Supplementary Fig. 1 – SyMAP dotplot of Pk-PacBio unitigs against PKNH. SyMAP was used to create a dot plot of the 50 PacBio unitigs against PKNH version 2 (2015) consensus chromosomal sequences. Default settings were used except for min_size which was set at 5,000.

Supplementary Fig. 2 – Hi-C for each scaffold. Intra-scaffold Hi-C contact maps (normalized counts, 10 kb resolution) from all scaffolds in our new assembly. Scaffolds 6 and 14 are displayed in Fig. 1C.

Supplementary Fig. 3 – PacBio support for observed rearrangements relative to PKNH. Each panel is a plot of the PacBio read depth before, after and through a junction rearrangement region. A) Region of PKNOH_scf8 flanking the fusion region indicated by Hi-C. The PKNOH sequence on the left of the fusion region maps to PKNH_chr13 and the sequence on the right maps to an inverted PKNH_chr4; B) Region of PKNOH_scf9 flanking the fusion region indicated by Hi-C. The PKNOH sequence on the left of the fusion region maps to PKNH_chr2 and the sequence on the right maps to an inverted PKNH_chr12; C) Region of PKNOH_scf4 flanking the fusion region indicated by Hi-C. The PKNOH sequence on the left maps to PKNH_chr13 and the sequence on the right maps to PKNH_chr5.

Supplementary Fig. 4 – PacBio support for PKNOH contig gaps and PKNH gap closure. A) Screenshot of PacBio read alignment in the area of a gap present on PKNOH_scf8 at nt 593,400. The black lines represent the visualized PacBio reads, the light blue graph above the reads represents the read coverage depth. B) Screenshot of a sequence alignment demonstrating that PacBio reads from PKNOH_scf13 nucleotide positions 965,722 - 966,022 are able to close a scaffold gap present in PKNH chr11 located at nt positions 955,512 - 955,622.

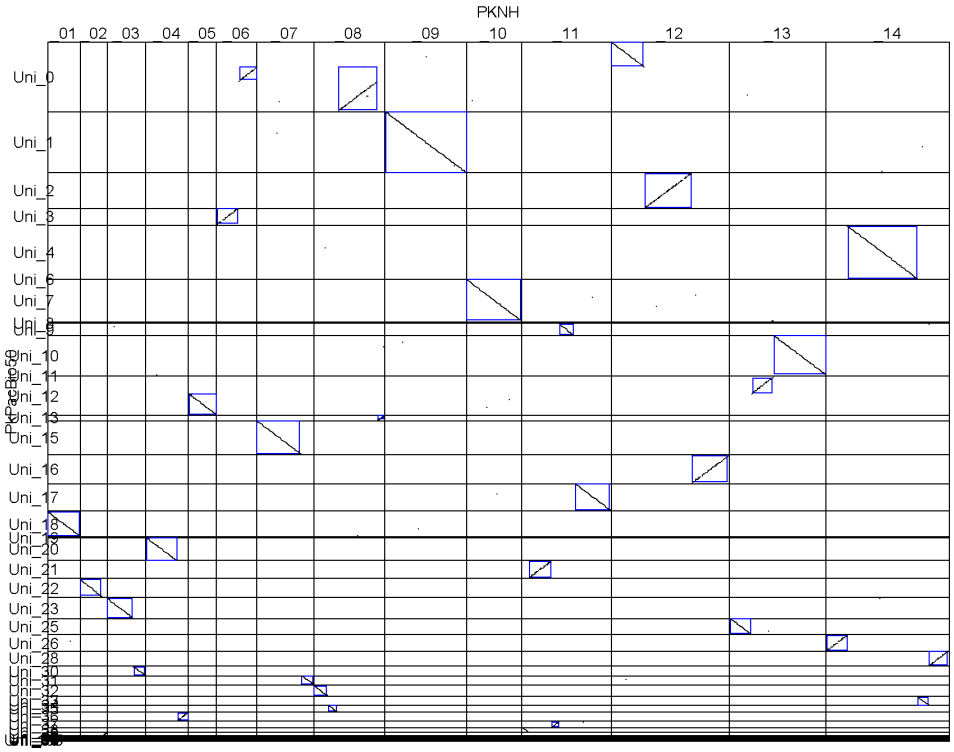
Supplementary Fig. 5 – DotPlot of PKNH chr 13 52 kb region to itself. Dotplot of 52 kb region of PKNH chr13 with no synteny in PKNOH or *P. coatneyi* to itself. Even with a window size of 50 nt, the repetitive structure of this sequence is highly evident in the numerous hits across the length of the sequence in addition to the diagonal.

