

Certificate of Analysis & Product Manual



Triple Repeat Disorders Genotyping

Fragile X, Myotonic Dystrophy, Friedreich's Ataxia, Huntington's disease, Spinocerebellar Ataxia's
Fluorescent Probes, siRNA, Hybridization and Detection Reagents

SCA2 CAG Repeat Genemer™ Control Template

Spinocerebellar Ataxia Type 2 (SCA2) CAG Repeats Control Templates

Catalog No. 40-2038-01

Storage Condition: See Material Supplied List

For Research Use Only. Not for use in diagnostic procedures for clinical purposes

Important Information

All Gene Link products are for research use only.

Not for use in diagnostic procedures for clinical purposes.

Product to be used by experienced researchers appropriately trained in performing molecular biology techniques following established safety procedures. Additional qualification and certification is required for interpretation of results.



Material Supplied

SCA2 CAG Repeat Genemer™ Control Template

Spinocerebellar Ataxia Type 2 (SCA2) CAG Repeats Control Templates

A tube containing 500 ng of lyophilized DNA segment of the specified CAG repeat fragment spanning the CAG repeat of the *ATXN2* gene.

Content	Catalog No.	Description	Size
□	40-2038-01	SCA2 22 repeat (19 CAG + 3 CAA) GScan™ & Genemer™ Control DNA	500 ng

Storage Condition

Store at -20°C.

Certificate of Analysis & Product Specifications

The SCA2 CAG repeat region control template DNA's are cloned products. These have been developed by inserting varying number of CAG repeats to serve as control DNA specifically for use with the SCA2 Genemer™ detection system. Using Gene Link's SCA2 Genemer primer pair (Catalog#: 40-2038-10) a fragment of approximately 130 bp is amplified. The above control DNA is an ideal genotyping template for optimizing and performing control amplification.

The size of the CAG repeats has been determined by sequencing and gel electrophoresis. The stability of size repeats upon cloning and amplification has NOT been determined. Thus, the size should be considered approximate and there is no claim for each fragment to contain the exact number of CAG repeats. These control DNA's are sold with the express condition that these NOT be used for exact CAG size determination of DNA of unknown genotype. The control DNA should be used for determining the performance of specific Gene Link Genemer™ products.

Appropriate nuclease free handling, dispensing and storage conditions required.

Manufacturing lot numbers are stated on the label of each product and accompanying packing slip.

Genemer™ Kits Product Ordering Information

Gene Link's Genemer™ kits contain optimized PCR amplification components for convenient agarose or polyacrylamide genotyping of triple repeat disorders and other genetic disorders. These are safe, convenient and sensitive, and afford rapid screening of samples. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. Included in these kits are ready-to-run control samples of various repeats of the specific triple repeat disorder.

The Genemer™ kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed.

Product	Unit Size	Catalog No.
Fragile X Genemer™ V2 Kit for gel based detection; 100 reactions kit	1 kit	40-2004-11
FRAXE/FMR2/AFF2 Genemer™ Kit for gel based detection; 100 reactions kit	1 kit	40-2054-11
Huntington's Disease Genemer™ V2 Kit for gel based detection; 100 reactions kit kit	1 kit	40-2025-11
Myotonic Dystrophy Genemer™ Kit for for gel based detection; 100 reactions kit	1 kit	40-2026-11
Friedreich's Ataxia Genemer™ Kit for gel based detection; 100 reactions kit	1 kit	40-2027-11
SCA2 Genemer™ V2 Kit for gel based detection; 100 reactions kit	1 kit	40-2038-11

GScan™ Related Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument. Included in these kits are ready-to-run control samples of various repeats of the triple repeat disorder kit. These control samples are for calibration with the molecular weight markers for accurate size determination of the amplified fragments.

The GScan™ kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed.

Product	Unit Size	Catalog No.
Fragile X GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15FM
Fragile X GScan™ V2 Kit for fluorescent detection; 20 reactions kit	1 kit	40-2004-15FMS
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2054-15FM
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2054-15FMS
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15FM
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 20 reactions kit	1 kit	40-2025-15FMS
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2026-15FM
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2026-15FMS
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2027-15FM
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2027-15FMS
SCA2 GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2038-15FM
SCA2 GScan™ V2 Kit for fluorescent detection; 20 reactions kit	1 kit	40-2038-15FMS

All Gene Link products are for research use only

Current pricing are posted at <http://www.genelink.com/>

Spinocerebellar Ataxia Type 2 (SCA2) Genotyping

Background

The autosomal dominant cerebellar ataxias (ADCA) are a heterogeneous group of neurodegenerative disorders characterized by progressive degeneration of the cerebellum, brain stem and spinal cord. Spinocerebellar ataxia (SCA) type 2 is characterized by deterioration in balance and coordination, slow saccadic eye movement, and in some individuals ophthalmoparesis.

People with this condition initially experience problems with coordination and balance (ataxia). Other early signs and symptoms of SCA2 include speech and swallowing difficulties, rigidity, tremors, and weakness in the muscles that control eye movement (ophthalmoplegia). Eye muscle weakness leads to a decreased ability to make rapid eye movements (saccadic slowing).

Over time, individuals with SCA2 may develop loss of sensation and weakness in the limbs (peripheral neuropathy), muscle wasting (atrophy), uncontrolled muscle tensing (dystonia), and involuntary jerking movements (chorea). Individuals with SCA2 may have problems with short term memory, planning, and problem solving, or experience an overall decline in intellectual function (dementia).

