MBD of Samples for Methylation Comparison

Methyl-Binding Domain Enrichment of Samples for Methylation Comparison

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.

Shearing Samples to ~500bp

1. Prepare samples for 1μg in 80μl of Tris HCl

Sample	Volume DNA	Volume Tris HCl
1041	16.03	63.97
1471	18.25	61.75
1637	18.25	68.48
1101	36.9	43.1

Sample	Volume DNA	Volume Tris HCl
1548	43.29	36.71
1628	58.82	21.18

1. Prep Qsonica following protocol
2. Set time for 1min 30 sec, 15 second on 15 seconds off, 25% amplitude
3. Check samples on tapestation after results 1 2
4. Samples 1637 and 1628 were in the more 600bp range average so were sonicated for 15 seconds more

MBD Enrichment

Made 43000µl of 1X Wash/Bind buffer from 5X concentrate before starting

Preparing Beads

1. Pipetted up and down the Dynabeads M-280 Streptavidin to resuspend them
2. Made 6 1.5mL tubes, each with 10µl of Dynabeads (the amount recommended for less than or equal to 1µg of DNA input)
3. Brought volume up to a total of 100µl with 90µl of 1X bind/wash buffer to each tube
4. Pipetted to mix
5. Placed tubes on long magnet rack and removed and discarded supernatant when clear
6. Removed tubes from rack and resuspended beads in 100µl 1X bind/wash buffer
7. Placed tubes on long magnet rack and removed and discarded supernatant when clear
8. Removed tubes from rack and resuspended beads in 100µl 1X bind/wash buffer

Coupling MBD-Biotin Protein to the Beads

1. Thawed MBD-Biotin protein from -80 on ice
2. Made 6 new 1.5mL tubes, each with 7µl of MBD-Biotin protein (amount recommended for 1µg of DNA input)

3. Added 93µl of 1X bind/wash buffer to each tube to get up to a total of 100µl
4. Transferred diluted protein to the washed bead tubes for a total volume of 200µl in each of 2 tubes
5. Put samples on the rotisserie mixer for 1 hour at room temp

Washing MBD-Biotin-Coupled Beads

1. Spun down **briefly** tubes from above
2. Placed tubes on magnet rack for 1 minute
3. Removed supernatant when clear
4. Resuspended beads in 100µl 1X bind/wash buffer and pipetted to mix
5. Mixed beads on rotisserie mixer for 5 minutes at room temp
6. Repeated: Spun down tubes **briefly**
7. Placed tubes on magnet rack for 1 minute
8. Removed supernatant when clear
9. Resuspended beads in 100µl 1X bind/wash buffer and pipetted to mix
10. Mixed beads on rotisserie mixer for 5 minutes at room temp
11. Repeated again: Spun down tubes **briefly**
12. Placed tubes on magnet rack for 1 minute
13. Removed supernatant when clear
14. Resuspended beads in 100µl 1X bind/wash buffer and pipetted to mix
15. Mixed beads on rotisserie mixer for 5 minutes at room temp
16. Finally: Spun down tubes **briefly**
17. Placed tubes on magnet rack for 1 minute
18. Removed supernatant when clear
19. Resuspended beads in 100µl 1X bind/wash buffer and pipetted to mix

Capture Reaction

1. To 6 new 1.5mL tubes, added 20µl each of **5X** bind/wash buffer
2. To each appropriate tube, added the 80µl of the sheared DNA samples (above)
3. Transferred all of each diluted DNA sample to separate tubes with the MBD-Biotin bound beads for a total of 200µl in each tube and pipetted to mix
4. Mixed on rotisserie mixer overnight at 4 degrees C in the cold room

