

Analysis Tutorial Prospectus

1. **Title:** NMD-Mediated Regulation of Arsenic Detoxification Genes Under Different Growth Conditions.
2. **Research Questions:** Does the regulation of mRNAs involved in arsenic detoxification by NMD vary across different growth conditions?
3. **Objective:** To develop an R script that analyzes RNA-Seq data to determine whether NMD-mediated regulation of arsenic detoxification genes (ARR1, ARR2 and ARR3) varies under different growth conditions (normal, low iron, and low/high copper stress). Generate clear visual representations of expression trends to support conclusions.
4. **Approach:**
 - **Data Collection & Identification of NMD-Regulated Genes**
Extract RNA-Seq data from wild-type and NMD mutant strains (upf1 Δ , upf2 Δ , upf3 Δ). Identify differentially expressed genes (DEGs) regulated by NMD, focusing on arsenic detoxification genes (ARR1, ARR2, ARR3, ACR3) with R.
 - **Comparative Expression Analysis with R**
Compare wild-type vs. NMD mutants to determine how NMD affects arsenic-related gene expression under normal conditions.
Integrate RNA-Seq datasets from low iron and copper stress conditions to observe changes in gene expression.
 - **Statistical Analysis & Functional Interpretation with R**
Use log₂ fold change (LFC) and adjusted p-values (padj < 0.05) to determine significant differential expression.
 - **Assess whether NMD regulation of arsenic genes is affected by metal stress conditions (iron, copper).**
 - **Development of Functional Code in R with the help of chat GPT**
Write R scripts to process and analyze RNA-Seq data.
Implement data filtering, differential expression analysis, and visualization (e.g., bar plots, heatmaps) to interpret results.

References

- 1) Celik A, Baker R, He F, Jacobson A. High-resolution profiling of NMD targets in yeast reveals translational fidelity as a basis for substrate selection. RNA. 2017 May;23(5):735-748. doi: 10.1261/rna.060541.116. Epub 2017 Feb 16. PMID: 28209632; PMCID: PMC5393182.
- 2) Unpublished Iron upregulated and downregulated data (Kebaara Lab data).

- 3) Xinyi Zhang, Sunday Olaniyan, Xiayan Li, Bernd Zechmann, Mary Lauren Benton, Bessie Kebaara. Global effect of copper excess and deficiency in *Saccharomyces cerevisiae* proficient or deficient in nonsense-mediated mRNA decay. *Genomics*, Volume 117, Issue 2, 2025, 111020, ISSN 0888-7543, <https://doi.org/10.1016/j.ygeno.2025.111020>.
- 4) Anders S, Huber W. 2010. Differential expression analysis for sequence count data. *Genome Biol* 11: R106.