Certificate of Analysis & Product Manual

Triple Repeat Disorders Genotyping
Fragile X, Myotonic Dystrophy, Friedreich's Ataxia, Huntington's disease
Fluorescent Probes, siRNA, Hybridization and Detection Reagents

Huntington's Disease Genemer™ Ver2 Kit

Huntington Disease CAG triple repeat genotyping

Catalog No. 40-2025-11

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.

20 years
results you can rely on
Gene Link™

Material Supplied

Huntington Disease CAG Triple Repeat Genotyping Genemer™ V2 Kit			
Kit	Catalog No.	HD Genemer™ Kit 100 X 25 µL Reactions	Size
□	40-2025-11	Huntington Disease Genotyping Genemer™ Kit	100 rxns

Huntington Disease Genotyping Genemer™ V2 Kit 100 Reactions Components			
Content	Catalog No.	HD Genemer™ V2 Kits 100 X 25 µL Reactions	Size
□	40-2025-11F	HD Genemer™ Component F; CAG repeat genotyping	600 µL
□	40-2025-11E	HD GScan™ V2 Component E	800 µL
□	40-2025-11M	HD GScan™ V2 Component M	450 µL
□	40-2025-03S	HD Genemer™ ~44 CAG Repeats control DNA, 2 ng/ µL	100 µL

Storage Condition

Store at -20°C.

Certificate of Analysis & Product Specifications

The Huntington 's disease Genemer™ V2 kit components supplied has been validated to amplify the CAG triple repeat spanning region in the first exon of the *IT15* gene. The length of CAG triple repeat amplification routinely obtained by using standard Taq polymerase is greater than 50 CAG repeats.
Appropriate nuclease free handling, dispensing and storage conditions required.

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

GeneProber™ 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 ^{32}P labeled southern blots. **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™ Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data for routine triple-repeat genotyping 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
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2054-15FM
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2026-15XX
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2027-15XX

Genemer™ Kits Product Ordering Information

Gene Link's Genemer™ kits contain optimized PCR amplification components for convenient agarose gel genotyping of triple repeat disorders and other genetic disorders. These are safe, convenient and sensitive, and afford rapid screening of samples 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

All Gene Link products are for research use only **Current pricing are posted at http://www.genelink.com/**

Huntington Disease Genotyping

Background

Huntington disease (HD) is an autosomal dominant, progressive neurodegenerative disorder with a prevalence rate of about 5-10 affected persons per 100,000 in most western populations. The disorder presents with motor impairment, cognitive deterioration, and psychiatric symptoms.

HD is caused by a CAG trinucleotide expansion within the first exon of the ITI5 gene on chromosome 4p16. The expanded CAG repeats are translated into a polyglutamine tract in the Huntington protein, which is believed to cause a dominant gain of function, leading to neuronal dysfunction and neurodegeneration.

The number of CAG repeats correlates inversely with the age of onset of symptoms. The American College of Medical Genetics/American Society of Human Genetics/ Huntington Disease Genetics Testing Working Group divided genotype/phenotype correlation in the following four categories for CAG repeat lengths:

CAG repeats	Genotype/Phenotype	Clinical Condition	Comments
≤ 26 repeats	Normal allele	unaffected	Normal
27-35 repeats	Intermediate allele	unaffected	Intermediate allele, mutable normal allele generating a normal phenotype
36-39 repeats	HD allele with reduced penetrance	normal or HD phenotype	HD allele with reduced penetrance, generating a normal or HD phenotype
≥ 40 CAG	HD allele	HD phenotype	Generating a HD phenotype.

The CAG trinucleotide expansion is unstable and can lengthen during transmission from parents to offspring. Thus, the stage of onset can decrease from one generation to the next, a phenomenon known as anticipation. HD anticipation is more intense in paternal transmission.

