

Background

DNA extraction kits are traditionally developed to work with liquid tissues such as blood, saliva, and swabs, but some have been proposed to work with solid tissues. Somatic variation in cancers can be important for tumor subtyping and treatment guidance, including ocular tumors. Additionally, epigenetic marks such as 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) are tissue-specific and change in disease states, particularly evident in diabetic retinopathy and age-related macular degeneration. Commercial DNA extraction kits are available from several vendors, but the various kits have different strengths and weaknesses, and the removal of PCR inhibitors will vary with each kit. This project investigates the yield and purity of DNA from ocular tissues using commercial DNA extraction kits.

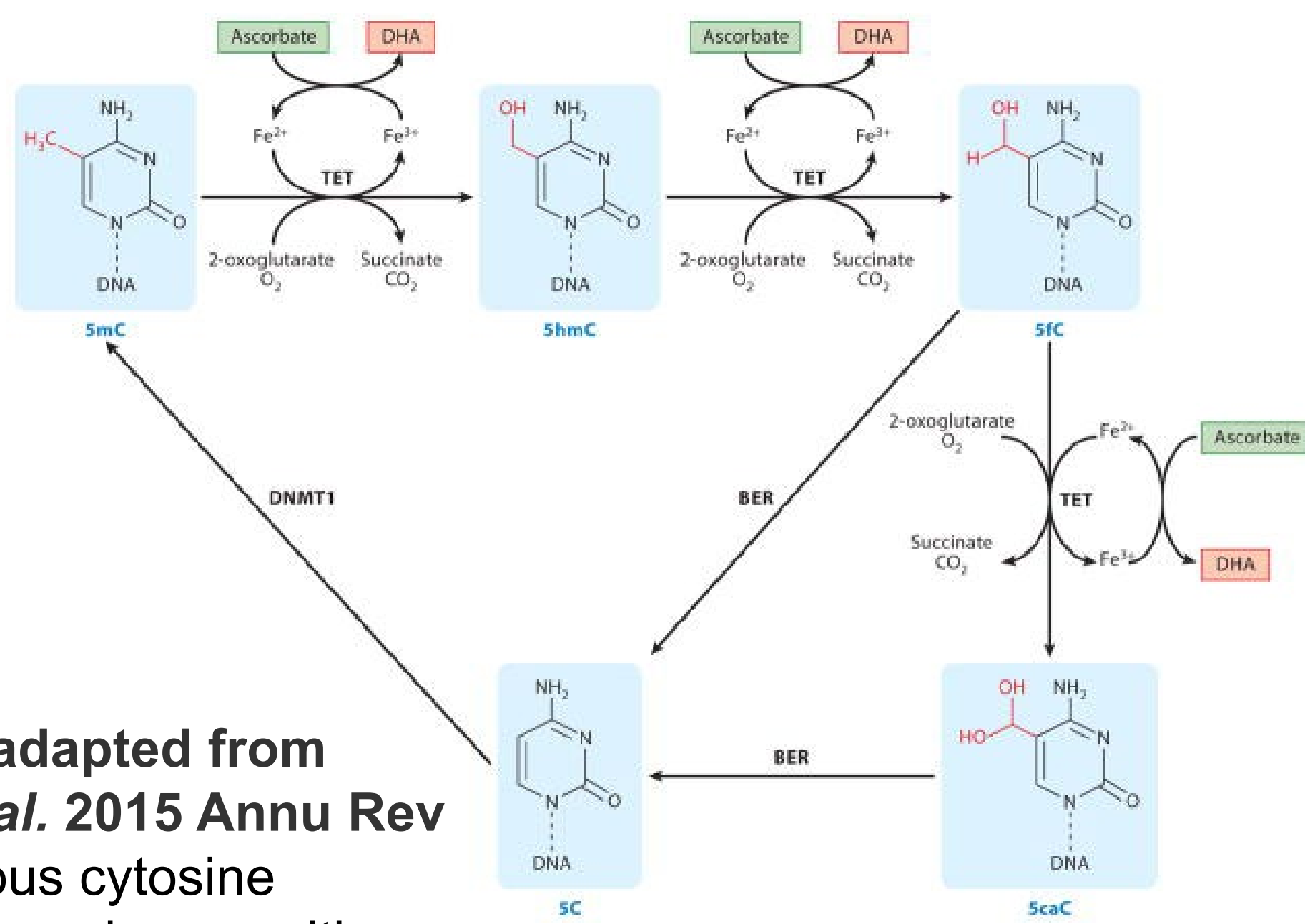
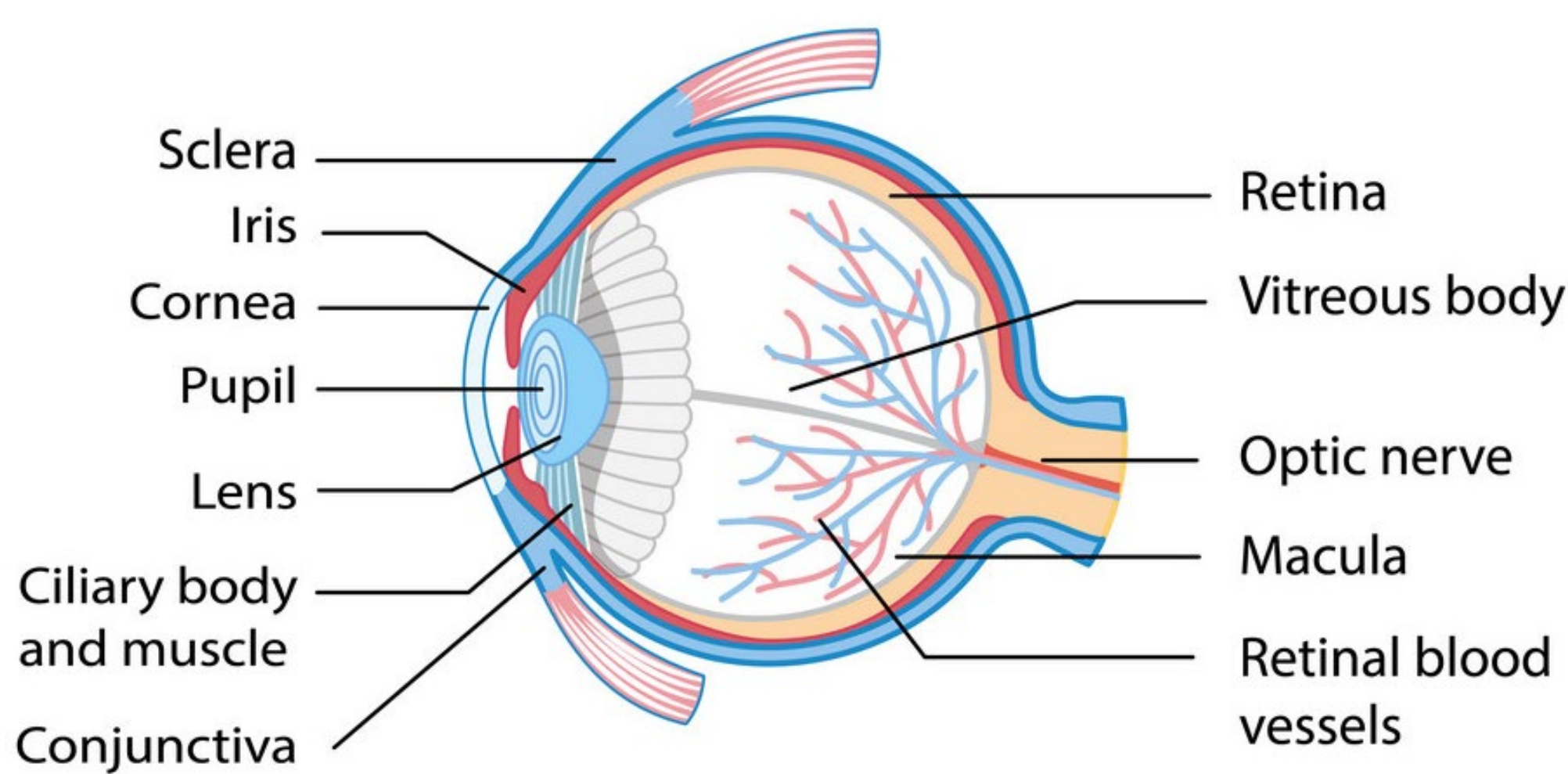


