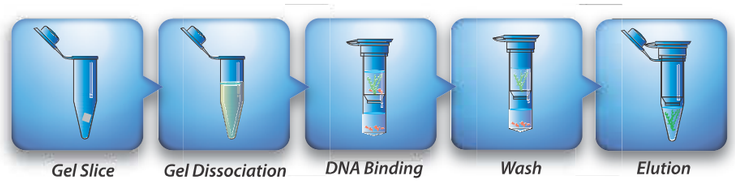
Real Genomics™

Store at room temperature 15°C~25°C



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HiYield Plus™ Gel Extraction Kit Protocol Book

Rapidly recover DNA fragments (70 bp - 20 kb) from agarose gel

Cat. No. **QGE100 / QGE300**

Real Genomics™

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HiYield Plus™ Gel Extraction Kit

HiYield Plus™ Gel Extraction Kit

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Cat. No. **QGE100**
100 mini preps / kit
QGE Buffer: 80 ml
3M Sodium Acetate: 200 µl*
W1 Buffer : 45 ml
Wash Buffer (concentrated): 25 ml**
Elution Buffer: 6 ml
QGE Column: 100 pcs
2 ml Collection Tube: 100 pcs

Cat. No. **QGE300**
300 mini preps / kit
QGE Buffer: 240 ml
3M Sodium Acetate: 200 µl*
W1 Buffer : 130 ml
Wash Buffer (concentrated): 75 ml **
Elution Buffer: 30 ml
QGE Column: 300 pcs
2 ml Collection Tube: 300 pcs

Sample: Up to 300 mg of agarose gel
DNA fragment size: 70 bp-20 kb
Recovery: Up to 90%
Format: Spin column
Operation Time: 20 minutes
Elution Volume: 20-50 µl

* QGE Buffer contains an integrated pH Indicator, allowing easy determination of the optimal pH for DNA binding and easy observation of undissolved agarose gel. Efficient DNA adsorption requires a pH≤7.5,and the pH Indicator in QGE Buffer will appear yellow in this range. If the pH is >7.5, the binding mixture turns violet. Violet color indicates DNA adsorption will be inefficient. The pH of the binding mixture can easily be corrected by addition of a small volume of 3M Sodium Acetate (pH5.0).
** Add absolute ethanol (see the bottle label for volume) to Wash Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

Description
HiYield Plus™ Gel Extraction Kit is designed to rapid recover or concentrate DNA fragments (70 bp-20 kb) from agarose gels within 20 minutes. QGE Buffer contains an integrated pH Indicator. Efficient DNA adsorption requires a pH≤7.5,and the pH Indicator in QGE Buffer appears yellow in this range. If the pH is >7.5, the mixture turns violet. Violet color indicates DNA adsorption will be inefficient. The pH of the mixture can easily be corrected by addition of a small volume of 3M Sodium Acetate (pH5.0), which is also included in this kit. The color of the mixture allows easy visualization of any unsolubilized agarose, ensuring complete solubilization and maximum yields. Typical recovery is up to 90%. The pH indicator, salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture without phenol extraction or alcohol precipitation and the purified DNA is ready for use in many downstream applications.

Features
Rapid purification of up 95% recovery of ready-to-use DNA within 20 minutes.
pH Indicator provides visual identification of optimal pH and ensures complete solubilization and maximum yields.
Suitable for wide range of DNA fragments (70 bp-20 kb).

Applications
Purified DNA is ready for direct use in restriction enzyme digestion, ligation, transformation, PCR, DNA sequencing, preparation of PCR probes for microarrays, DNA library screening and analysis.

Quality Control
The quality of HiYield Plus™ Gel Extraction 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 agarose gels. The purified DNA is checked by agarose gel analysis.

Storage
HiYield Plus™ Gel Extraction Kits should be stored dry at room temperature (15–25°C) for up to 12 months without showing any reduction in performance and quality.

Important Notes
Please read the entire notes before starting any of the protocol procedures.

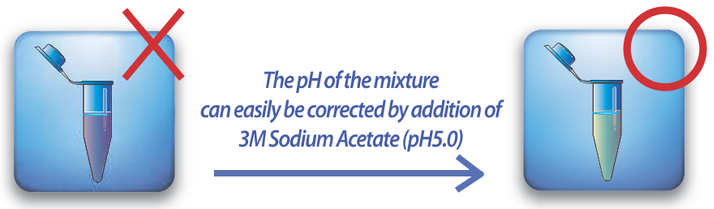
Gel Sample Preparation
1. Record the weight of an empty 1.5 ml microcentrifuge tube for the gel dissociation step. Once the gel has been transferred to the tube, record the weight again. Subtract the empty tube weight from the total weight to determine the actual gel weight.
2. If using more than 300 mg of gel slice, separate it into multiple 1.5 ml microcentrifuge tubes.
3. Perform gel purification when primer dimers are highly visible or add an additional 80% ethanol wash to avoid primer dimer contamination.

