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AxyPrep magnetic Bead normalization and PCR clean up For dual index

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ALEXANDER J BRADSHAW[1]

[1]Natural History Museum of Utah

Dentinger Lab

ALEXANDER J BRADSHAW
University of Utah

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

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Abstract

In-house protocol used to prepare PCR amplicons with AxyPrep beads for Illumina sequencing.

Troubleshooting

1 Place Elution buffer, Magnetic Beads, and binding buffer on the bench and allow each to reach room temperature before using.

Room temperature

2 Mix magnetic beads until homogenous (no seriously, vortex the shit out of them)

3 Add 10ul of magnetic beads to each well. Pipette Up and down 5 times to mix completely

(10ul collects 200ng of DNA, adjust accordingly if more or less is desired)

4 add 100 ul of binding buffer (BB) to each well. Pipette Up and down 5 times to mix completely

5 Incubate samples at room temperature for 10 minutes 10m

Room temperature 00:10:00

6 place on magnetic separation device for 4 minutes or until the solution clears 4m

00:04:00

7 with the sample plate still, on the magnet, remove and discard the supernatant by pipetting.

8 Remove the sample plate from the magnetic separation device. 2m

Add 150 µl freshly prepared 70% ethanol to each well

pipet mix 5 times and incubate for 2 minutes at room temperature.

00:02:00

9 Place the sample plate on the 96 magnetic separation device for 4 minutes or until the solution clears. 4m

00:04:00

10 With the sample plate still on the magnet, remove and discard the supernatant by pipetting.

11 Dry the beads by incubating the plate for 2 minutes at room temperature with the plate still on the magnetic separation device. 2m

.Remove the sample plate from the magnetic separation device.

00:02:00 Room temperature

12 Add 50µl of EB-N Elution Buffer to each well and pipet up and down 5 times to mix. 2m

Incubate the sample plate for 2 minutes at room temperature.

00:02:00 Room temperature

13 Place the sample plate back on the magnetic separation device and wait 4 minutes or until the magnetic beads clear from the solution. 4m

00:04:00

14 Transfer the eluate (cleared supernatant) to a new plate/tube for storage or for subsequent applications.

For us, we will pool all 50ul of each sample into a single tube. If you have a lot of samples this could be a 50mL conical tube