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# AxyPrep magnetic Bead normalization and PCR clean up For dual index

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Protocol status: Working

We use this protocol and it's working

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Keywords: pcr amplicons with axyprep bead, axyprep magnetic bead normalization, axyprep bead, pcr amplicon, axyprep,

magnetic bead normalization, pcr, magnetic bead, bead

#### **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  |
|   | <b>©</b> 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.  |     |
|   | <b>©</b> 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

- With the sample plate still on the magnet, remove and discard the supernatant by pipetting.
- Dry the beads by incubating the plate for 2 minutes at room temperature with the plate still on the magnetic separation device.

2m

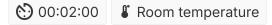
.Remove the sample plate from the magnetic separation device.



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

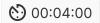
2m

Incubate the sample plate for 2 minutes at room temperature.



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

4m



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