Deeply sequenced infectious clones of key cassava begomovirus isolates from Cameroon

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Running head: Full sequences for key clones of ACMV and EACMCV

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Abstract

We Illumina-sequenced two pairs of widely used infectious clones (4 plasmids) of the bipartite begomoviruses African cassava mosaic virus (ACMV) and East African cassava mosaic Cameroon virus. The ACMV clones were quite divergent from published expectations. Raw reads, consensus plasmid sequences, and the infectious clones themselves are all publicly available.

Main text

Infectious clones are the central tool of molecular virology. Cloned isolates of African cassava mosaic virus (ACMV) and East African cassava mosaic Cameroon virus (EACMCV) provided conclusive proof of synergy between two major clades of cassava begomoviruses (1), which is a defining feature of the epidemic of mosaic disease that has devastated cassava production in sub-Saharan Africa (2, 3). Herein we announce new sequence resources for these frequently used clones, which confirm the EACMCV clones and clarify the identity of the ACMV clones.

Complete and accurate plasmid sequences considerably simplify molecular analysis of infectious clones and design of new constructs. To confirm the sequences of the four plasmids of interest, libraries

40 were prepared from Covaris-sheared plasmid DNA in triplicate with
41 the NEBNext Ultra II kit and sequenced on the Illumina NextSeq 500
42 platform in the 150 bp paired-end read configuration.

43 These plasmids (1, 4, 5) had not been fully sequenced, but deduced
44 sequence maps (including partial-tandem-dimer virus segment inserts)
45 were available. Reads were trimmed with CutAdapt 1.16 (6) and
46 aligned to these sequences with the Burrows-Wheeler Aligner (BWA-
47 MEM v0.7.13 (7)). Variants relative to the reference sequence were
48 identified with samtools v1.8 (8) and VarScan v2.4.4 (9).

49 The EACMCV DNA-A and DNA-B clones had four and two small
50 differences relative to their corresponding GenBank accessions
51 (AF112354.1 and FJ826890.1). The consensus sequences of the
52 ACMV clones, however, were 3.1% and 5.8% divergent from the
53 sequences (AF112352.1 and AF112353.1) originally reported by
54 Fondong et al. (1), as calculated with Sequence Demarcation Tool v1.2
55 (10). This difference was not entirely unexpected, because of the
56 parallel history of two sets of ACMV clones: infectious partial-
57 tandem-dimer clones were made via restriction-digestion/ligation from
58 sap-inoculated *Nicotiana benthamiana* plants whereas the monomer-
59 segment-unit clones were cloned with PCR from the same original
60 cassava field sample (1). We expect these complete infectious clone
61 sequences will be of great utility to the community, given that many

62 follow-up publications (11–22) specifically referenced the related but
63 non-identical monomer sequences (AF112352.1 and AF112353.1).

64 We have deposited consensus sequences for the four plasmids and the
65 relevant ACMV segments and have additionally deposited the
66 plasmids with Addgene to simplify sharing and ensure their continued
67 availability. The deep coverage (over 20,000-fold; Figure 1), which
68 was consistent across three technical sequencing replicates, assures the
69 correctness of the partial-tandem-dimer sequences.

70 Our results underscore the value of confirming the sequence of
71 molecular clones. An additional example from the geminivirus
72 literature is the Nigerian Ogoroco clone of ACMV (23). Kittelmann et
73 al. (24) thoroughly documented that the infectious DNA-A clone is not
74 100% identical to the related GenBank accession (AJ427910.1). We
75 do not believe the large literature on the Cameroonian ACMV isolates
76 requires dramatic reinterpretation, but we recommend using the
77 revised sequences described here for future analyses.

78 **Data availability:** Plasmids will be made publicly available via
79 Addgene (plasmid # 159134 to 159137). Sequences for full plasmids
80 (MT856192 to MT856195) and ACMV segments (MT858793 and
81 MT858794) are available in GenBank. Raw Illumina data are available
82 from the NCBI Sequence Read Archive (PRJNA649777).

