

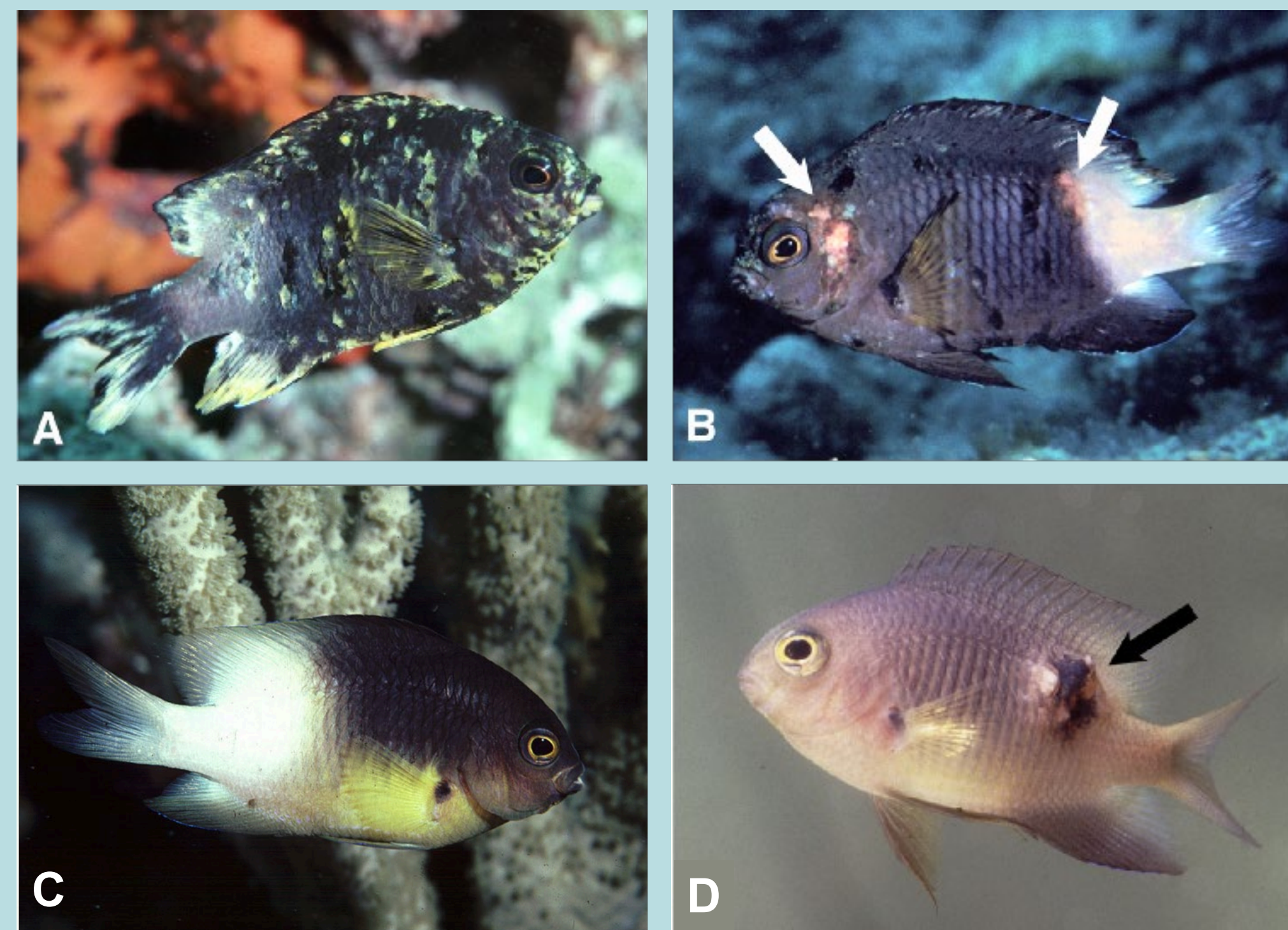
Effects of Damselfish Neurofibromatosis on Cell Respiration and DNA Copy Number in Bicolor Damselfish (*Stegastes Partitus*)