Signs and symptoms of the disorder typically begin in mid-adulthood but can appear anytime from childhood to late adulthood. People with SCA2 usually survive 10 to 20 years after symptoms first appear. SCA2 is inherited in an autosomal dominant manner. Offspring of an affected individual have a 50% chance of inheriting the gene mutation.

Mutations in the *ATXN2* gene cause SCA2. The *ATXN2* gene provides instructions for making a protein called ataxin-2. This protein is found throughout the body, but its function is unknown. Ataxin-2 is found in the fluid inside cells (cytoplasm), where it appears to interact with a cell structure called the endoplasmic reticulum. The endoplasmic reticulum is involved in protein production, processing, and transport. Researchers believe that ataxin-2 may be involved in processing RNA, a chemical cousin of DNA. Ataxin-2 is also thought to play a role in the production of proteins from RNA (translation of DNA's genetic information).

The *ATXN2* gene mutations that cause SCA2 involves a DNA segment known as a CAG trinucleotide repeat. This segment is made up of a series of three DNA building blocks (cytosine, adenine, and guanine) that appear multiple times in a row. Normally, the CAG segment is repeated approximately 22 times within the gene, but it can be repeated up to 31 times without causing any health problems. Individuals with 32 or more CAG repeats in the *ATXN2* gene develop SCA2. People with 32 or 33 repeats tend to first experience signs and symptoms of SCA2 in late adulthood, while people with more than 45 repeats usually have signs and symptoms by their teens.

It is unclear how the abnormally long CAG segment affects the function of the ataxin-2 protein. The abnormal protein apparently leads to cell death, as people with SCA2 show loss of brain cells in different parts of the brain. Over time, the loss of brain cells causes the movement problems characteristic of SCA2.

The mutation in all identified SCA genes is the expansion of an unstable CAG repeat encoding a polyglutamine tract. Similar to other trinucleotide repeat disorders, such as Huntington disease and spinal and bulbar muscular atrophy, the SCAs show anticipation and different degrees of expansion in maternal or paternal transmission. There is a direct correlation between the size of the CAG repeat and the onset and severity of the disease. Affected adult individuals have alleles with 36-64 CAG trinucleotide repeats, while infantile- and juvenile-onset SCA2 is associated with expansions of 130 to more than 200 CAG trinucleotide repeats.

The SCA2 locus has been mapped to chromosome 12q24. Several SCA genes have been cloned and shown to contain CAG repeats in their coding regions. Spinocerebellar ataxia (SCA) 1, 2, 3, 4 and 6, are assigned to five different chromosomes.

Table 1. Trinucleotide Repeats in Human Genetic Disease

Disease	Repeat ^a	Normal Length ^b	Intermediate Length (Premutation) ^{a,b}	Full Disease Length ^b
Fragile XA (FRAXA)	(CGG) _n	6-52	59-230	230-2,000
Fragile XE (FRAXE)	(CCG) _n	4-39	? (31-61)	200-900
Fragile XF (FRAXF)	(CGG) _n	7-40	?	306-1,008
FRA16A	(CCG) _n	16-49	?	1,000-1,900
Jacobsen Syndrome (FRA11B)	(CGC) _n	11	80	100-1,000
Kennedy Syndrome (SMBA)	(CAG) _n	14-32	?	40-55
Myotonic Dystrophy (DM)	(CTG) _n	5-37	50-80	80-1,000; congenital, 2,000-3,000
Huntington disease (HD)	(CAG) _n	10-34	36-39	40-121
Spinocerebellar ataxia 1 (SCA1)	(CAG) _n	6-39	None Reported	40-81
Spinocerebellar ataxia 2 (SCA2)	(CAG) _n	14-31	None Reported	34-59
Spinocerebellar ataxia 3 (SCA3)/Machado Joseph disease (MJD)	(CAG) _n	13-44	None Reported	60-84
Spinocerebellar ataxia 6 (SCA6)	(CAG) _n	4-18	None Reported	21-28
Spinocerebellar ataxia 7 (SCA7)	(CAG) _n	7-17	28-35	38-130
Haw River syndrome (HRS; also DRPLA))	(CAG) _n	7-25	?	49-75
Friedreich ataxia (FRDA)	(GAA) _n	6-29	? (>34-40)	200-900

^a Typically, repeats tracts contain sequence interruptions. See Pearson and Sinden (1998a) for a discussion of the sequence interruptions.

^b No. of triplet repeats.

^c A question mark (?) indicates potential mutagenic intermediate length, and an ellipsis (...) indicates none. Not all diseases are associated with a premutation length repeats tract or premutation disease condition.

Molecular Analysis

ATXN2 is the only gene known to be associated with SCA2. One hundred percent of individuals affected with SCA2 have a CAG trinucleotide repeat expansion. The presence of one abnormal allele is diagnostic. Normal alleles CAG repeats are below 31. DNA analysis can detect 100% of expanded alleles.

SCA2 genotyping can be done by direct PCR amplification of the CAG trinucleotide repeats region or by southern analysis. In most cases both methods are used to complement the results.

Gene Link offers safe and reliable chemiluminescent detection methods as an alternate to radioactive based detection methods. PCR-Prober™, GScan™ and GeneProber™ line of products replaces radioactive based methods. Gene Link's GScan Ver2 kit is for PCR amplification followed by fluorescent detection of the specific triple repeat fragment size and routinely detects greater than 120 CGG repeats.

