

Determining the Vitamin Supplementation Requirements of the Methanogenic Archaeon *Methanosarcina acetivorans*

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Abstract

Obligate anaerobic archaea known as methanogens produce methane by reducing carbon-containing substrates in a process known as methanogenesis. *Methanosarcina acetivorans* are of particular interest due to their adaptable genome and use of several methanogenesis pathways, resulting in high bioengineering potential for biofuels and industrial products. Laboratory cultures of methanogens are grown in Balch tubes with a high-salt growth media to provide the environment and substrates for metabolism. This generalized HS media includes the exogenous addition of ten distinct vitamins to assist growth. However, the exact function of these vitamins during methanogenesis has yet to be fully explained. As such, this project aims to identify the most metabolically stimulating vitamins in the growth media to streamline resources and optimize the growth of the utilized *Methanosarcina acetivorans*. This would be beneficial in lowering materials costs and increasing efficiency for industrial-scale production. Furthermore, this project aims to contribute to closing knowledge gaps about methanogenesis biosynthetic pathways by explicating the relevant genes used in the pathways. This would serve to promote future research questions and increase understanding of methanogen biochemistry.

Research Questions

- Which vitamins are required, stimulatory, or neutral to the growth of *Methanosarcina acetivorans*?
- Can the genes required for vitamin uptake, biosynthesis, and metabolism be identified by comparing gene expression patterns between growth conditions?

Methods

- Four starting cultures inoculated from the parental strain NB34 under different substrate and vitamin conditions are grown: methanol no-vitamin, methanol full-vitamin, acetate full-vitamin, and acetate no-vitamin.
- M. acetivorans* is capable of surviving with no vitamins as a prototroph.

Figure 1. Chemical structures of the two substrates, methanol(MeOH) and acetate, and methane. Both substrates are reduced to methane by the methylotrophic methanogenesis pathway.

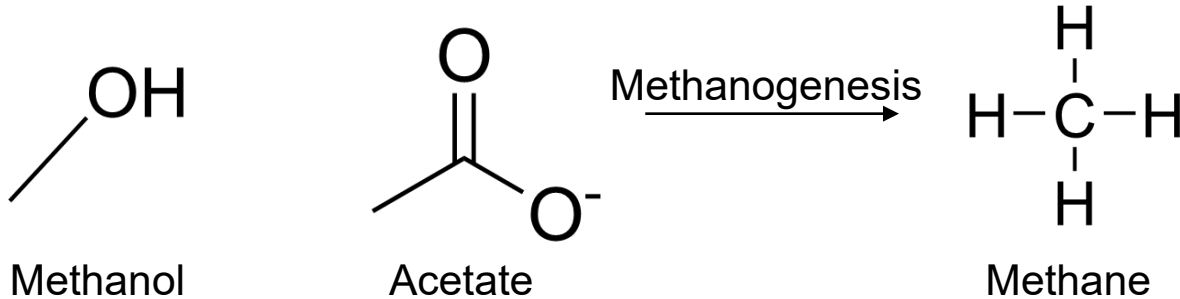


Figure 2. Cultures are grown and passaged to the 5th passage to eliminate vitamin carryover in no-vitamin cultures before inoculation into 96-well plates.

