Quantification of DNA products using ion-pair reverse phase liquid chromatography

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The transcription of DNA via RNA polymerases is a fundamental process in cellular systems. In eukaryotic cells, we observe transcription in the nucleus (via genomic DNA) as well as in the mitochondria (via mitochondrial DNA). There are many tools to available to investigate nuclear transcription; however, few tools exist to study mitochondrial transcription even though the mitochondrial DNA encodes several essential proteins. Recently an in vitro transcription system using purified mitochondrial transcription proteins, including the mitochondrial RNA polymerase, and linear mitochondrial DNA templates has been developed. Quantitative analysis of the DNA templates can be done via ion-pair reverse-phase high performance liquid chromatography (IP-RP HPLC), a high-resolution technique in separating DNA based on size. Using IP-RP HPLC our aim is to assess the lower limits of separation, and our quantification method is based on measuring peak area and the peak height.

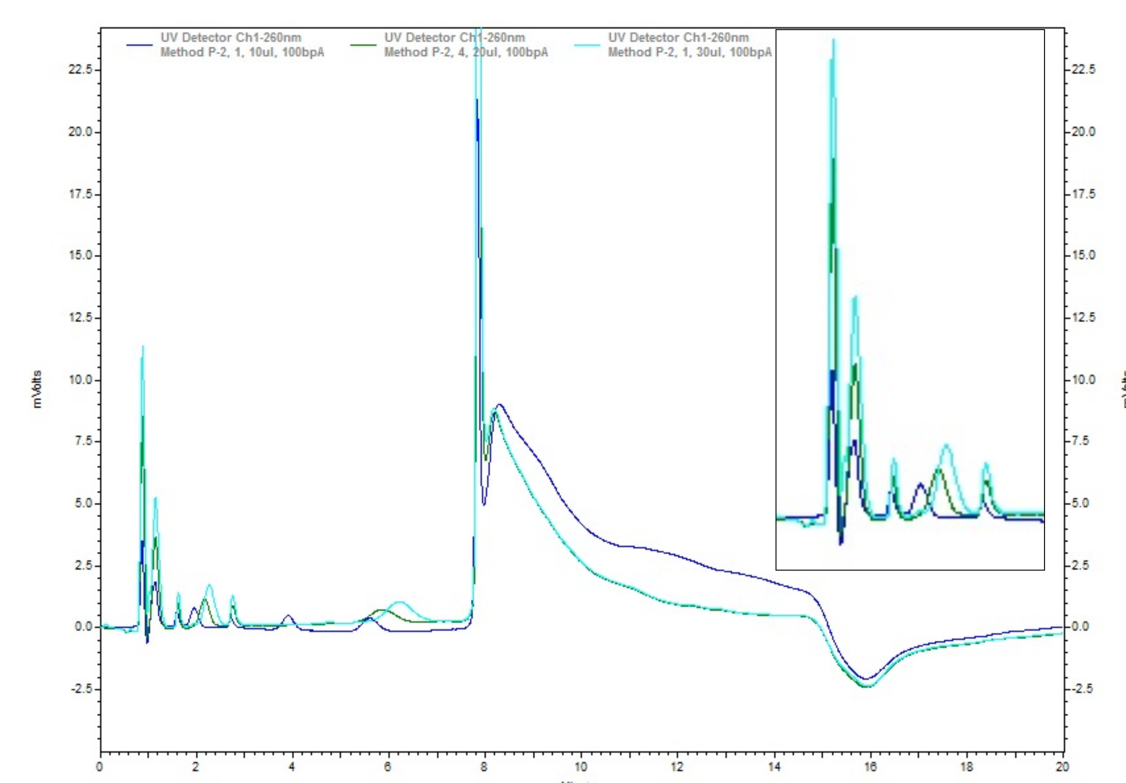
Introduction

The transcription of DNA to RNA via RNA polymerases is a fundamental process in cellular systems. In eukaryotes, we observe transcription in the nucleus (genomic DNA) as well as in the mitochondrial matrix (mitochondrial DNA). There are many tools available to investigate nuclear transcription; however, few tools exist to study mitochondrial transcription. Recently an in vitro transcription system using recombinantly expressed and purified mitochondrial transcription proteins has been developed (*1*). Ion-pair reverse-phase high performance liquid chromatography (IP RP HPLC) has long been used as a high-resolution technique in separating DNA based on size (*2,3*). *Our aim is to develop a method using IP RP HPLC to separate DNA based on size and provide a* ***quantitative analysis*** *of the separations*. Ultimately we would like to apply this method to RNA and the mitochondrial in vitro transcription system.

