The Effects of Collection Procedures on Telomere Length Measurement in Population-based Surveys

NIA-Sponsored Biomarker Network Meeting

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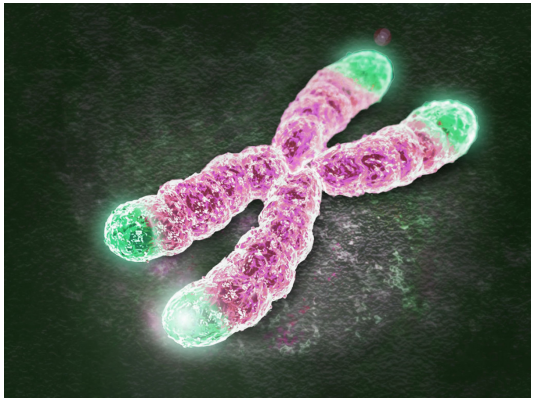
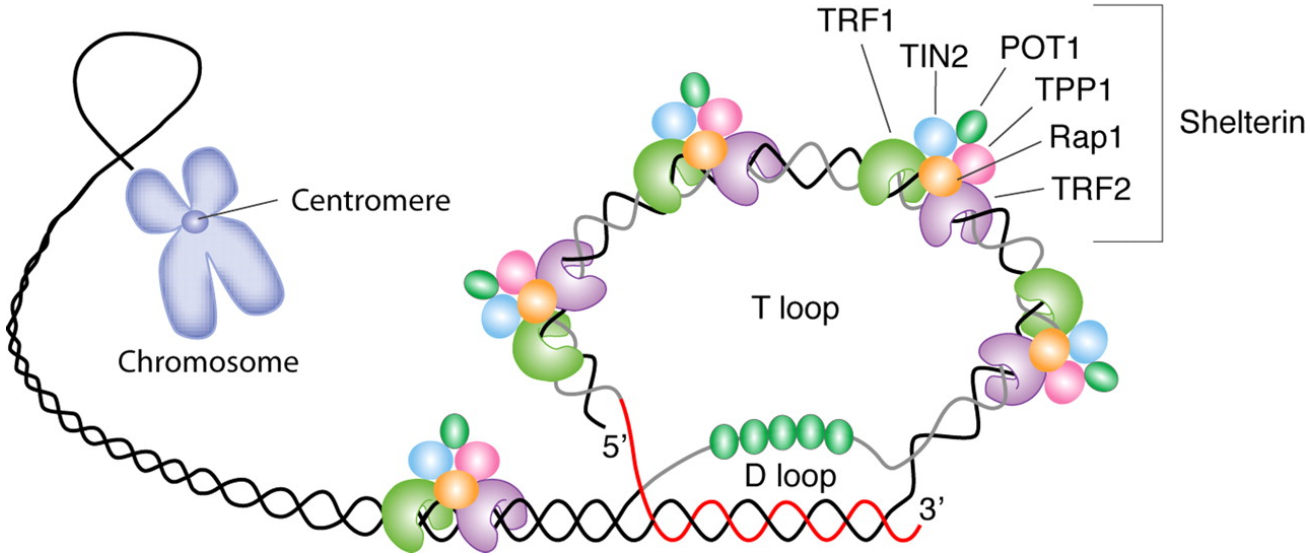
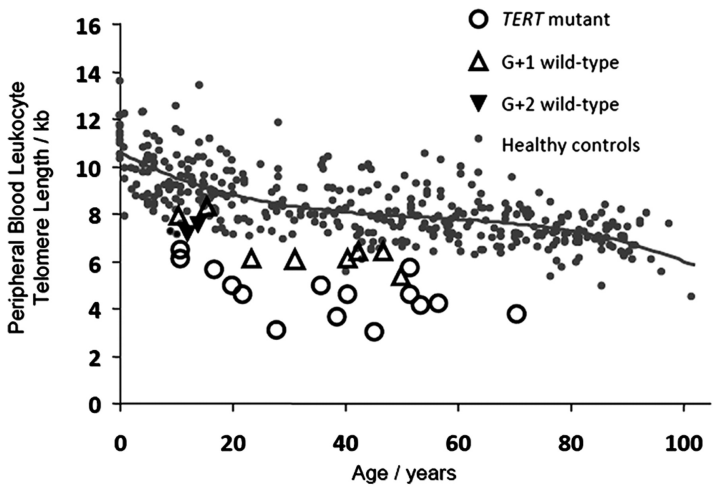
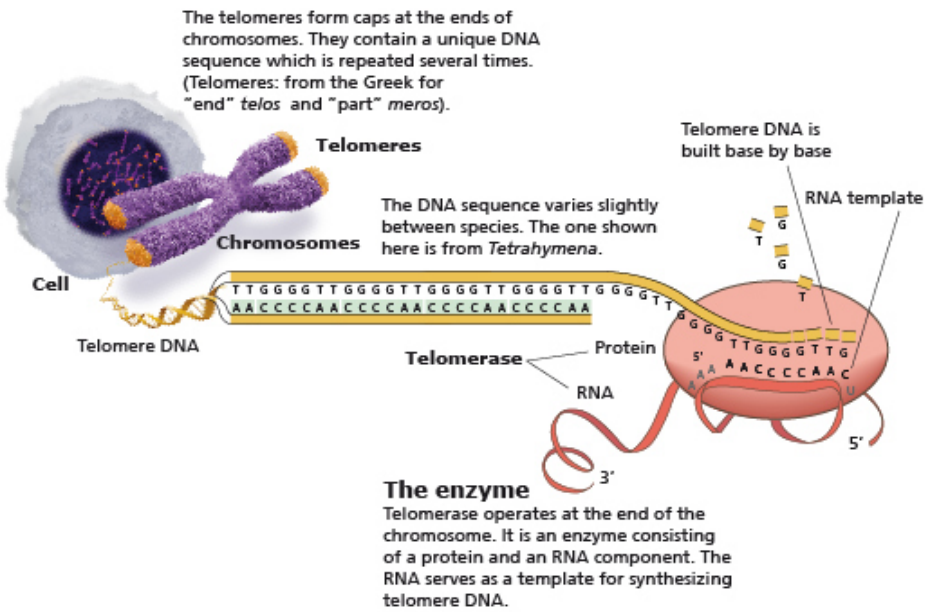
THE TELOMERE'S STRUCTURE AND BIOLOGY IS MUCH MORE COMPLEX THAN COMMONLY PORTRAYED





Telomere length at age represents a balance between cell division and telomerase activity. Other factors may disturb this balance or damage telomeric DNA, resulting in shortening or lengthening of the telomere. It is this 'disturbance' that makes the telomere length an interesting biomarker.





