Using the Agilent Bioanalyzer to Diagnose and Troubleshoot Unexpected Shearing Profiles

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Background

The Agilent 2100 BioAnalyzer is a microfluidics – based system for gel electrophoresis. The Agilent is used as a QC for size distribution after Covaris shearing and PCR enrichment. Using our Agilent results, we can determine whether to continue, rework or abandon a sample early in the process.

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Inconsistent Results
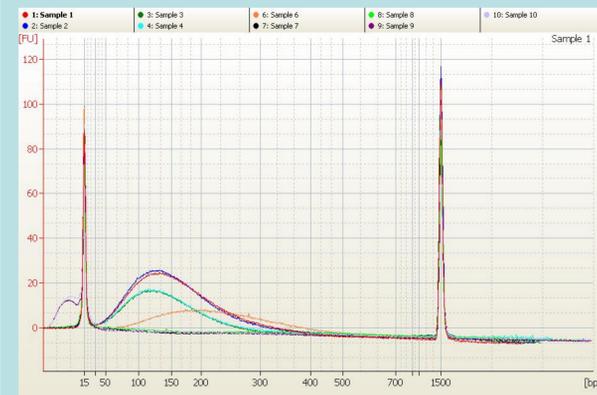
New shearing protocols require rigorous validation to catch instances of variation. These samples were all sheared together with the same conditions (machine settings, vessel type, holder type, input concentration and volume) yet have greatly variable results (differing size ranges and greatly variable yields) . New shearing conditions are not implemented until extensive validation is performed.

## Over-Shearing: Shearing Down to Small Fragments
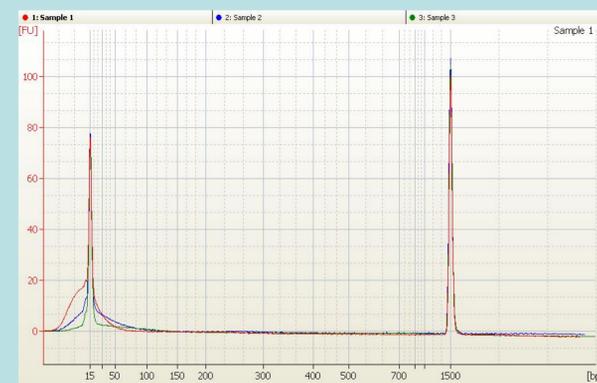
Observed when testing a new shearing vessel. Possibly attributed to the increased pressure from a new holder (a plate pad and a screw-top combination) or the plastic tube becoming much hotter than expected while running a protocol.

Sample Loss

Sample loss is an issue and is greatly effected by vessel and holder type as well as bath level. Here, when testing a new capping method and holder we started with 3ug and ended up with essentially nothing.
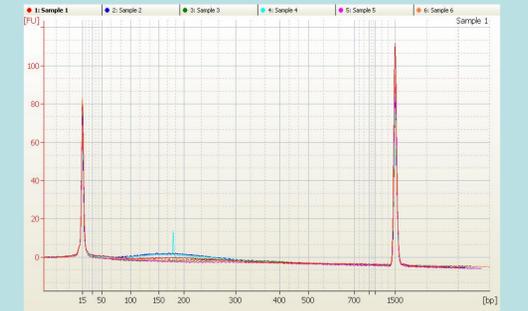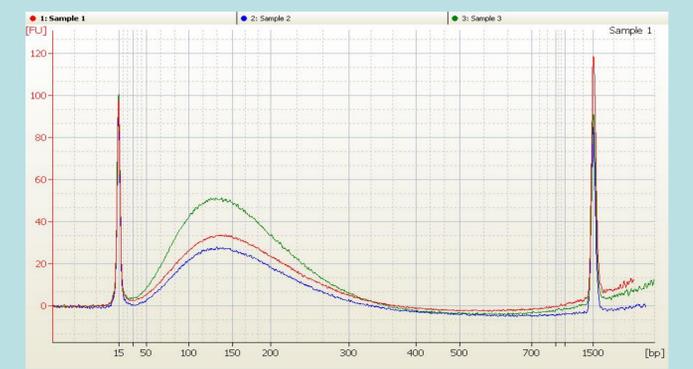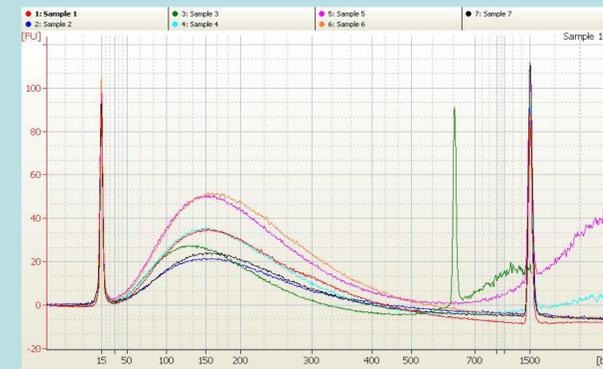
Importance of Validation: Volume Dependent Process
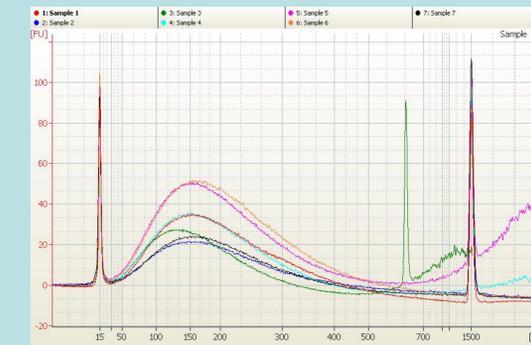
Sample volume directly impacts shearing size and consistency. Here, we changed just the volume from 100ul in our standard protocol to 50ul .The samples displayed two different peaks: 1 in the correct size range and 1 at a much bigger size range.

Rework Loop: Salvaging insufficiently sheared samples

One potential fail mode is insufficient shearing. The Agilent allows us to catch this issue and rework our sample. If a sample is not completely sheared to the desired size range, we can reshear to improve the size distribution. More sample is lost during re-shearing but more sample will also be in the desired size range.