Methods and Materials

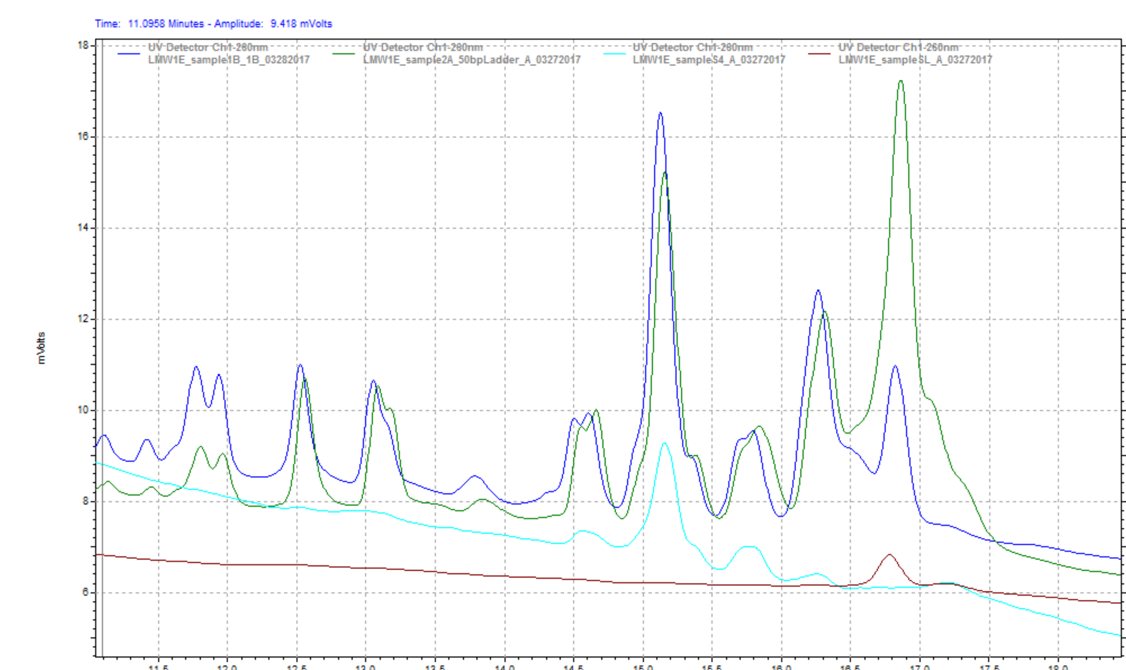
All samples were run using either a Xbridge Oligonucleotide BEH C18 (130A, 2.5 µm, 4.6 x 50mm), or a YMC Basic S-3 (4.6 x 150mm) column. The column was run on an LC-2010HT Liquid Chromatography System by Shimadzu. To develop an method for IP RP HPLC using our system, we used both high molecular weight DNA and low molecular weight DNA ladders (NEB 100bp DNA ladder, and NEB low molecular weight DNA ladder). All samples were heated at 65°C for 5 minutes, then directly set in ice for one minute. For 100bp DNA ladder the column was equilibrated at 90% solvent A and 10% solvent B at 60°C. Solvent A: 0.1M triethylammonium acetate (TEAA), in diethyl pyrocarbonate (DEPC) treated water, and solvent B: 0.07M TEAA with 25% acetonitrile (ACN). For the low molecular weight ladder, the column was equilibrated to 65% solvent A and 35% solvent B.

