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Lab 7 Notebook

Forked from Lab 6 Notebook

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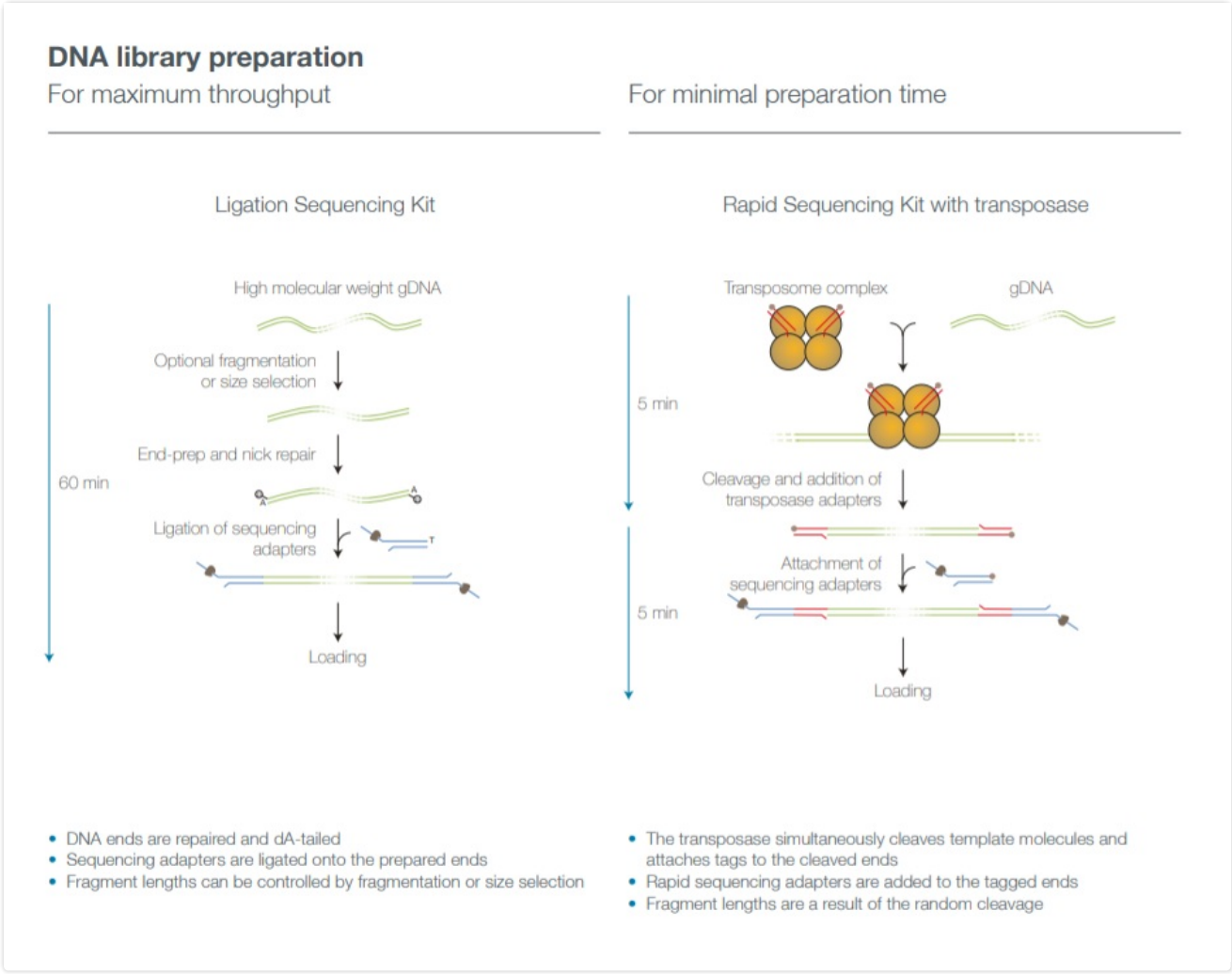
Abstract

Pre-Lab

Read the RAD-004 and LSK-109 protocol prior to starting the lab and answer the following questions.
RAD-004 Protocol
LSK-109 Protocol



There are numerous protocols to prepare a sample into what is known as a DNA library for sequencing on the nanopore. For this class, we will use the RAD-004 protocol. This protocol utilizes transposases to fragment the DNA and attach transposase adapters to each DNA fragment. Once the transposase complex is broken, rapid sequence adapters are added to the ends of the transpose adapters. The simplicity and "two-step" process is best utilized for those in situations where users desire a fast and simple library preparation time and lack access to ideal lab equipment. Since we will be sequencing in a DIY lab, this is the most ideal approach we can take.

1. Is DNA sequenced in a single strand or in a duplex?
2. What's responsible for pulling the DNA through the pore?
3. What is the significance of the transposase complex?
4. How is the sequencing information interpreted by the MinKNOW software?
5. Draw a picture of how you think Nanopore sequencing works and write 2-3 sentences explaining it.

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Contents	Description	No. of tubes
LMD (yellow cap)	Lambda DNA Identical to that found in the SQK-RAD001 kit	1
FRA (amber cap)	Fragmentation Mix Contains the transposase with transposase adapters	1
RAP (green cap)	Rapid Adapter Contains leader adapters with loaded motor protein; this is a direct replacement of the AMX tube in the Ligation Sequencing kits	1
SQT (violet cap)	Sequencing Tether	1
LB (pink cap)	Loading beads	1
SQB (red cap)	Sequencing buffer	1

This is a list of all the reagents that come with the RAD-004 kit as well as a description of what they do to help with the library preparation and loading process.

Lab Results

1. What protein is this? What's its significance?
2. What is the most prevalent variant shown? Report it's the location and the quality score.
3. What amino acid change would occur in respect to the reference genome at the location of this variant?
4. What gene in Sars-CoV 2 is the variant affecting?
5. What strains do you notice have a similar variation to the one you saw in your data?
6. Were there other variants reported at this location by IgV? If so, how frequently were they?
7. How many insertions and deletions were reported?

Resources

- DIY DNA Sequencing: Nanopore Attempt 1 (video)
- Nanopore MinION starter-pack video (video)

Disclaimer:

The information provided on this document is intended for the educational purposes of the BME 22L laboratory course. It is worth noting that the information listed on this document is subject to change and is not finalized. Therefore, the information on this document should not be used outside of this course.

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