Addendum to Stimulus File tutorial:

Stimulus Files are used in Clampex when the Waveform Editor cannot create the desired waveform. The Stimulus File waveform of choice originates from one of two types:

1 – Waveform from a previously recorded file: The intended Stimulus file format is already ABF. The file will be either episodic or gap-free. In some cases gap-free files need to be treated a bit differently before they can be used. (see below about how to address this).
2 – Waveform is completely artificial: Created using a mathematical equation or it manually edited in a program like Microsoft Excel.

In the first case, if the waveform comes from a file recorded in Episodic acquisition mode, setting up the Stimulus waveform is straightforward. Let's use an Episodic file with a single sweep recorded at 10 kHz (a 100 microsecond sampling interval) with 2064 samples. Typically one would use as many samples in the Stimulus File as are chosen in the Protocol Editor under Samples / Sweep / Signal (see Figure 1). If fewer samples are used in the protocol (e.g. 1032; see Figure 2), then the remaining samples from the Stimulus file will be ignored. If more samples are used (e.g. 5128; see Figure 3), the remaining samples will be 'filled in' with the last value of the Stimulus File. If the last sample is at −79 mV, the remaining samples will be at this potential.
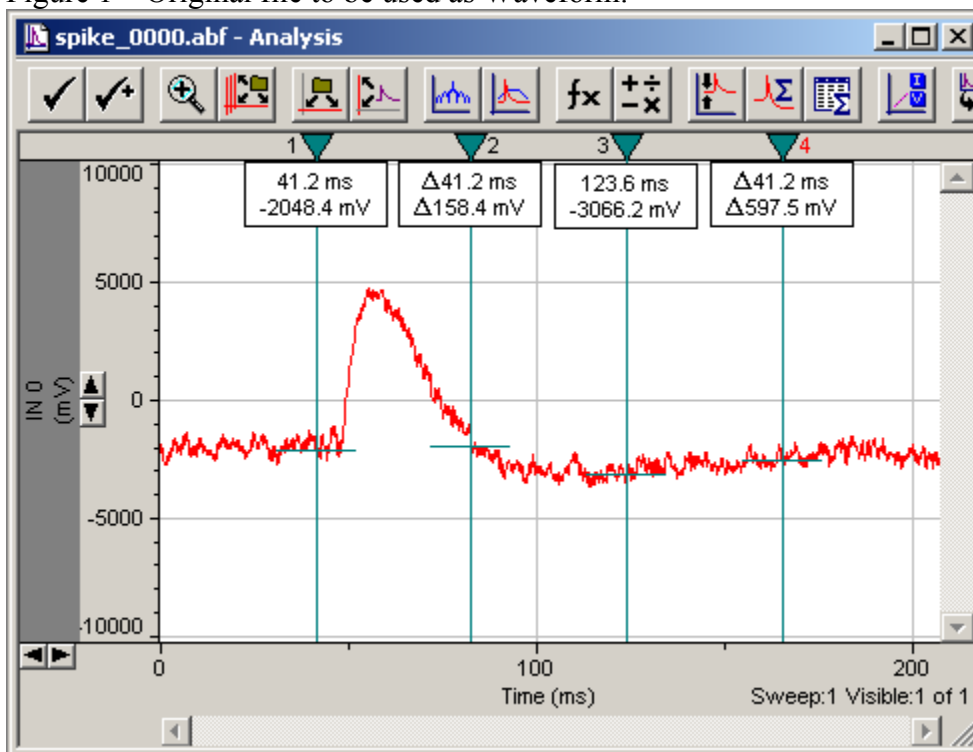
Figure 1 – Original file to be used as Waveform:

Figure 2 – Using fewer samples in the Samples / sweep / signal box (1032): The Stimulus
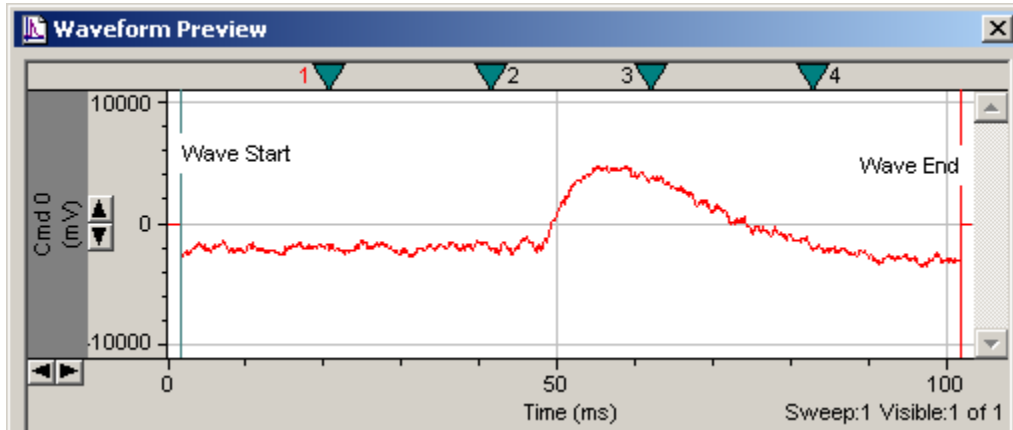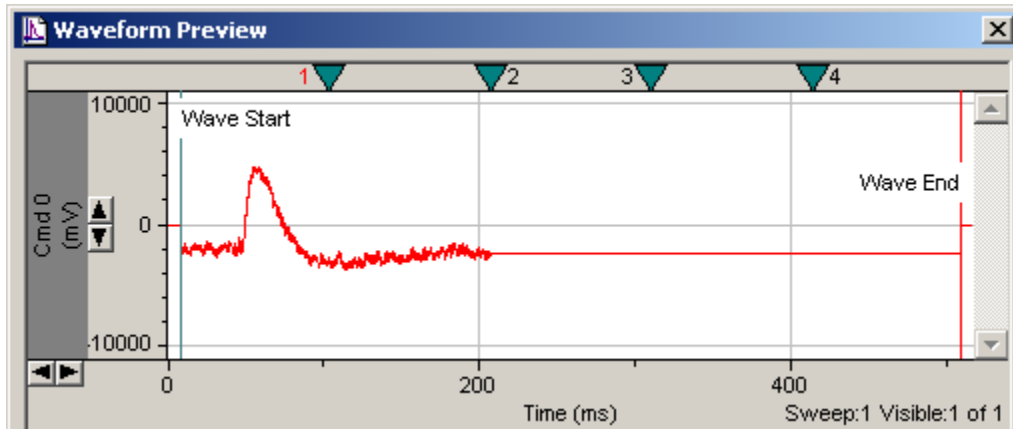File waveform is truncated.

Figure 3 – Using an excess of samples (5160): The Stimulus File is used in its entirety,
but in order to fill all samples beyond the 2064, the last sample from the Stimulus File is
used to fill in the remainder.

The second point to consider is the First and Last Holding samples. Clampex always
reserves 1/64th of the total samples in a sweep for the First and Last Holding levels. In
the example above, with 2064 samples, the first 32 and last 32 samples will be held at the
Holding Command level of the protocol (this is determined in the Outputs tab of the
Protocol Editor). The consequence is that the first and last 32 samples of the Stimulus
File are ignored. Remember that 32 samples with a 100 microsecond First holding
interval (10 kHz sampling rate) means that the first 3.2 ms of the waveform are at the
Holding command. So make sure that the stimulus proper begins after the first 1/64th
samples and ends before the last 1/64th samples. In case you constructed a waveform
without a baseline preceding the waveform, you will need to add some dummy points at
the start.

As an example, let us assume that the Holding command is set at 0 mV. You can see what it is by selecting the Outputs tab in the Protocol Editor and looking at the value for the Analog output.   Figure 4 illustrates the effect of the Holding command on the First holding samples.