100bp DNA Ladder Sample



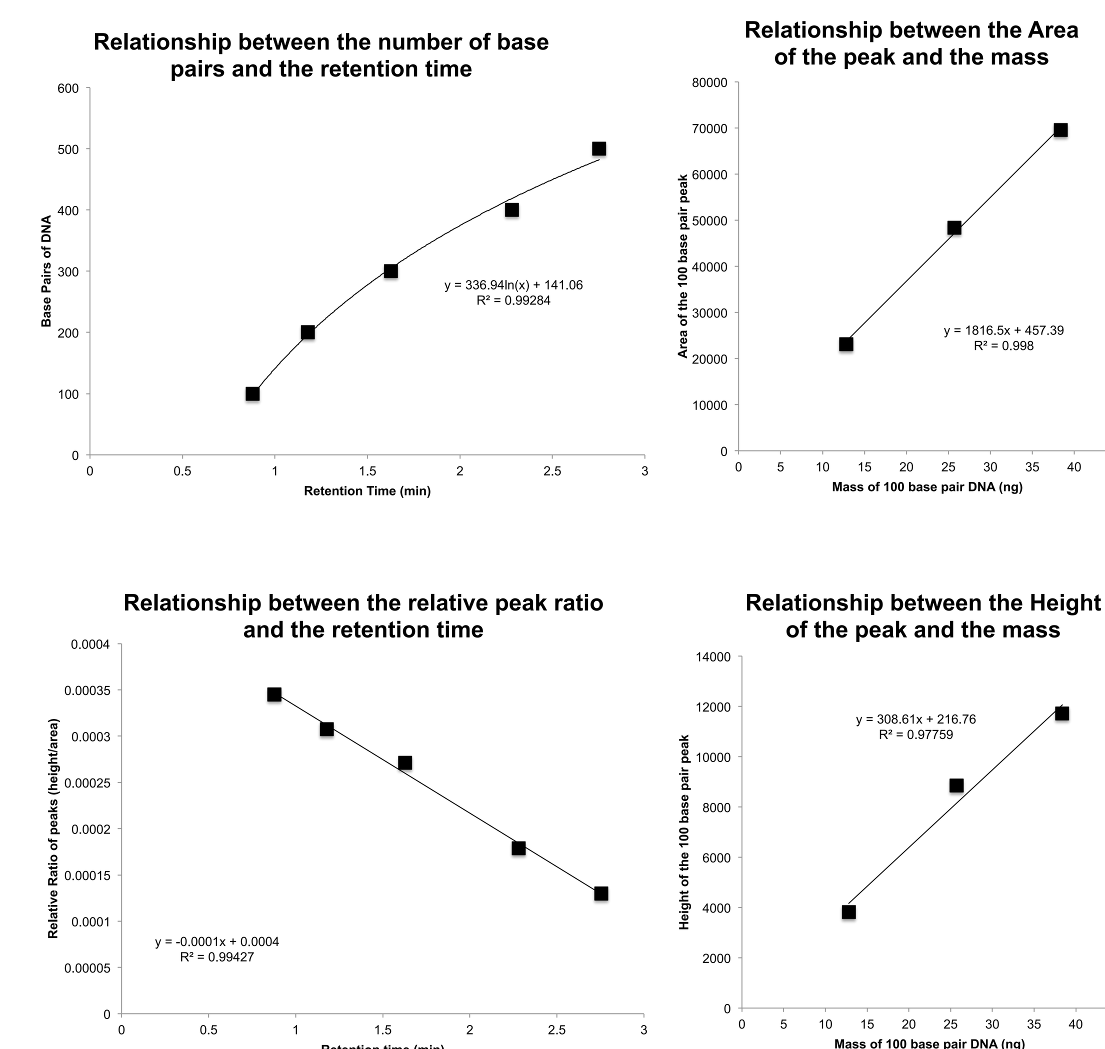
Absorption traces at A_{260} of three different amounts of DNA 100base pair ladder vs. the retention time. Dark blue represents 133.8ng sample of DNA ladder injection, green represents 269.0ng sample of DNA ladder injection, light blue represents 401.5ng sample of DNA ladder injection. Inset is an enlargement of the key peaks from retention times 0-3.5 minutes.

