



Ordering information

DNA extraction from plant material is recognized as a difficult task with haphazard results. Make your DNA isolation from plant samples easier on you and easier on your samples, while achieving high-yield, high-purity results with Invitrogen's plant molecular biology reagents. Order today.

For complete information about Invitrogen's nucleic acid purification and quantitation products, visit www.invitrogen.com/naprep.

Product	Quantity	Cat. no.
For DNA purification		
ChargeSwitch® gDNA Plant Kit	96 preps	CS18000
	960 preps	CS18000-10
MagnaRack™ Magnetic Separator (for 1.5 ml tubes)	1	CS15000
96-Well Magnetic Separator	1	CS15096
96 Deep Well Block	50	CS15196
For finding the optimal PCR enzyme		
PCR Enzyme Selection Kit—High Specificity	4 × 50 rxns	12567-012
PCR Enzyme Selection Kit—High Fidelity	4 × 50 rxns	12567-020
Additional plant nucleic acid purification products		
PureLink™ Genomic Plant DNA Purification Kit	50 preps	K1830-01
Plant DNazol® Reagent	100 ml	10978-021
Plant RNA Isolation Reagent	100 ml	12322-012

The easiest way to
get the purest, highest
yields of plant DNA

ChargeSwitch® gDNA Plant Kit





Successful plant DNA extraction that's easy on you

ChargeSwitch® gDNA Plant Kit

- Simple, fast process—magnetic bead format allows multiple samples to be processed with less hands-on time, increasing throughput
- Pure DNA yields—organic solvent-free procedure successfully removes polysaccharides, polyphenols, and other inhibitors
- Fully automatable—processes more samples in less time while maintaining high yields and purity

Plant tissue itself is difficult enough to work with due to the high levels of polysaccharides and polyphenols present. Why compound the complexity with tedious, inefficient processes? Cetyl trimethyl ammonium bromide (CTAB) methods require excessive time and handling, limiting your throughput. Silica membrane- or magnetic bead-based protocols rely on guanidine and ethanol for DNA capture, but these reagents often don't remove inhibitors inherent in plant samples that can carry over into the final product and interfere with downstream PCR applications. This leads to frequent sample processing failure, causing you to repeat the purification—if you have the time and sample to spare. Now Invitrogen offers you products specifically designed for easy, high-yield, high-purity DNA purification from plant samples. Inhibitors are removed for reliable downstream results the first time. In addition, the ChargeSwitch® DNA isolation method is automation friendly so you can process hundreds of samples while obtaining the purity and yield you need.

How ChargeSwitch® technology works

The ChargeSwitch® gDNA Plant Kit provides the most innovative and successful method for plant DNA extraction—even when working with difficult plant samples. The basis of ChargeSwitch® nucleic acid purification is a novel surface-charge technology (Figure 1). In conditions of pH <6.5, the ChargeSwitch® surface has a positive charge that selectively binds the negatively charged nucleic acid backbone. Proteins and other contaminants are

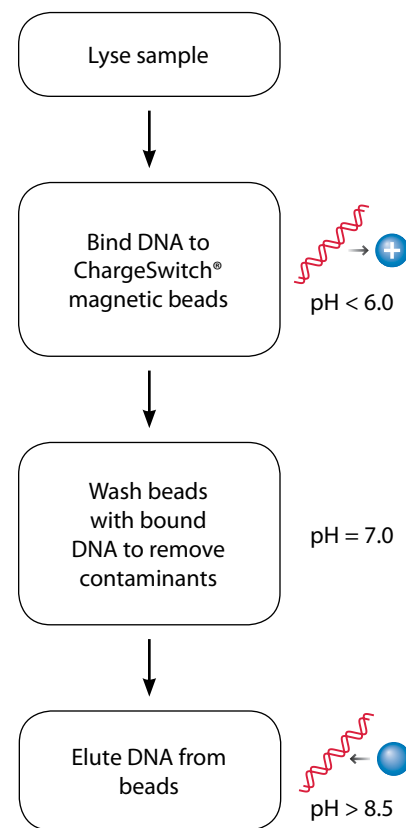


Figure 1—The ChargeSwitch® procedure is easy to perform.

removed in an aqueous wash buffer. For elution, the charge on the ChargeSwitch® surface is switched off by raising the pH to 8.5. Purified nucleic acid elutes instantly and is ready for downstream use. From binding DNA to eluting, the protocol takes just 20 minutes. The ChargeSwitch® method uses low-salt, aqueous buffers and avoids the introduction of organic solvents, ethanol, or concentrated chaotropic salts that may interfere with downstream experiments. The simplified protocol requires only four basic steps, making it ideal for high-throughput processes.