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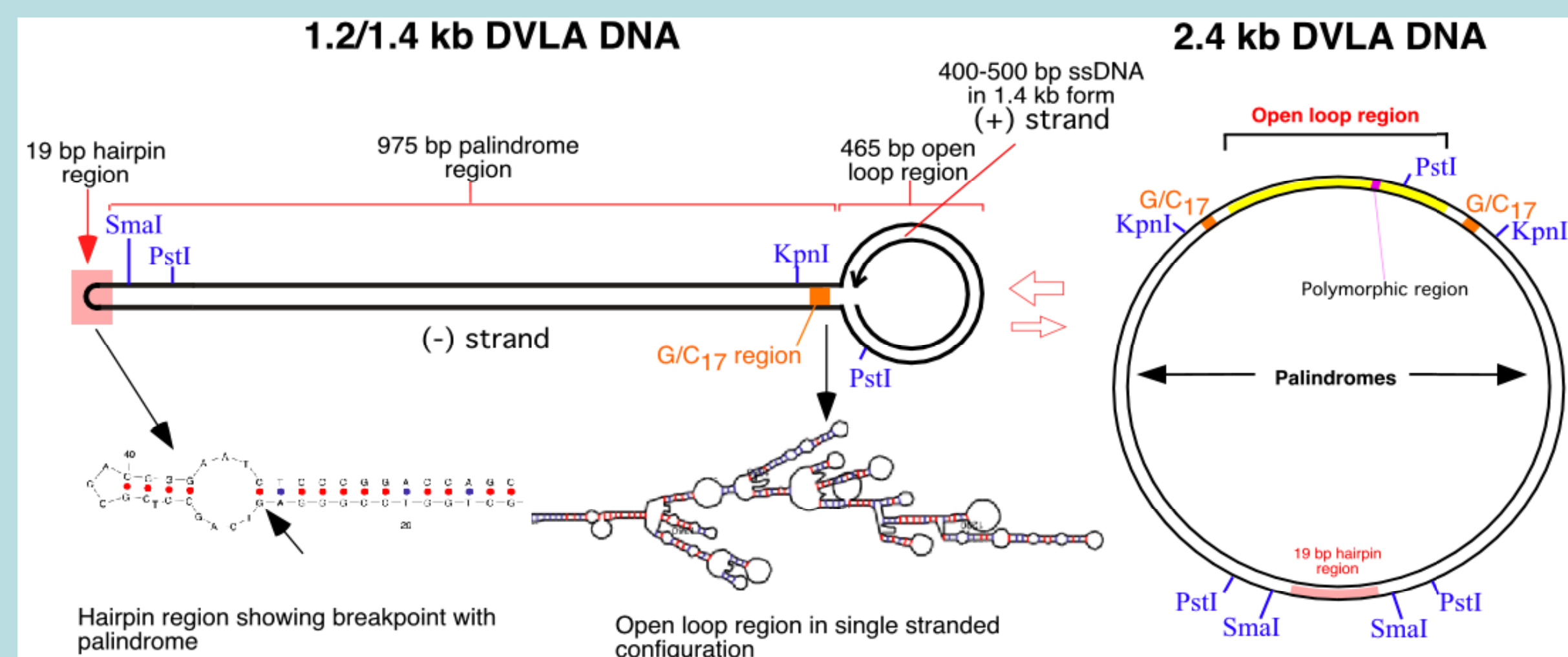
Damselfish Neurofibromatosis

Damselfish neurofibromatosis (DNF) is a transmissible, neoplastic disease affecting bicolor damselfish (*Stegastes partitus*) on South Florida reefs leading to the formation of malignant peripheral nerve sheath tumors, chromatophoromas and neurofibromas. The cause of DNF is a virus-like agent (termed the damselfish virus-like agent or DVLA) which has a 2.4kb DNA genome and does not resemble any known family of DNA virus in either sequence or secondary structure. Several lines of evidence indicate that this agent has a unique life cycle which involves replication within the mitochondria of infected cells while having no detectable association between DVLA and the cell nucleus – the area where all known small DNA viruses replicate. This life cycle suggests novel mechanisms of tumorigenesis and pathways by which foreign DNA may gain access to mitochondria. Development of an *in vitro* infection model will facilitate the investigation of changes in mitochondria associated with neoplastic transformation of cells in DNF.