PNAS June 1, 2010. 107 (22) 10148-10153

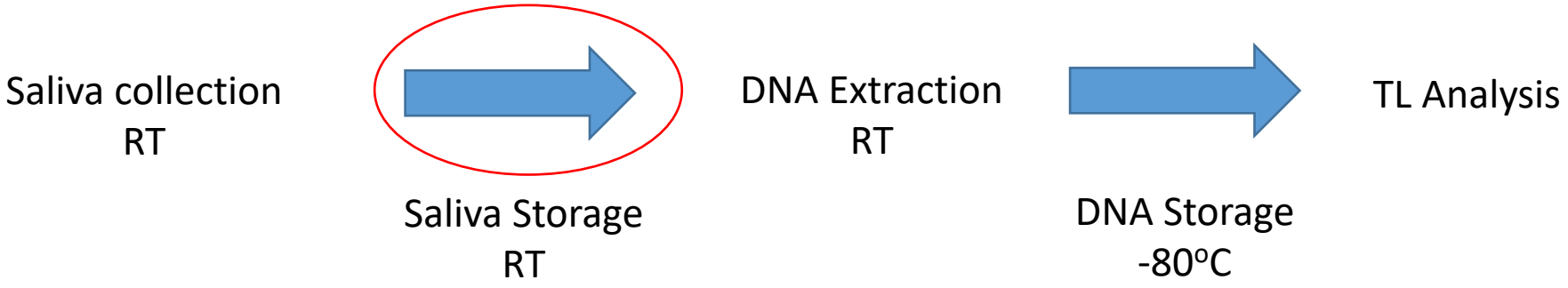
Nobelprize.org

BACKGROUND

- Studies show associations between shorter TL and age-related diseases such as: cardiovascular disease (e.g., stroke, ASHD), cancer, and diabetes as well as osteoporosis, cognitive function, dementia, depression, and autoimmune diseases.
- TL is also measured cross-sectionally and longitudinally to characterize positive or negative effects of social environment, nutritional or behavioral factors.
- Measuring telomere length (TL) accurately has become increasingly important as interest grows in TL as a biomarker of aging and stress – which differences are real and which are measurement error?

SIGNIFICANCE

- Many past TL studies collected samples in a lab, but in the extension to field studies—with longer delays between collection and DNA extraction—some fundamental issues were left untested.
- This project examines one possibly strong confounder of measured TL—time stored at room temperature <u>prior</u> to DNA extraction and storage at -80° to -196°C.
- Does storage time affect TL outcomes?



PROTOCOL

- Saliva collected in lab using from 30 volunteers (18-65 years, avg. 35 y; 13 F) over 3 days
- Subject spits into a 50 ml conical tube
- Saliva divided into 6 DNA Oragene saliva collection kits and mixed with Oragene solution
- DNA isolated from 1 kit (time 0) and stored at -80°C
- The remaining aliquots stored at room temperature for 1, 2, 6 or 12 months after which time DNA isolated and frozen.

ANALYSIS

- At 1 year, all samples analyzed simultaneously using quantitative PCR (Mitchell et al., 2014; O'Callaghan 2011) in the Notterman Lab at Princeton
- DNA quantified by picogreen (measures dsDNA)
- Telomere length from all samples measured in triplicate by qPCR at 12 months (c.v. assay < 0.1).
- Significance tested by ANOVA, Tukey.

> What is genius but the power of expressing a new individuality?
> — Elizabeth Barrett Browning

The Maharashtra Herald, Saturday, January 26, 2008

World Spitathon Champs

This ancient Indian sport needs to get its due recognition

GUEST COLUMN

HARSHAD OAK

Indians might have the strongest lungs on the planet. Not because of any genetic reasons but because of the exercise that they make their lungs undergo by firing spit missiles every couple of minutes. After generations of spitting projectiles, it no longer is just a habit but is a national sport and a cherished art. Across the country we have great exponents of this art, hard at work on every street. While walking, driving, through cars, buses and every other vehicle you will find spit missiles fired with great dexterity and regularity. Although the best performances come from tobacco consumers, even those who are not, often put in scintillating performances.

One of the reasons why this art thrives in India is the conducive environment. Pollution in the cities ensures that most are suffering from cold, cough and throat problems, so firing spit missiles comes very naturally to them. Practising the art all day long can also then be categorised as a health related exercise. Widespread tobacco consumption also works as a great stimulant to participate in the sport in both the urban and rural areas.

Occasionally the government, social organisations and even movies like *Lage Raho Munnabhai* play spoilsport and try to discourage people from practising the sport. However in spite of these minor hurdles it continues to flourish in India. Even our cricketers, despite their global lifestyle haven't lost touch with their roots and are often caught on camera trying out their hand or rather mouth at the great art. Youngsters are quick to pick up not just cricketing skills but even this expertise from our men in blue. Discrimination amongst the sexes is a much-debated subject in sport; however that isn't true when it comes to the sport of spitting on the streets. Both men and women are just as competent and get equal opportunities.

In my effort to research this great art further, I spoke to some promising spitters in Pune about why they spit on the streets and how they began. The reasons I got ranged from "Cough problems first got me interested in the sport and I have been hooked since" to "It's fun and the man thing to do." Some just couldn't come to terms with the question. Their expression suggested that they thought my question was as ridiculous as "Why do you eat everyday?" When I asked if these prolific spitters also practised at home, most complained that modern buildings just do not have necessary provisions or ambiance. When I asked about a common belief that practitioners of this sport are inconsiderate and inconvenience others, the spitters were unanimous in saying that this was unfair of society. They asked: "If a cricketer hits a sixer that also knocks out a spectator, don't we still applaud; so why blame a spitter who not just hits his target but also the clothes of bystanders?"