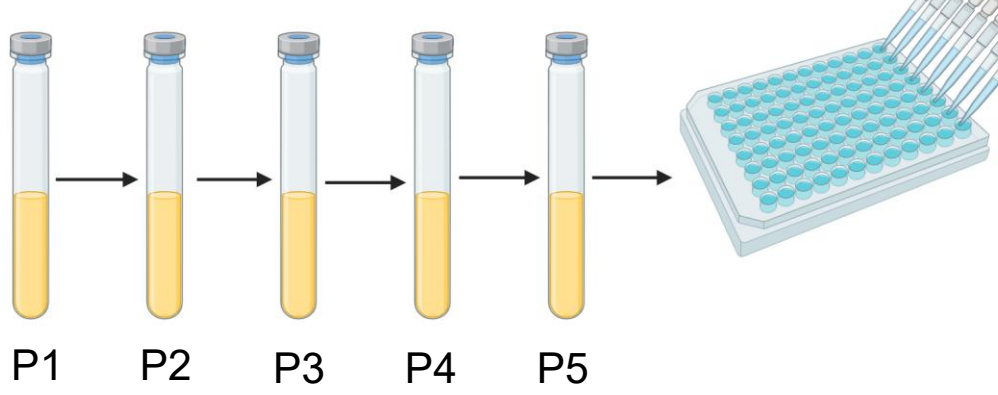


Figure 3. The absorbance of the plate wells is read every 30 minutes, with agitation and temperature control maintained throughout. Total time spent for readings varies by substrate.

Figure 4. Plate layout of the 10 listed vitamin conditions and controls. Water in the surrounding outer wells to prevent samples from drying out. Replicate data blanked using the no cell, no vitamin blank wells.

