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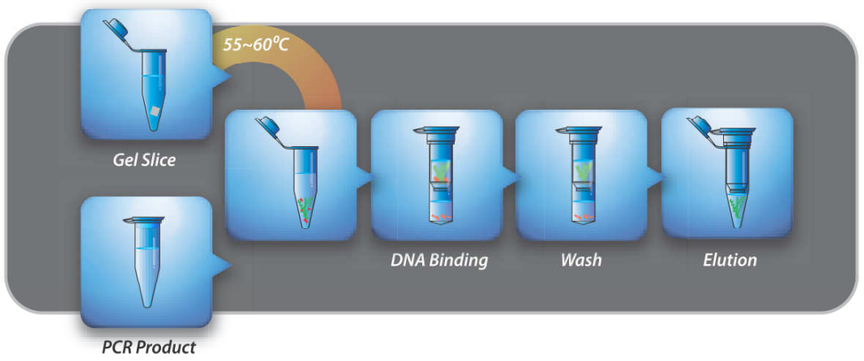
Real Genomics™

Store at room temperature 15°C~25°C



HiYield™ Gel/PCR DNA Mini Kit Protocol Book

Optimized for DNA Fragments between 50 bp and 10 kb

Cat. No. **YDF100 / YDF300**

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HiYield™ Gel/PCR DNA Mini Kit

HiYield™ Gel/PCR DNA Mini Kit

Store at room temperature 15°C~25°C

Cat. No. **YDF100**
100 mini preps / kit
DF Buffer: 80 ml
W1 Buffer: 45 ml
Wash Buffer (concentrated): 25 ml *
Elution Buffer: 6 ml
DF Column: 100 pcs
2 ml Collection Tube: 100 pcs

Cat. No. **YDF300**
300 mini preps / kit
DF Buffer: 240 ml
W1 Buffer: 130 ml
Wash Buffer (concentrated): 75 ml **
Elution Buffer: 30 ml
DF Column: 300 pcs
2 ml Collection Tube: 300 pcs

Sample: Up to 300 mg of Agarose Gel, Up to 100 µl of PCR Product
Yield: Gel Recovery up to 90% / PCR Recovery up to 95%
Format: Spin Columns
Operation: Centrifuge or Vacuum
Operation Time: 20 Minutes for Gel Extraction / 15 Minutes for PCR Cleanup

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* Add 100 ml of ethanol (96~100%) to 25 ml of Wash Buffer prior to the initial use.
** Add 100 ml of ethanol (96~100%) to 25 ml of Wash Buffer prior to the initial use.
Add 200 ml of ethanol (96~100%) to 50 ml of Wash Buffer prior to the initial use.

Description

HiYield™ Gel/PCR DNA Mini Kit is designed to recover or concentrate DNA fragments (50 bp-10 kb) from agarose gels, PCR or other enzymatic reactions. The unique dual purpose application and high yield DF column make this kit exceptional value. Salts, enzymes and unincorporated nucleotides could be effectively removed from reaction mixtures without phenol extraction or alcohol precipitation.

Features

Up to 95% recovery of ready-to-use DNA.
Unique dual purpose application(Gel/PCR DNA Extraction).
Without phenol extraction or alcohol precipitation.

Applications

Purified DNA is ready for direct use in PCR, DNA Sequencing, DNA Library Screening and Analysis, Restriction Digestion, DNA Labeling, Preparation of PCR Probes for Microarrays, Ligation and Transformation.

Quality Control

The quality of HiYield™ Gel/PCR DNA Mini Kits are tested on a lot-to-lot basis. The efficiency of DNA recovery are tested by isolation of DNA fragments of various sizes from either aqueous solution or agarose gel. The purified DNA is checked by agarose gel analysis.

