# SARS-CoV-2 cDNA library construction using a reduced reaction volume protocol on the mosquito® HV genomics and dragonfly® discovery nanoliter liquid dispensing instruments

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## SUMMARY

The identification of SARS-CoV-2 viral variants remains a critical step in controlling the future spread of infection. Using genome sequencing from isolated viral RNA, it is possible to detect and quantify circulating viral lineages (including novel, potentially more transmissible and/or pathogenic) in the human population. This has important implications regarding the surveillance of potentially more infectious and dangerous viral variants and the ability to proactively implement specific public health measures to help control variant spread. To meet the demand for high throughput genomic sequencing, the Interdisciplinary Center for Biotechnology Research (ICBR) miniaturized a standard protocol for SARS-CoV-2 library construction using the SPTLabtech **mosquito® HV Genomics** and **dragonfly® Discovery** liquid handling platforms with the Illumina COVIDSeq emergency use authorized test protocol.

We first manually tested reducing the library synthesis reaction volume to half and quarter volumes with different inputs amounts of total RNA. Results showed that in comparison to the full volume reaction, DNA library product of sufficient quality and quantity can be consistently generated with 10ng input RNA at ¼ reduced reaction volume (RRV). Adapting the COVIDSeq library prep onto the SPTLabtech **mosquito®** nanoliter liquid dispensing instrument allowed further reaction volume reduction to 1/5 scale.

To assess what effects if any the RRV protocol has on DNA sequencing, the same 94 RNA samples obtained from SARS-CoV-2 positive patients were processed manually using the full-scale reaction protocol, and on the mosquito using the 1/5 reaction volume. DNA sequencing was performed on the Illumina NovaSeq6000, S1 flow cell, using the 2x100 configuration. The sequencing results showed no difference in SARS-CoV-2 genome coverage or in variant identification rate. The RRV protocol using the **mosquito® HV Genomics** and **dragonfly® Discovery** facilitates the generation of 384 high quality COVIDSeq libraries per day with significant reagent cost savings.

## MATERIALS AND METHODS

Genomic libraries were generated manually using 10ng, 25ng and 50ng of input SARS-CoV-2 RNA using the standard Illumina COVIDSeq emergency use authorized test protocol according to the manufacturer's recommendations, at either full-scale or one quarter-scale reaction volumes. Determination of reaction volume limits and RNA input requirements led to a standardized 5-fold reduced reaction volume (RRV) library prep protocol for adaptation onto the SPTLabtech **mosquito** in a 384-well format. The SPTLabtech **dragonfly** was introduced into the workflow for reagent preparation and dispensing

## RESULTS AND DISCUSSION

### 1. Manual COVIDSeq Library Reaction Volume Reduction

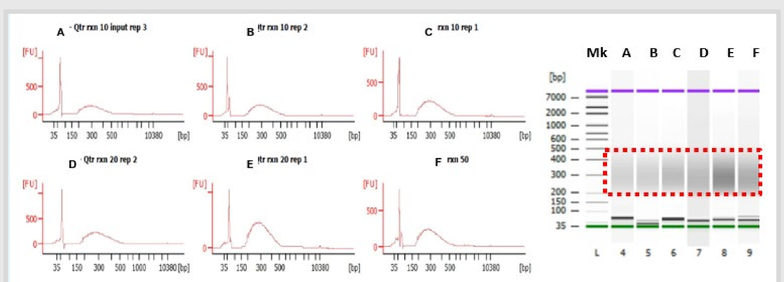



*Figure 1. Bioanalyzer traces of cDNA libraries constructed using the COVIDSeq manual library preparation at varying RNA input amounts. Result shown are from samples using one-quarter the standard reaction volume.*

Bioanalyzer traces of cDNA reaction indicates sufficient library products with sizes between 200 and 500 bases at the minimum viral RNA input (10 ng) and a reduced reaction volume (RRV) of one quarter.

### 2. Adaptation of RRV protocol on mosquito

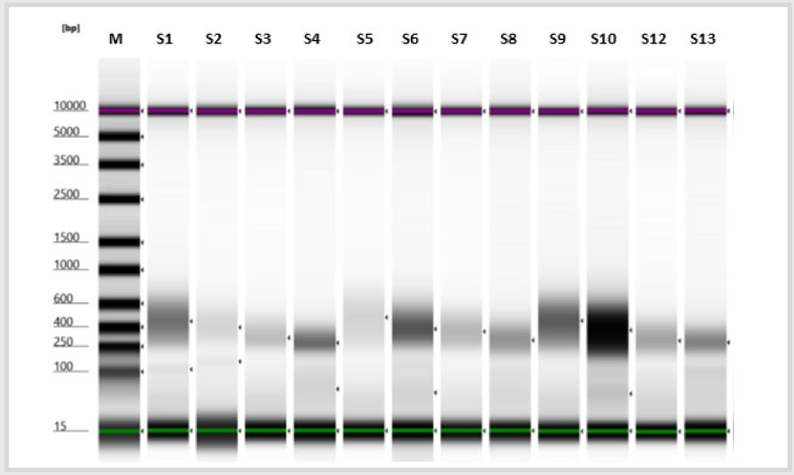



*Figure 2. Qualitative and quantitative assessment of initial RRV protocol on mosquito*

TapeStation analysis of 13 representative libraries (S1- S13) prepared from 235 RNA samples using the reduced volume protocol on the **mosquito** shows significant variation. Adaptation onto the **mosquito** enabled even lower reduction in reaction volume to one fifth and increased the overall efficiency and throughput of the library construction process. While an average operator could manually produce 96 cDNA libraries over a two-day period, miniaturization of the protocol on the **mosquito** results in a throughput of 384 libraries within the same two-day period.