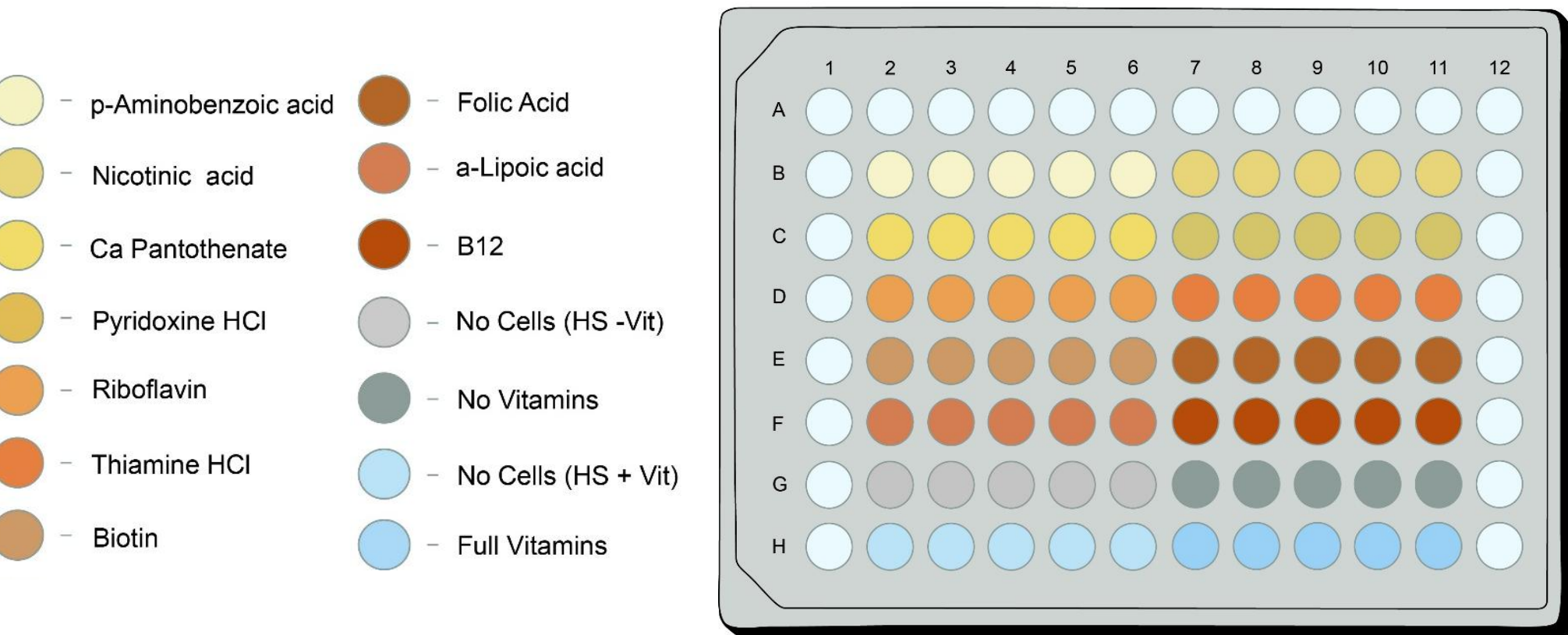


Plate Growth Curves

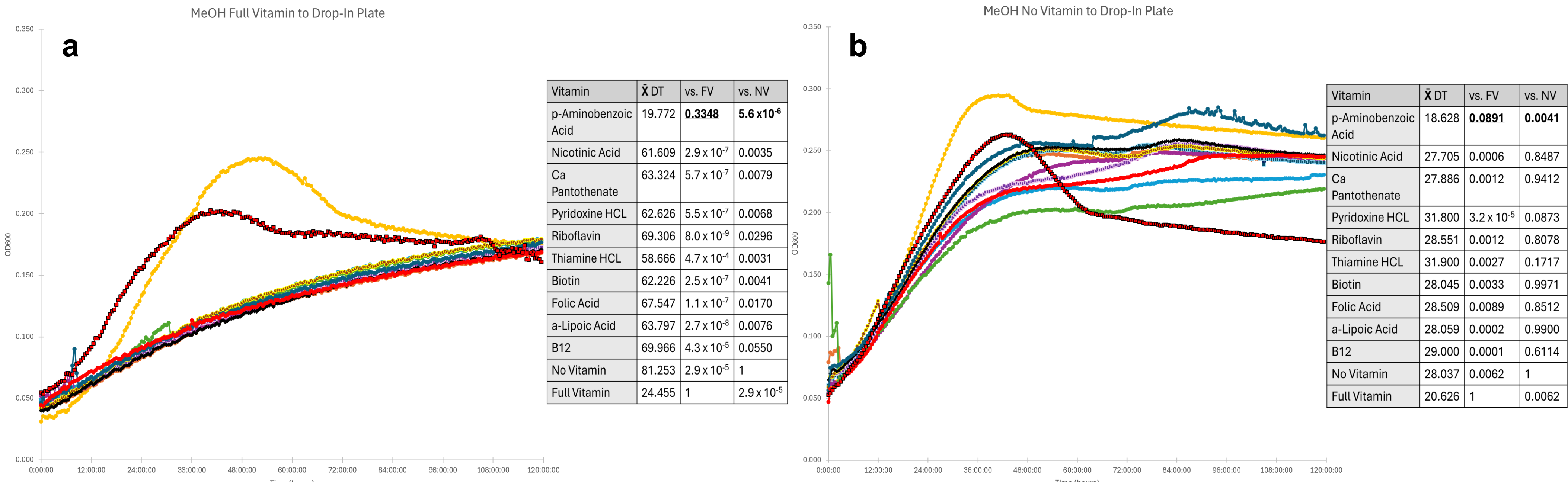
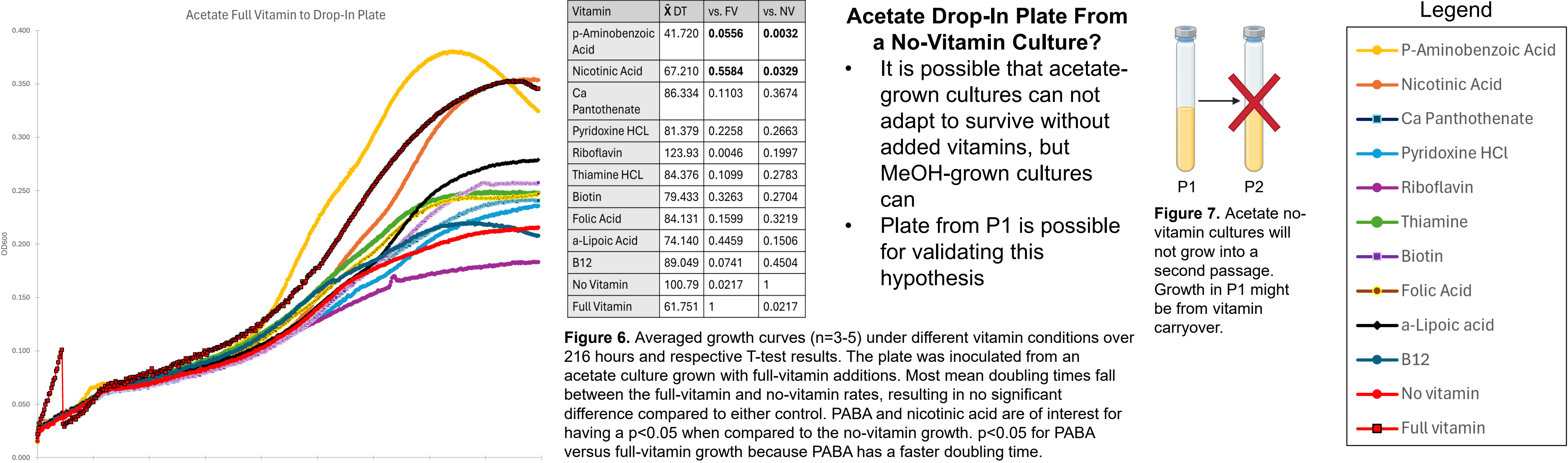


