Optimizing DNA purification from saliva for Next Generation Sequencing

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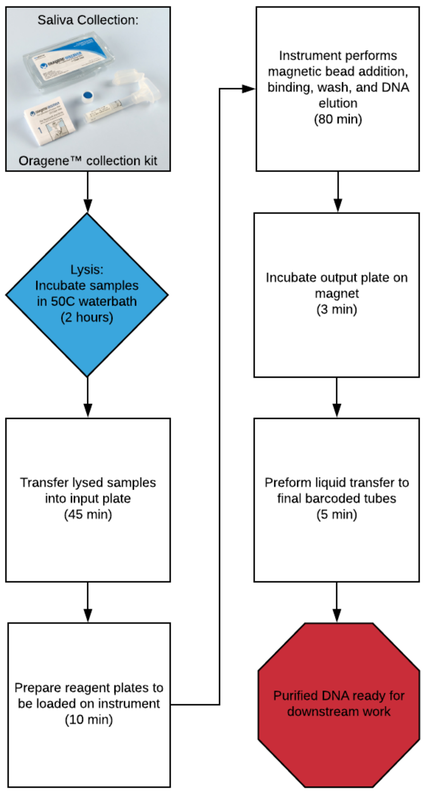
Impact of DNA from saliva

Relative ease of saliva collection paired with the direct-to-patient model has removed many technical barriers allowing for a new paradigm in genomic research. Count Me In research initiatives including; www.mbcproject.org, www.ascproject.org, www.mpcproject.org are now routinely using this model to collect saliva and solid tumor biopsy samples to sequence participants. Traditional methods of DNA collection of blood samples requires meeting with a trained phlebotomist and flash freezing the sample. Fresh blood can also be used, however it has a shorter shelf life than Oragene saliva collection tubes. Saliva will stay intact for years at room temperature and lysis can occur safely in the collection tube with minimal intervention. Optimization of saliva extraction methods is important in order to obtain substantial yield and purity from the sample. The following system is used to rapidly extract DNA from saliva at high throughput.

Chemagic MSM I



Saliva is collected and shipped to the Broad Institute for processing. Once lysis has occurred in the original sample collection tube the raw material is transferred into a plate for high throughput processing on the Chemagic MSM I and Agilent Bravo.

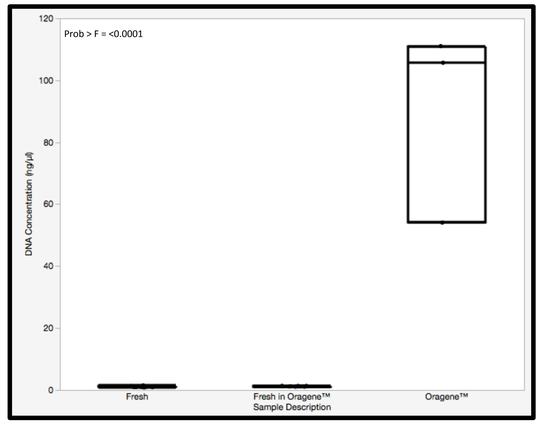
We routinely assess new methods available in both research and commercial spaces. These are the qualities we are looking for in a new approach:

- Automated workflow with walk-away instrumentation
- High throughput system capable of processing ≥ 96 samples
- Flexible and customizable user interface
- Ability to maintain sample chain of custody
- Ability to integrate reagent lot tracking
- DNA yield and purity sufficient for downstream sequencing

Investigation of types of experimental saliva samples

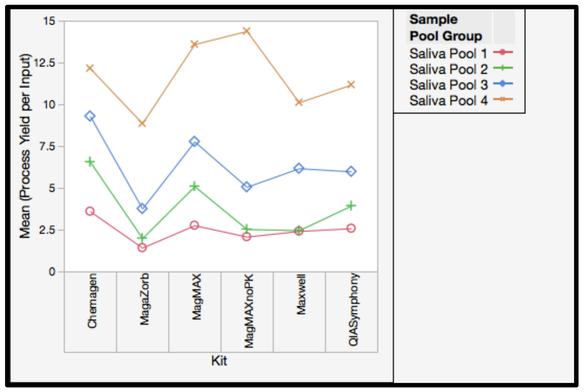
Initial testing of fresh saliva resulted in poor yields. An experiment was designed to determine if the samples used were of poor quality or if there were processing issues in the first tests. We evaluated several different starting materials on the same instrumentation with identical input and output volumes and determined that the fresh saliva was not appropriate for testing.

Input material analysis



Saliva types were extracted in parallel and the final DNA concentration was compared for fresh saliva, fresh saliva aliquoted to Oragene tubes, and Oragene™ collected saliva. The Oragene™collected saliva was significantly different than the other sample types.

