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Reconditioning PCR for removal of PCR bubbles in Illumina librarys V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes how to remove partly single-stranded PCR products ("PCR bubbles") from Illumina libraries. For more information about this phenomenon please see **this explanation from Illumina**. For metabarcoding libraries, it can be hard to estimate the optimal input template or the perfect amount of PCR cycles and therefore overamplification happens frequently. PCR bubbles cannot be quantified reliably with fluorometric-based methods and may look different on different capillary electrophoresis devices.

PCR bubbles can lead to failed sequencing runs due to over- or underloading the flowcell.

Guidelines

Follow general lab etiquette. Wear gloves to prevent contamination of samples. Clean the workspace before starting and after finishing with 80% EtOH.

Materials

Materials required:
Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable depending on the supply situation.

Chemicals:
QIAGEN Multiplex PCR Plus Kit **Qiagen Catalog #**206152

Primers:
Illumina P5 5' - AATGATACGGCGACCACCGAGATCT - 3'
Illumina P7 5' - CAAGCAGAAGACGGCATACGAGAT - 3'

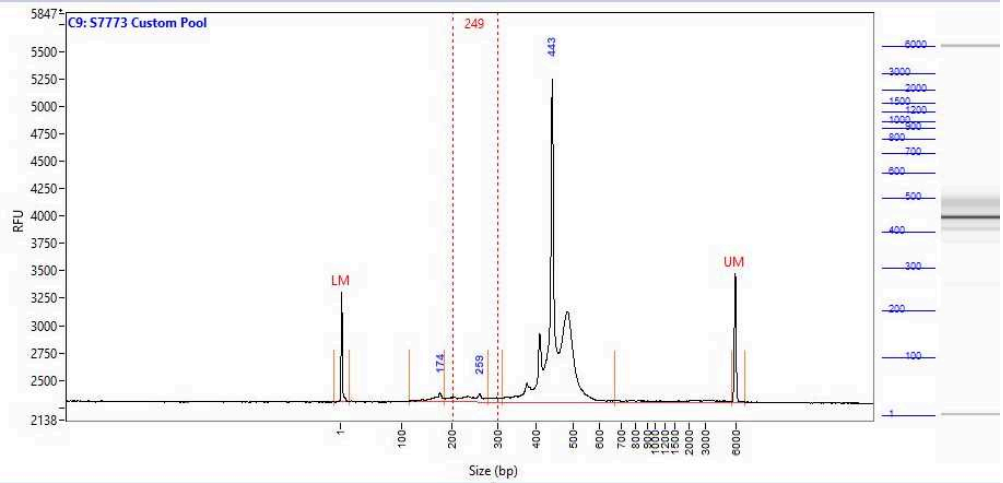
Troubleshooting

Safety warnings

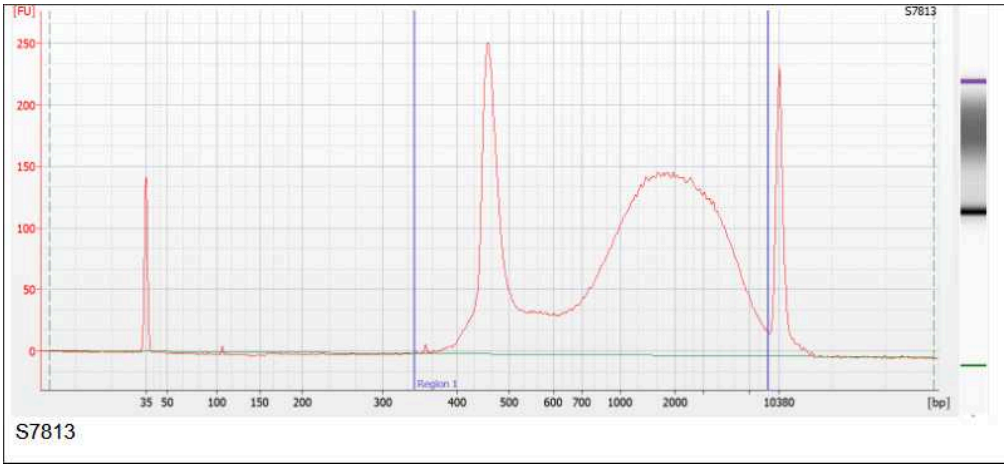
Buffers containing guanidine produce highly reactive compounds when mixed with bleach. Don't mix the extraction waste with bleach or solutions that contain bleach.
Reagents are potentially damaging to the environment. Dispose waste as mandated.