Reference: Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Note: DF Buffer contains guanidine thiocyanante which is harmful and an irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

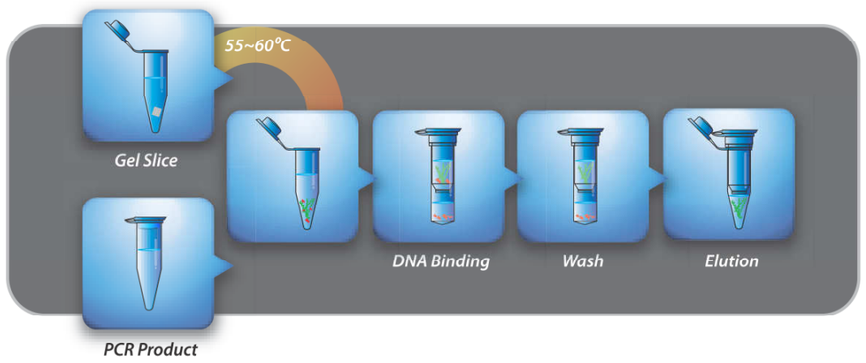
Gel Extraction Protocol

Additional requirements:

* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and microcentrifuge tubes.

Things to do before starting:

Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for details).



Gel Dissociation

1. Excise the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. (It's better to make the gel with TAE buffer than TBE buffer, because TBE buffer might affect the downstream experiment.)
2. Transfer up to 300 mg of the gel slice into a 1.5 ml microcentrifuge tube (not provided).
3. Add 500 µl of DF Buffer to the sample and mix by vortexing.
4. Incubate at 55~60°C for 10-15 minutes until the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 minutes.
5. Cool the dissolved sample mixture to room temperature.

DNA Binding

6. Place a DF Column in a 2 ml Collection Tube.
7. Apply 800 µl of the sample mixture from step 5 into the DF Column.
8. Centrifuge at full speed (approx. 13,000 rpm) for 30 seconds.
9. Discard the flow-through and place the DF Column back in the 2 ml Collection Tube.
   (If the sample mixture is more than 800 µl, repeat this DNA Binding Step.)

Wash

10. Add 400 µl of W1 Buffer into the DF Column.
11. Centrifuge at full speed (approx. 13,000 rpm) for 30 seconds.
12. Discard the flow-through and place the DF Column back in the 2 ml Collection Tube.
13. Add 600 µl of Wash Buffer (ethanol added) into the DF Column and let stand for 1 minute.
14. Centrifuge at full speed (approx. 13,000 rpm) for 30 seconds.

15. Discard the flow-through and place the DF Column back in the 2 ml Collection Tube.
    Note: For TAE gels, proceed to step 16. For TBE gel, repeat wash step 13-15. (Boric Acid is difficult to remove and can affect downstream applications, therefore double wash is recommended.)
16. Centrifuge again for 3 minutes at full speed (approx. 13,000 rpm) to dry the column matrix.

DNA Elution

17. Transfer the dried DF Column in a new 1.5 ml microcentrifuge tube (not provided).
18. Add 20 µl - 50 µl of Elution Buffer or TE into the center of the column matrix.
19. Let stand for 2 minutes until Elution Buffer or TE is absorbed by the matrix.
20. Centrifuge at full speed for 2 minutes to elute the purified DNA.

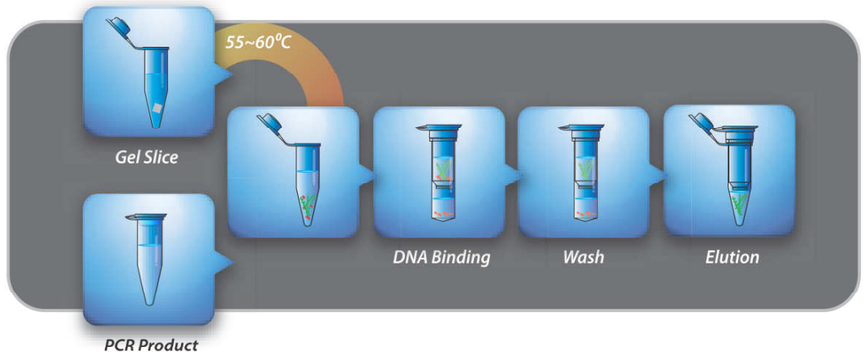
Gel Extraction (Sequencing) Protocol

Additional requirements:

* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and microcentrifuge tubes.

