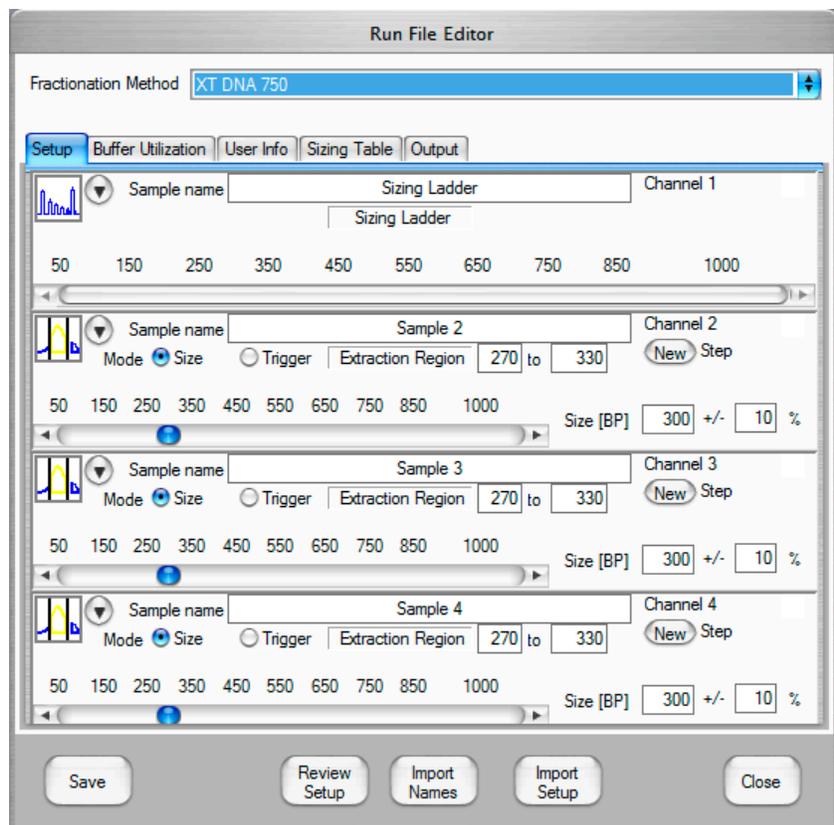


LabChip XT

Guide to Advanced Run File Options

Prior to starting a run on the LabChip XT it is necessary to set up a Run File to specify the extraction parameters for each channel on the chip. The Run File Editor is opened through the Tools menu.



Extraction of a specific size range from a sheared/fragmented DNA sample

This is the most basic use of the LabChip XT instrument. The **Setup** tab allows the user to specify an extraction for channels 2-4 by typing in the desired collection width (i.e. +/- 10%) and setting the target size by either moving the blue sliding bar or typing in the target size (i.e. 300 bp). The user can also type the desired range in the Extraction Region. In this type of run, the software sizes the sample DNA by referencing ladder peaks detected in channel 1 and automatically triggers the collection at the appropriate time. As soon as the extraction for a specific channel is complete, the run automatically pauses, the **Run Paused** window displays, and any channels that have completed the extraction display **COLLECT** in the Run Settings. The user can then remove the chip, pipette the extracted sample out of the collection well, close the lid, and resume the run by clicking the **Resume** button on the **Run Paused** window.

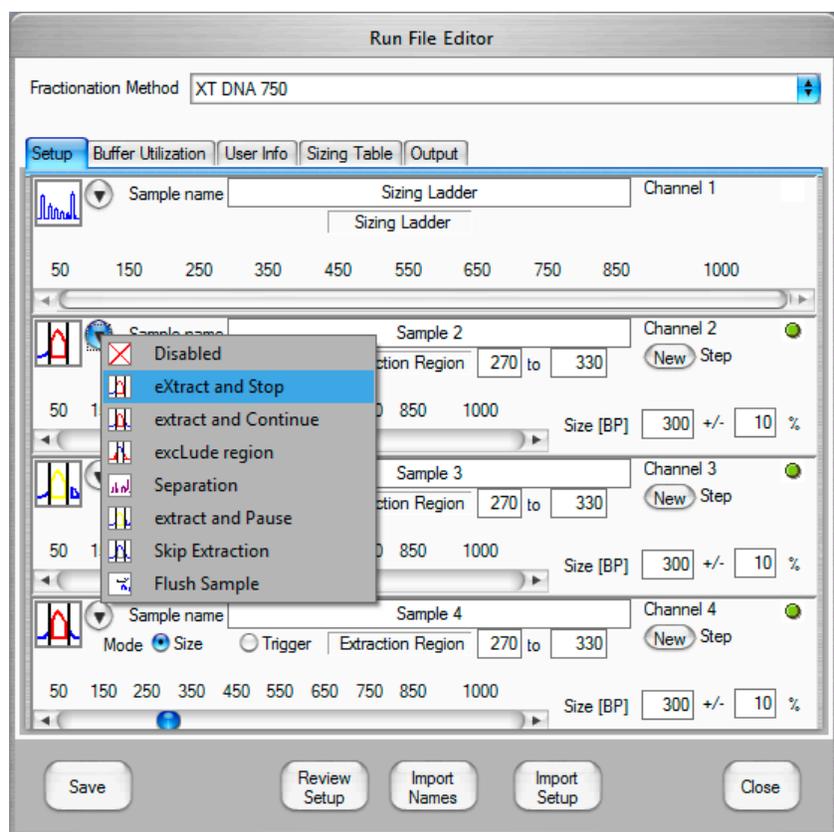
For the XT DNA 750 assay, the user can set the extraction size between 50 and 1000 bp. For the XT DNA 300 assay, the extraction size may be set between 25 and 500 bp. Extraction widths may be set

between +/- 3% and +/- 20%. For maximum extraction efficiency and even distribution of fragment sizes, Caliper recommends extraction widths of 5-15%. At less than 5%, the yield of extracted material decreases. At greater than 15%, the distribution of extracted material may be biased toward larger fragments.