Quality control

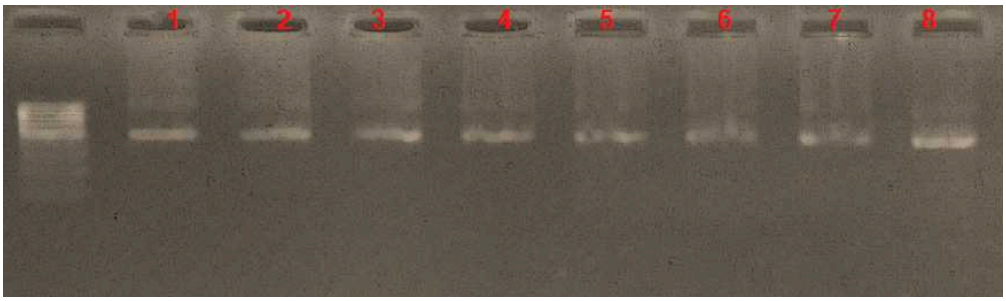
1 Perform a quality control of your library. PCR bubbles may look different depending on the method used for quality control. See below and example of the Fragment Analyzer, Bioanalyzer and an agarose gel.



Example result of the AgieInt fragment analyzer for a library that contains PCR bubbles. The desired peak is at 443 bp, the shoulder to the right of it is the PCR bubble.



The same library on the Agilent bioanalyzer. The shoulder is moved further to the right, also the relationship of library to PCR bubble changed significantly.



PCR reactions of the same library visualized on 1% agarose. Notice the faint band above the actual amplicon.

Library concentration (optional)

2 Concentrate your library by reducing the volume down to 100 µL . We usually do this with a spin-column based protocol, although this can be performed with magnetic beads as well.

Note

Please see:

Protocol

NAME

PCR cleanup and size selection with magnetic beads

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Preview

or

Protocol

NAME

Guanidine-based DNA extraction with silica-coated beads or silica spin columns

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Reconditioning PCR

3 Fill the concentration of your library and the project name into the Excel spreadsheet. The suggested master mix for the reconditioning PCR will be calculated accordingly. We usually go for 1250 ng of template input, however, this can be adjusted if necessary.

master mix

Note

You can download the Excel spreadsheet here:
Mastermix calculator reconditionin...

4 Perform the PCR with 4 reactions of 50 µL .

PCR clean-up

5 Pool the 4 PCR reactions.

6 Perform a column-based PCR clean-up to exchange the buffer. This can also be done with magnetic beads. Elute the DNA in 100 µL .

Protocol

NAME
Guanidine-based DNA extraction with silica-coated beads or silica spin columns

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Size-selection

7 Perform a size selection with a ratio of 0.7x to remove residual primer dimers. Elute the final library in 50 µL .

Protocol

NAME

PCR cleanup and size selection with magnetic beads

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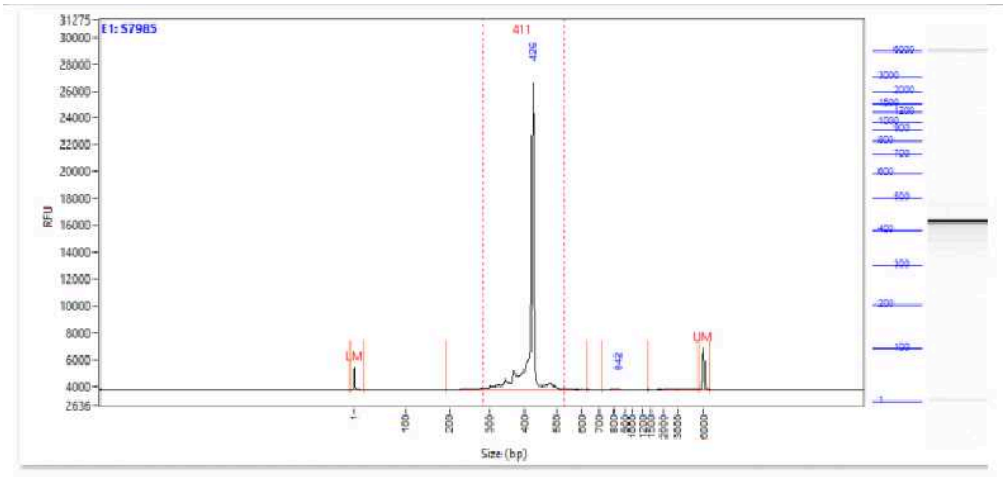
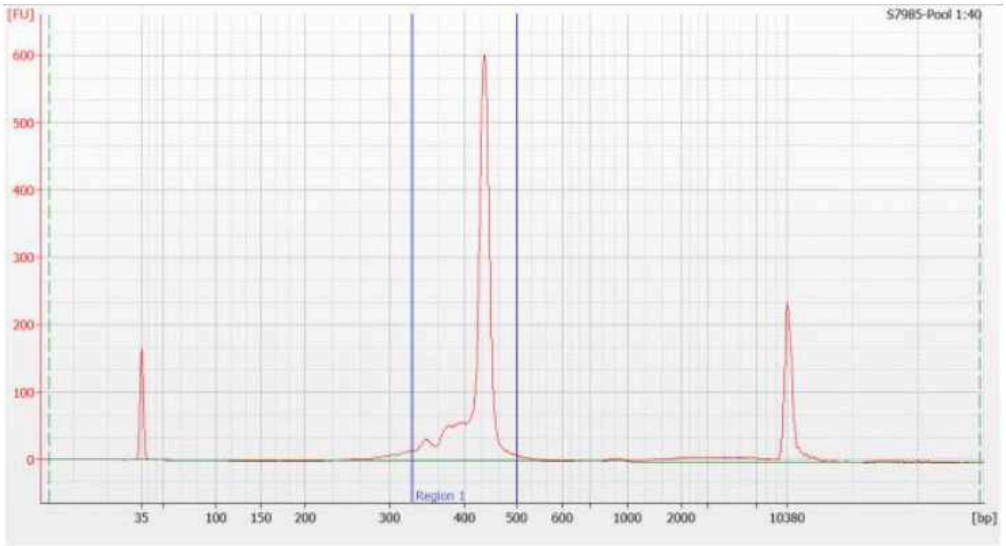
Perform final quality control