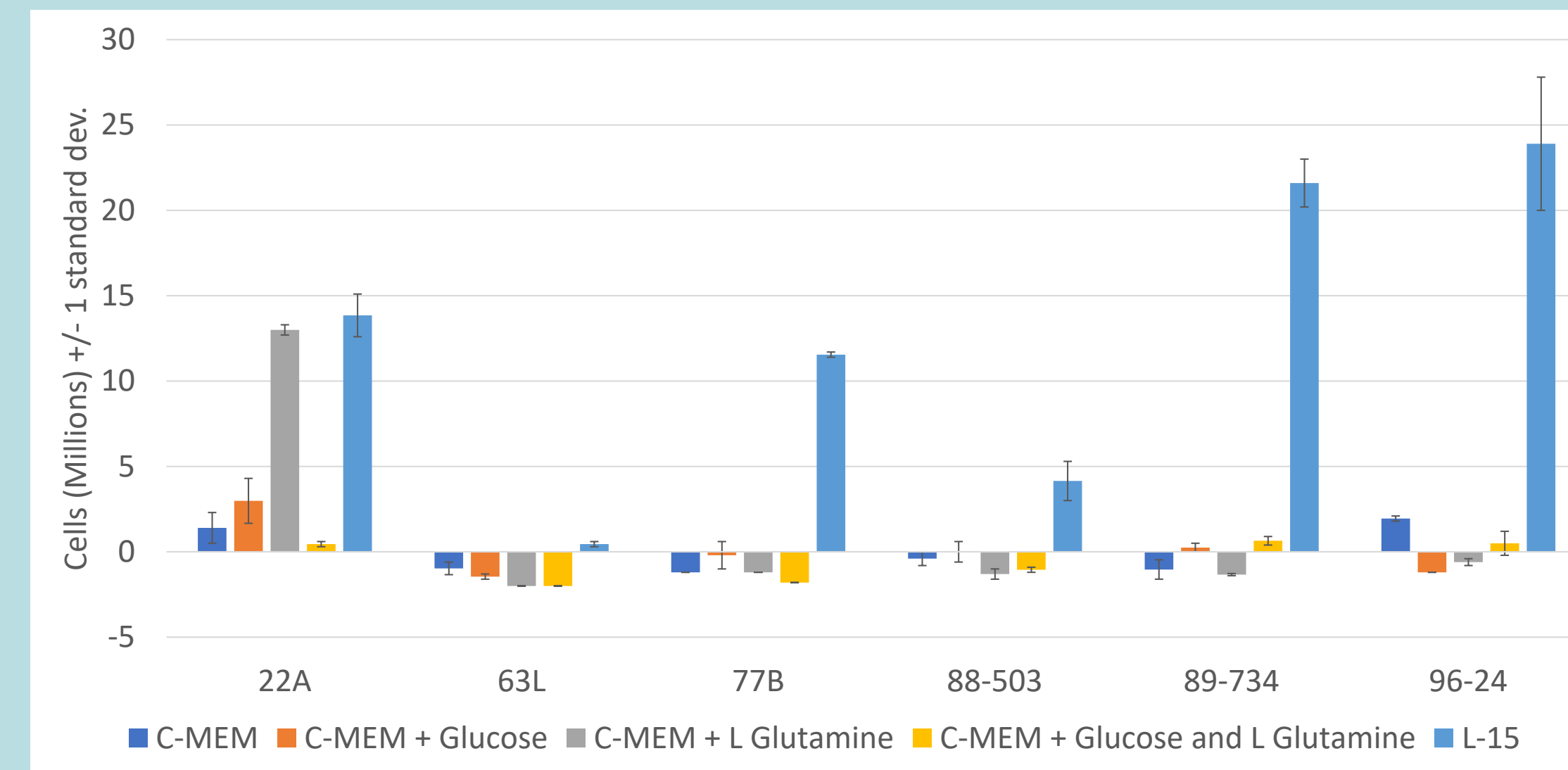
Bicolor damselfish (*Stegastes partitus*): A. & B. Spontaneously tumored fish in the wild showing numerous hyperpigmented lesions on the surface of the body and fins. Non-pigmented neurofibromas erupt through the skin (arrows) in some fish (B). C. Healthy bicolor damselfish. D. Fish with experimentally induced tumor near injection site on the flank (arrow).