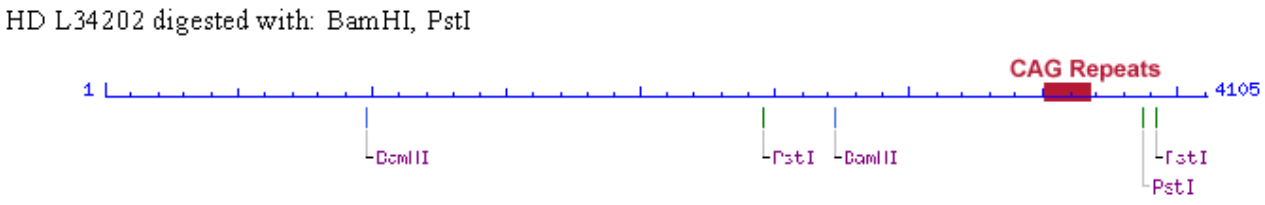


Table 1. Trinucleotide Repeats in Human Genetic Disease

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 (1998*a*) 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

The detection of expansion of a region of DNA sequence can be detected by PCR and Southern blotting procedures. These methods can be used for all disorders involving increase in size of a region of DNA. DNA analysis for direct detection of CAG expansion in Huntington Disease is based on enzymatic amplification of a fragment containing the CAG repeat sequence in exon I of the HD gene. This test detects the CAG expansion by the size of the amplified product; an increase in size is correlated with the corresponding number of CAG repeats and a calculated risk factor. Normal individuals have repeat numbers of up to 30, while individuals with a high probability of developing HD carry more than 37 repeats. Individuals with 30-37 repeats have a high probability of passing on repeats in the pathological size range.

Polymerase Chain Reaction (PCR) based methods are fundamentally similar. The two primers are constructed such that they span the region of the CAG trinucleotide repeat region. PCR is the most common method used to estimate the number of CAG repeats. Since the CAG repeats in the HD gene are immediately 5' of a CCG repeat which is also polymorphic in length, the PCR product of this primer pair excludes the known adjacent polymorphic CCG repeat that can contribute to an inaccurate determination of HD gene CAG repeat sizes in individuals who may have an HD gene CAG repeat allele close to the normal/affected boundary.

Reliable and consistent amplification of the CAG repeat region requires the use of PCR additives e.g. DMSO, TMAC, Betaine and polymerases that are able to amplify high GC rich region. Gene Link has a series of triple repeat genotyping products and kits that reliably amplifies triple repeats for genotyping.

Table 2 lists the size of PCR fragment in base pairs (bp) that can be expected when using the HD GScan™ V2 CAG primer mix F that has been provided. The formula for determining PCR fragment size is 186 + 3n, where n= the number of CAG repeats.

Table 2. HD CAG Fragment F Expected Length *			
$CAG_{(n)}$	**Fragment Size (bp)**	**$CAG_{(n)}$**	**Fragment Size (bp)**
1	189	36	294
2	192	37	297
3	195	38	300
4	198	39	303
5	201	40	306
6	204	45	321
7	207	50	336
8	210	55	351
9	213	60	366
10	216	65	381
11	219	70	396
12	222	75	411
13	225	80	426
14	228	85	441
15	231	90	456
16	234	95	471
17	237	100	486
18	240	105	501
19	243	110	516
20	246	115	531
21	249	120	546
22	252	125	561
23	255	130	576
24	258	135	591
25	261	140	606
26	264	145	621
27	267	150	636
28	270	155	651
29	273	160	666
30	276	165	681
31	279	170	696
32	282	175	711
33	285	180	726
34	288	185	741
35	291	190	756
*Size of PCR fragment in base pairs (bp) that can be expected when using the CAG primer mix F that has been provided. The formula for determining PCR fragment size is 186 + 3n, where n= the number of CAG repeats			

Amplification of CCG and CAG + CCG Regions

Proximal to the 3' end of the CAG trinucleotide repeat region is a second polymorphism that consists of a short sequence of 7-12 CCG trinucleotide repeats. As the presence of a second polymorphism would complicate the estimation of the CAG expansion, primers that amplify the CAG trinucleotide repeat region have been carefully designed to exclude the CCG trinucleotide repeat. However, when only a single allele is detected during amplification of the CAG repeat, inclusion of the CCG polymorphism becomes useful. Detection of a single allele could result from one of the following situations A.) the individual is homozygous for the CAG repeat; B.) a mutation in the region of primer binding precludes amplification of one allele; C.) one allele contains a very large CAG expansion that is not amenable to PCR amplification.