8 Perform a final quality control. Quantify the library concentration with a fluorometric-based method and perform quality control via electrophoresis. The shoulder should be gone and the library should be good for sequencing.



S7985 Fragment Analyzer

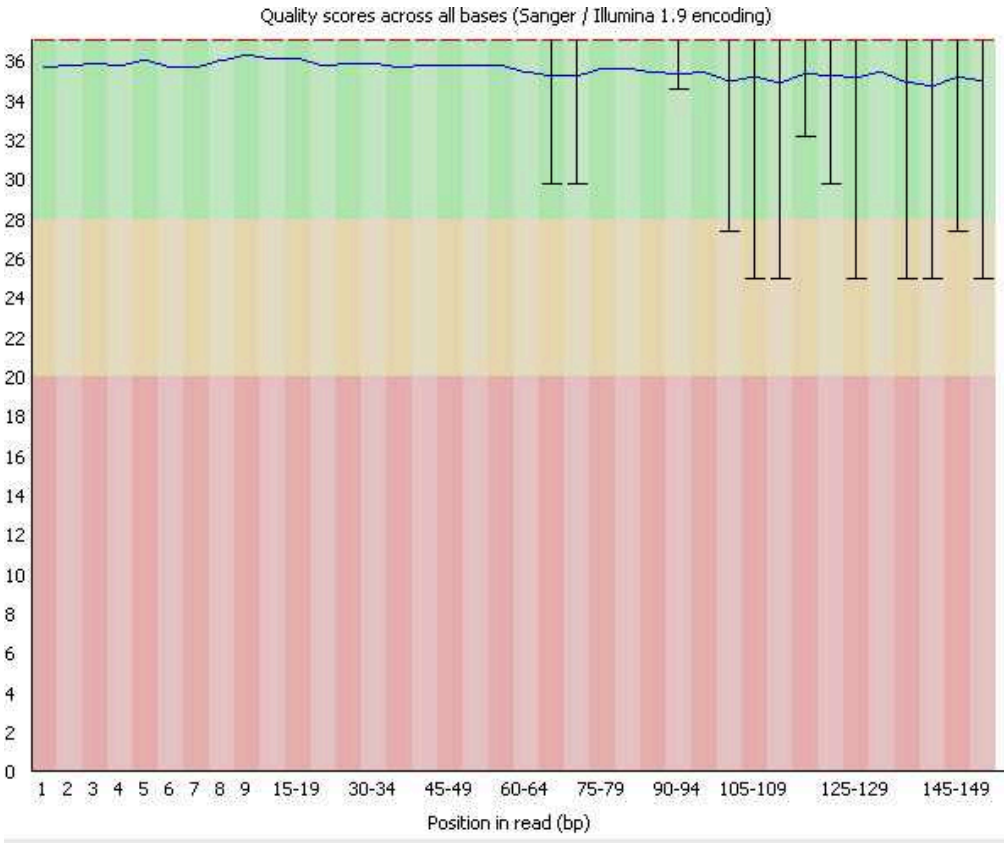
Example result of the AgieInt fragment analyzer for a library after the removal of PCR bubbles.



S7985 Bioanalyzer

The same library on the Agilent bioanalyzer after the removal of PCR bubbles.

Expected result



The quality of the sequencing run should be high after the reconditioning PCR.

Protocol references

https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000001918

https://dnatech.genomecenter.ucdavis.edu/faqs/my-libraries-show-peaks-larger-than-expected-can-i-still-sequence-these-pcr-bubbles/