Higher genomic DNA yield than from silica-based methods

Compared to plant genomic DNA extraction kits that use silica membranes or magnetic beads, the ChargeSwitch® gDNA Plant Kit purifies higher yields of high molecular weight DNA from a wide range of samples, including commercial grains, soft crops, and horticultural species (Figure 2). Even with seeds, notoriously difficult due to their rich polysaccharide interior, the ChargeSwitch® method is able to produce consistent DNA yields (Figure 3).

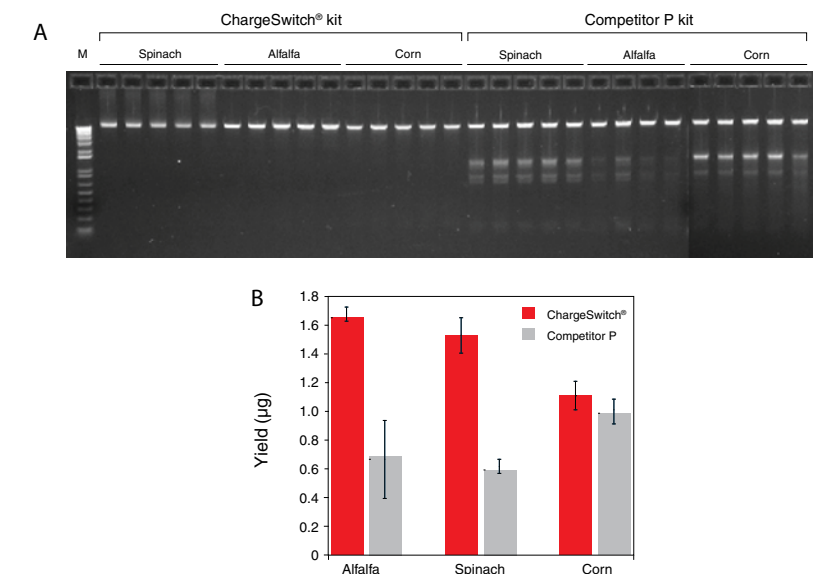


Figure 2—Consistently higher, cleaner DNA yields obtained with the ChargeSwitch® gDNA Plant Kit. DNA was extracted from 50 mg samples of alfalfa, spinach, and corn using the ChargeSwitch® gDNA Plant Kit or Competitor P's magnetic silica bead kit. A. Purified DNA (40 ng from each extraction) was loaded onto a 1% agarose gel. Yields were consistent among multiple preps. Lane M: 1 Kb DNA Ladder. B. DNA yields were measured using the Quant-iT™ PicoGreen® dsDNA Reagent.

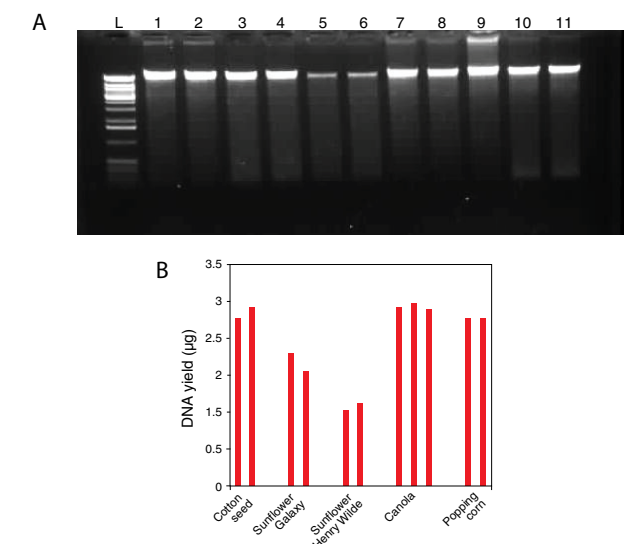


Figure 3—Consistent high molecular weight DNA recovery from seeds using the ChargeSwitch® Kit. DNA was extracted from 50 mg samples of seeds from cotton, two varieties of sunflower, canola, and popping corn using the ChargeSwitch® gDNA Plant Kit. A. 200 ng DNA from each extraction was analyzed by electrophoresis. Lane L: 1 Kb Extension DNA Ladder. Lanes 1, 2: Cotton seed. Lanes 3, 4: Sunflower seed (Galaxy). Lanes 5, 6: Sunflower seed (Henry Wilde). Lanes 7–9: Canola seed. Lanes 10, 11: Popping corn. B. DNA yields were measured using the Quant-iT™ PicoGreen® dsDNA Reagent.