Situations A and B can usually be resolved by amplification of the CAG + CCG region. Individuals that are homozygous for the CAG repeat may not be homozygous for the CCG repeat, thus allowing for detection of the second allele. To verify whether the individual is heterozygous for the CCG repeat, a primer mix for amplification of the CCG repeat region has been included in the kit.

For situation B, the mutations that interfere with primer binding have been shown to occur primarily in the 3' region of the CAG repeat and affect the reverse CAG primer. The reverse primer used for amplification of the CAG + CCG region binds to the DNA downstream from the mutable area and results in detection of the second allele.

In situation C both CAG and CAG + CCG amplification would detect only one allele. Detection of a second allele would be possible by amplification of the CCG region, but only if the individual were heterozygous for the CCG polymorphism. In the case of very large CAG expansions it is probably best to perform analysis by Southern blotting.

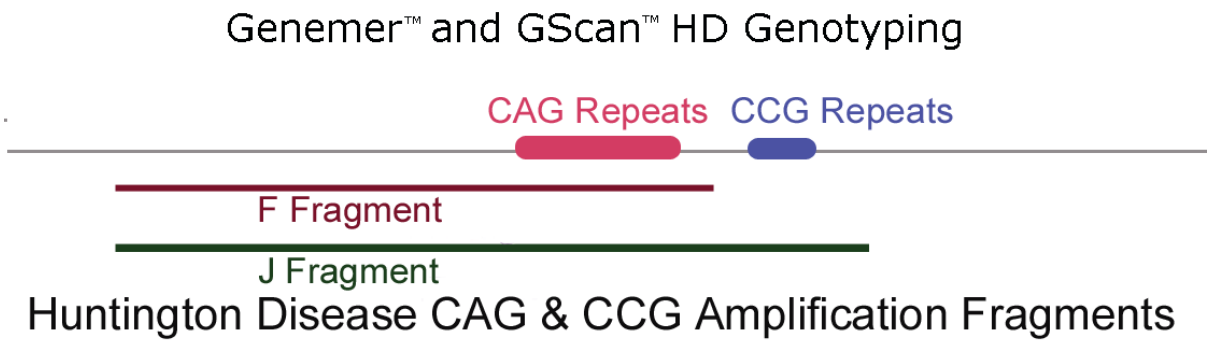


*Table 3. HD CCG Fragment G Expected Length	
$(CCG)_n$	Fragment Size (bp)
7	173
8	176
9	179
10	182
11	185
12	188
*Above table lists the size of PCR fragment in base pairs (bp) that can be calculated when using the CCG repeat region G primers. The formula for determining PCR fragment G size is 152 + 3n, where n= the number of CCG repeats.	

*Table 4. HD CAG + CCG Fragment J Expected Length						
(CAG)n	(CCG)n					
	7	8	9	10	11	12
	Fragment Size					
5	344	347	350	353	356	359
10	359	362	365	368	371	374
15	374	377	380	383	386	389
20	389	392	395	398	401	404
25	404	407	410	413	416	419
30	419	422	425	428	431	434
35	434	437	440	443	446	449
40	449	452	455	458	461	464
45	464	467	470	473	476	479
50	479	482	485	488	491	494
55	494	497	500	503	506	509
60	509	512	515	518	521	524
65	524	527	530	533	536	539
70	539	542	545	548	551	554
75	554	557	560	563	566	569
80	569	572	575	578	581	584
85	584	587	590	593	596	599
90	599	602	605	608	611	614
95	614	617	620	623	626	629
100	629	632	635	638	641	644
105	644	647	650	653	656	659
110	659	662	665	668	671	674
115	674	677	680	683	686	689
120	689	692	695	698	701	704
125	704	707	710	713	716	719
130	719	722	725	728	731	734
135	734	737	740	743	746	749
140	749	752	755	758	761	764
145	764	767	770	773	776	779
150	779	782	785	788	791	794

*Above table lists the size of PCR fragment in base pairs (bp) that can be expected when using the primer mix J that amplifies the region that includes both the CAG and CCG repeats. The formula for determining PCR fragment J size is $308 + 3(CAG)_n + 3(CCG)_n$, where n is the number of trinucleotide repeats