Figure 1. adapted from Young et al. 2015 Annu Rev Nutr. Various cytosine modifications change with tissues and in disease states, necessitating extraction of DNA directly from tissues.

Methods

Tissues from Cornea, neural retina, RPE/choroid layer, optic nerve, and capsular bag were collected and aliquoted into 15 mg aliquots. Extractions were performed using the following kits: DNEasy Blood and Tissue Kit (Qiagen;), GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific), Monarch HMW DNA Extraction Kit for Tissue (New England Biosciences), and genomicPrep Mini Spin Kit (Cytiva). DNA was quantified using the Qubit Fluorometer and molecular weight was checked by agarose gel. Several more kits are currently being tested.



Picture 1. Anatomy of the Human Eye. Obtained from VectorStock.

Several kits can be used to obtain high molecular weight DNA from ocular tissues

Results

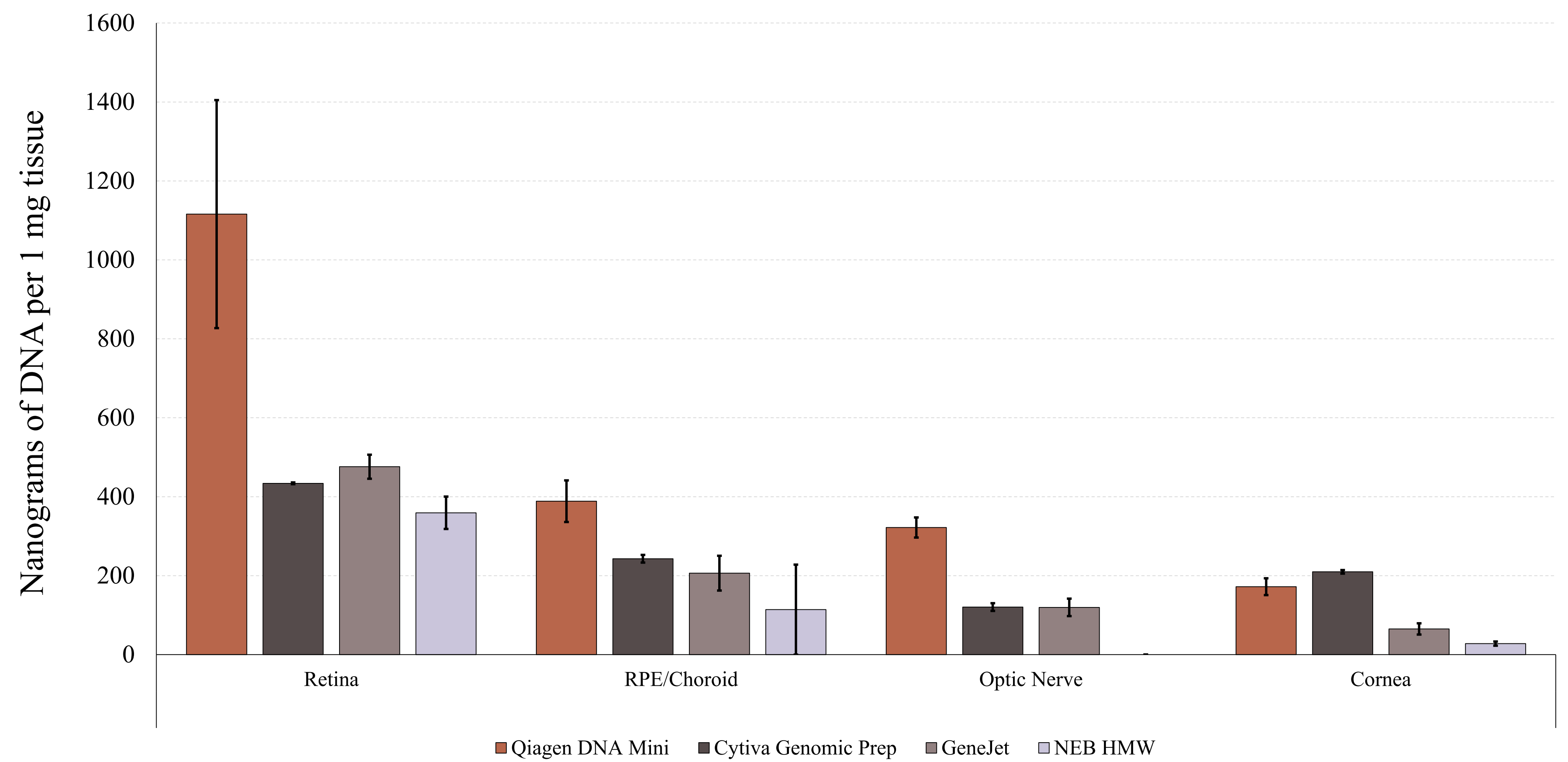


Figure 2. Yield of DNA from different extractions (ng of DNA from 1 mg of tissue). DNA was extracted from ocular tissues using four different DNA extraction kits: Qiagen DNA Mini, Cytiva Genomic Prep, Thermo Fisher GeneJet, N.E.B. High Molecular Weight Tissue Kit. Overall yields were highest from the Qiagen kit, but the Cytiva kit was the most consistent.

