparing treated vs control samples" 🡪 OK
* "What's the dispersion estimate for highly variable genes?" 🡪 OK
* "Display the size factors calculated by DESeq2" 🡪 OK

**Visualization & Dimensionality Reduction**

**Positive testing:**

* "Generate a PCA plot colored by treatment group" 🡪 OK
* "Show MDS plot with the first 500 most variable genes" 🡪 Just plotted the MDS
* "Display a heatmap of the top 50 differentially expressed genes" 🡪 KO (what is this)

**Negative testing:**

* "What percentage of variance is explained by PC1 and PC2?" 🡪 OK

**Gene Set Enrichment Analysis**

* " Run GSEA on hallmark pathways for the flattening yes vs no comparison in the late subset" 🡪 OK
* "Which GO biological processes are enriched in upregulated genes?"
* "Show me the normalized enrichment scores for immune-related hallmark pathways"
* "What molecular functions are over-represented in downregulated genes with padj < 0.05?"
* "Run ORA analysis using only genes with |log2FC| > 1.5"

**Complex Multi-Step Queries**

* "Compare the expression of cell cycle genes between timepoints and show their enrichment"
* "Find genes correlated with CD8A expression and test for T-cell pathway enrichment"
* "Identify the most variable genes in the control group and run GO analysis"
* "Show me differentially expressed transcription factors and their target pathway enrichment"

**Edge Cases & Error Handling**

* "What's the expression of NONEXISTENTGENE123?"
* "Run DESeq2 comparing group\_X vs group\_Y" (non-existent groups)
* "Show correlation between samples with only 2 replicates"
* "Run GSEA with an empty gene list"
* "Display PCA with categorical variables that have only one level"

**Ambiguity & Context Resolution**

* "Show me the top genes" (which comparison? how many? by what metric?)
* "Are there any significant pathways?" (which analysis? what threshold?)
* "Compare the groups" (which groups? what type of comparison?)
* "Show me the results" (which results from which analysis?)

**Technical Specificity Tests**

* "Use Benjamini-Hochberg correction with alpha = 0.01 for multiple testing"
* "Run DESeq2 with independent filtering disabled"
* "Show genes with baseMean > 100 and |log2FoldChange| > 2"
* "Use the top 1000 most variable genes for PCA"
* "Run GSEA with 10,000 permutations and weighted enrichment statistic"

**Cross-Analysis Integration**

* "Show the PCA loadings for genes that are enriched in oxidative phosphorylation"
* "Are the top PC1 genes enriched for any hallmark pathways?"
* "Compare the expression correlation patterns between treatment groups"
* "Which differentially expressed genes contribute most to sample clustering?"

**Biological Context & Interpretation**

* "What pathways are dysregulated in the disease samples?"
* "Are stress response genes consistently upregulated across replicates?"
* "Show me immune-related genes that are differentially expressed"
* "Which metabolic pathways show coordinated expression changes?"

**Performance & Scale Testing**

* "Correlate all genes with the first principal component"
* "Run ORA on all significantly changed genes (thousands of genes)"
* "Generate correlation matrix for all samples simultaneously"
* "Perform GSEA on multiple comparisons sequentially"

**Data Export & Summary Requests**

* "Export the DESeq2 results table with gene annotations"
* "Summarize the overall experiment design and sample composition"
* "What are the key findings from this RNAseq experiment?"
* "Create a methods summary for the statistical analyses performed"