Things to do before starting:

Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for details).



Gel Dissociaton

1. Excise the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. (It's better to make the gel with TAE buffer than TBE buffer, because TBE buffer might affect the downstream experiment.)
2. Transfer up to 300 mg of the gel slice into a 1.5 ml microcentrifuge tube (not provided).
3. Add 500 µl of DF Buffer to the sample and mix by vortexing.
4. Incubate at 55~60°C for 10-15 minutes until the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 minutes.
5. Cool the dissolved sample mixture to room temperature.

DNA Binding

6. Place a DF Column in a 2 ml Collection Tube.
7. Apply 800 µl of the sample mixture from step 5 into the DF Column.
8. Centrifuge at full speed (approx. 13,000 rpm) for 30 seconds.
9. Discard the flow-through and place the DF Column back in the 2 ml Collection Tube.
   (If the sample mixture is more than 800 µl, repeat this DNA Binding Step.)

Wash

10. Add 600 µl of Wash Buffer (ethanol added) into the DF Column and let stand for 1 minute.
11. Centrifuge at full speed (approx. 13,000 rpm) for 30 seconds.
12. Discard the flow-through and place the DF Column back in the 2 ml Collection Tube.
13. Add 600 µl of Wash Buffer (ethanol added) into the DF Column and let stand for 1 minute.
14. Centrifuge at full speed (approx. 13,000 rpm) for 30 seconds.

15. Discard the flow-through and place the DF Column back in the 2 ml Collection Tube.
    Note: For TAE gels, proceed to step 16. For TBE gel, repeat wash step 13-15. (Boric Acid is difficult to remove and can affect downstream applications, therefore double wash is recommended.)
16. Centrifuge again for 3 minutes at full speed (approx. 13,000 rpm) to dry the column matrix.

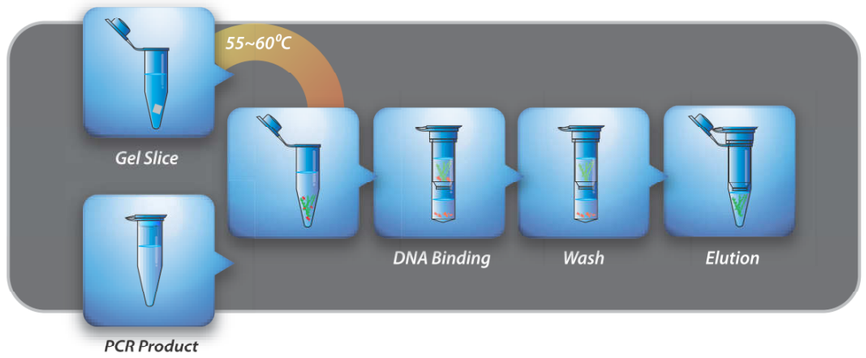
DNA Elution

17. Transfer the dried DF Column in a new 1.5 ml microcentrifuge tube (not provided).
18. Add 20 µl - 50 µl of Elution Buffer or TE into the center of the column matrix.
19. Let stand for 2 minutes until Elution Buffer or TE is absorbed by the matrix.
20. Centrifuge at full speed for 2 minutes to elute the purified DNA.

PCR Clean Up Protocol

Additional requirements:
* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and microcentrifuge tubes.

Things to do before starting:
Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for details).



