TARDIGARD

protocol

iGEM Team SIAT—SCIE
Email：siatxscie@gmail.com

PCR purification

dNTPs, primers, enzymes, and Mg ion, these components are required for the PCR to take place but they must be removed after PCR reaction is completed. Since they are now considered as "contaminants" due to its ability to interfere with subsequent manipulations such as restriction digests.

1.1 Principle

QIAquick Kits contain a silica membrane assembly for binding of DNA in high-salt buffer and elution with low-salt buffer or water. The purification procedure removes primers, nucleotides, enzymes, mineral oil, salts, agarose, ethidium bromide, and other impurities from DNA samples. Silica-membrane technology eliminates the problems and inconvenience associated with loose resins and slurries. Specialized binding buffers are optimized for specific applications and promote selective adsorption of DNA molecules within particular size ranges. [1]

1.2 Materials

autoclavated H2O
minipin centrifuge
PCR tube with PCR product
QIAquick PCR Purification Kit

PB	High salt, low pH, binding buffer used in Step 1. Contain chaotropic salts (probably gua chemical reagent
PE	***Must add ethanol to PE before use. Wash buffer used in Step 5, remove any remaining contaminants. We usually leave the second wash so that the ethanol can evaporate.
EB	

Elution Buffer used in Step 8 as an alternative to water.

We are using QIAquick PCR Purification Kit, Cat No./ID: 28104

https://www.qiagen.com/us/shop/sample-technologies/dna/dna-clean-up/qiaquick pcr-purification-kit/#productdetails

However, we use a version based on this and modified a little part of it

1. Mix 1 volume of the PCR sample with 5 volumes of buffer PB.
2. Load the sample on a QIAquick spin column which is inserted in a collection tube (with a maximum of 800 µl per run).
3. Centrifuge the sample for 1 minute at 13,400 rpm. Weight-balance the sample well.
4. Discard the flow-through.
5. Wash the sample with 750 µl of PE buffer and centrifuge for 1 minute at 13,400 rpm.
6. Discard the flow-through.

7. Dry spin the sample for 1 minute at 13,400 rpm.
8. Transfer the spin column to a new autoclaved Eppendorf tube.
9. Leave it open for 15 minutes to let the ethanol evaporate (this step can reduce salt contaminant, which may affect the following reaction)
10. Pre heat ddH2O in 60 degree Celsius
11. Load 30 µl of autoclaved H2O on the column (pipette drops in the middle of the membrane, do not touch the membrane). Incubate for 10 minute and centrifuge for 1 minute at 13,400 rpm.
12. The resulting elution product will contain purified PCR product.