DVLA Genome

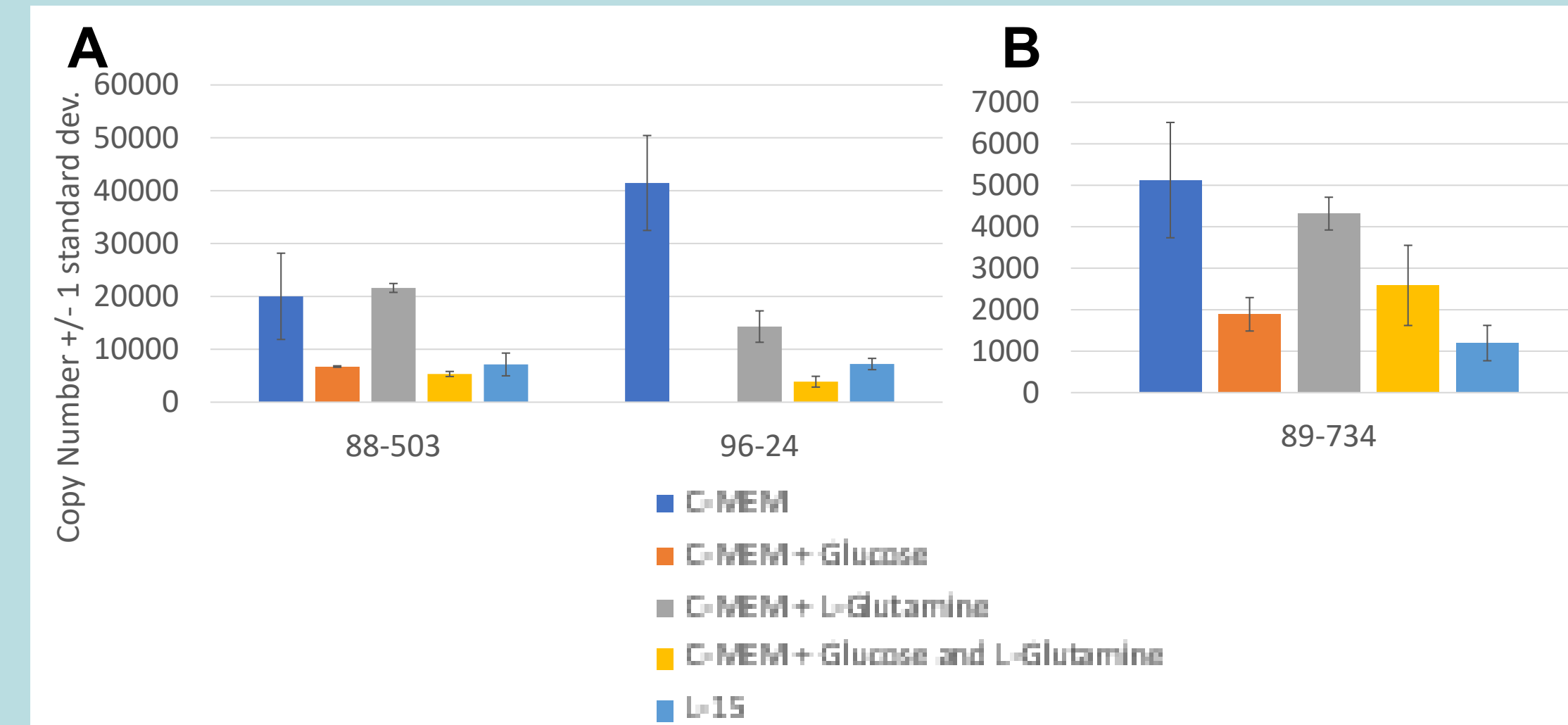


Genome of DVLA - Key features:

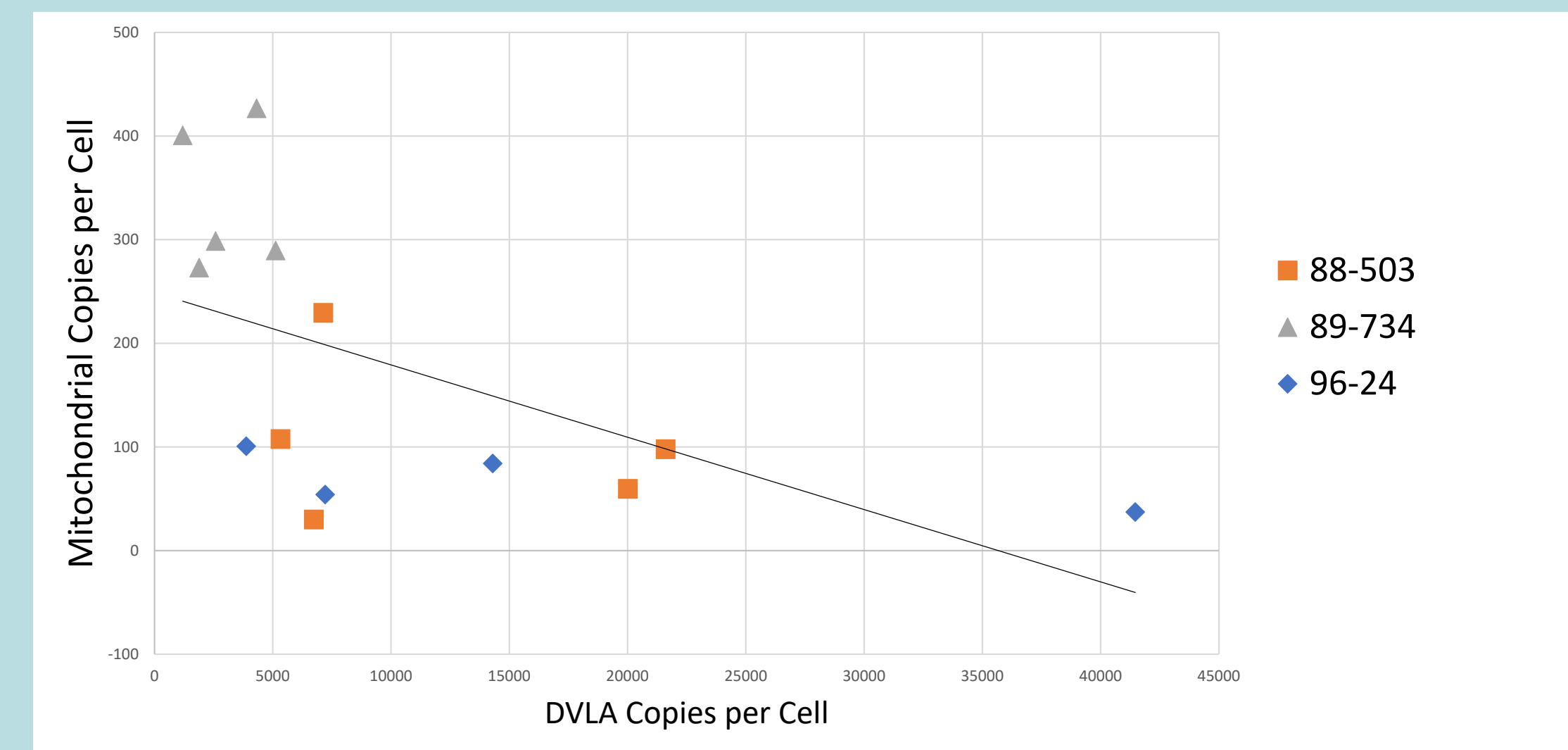
2,434 bp genome	No clear sequence homologies in GenBank
Single and double stranded circular DNA	Most sequence appears highly conserved/stable
Distinct + and – strands	Lacks obvious, realistic open reading frames
Sequence dominated by inverted repeats	No detectable integration into cellular DNA