Note: *The symbols on the left of the window indicate the operation mode selected for each channel* (see chart on page 14 for a guide to symbols). The default mode is Extract and Pause. However, if a user has changed the operation mode for one or more channels for a previous run, the operation mode in this setup screen may not be set to Extract and Pause. In this case, the user can click the gray drop-down arrow to select the desired operation mode.

Operation Modes

The Setup Tab on the Run File Editor window offers options for setting up alternative extraction methods. These include various **Operation Modes** (pull-down menu) and various **Extraction Modes** (radio buttons), which can be set independently for each channel. The most appropriate operation mode(s) and extraction mode(s) for a given run depend on the type of sample and the goal of the run. Examples illustrating the use of different options are described below. **Separation, Exclude region** and **Skip Extraction** operation modes are available on the XT only.



Changing the Run File during a run

To manually pause a run, hover the cursor over the instrument diagram in the upper left window, and click on the orange Pause button that appears. During the pause, a **Modify Run Settings** window opens. The user can choose to resume the run without changes, modify the run file and then resume the run, or stop the run. The same Modify Run Settings window opens if the user selects **Edit or Stop Run** in the window displayed during a pause associated with an Extract and Pause operation.

Note: *When editing a run during a pause, the Operation Mode for each channel cannot be changed.* The Size Region, Extraction mode, Threshold, and Collection Width settings can be altered. If the channel is set to Extract and Pause or Extract and Continue, new steps can be added during the pause. If the channel is set to Extract and Stop, Flush sample can be added during the pause. Steps cannot be deleted once the run has been started.

Changing the ladder channel or running without a ladder

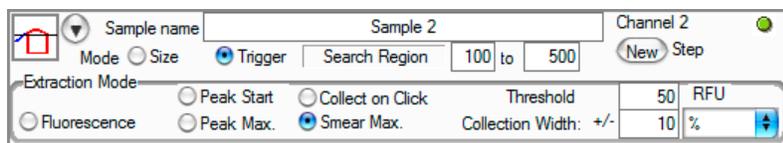
The default Run File designates Channel 1 as a ladder channel. To change which channel is used for the ladder, first change the **Operation Mode** for Channel 1 by selecting a different mode from the pull-down menu (e.g. Extract and Stop). Then set the operation mode to **Ladder** for the channel in which the ladder will be run. Only one channel in the run file can be set to the Ladder operation mode.

It is not necessary to run a ladder on every chip; an extraction operation can be set on all channels. Caliper recommends running a ladder to get the most accurate sizing/extraction when using the Size Range extraction mode. Running the Size Range extraction mode without a ladder channel is a good option if the goal of the extraction is to ensure that the collected sample contains a specific range of fragment lengths, but the exact size of the extracted region is somewhat flexible. In addition, optically assisted extraction modes (e.g. Skip Extraction or Peak Start) can be used for precise extraction of specific bands without requiring the use of a physical ladder.

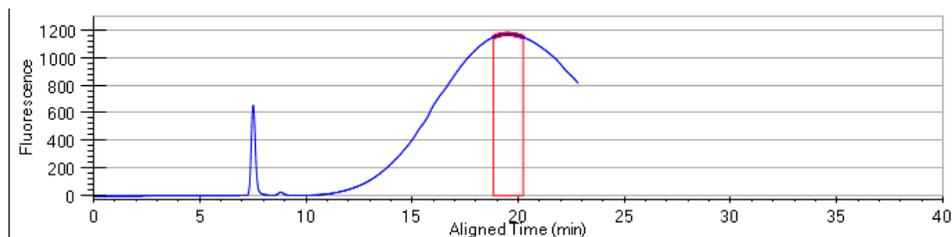
Single extractions per channel

For maximum extraction efficiency, the operation mode for each channel should be set to Extract and Stop or Extract and Pause. With **Extract and Stop**, the current in a channel is shut off immediately after extraction, and the channel ceases to collect data. The extracted material stays in the collection well while other channels complete extraction. **Extract and Pause** can be used when the user wants to generate a complete electropherogram for the sample without compromising recovery of the extracted material. The run pauses when each of the extraction steps is complete, allowing the user to collect the extracted sample, replace the collection buffer, and resume the run. Additional extraction steps may also be adding with Extract and Pause. It is possible to run single extractions with **Extract and Continue**, but the user should expect reduced recovery of extracted material due to slow migration out of the collection well.