Low Molecular Weight DNA Ladder

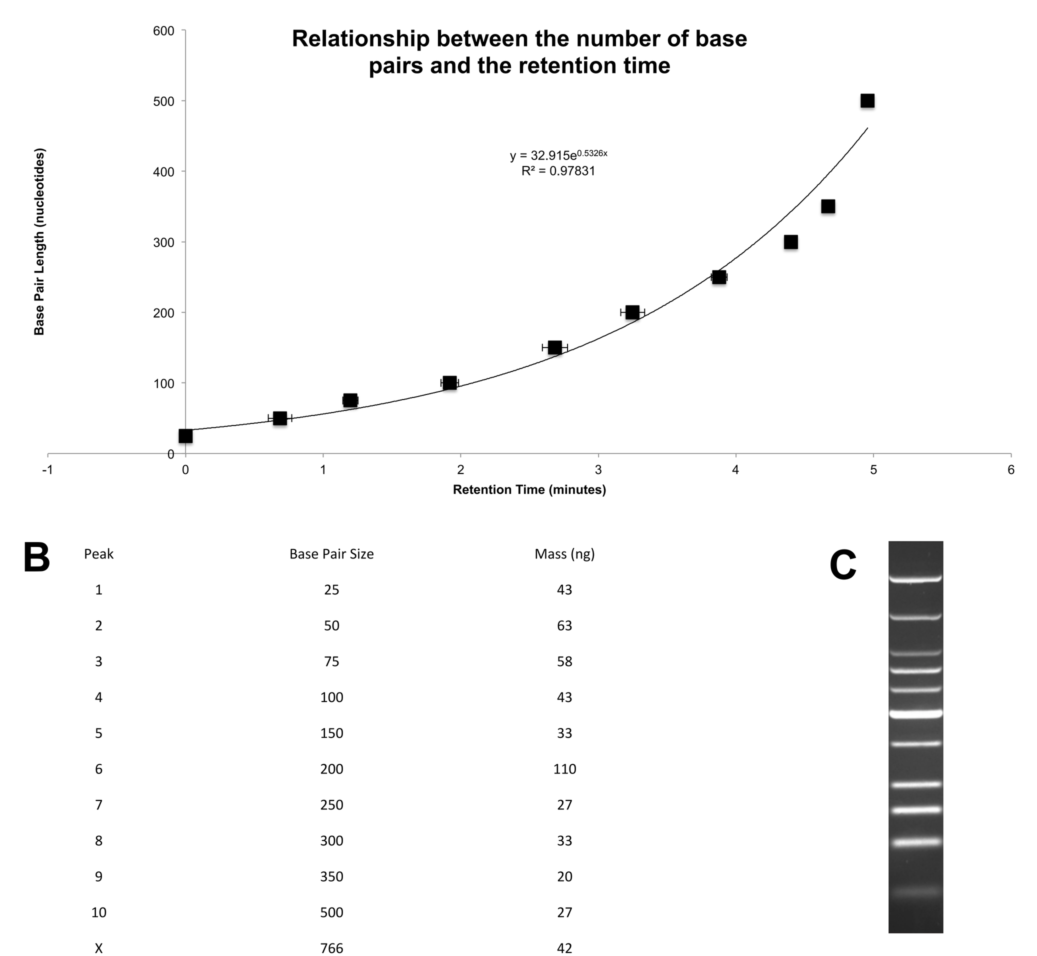


Absorption traces at A_{260} of several samples of DNA vs. the retention time. Dark blue represents the Low Molecular Weight DNA Ladder, green represents the Low Molecular Weight Ladder doped with a 50 Base Pair Ladder, light blue represents the 200 base pair section of the Low Molecular Weight Ladder, and the red represents the 500 base pair section of the Low Molecular Weight Ladder.