Genemer™ Kit Agarose Gel Analysis

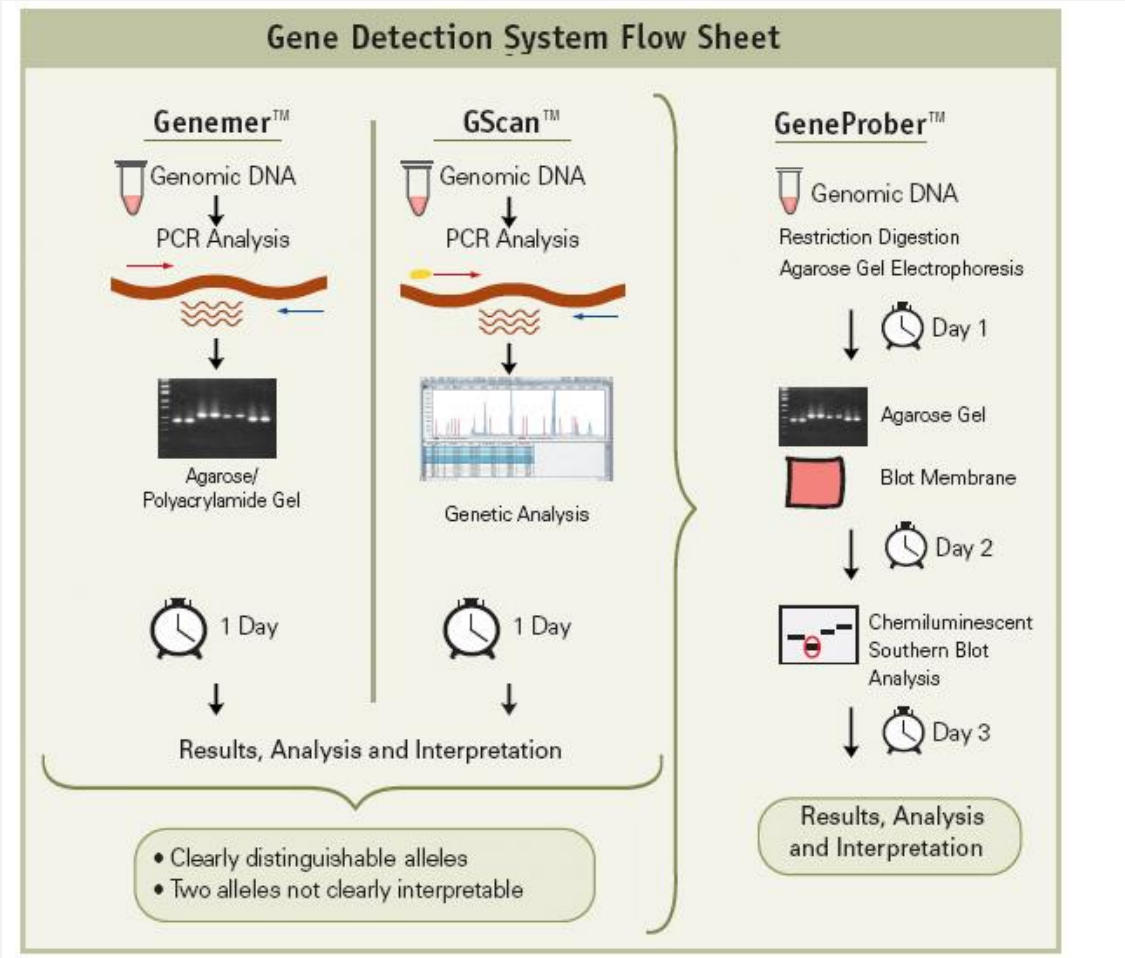
Genemer™ kit contains optimized components for PCR amplification of greater than 100 triple repeats using standard Taq polymerase. Amplified samples are resolved by agarose gel electrophoresis. This Genemer™ method or GScan™ fluorescent detection is recommended for initial screening of all samples.

GScan™ Kit

GScan™ kit contains optimized components for PCR amplification of greater than 100 triple 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.



Procedure

Huntington Disease CAG Repeats Analysis by PCR

Thaw individual components. *Promptly store at -20°C after use*. Prepare ***fresh*** before use enough PCR premix for the number of reactions to be performed. Prepare 10% more for pipetting allowance. Prepare premix following the volumes given below. Follow the same ratio for preparing other final volumes.