Extract and Stop



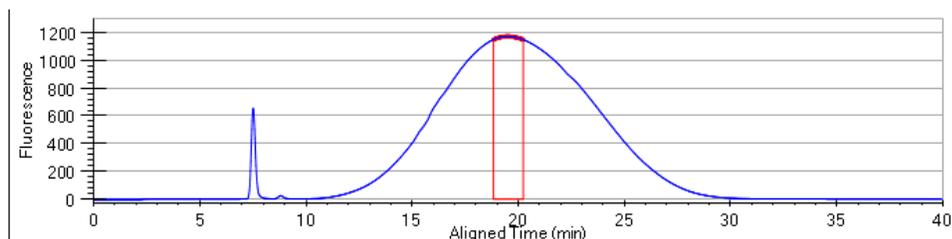
Sample name	Sample 2	Channel 2	<input checked="" type="radio"/> Trigger	Search Region	100 to 500	<input type="button" value="New Step"/>
Extraction Mode	<input type="radio"/> Peak Start	<input type="radio"/> Collect on Click	Threshold	50	RFU	
<input type="radio"/> Fluorescence	<input type="radio"/> Peak Max.	<input checked="" type="radio"/> Smear Max.	Collection Width: +/-	10	%	



Extract and Continue

Sample name: Sample 3 Channel 3 ●
 Mode: Size Trigger Search Region: 100 to 500 New Step

Extraction Mode:
 Peak Start Collect on Click Threshold: 50 RFU
 Fluorescence Peak Max. Smear Max. Collection Width: +/- 10 %



Note: Caliper recommends promptly removing extracted material from the chip when a run is paused or upon completion of the run. Allowing samples to remain on the chip in the absence of applied current allows diffusion and may result in reduced recovery and/or reduced resolution.

Multiple extractions per channel

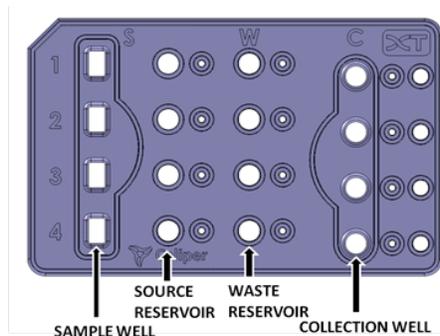
When the operation mode is set to **Extract and Pause** or **Extract and Continue**, the user has the option of including additional extraction steps by clicking on the **New** button. A step may be removed by clicking on the **X** symbol above the step number.

Sample name: Sample 2 Channel 2 ● X
 Mode: Size Trigger Extraction Region: 315 to 385 New Step 2 X

50 150 250 350 450 550 650 750 850 1000 Size [BP]: 350 +/- 10 %

When running with multiple extraction steps in one or more channels, it is important that the user monitor the run. When an individual **Extract and Pause** step is completed, the run pauses and the software prompts in a pop-up window for a **Resume** or **Edit or Stop Run** command. The run status window indicates the channel(s) for which extraction has been completed with COLLECT. The extracted sample(s) should be removed from the collection well and replaced with fresh collection buffer (with or without a wash step as described on page 7). If desired, the user may select Edit Run File to change a subsequent extraction setting on the same or a different channel. The run should be resumed as soon as possible.

Note: When preparing for a run with multiple extractions, running buffer from the chip should be set aside for replacing buffer in collection wells and/or washing collection wells. Before starting the run, up to 60 uL of running buffer can be collected from each channel's source reservoir and/or waste reservoir (prior to adding dye to the chip).



Note: Each assay has a maximum recommended total collection time for an individual channel. The downstream electrode in the collection arm of the channel accesses a separate buffer chamber than the downstream electrode in the waste arm of the channel. The buffer capacity of this smaller chamber is limited and currents may drop after excessive application of voltage to the collection arm electrode. For the DNA 750 assay the maximum recommended collection time is 6 minutes; For the DNA 300 assay it is 8 minutes. The **Buffer Utilization** window in the Run File Editor tracks the estimated total extraction time for each channel. A green check symbol indicates that the total extraction time will be under the limit. A red X symbol indicates that the limit will be exceeded. The user will be allowed to start the run, but should be aware that collection arm buffer capacity may not support the final extraction step(s). For collection modes for which the software cannot predict the exact buffer utilization in advance (e.g., peak start, peak max, click to collect), the software reports a warning message if and when the buffer utilization limit is exceeded.