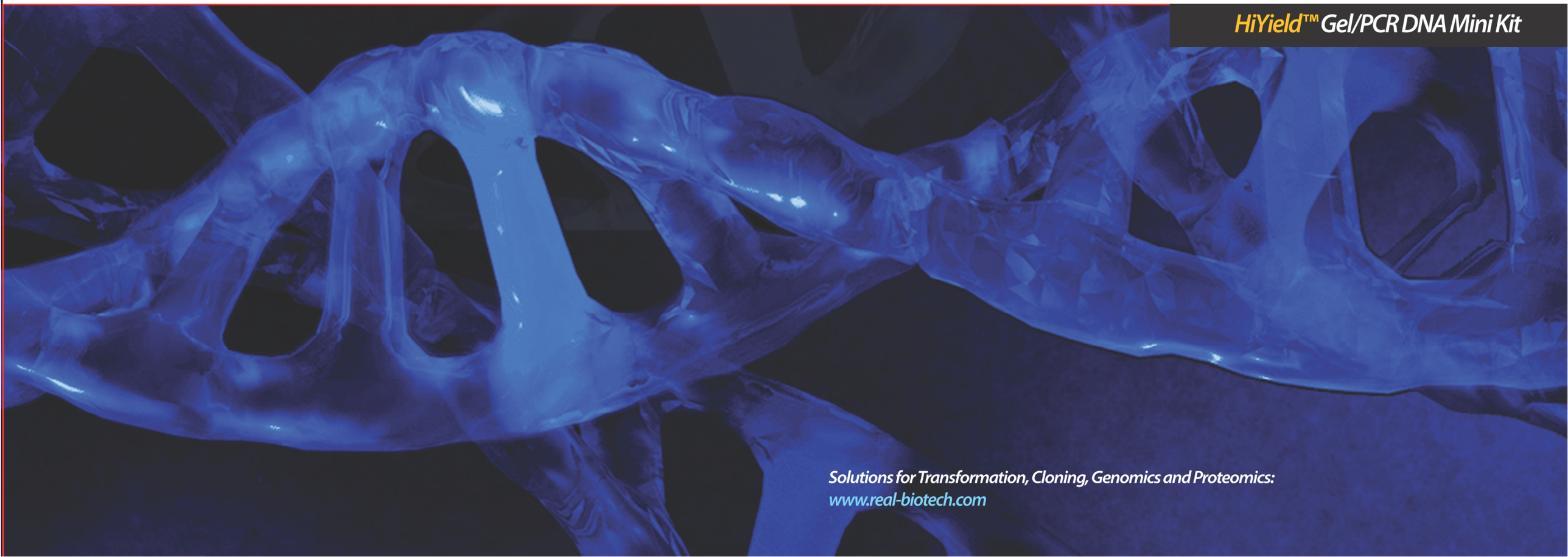
Sample Preparation
1. Transfer up to 100 µl of a reaction product to a 1.5 ml microcentrifuge tube (not provided).
2. Add 5 volumes of DF Buffer to 1 volume of the sample and mix by vortexing.

DNA Binding
3. Place a DF Column in a 2 ml Collection Tube.
4. Apply the sample mixture from step 2 into the DF Column.
5. Centrifuge at full speed (approx. 13,000 rpm) for 30 seconds.
6. Discard the flow-through and place the DF Column back in the 2 ml Collection Tube.

Wash
7. Add 600 µl of Wash Buffer (ethanol added) into the center of the DF Column and let stand for 1 minute.
8. Centrifuge at full speed (approx. 13,000 rpm) for 30 seconds.
9. Discard the flow-through and place the DF Column back in the 2 ml Collection Tube.
10. Centrifuge again at full speed (approx. 13,000rpm) for 3 minutes to dry the column matrix.

DNA Elution
11. Transfer the dried DF Column in a new 1.5 ml microcentrifuge tube (not provided).
12. Add 20 µl-50 µl of Elution Buffer or TE into the center of the column matrix.
13. Let stand for 2 minutes until Elution Buffer or TE is absorbed by the matrix.
14. Centrifuge for 2 minutes at full speed to elute the purified DNA.

HiYield™ Gel/PCR DNA Mini Kit



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