Buffer Notes
1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Most buffers contain irritants. Always wear a lab coat, disposable gloves, and protective goggles when handling these buffers.
3. QGE Buffer contains an integrated pH Indicator, allowing easy determination of the optimal pH for DNA binding and easy observation of undissolved agarose gel. Efficient DNA adsorption requires a pH≤7.5,and the pH Indicator in QGE Buffer will appear yellow in this range. If the pH is >7.5, the binding mixture turns violet. Violet color indicates DNA adsorption will be inefficient. The pH of the binding mixture can easily be corrected by addition of a small volume of 3M Sodium Acetate (pH5.0).

Centrifugation Notes
All centrifugation steps are carried out at 14,000 - 1,6000 x g in a conventional, table-top microcentrifuge at room temperature (15–25°C).

Elution Notes
1. Ensure that the elution buffer, TE or water is dispensed directly onto the center of the QGE Column in a 2 ml Collection Tube.
2. To increase DNA yield, use a higher elution buffer volume. To increase DNA concentration, use a lower elution buffer volume.
3. If plasmid DNA are larger than 5 kb, use pre-heated Elution Buffer (60~70°C).
4. Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. If plasmid DNA are larger than 5 kb, use pre-heated TE (60~70°C).
5. If using water for elution, ensure its pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the QGE Column matrix and is completely absorbed. If plasmid DNA are larger than 5 kb, use pre-heated water (60~70°C). DNA eluted in water should be stored at -20°C to avoid degradation.

How pH Indicator and 3M Sodium Acetate Work
QGE Buffer contains an integrated pH Indicator. Efficient DNA adsorption requires a pH≤7.5,and the pH Indicator in QGE Buffer appears yellow in this range. If the pH is >7.5, the mixture will turn violet. Violet color indicates DNA adsorption will be inefficient. The pH of the mixture can easily be corrected by addition of a small volume of 3M Sodium Acetate (pH5.0), which is also included in this kit.



Gel Extraction Protocol

Please read the entire important notes before starting any of the protocol procedures.

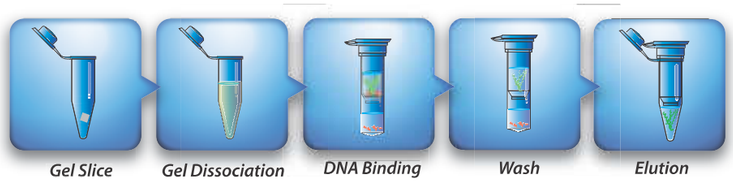
This protocol is designed to rapid recover or concentrate DNA fragments (70 bp-20 kb) from agarose gels using HiYield Plus™ Gel Extraction Kit. Typical recovery is up to 90%. If the DNA fragment is larger than 5 kb, preheat the Elution Buffer, TE, or water to 70°C prior to the Elution Step.

Things to do before starting

1) Add absolute ethanol (see the bottle label for volume) to Wash Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2) Record the weight of an empty 1.5 ml microcentrifuge tube for the gel dissociation step. Once the gel has been transferred to the tube, record the weight again. Subtract the empty tube weight from the total weight to determine the actual gel weight.
3) Perform gel purification when primer dimers are highly visible or add an additional 80% ethanol wash to avoid primer dimer contamination.

Additional Requirements

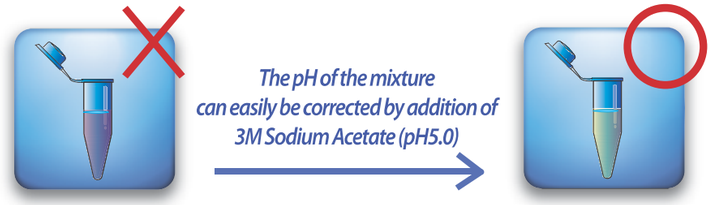
1) Absolute ethanol.
2) Sterile, DNase-free pipette tips and microcentrifuge tubes.



Gel Dissociation

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose. Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube.
2. Add 500 µl of QGE Buffer to the sample then mix by vortex. Incubate at 55-60°C for 10-15 minutes or until the gel slice is completely dissolved. During incubation, invert the tube every 2-3 minutes.
3. If the color of the mixture has turned from yellow to purple, add 10 µl of 3M Sodium Acetate (pH5.0) and mix thoroughly. This will adjust pH and the color will return to yellow. Make sure the color of the mixture is yellow then proceed with the next step.
4. Cool the dissolved sample mixture to room temperature.

