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Size selection (Purification)



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Protocol status: Working

We use this protocol and it's working



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Abstract

Size selection (Purification)

Troubleshooting

- 1 Calculate the amount of magnetic beads you need and **DISTRIBUTE in a new 1.5 mL eppendorf tube** (Mix the bottle **VIGOROUSLY** to make sure it's homogeneous).

For example, if you have 5 samples to clean up, distribute 49.5 µl in a new 1.5 mL eppendorf tube.



	A	B	C
	Sample	Magnetic beads need	Magnetic beads need *1.1
	1	9 µl	9.9 µl
	2	18 µl	19.8 µl
	3	27 µl	29.7 µl
	4	36 µl	39.6 µl
	5	45 µl	49.5 µl
	6	54 µl	59.4 µl
	7	63 µl	69.3 µl
	8	72 µl	79.2 µl

Note

The ratio is **20µl of sample** use **9µl of magnetic beads** to clean up (0.45X to keep long DNA fragments).

Note

Magnetic bead: BeaverBeads™ DNA Select Isolation

- 2 In each  20 µL sample, add  9 µL magnetic beads in.



Note

Pipetting thoroughly every time before adding to make sure all magnetic beads mixed well.


- 3 Mix sample and beads by **gently flicking** the tube for 5mins  00:05:00

5m

Note

Be gentle, mix too aggressive will make DNA fragmented

- 4 Transfer the tube to the 8-strip magnetic rack.
After all the magnetic beads are arrested, remove the supernatant.

- 5 Add  200 μL 80% ethanol, flip the magnetic rack around to clean the pellet.
Wait until all the magnetic beads are arrested, remove the supernatant.

Note


FRESHLY make 80% ethanol

- 6 Repeat the **step 5**.

- 7 Flash spin the tube, and put on the magnetic rack.
Remove **ANY trace of liquid residuals** using 10 μL pipette.

Note


Caution: DO NOT let the beads crack


- 8 Add  10 μL elution buffer.
Mix gently by flicking the tube and make sure all magnetic beads are dissolved in buffer, then flash spin down the sample.



- 9 Incubate the tube in PCR thermal cycler using "**37_incubation**" program for 10min

10m

 00:10:00 .

- 10 Transfer the tube to the magnetic rack.
After all the magnetic beads are arrested, collect the  10 μL supernatant to a 200 μL PCR tube or a 8-strip PCR tube.
- 11 The clean-up sample is now ready for 2' PCR.