Growth patterns per media type from start to finish of the 2-week experimental media period. It was obtained by subtracting the initial cell count of 2 million cells from the final cell count. These values are per media, so it displays the average of two replicate flasks growth patterns..



DVLA Loop copies per cell for the three DVLA positive cell lines. This is displayed in two graphs due to the large variation between 88-503/96-24 (A) and 89-734 (B) in copies per cell.

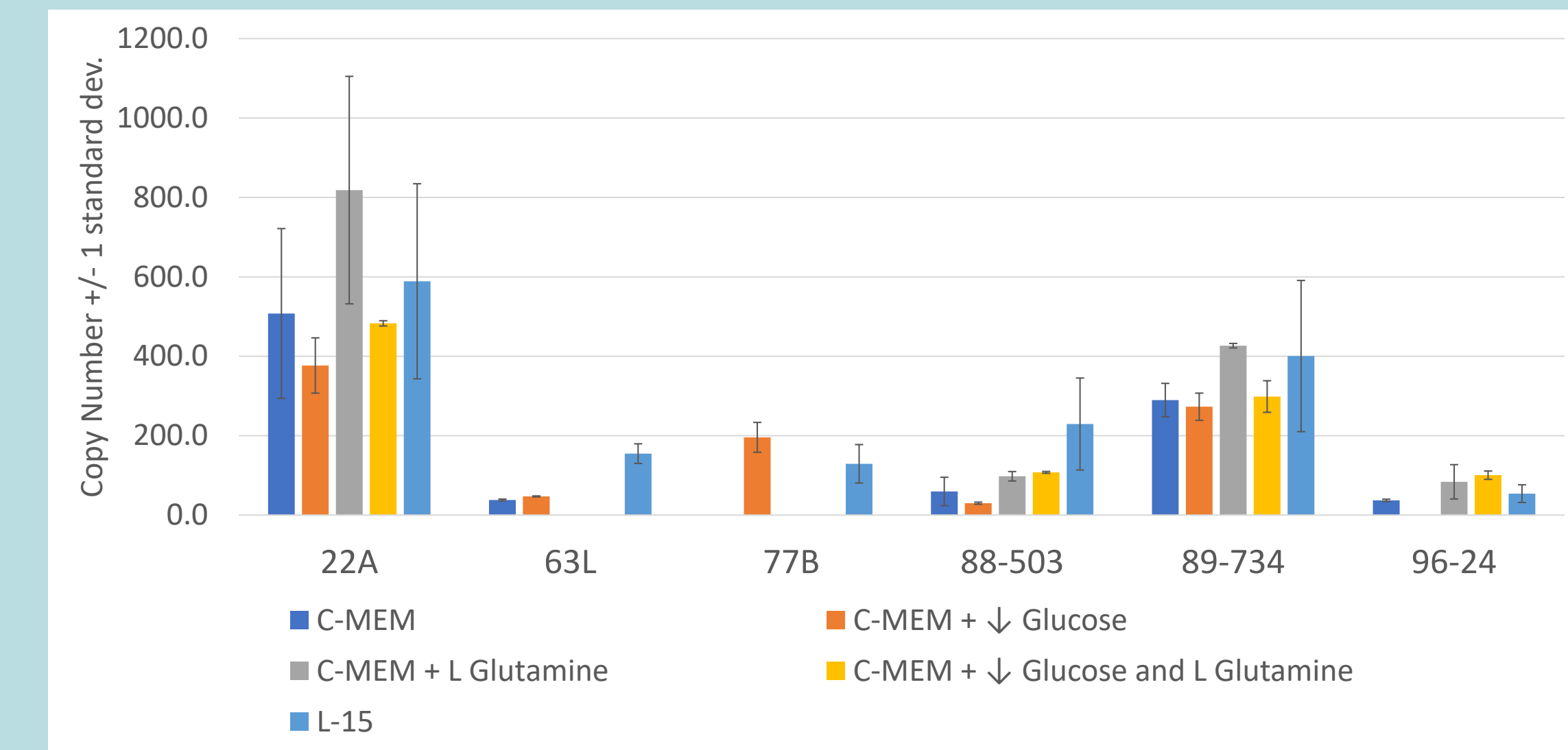


Copies per cell of DVLA DNA vs. Mitochondrial DNA in tumor derived cell lines. Plotted values come from all 5 media types, except 96-24 C-MEM + Glucose. Values were gathered via qPCR for each type of DNA

