Troubleshoot and Redo Some Samples for Methylation Comparison With Pico Methyl Seq Library Prep Kit

Additional amplifications of already made libraries and two re-tries of the full library prep with samples from the previous post. Using the Zymo Pico Methyl Seq Kit for the purpose of comparing to RRBS preparations

Samples for methylation comparison are from the Holobiont Integration Project, and were extracted by Emma Strand or myself, see her notebook posts for the extraction specifications: 20190805 and 20180823, 20190718 and 20190903.

Re-Amplification on 10-2-19

Original amplifications of library preps could have been low for all samples because we added too much primer (see primer dimer present in all sample traces in previous post). All samples except WGBS 1471 were amplified again and then bead cleaned to be sure to

remove any primer dimer. Samples were also bead cleaned before amplification to ensure remaining primer was not inhibiting anything.

1X bead clean

- 11*µ*l of KAPA pure beads to each sample (1X)
- Performed normal clean up with fresh 80% EtOH
- Resuspended and eluted in 12*µ*l of DNA elution buffer from the Pico Methyl-Seq kit

Re-amp

- Some already made libraries needed more amplification than others, they were split into an extra 4 cycle PCR or extra 2 cycle PCR
- The same index primers were used but only 0.5*µ*l of each were added this time
- Set up new strip tubes with:

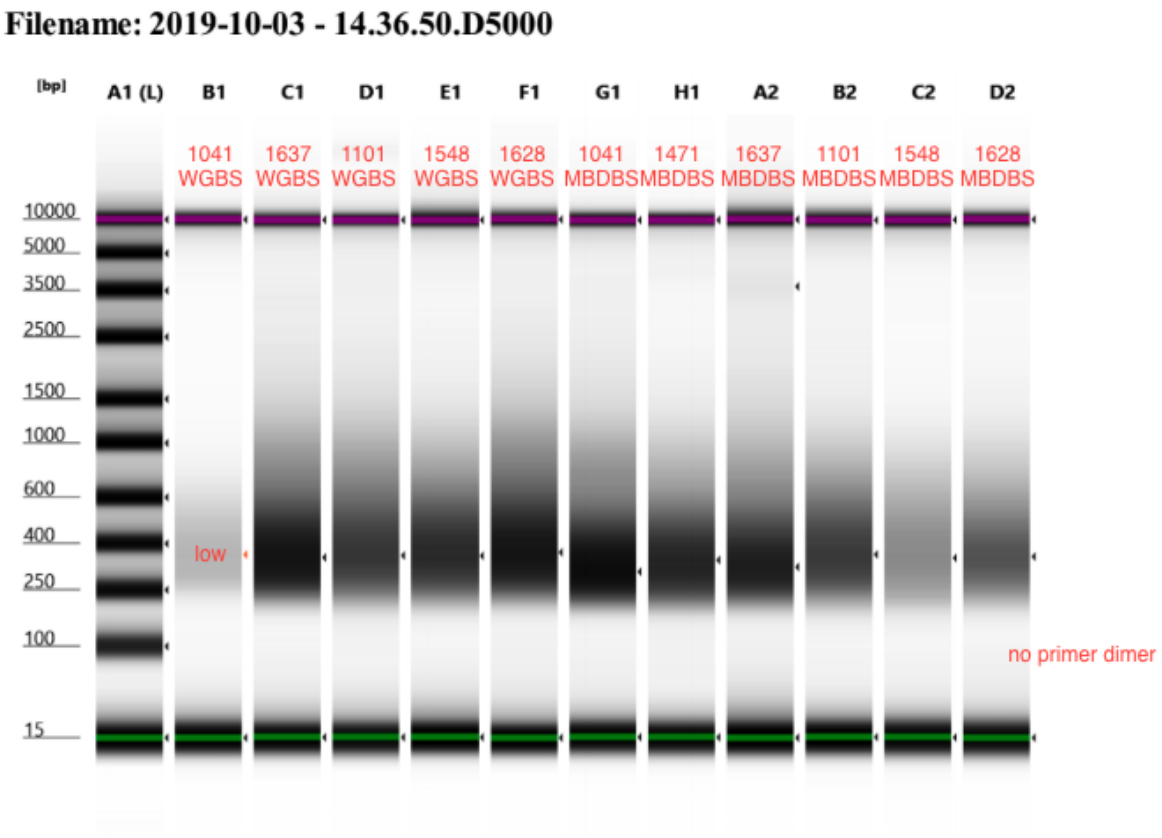
Sample	vol library needed	vol lib amp master mix	vol i5 index	vol i7 index	# of cycles
1041 WGBS	12*µ*l	13*µ*l	.5*µ*l 1	.5*µ*l 1	4
1101 WGBS	12*µ*l	13*µ*l	.5*µ*l 10	.5*µ*l 11	4
1101 Captured MBD	12*µ*l	13*µ*l	.5*µ*l 16	.5*µ*l 16	4
1548 Captured MBD	12*µ*l	13*µ*l	.5*µ*l 17	.5*µ*l 17	4
1628 Captured MBD	12*µ*l	13*µ*l	.5*µ*l 18	.5*µ*l 18	4
1637 WGBS	12*µ*l	13*µ*l	.5*µ*l 3	.5*µ*l 3	2
1548 WGBS	12*µ*l	13*µ*l	.5*µ*l 11	.5*µ*l 10	2