Supplementary Fig. 6 – Conservation of protein orthology across three *P. knowlesi* genome sequence and annotation versions. Venn diagram of OrthoFinder results. Genome sequence and annotation versions are as in Table 2.

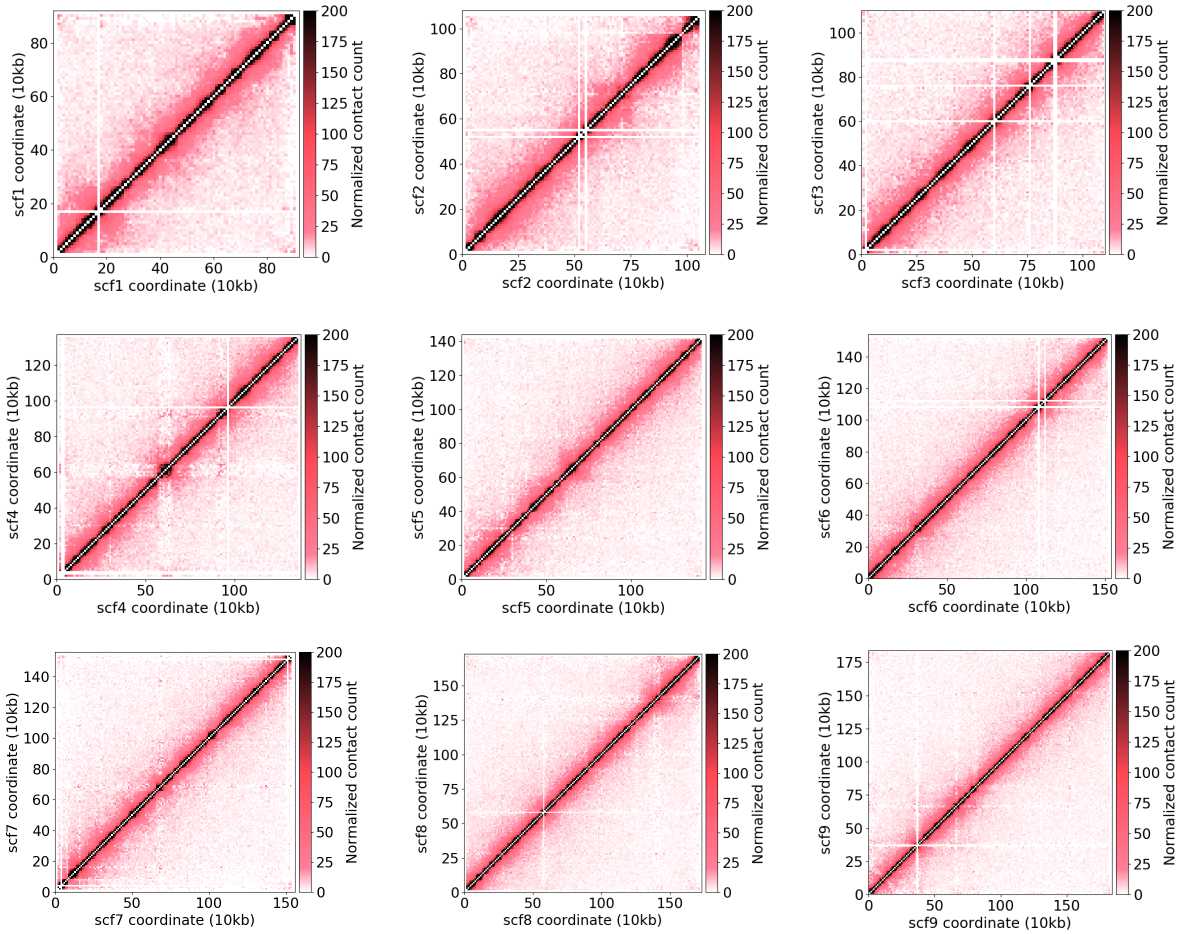
Supplementary Fig. 7 – Predicted protein lengths in several *P. knowlesi* genome sequence and annotation versions. Lengths are in amino acids. A) Full distribution of observed protein lengths. B) Re-scaled axis to focus on proteins under 1,000 amino acids. *P. knowlesi* genome annotations are as indicated here and in Table 2.

