DNA Purification

Purify DNA (Using QIAquick PCR purification)

1. Add 5 volumes of Buffer PB1to 1 volume DNA and mix
2. Apply DNA sample to QIA quick column
3. Spin DNA into column at 13K rpm for 1 minute
4. Discard flowthrough
5. Wash DNA with 0.75 ml Buffer PE, spin through column at 13K rpm for 1 minute
6. Discard flowthrough
7. Spin residual liquid from column at 13K rpm for 1 minutes
8. Elute DNA; apply 40ul Buffer EB to column, incubate at room temperature for 2 minutes before spinning DNA out of column at 13K rpm for 1 minute

Separate DNA by size on an agarose gel

1. Make an agarose gel at 0.8<= gel density <= 1.5
2. Add loading dye to samples (5 ul dye/50 ul sample)
3. Load samples and a ladder (5 ul) into gel wells
4. Run samples through gel (negative to positive) at 100 V for 40 minutes at room temperature
5. Visualize DNA under UV light

Purify DNA from Gel (Using QIAquick Gel Extraction Kit

1. Excise gel piece containing DNA with a new razor
2. Add three volumes of Buffer QG to 1 volume gel
3. Incubate at 50C for 15 minutes, or until gel is solublized, mixing frequently
4. Make sure dissolved solution is yellow
5. If the DNA fragment is <500bp and >4kb, add 1 gel volume isopropanol to increase the yield
6. Apply sample to QIAquick spin column in 700 ul aliquots
7. Spin sample into column at 13K rpm for 1 minute
8. Discard flowthrough
9. Spin 0.5 ml Buffer QG through column at 13K rpm for 1 minute to solublize any remaining gel chunks
10. Discard flowthrough
11. Wash column with 0.75 ml Buffer PE, spinning through column at 13K rpm for 1 minute
12. Discard flowthrough
13. Spin out residual liquid from column at 13K rpm for 1 minute
14. Elute DNA by applying 40-50ul Buffer EB to column, incubate at room temperature for 2 minutes, spin DNA out of the column at 13K rpm for 1 minute into a clean microfuge tube