Considering the history and tradition of this skill and the fact that it continues to thrive and prosper in India, one would think that India is best suited to conduct the first World Spitathons. The rules are simple; spit a glass of coloured water as far as possible. For best results, the competition arena should have traffic signals installed; for some unknown reason a red light always induces *Aaaak Thuuus* all around. Once the field gets really competitive, we could see research on what kind of liquid gets you the most distance, etc. Adidas and Nike might even start selling special sports gear that optimises athlete posture, trajectory and performance. Like Athens is considered the home of the Olympics, considering the talent in the city, Pune could very well be the home of the Spitathons. Since most Indians practise the sport, it is sure to get huge viewership. Millions in sponsor money will flow in, particularly from the tobacco companies. Imagine the 'World champ M S Spithoni' on TV all day in ads for Spitichand mineral water.

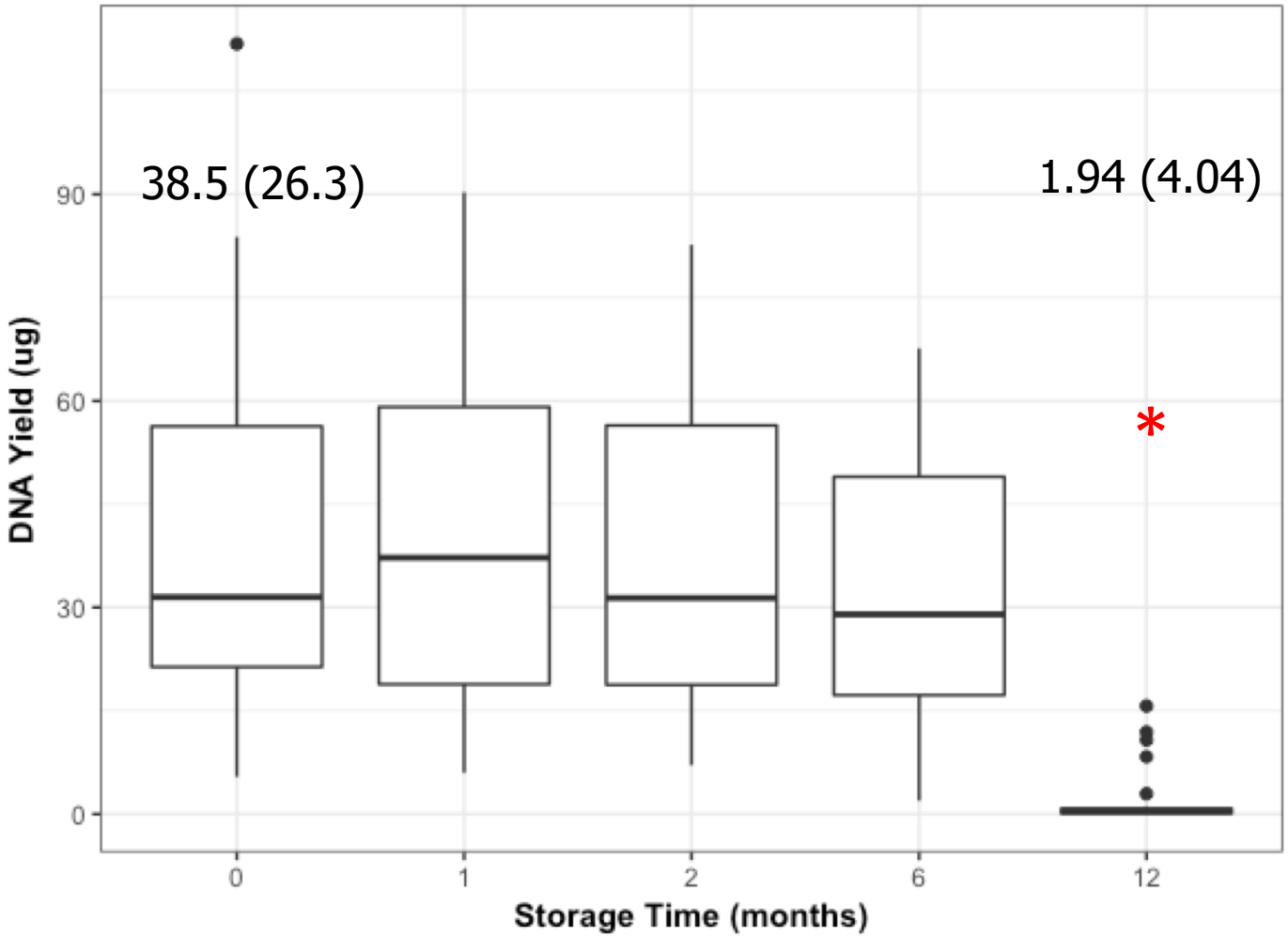
Considering the cultural advantage we enjoy, at least in the initial years the world spitathon champs will be Indians. The Chinese would probably soon create super spitters who will beat us silly, but for a short period we can bask in the glory of being world champs.

The only major hurdle to the growth of the sport are a handful of people who have different notions of cleanliness and resent good old Indian habits like spitting every 30 seconds on the nearest public property available. Not only do these people never spit on the streets but also actually have the audacity to ask others to give up the habit. Stringent measures are necessary to control this lot. So anytime one encounters such a person with contradictory opinion to the masses, he should be beaten up or at least humiliated in public. Fortunately all groups performing such acts of public beating, humiliation or destruction enjoy automatic immunity in India.

So now that you have been initiated in the Spitathons, keep aside this paper and fire your first spit missiles. Ensure that you are spitting on public property as that's the basic rule of the sport. Anything beyond five feet is good. You never know, you might just become the first spitathon champion of the world.