Run File Editor

Fractionation Method: XT DNA 300

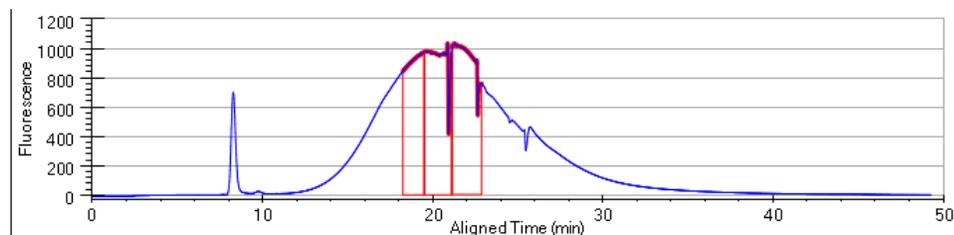
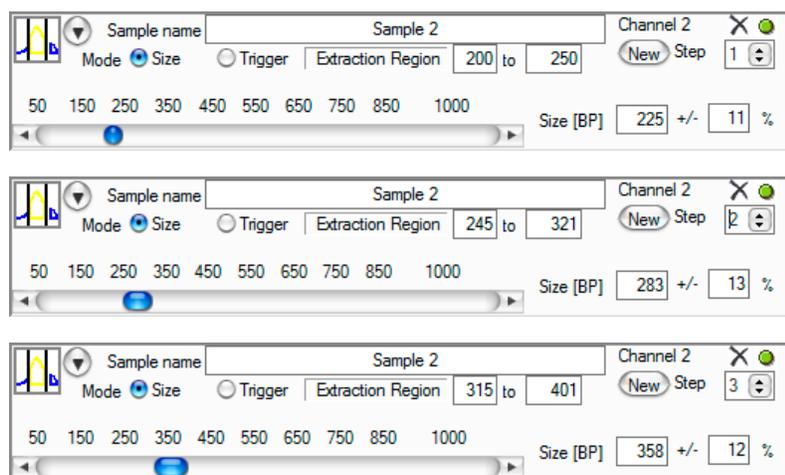
Setup | **Buffer Utilization** | User Info | Sizing Table | Output

Channel	Estimated extraction time. (Buffer limit is 8.1 min.)	State
1	Not applicable	
2	Between 6.4 and 10.5 min.	⚠
3	Between 2.0 and 6.1 min.	✅
4	10.9 min.	❌

Using multiple collections to achieve wider extraction widths

The maximum extraction width allowed for a single extraction is +/-20% of the target size. If a single extraction is allowed to continue beyond this limit, fragments move out of the collection well into the gel in the collection arm of the channel, where they cannot be recovered.

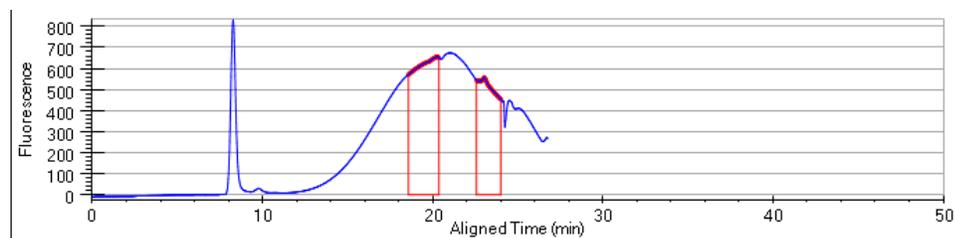
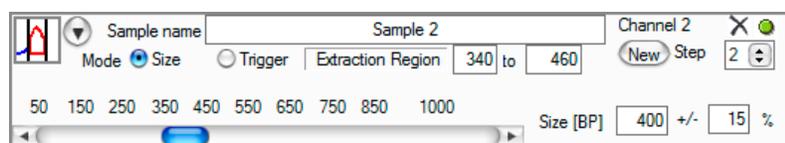
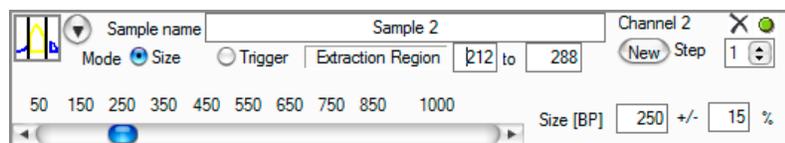
To collect a larger region of DNA, the Run File should be set up with multiple extraction steps using the **Extract and Pause** operation mode. For example, to collect the entire region from 200 bp to 400 bp from a fragmented DNA sample in Channel 2, the extraction should be set up in 3 steps. Each step is set to collect from a region representing the median size +/- 12-15% using **Extract and Pause** as the operation mode. When each extraction step is completed, the run will pause, allowing the user to open the lid, pipette the collected material out of the collection well for channel 2, replace the collection buffer, and continue the run. This strategy ensures that the maximum collection efficiency is achieved across the entire size range. If the collected samples are to be mixed, there is no need to rinse the collection well between steps.



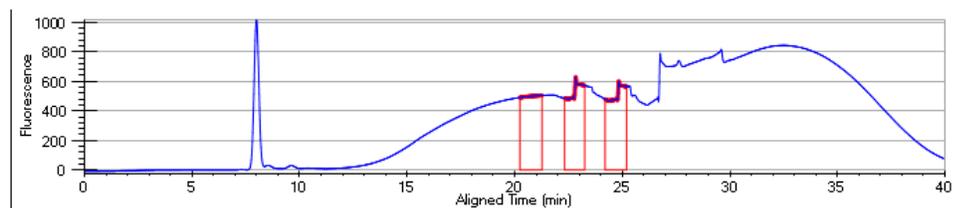
Note: Collection size ranges may be set with 5-10 bp overlap to ensure continuous extraction across the entire region. The full range of the first extraction will complete, and then the second extraction will begin immediately upon resuming the run.

Using multiple collections to extract discrete fractions

The **Extract and Pause** operation mode can be used to collect from multiple regions within a sample. At each pause, the user should remove the extracted sample from the collection well, rinse the well one time with 20 uL running/collection buffer, add 20 uL running/collection buffer, and resume the run. As noted on page 5, additional buffer for rinsing and/or collection can be taken from the running buffer reservoir(s) of the chip before the run. Minimal carryover is observed when extracted regions are separated by at least 100bp.



