Genomics Assembly and Analysis Training Module

ORIGINAL ARTICLE BRIEF REPORT

Molecular Evidence of Sexual Transmission of Ebola Virus

Suzanne E. Mate, Ph.D., Jeffrey R. Kugelman, Ph.D., Tolbert G. Nyenswah, L.L.B., M.P.H., Jason T. Ladner, Ph.D., Michael R. Wiley, Ph.D., Thierry Cordier-Lassalle, M.B.A., D.E.S.S., Athalia Christie, M.I.A., Gary P. Schroth, Ph.D., Stephen M. Gross, Ph.D., Gloria J. Davies-Wayne, R.N., M.P.H., Shivam A. Shinde, M.B., B.S., Ratnesh Murugan, M.B., B.S., et al.

- In Liberia, the partner of an Ebola survivor became sick.
- Did the partner contract Ebola through sexual transmission? Or through some other means?
- How can we tell?
- **These questions can be answered by sequencing.**

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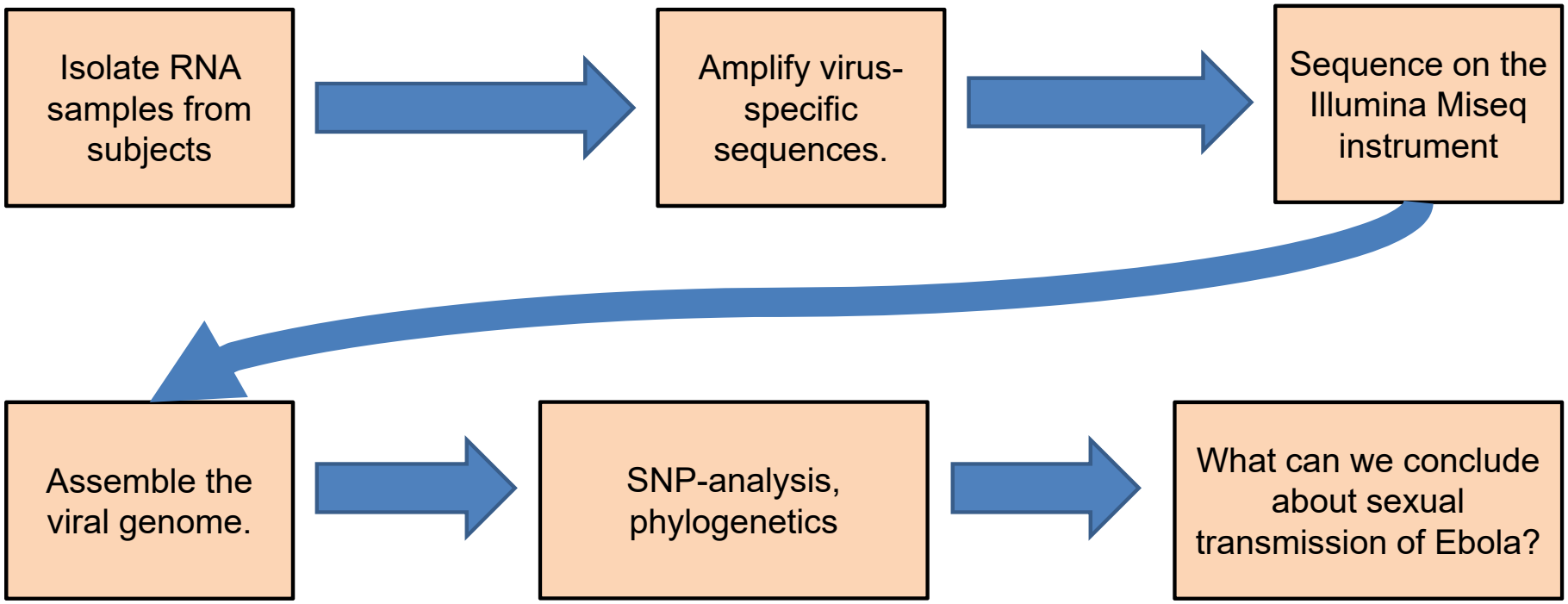
To answer these questions, we can:

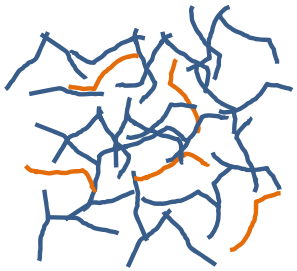
- Isolate virus from the survivor and their partner.
- Sequence the virus to discover the complete, accurate genome of each sample.
- Compare these sequences to each other and to other virus samples from this outbreak.
- Is the partner sample more similar to the survivor sequence? Or to the other samples from this outbreak?

