**{diffloop:} a computational framework for identifying and analyzing differential DNA loops from sequencing data**

Response to Reviewer Comments

Reviewer: 4

Comments to the Author

**The authors have done a good job of responding to the original set of reviews. The change to an application mote more accurately reflects the main goal of this manuscript.  I have only a few remaining comments.**

**I don't think the authors understood the first major point in my initial review.  I was concerned about controlling for biases like mappability and GC content, but the response says "this bias is not specific to the loop data that we've proposed examining using diffloop but a product of next-generation sequencing data as a whole." I was not talking about the fact that chromatin conformation data is bigger than RNA-seq data.  The application note should mention whether the failure to control for these biases will negatively impact the analysis of, e.g., Hi-C data.**

We apologize for the misunderstanding of the concern. We agree that loop read counts will definitely be biased by mappability, GC content and other factors. Due to space constraints in the main text we have added the following section to the supplementary material:

**Impact of GC content and mappability on differential looping analysis**

Factors such as GC content and mappability will affect read counts at ChIP peaks (i.e. loop anchors) will also bias loop read counts. Many of these factors are relatively constant across samples, and are therefore not expected to have a major impact on the fold change between samples. It should be noted, however, that this bias will affect the power to detect fold change differences. In particular we would expect lower sensitivity to detect strength differences for loops with low average read counts.

**In their response to one of the other reviewers, the authors claim that they discuss diffHiC.  I see a citation of this work (Lun and Smyth 2015) but no actual citation or mention of the work in the main text.  This, and diffBInd, should be mentioned.**

The following sentence has now been added to the Introduction to introduce these tools—

While existing tools such as \texttt{DiffBind} (\citealp{diffBind}) and \texttt{diffHiC} (\citealp{diffHiC}) provide functionality for identifying differential features from ChIP-seq and Hi-C experiments, \texttt{diffloop} provides a suite of functions tailored to chromatin loop data.

**p. 2: The Results section starts immediately talking about "All four Pol2 ChIA-PET samples," but these samples have not yet been mentioned.**

We have modified the text (“All four Pol2 samples” - > “POL2 ChIA-PET data from two MCF7 and two K562 samples”) noting that the samples were briefly introduced in the first section “… comparing chromatin interactions inferred by ChIA-PET replicates between the MCF7 and K562 cell lines.”