Do not scale QGE Buffer if using less than 300 mg of gel slice. If using more than 300 mg of gel slice, separate it into multiple 1.5 ml microcentrifuge tubes.



DNA Binding

5. Place a QGE Column in a 2 ml Collection Tube. Transfer 800 µl of the sample mixture to the QGE Column.
6. Centrifuge at 14,000-16,000 x g for 30 seconds. Discard the flow-through then place the QGE Column back in the 2 ml Collection Tube.

If the sample mixture is more than 800 µl, repeat the DNA binding step.

Wash

Wash steps for DNA be used for direct sequencing, in vitro transcription, or microinjection

7. Add 600 µl of Wash Buffer (absolute ethanol added) into the QGE Column and let stand for 1 minute. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the QGE Column back in the 2 ml Collection Tube.
8. Add 600 µl of Wash Buffer (absolute ethanol added) into the QGE Column and let stand for 1 minute. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the QGE Column back in the 2 ml Collection Tube.
9. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

Standard wash steps

7. Add 400 µl of W1 Buffer into the QGE Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the QGE Column back in the 2 ml Collection Tube.
8. Add 600 µl of Wash Buffer (absolute ethanol added) into the QGE Column and let stand for 1 minute. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the QGE Column back in the 2 ml Collection Tube.
9. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

DNA Elution

Transfer the dried QGE Column to a new 1.5 ml microcentrifuge tube. Add 20-50 µl of Elution Buffer[1], TE[2] or water[3] into the CENTER of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 14,000-16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1] If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 100 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the column matrix and is completely absorbed. If DNA fragment is larger than 5 kb, use pre-heated Elution Buffer (60~70°C).

[2] Using TE is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Check elution notes listed in page 4 for more details.

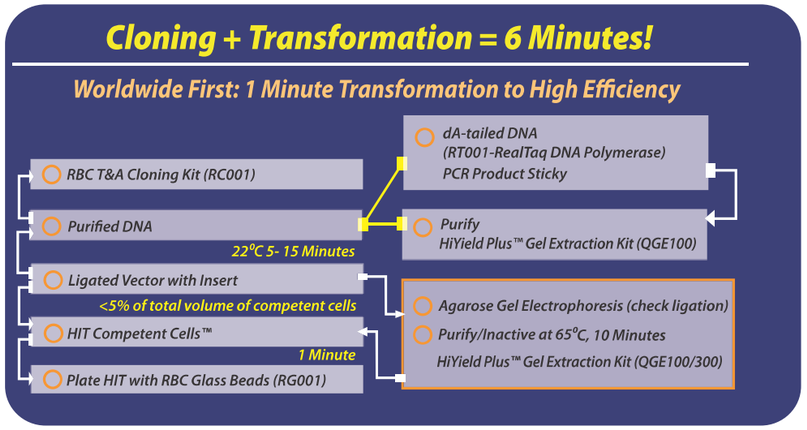
[3] If using water for elution, check elution notes listed in page 4 for more details.

Troubleshooting

Problem	Possible Reasons / Solution
Low Yield	**Gel slice did not dissolve completely .** 1. If using more than 300 mg of agarose gel, separate it into multiple 1.5 ml microcentrifuge tubes. 2. Ensure the agarose gel was melted/dissolved completely between 55-60°C for 10-15 minutes, or until no gel is visible. If undissolved agarose remains in the sample, the column could clog and some DNA will be unrecoverable. DNA can be denatured if the incubation temperature exceeds 60°C.
	Incomplete Wash Buffer preparation. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
	Incorrect DNA elution step. Ensure that Elution Buffer, TE or water is added into the CENTER of the Column matrix and is completely absorbed. If DNA fragments are larger than 5 kb, use pre-heated Elution Buffer, TE, or water (60~70°C). If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.
Purified DNA does not perform well in downstream applications	**DNA was denatured (a smaller band appeared on gel analysis).** Ensure the agarose gel was melted/dissolved completely between 55-60°C for 10-15 minutes, or until no gel is visible. DNA can be denatured if the incubation temperature exceeds 60°C. Incubate the eluted DNA at 95°C for 2 minutes then cool down slowly to reanneal the denatured DNA.