Genemer™ Kit Agarose Gel Analysis

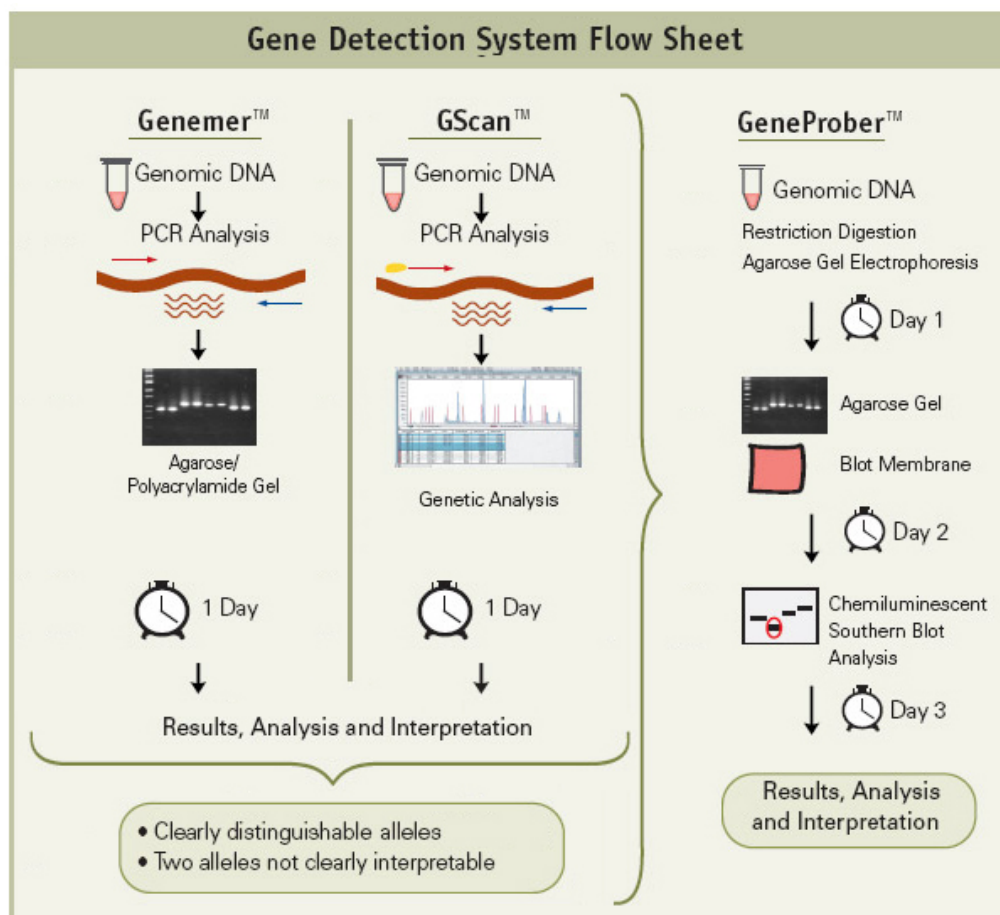
Optimized Genemer™ kit with components for PCR amplification of up to 130 Fragile X CGG repeats using standard Taq polymerase. Amplified samples are resolved by agarose gel electrophoresis. This Genemer™ method or GScan™ fluorescent detection is recommended for initial screening of all samples.

GScan™ Kit

Optimized GScan™ kit with components for PCR amplification of up to 130 Fragile X CGG repeats using standard Taq polymerase. Amplified samples are resolved by genetic analyzers capable of fluorescent detection or agarose gel electrophoresis. This Genemer™ Kit or GScan™ kit for fluorescent detection is recommended for initial screening of all samples.

GeneProber™ Probes for Southern Blot Analysis

Digoxigenin labelled probes for chemiluminescent Southern blot detection method or unlabeled probe for end user to perform radioactive label. Gene Link offers safe and reliable chemiluminescent detection methods as an alternate to radioactive based detection methods.



Material Supplied/Procedure

SCA2 22 repeat (19 CAG + 3 CAA) GScan™ & Genemer™ Control DNA

A tube containing 500 ng of lyophilized DNA segment of the specified CAG repeat fragment spanning the CAG repeat region of the *ATXN2* gene. The quantity supplied is sufficient for 1000 regular 50µl PCR** reaction.

This Genemer™ product contains control template DNA of specific CAG repeats. The fragment containing the CAG repeats can be amplified by the corresponding Genemer™ primer pair or Genemer™ kit.

Reconstitution

Stock Solution: Add 100 µL sterile water to the tube containing the lyophilized DNA to yield a solution of 5 ng/µL.

Working Solution: Dilute 1:10 an aliquot of the stock solution.

Usage: Initially use 1 µL each of the stock and working template solution for amplification and optimization of the reaction. Dilute further based on results obtained. Use 1 µL of template at the lowest concentration.

Other Required Components

The Genemer™ Control Template DNA product is specifically designed to be used with the following Gene Link products.