Huntington's Disease Genemer™ Control DNA

□	40-2025-03S	HD Genemer™ ~44 CAG Repeats control DNA, 2 ng/ µL	100 µL

Huntington's Disease control DNA containing ~44 CAG repeats [40-2025-03] is included in this kit. Use 1 to 2 µL of this DNA as control amplification. A fragment will be amplified with ~44 CAG repeats.

PCR Thermal Cycler Files: Program the following PCR thermal cycler files

Hot Start File		
Step	Time and Temperature	Cycles
Denaturation	5 minutes at 98°C	1
Hold	Hold for infinity at 62°C	Hold

HD CAG & CCG Amplification File		
Step	Time and Temperature	Cycles
Initial Denaturation	5 minutes at 95°C	1
Denaturation	30 seconds at 94°C	30
Annealing	30 seconds at 65°C	
Extension*	60 seconds at 72°C	
Fill up	7 minutes at 72°C	1
Hold	Hold for infinity at 4°C	Hold for infinity
*For CAG expansion greater than 70, the extension time can be increased to 1 minute.		

Protocol

A. PCR premix preparation

Given below is a protocol for preparing a PCR premix for 25 µL reactions. This can be scaled up as required.

F Fragment (CAG) Only PCR Premix Preparation		
Component	1 x 25 µL rxn.	10 x 25 µLrxns.*
Sterile water	8.0 µL	80 µL
HD GScan™ Component F	5.5 µL	55 µL
PCR Component E	7.5 µL	75 µL
PCR Component M	4.0 µL	40 µL
Total	**25 µL**	**250 µL**
*Take into account that 3 µL/reaction will be used to prepare Taq polymerase mix (Enzyme Mix, EM)		

B. Enzyme premix (for 10 reactions). Label tube as "EM" (Enzyme mix)

Enzyme Mix Preparation-EM	
Component	Volume
PCR premix (from above)	30 µL
Taq. Polymerase*	2.5 µL
Total	**32.5 µL**

*The kit is optimized for regular Taq Polymerase. Any good quality Taq polymerase that is regularly used in the laboratory can be used.

C. Initial Denaturation/ Hot Start PCR

For each sample add the following

Hot Start PCR	
Component	Volume
PCR premix (above)	21 µL
DNA Template (~100ng chromosomal DNA)	1 µL

Transfer all sample tubes to thermal cycler and start "Hot Start" file. This step is used when using standard Taq polymerase. This step can be skipped if using hot start Taq polymerase.

D. PCR Amplification

After initial denaturation while thermal cycler is 'holding' at 62^{o}C add Enzyme Mix-EM as follows to each tube.

PCR Amplification	
Component	Volume
Enzyme Premix-EM (from step B above)	3 µL

Start HD CAG & CGG thermal cycling amplification file.

E. Analysis

PCR products can be analyzed by 2.5% agarose gel electrophoresis or by 10% polyacrylamide gel electrophoresis. An aliquot of 5-10 µL can be directly analyzed by adding non-denaturing loading (Gene Link product; Loading buffer 10X BPB/XC non-denaturing; 15 mL**;** Catalog #: 40-3003-15).