Figure 5. Averaged growth curves (n=4-5) under different vitamin conditions over 120 hours and respective T-test results. Plates were inoculated from a MeOH culture grown with a) full-vitamin additions or b) no vitamin additions. Mean doubling times were calculated from the slope of the ln growth and compared to the full-vitamin and no-vitamin controls via a T-test of significance. Both plates indicate p-aminobenzoic acid(PABA) as an important vitamin for growth with MeOH as a substrate. Starting from a full-vitamin culture causes vitamin carryover in the plates, which can affect the results of a).



Balch Tube Growth Curves

- Based on the plate results, the growth of p-aminobenzoic acid (PABA) in MeOH was scaled up for observation in Balch tubes.
- PABA only, full-vitamin, and no-vitamin replicates inoculated from the same tube.
- Essentially, a scaled-up version of MeOH plate b) focusing on PABA only

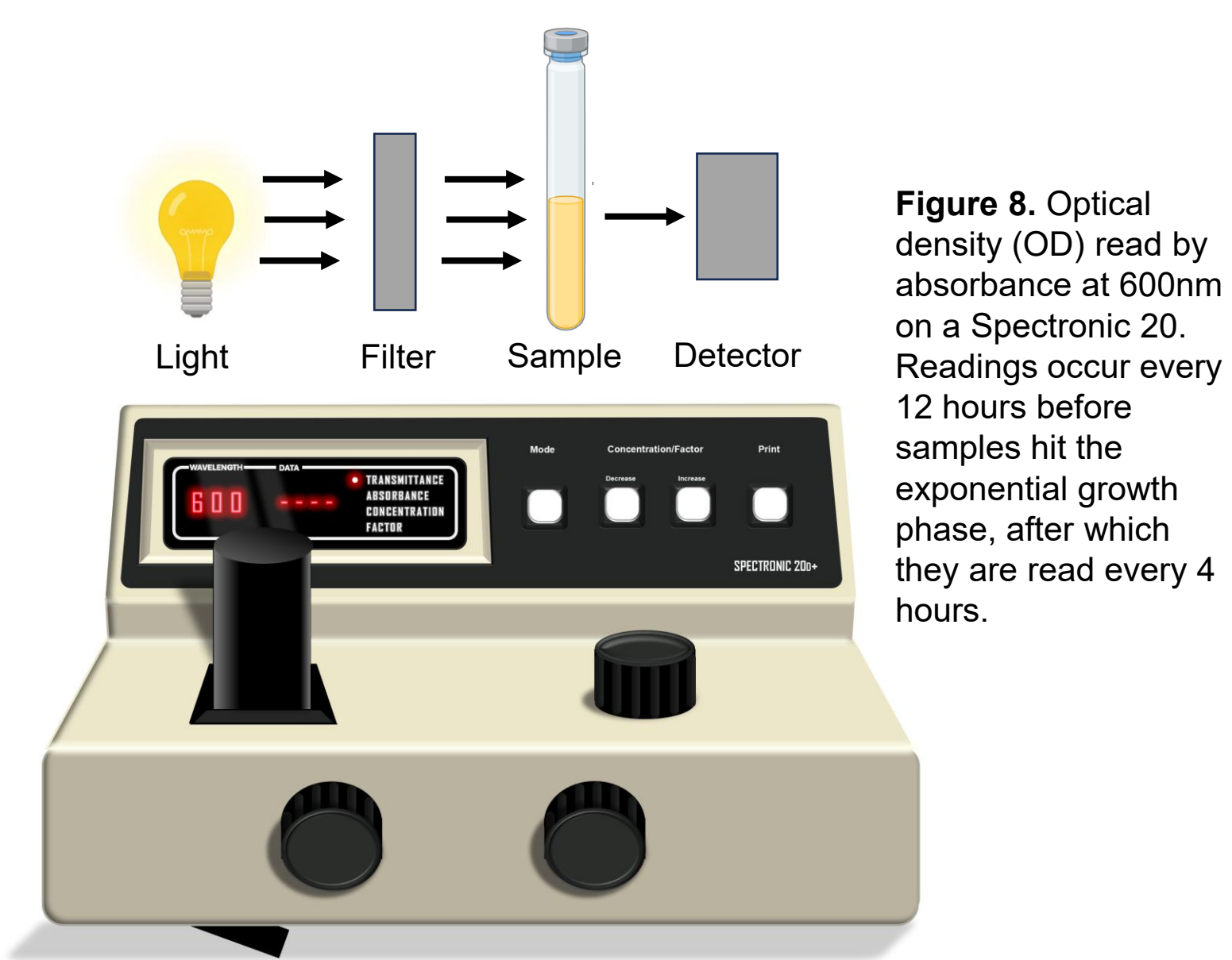
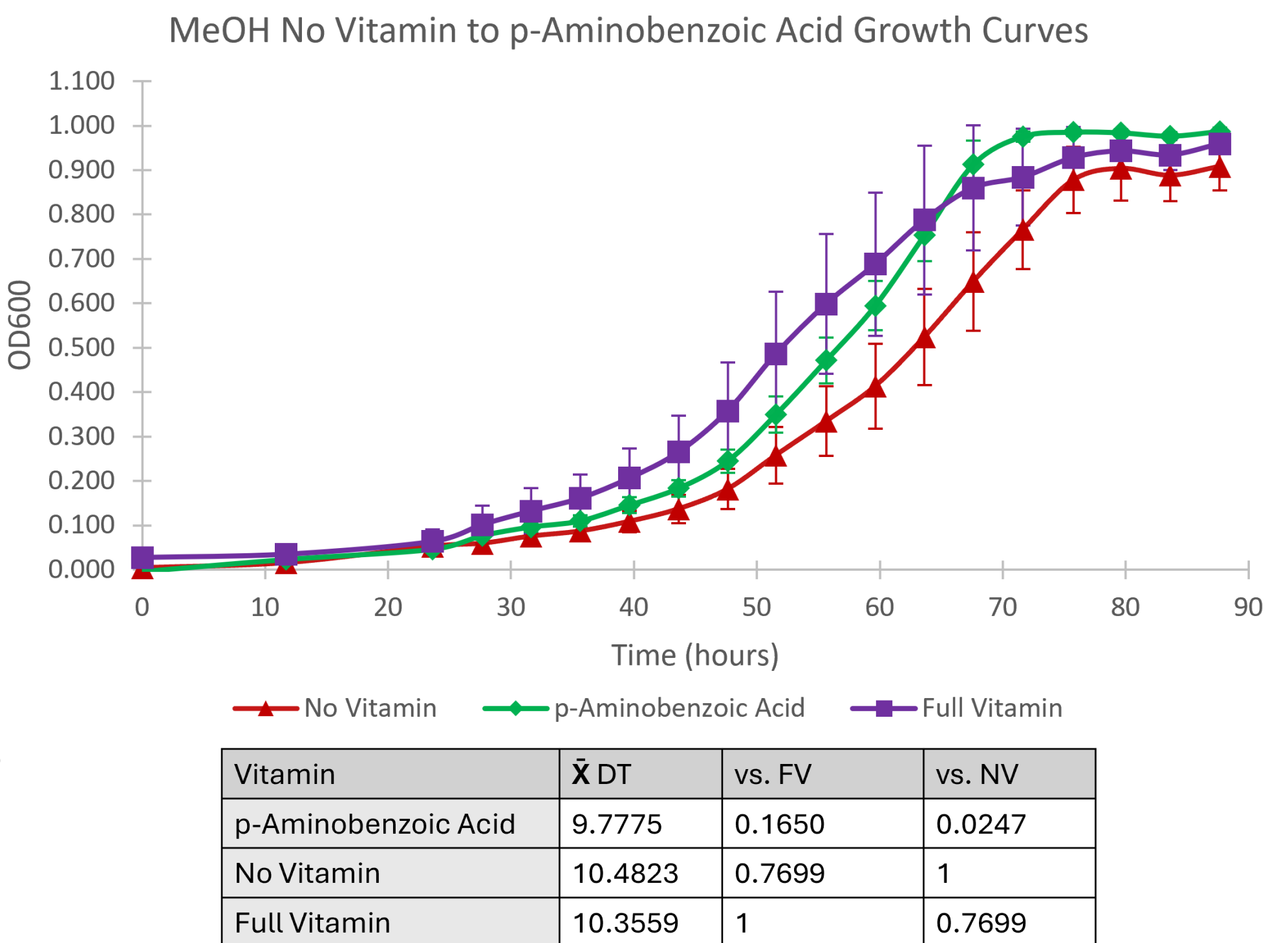