Higher-purity DNA isolated using the ChargeSwitch® gDNA Plant Kit

DNA extracted using the ChargeSwitch® method is of higher purity than silica-prepared DNA, which is typically contaminated with guanidine salts (Figure 4). Purity is further demonstrated by consistent PCR amplification of single-copy and multiple-copy genes from ChargeSwitch® gDNA Plant Kit–extracted DNA, including difficult samples such as phenolic compound–rich rhododendron leaves (Figure 5), and the single peaks and nearly identical C_t values achieved in melting curve analysis of replicate samples (Figure 6). DNA purity is so high compared to other methods that the ChargeSwitch® kit can be routinely used for the most sensitive applications, including genetically modified organism (GMO) screening (see below).

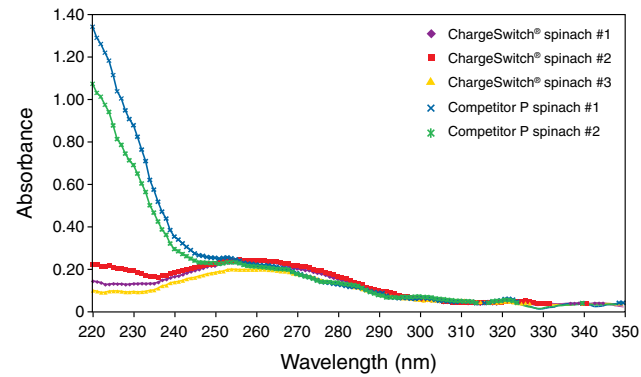


Figure 4—Higher-purity DNA obtained using the ChargeSwitch® kit vs. silica-based kits. DNA samples were isolated from spinach using the ChargeSwitch® gDNA Plant Kit and Competitor P’s silica magnetic bead kit. UV spectral analysis of the gDNA shows traces of impurities (high absorbance at 220–230 nm is indicative of guanidine salt contamination) in the samples purified using Competitor P’s kit.