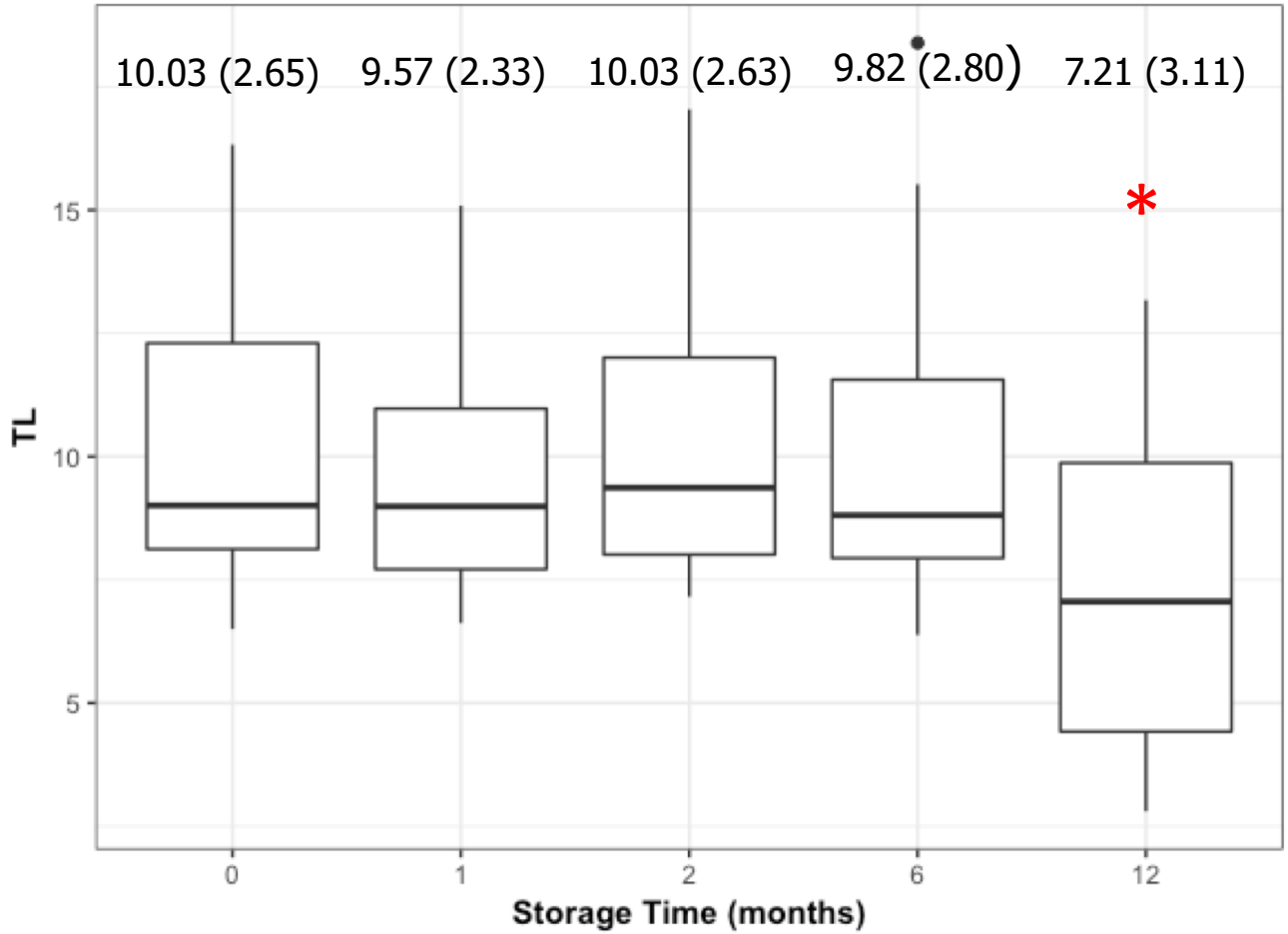
(The columnist can be reached at harshad@rightrix.com)