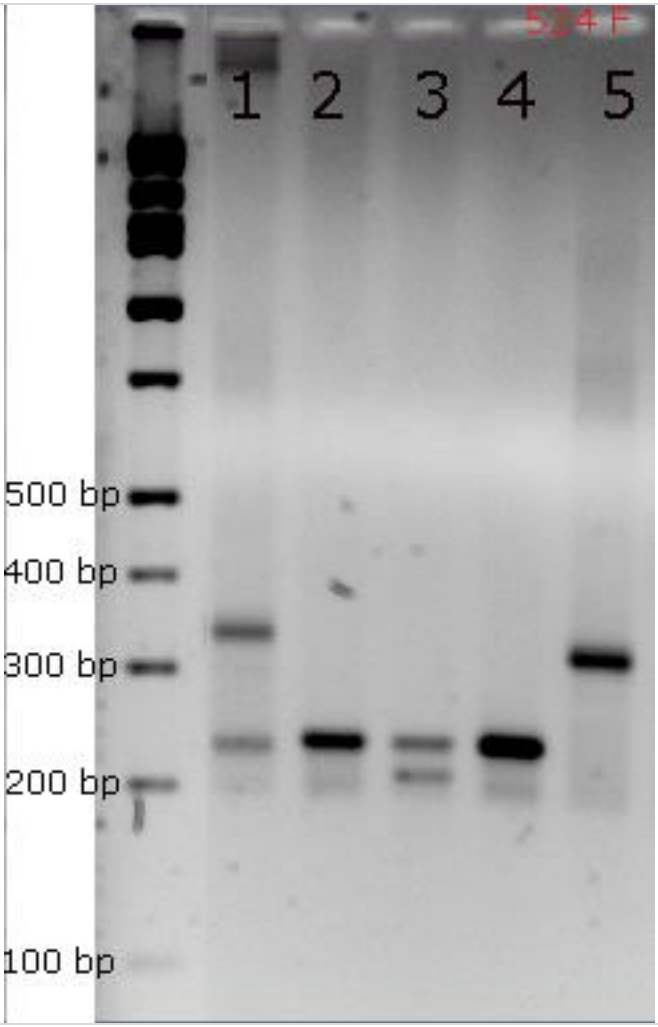
F. CAG Size Standards

Due to their high GC content and the increased probability of secondary structure, fragments containing the CAG expansion tend to migrate faster during gel electrophoresis than corresponding molecular weight standards that usually have random GC contents. Furthermore, electrophoretic mobility increases with the size of the CAG expansion.

To correct for the anomalous migration rate of the CAG fragments, Gene Link provides control DNA samples and GScan™ ready to run samples of various CAG sizes. Please refer to product information page for details. The CAG size standards were generated by PCR using the primers provided with this kit and template DNA from exon I of the HD gene that contained CAG repeats of known size. The number of CAG repeats was verified by sequence analysis. 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 triple repeats. These control DNA's are sold with the express condition that these NOT be used for exact triple repeat size determination of DNA of unknown genotype. The control DNA should be used for determining the performance of specific GScan™, Genemer™ and PCRProber™ Gene Link products.

Results and Interpretation

A representative 2.5% agarose gel electrophoresis ethidium bromide stained gel photograph is shown below.



The Huntington primer pair (HD-Seq1/HD3N2FU) was used using GScan™ reaction conditions (40-2025-15FM) to amplify F fragment spanning the CAG triple repeat region.
Lane 1. Coriell Institute reference HD affected female genomic DNA sample NA13506. 17 & 48 CAG repeats, ~ 237 and 330 bp fragments.
Lane 2 & 3. Normal genomic DNA sample, ~ 220 and 240 bp fragments.
Lane 4. Huntington Disease 18 CAG Genemer Control DNA (40-2025-01), ~ 240 bp fragment.
Lane 5. Huntington Disease 44 CAG Genemer Control DNA (40-2025-03), ~ 318 bp fragment.

References

1. Kremer, B et al. (1993) N. ENG. J. Med. 330: 1401-1406.
2. The American College of Medical Genetica/American Society of Human Genetics Huntington Disease Genetic Testing Working Group (1998) Am. J. Hum. Genet. 62: 000-000
3. Reiss O, Noerremoelle A, Soerensen SA, Epplen JT. Hum Mol Genet (1993) 2: 637-642.
4. Yu S, Fimmel A, Fung D, Trent RJ. Clin. Genet. (2000) 58: 469-472.
5. Williams LC, Hedge MR, Herrera G, Stapleton PM, Love DR. Mol. and Cell. Probes (1999) 13: 283-289.

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
Rapid DNA Purification Protocol for 300 µl Whole Blood

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 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 Tm and the complex secondary structure, thus the duplex stability (1-5). Tetramethyl ammonium chloride (TMAC) actually increases the specificity of hybridization and increases the Tm. 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]trimethylammonium)	Reduces Tm 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 µg/µl to 0.1 µg/ µl 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 *Taq* 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 stabilise *Taq* polymerase and may also supress 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 *Taq* 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 Tm 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.