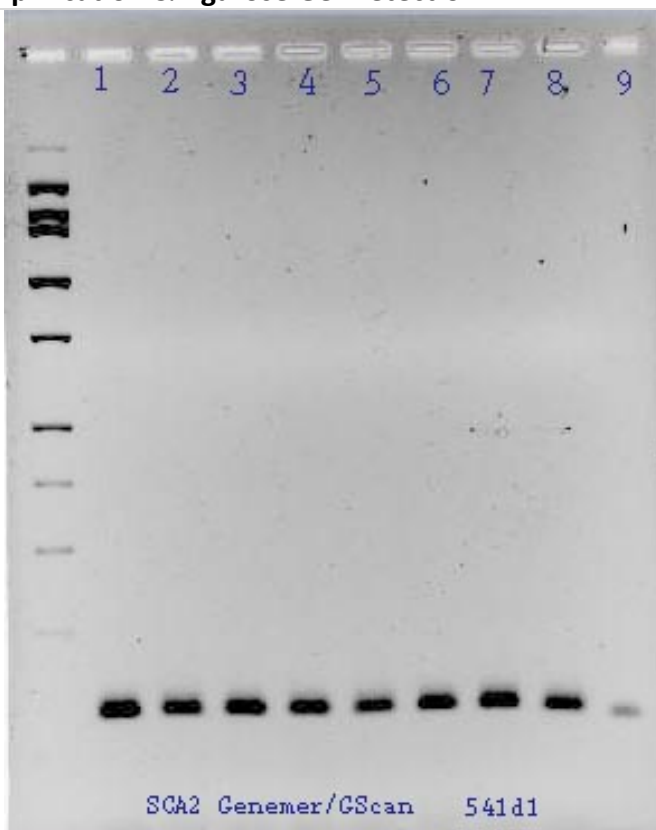
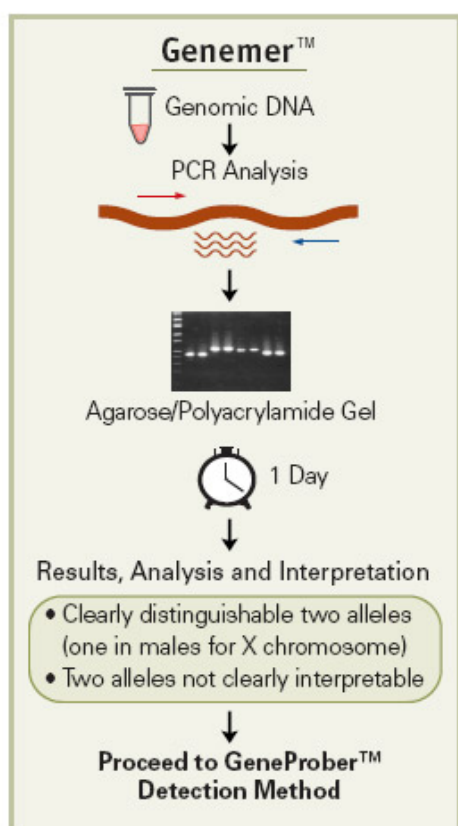
1. SCA2 Genemer™ Primer Pair. Catalog Number: 40-2038-10
2. SCA2 Genemer™ Kit. Catalog Number: 40-2038-11
3. SCA2 GScan™ Kit. Catalog Number: 40-2038-15

Refer to the product manuals of the above listed products for specific use instructions

SCA2 Genotyping Results and Interpretation

SCA Type	Normal Repeats	Intermediate Length	Full Disease Length
Spinocerebellar ataxia 1 (SCA1)	6-39	None Reported	40-81
Spinocerebellar ataxia 2 (SCA2)	14-31	None Reported	34-59
Spinocerebellar ataxia 3 (SCA3)/ Machado Joseph disease (MJD)	13-40	None Reported	60-84
Spinocerebellar ataxia 6 (SCA6)	4-20	None Reported	21-28
Spinocerebellar ataxia 7 (SCA7)	7-19	28-35	38-220

SCA2 GScan™ [40-2038-15FM] & Genemer™ V2 Kit [40-2038-11] PCR Amplification & Agarose Gel Detection



SCA2 X GScan™ V2 [40-2038-15FM] and SCA2 Genemer™ V2 Kit [40-2038-11] was used to amplify normal human genomic samples (lanes 1-8) and the SCA2 Genemer control template DNA (lane 9) containing 19 CAG and 3 CAA repeat. Approximately 130 bp fragment was amplified from the control DNA template and all normal fragment sizes from the 8 human genomic samples. A 25 µL volume PCR amplification was performed using SCA2 GScan™ kit. After PCR 10 µL samples were applied to a 2.5 agarose gel for electrophoresis. Gel picture is of an ethidium bromide stained gel.

Appendix: Protocols

Genomic DNA Purification

Genomic DNA is usually extracted from blood. A simple procedure is given below that purifies ~10 µg DNA from 300 µl blood using a 30 minute procedure.

Omni-Pure™ Genomic DNA Purification System Catalog Number: 40-4010-01

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 900 µl GD-1 solution (RBC Lysis Solution) to one tube for each sample.
3. Add 300 µl Isopropanol (2-propanol) to one tube for each sample. Cap the tubes.

B. Cell Lysis

1. To the tube containing 900 µl GD-1 solution (RBC Lysis Solution) using a filter tip pipet transfer 300 µl whole blood. Cap and gently mix by inversion. Incubate for 1-3 minutes at room temperature. Mix by inversion a few times during this incubation period. Incubate longer for fresh blood cells as they are intact and not lysed already.
2. Centrifuge at 3 K rpm for 20 seconds to pellet the white blood cells. A reddish white pellet should be clearly visible. Decant and discard supernatant leaving behind the last few droplets. Do not totally remove the supernatant.
3. Completely resuspend the white blood cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.
4. To the resuspended cells add 300 µl GD-2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample. Samples may be stored at this stage for processing later. It has been shown that the samples are stable in Cell Lysis Solution for at least 2 years at room temperature.

