Techsupport Quick Guide:

How to Generate FASTQ data from an Incomplete Sequencing Experiment

To rescue your sequencing data and generate FASTQ files from a prematurely terminated sequencing experiment, you can use either Local Run Manager installed on your sequencer (NextSeq, MiSeq, MiniSeq, or iSeq) or a desktop version running on your computer.

Before You Start:

- **Determine the Last Completed Cycle:** Identify the last cycle with a full set of BCL files in the BaseCalls directory within your experiment folder. The number of BCL files per cycle varies by instrument. If the run was prematurely terminated due to performance issues, first check the run in the Sequencing Analysis Viewer to identify the last cycle with good base call quality.
- **Ensure No Sequencing is in Progress:** Make sure no sequencing is in progress if you are using Local Run Manager on the instrument.
- **Using BaseSpace Sequence Hub:** If you use BaseSpace Sequence Hub for analysis and generating FASTQ instead of Local Run Manager, contact techsupport@genetica-group.com for further support.
- **Index Read Completion:** If the run stops before completing the index read, you cannot demultiplex and identify samples, and data cannot be retrieved.
- **Check for RTAcomplete.txt File:** Ensure that the RTAcomplete.txt file is not present in the run folder.

Steps to Follow:

1. **Identify the Last Completed and Usable Cycle:**
    - Calculate how many cycles were successfully completed and extracted, and determine the cycle distribution. For example, if the original run setup was paired-end 151 cycles with 8 cycles of dual index reading and the last usable cycle is cycle 217, the new sequencing distribution would be 151 cycles for read 1, 8 cycles for index 1, 8 cycles for index 2, and 50 cycles for read 2.
2. **Backup and Modify Files:**
    - Backup the original RunInfo.xml file in the experiment folder by copying and renaming it to RunInfo.xml.BAK. Do the same for the SampleSheet.csv file if it exists, renaming it to SampleSheet.csv.BAK.

- Using a text editor such as Notepad, modify the Reads section of the RunInfo.xml file in the experiment folder to use data only up to the last usable cycle. For example:

Original RunInfo.xml:

```
<Reads>
 <Read Number="1" NumCycles="151" IsIndexedRead="N" />
 <Read Number="2" NumCycles="8" IsIndexedRead="Y" />
 <Read Number="3" NumCycles="8" IsIndexedRead="Y" />
 <Read Number="4" NumCycles="151" IsIndexedRead="N" />
</Reads>
```

Modified RunInfo.xml:

```
<Reads>
 <Read Number="1" NumCycles="151" IsIndexedRead="N" />
 <Read Number="2" NumCycles="8" IsIndexedRead="Y" />
 <Read Number="3" NumCycles="8" IsIndexedRead="Y" />
 <Read Number="4" NumCycles="50" IsIndexedRead="N" />
</Reads>
```

Following these steps will help you generate the FASTQ files from the available incomplete data. If you encounter any issues, don't hesitate to contact GeneTiCA Group Techsupport at techsupport@genetica-group.com.