# Polymerase Chain Reaction Purification

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- **What is PCR purification?**

PCR Purification Protocol is designed to purify double stranded DNA fragments from PCR and other enzymatic reactions. Fragments from 100bp to 10kb are purified from primers, nucleotides, polymerases and salts using QIAquick spin columns in a microcentrifuge. Even if distinct bands of the expected size are observed, primer dimers should be removed by PCR purification method. In the absence of the purification, the proofreading activity of DNA polymerase will degrade the PCR fragments or remove the 3' terminal deoxyadenosine from the vector during ligation reaction.

- **How does it work?**

Considering the significance of purification step, PCR Purification Protocol is optimized using QIAquick PCR Purification Kit and for that, 5 volumes of buffer PB is added to 1 volume of the PCR sample and mixed. For example, 500µl of buffer PB is added to 100µl PCR sample excluding oil. It is not necessary to remove mineral oil or kerosene. A QIAquick spin column is placed in a provided 2ml collection tube. The sample is applied to the column and centrifuged for 60sec. All centrifuge steps are at 18928 rpm in a conventional tabletop microcentrifuge. The flow through is discarded and QIAquick column is placed back into the same tube. The collection tubes are reused to reduce plastic waste. To wash, 750µl buffer PE is added to the QIAquick column and centrifuged for 60sec. Absolute ethanol is added to buffer PE according to bottle label. This washing step is repeated with guanidinium chloride 37% to increase efficiency. The flow through is discarded before an additional 1min centrifugation to completely remove residual ethanol from buffer PE. Residual ethanol from buffer PE will not be completely removed unless the flow through is discarded before this additional centrifugation. QIAquick column is placed in a clean 1.5ml microcentrifuge tube. To elute DNA, 30µl buffer EB (10mM Tris-Cl, pH 8.5) or $H_2O$ is added to the center of the QIAquick membrane, stood for 10min and centrifuged for 1min. The elution buffer should dispense directly onto the QIAquick membrane for complete elution of bound DNA. To increase elution efficiency, this step is repeated in a new microcentrifuge tube for a second elution. The average eluate volume is 48µl from 50µl elution buffer volume and 28µl from 30µl elution buffer.

- **What is it used for?**

An aliquot of the purified product is analyzed on 1% agarose gel at 120v for 30min. If the intensity of the band is enough, the product is prepared at 300ng/µl for direct sequencing; otherwise the product is used in the ligation reaction for cloning purpose.

- **When does PCR purification fail or succeed?**

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, it should be ensured that the pH value is within this range. DNA should also be stored at -20°C as it may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10mM Tris-Cl, 1mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

- **Contact us**

For more information or troubleshooting on your PCR purification, please do not hesitate to contact us at *ijpp@iau-saveh.ac.ir*. You can simply mention your problem by attaching your gel picture. We look forward to hearing from you soon.

- **Read more on**:

http://www.qiagen.com/Products/DnaCleanup/GelPcrSiCleanupSystems/QIAquickPCRPurificationKit.aspx?r=926

http://www.lab-manual.com/lm_193.htm

http://www.promega.com/resources/protocols/technical-bulletins/101/wizard-sv-gel-and-pcr-cleanup-system-protocol/