C. Protein Precipitation

1. Add 100 µl GD-3 solution (Protein Precipitation Solution) to the sample in cell lysis solution.
2. Vortex vigorously for 20 seconds. Small particles of brown color will appear and be visible at this stage.
3. Centrifuge at 5 K rpm for 1 minute to pellet the precipitated proteins. A clearly visible brown pellet containing proteins should be collected at the bottom of the tube.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 300 µl 100% Isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Mixing by inversion 30-40 is usually sufficient.
3. Centrifuge at 6 K rpm for 1 minute to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 6 K rpm for 1 minute to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate tube so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

E. DNA Reconstitution & Use

1. Add 100 µl of GD-4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4 °C. For long-term storage, place sample at -20 °C or -80 °C.
3. Average yield of 10 µg is expected from 300 µl blood DNA. The range is between 5 µg to 15 µg.
4. The 100 µl of purified DNA obtained will have an average concentration of ~ 100 ng/µl.
5. For PCR amplification use 1-2 µl.
6. Use 100 µl for restriction digestion followed by Southern blot analysis.
7. It is convenient to perform multiple 300 µl blood DNA purification instead of scaling up the procedure.

PCR Enhancers & Additives

DNA polymerases need to elongate rapidly and accurately to function effectively *in vivo* and *in vitro*, yet certain DNA regions appear to interfere with their progress. One common problem is pause sites, at which DNA polymerase molecules cease elongation for varying lengths of time. Many strong DNA polymerase pauses are at the beginnings of regions of strong secondary structure such as template hairpins (1). *Taq* polymerase used in PCR suffers the same fate and GC-rich DNA sequences often require laborious work to optimize the amplification assay. The GC-rich sequences possess high thermal and structural stability, presumably because the high duplex melting temperature that permits stable secondary structures to form, thus preventing completion of a faithful replication (2).

Nucleotide analog 7-deaza dGTP is effective in reducing the secondary structure associated with GC rich region by reducing the duplex stability (4). Betaine, DMSO and formamide reduces the T_m and the complex secondary structure, thus the duplex stability (1-5). Tetramethyl ammonium chloride (TMAC) actually increases the specificity of hybridization and increases the T_m . The use of TMAC is recommended in PCR conditions using degenerate primers.

These PCR additives and enhancing agents have been used to increase the yield, specificity and consistency of PCR reactions. These additives may have beneficial effects on some amplification and it is impossible to predict which agents will be useful in a particular context and therefore they must be empirically tested for each combination of template and primers.

PCR Additives		
Additive	Purpose & Function	Concentration
7-deaza-2'-deoxyguanosine; 7-deaza dGTP	GC rich region amplification. Reduce the stability of duplex DNA	Totally replace dGTP with 7-deaza dGTP; or use 7-deaza dGTP: dGTP at 3:1
Betaine (N,N,N-trimethylglycine = [carboxymethyl]trimethylamm onium)	Reduces T_m facilitating GC rich region amplification. Reduces duplex stability	Use 3.5M to 0.1M betaine. Be sure to use Betaine or Betaine (mono)hydrate and not Betaine HCl.
BSA (bovine serum albumin)	BSA has proven particularly useful when attempting to amplify ancient DNA or templates, which contain PCR inhibitors such as melanin.	BSA concentration of 0.01 $\mu\text{g}/\mu\text{l}$ to 0.1 $\mu\text{g}/\mu\text{l}$ can be used.
DMSO (dimethyl sulfoxide)	DMSO is thought to reduce secondary structure and is particularly useful for GC rich templates.	DMSO at 2-10% may be necessary for amplification of some templates; however 10% DMSO can reduce <i>Taq</i> polymerase activity by up to 50% so it should not be used routinely.
Formamide	Reduces secondary structure and is particularly useful for GC rich templates.	Formamide is generally used at 1-5%. Do not exceed 10%.
Non-ionic detergents e.g. Triton X-100, Tween 20 or Nonidet P-40 (NP-40)	Non-ionic detergents stabilize <i>Taq</i> polymerase and may also suppress the formation of secondary structure.	0.1-1% Triton X-100, Tween 20 or NP-40 may increase yield but may also increase non-specific amplification. As little as 0.01% SDS contamination of the template DNA (left-over from the extraction procedure) can inhibit PCR by reducing <i>Taq</i> polymerase activity to as low as 10%, however, inclusion of 0.5% Tween-20 or -40 will effectively neutralize this effect.
TMAC (tetramethylammonium chloride)	TMAC is used to reduce potential DNA-RNA mismatch and improve the stringency of hybridization reactions. It increases T_m and minimizes mis-pairing.	TMAC is generally used at a final concentration of 15-100 mM to eliminate non-specific priming.

Purification of PCR Product

Various purification methods are available for the purification of PCR products. The selection of a particular method over another is based on the downstream application and the initial robustness of the amplification. Usually no further purification is required for most cloning experiments if a single fragment is amplified, whereas for sequencing applications the amplified product should be purified from the primers and any other minor amplification products.

For fragment analysis of PCR products the preferred method of purification to eliminate primers, primer dimers and salts is the Omni-Clean™ Purification System available from Gene Link. Catalog No. 40-4130-10 for bead based system; 40-4140-10 for spin column based system. Gene Link recommends the beads system as recovery of the amplified PCR product is critical. Please refer to product insert for detailed protocol or visit www.genelink.com

A. Purification of DNA from solution using glass beads. Provides removal of salts, primers and dNTP.

[Omni-Clean™ DNA Beads Concentration System; Catalog No. 40-4130-10]

Protocol

1. Determine volume of DNA solution and add 2 volumes of OCC-2 solution and mix by vortexing.
2. Add 1 µl of glass bead suspension per µg of DNA and mix by vortexing.
3. Centrifuge at 4K rpm for 20 seconds to pellet glass bead/DNA complex. Discard all traces of supernatant.
4. Re-suspend pellet in 400 µl Omni-Clean™ G3 wash buffer.
5. Centrifuge at 4K rpm for 20 seconds and discard wash buffer.
6. Pipet out any remaining buffer in the tube.
7. Repeat steps 4-6 twice.
8. Add 20 µl water or TE; re-suspend pellet by vortexing and centrifuge at 4K rpm for 20 seconds.
9. The supernatant contains the purified DNA. Using a pipet, collect the supernatant and transfer to a new appropriately labeled tube.