Study overview



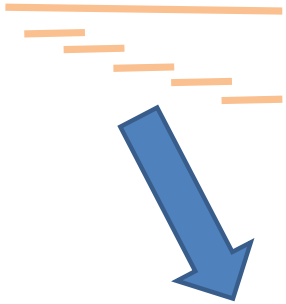
Prepare for sequencing.

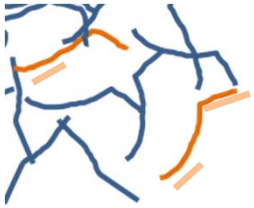
Collect samples



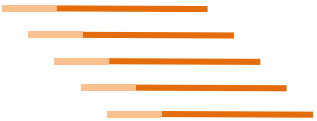
Extract RNA, convert to DNA. Most of the material will be from the host, and highly fragmented.

Make primers that span known filoviruses (including Ebola)



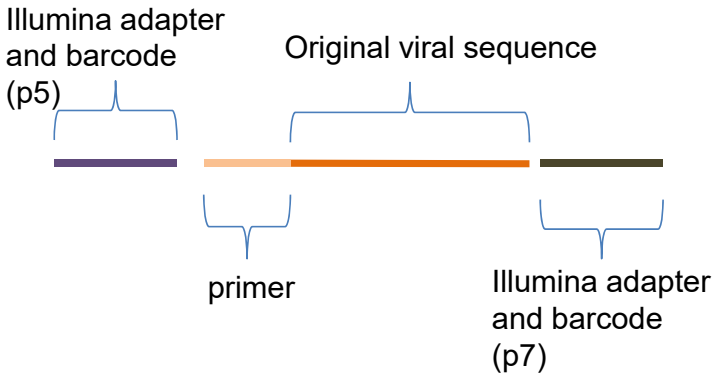


Amplify viral DNA, using primers specific to the Ebola genome.



End up with a lot of viral DNA fragments.

Library Preparation



Add adapters, which are DNA sequences necessary for sequencing on the Illumina Miseq.

Library Preparation

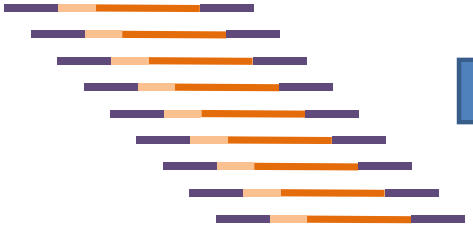
Illumina adapter and barcode (p5)

Original viral sequence

primer

Illumina adapter and barcode (p7)

Each piece of DNA is sequenced twice, starting from either end (Paired end sequencing). The products of a sequencing run are called “reads.” The sequence and quality information are stored in .fastq files.

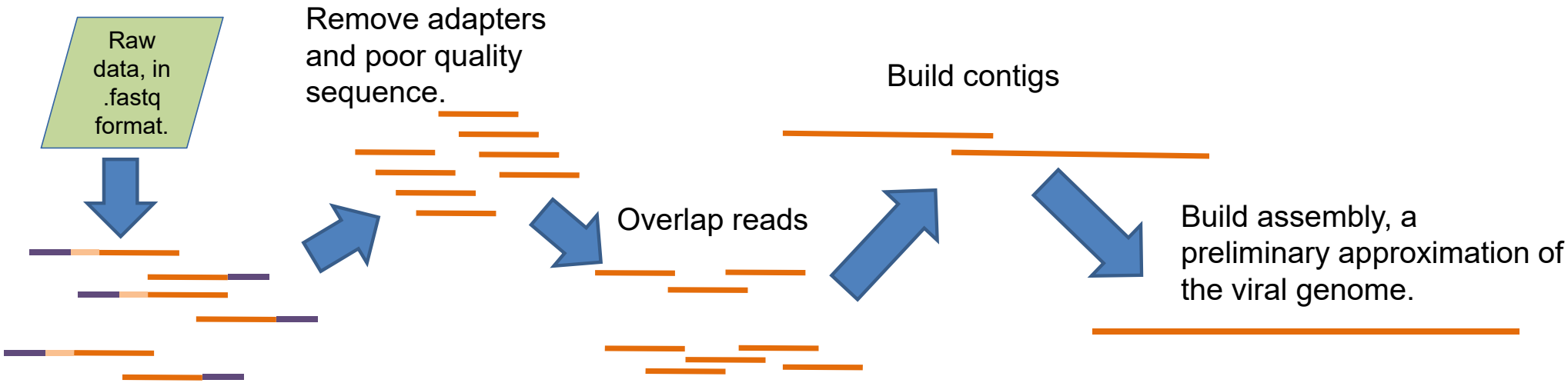


Library-DNA fragments ready to be sequenced

Illumina MiSeq

Raw data, in .fastq format.

Assembly: Broad Overview



Raw data consists of sequences containing fragments of the Ebola genome. Ultimately, we need to take these fragments and assemble them into the complete genome.