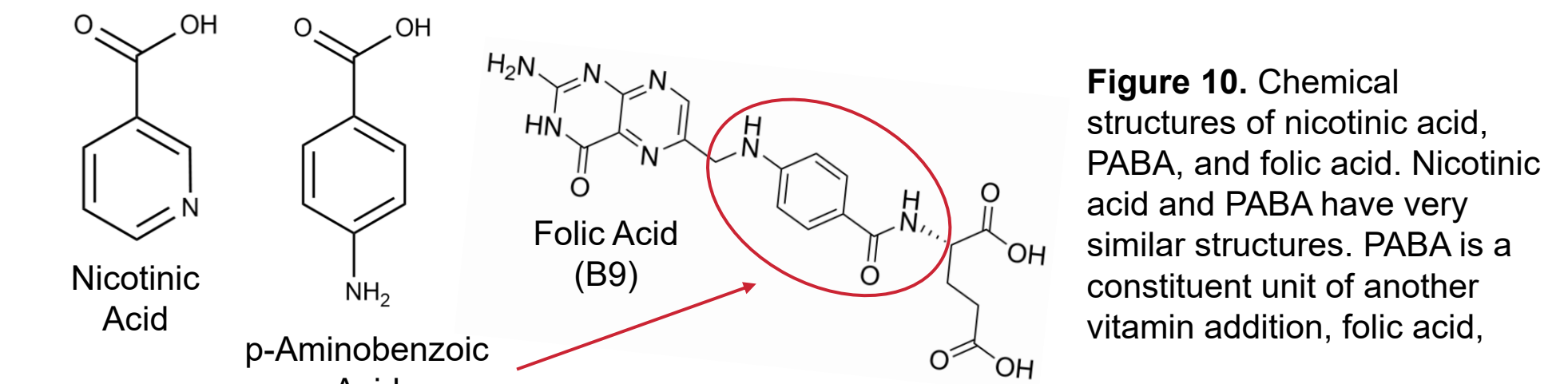
Figure 8. Optical density (OD) read by absorbance at 600nm on a Spectronic 20. Readings occur every 12 hours before samples hit the exponential growth phase, after which they are read every 4 hours.