B. Purification of DNA from solution using spin column. Provides removal of salts, primers and dNTP.

[Omni-Clean™ DNA Spin Column Concentration System; Catalog No. 40-4140-10]

Protocol

1. Determine volume of DNA solution and add 2 volumes of OCC-2 solution and mix by vortexing.
2. Add the above solution to the spin column assembled on a collection tube.
3. Let the solution flow by gravity or centrifuge at 2K rpm for 20 seconds. Discard flow through collected in the collection tube.
4. Add 400 µl Omni-Clean™ G3 wash buffer to the spin column. Centrifuge at 2K rpm for 2 minutes and discard wash buffer collected in the collection tube.
5. Replace the collection tube with a new appropriately labeled 1.5ml tube.
6. Add 25 µl water or TE to the spin column. Let sit for 3 minutes.
7. Centrifuge at 2K rpm for 2 minutes.
8. The collection tube contains the purified DNA.

SCA2 Genotyping Product Ordering Information

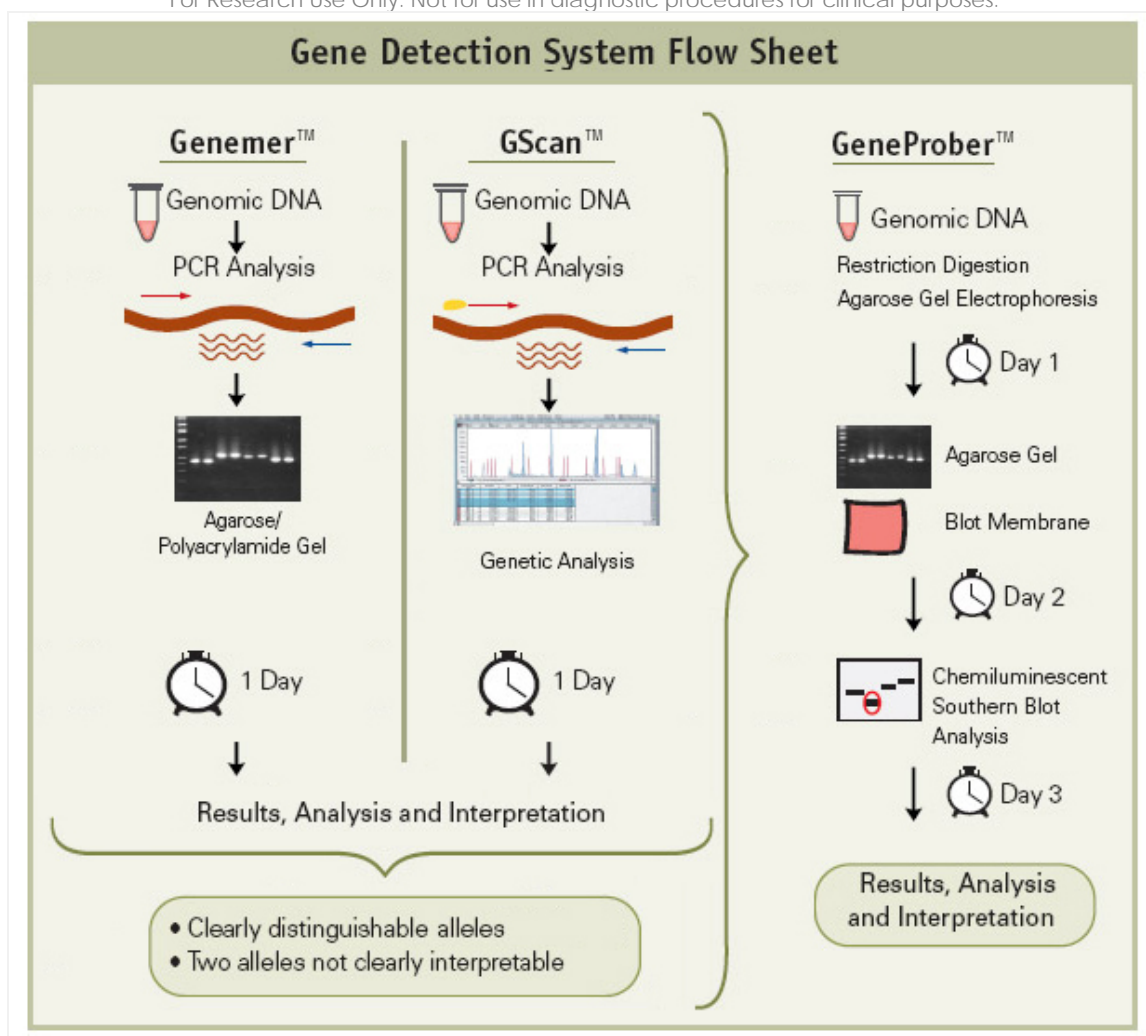
Product	Unit Size	Catalog No.
SCA2 Genemer™ Kit for gel based detection. Kit for performing PCR amplification and gel based detection.	1 Kit [100 rxns]	40-2038-11
SCA2 GScan™ Kits for Fam fluorescent detection Kit for performing fluorescent PCR amplification based detection.	1 Kit [100 rxns]	40-2004-15FM
SCA2 Genemer™ Primer pair Primers for amplification of CGG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µL PCR reactions.	10 nmols	40-2038-10
SCA2 22 repeat (19 CAG + 3 CAA) GScan™ & Genemer™ Control DNA	500 ng	40-2038-01