Supplementary Fig. 8 – Example of a misassigned pseudogene due to an unannotated intron, containing a common polymeric (A/T) nucleotide sequence. Example from Artemis of a gene that had been misassigned in the PkNOH automated annotation as a pseudogene, due to an unannotated intron (highlighted in green), characterized by polymeric (A/T) base pair sequence. Manual annotation rectified this and other such gene sequences that had been noted as pseudogenes.

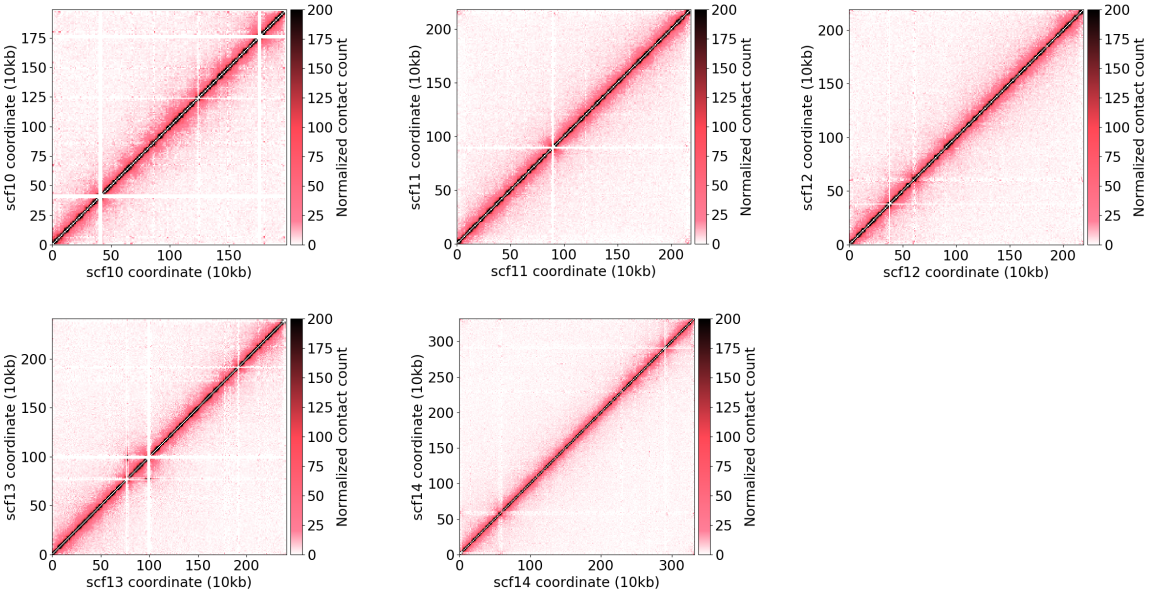
Supplementary Figure 1



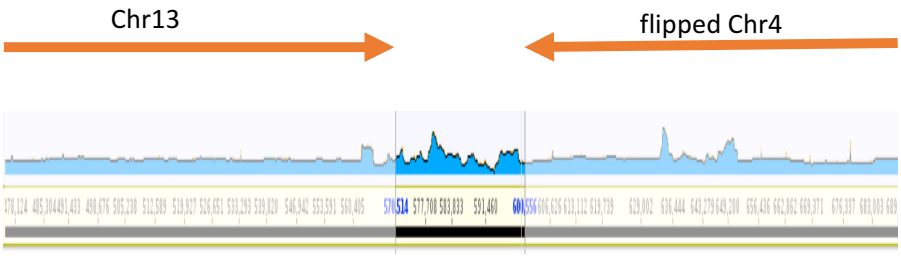
Supplementary Figure 2



Supplementary Figure 2, (cont.)

