# Next-generation sequencing of the Chacma Baboon and Drill Monkey Cytomegalovirus Genomes

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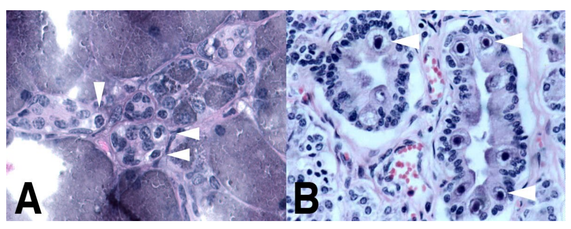
## Background

*Cynocephalus ursinus*, Chacma Baboon, has natural and common infections of baboon cytomegalovirus (BaCMV). *Mandrillus leucophaeus*, Drill monkey, are an endangered species that also carries cytomegalovirus (DrCMV). Laboratories study the BaCMV and DrCMV as they share many features with human cytomegalovirus (HCMV) and thus is can be used as a model virus for HCMV research. Nonhuman primate cytomegalovirus is also studied to develop diagnostic assays to help primate colony health. With the introduction of next-generation sequencing we now have the capability of determining specific Chacma Baboon and Drill Monkey cytomegalovirus strain genomes in order to further this research.

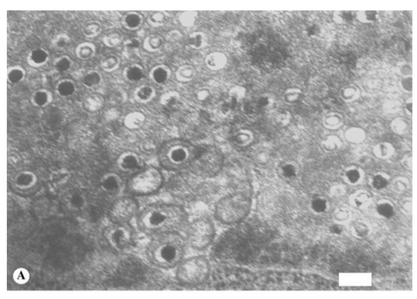

**Chacma Baboon**




H&E Stain: (Left) Human Right: Baboon CMV Salivary Gland

Courtesy of Cyril Ruoso

**Drill Monkey**



DrCMV Electron micrograph OCOM6-2. Scale bar 200 nm

## Methods/Results

Chacma Baboon isolate

University of Oklahoma Health Sciences Center isolated BaCMV strain OCOM4-52 in 1996 from a Chacma Baboon in their colony. This isolate was from a chemically-immunosuppressed baboon oral-pharyngeal swab or saliva sample. Baboon samples were processed within 6 hours of collection, half of the sample were 0.45 µM filtered and other half unfiltered, and then inoculated onto Human foreskin fibroblast (HFF). Upon electron microscopy, BaCMV virions were typical of CMV morphology but the capsid was 100-101 nm and the virus particle 140-219 nm in diameter. (Blewett 2001)

Drill Monkey isolate

DrCMV strain OCOM6-2 was provided by University of Oklahoma Health Sciences Center. This isolate was from a captive-born Nigerian drill oral-pharyngeal swab. Samples inoculated on Human embryonic lung fibroblasts (MRC-5). Upon electron microscopy, DrCMV virions also were typical of CMV morphology but the capsid was 95 nm and the virus particle 220-230 nm in diameter. (Blewett 2003)

Roche 454 GS Junior

Viral DNA was purified by Oklahoma State University and submitted to University of North Carolina Vironomics Core for sequencing (http://www.med.unc.edu/vironomics). A low-input of 500ng of DNA of each isolate was used for making the separate sequencing Roche Rapid Libraries, emulsion Lib-L PCR, and then shotgun next-generation sequencing was performed on a Roche 454 GS Junior.

Assembly

The sequencing data was assembled de novo using Roche GS De novo assembler (Newbler) software. We were able to assemble the entire Chacma ~226,000 bp and Drill ~223,000 bp genomes without the need for further sequencing. There isn't a reference sequence available for BaCVM or DrCMV so Cercopithecine herpesvirus 5 strain 2715 NC_012783 was used. Each of the largest contigs of the isolates (OCOM4-52, OCOM6-2) were aligned to the reference using Software CLC Bio genome finishing module and annotated. Afterward, these three genomes were aligned using PipMaker (Pennsylvania State University) against other CMV genomes for comparison. It was determined that this BaCMV viral genome is significantly different compared to the other Baboon CMV DNA sequences.

### GUIDELINES FOR SEQUENCING SAMPLE SUBMISSION

***Rapid Library Preparation***

**DNA type**– gDNA, Plasmid, Cosmid, BAC, and Long Range PCR (>1.5 kb)

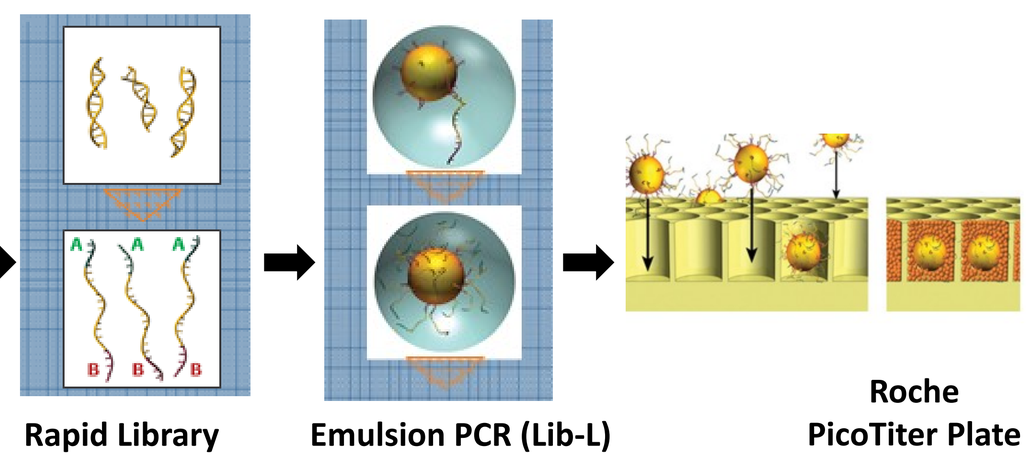
**DNA Quantity for Library Preparation**