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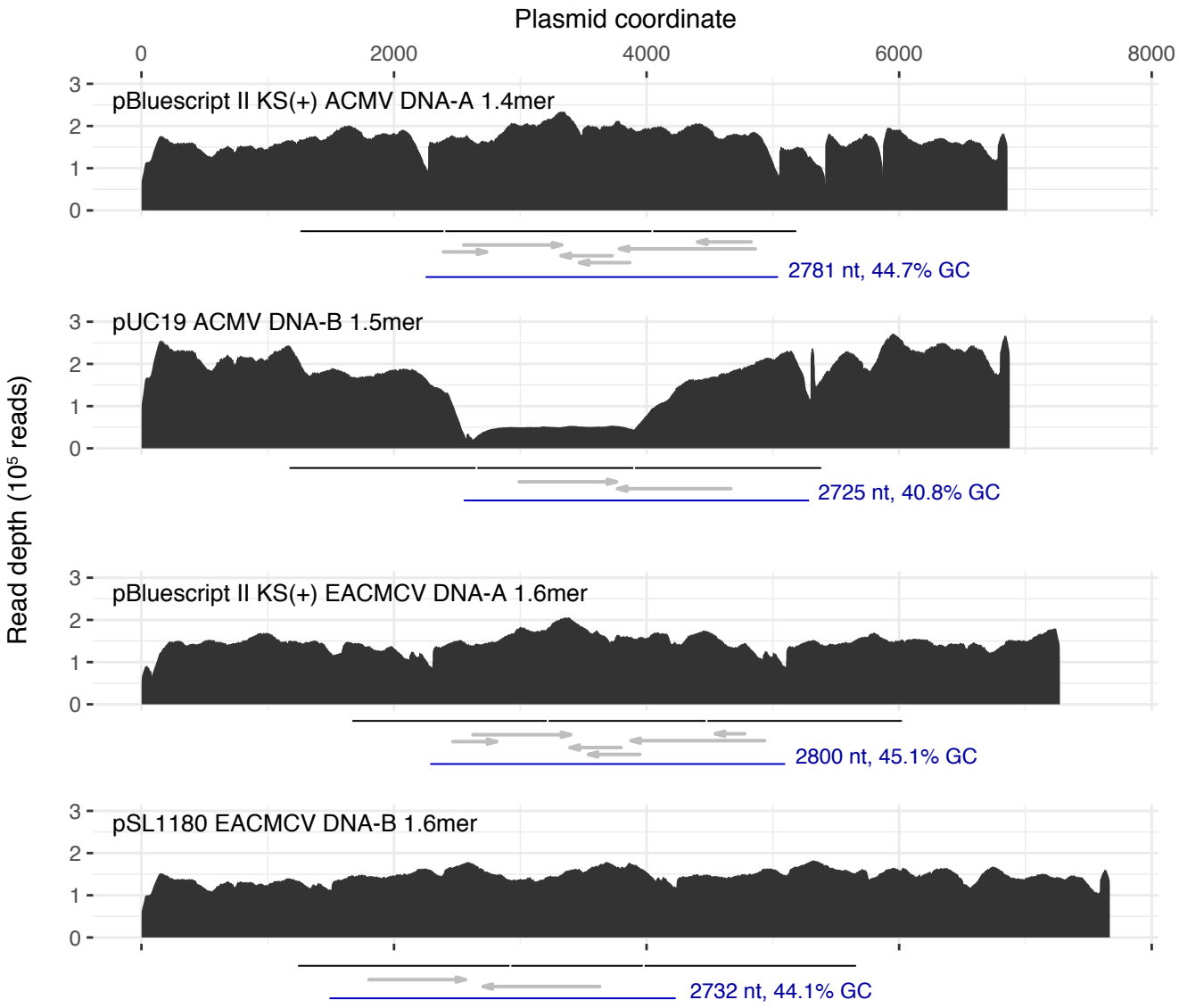

Figure 1. Plots of Illumina read depth across the length of the four infectious clone plasmids. The region in each plasmid corresponding each virus segment partial-tandem-dimer unit is indicated with a black line under each graph. White lines demarcate the boundaries of the unique and duplicated regions of the concatamer. Each virus segment monomer unit (in between two replication-origin nick sites) is shown in blue. Canonical virus genes are indicated with gray arrows, left to right for virus sense (AV1, AV2, BV1) and right to left for complementary sense (AC1 to AC4, BC1). Uneven read depth for the ACMV DNA-B plasmid is due to instability (truncation) in *E. coli*, which is evident in single-cut restriction digests.

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