Troubleshooting

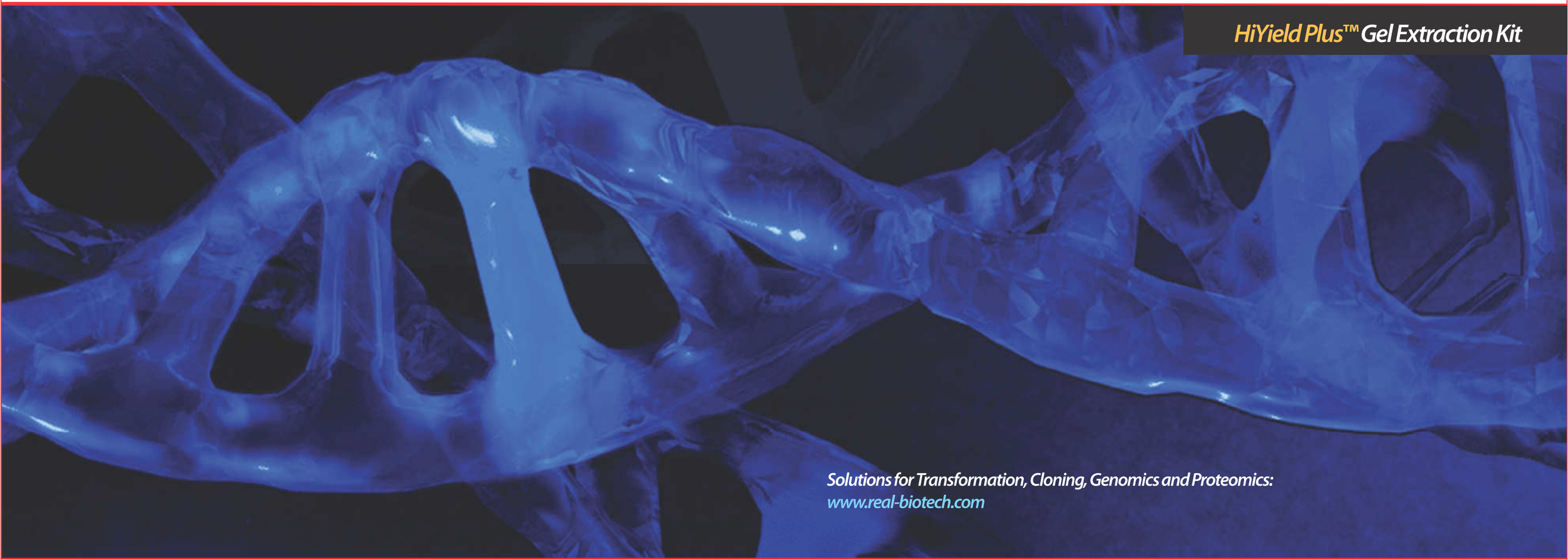
Problem	Possible Reasons / Solution
Purified DNA does not perform well in downstream applications	**Primer dimer contamination in the final PCR elution product.** Gel purification should be performed if primer dimers are visible in the agarose gel following PCR reactions. Simply cut the PCR product from the gel and avoid the primer dimer. Using an additional 80% ethanol wash will reduce primer dimer contamination when performing PCR cleanup.



Ordering Information

		Cat. No.	Size	Items
Related products	Taq	RT001	S	RealTaq™ DNA Polymerase w/ dNTP, 500U/kit
		RTT01	L	RealTaq™ DNA Polymerase w/ dNTP, 2500U/kit
	Cloning	RC001	S	RBC™ T&A Cloning Kit, 20 reactions/kit
		RC013	S	RBC™ T&A Cloning Vector, 20 reactions/kit
		RC011	S	RBC™ Rapid Ligation Kit, 100 reactions/kit
	Gel Extraction PCR Cleanup	QPP100	S	HiYield Plus™ PCR Purification Kit, 100 preps/ kit
		YDF100	S	HiYield™ Gel/PCR DNA Mini Kit, 100 preps/ kit
		QDF100	S	HiYield Plus™ Gel/PCR DNA Mini Kit, 100 preps/ kit
		YDS100	S	HiYield™ Gel/PCR Small DNA Mini Kit, 100 preps/ kit
		YDL100	S	HiYield™ Gel/PCR Large DNA Mini Kit, 100 preps/ kit
		YDM10	S	HiYield™ Gel/PCR DNA Maxi Kit, 10 preps/ kit
		YDF96B-2	S	HiYield™ 96-Well Gel/PCR DNA Extraction Kit, 2 preps/ kit
	Transformation	RH617	S	HIT Competent Cells™ -DH5alpha Value 10^8, 100 µl x 10 vials, strain: DH5 alpha
		RH717	S	HIT Competent Cells™ -JM109 Value 10^8, 100 µl x 10 vials, strain: JM109
		RH117	S	HIT Competent Cells™ -Blue Value 10^8, 100 µl x 10 vials, strain: XL1-Blue
		RH217	S	HIT Competent Cells™ -BL21 Value 10^8, 100 µl x 10 vials, strain: BL21(DE3)
		RH517	S	HIT Competent Cells™ -DH10B Value 10^8, 100 µl x 10 vials, strain: DH10B
		RH317	S	HIT Competent Cells™ -GM2163 Value 10^8, 100 µl x 10 vials, strain: GM2163

HiYield Plus™ Gel Extraction Kit



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