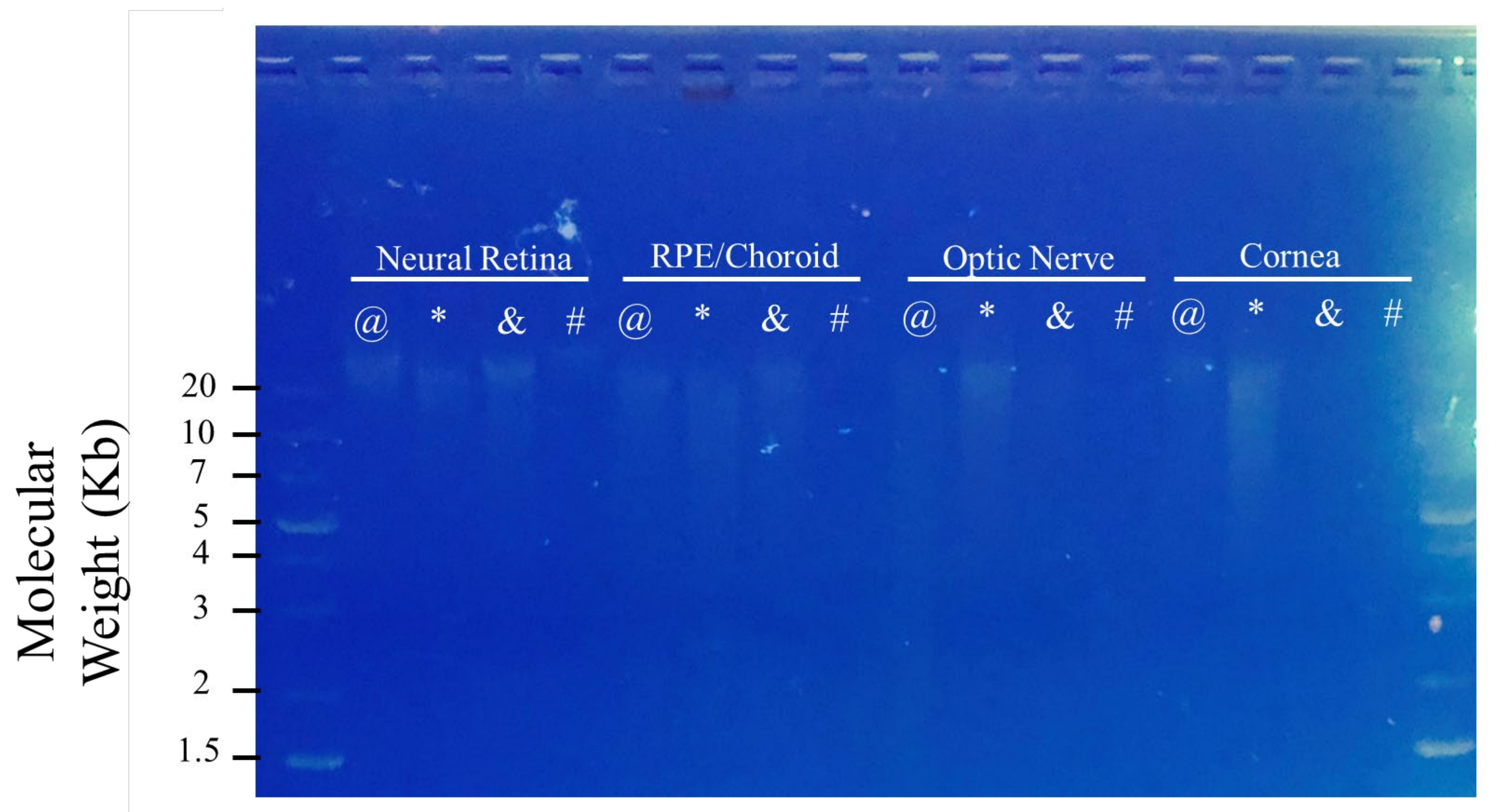


Figure 3 Agarose gel showing molecular weights. Agarose gel (1% agarose) was used to determine the average molecular weight of the DNA extracted from various gels and tissues. Symbols: @ Qiagen DNA Mini; * Cytiva Genomic Prep; & Thermo Fisher GeneJet; # N.E.B. High Molecular Weight Tissue.

Conclusion

Of the kits tested, all are sufficient to obtain significant amounts of DNA from all ocular tissues aside from the capsular bag. The Monarch HMW yielded the highest molecular weight, but significantly lower quantities of DNA than the other kits, indicating that it may not be ideal for most purposes. Protocol development for the capsular bag is still underway.