Sample	vol library needed	vol lib amp master mix	vol i5 index	vol i7 index	# of cycles
1628 WGBS	12*µ*l	13*µ*l	.5*µ*l 12	.5*µ*l 12	2
1041 Captured MBD	12*µ*l	13*µ*l	.5*µ*l 7	.5*µ*l 7	2
1471 Captured MBD	12*µ*l	13*µ*l	.5*µ*l 8	.5*µ*l 8	2
1637 Captured MBD	12*µ*l	13*µ*l	.5*µ*l 9	.5*µ*l 9	2

- PCR programs were copied from the last amplification program in the Pico Methyl-Seq protocol and # of cycles was adjusted

After-amp 1X bead clean

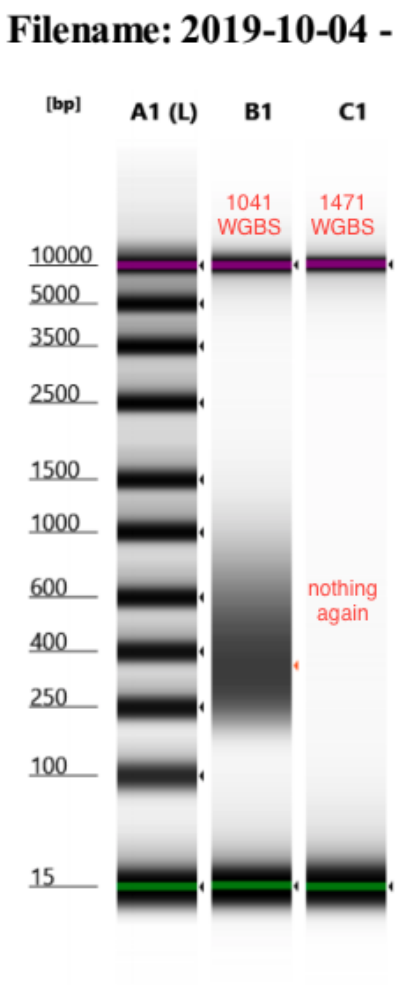
- 26*µ*l of KAPA pure beads to each sample (1X)
- Performed normal clean up with fresh 80% EtOH
- Resuspended and eluted in 14*µ*l of DNA elution buffer from the Pico Methyl-Seq kit
- Ran D5000 tapestation



- 1041 WGBS looks like it didn't amplify well again

Re-Library prep 1041 and 1471 WGBS

- Re-doing the library prep for 1041 which did not amplify well and 1471 which did not work at all
- Followed exact same steps as in this library prep except did a 1X bead clean up after final amplification instead of doing the DCC
- Ran D5000 Tapestation

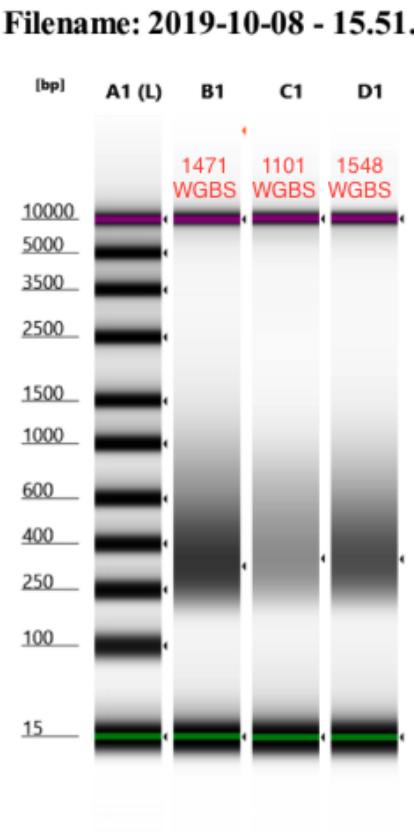


- Once again 1471 WGBS did not work

One more try of 1471 WGBS and re-do of 1101 and 1548 WGBS with *correct paired* indexes this time

- Re-diluteded i5 #2 and i7 #2 indexes to 10uM to see if the primers were the issue
- Followed exact same steps as in this library prep except did a 1X bead clean up after final amplification instead of doing the DCC

    - 1101 WGBS now has paired #10 i5i7 indexes and 1548 now has paired #11 i5i7 indexes
- Ran D5000 Tapestation



Library concentrations for all completely libraries for the methylation methods comparison project. All libraries are in 13μl DNA elution buffer from the Zymo kit

Sample	Library Concentration
1041 WGBS	10.5ng/ul
1471 WGBS	11.9ng/ul
1637 WGBS	18.7ng/ul

Sample	Library Concentration
1101 WGBS	18.1ng/ul
1548 WGBS	15.6ng/ul
1628 WGBS	13.7ng/ul
1041 MBDBS	5.95ng/ul
1471 MBDBS	10.2ng/ul
1637 MBDBS	18.4ng/ul
1101 MBDBS	12.3ng/ul
1548 MBDBS	5.61ng/ul
1628 MBDBS	9.39ng/ul

Written on October 8, 2019