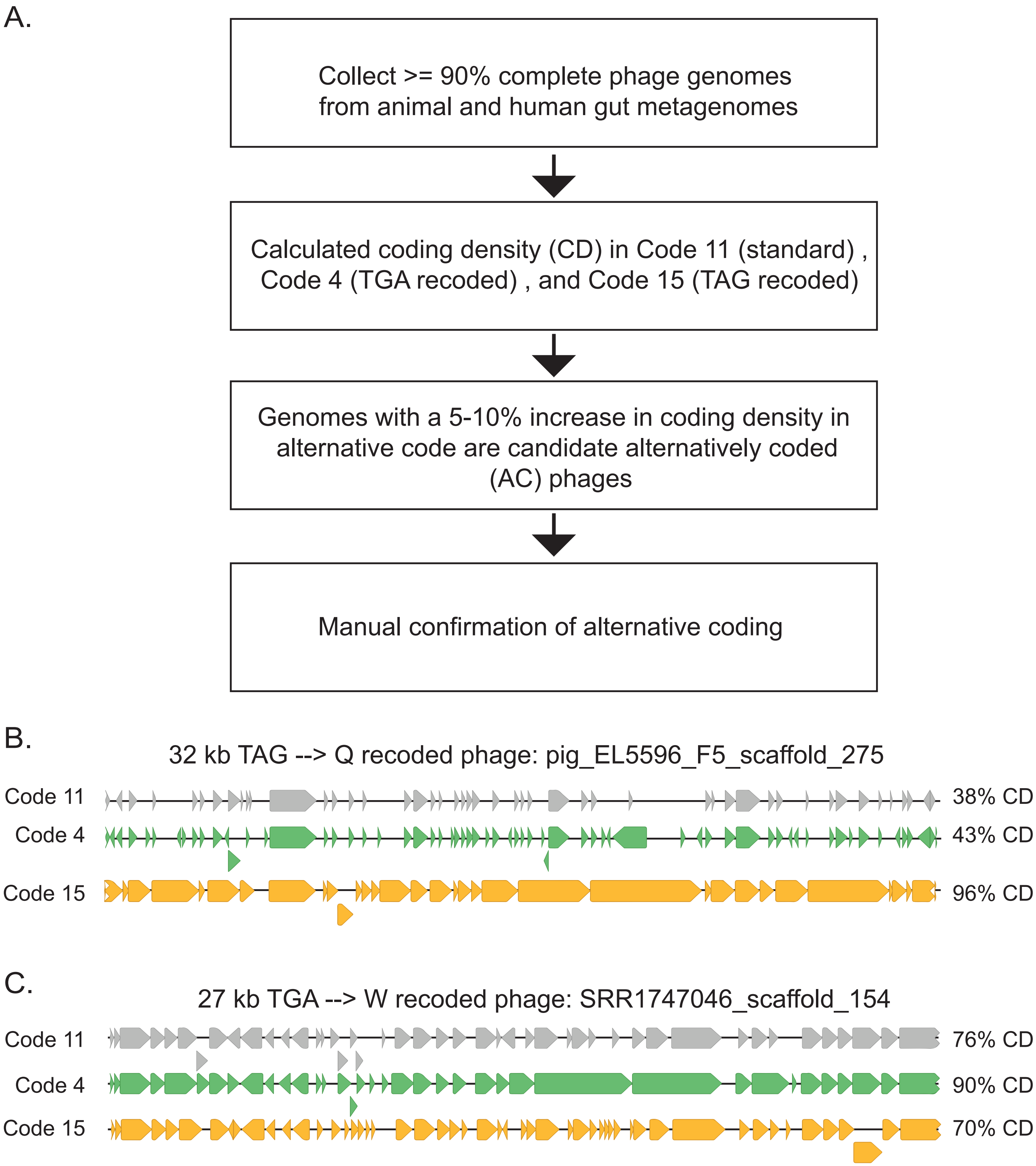
Supplementary Figure 1



Collect >= 90% complete phage genomes from animal and human gut metagenomes

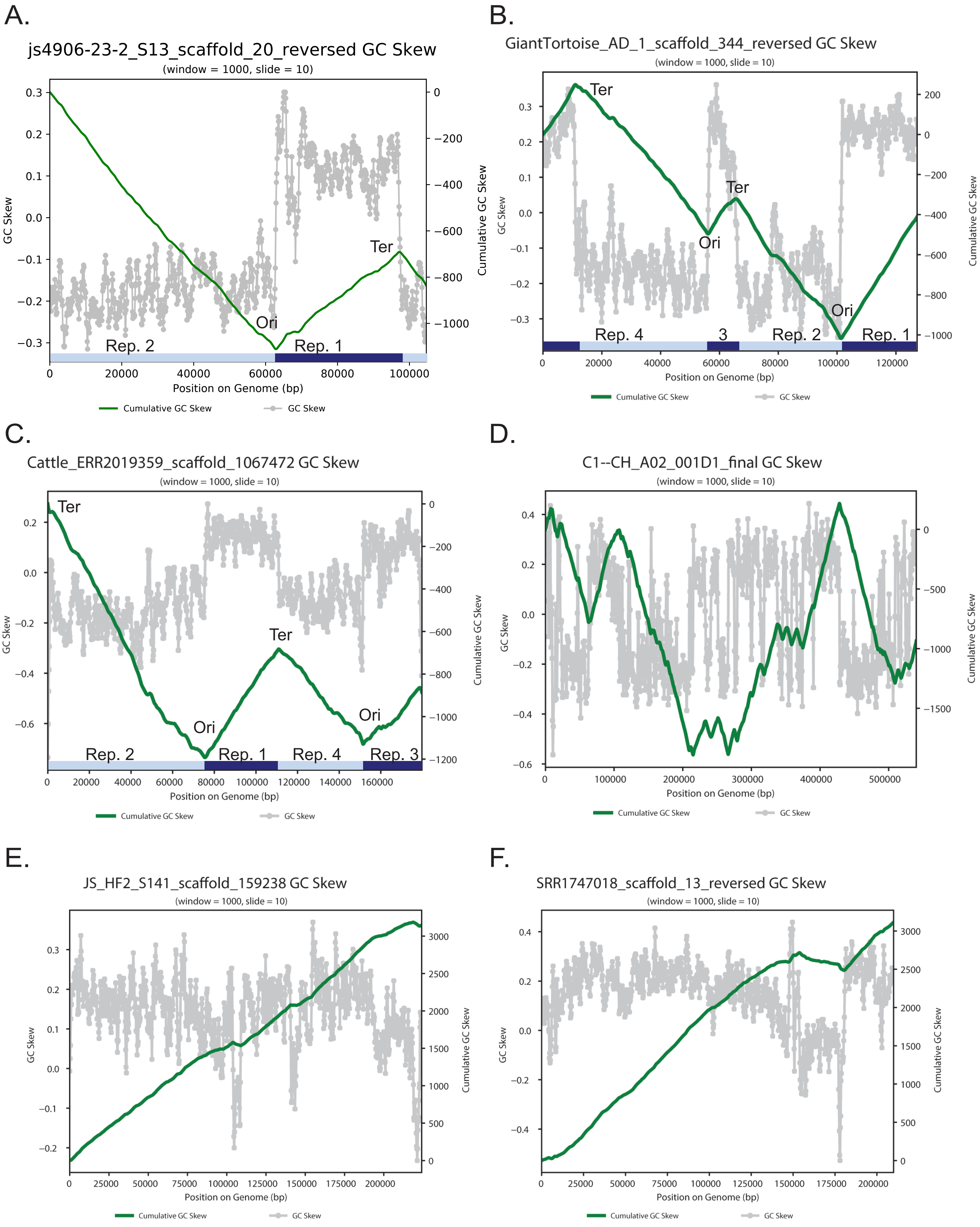
Calculated coding density (CD) in Code 11 (standard) , Code 4 (TGA recoded) , and Code 15 (TAG recoded)

Genomes with a 5-10% increase in coding density in alternative code are candidate alternatively coded (AC) phages

Manual confirmation of alternative coding

Fig S1. Identification of alternatively coded bacteriophages. A. Workflow for identifying bacteriophages that have recoded the TAG or TGA stop codons. Stop codon recoding results in low coding density when genes are predicted in standard code. Candidate alteratively coded genomes were genomes 5-100 kb that had at least a 10% increase in coding density in code 4 or code 15, or genomes > 100 kb that had a coding density increase of at least 5%. Alternative coding was then confirmed by manual inspection. **B**. Gene predictions and coding denisty (CD) using Code 11 (standard code), Code 4 (TGA recoded) and Code 15 (TAG recoded) for a 32 kb TAG recoded phage. **C**. Gene predictions and coding density (CD) using Code 11 (standard code), Code 4 (TGA recoded) and Code 15 (TAG recoded) for a 27 kb TGA recoded phage.

