Optimizing DNA purification from saliva for Next Generation Sequencing

JACQUELINE DION1, ALYSON REARDON1, MICHELLE CIPICCHIO1, KATIE LARKIN1, NIAWL LENNON1, STACEY GABRIEL1
1Broad Institute of MIT and Harvard, 320 Charles St, Cambridge, MA 02141

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.mitcsproject.org, www.ascproject.org, www.mcpproject.org are now routinely using this model to collect saliva and solid tumor biopsies 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

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.

Chemagic MSM I

Investigation of types of experimental saliva samples

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 Protease 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 (MagMAX/noPK) and found that it performed slightly better without its use. However, the overall performance of the kit compared to others was unremarkable.

Average sample performance

Average purity

Workflow evaluation

Ease of use assessment

Conclusion

This testing showed us that the sample collection methods can significantly impact the yield from DNA extraction. We learned that the addition of Protease 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.

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