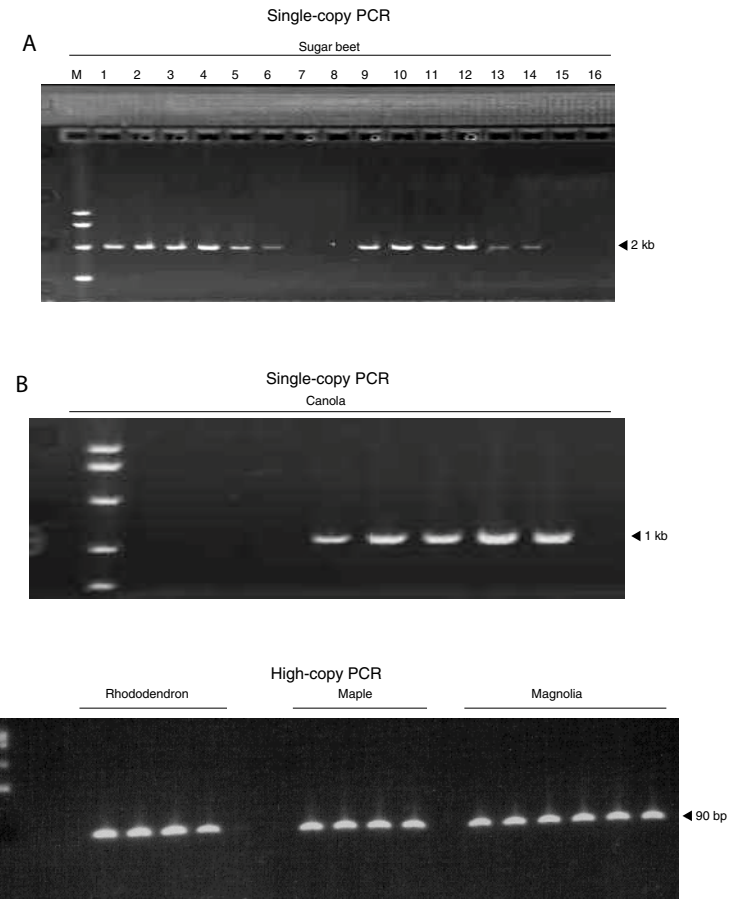


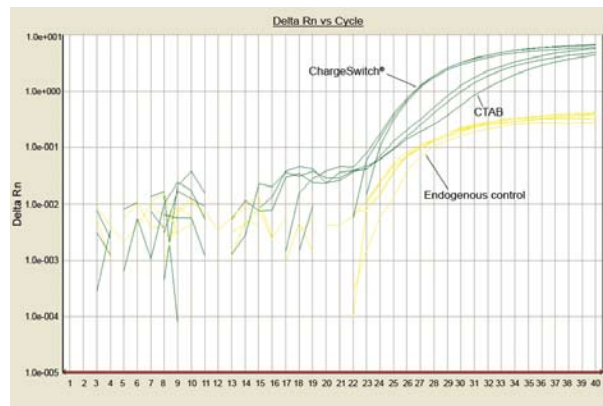
Figure 5—Consistent PCR amplification of ChargeSwitch® kit–extracted DNA. DNA was extracted from various samples using the ChargeSwitch® gDNA Plant Kit. PCR was performed with 50 ng purified DNA in a 50 µl volume. A. The 2 kb sucrose-6-phosphate synthetase gene from sugar beet DNA. B. The 1 kb acetyl CoA carboxylase gene from canola seed DNA. C. The 90 bp fragment of the cytochrome oxidase (cox) multicopy gene from rhododendron, maple, and magnolia DNA.

GMO testing using the ChargeSwitch® gDNA Plant Kit

by Satish Rai, Seed Science Center, Iowa State University

Testing for adventitious presence of biotechnology traits in seed and grain [recently] became an integral part of crop development, production, stewardship, and regulation. Presently, government regulators, seed companies, grain suppliers, interest groups, contract laboratories, and academia are all involved in testing of biotech traits. Sensitive methods of extracting genomic DNA from leaf tissue and seeds are needed to quantify GMOs accurately. DNA extraction followed by PCR amplification using GMO-specific primers is the traditional method for GMO screening.

The ChargeSwitch® gDNA Plant Kit was compared to the traditional CTAB method for extraction and detection of GMO levels using real-time PCR. Corn spiked with 0.5% GMO was ground to flour and subjected to DNA extraction according to each method’s standard protocol. The C_t values for ChargeSwitch® kit–extracted DNA were 3 cycles lower (figure), indicating a 10-fold increase in sensitivity, and the replicates were tighter, indicating the high level of DNA purity and consistency of sample processing using the ChargeSwitch® kit.



Ten-fold increase in sensitivity using DNA extracted using the ChargeSwitch® gDNA Plant Kit. GMO testing of corn flour samples, using DNA extracted with the ChargeSwitch® gDNA Plant Kit or the traditional CTAB method and used for qPCR with GMO-specific primers. For qPCR, 10 µl of sample (200 ng) was used in a 50 µl PCR using an Eppendorf real-time master mix kit and ABI 7000 instrument. The C_t values for the material processed with the ChargeSwitch® kit were 3 cycles lower compared to those with the CTAB method of purification. Data kindly provided by Satish Rai, Seed Science Center, Iowa State University.