- 500ng DNA
- Quantitation should be performed using a PicoGreen assay. *
- ***NanoDrop UV and NanoDrop Fluorometry are NOT compatible with this protocol.***

**DNA Quality for Library Preparation**

- Must be double-stranded.
- Should not be a product of whole genome amplification.
- Should be in fragments greater than 1.5 kb – Verified by agarose gel.
- Should contain no particulate matter.
- Should have an OD260/280 ratio of ~1.8 ***(NanoDrop NOT compatible)***
- Sample should have a minimal concentration of 5 ng/µl, in TE or Tris.

**500ng DNA of Isolate (virus)**

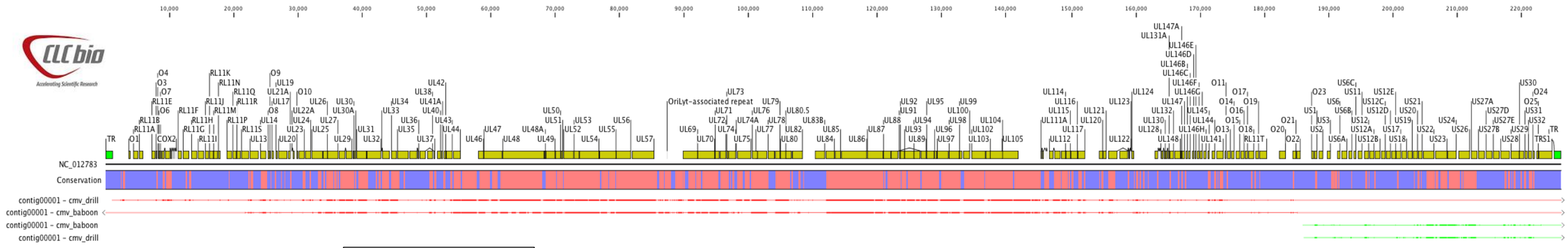



**Rapid Library** **Emulsion PCR (Lib-L)** **Roche PicoTiter Plate**

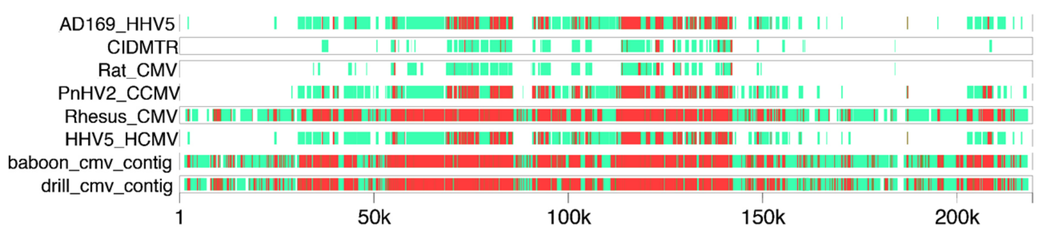
GS De Novo Assembler

**aka "Newbler"**

| | Roche GS Junior | Chacma Baboon | Drill Baboon |
|---|---|---|---|
| ave base pair length of output reads | 400-550 | 395 | 376 |
| amount of DNA for library (ng) | 500 | 500 | 500 |
| Hands on time (estimation) | 10 hrs (2 day split) | 10 hrs | 10 hrs |
| Time sequencing instrument takes to run | 9 hrs | 9 hrs | 9 hrs |
| # of reads per run | >100,000 | 111,899 | 82,070 |
| # runs | 1 | 1 | 1 |
| # contigs | | 66 | 56 |
| # aligned reads to reference | | 52,180 | 28,535 |
| # reads used for longest contig | | 42,047 | 19,140 |
| largest contig length | | **226,084** | **223,083** |




**Legend**
Conserved
Not conserved




**PipMaker**

## Conclusion

Both OCOM4-52 BaCMV and OCOM6-2 DrCMV strains were able to be sequenced and assembled using the Roche 454 GS Junior instrument. From this genome, homology of BaCMV orfs to other CMV's was determined along with the genome structure. Although, subsequent analysis shows that these genomes of BaCMV and HCMV are strongly conserved, this BaCMV genome has also unearthed differences which include: an inversion of unique short regions, decreased and increased numbers of repetitive sequences, and retention of homologs to the HCMV strain Toledo but not HCMV strain AD169 (Blewett AVS2014). Annotation and detailed analysis of the BaCMV and DrCMV genomes are still in progress.



## References

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