### 3. Optimized RRV cDNA Synthesis on mosquito

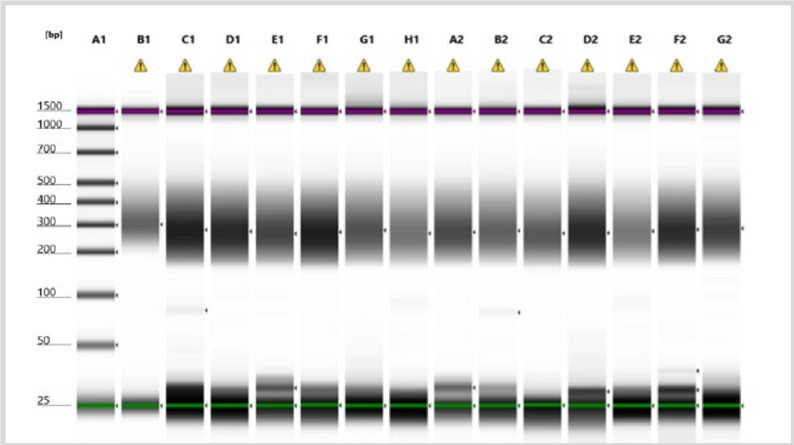
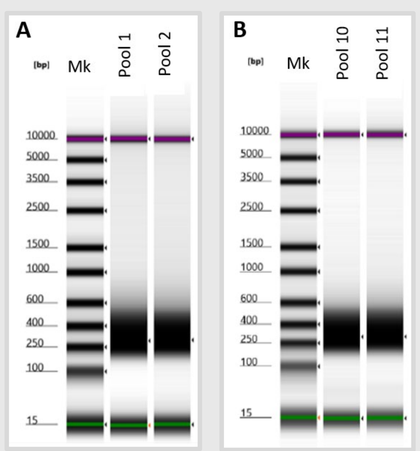



*Figure 2. Qualitative and quantitative assessment of initial RRV protocol on mosquito*

As shown in Figure 3, consistency was greatly increased between individual RNA samples following optimization and adjustment to the initial protocol. This protocol used the **mosquito** alone and required the preparation of a 384-well reagent plate.

### 4. mosquito and dragonfly combined Protocol




The addition of the **dragonfly** to the workflow reduced reagent dispensing time from 12 minutes per 384 samples to ~1.5 minutes. As shown in Figure 4, consistency of final pools (P1 & P2; P10 & P11) is achieved across separate 384 library preparations. Individual libraries are pooled using fixed volumes and are purified as a pool of 96 samples. The **mosquito** and **dragonfly** combined RRV protocol allows for consistent COVID-Seq library preparation at a one-fifth reaction volume scale within one 8-hour workday with minimum hands-on time by laboratory staff.

*Figure 4. Tapestation Gel Images of pools (Panel A, Pools 1 & 2; Panel B, Pools 10 &11) made from separate RRV library runs of 384 samples occurring 3 months apart using the mosquito and dragonfly.*

### 5. Comparison of mosquito and manual library preparation

#### A. Converge Correlation Comparison

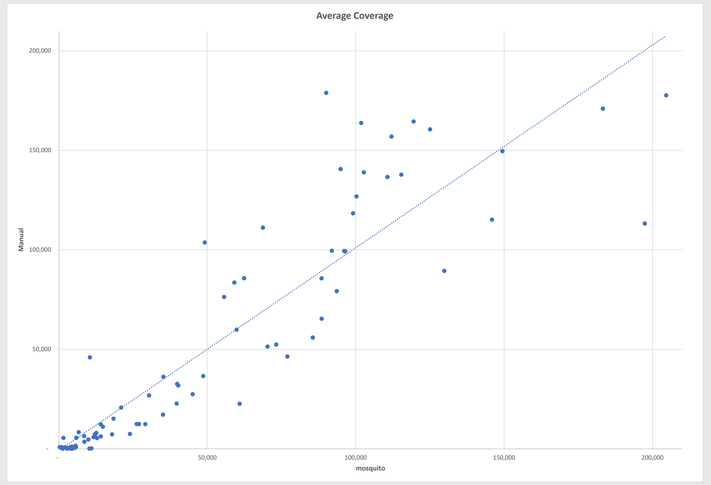



*Figure 5. Average SARS-CoV-2 genome coverage in mosquito and manual sample preparation.*

94 COVIDseq libraries for the same samples were prepared manually and using the mosquito protocol and sequenced on an Illumina NovaSeq 6000. Average coverage (normalized by total number of reads) was highly correlated in mosquito vs manual library preparation (correlation = 91%, $r^2 = 0.83$).

#### B. Lineage Comparison

Sequencing results were processed through our standard COVIDseq analysis pipeline to produce a consensus sequence for each sample. Pangolin was then used to assign a lineage to each sample.

| | |
|---|---|
| Total number of samples: | 94 |
| Samples with lineage – Manual: | 63 |
| Samples with lineage – mosquito: | 85 |
| Lineage in Manual only: | 0 |
| Lineage in mosquito only: | 30 |
| Samples with same lineage: | 62 |

mosquito-prepared libraries gave a lineage for 90% of samples, compared with 67% for manual libraries. 63 samples received a lineage from both library preparations, and in all cases but one the lineage assignment was identical.

## CONCLUSIONS

- We validated a protocol for COVIDSeq library generation using a one-fifth RRV utilizing the SPTLabtech **dragonfly** and **mosquito** liquid handling instruments.
- The resulting libraries show the same SARS-CoV-2 genome coverage and variant calling as manual preparation, or better.
- This protocol reduced reagent supply costs and increased throughput.
- The RRV method reduced operator hands-on time facilitating the generation of 384 libraries within an 8-hour workday.

## ACKNOWLEDGEMENTS

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