Figure 9. Averaged growth curves (n=5-10) under different vitamin conditions over 90 hours and respective T-test results. Samples were inoculated from a MeOH culture grown with no-vitamin additions. Mean doubling times were calculated from the slope of the ln growth and compared to the full-vitamin and no-vitamin controls via a T-test of significance. A p>0.05 for full-vitamin versus no-vitamin growth indicates culture adaptation to the no-vitamin environment. p<0.05 for PABA versus no-vitamin, and the doubling time is shorter than that of the full-vitamin control.

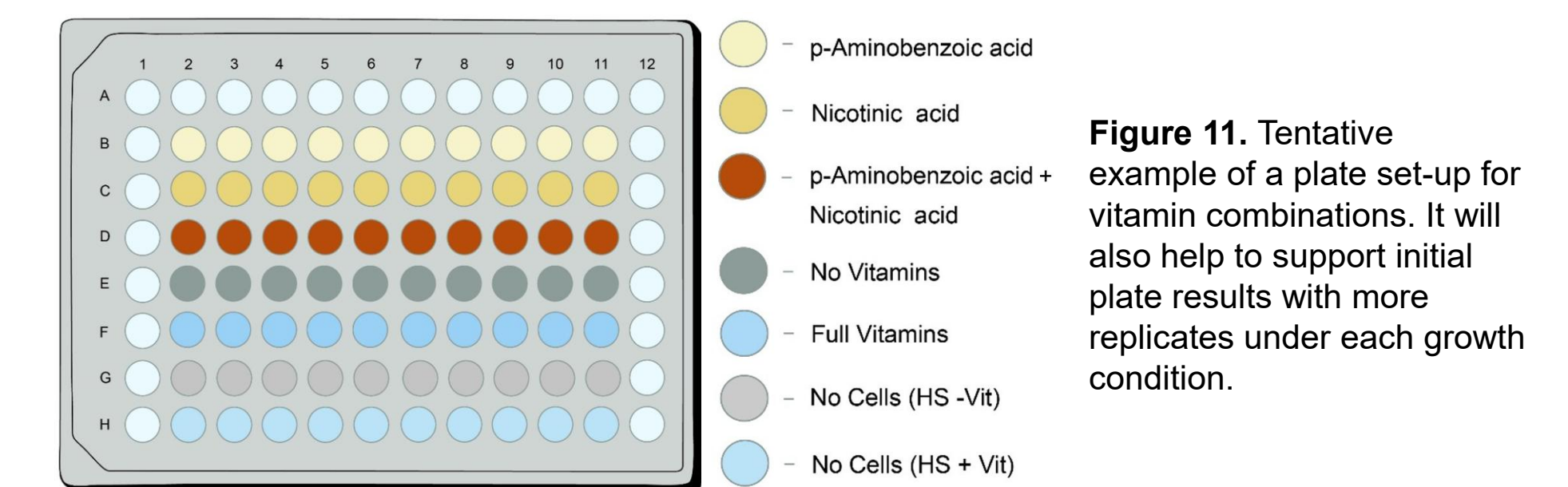


Future Work

- Investigate the theoretical basis behind why p-aminobenzoic acid(PABA) and nicotinic acid might be significant in methanogenesis.



- Complete an acetate no vitamin drop-in plate from P1. Do further acetate full vitamin drop-out plates with vitamin combinations.



