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# PCR Cleanup

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1 Works for me dx.doi.org/10.17504/protocols.io.bpvqmn5w

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ABSTRACT

This protocol explain how to clean PCR products.

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ABSTRACT

This protocol explain how to clean PCR products.

IMPORTANT: We now are performing clean up with our sequencing servicers GeneWiz and Eurofins. This removes the need for clean up, as it is cheaper for them to do it by themselves.

Use the following only with a single band PCR. With more bands, the only option is gel extraction & purification.

**Exo-Sap-IT**
Keep Exosap on ice and don't keep out of freezer longer than necessary. This product doesn't freeze so it is always ready to use straight out of the freezer. Keep tubes in ice block until ready to put into thermocycler.

1)Per sample for bidirectional sequencing, use an ice block and in each PCR tube mix:
·0.5uL PCR grade water
·2uL PCR product
2) After all tubes are prepped, get tube of Exosap out of freezer and promptly add 1 uL to each tube. Immediately return the tube of Exosap to the freezer.
3) After spinning down, run ExoSap program on thermocycler (37C for 15 min, and 80C for 15 min). Depending on the thermocycler, you may need to change the volume of product in the program settings (or when prompted) to 3 uL.
4) When the cycle is finished, spin down again and put half the contents of each PCR tube (1.75 uL) into a 1.5 mL tube, and the remaining half into a second 1.5 mL tube. These will be your forward and reverse submissions for sequencing.

5) Label tubes with the appropriate database contig ID number (format ####-####) (not just the sequencing ID number) and update information in database.

**Gel extraction and clean-up**

Our choice:**E.Z.N.A. Gel Extraction Kit, Omega Bio-tek**

·1) cut out gel slice, put in a tube, and weigh it (actual weight minus one empty tube (0.90 g))
·2) add equal volume of**Binding Buffer**(*x*g gel =*x*ml Buffer)
·3) incubate at 55-60 C for 7 min (or until gel dissolves)
·4) add 700uL to spin column, centrifuge at 10,000g for 1 min
·(*5) keep adding 700uL of solution to the spin column and spinning until all added*)
·6) add 300uL of fresh**Binding Buffer**to the column, spin at 10,000 for 1 min
·7) add 700uL of '*Wash Buffer*, centrifuge at 10,000g for 1 min, discard flow-through
·8) same thing again: add 700uL of**Wash Buffer**, centrifuge, discard liquid
·9) discard liquid, centrifuge the empty column for 2 min at max speed
·10) put column in clean tube, add 30uL of**Elution Buffer**
·11) let soak for 1 minute, then centrifuge at max speed for 1 min
·(*12) (if maximum yield is more important than concentration, add another 30uL of**Elution Buffer**, centrifuge at max speed.*)

Notes: When DNA yield is important, use fresh running TAE buffer. Old buffers have high pH and decrease yield.
If Buffer turns orange or red during incubation, pH is too high!
If you need to load and separate the whole PCR reaction, make a thick gel.

**AMPure XP PCR Cleanup with Ring Magnet Plate**

Manufacturer says to store AMPure refrigerated (do not freeze) and use within12 months.
Our Eppendorf skirted plates are NOT compatible with the magnetic ring plate. All other brands of 96 well plates we have on hand at the time of writing (VWR, Fisherbrand, Applied Biosystems) are compatible.
Magnet is very strong:
·Must be kept well away from computers, thermal cyclers, centrifuge, cell phones and other electrical equipment, credit cards, etc.
·Must be kept away from people with pacemakers and defibrillator implants.

Do not autoclave magnet plate – looses magnetic strength if heated above 80 C.
1.Gently shake the AMPure bottle to resuspend any magnetic beads that may have settled (color should be uniform)
2.In a 96 well PCR plate (note compatible brands above) add 1.8 uL AMPure for every 1 uL of PCR product (e.g. add 45 uL Ampure to a 25 uL PCR reaction =70 uL total volume) Keep PCR plate off magnetic plate until step 4.
3.Mix AMPure and PCR product by pipetting 10x, then incubate at room temperature for 5 min.
4.Following incubation, place PCR plate into the magnetic ring plate, let sit for 2 minutes or until solution is clear.
5.Keeping the plate magnetized, remove and discard the clear solution via pipetting (do not disturb the ring of beads around the sides).
6.Add 200 uL 70% ethanol, let sit for 30 seconds, then remove and discard all ethanol without disturbing the beads. Repeat this step for a total of two washes.
7.Let stand for about 5 minutes for any remaining traces of ethanol to evaporate. Avoid allowing the ring of beads to dry for significantly longer, which will create a crackled appearance and inhibit elution.
8.Remove from magnetic plate and add a minimum of 40 uL of elution buffer (=whatever you are resuspending your product in – molecular grade water, TE, etc). Mix by pipetting 10x. All the beads do not need to go back into solution for full elution.
9.Place the plate back on the magnet and let sit for 1 minute to draw the beads back out of suspension.
10.Without disturbing the ring of beads, transfer your samples to a new plate/tubes for storage.



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