Quantification of 100bp DNA Samples



Quantification of Low Molecular Weight DNA Ladder



Peak	Base Pair Size	Mass (ng)
1	25	43
2	50	63
3	75	58
4	100	43
5	150	33
6	200	110
7	250	27
8	300	33
9	350	20
10	500	27
X	766	42

A) The correlation between the number of base pairs and the retention time using the low molecular weight method. **B)** The peak number, base pair size and the mass of DNA per base pair size generated from figure C. **C)** The Low Molecular Weight DNA Ladder from New England Bio Labs.

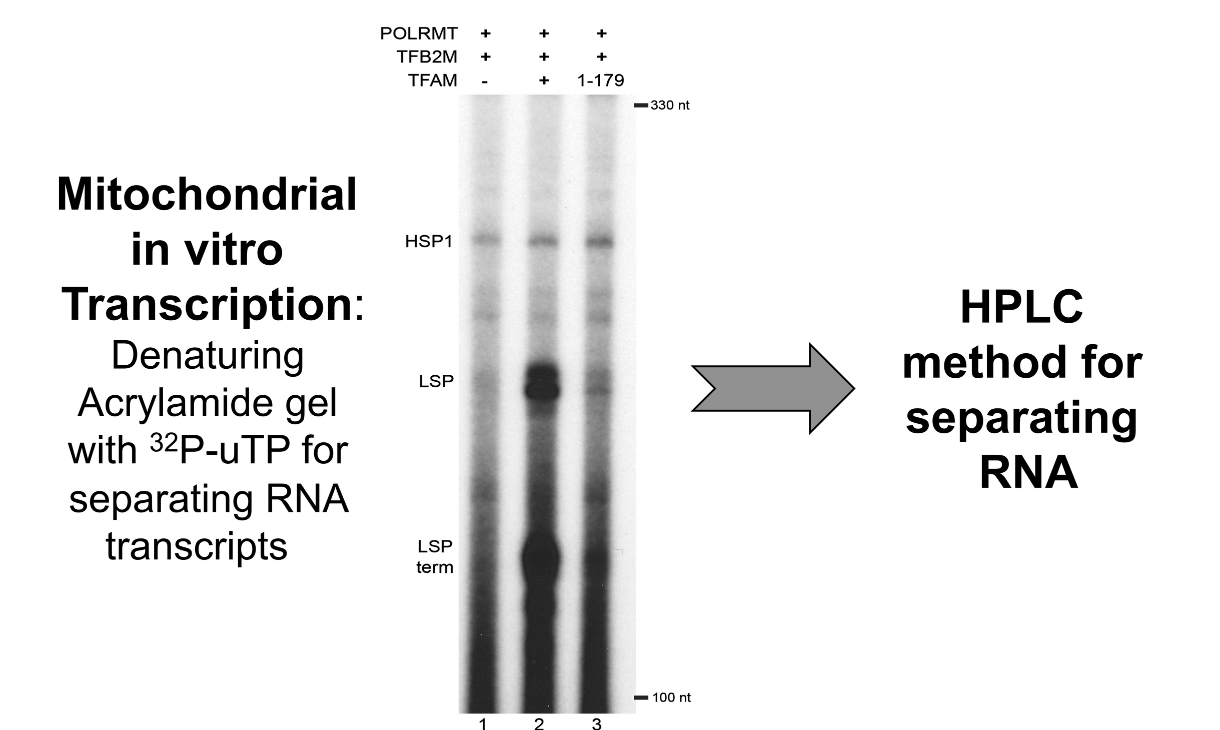
Conclusions

- **Samples of mixed DNA and mixed RNA can be separated based on size using Ion-pair reverse-phase high performance liquid chromatography.**
- **Quantitative relationships can be made between retention time and size, as well as peak height and area to he amount of nucleotides present in a sample.**

Future Directions

Apply the developed IP RP HPLC method to additional in vitro transcription systems:

- other simple systems with alternate RNA polymerases
- more complex systems such as mitochondrial in vitro transcription



Acknowledgements

Linfield Chemistry Department
Linfield Student-Faculty Collaborative Research Grant
The Wendell L. Foote Fund

References

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