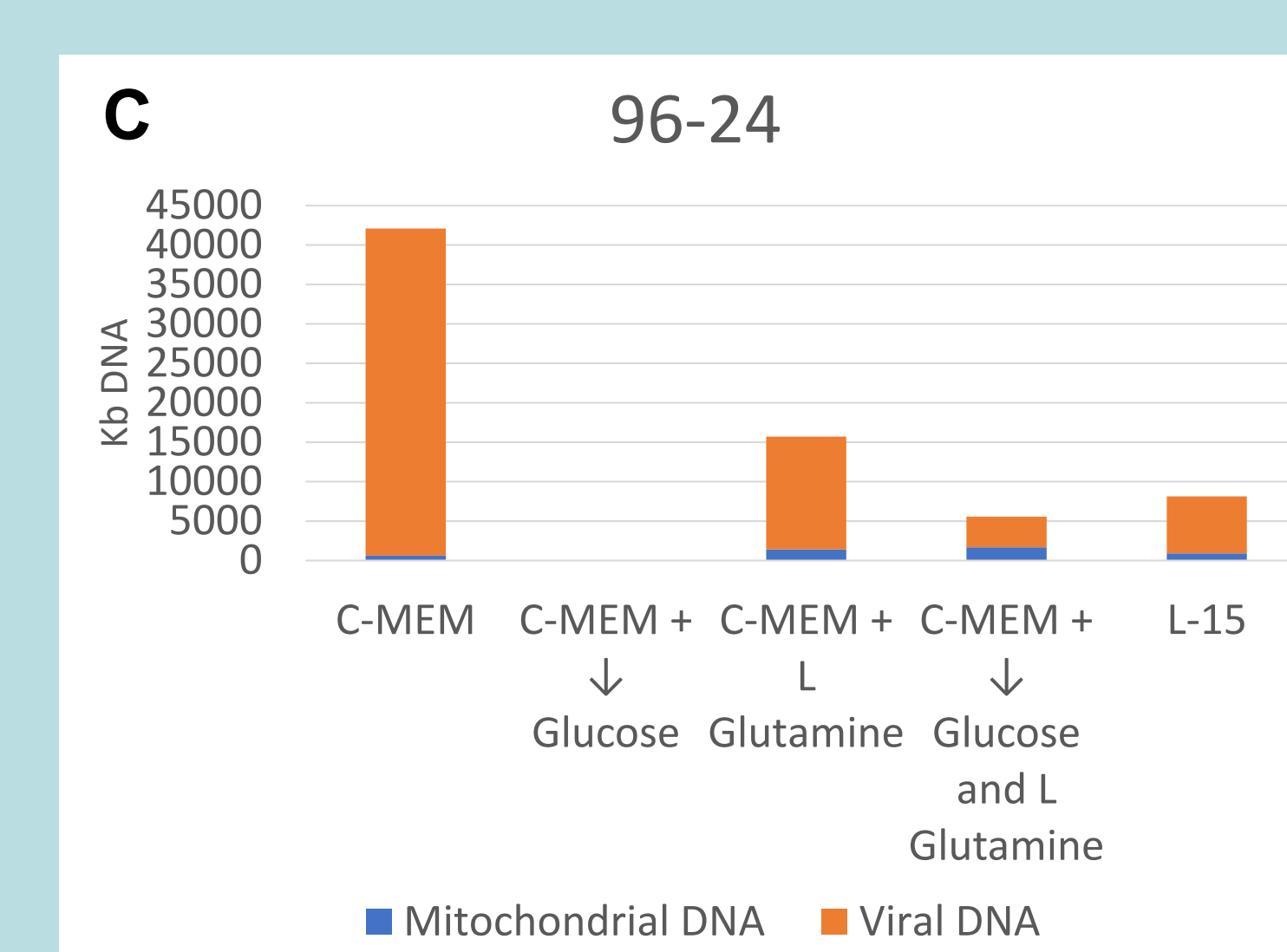
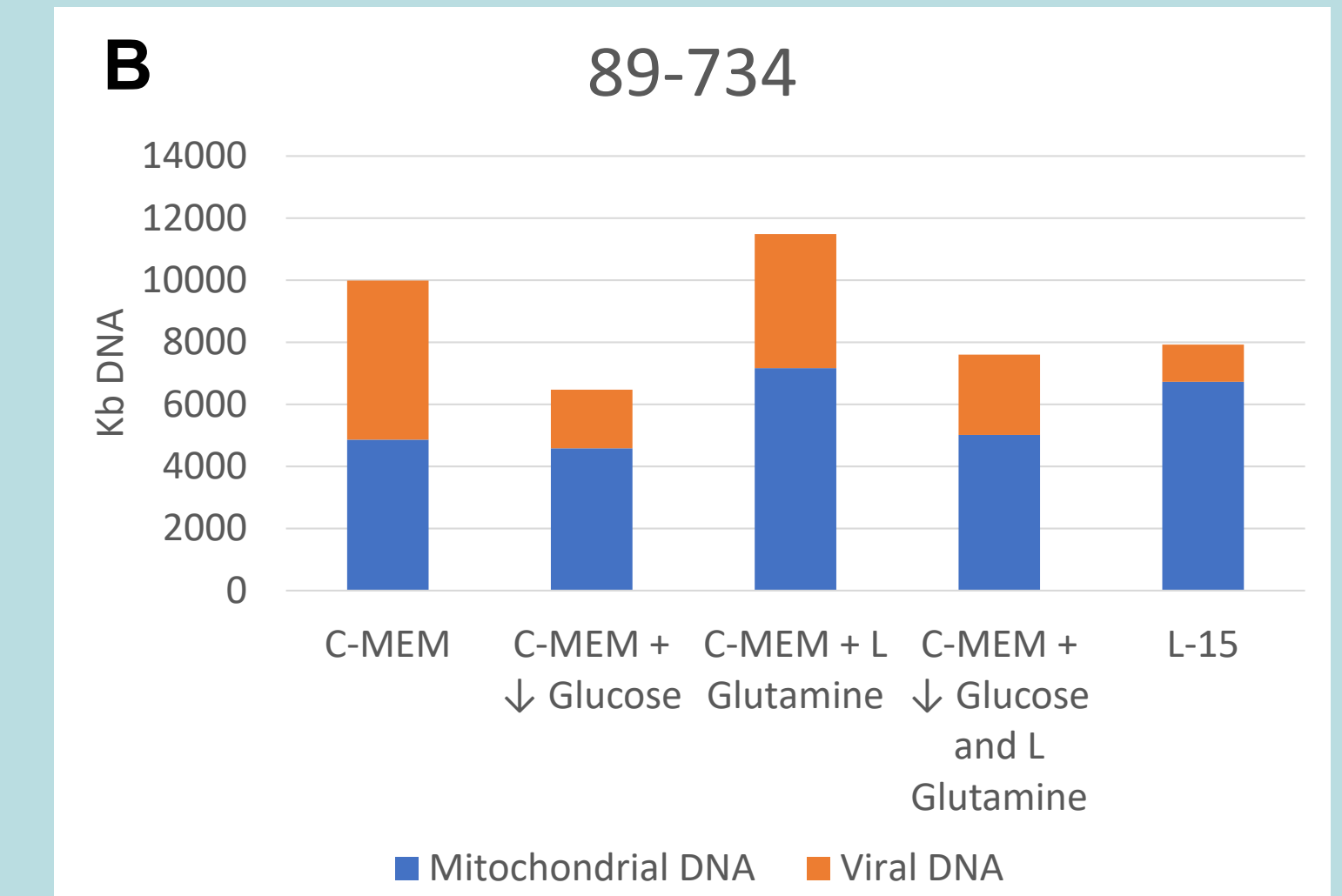