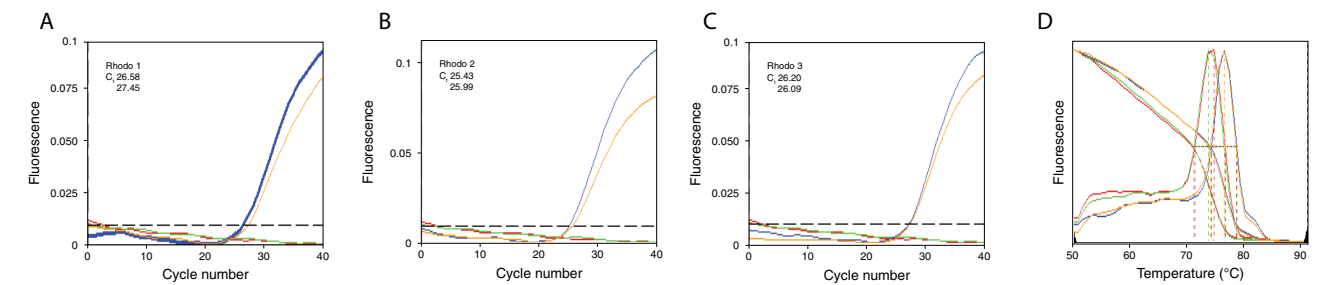


Figure 6—Single peaks obtained in melting curve analysis indicate single PCR products in starting material. DNA isolated with the ChargeSwitch® gDNA Plant Kit was used in real-time PCR. qPCR was performed with 20 ng purified DNA in a 50 µl volume. A–C. Amplification of the COX gene using TaqMan® primers shows C_t values that are consistent between replicates and different runs with rhododendron DNA. Consistency of the C_t values is indicative of the purity. D. Melting curve analysis performed with SYBR® Green dye shows one clean peak for the cox gene and another peak for the trnL gene, indicating amplification was robust and specific. No nonspecific amplification was observed, demonstrating the purity and quality of gDNA.



Find the amplification enzyme that's right for you

Once you've isolated DNA from your plant sample, the next step is often amplification. Plant DNA tends to be GC-rich and high in secondary structure, making this step difficult. Using the optimal enzyme is critical to your success (Figure 7, Table 1). The PCR Enzyme Selection Kits provide you with a convenient way to sample the best Invitrogen™ PCR enzymes available and choose the one that works best in your experiment. Two PCR Enzyme Kits are currently available. Each kit contains four PCR enzymes (50 reactions each), the corresponding buffers, and dNTPs. The PCR Selection Kit—High Specificity includes Platinum® *Taq* DNA Polymerase, Platinum® *Taq* DNA Polymerase High Fidelity, AccuPrime™ *Taq* DNA Polymerase, and Platinum® PCR SuperMix (containing Platinum® *Taq* DNA Polymerase). The PCR Selection Kit—High Fidelity includes *Pfx50*™ DNA Polymerase, AccuPrime™ *Pfx* DNA Polymerase, Platinum® *Taq* DNA Polymerase High Fidelity, and Platinum® PCR SuperMix High Fidelity (containing Platinum® *Taq* DNA Polymerase High Fidelity). Learn more at www.invitrogen.com/pcr.

Table 1—PCR enzyme selection guide.

Invitrogen™ PCR enzyme	Product size	Yield	Specificity	Fidelity	Convenience	GC-rich templates
<i>Taq</i> DNA Polymerase	<5 kb	●	●	●	●	●
<i>Tfi</i> DNA Polymerase	<5 kb	●	●	●	●	●
Platinum® <i>Taq</i> DNA Polymerase	<5 kb	●●●	●●	●	●●	●
Platinum® <i>Tfi</i> DNA Polymerase	<3kb	●●●	●●	●	●●	●
AccuPrime™ <i>Taq</i> DNA Polymerase	<5 kb	●●●	●●●	●●	●●●	●
<i>Pfx50</i> ™ DNA Polymerase	<6 kb	●●●	●	●●●●●	●●●	●
Platinum® <i>Pfx</i> DNA Polymerase	<12 kb	●●	●●	●●●●	●●●	●
AccuPrime™ <i>Pfx</i> DNA Polymerase	<12 kb	●●●	●●●	●●●●	●●●	●
Platinum® <i>Taq</i> DNA Polymerase High Fidelity	<20 kb	●●●●	●●	●●●	●●	●
AccuPrime™ <i>Taq</i> DNA Polymerase High Fidelity	<20 kb	●●●●	●●●	●●●●	●●●	●
AccuPrime™ GC-Rich DNA Polymerase	<5 kb	●●●	●●●	●●	●	●●●●

DNA fingerprinting?

Check out the Amplified Restriction Fragment Length Polymorphism (AFLP®) primers designed specifically for plant analysis, at www.invitrogen.com.

AFLP® is a trademark of Keygene NV Corporation.

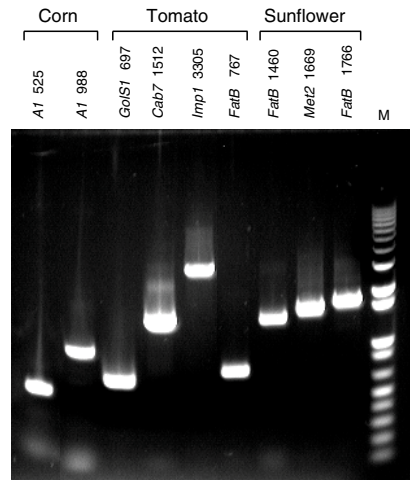


Figure 7—Successful amplification from plant DNA samples. DNA was extracted from corn, tomato, and sunflower samples with the ChargeSwitch® gDNA Plant Kit and then used in PCR to amplify the indicated genes using AccuPrime™ *Taq* DNA Polymerase. The indicated amount of each PCR was then run on a 1.2% E-Gel® precast gel. Lanes 1, 2: 15 ng corn DNA. Lanes 3–5: 15 ng tomato DNA. Lane 6: 11 ng tomato DNA. Lanes 7–9: 6 ng sunflower DNA. Lane M: 5 µl TrackIt™ 1 Kb Plus DNA Ladder.