The preferred method of purification of an amplified fragment is the excision of the fragment band after agarose gel electrophoresis. This method yields the purification of a single fragment; as such care should be taken to excise a gel piece containing a single electrophoretically resolved fragment. The Omni-Clean™ Purification System available from Gene Link can be used for this purpose. Catalog No. 40-4110-10 for bead based system; 40-4120-10 for spin column based system and 40-4130-10 for DNA concentration. Please refer to product insert for detailed protocol or visit **www.genelink.com**.

A. **Purification of DNA from gel slices using glass beads. Provides purified single fragment.**
[Omni-Clean™ Gel DNA Beads Purification System; Catalog No. 40-4110-10]

Protocol

1. By weight, determine the volume of the excised DNA fragment.
2. Add 3 volumes of NaI solution and heat to 55 °C. Visually determine the dissolution of gel pieces.
3. Add 1 µl of glass bead suspension per µg of DNA and vortex.
4. Centrifuge at 2K rpm for 20 seconds to pellet glass bead/DNA complex. Discard supernatant.
5. Re-suspend pellet in 400 µl Omni-Clean™ wash buffer. Centrifuge at 2K rpm for 20 seconds and discard wash buffer.
6. Pipet out any remaining buffer in the tube.
7. Add 25 µl water or TE; re-suspend pellet and centrifuge at 2K rpm for 20 seconds.
8. 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 gel slices using spin column. Provides purified single fragment.**
[Omni-Clean™ Gel DNA Spin Column Purification System; Catalog No. 40-4120-50]

Protocol

1. By weight, determine the volume of the excised DNA fragment.
2. Add 3 volumes of NaI solution and heat to 55 °C. Visually determine the dissolution of gel pieces.
3. Add the above solution to the spin column assembled on a collection tube.
4. Let the solution flow by gravity or centrifuge at 2K rpm for 20 seconds. Discard flow through collected in the collection tube.
5. Add 400 µl Omni-Clean™ wash buffer to the spin column. Centrifuge at 2K rpm for 2 minutes and discard wash buffer collected in the collection tube.
6. Replace the collection tube with a new appropriately labeled 1.5ml tube.
7. Add 25 µl water or TE to the spin column. Let sit for 3 minutes.
8. Centrifuge at 2K rpm for 2 minutes.
9. The collection tube contains the purified DNA.

C. **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 3 volumes of NaI solution.
2. Add 1 µl of glass bead suspension per µg of DNA.
3. Centrifuge at 2K rpm for 20 seconds to pellet glass bead/DNA complex. Discard supernatant.
4. Re-suspend pellet in 400 µl Omni-Clean™ wash buffer.
5. Centrifuge at 2K rpm for 20 seconds and discard wash buffer.
6. Pipet out any remaining buffer in the tube.
7. Add 25 µl water or TE; re-suspend pellet and centrifuge at 2K rpm for 20 seconds.
8. The supernatant contains the purified DNA. Using a pipet, collect the supernatant and transfer to a new appropriately labeled tube.

D. **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 3 volumes of NaI solution.
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™ 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.

PEG Precipitation

Primers and salts are efficiently removed by a simple PEG precipitation. This method is recommended for downstream DNA sequencing application. This method is generally used for plasmid DNA.

Protocol

1. To 50 µl of amplified PCR reaction add 6.0 µl of 5 M NaCl and 40 µl of 13% (w/v) PEG 8000. Incubate the mixture on ice for 20-30 minutes.
2. Collect the DNA precipitate by centrifugation at maximum speed for 15 minutes at 4 °C in a microfuge. Carefully remove the supernatant by gentle aspiration.
   The pellet of DNA is translucent and generally invisible at this stage.
3. Rinse the pellet with 500 µl of 70% ethanol.
   The precipitate changes to a milky-white color and becomes visible.
4. Carefully pour off the 70% ethanol. Rinse the DNA pellet once more with 70% ethanol. Store the tube in an inverted position at room temperature until the last visible traces of ethanol have evaporated.
5. Dissolve the DNA in 20 µl of H_20.
6. Run an aliquot on an agarose gel to confirm the presence of the correct amplified product. The purified DNA is sequence grade and can be used directly for sequencing.

Gel Filtration

Primers and salts are efficiently removed by gel filtration using Sephadex G-50. This method is recommended for downstream DNA sequencing application.