Fragile X Genotyping Product Ordering Information

Product	Unit Size	Catalog No.
Fragile X Genemer™ Kit for gel based detection. Kit for performing PCR amplification and gel based detection.	1 Kit [100 rxns]	40-2004-11
Fragile X GScan™ Kits for fluorescent detection Kit for performing fluorescent PCR amplification based detection. Various dye kits. XX=FM for 6-Fam; HX for Hex; TT for Tet; C3 for Cy3 and C5 for Cy5.	1 Kit [100 rxns]	40-2004-15XX
Fragile X GeneProber™ GLFX1 Probe unlabeled Probe for radioactive labelling and Southern blot analysis	500 ng	40-2004-40
Fragile X GeneProber™ GLFX1 Probe Digoxigenin labeled Probe for non-radioactive chemiluminescent Southern blot analysis	110 µL	40-2004-41
Fragile X Genemer™ Primer pair Primers for amplification of CGG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µL PCR reactions.	10 nmols	40-2004-10
Fragile X PCRProber™ AP labeled probe Alkaline phosphatase labeled probe	12 µL	40-2004-31
Fragile X PCRProber™ Kit for chemiluminescent detection Kit for performing PCR amplification and chemiluminescent based detection.	5 blots [50 rxns]	40-2004-32

FRAXE/FMR2/AFF2 Genotyping Product Ordering Information

Product	Unit Size	Catalog No.
FRAXE/FMR2/AFF2 GeneProber™ AFF2-AJ31Dig1 Probe Digoxigenin labeled Probe for non-radioactive chemiluminescent Southern blot analysis	110 µL	40-2054-41
FRAXE/FMR2/AFF2 Genemer™ Kit for gel based detection Kit for performing PCR amplification & gel based detection	1 Kit [100 rxns]	40-2054-11
FRAXE/FMR2/AFF2 GScan™ Kits for fluorescent detection Kit for performing fluorescent PCR amplification based detection, Fam labeled	1 Kit [100 rxns]	40-2054-15FM
Fragile X ~16 CGG repeat Genemer Control Template DNA	500 ng	40-2004-01
Fragile X ~29 CGG repeat Genemer Control Template DNA	500 ng	40-2004-02
Fragile X ~40 CGG repeat Genemer Control Template DNA	500 ng	40-2004-03
Fragile X ~60 CGG repeat Genemer Control Template DNA	500 ng	40-2004-04
Fragile X ~90 CGG repeat Genemer Control Template DNA	500 ng	40-2004-05



Genemer™ Kits Product Ordering Information

Gene Link's Genemer™ kits contain optimized PCR amplification components for convenient agarose or polyacrylamide genotyping of triple repeat disorders and other genetic disorders. These are safe, convenient and sensitive, and afford rapid screening of samples. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. Included in these kits are ready-to-run control samples of various repeats of the specific triple repeat disorder.

The Genemer™ kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed.

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FRAXE/FMR2/AFF2 Genemer™ Kit for gel based detection; 100 reactions kit	1 kit	40-2054-11
Huntington's Disease Genemer™ V2 Kit for gel based detection; 100 reactions kit kit	1 kit	40-2025-11
Myotonic Dystrophy Genemer™ Kit for for gel based detection; 100 reactions kit	1 kit	40-2026-11
Friedreich's Ataxia Genemer™ Kit for gel based detection; 100 reactions kit	1 kit	40-2027-11

All Gene Link products are for research use only

Current pricing are posted at <http://www.genelink.com/>

GeneProber™ Related Product Ordering Information

The GeneProber™ product line is based on the chemiluminescent Southern blot detection method. Gene Link's non-radioactive detection systems for genotyping of triple repeat disorders are rapid, reliable and as sensitive as the ³²P labeled southern blots. No more decayed probes and radioactive exposure. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders.

Unlabeled GeneProber™ probes are also available for radio labeling and radioactive based detection. Gene Link strongly recommends the use of non-radioactive gene detection systems. Consider switching to Gene Link's product line of non-radioactive detection systems.

Product	Unit Size	Catalog No.
Fragile X GeneProber™ GLFX1 Probe unlabeled	500 ng	40-2004-40
Fragile X GeneProber™ GLFXDig1 Probe Digoxigenin labeled	110 µL	40-2004-41
FRAXE/FMR2/AFF2 GeneProber™ AFF2-AJ31Dig1	110 µL	40-2054-41
Huntington's Disease GeneProber™ GLHD14 Probe unlabeled	500 ng	40-2025-40
Huntington's Disease GeneProber™ GLHDDig2X Probe Digoxigenin labeled	110 µL	40-2025-41
Myotonic Dystrophy GeneProber™ GLDM1 Probe unlabeled	500 ng	40-2026-40
Myotonic Dystrophy GeneProber™ GLDMDig2 Probe Digoxigenin labeled	110 µL	40-2026-41
Friedreich's Ataxia GeneProber™ GLFRDA21 Probe unlabeled	500 ng	40-2027-40
Friedreich's Ataxia GeneProber™ GLFRDADig21 Probe Digoxigenin labeled	110 µL	40-2027-41

GScan™ Kits Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument. Included in these kits are ready-to-run control samples of various repeats of the triple repeat disorder kit. These control samples are for calibration with the molecular weight markers for accurate size determination of the amplified fragments.