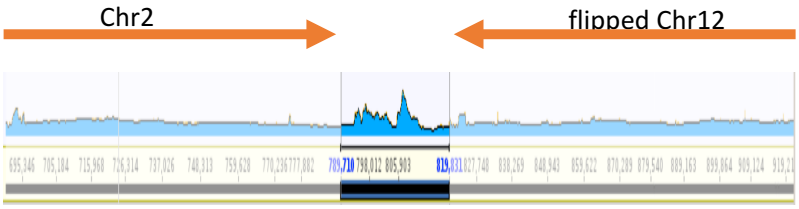


Supplementary Figure 3A



Scaffold_8 junction uniting PKNH Chr13 and flipped PKNH Chr4
PacBio reads = 249.7X coverage

Supplementary Figure 3B

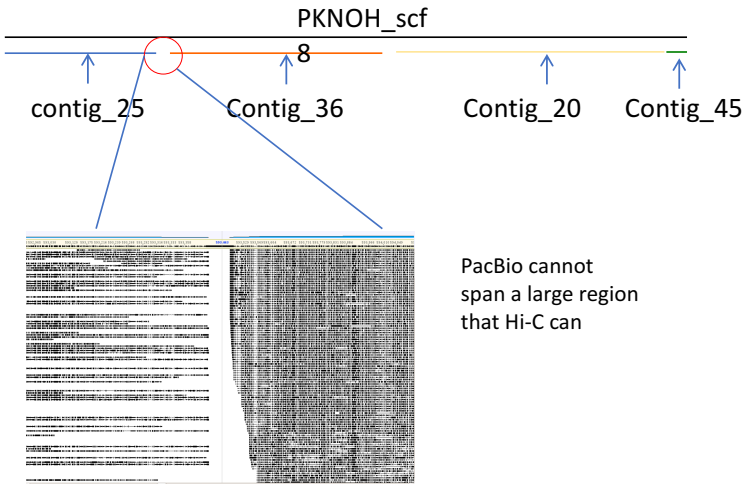


Scaffold_9 junction uniting PKNH Chr2 and flipped PKNH Chr 12
PacBio reads = 249X coverage

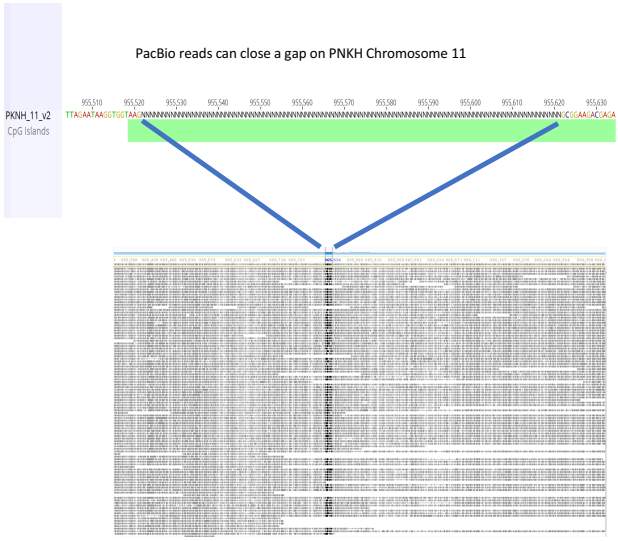
Supplementary Figure 3C

Scaffold_4 junction uniting PKNH Chr13 and PKNH Chr5
PacBio reads = 279X coverage