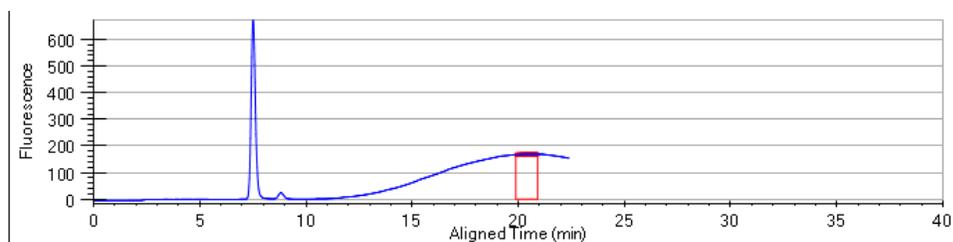
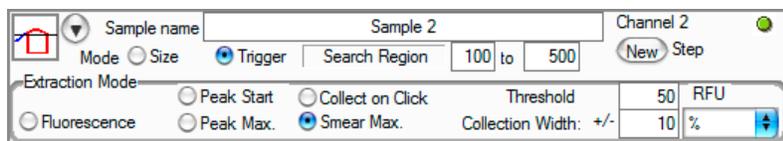
Note: *Optics artifacts will often be observed in electropherogram data when running multiple extractions.* During the time that the downstream collection arm electrode is activated, no dye is drawn into the gel from the waste buffer chamber. This can result in a temporary reduction in the concentration of dye near the optical detection point in the main channel. When the downstream voltage is switched back to the waste well electrode, the concentration of dye is restored, resulting in a spike in signal. This signal spike can occur several minutes after the current switch. Also, if the user removes the chip from the instrument while collecting sample and replacing the collection buffer, the altered positioning of the chip upon reinsertion can result in altered signal intensity. An example of an electropherogram showing these optics artifacts is shown below.



Using Smear Max to collect the most concentrated range within a smear (Available on the XT only)

To recover the maximum amount of tightly sized DNA (irrespective of the exact size of the fragments) from a sheared/fragmented sample, the Smear Max mode should be used (XT only). The Smear Max mode is ideal for samples that are sheared to a broad distribution in which no defined peak can be found. When this extraction mode is selected, the **Extraction Region** field will change to **Search Region**. In addition, fields will activate allowing the user to set a threshold value, collection width, and collection width mode.

In **Smear Max** mode, the software searches within the specified region for the point at which two consecutive conditions have been met: 1) the signal has reached or surpassed the specified threshold signal (RFU) and 2) the signal (averaging over 3 consecutive data points) is decreasing. Once the smear max has been identified through these criteria, the instrument sets the collection window to +/- the specified % size of the peak maximum or to the specified number of base pairs centered on the peak maximum. Alternatively, the user may select **Manual** to activate the option to manually trigger the end of the collection step. In this case the collection is set to start the specified -% size before the maximum. Extraction will continue until the user triggers the STOP command. If the user has not stopped the extraction when the end of the search region has been reached, the extraction will end automatically there. With any of the collection width settings, if no maximum is found within the search region, but the specified threshold signal (RFU) is exceeded, extraction will occur centered at the end of the search region. If multiple peaks exceeding the threshold are present in the search region, the first local maximum (ie first peak) will be extracted.



Using Optically Assisted extraction modes to collect discrete fragment peaks

When a sample contains one or more discrete fragments (i.e. PCR products), individual peaks may be extracted using either **Peak Start (XT or XTe)** or **Peak Max (XT only)** as the extraction mode. When one of these extraction modes is selected, the **Extraction Region** field changes to **Search Region**. In addition, fields activate allowing the user to set a threshold value, collection width, and collection width mode. The **Extract and Pause** operation mode can be used to set up extractions from multiple regions within a sample.

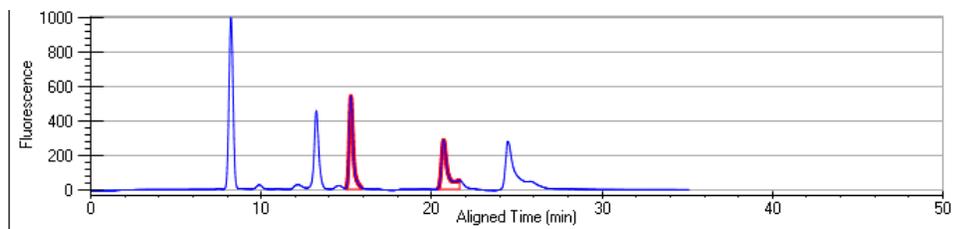
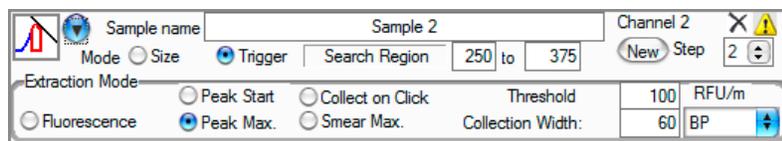
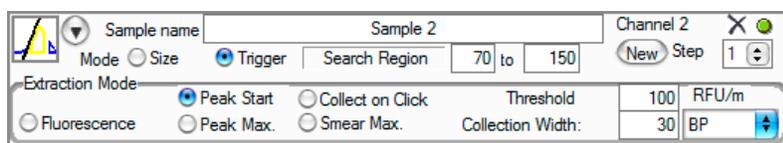
In **Peak Start** mode, the software searches within a specified region for the point at which the signal slope reaches the threshold value (RFU/min). Collection continues for the specified collection width, which may be set in **BP** or **+/-%**. If **BP** is chosen, collection occurs from the point the threshold slope is reached through the specified number of BP. If **+/-%** is chosen, collection occurs **+/-** the specified percentage from the projected center of the collected region. Alternatively, the user may select **Manual** to actively trigger the end of the collection step during the run. If the user has not stopped the extraction when the end of the search region has been reached, the extraction will end automatically there.