dsDNA Yield is dramatically reduced between 6 and 12 months of storage



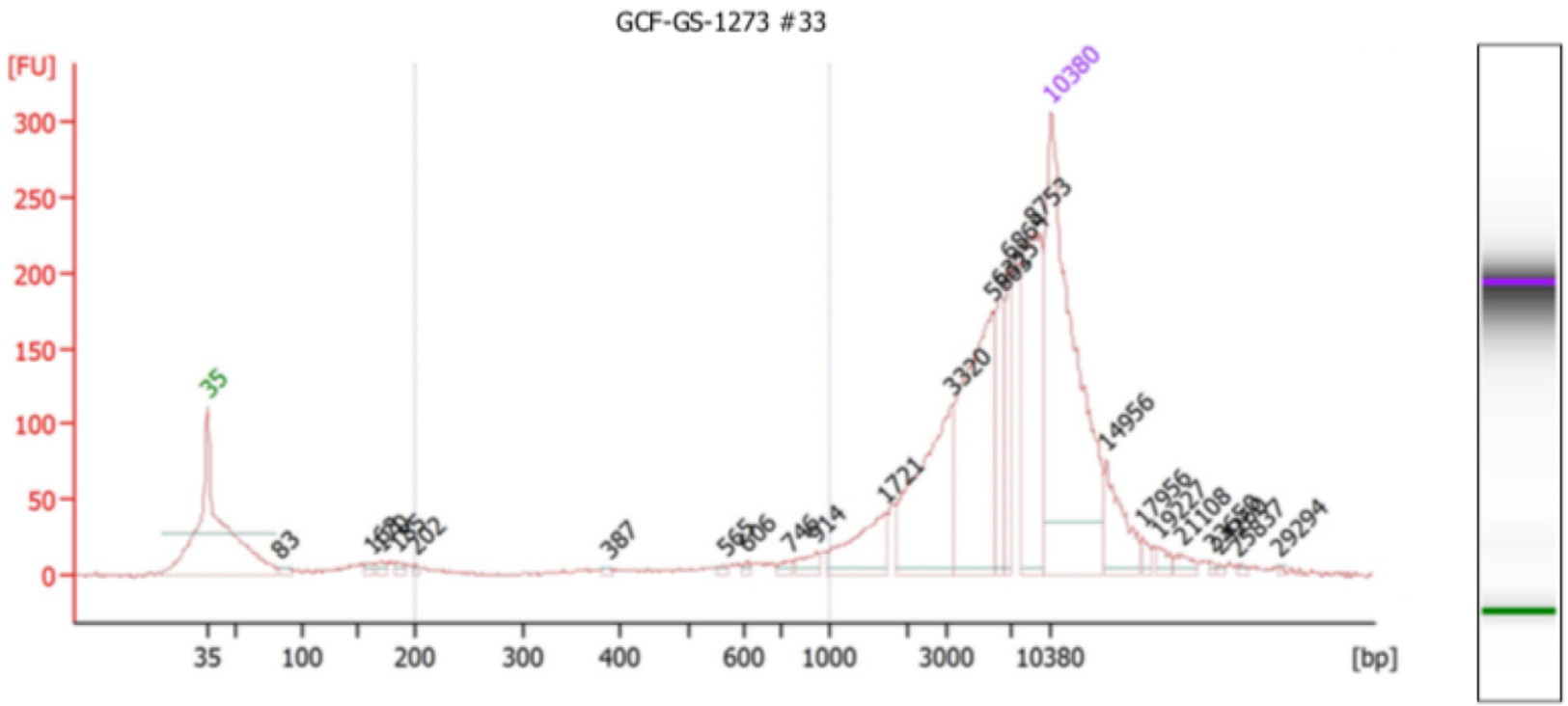
* $p<1*10^{-6}$

Measured TL decreases between 6 and 12 months of storage

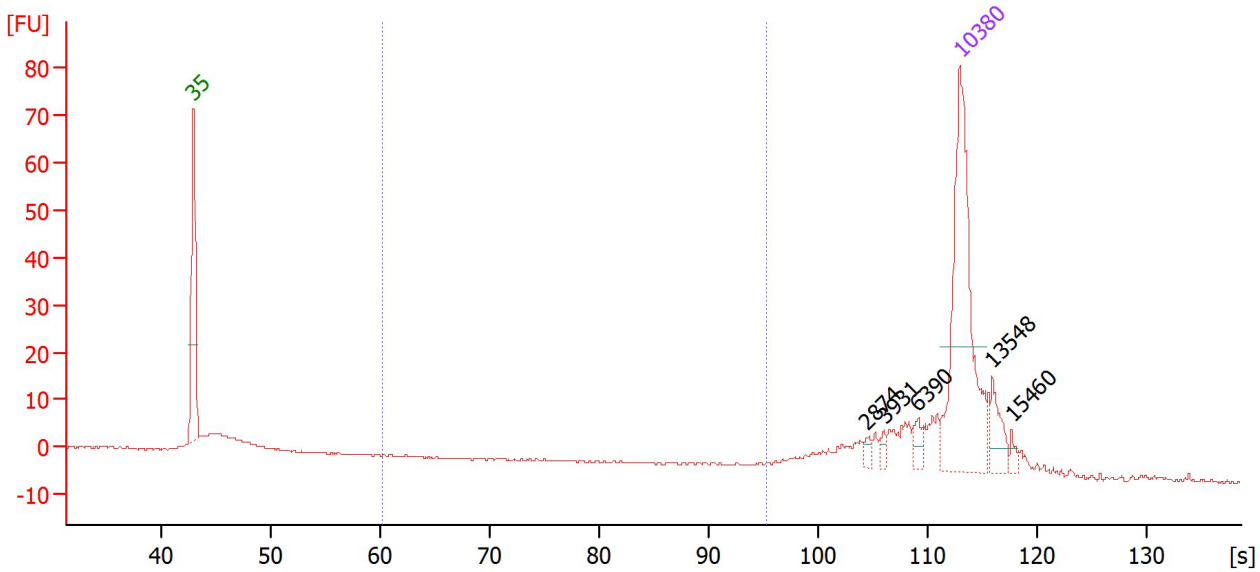


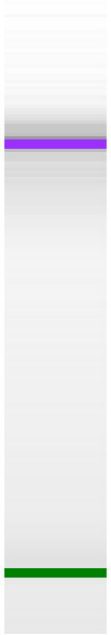
* $p < 10^{*-3}$

DNA distribution at 12 months in stable sample

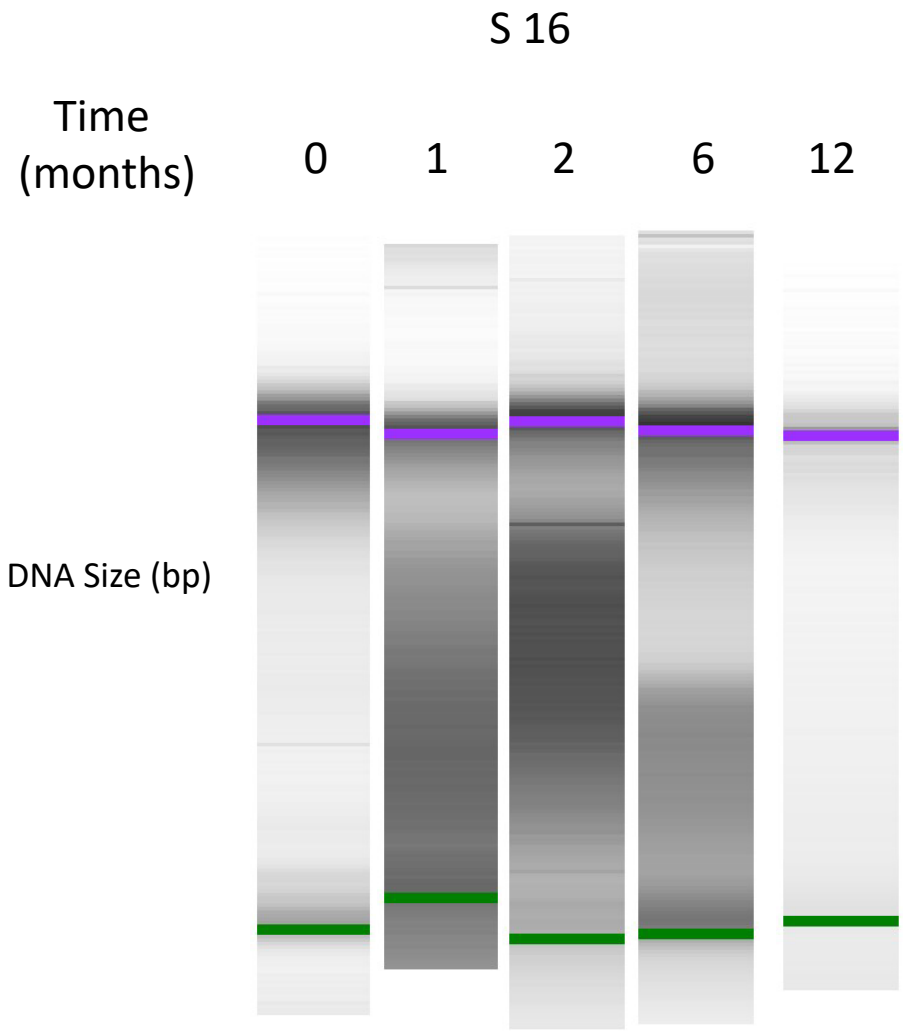