Protocol

1. Hydrate Sephadex G-50 ahead of time in sterile water or TE (10mM Tris pH 8, 1 mM EDTA). Take out from fridge if already stored hydrated. Bring to room temperature.
2. Assemble a spin column on a collection tube.
3. Add 700 µl of hydrated Sephadex G-50 to each spin column, initiate flow using rubber bulb or any other method.
4. Allow flowing by gravity till there is no more fluid left above the Sephadex G-50 bed. Discard flow through from the collection tube.
5. Spin the spin column placed inside the collection tube for 2 minutes at 3 K rpm.
6. Change collection tube to new 1.5 ml tube appropriately labeled with sample name.
7. Apply up to 50 µl sample gently to the G-50 bed of the column.
8. Spin for 2 minutes at 3 K rpm.
9. Purified sample is collected in the collection tube. The eluent collected in the 1.5 ml tube is free of salts and primers shorter than 35-40mer.

References

1. Kovarova, M; and Draber, P; (2000) New Specificity and yield enhancer for polymerase chain reactions (2000) Nucl. Acids. Res. 28: e70.
2. Henke, W., Herdel, K., Jung, K., Schnorr, D. and Stefan A. Loening, S. (1997) Betaine improves the PCR amplification of GC-rich DNA sequences. Nucl. Acids Res. 25: 3957-3958.
3. Daniel S. Mytelka, D.S., and Chamberlin, M.J.,(1996) Analysis and suppression of DNA polymerasepauses associated with a trinucleotide consensus. Nuc. Acids Res.,. 24:2774–278.
4. Keith, J. M., Cochran, D.A.E., Lala, G.H., Adams, P., Bryant, D.and Mitchelson, K.R. (2004) Unlocking hidden genomic sequence. Nucl. Acids Res. 32: e35.
5. Owczarzy, R., Dunietz, I., Behlke, M.A., Klotz, I.M. and Joseph A. Walder. (2003) Thermodynamic treatment of oligonucleotide duplex–simplex equilibria. PNAS, 100:14840-14845.

Huntington's Disease Product Ordering Information

Product	Unit Size	Catalog No.
Huntington's Disease Genemer™ Primer pair Primers for amplification of CAG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µL PCR reactions.	10 nmols	40-2025-10
Huntington's Disease Genemer™ Kit Primers for amplification of CAG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µL PCR reactions.	100 rxns	40-2025-11
Huntington's Disease PCRProber ™ AP labeled probe Alkaline phosphatase labeled probe	12 µL	40-2025-31
Huntington's Disease PCRProber ™ Kit for chemiluminescent detection Kit for performing PCR amplification and chemiluminescent based detection.	5 blots [50 rxns]	40-2025-32
Huntington's Disease Genemer™ Kit for Radioactive Detection Kit for amplification and radioactive detection of Huntington's Disease CAG triple repeat region amplified PCR products using ^{35}S or ^{32}P. 100 Reactions.	1 Kit [100 rxns]	40-2025-20
Huntington's Disease GScan Kit 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]	40-2025-15XX
Huntington's Disease GeneProber™ GLHD14 Probe unlabeled Probe for radioactive labelling and Southern blot analysis	500 ng	40-2025-40
Huntington's Disease GeneProber™ GLHDDig2X Probe Digoxigenin labeled Probe for non-radioactive chemiluminescent Southern blot analysis	110 µL	40-2025-41

Genemer™ GScan Control DNA Cloned fragment of the mutation region of a particular gene. These control DNAs are ideal genotyping templates for optimizing and performing control amplification with unknown DNA. The size of the triple 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 triple repeats. These control DNAs are sold with the express condition that these NOT be used for exact triple repeat size determination of DNA of unknown genotype. The control DNA should be used for determining the performance of specific Genemer™ and PCRProber™ Gene Link products.

Huntington's Disease 7 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-05HX
Huntington's Disease 18 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-01HX
Huntington's Disease 31 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-07HX
Huntington's Disease 34 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-02HX
Huntington's Disease 37 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-08HX
Huntington's Disease 44 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-03HX
Huntington's Disease 49 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-09HX
Huntington's Disease 89 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-04HX
Huntington's Disease 116 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-06HX
Huntington's Disease 134 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-61HX
Huntington's Disease 182 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-62HX

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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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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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Notes:

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