For typical single PCR product peaks, the collection width should be set to a BP value that represents 10% of the target size (eg 30bp for a 300bp fragment). If the baseline is expected to be noisy relative to the target peak signal, the user should take care not to set the threshold too low, as noise in the baseline may trigger collection prematurely. The appropriate threshold for peak start extractions will depend on the concentration of the target fragment in the sample. The following can be used for guidance:

0.5ng-5ng DNA: 25RFU/min

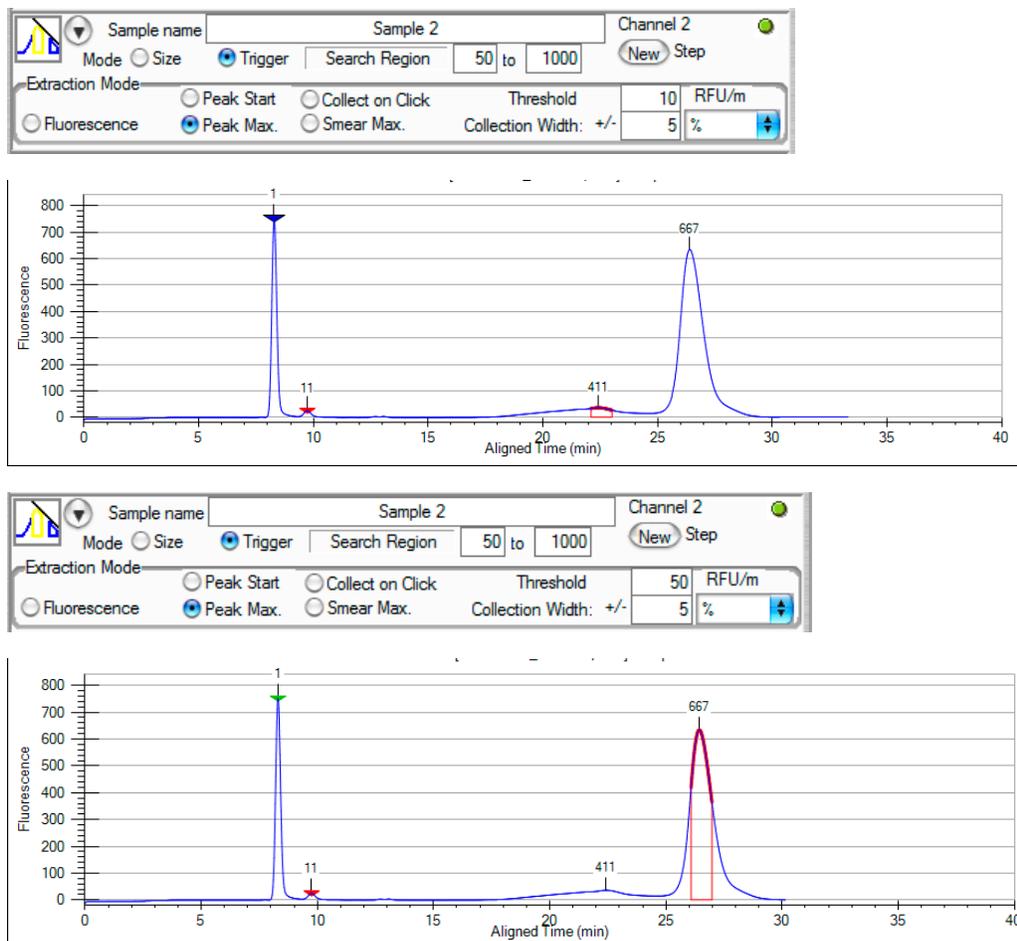
5ng-10ng DNA: 100RFU/min

10ng-50ng DNA: 500RFU/min



The **Peak Max** mode is ideal for samples with a narrow distribution (i.e., samples that have previously been size selected). In **Peak Max** mode, the software searches within the specified region for the point at which two consecutive conditions have been met: 1) the signal slope has reached or surpassed the specified threshold (RFU/min) and 2) the signal has switched to a negative slope surpassing the specified threshold in magnitude. Once the peak max has been identified through these criteria, the instrument sets the collection window to **+/-** the specified % size of the peak maximum or to the specified number of base pairs centered on the peak maximum. Alternatively, the user may select **Manual** to activate the option to manually trigger the end of the collection step. In this case the collection is set to start the specified **-%** size before the maximum. Extraction will continue until the user triggers the **STOP** command. If the user has not stopped the extraction when the end of the search region has been reached, the extraction will end automatically there.

