

Below are some errors that may arise in the terminal window when running AftrRAD, and possible reasons for the error. If you find an error message that has a relatively simple solution, feel free to pass it along and we'll get it added to the list.

**Can't exec "./ACANA": No such file or directory at AftrRAD.pl line 1687.
readline() on closed filehandle TEMPALIGNFILE at AftrRAD.pl line 1693.**

Check to make sure that a working version of ACANA, along with the dnaMatrix file, are in your working directory.

No such file or directory at AftrRAD.pl line 503.

AftrRAD can't find a data file corresponding to one of the files in the Barcodes folder. Check to make sure that your barcode file(s) and the corresponding data file(s) have the exact same name, including the extension (i.e. RAD1.txt and RAD1.fastq are different). Another possibility is that there are hidden files in the Barcodes folder. Any hidden files (usually with '~' at the end of the file name) need to be removed.

Creating file to test mean read counts.

**Use of uninitialized value \$Middle7C[5] in join or string at AftrRAD.pl line 1530,
<VARIANCEOUT> line 83810.**

**Use of uninitialized value \$Middle7C[0] in join or string at AftrRAD.pl line 1530,
<VARIANCEOUT> line 83810.**

You may have sequences of different lengths in the dataset. One way to check this is by scrolling through the file TempFiles/ErrorReadTest/ErrorTestOut.txt

Performing heuristic search of all pairwise comparisons to identify potentially allelic pairs.

Use of uninitialized value \$ReadLength in numeric lt (<) at AftrRAD.pl line 1506.

One possible reason for this error could be that no sequences made it through the upstream error filtering. Check the Report file in Output/RunInfo to see how many reads made it through the initial quality filtering. AftrRAD is generally conservative about throwing out potential error reads, and this error could be due to a generally low-quality sequencing run. Alternatively, the minimum Phred score value could be misspecified (i.e. using a Phred score value based on Phred64 when the sequencing is based on Phred33).