Optimized ChargeSwitch® gDNA Plant Kit protocol

To obtain superior results when working with plant samples particularly rich in phenolics and polysaccharides, follow this enhanced protocol.

Preparing lysate from up to 100 mg plant tissue

Prepare 10 ml Reagent A (300 mM CaCl₂, 15% polyvinylpyrrolidone) fresh by adding 0.441 g CaCl₂ and 1.5 g PVP (10,000 average MW) to 10 ml ChargeSwitch® Lysis Buffer (L18).

Prior to use for sample preparation, add 100 µl Reagent A to 900 µl ChargeSwitch® Lysis Buffer (L18) supplied in the kit.

Chill the Precipitation Buffer (N5) on ice.

- For **hard plant tissue**, freeze the tissue in liquid nitrogen and grind frozen tissue to powder using mortar and pestle. Let liquid nitrogen evaporate before proceeding to Step 2. For **soft, nonfibrous plant tissue**, cut the tissue into small pieces. For lyophilized samples, proceed directly to Step 2.
- At room temperature, add 1 ml ChargeSwitch® Lysis Buffer (L18) to the tissue from Step 1, or, for samples rich in polysaccharides and polyphenolics, add 1 ml ChargeSwitch® Lysis Buffer containing Reagent A (see above for recipe) to the tissue from Step 1. **Note:** If the solution is very viscous, add more Lysis Buffer (L18).
- Add 2 µl RNase A to the samples.
- Prepare lysate by homogenizing the pieces of soft tissue with a tissue homogenizer or grinder or by vortexing the ground tissue/lyophilized sample until sample is completely resuspended.
- Add 100 µl 10% SDS for each 1 ml of plant lysate and mix by pipetting. If different amounts of ChargeSwitch® Lysis Buffer (L18) are used, maintain a ratio of 10:1 of Lysis Buffer to SDS.
- Incubate at room temperature for 5 min.
- Add 400 µl ChargeSwitch® Precipitation Buffer (N5) for each 1 ml of lysate. Mix by inversion or vortexing for 10 sec until a precipitate forms. **Note:** If an increased amount of ChargeSwitch® Lysis Buffer (L18) was used, add more N5 to maintain a ratio of 10:4 of Lysis Buffer to Precipitation Buffer.
- Centrifuge at maximum speed for 5 min at room temperature to produce a clear lysate.

- Transfer the clear lysate to a new, sterile 1.5 ml microcentrifuge tube for manual purification or to a 96 × 2 ml Deep Well Block (Cat. no. CS15196) for automated purification using the 96-Well Magnetic Separator (Cat. no. CS15096).

Binding the DNA

- Thoroughly vortex the tube containing the ChargeSwitch® Magnetic Beads.
- Add 100 µl ChargeSwitch® 10% Detergent to the lysate.
- Add 40 µl resuspended ChargeSwitch® Magnetic Beads.
- Mix gently by pipetting up and down 5 times.
- Incubate at room temperature for 1 min.
- Place tubes on the MagnaRack™ Magnetic Separator (Cat. no. CS15000) for 1.5 ml microcentrifuge tubes or on the 96-Well Magnetic Separator (Cat. no. CS15096) for deep-well plates until the beads have formed tight pellets.
- Remove and discard the supernatant.

Washing the beads

- Remove tubes from the magnet.
- Add 1 ml of Wash Buffer (W12) and mix.
- Place tubes on the magnet until the beads have formed tight pellets.
- Remove and discard the supernatant.
- Repeat steps 17–20.

Eluting the DNA

- Remove the tubes containing the pelleted magnetic beads from the magnet.
- Add 150 µl of Elution Buffer (E6).
- Pipet up and down gently 15–30 times.
- Incubate at room temperature for 1 min.
- Place tubes on the magnet until the beads have formed tight pellets.
- Transfer the eluate containing the DNA to a sterile microcentrifuge tube.