Figure 4 – Initial first holding of Stimulus waveform will be at this Holding command: This graphic shows an expanded view of the initial portion of the Stimulus waveform. Note how the waveform proper should start at about –3000 mV, but the First Holding is kept at 0 mV.
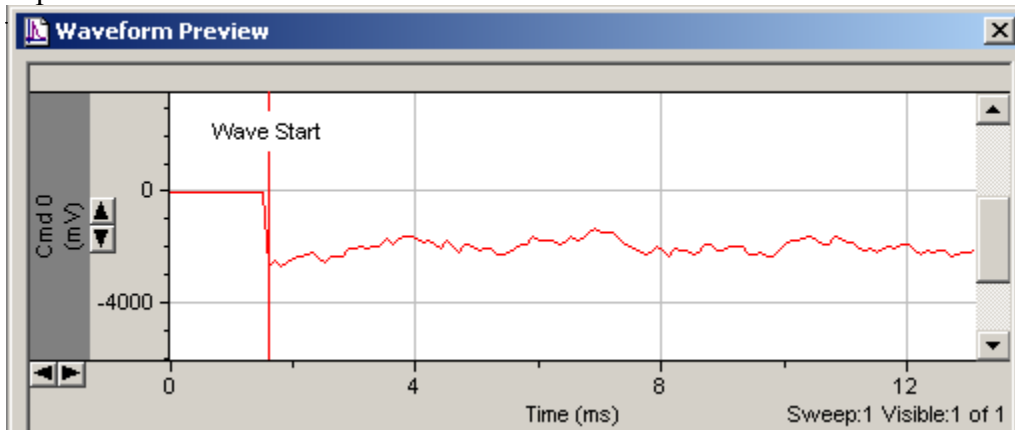
If the original file was a gap-free file it is not readily accepted as a Stimulus File. It needs to be converted to an Axon Text File (ATF) using the File / Save As option.

If you constructed an artificial waveform, the first step that needs to be taken is to transform it to a format that Clampex will recognize. The easiest way of doing this is to use Clampfit. Typically, such a waveform will be composed of columns of data. The first column will be (equally spaced) time intervals, and further columns will contain the waveform(s). With these data active, open Clampfit and go to the Results window. Select the first general-purpose empty sheet (Sheet 8 in Clampfit 8; Sheet 14 in Clampfit 9). Copy the data from the source program; this is usually done using the CTRL+C keyboard shortcut. Then paste it into the Clampfit Sheet using CTRL+V. This information can now be saved into an ATF file. In Clampfit select File / Save As and select ATF as the file format. This file is now a ready-to-use Stimulus File for Clampex.

NOTE: If you created an ATF file with a single sweep AND you want to repeat this sweep by selecting multiple Sweeps / Run in the Protocol Editor then you must change the header of the ATF file.
Example:
Open the Stimulus ATF file and re-save it under a different name. This will create the full ATF header.

Change the words 'Gap-Free' to 'Episodic Stimulation'
Figure 5 – Modified header:

	A	B	C	D
1	ATF	1		
2	7	2		
3	AcquisitionMode=Episodic stimulation			
4	Comment=			
5	YTop=4765.68			
6	YBottom=-3619.75			
7	SweepStartTimesMS=0.000			
8	SignalsExported=amplitude			

(Note that this file will still have a time column and a single data column. The multiple sweeps are chose in the Mode / Rate tab of the Protocol Editor.)
Save this file using the ATF extension and open it in Clampfit. Select File / Properties to ensure that the file is indeed viewed as Episodic.

Figure 6 – File Properties highlights:

```
Data File: superjunk2.atf
File format: ABF V1.80
Created: Feb 4, 2003, at 08:48:53 [00:00:00]
Application version number: pre-9.0
Acquisition mode: episodic stimulation.
Axon binary file (floating point)
2064 samples in this file.
Comment: n/a
```

Save this save as an ABF file, and use the new ABF file as the Stimulus File. You will now be able to repeat this sweep multiple times.