Average sample performance



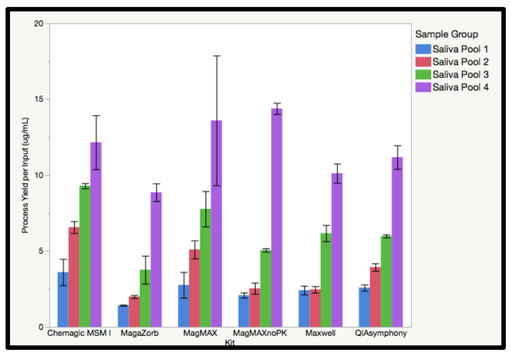
Each pool group of Oragene collected saliva had similar performance across kits. This displays each pool group's mean process yield per input across each kit that was tested. There is no significant difference between pool groups.

The low yield analysis of fresh frozen saliva vs Oragene collected saliva triggered the use of Oragene collected saliva pool groups for the subsequent experiments. As most samples arrive in this format it was logical to continue testing with saliva that was originally collected in these tubes. Samples were obtained anonymously and pooled into four groups based on total volume available. This was achieved by donors not being identified from a larger audience and having a drop off location that maintained autonomy.

Kit chemistry metrics are highly comparable

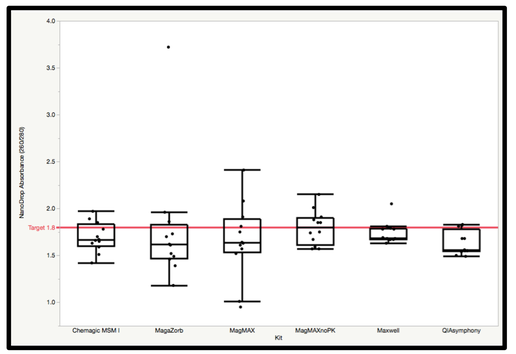
Six saliva extraction methods were investigated using maximum input and maximum elution volume based off of manufacturer recommendation. Four saliva sample pools were used in triplicate for each experiment performed. It is important to note that Proteinase K is commonly used in DNA extraction. With Oragene tubes the lysis occurs in the preservative buffer provided. Therefore it was recommended to not use Proteinase K by some manufacturers. We tested the MagMAX kit with and without the enzyme (MagMAXnoPK) and found that it performed slightly better without its use. However, the overall performance of the kit compared to others was unremarkable.

Average yield



Yield analysis was performed using a picogreen quantification assay to determine the quantity of DNA in the elution buffer. Variable volumes were used so yields were normalized using total process yield (ug) per input (mL). All kits were tested using the maximum volumes recommended by the manufacturer. There was no significant difference in yield. Each error bar is constructed using 1 standard deviation from the mean.

Average purity



NanoDrop spectrophotometer was used to determine the A260/A280 ratio for each sample. The A260/A280 ratio can be used to evaluate DNA purity. When the A260/A280 ratio is close to 1.8 it is generally accepted as representative of a high-quality DNA sample. The MagMAX without the use of Proteinase K and the Maxwell kit had average A260/A280 ratios close to 1.8.

Workflow evaluation

Ease of use assessment

	Manual Processing Time	Preparation Required	Execution of SOP	Clean Up	Risk Assessment	Total Score
Chemagic MSM I	4	2	5	2	1	14
MagaZorb	2	4	5	4	4	19
MagMAX	1	3	2	4	4	14
MagMAX (No Pro K)	2	4	3	4	4	17
Maxwell	1	4	1	4	4	14
QIAsymphony	5	3	5	2	1	16

Ease of use assessment is based off subjective analysis in the ability to execute the chemistries in the lab. It uses a scale of 1-5 where 5 is the ideal score. The coloring scheme increases progressing from light to dark; lowest possible score to highest. This total score system was used as a guide to determine which chemstries would be best to investigate further.

An ease of use subjective analysis was performed to evaluate the workflow for processing samples in the lab. The MagMAX, MagaZorb, and Maxwell kits can be placed on high-throughput automation for increased scalability. To determine chemistry efficiency and quality, the manual versions of the manufacturer protocols were used. Since the Chemagic MSM I and QIAsymphony are automated systems they both received higher ease of use scores. It is expected that this will level out when evaluating the manual kits using automation options.

From this testing it was determined that all kits had comparable total process yield per input and purity scores. Here we found that the MagaZorb system was easier to use in the lab manually. However, further testing is needed with this chemistry on an automated solution to determine its comparability to other automation options. An overall evaluation of the workflow, timing, throughput capacity, purity, and yield helped to evaluate which chemistry methods should be investigated further with liquid handlers incorporated in the testing.

Conclusion

This testing showed us that the sample collection methods can significantly impact the yield from DNA extraction. We learned that the addition of Proteinase K is not necessary for saliva collected in Oragene tubes. We discovered that yield and purity were highly comparable between our current method and other methods on the market. Further investigation is needed in the workflow, reliability, and ease in customization, from each available automation system. As a result of our experiences with other automation systems we generated an automation best practices checklist which will guide us in choosing which instrumentation will run the DNA extraction chemistry from saliva. Our next steps are to evaluate available options to find instrumentation that better suits our current needs and will be adaptable to modern DNA extraction chemistry.

Acknowledgments

Data used in this poster was generated at the Broad Institute, for more information please visit: http://genomics.broadinstitute.org/