DNA distribution at 12 months in an unstable sample





DNA Degradation over 12 months of Saliva Storage at RT



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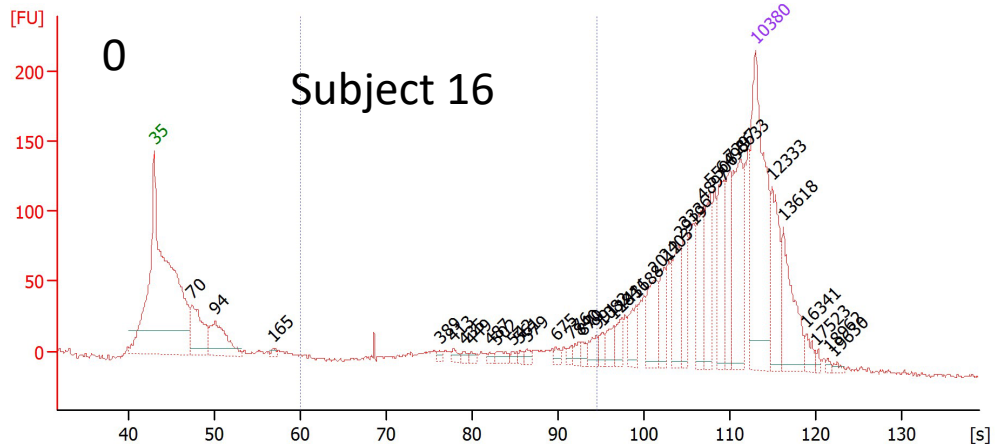
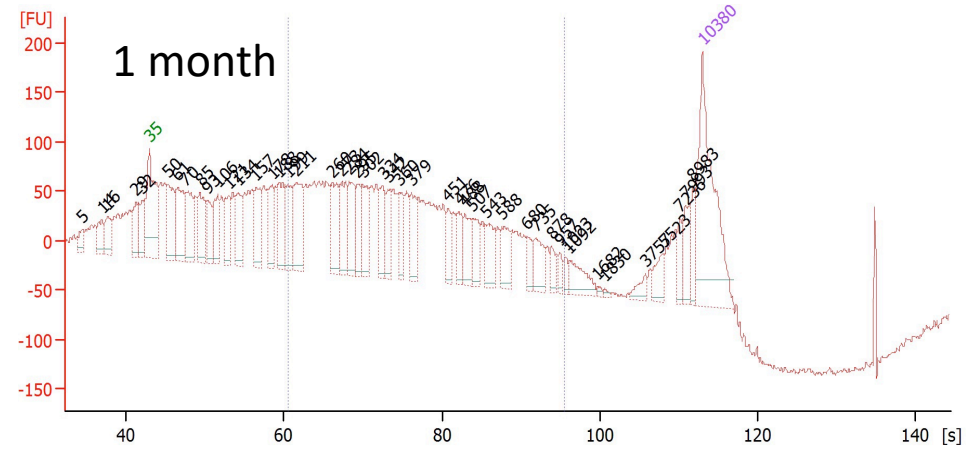
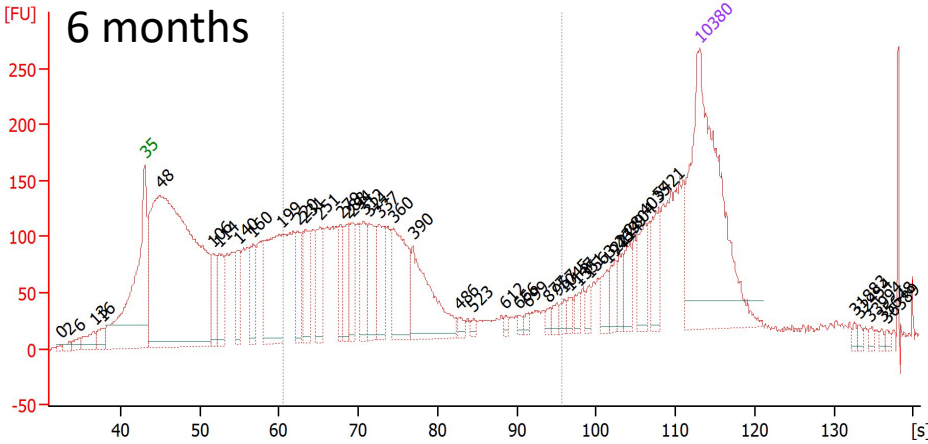
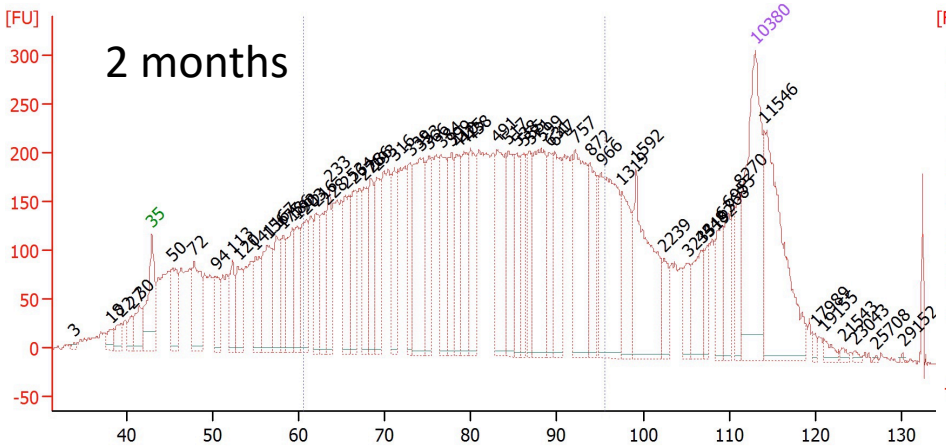
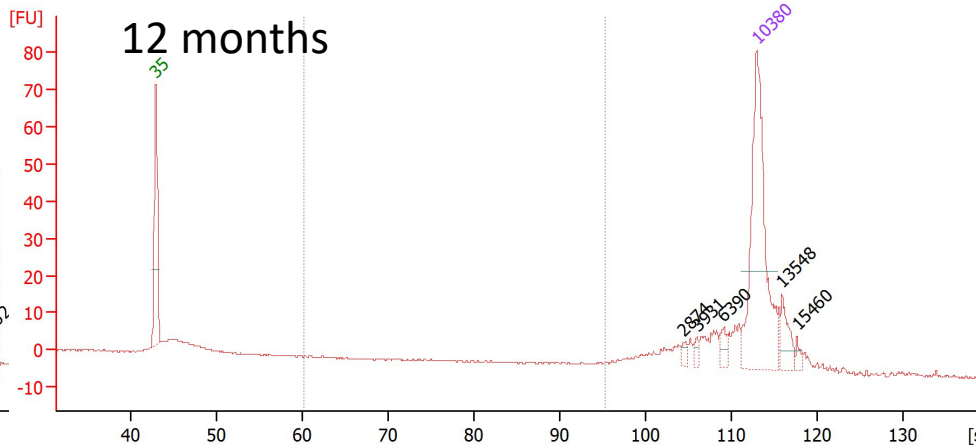