Since Peak Max has more stringent trigger requirements than Peak Start, it is less sensitive to noise in the electropherogram. Thresholds as low as 10RFU/min can be used. For samples with more noise, a higher threshold (50-100RFU/min) can be used.



Note: In multiple extraction runs, Peak Start and Peak Max should only be used with samples containing well separated peaks. If peaks are close together or overlapping, it may be difficult to identify the correct collection width that maximizes recovery while extracting material from only the desired peak. Search for a subsequent peak cannot begin until extraction of the first peak is completed. In addition, optics artifacts may interfere with extraction by triggering collection at inappropriate points in the run.

Avoiding excessive numbers of pauses when running multiple extractions in multiple channels

When all sample channels are set to run the same extraction steps, each channel will run at a slightly different speed. Extraction of coinciding regions will not be completed at exactly the same time. However, the user does not necessarily need to collect sample at the completion of every extraction. If two or more channels are to complete extraction within 60 seconds of each other, the software automatically applies the pause step and prompts for collection from multiple channels at the same time.

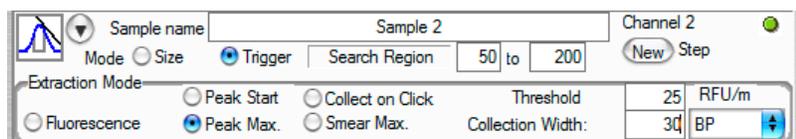
If running different extraction steps in different channels, the user may manually skip a pause step or may use Extract and Continue to avoid pause steps. If all channels are set to Extract and Pause, the user may resume a run without opening the lid or collecting sample at the completion of an extraction in a given channel. When the next channel has completed the extraction, the user may open the lid and collect samples from multiple channels prior to resuming the run. Alternatively, the user may set an earlier extraction step in one channel to Extract and Continue and a later extraction step in another channel to Extract and Pause. This prevents the software from pausing the run independently for each channel. A final option is to set all channels to Extract and Continue and manually pause the instrument at appropriate times during the run.

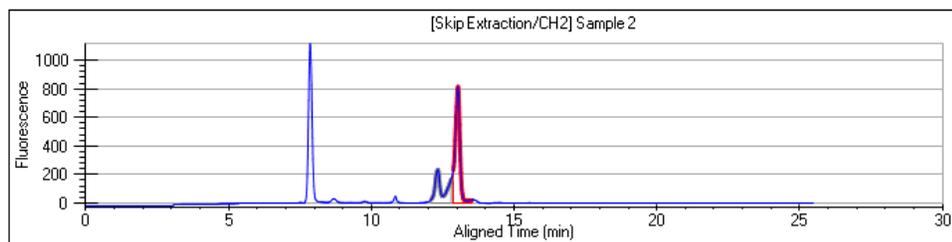
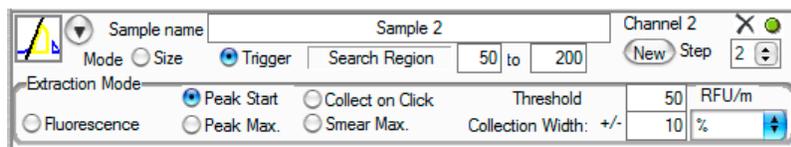
When using any of these strategies, the user must ensure that extracted material does not remain in the collection well for extended time periods. For example, if channel 2 and channel 3 are set to extract +/- 10% widths at 250 bp and 300 bp, respectively, then channel 2 may be set to Extract and Continue and channel 3 to Extract and Pause with little loss in collection efficiency for channel 2. However, if channel 2 is set to extract at 250 bp and channel 3 at 500 bp, it is recommended to set both channels to Extract and Pause.

Note: Whenever Extract and Continue is used in a channel, no subsequent extraction in that channel will start until the instrument is paused and the lid is opened. For example, if there are two extractions planned for channel 2 and the first extraction is set to Extract and Continue, then the software will not trigger the second extraction until the instrument goes into pause mode and the lid is opened (either by an Extract and Pause step in another channel, or by manually pausing the run).

Using skip extraction to avoid collection of a certain region and extract from a neighboring region

With some sample types, such as dsDNA libraries containing sequences derived from small RNA fragments, it is necessary to extract material that is very close in size to unwanted material. The **Skip Extraction** mode allows the user to identify and exclude the peak of unwanted material (containing no insert) from the slightly larger peak containing the fragments of interest. The first step is set to Skip Extraction using Peak Max as the extraction mode. Define the search region as 50-200bp (for samples that contain large primers due to barcode sequences, search region may need to be adjusted to 100-200bp), set the threshold as 25RFU/Min, and set the collection width as 25-35bp (depending on the width of the adapter-dimer peak). The software automatically identifies the peak within the Search Region, and prevents the next step from triggering through the size range designated by the Collection Width. The peak identified in the Skip Extraction step is marked in grey on the electropherogram. The second step, **Extract and Pause** for the peak of interest, should be set with an overlapping Search Region. Define the search region as 50-200bp (the same Search Region as step1), set the threshold as 50RF/min using Peak Start and set the collection Width as 5 - 10% (depending on width of the library peak). Since individual peaks are identified using the optically assisted extraction modes, the use of a physical ladder is not required.