September 24th 2019, Removing Non-Captured DNA

1. Again started by writing out protocol to calculate how much 1X bind/wash buffer to dilute (25000μl needed and appropriately diluted)
2. Took tubes out of the cold room rotisserie and spun down **briefly**
3. Placed tubes on magnet rack for 1 minute
4. Removed clear supernatant and **SAVED** in tubes labeled non-captured DNA and sample number
5. Resuspended beads in 200μl of 1X bind/wash buffer and placed on rotisserie mixer for 3 minutes
6. Spun down tubes **briefly**
7. Placed tubes on magnet rack for 1 minute
8. Removed clear supernatant and **SAVED** in tubes labeled wash and the sample number
9. Repeated: added 200μl 1X bind/wash buffer and placed on rotisserie mixer for 3 minutes
10. Spun down tubes **briefly**
11. Placed tubes on magnet rack for 1 minute
12. Removed clear supernatant and **SAVED** in the same tubes labeled wash and the sample number for a total of 400μl in each tube

Single Fraction Elution

1. Resuspended beads in 200μl each High Salt Elution buffer
2. Incubated on rotisserie mixer for 3 minutes
3. Spun down tubes **breifly** and placed on magnet rack for 1 minute
4. Removed supernatant when clear and **SAVED** in a new 1.5mL tube labeled captured DNA and the sample number
5. Repeated: added 200μl High Salt Elution Buffer to resuspended beads in each tube
6. Incubated tubes on rotisserie mixer for 3 minutes
7. Spun down tubes **briefly** and placed on magnet rack for 1 minute
8. Removed clear supernatant and **SAVED** in each sample tube for captured DNA, for a total of 400μl in each tube

Ethanol Precipitation

Each tube for ethanol precipitation gets 1μl of glycogen (co-precipitator), 1/10th the volume of the sample of 3M sodium acetate pH 5.2, and 2 volumes of the sample 100% ethanol:

note, 3M sodium acetate is kept in the acid cabinet and volumes were added to samples in the hood

Sample	**vol glycogen (μl)**	**vol sodium acetate (μl)**	**vol 100% EtOH (μl)**
1041 Captured	1	40	800
1471 Captured	1	40	800
1637 Captured	1	40	800
1101 Captured	1	40	800
1548 Captured	1	40	800
1628 Captured	1	40	800

1. Vortexed to mix and spun down
2. Placed tubes in the -80 freezer for 3.5 hours
3. Set centrifuge to 1 degrees C with about 15 min time left and let it run to get down to temp
4. Put 70% EtOH in -20 to chill down
5. Took sample tubes out of -80 and centrifuged for **15 minutes** at 1 degrees C and 14,000 rcf
6. Took tubes out of centrifuge **one at a time** to keep cold, looked at tubes to see if there was a pellet. The non-captured 1431 sample did not have a visible pellet. In all these steps tubes were taken out of the centrifuge one at a time to keep them cold

7. Removed the supernatant and discarded **very carefully**. All samples had a very very small visible pellet
8. Added 500*µ*l of **cold** 70% EtOH to each tube carefully
9. Centrifuged for **5 minutes** at 1 degrees C and 14,000 rcf
10. **Very carefully** removed supernatant from pellet
11. Centrifuged tubes for **5 minutes** at 1 degrees C at 14,000 rcf
12. Removed any remaining supernatant as best as possible with the smallest pipette tip and making sure to not removed the pellet
13. Air dried pellet for 3 minutes
14. Resuspended pellets in 25*µ*l of ultra pure water

High Sensitivity DNA Qubit

Followed Qubit protocol for HS reagents

Sample	Standard 1 (RFU)	Standard 2 (RFU)	1st reading (ng/ul)	Second reading (ng/ul)	Average ng/ul
1041 Captured	44.62	25969	0.998	0.993	0.995
1471 Captured	44.62	25969	0.846	0.85	0.848
1637 Captured	44.62	25969	2.1	2.1	2.1
1101 Captured	44.62	25969	0.14	0.14	0.14
1548 Captured	44.62	25969	0.172	0.174	0.173
1628 Captured	44.62	25969	0.13	0.132	0.131

Written on September 23, 2019