- Compare gene expression under different vitamin conditions

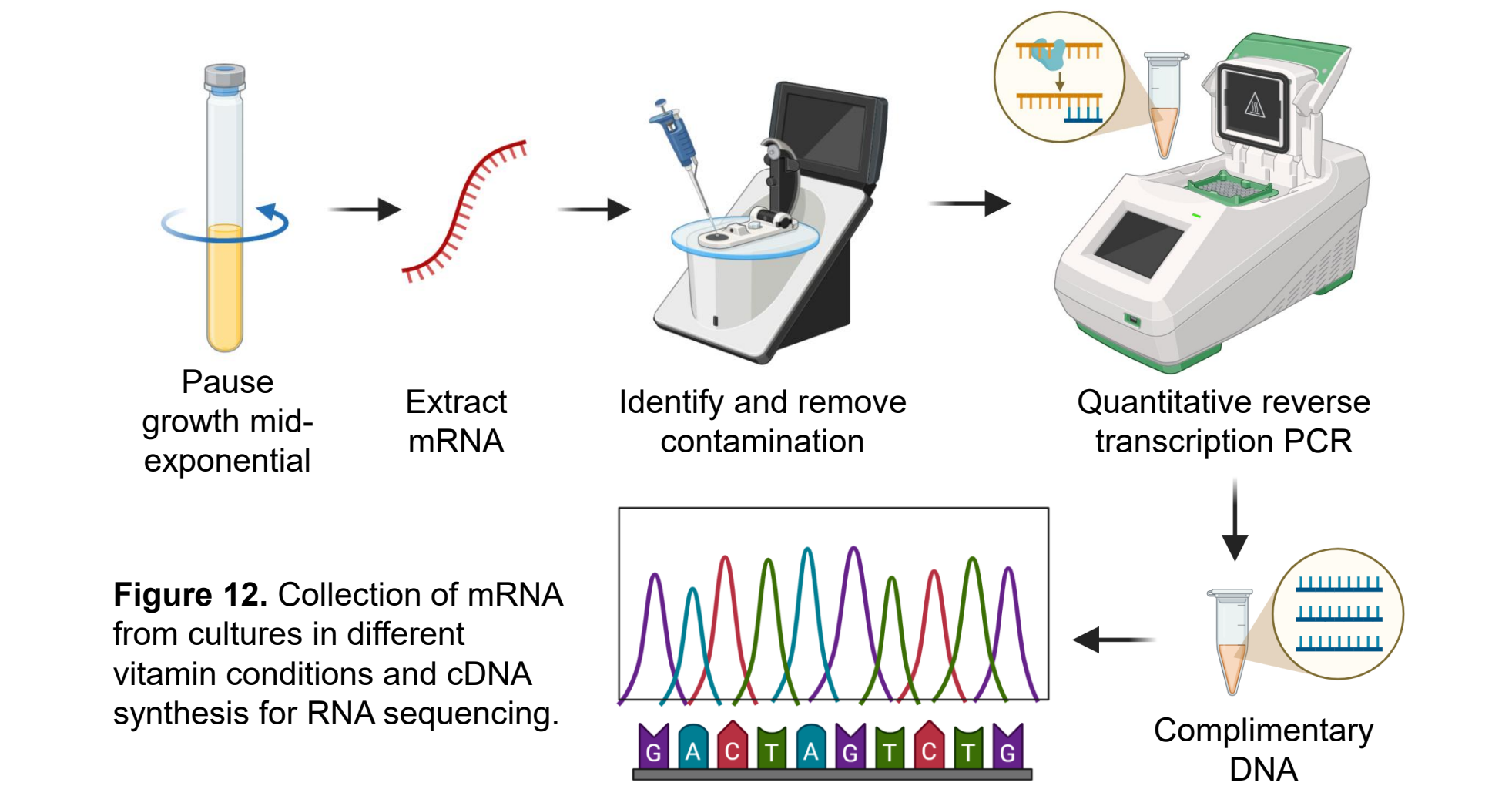


Figure 12. Collection of mRNA from cultures in different vitamin conditions and cDNA synthesis for RNA sequencing.

Acknowledgements

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References

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