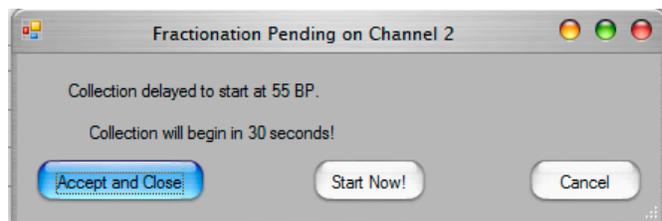


Using Collect on Click to manually select an extraction region

When **Collect on Click** is selected as the extraction mode, the user must watch the data during the run and manually trigger collection at the appropriate point in the electropherogram. The Search Region is extended to the full range of the assay, (eg, 50-1000 bp). When the trigger collection option becomes available for a given channel (data tracing has reached 50bp), the Run Setup window shows a pink **CLICK** box.

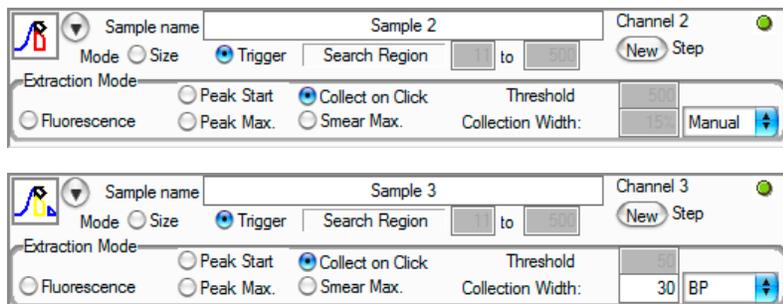
C	S	Mode	Size Range [BP]	Trigger At
1		Lddr		
2	1	X&S	50-1000 [+?]	CLICK
3	1	X&P	50-1000 [+30]	CLICK
4	1	X&P	50-1000 [+?]	CLICK

Upon selecting **CLICK** for a given channel, the software opens a **Fractionation Pending** window. Because the data in the electropherogram is collected at a point in the channel in front of the collection switch point, there will be a time delay before collection begins (5-10% of the elapsed separation time, depending on assay). If this is the collection intended, the user need not do anything. The Fractionation Pending window can be left open, or it can be closed to allow a **CLICK** on another channel. The Run Setup window shows **Pending** for the specific channel.



If **Cancel** is selected, the collection does not occur. The user may select **CLICK** again later in the run. If **Start Now!** is selected, collection begins immediately. This allows collection of material that has already passed the detection point starting at approximately -5-10% (depending on assay) of the elapsed run time shown in the electropherogram.

When using Collect on Click, a given extraction step can be set to stop extraction at a specific extraction width (+/- % of the projected center of the point marked or forward from the region marked in **BP**). Alternatively, the user can select **Manual** to trigger the stop point based on the electropherogram data for the channel.



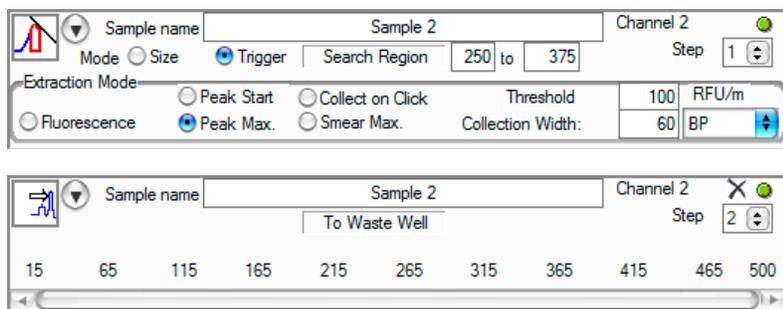
On the electropherogram, the extracted region is marked in red when the extraction is occurring in real time. If **Manual** has been selected, the Run Setup window shows a pink **STOP** box during the time the collection is actually occurring. The user should select STOP when the red region has marked the desired point in the electropherogram.

Note: START collection should be triggered based on the blue electropherogram data trace representing detected DNA. STOP collection should be triggered when the red portion of the electropherogram matches the region the user wants to collect.

Using Sample Flush to recover unextracted sample from waste well

If the user wants to use the Extract and Stop operation mode for the highest collection efficiency, but also wants to be able to recover the entire unextracted sample from the waste well at the end of the run, the Flush Sample operation should be added at the start of the run. The Flush Sample operation is the only step permitted to follow the Extract and Stop step. The Flush Sample operation mode will move the remaining sample into the waste well after all extraction steps in the channel are completed.

The Sample Flush step only begins operation after the run is paused and the instrument lid has been opened to remove the extracted material from the collection well. If all sample channels in the chip are set to Extract and Stop, the software automatically pauses when all extractions are complete. To run the Sample Flush steps, the user must open the instrument lid and remove the extracted material from the collection well before resuming the run. The run continues until each channel has run for the full method time.



Mode Symbols

Each combination of Operation and Extraction Mode is presented on the left side of the channel in the Setup Tab as a different symbol to help user quickly review the run setup. The symbols used are presented in tabular form below:

Extraction Mode / Operation	Size Range	Fluorescence Trigger	Peak Start	Peak Maximum	Click to Collect	Smear Maximum
Disabled						
Ladder						
Extract and Stop						
Extract and Continue						
Exclude Region						
Separation						
Extract and Pause						
Skip Extraction						
Flush Sample						