Supplementary Figure 2



Supplementary Figure 2, continued

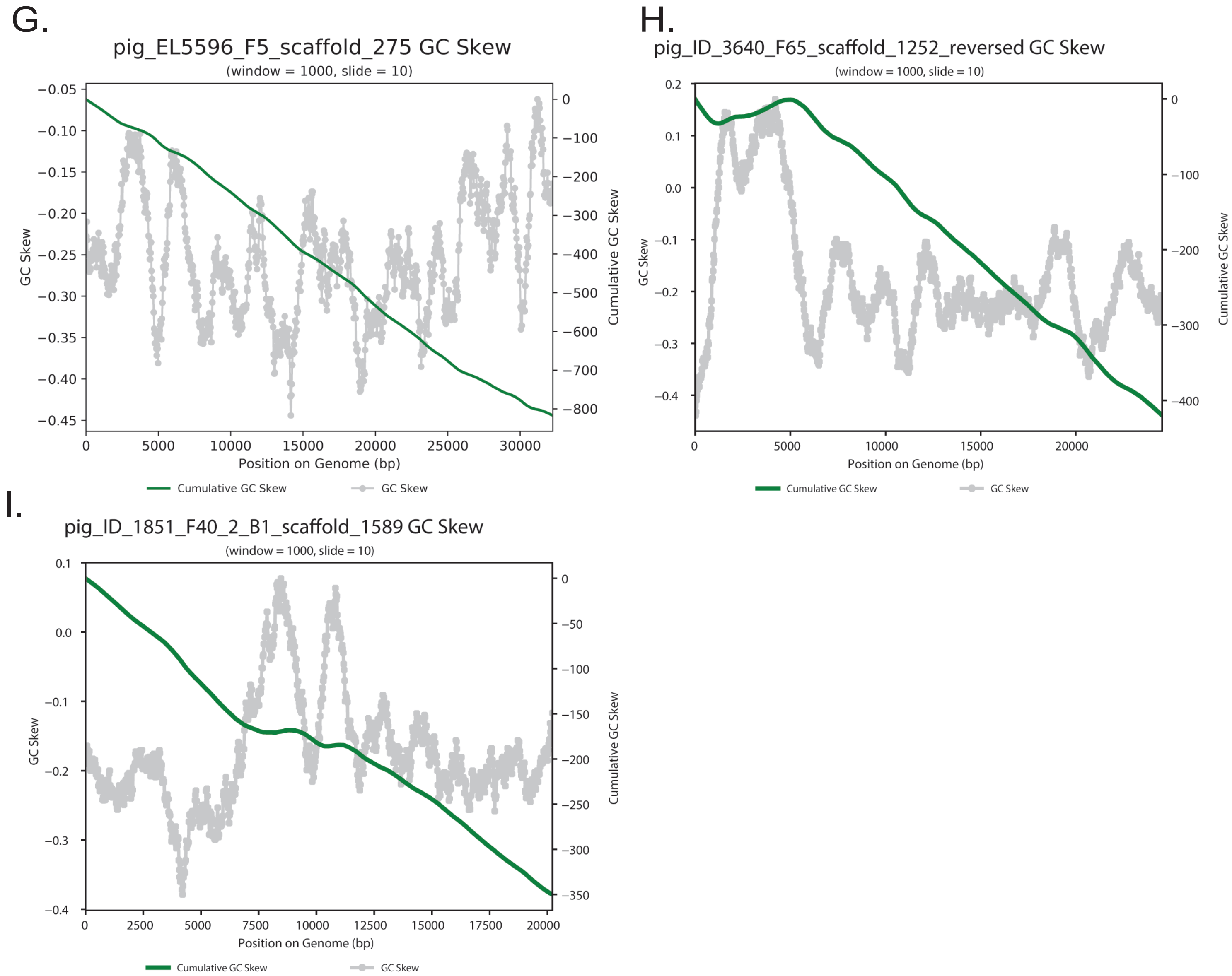


Fig. S2. GC skew plots of for representatives of the alternatively coded phage clades.
A. The crAss-like phage genome from Figure 4A has a single origin and terminus, giving two replichores. **B-C**. The Agate phage genomes from Figure 4 B (B) and Extended Data 4C (C) each have two origins and two termini, for a total of four replichores each. **D.** The Lak phage genome likely uses some complex form of bidirectional replication. There are many putative origins and termini (valleys and peaks, green line), making it difficult to map exact replichore boundaries. **E-I.** Jade (E), Sapphire (F) , Amethyst (G), Garnet (H) and Topaz (I) phage genomes exhibit GC skew patterns most consistent with unidirectional genome replication.