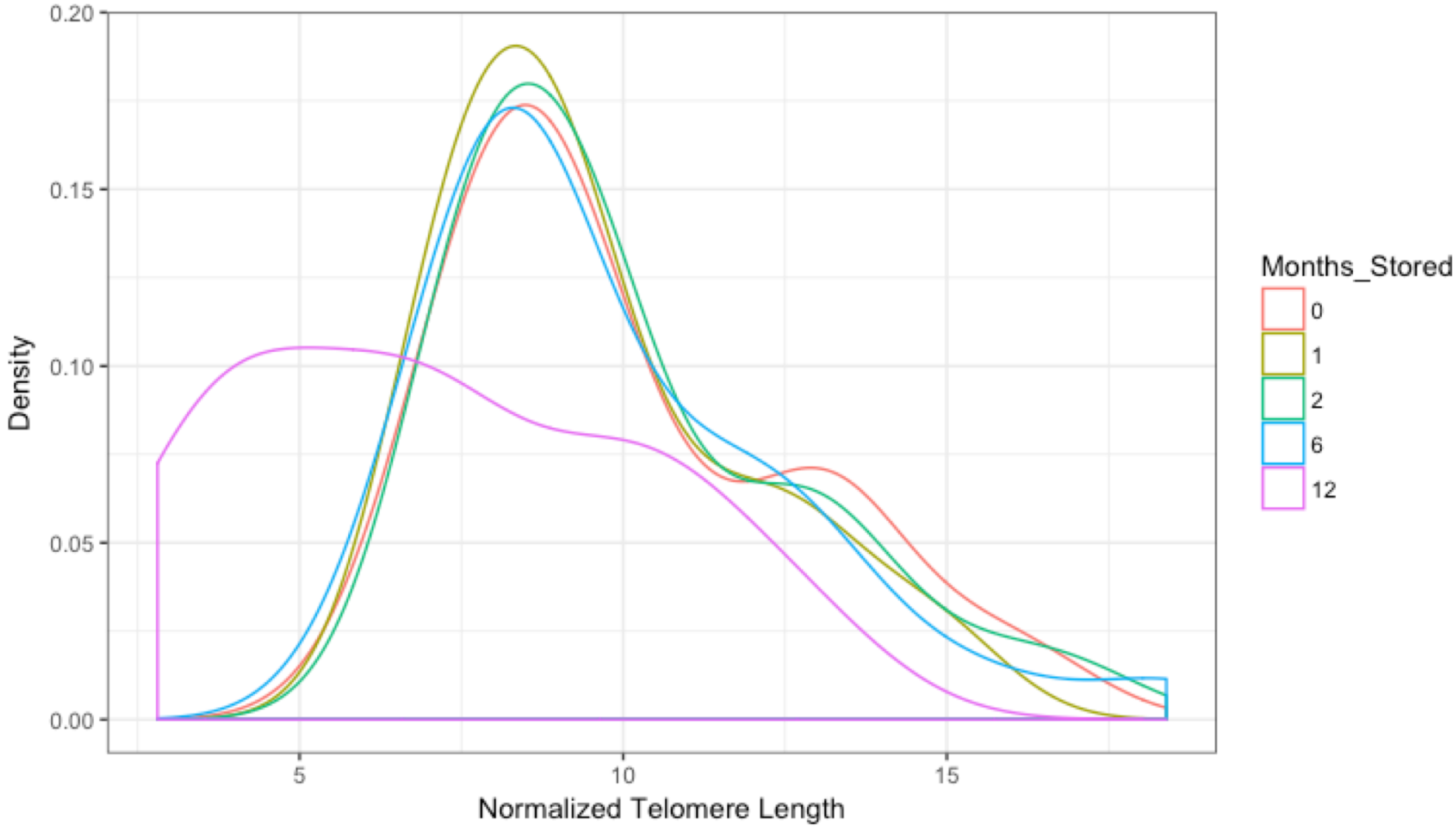




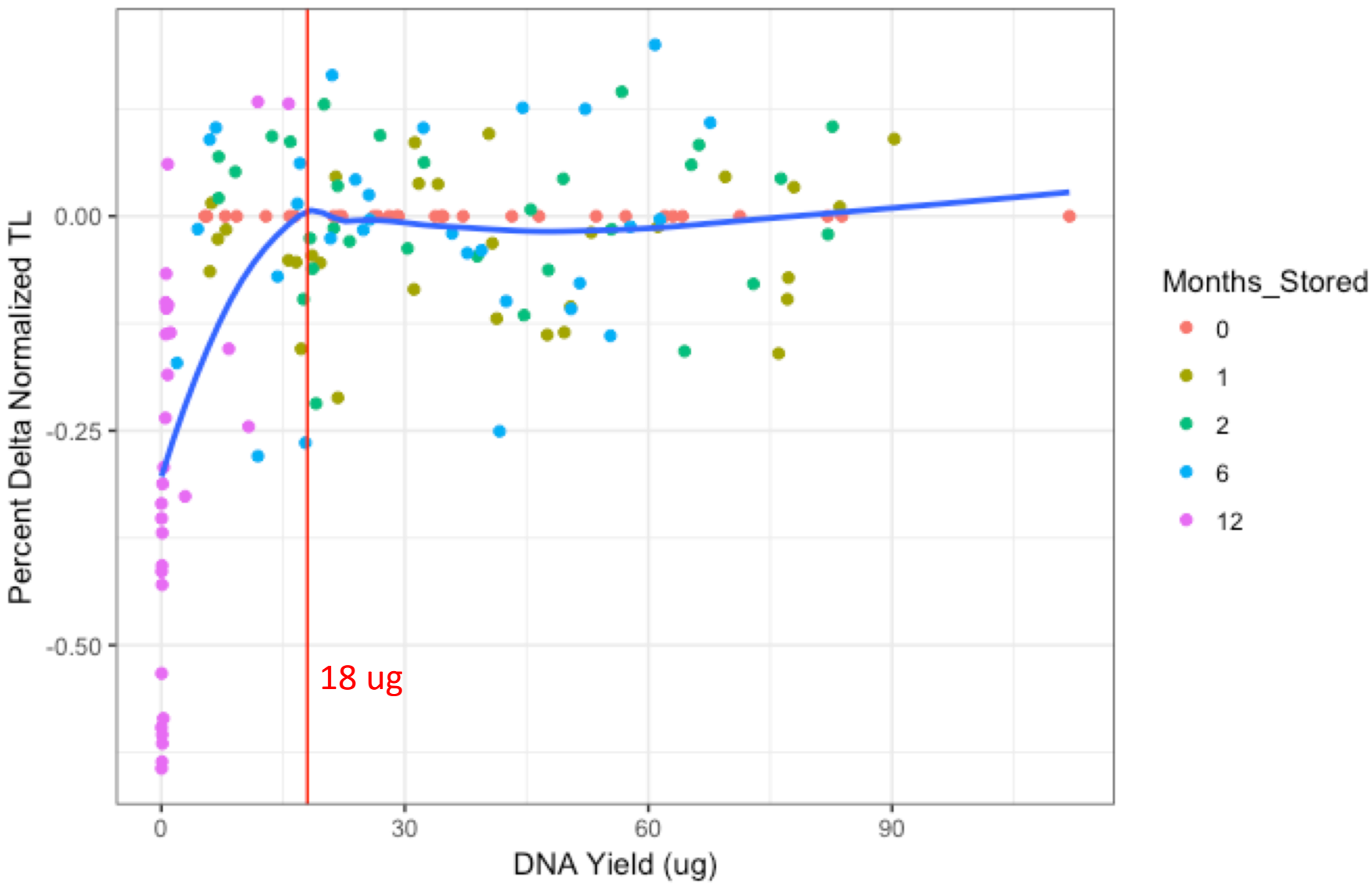




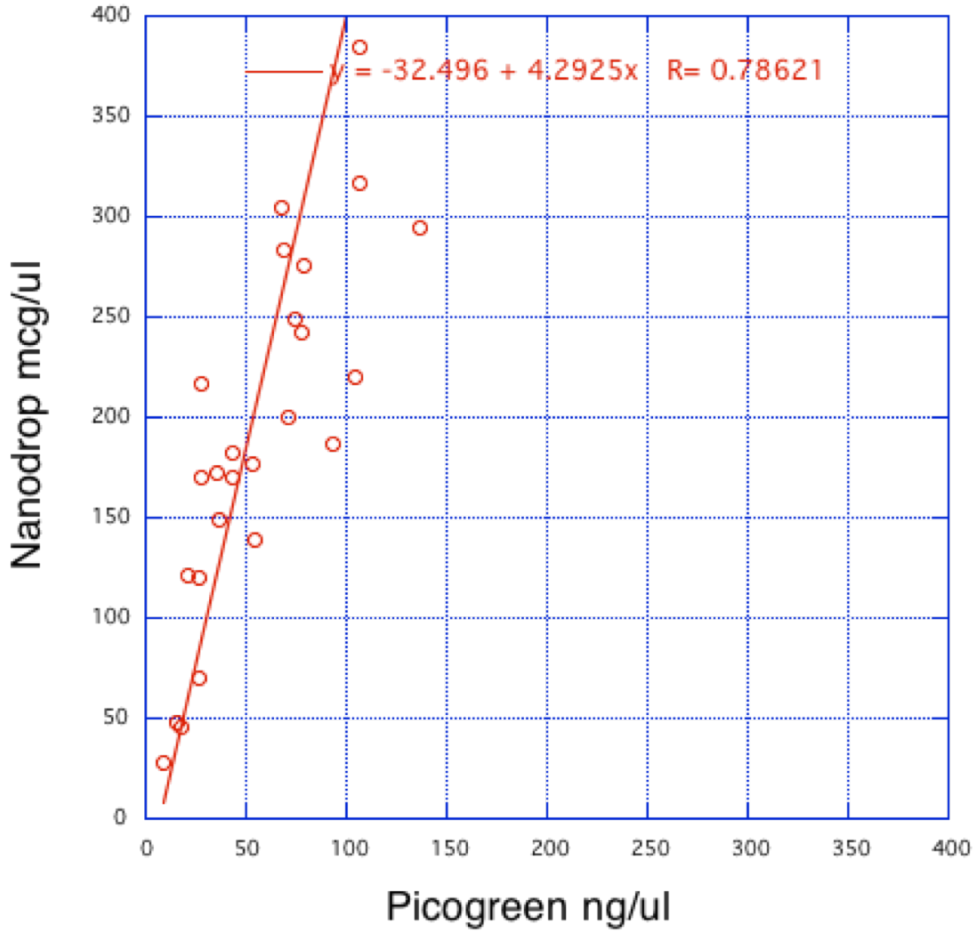
Density plot—TL stable until 6-12 months



Percent change in TL vs DNA Yield: at ~50% loss of yield measured TL decreases sharply



Picogreen (dsDNA) vs Nanodrop (all nts) measurement of DNA Yield



RECOMMENDATIONS

- Saliva sample storage duration at room temperature should be minimized, even when using a stabilizing kit. Our lab processes samples within 2 weeks to a month.
- Determine DNA yield following extraction, and reject samples with substantially less DNA than expected (~18 µg in this experiment; ~50% of original yield).
- Extracted samples stored in several aliquots at -80°C. Do not freeze, thaw, re-freeze samples.
- Preprocessing stability of blood and blood spot samples should be prospectively evaluated.
- Long term goal: possible mechanisms for adjusting already collected material if time at room-temperature is known.
- Further research on improved room temperature stabilizing solutions.

ACKNOWLEDGEMENTS

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