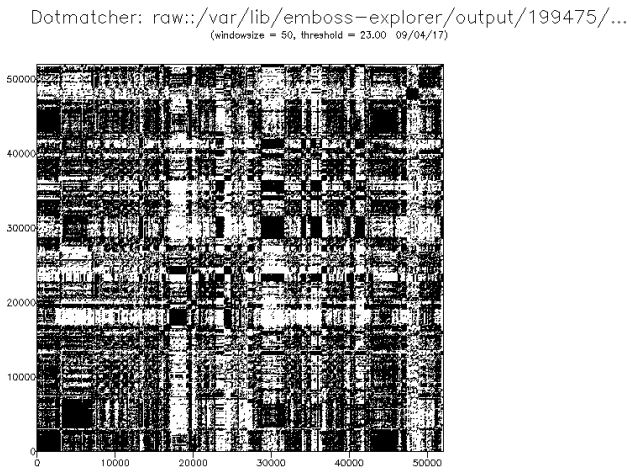
Supplementary Figure 4A



Supplementary Figure 4

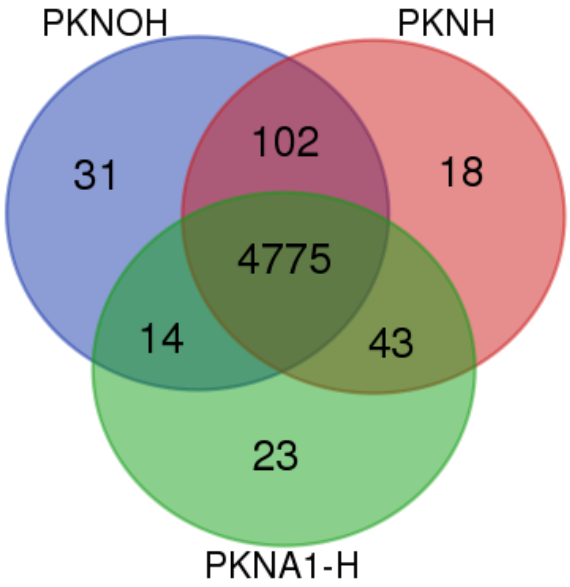


Supplementary Figure 5



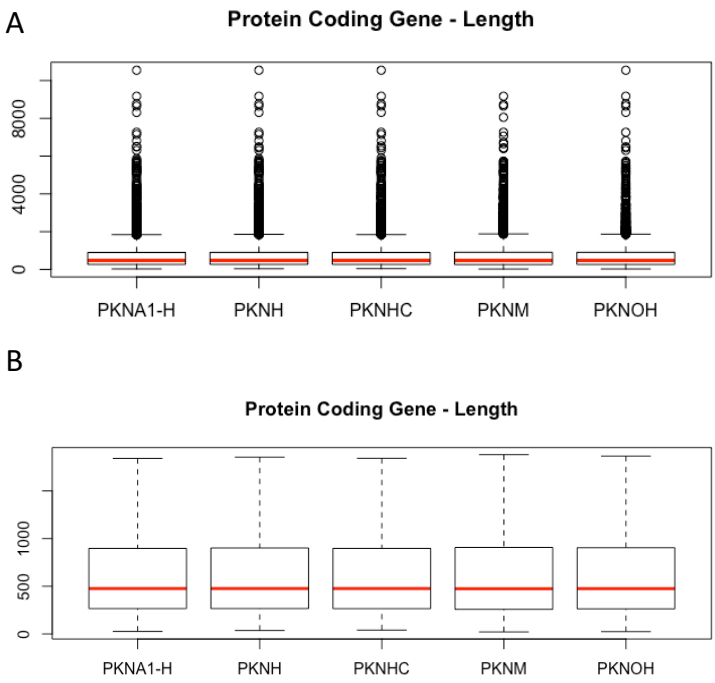
Dotplot of 52kb region of PKNH chr13 with no synteny in PKNOH or *P. coatneyi* to self. Even with a window size of 50nt The repetitive structure of this Sequence is highly evident.

Supplementary Figure 6



Supplementary Figure 7

PKNH**C** = **C**ompanion PKN**M** = **M**aker2



Supplementary Figure 8

Supplementary figure #. Example of a misassigned pseudogene due to an unannotated intron, characterized by polymeric (A) base pair sequence.