Metagenomic approaches for arbovirus surveillance in Brazil

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Introduction

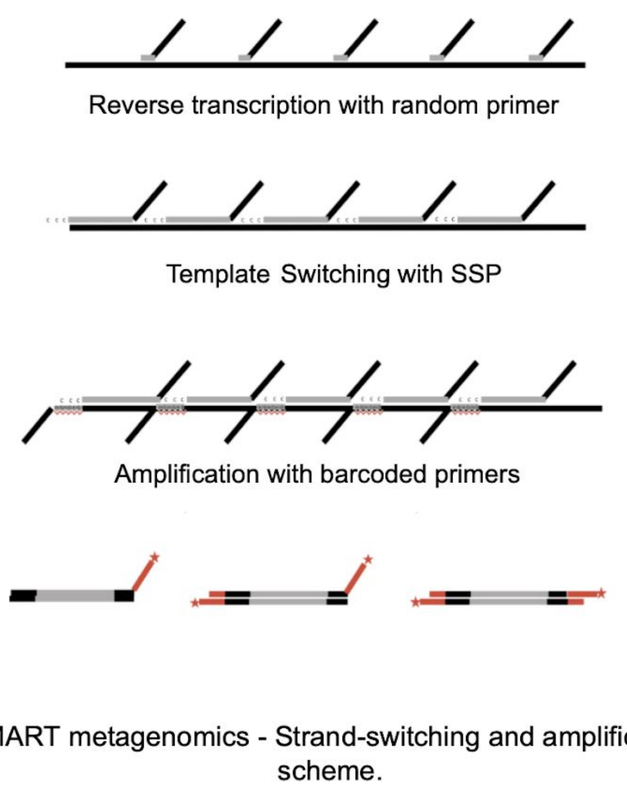
Emerging and re-emerging viruses transmitted by arthropods (arboviruses) are a global concern to human health especially in tropical and subtropical regions.

Recently, Brazil has been affected by a wave of severe overlapping arbovirus epidemics including the viruses Zika (ZIKV), Chikungunya (CHIKV), Dengue (DENV) and Yellow Fever (YFV). However, epidemiological surveillance is limited due to the difficulty of making definitive clinical diagnoses, coupled with limited availability of molecular diagnostic capacity in Brazil. We have previously employed specific pathogen-targeted sequencing approaches coupled with mobile diagnostic surveillance to help collect important epidemiological information. However, such approaches are only suitable for detecting already known viral outbreaks.

Recently, improvements in yield of nanopore sequencing has meant that untargeted metagenomic sequencing of clinical samples is now viable, potentially meaning that new viruses and lineages can be detected with a single, universal test. We describe here a full-length, low-input, long-read approach we term "SMART-metagenomics" to sequence RNA arboviruses directly from clinical samples by MinION nanopore sequencing.

Methods

Workflow for generation of SMART-metagenomics libraries from clinical samples:
RNA is extracted from clinical samples using the Zymo viral RNA kit, then treated with DNase I to remove residual DNA before being cleaned up using Zymo RNA clean & concentrator-5.
cDNA is produced using a tagged 9N random primer for initiation of RT, then taking advantage of the template switching mechanism of MMLV type RTases a 5' tag is added to the end of the cDNA via a strand switching primer (SSP). These double tagged cDNA products are then amplified by single primer PCR. The amplification products are cleaned up and barcoded using the Native Barcoding kit by Oxford Nanopore Technologies. This protocol can be performed in three hours in single tube.
The method was used during the ZiBRA 2 trip in Midwest Brazil and was used to process 68 clinical samples negatives by qPCR and 5 CHIKV positive samples. Those samples were sequenced with the standard tiling, multiplex PCR amplicon sequencing approach, to obtain a comparison between the methods.



SMART metagenomics - Strand-switching and amplification scheme.

Results

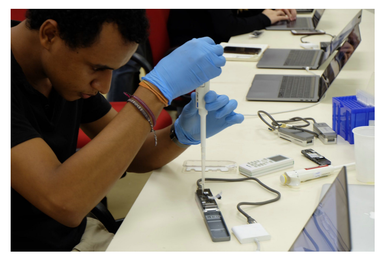
Figure 1. Tablet coverage of ZIKV genome for reference material - we were able to recover a **10.2 kb** read spanning the Zika virus genome sequenced from 5 pg input material.

YFV sample	Ct	#Reads	%Mapped	Coverage depth	Genome coverage	% Identity	N50
1053	33	35,662	9.1	248	100%	92.1	1,008
1045	23	19,998	10.3	156	100%	92.2	990
1029	17	141,005	66.9	10,556	100%	93.0	1,458
1030	8	347,744	92.0	25,602	100%	93.1	1,142

Table 1. Sequencing summary results for YFV positive clinical samples sequenced using the SMART-metagenomics method during development.

With the metagenomics approach we could recover 4 of the 5 CHIKV genomes at higher genome coverage than the amplicon approach. We also recovered 1 further CHIKV genome from a sample negative by qPCR. In addition to the 5 CHIKV genomes, we also found 20 samples potentially containing hepatitis virus G, 1 hepatitis virus A and 1 parvovirus.

Comparison between PCR Multiplex and SMART-metagenomics.



Conclusions

Here we describe a workflow which takes advantage of long-read nanopore sequencing technology by generating long (up to 10 kb) cDNA amplification products for viral metagenomics. The method was successfully trialled on the ZiBRA 2 road trip in midwest Brazil and was shown to recover full genomes for CHIKV while also detecting 3 additional pathogens. Future work will focus on the barcoding workflow to reduce sample processing time.

Bibliography

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