Lastly, we need to point out that the time column from the Stimulus File will be ignored in favor of the First Interval that is specified in the Protocol Editor of Clampex. This point is better illustrated by an example. Let us assume that you created an artificial waveform containing 5160 points (samples). You were careful to leave the first and last 80 samples at the holding level. You chose an interval of 0.1 ms (100 microseconds; a sampling frequency of 10 kHz, see Figure 7); saved the file and are ready to use it as a stimulus file. Remembering the caveats, you open the Protocol Editor in Clampex and choose 5160 samples / sweep / signal. By mistake, you leave the sampling interval at 200 microseconds (5 kHz sampling frequency). What will happen? The First Interval of 200 microseconds will take over the Stimulus File's own sampling interval. The actual protocol will run with the correct number of 5160 points, but using 200 microseconds between samples (Figure 8). Note that the total recording time is reduced. This property can sometime be used to one's advantage. Make sure that you are aware of this behavior.
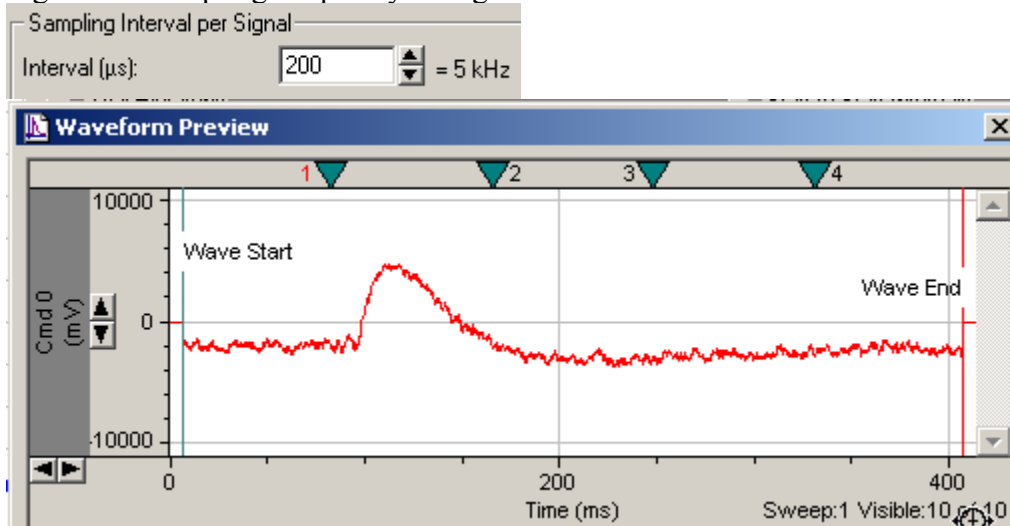
Example:

Figure 7 – Sampling frequency set at 10 kHz:
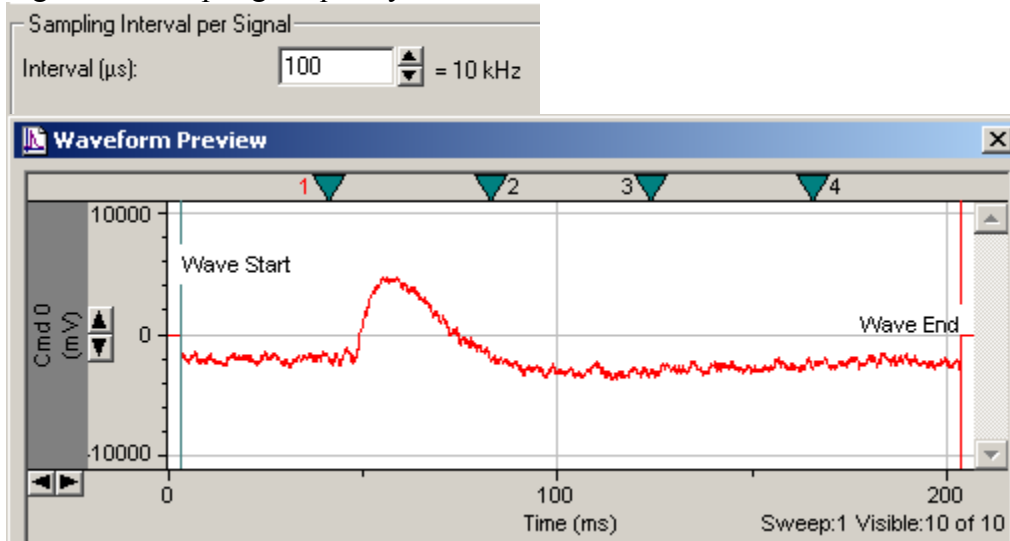
Note the time base.

Figure 8 – Sampling frequency changes to 5 kHz:

The waveform is the same as above, but the time base has been slowed by a factor of 2.