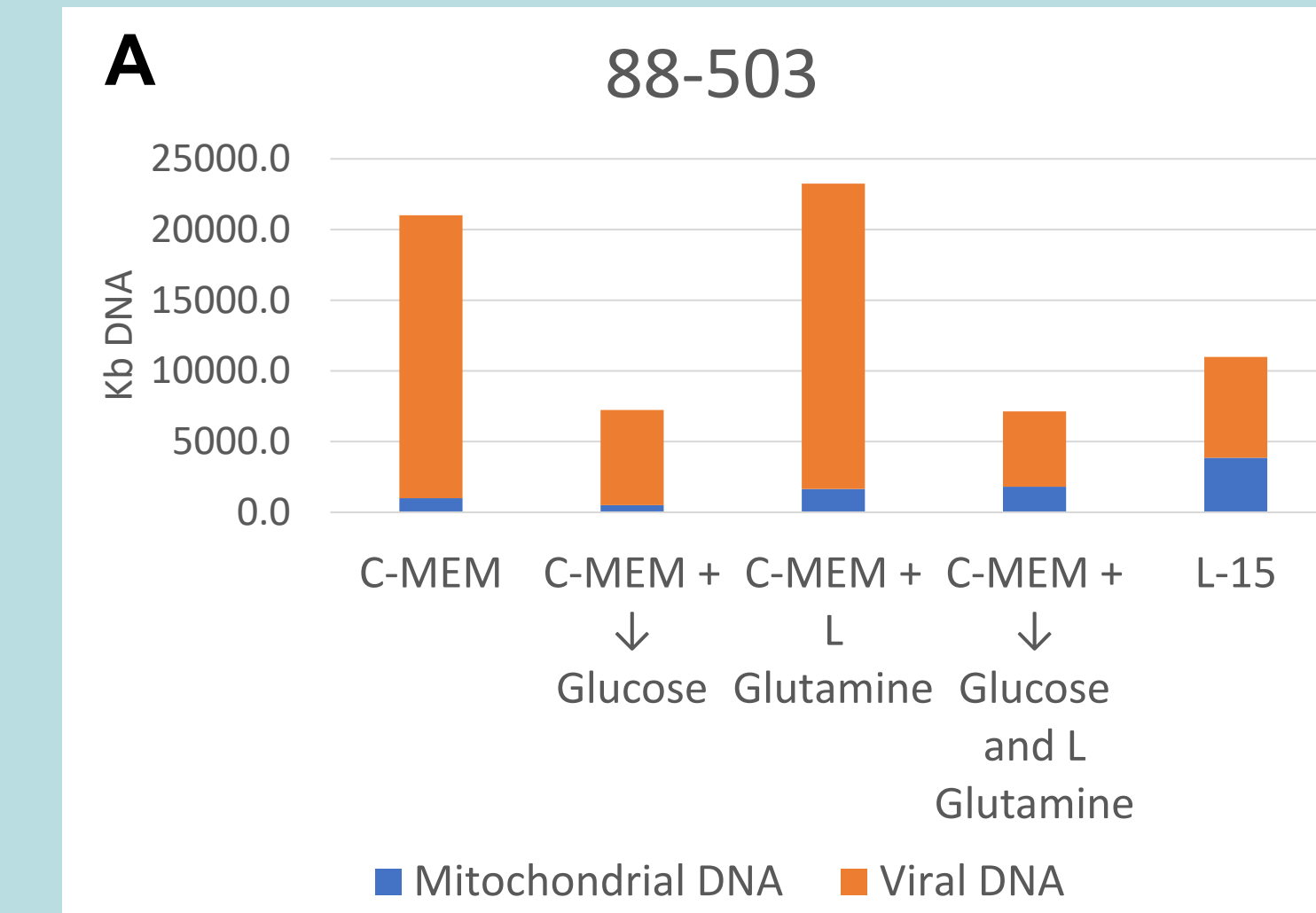
Methodology