The GScan™ kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed.

Product	Unit Size	Catalog No.
Fragile X GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX
Fragile X GScan™ V2 Kit for fluorescent detection; 20 reactions kit	1 kit	40-2004-15FMS
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2054-15FM
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2054-15FMS
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 20 reactions kit	1 kit	40-2025-15FMS
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2026-15XX
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2026-15FMS
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2027-15XX
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2027-15FMS

All Gene Link products are for research use only

Current pricing are posted at <http://www.genelink.com/>

Spinocerebellar Ataxia Type 2 (SCA2) Genemer™ CAG Repeat Control Template DNA

For Research Use Only. Not for use in diagnostic procedures for clinical purposes.

Southern Blot Buffers & Reagents

Product	Catalog No.	Unit Size
Agarose Tablets, 0.5 gm each; 100 tablets	40-3011-10	100 tablets
Agarose LE Molecular Biology Grade; 100 g	40-3010-10	100 g
Agarose LE Molecular Biology Grade; 500 g	40-3010-50	500 g
Hybwash A, Hybridization Wash Solution (20X SSC); 200 mL	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution (10% SDS); 100 mL	40-5021-10	100 mL
TAE Buffer; 50 X Concentrate; 100 mL	40-3007-01	100 mL
TAE Buffer; 50 X Concentrate; 1 L	40-3007-10	1 L
TBE Buffer; 5 X Concentrate; 1 L	40-3008-10	1 L
Buffer M 10X (Maleic Acid buffer); 100 mL	40-5025-10	100 mL
10% Blocking solution; 100 mL	40-5026-10	100 mL
Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL	40-5027-10	1 mL
10x AP Detection buffer (alkaline phosphatase detection buffer); 100 mL	40-5031-10	100 mL
Lumisol™ I Hybridization Solution; contains formamide; 200 mL	40-5022-20	200 mL
Lumisol™ II Hybridization Solution; for non-toxic hybridizations; 200 mL	40-5023-20	200 mL
Lumisol™ III Hybridization Solution; for oligo probes; 200 mL	40-5024-20	200 mL
CDP-Star® Substrate; Ready-to-Use 0.25 mM in spray bottle; 10 mL	40-5010-10	10 mL

Loading Buffers

Product	Catalog No.	Size
Gel Loading Buffer 5X BPB/XC non-denaturing; 1 mL	40-3002-10	1 mL
Gel Loading Buffer 5X BPB/XC non-denaturing; 15 mL	40-3002-15	15 mL
Gel Loading Buffer 10X BPB/XC non-denaturing; 1 mL	40-3003-10	1 mL
Gel Loading Buffer 10X BPB/XC non-denaturing; 15 mL	40-3003-15	15 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing; 1 mL	40-3004-10	1 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing; 15 mL	40-3004-15	15 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL	40-5027-10	1 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 15 mL	40-5027-15	15 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer ; 1 mL	40-5028-10	1 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer; 15 mL	40-5028-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 1 mL	40-5029-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 15 mL	40-5029-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide ; 1 mL	40-5030-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide; 15 mL	40-5030-15	15 mL

Omni-Marker™

Product	Catalog No.	Size*
Omni-Marker™ Universal unlabeled; 1 mL	40-3005-10	1 mL
Omni-Marker™ Low unlabeled; 1 mL	40-3006-10	1 mL
Omni-Marker™ GScan™-2 Tamra labeled 50 bp - 600 bp; 500 µL	40-3062-05	500 µL

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Spinocerebellar Ataxia Type 2 (SCA2) Genemer™ CAG Repeat Control Template DNA

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Related Products Ordering Information

Omni-Pure™ DNA & RNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Blood DNA Purification System	40-4010-01	100
Omni-Pure™ Blood DNA Purification System	40-4010-05	500
Omni-Pure™ Blood DNA Purification System	40-4010-10	1000
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100
Omni-Pure™ Tissue DNA Purification System	40-4050-05	500
Omni-Pure™ Tissue DNA Purification System	40-4050-10	1000
Omni-Pure™ Plant DNA Purification System	40-4060-01	100
Omni-Pure™ Plant DNA Purification System	40-4060-05	500
Omni-Pure™ Plant DNA Purification System	40-4060-10	1000
Omni-Pure™ Viral DNA Purification System	40-3720-01	100
Omni-Pure™ Viral DNA Purification System	40-3720-05	500
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
Omni-Pure™ Viral RNA Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Purification System	40-3650-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Clean™ Gel DNA Purification and Concentration Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Clean™ Gel DNA Beads Purification System	40-4110-10	100
Omni-Clean™ Gel DNA Beads Purification System	40-4110-50	500
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-10	100
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-50	500
Omni-Clean™ DNA Beads Concentration System	40-4130-10	100
Omni-Clean™ DNA Beads Concentration System	40-4130-50	500
Omni-Clean™ DNA Spin Column Concentration System	40-4140-10	100
Omni-Clean™ DNA Spin Column Concentration System	40-4140-50	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Pure™ Plasmid DNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System	40-4020-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

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Gene Link, Inc.
190 Saw Mill River Road
Hawthorne,
NY 10532
USA

Tel: (914)769-1192
Email: support@genelink.com
www.genelink.com