Six total cell lines were originally obtained from bicolor damselfish *Stegastes partitus* collected on South Florida reefs. Three healthy cell lines collected from healthy fish and three DVLA positive cell lines collected from fish with tumors were chosen for this study. Cell lines were grown in 5 different media types containing different variations of metabolic substrates. The basal media used in experimental treatments was a custom minimal essential media (MEM, ScienceCell Research Labs), being comprised of a standard MEM formulation with Hank's salts and lacking any glucose, glutamine, or pyruvate; the C-MEM did contain 10% FBS, dH₂O, and antibiotics. The three other experimental medias were tested: C-MEM with the addition of glucose, L-glutamine, or both nutrient sources. The fifth media was a modified Leibowitz L-15 (Thermo-Fisher) medium with added ampicillin, Pen/Strep/ Amphotericin B, NaCl, and 10% Fetal Bovine Serum. Each flask was fed at the 7 day mark and then collected after 14 days. Upon harvesting of the cells, the DNA of each flask was collected and tested via RT-qPCR for copy number. Student t-tests and a Pearson Correlation test were used to test for significance.

Results



Mitochondrial DNA copies per cell for all six examined cell lines. Missing data points represent samples with DNA concentration too low to run in a qPCR.



Combined DNA total mass. Graph of each of the DVLA positive cell lines, 88-503 (A), 89-734 (B), and 96-24 (C) displaying total mitochondrial and viral DNA mass in a cell of each sample. These values were obtained by taking the viral copy number and multiplying it by 2.4, and taking the mitochondrial copy number and multiplying it by 16.8. These values represent the total Kilobases per one copy of each DNA type

Conclusion

Overall growth was much better in L-15 media than any other media across all cell lines. There didn't appear to be any pattern to growth across cell lines, however each cell line was consistent in how its replicate flasks acted. Only the healthy cell line of 22A had success growing in a majority of the experimental medias tested. In tumor cell line 96-24, the C-MEM flasks outperformed all medias containing metabolic substrates besides L-15. Copy number analysis by qPCR displays an interesting possible relationship between cell growth and viral copy number in the C-MEM and C-MEM + L-Glut flasks. These two media types had the worst growth curves in viral cell lines, however, presented the highest viral copy numbers per cell. The more viral copies that were produced, the less mitochondrial copies there were. A relationship like this could assist an understanding of the mechanisms by which tumor cells are still able to utilize oxidative phosphorylation at a reduced capacity while still propagating its own production via glycolysis (1. 2)

References

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- Alan, Md Maksudul & Lal, Sheha & Fitzgerald, Keely & Zhang, Li. (2018). Correction to: A holistic view of cancer bioenergetics: mitochondrial function and respiration play fundamental roles in the development and progression of diverse tumors. *Clinical and Translational Medicine*. 7. 10.1186/s40169-018-0186-5.