

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 CAG Repeat Genotyping GScan™ Ver3 Kits

Huntington Disease CAG triple repeat fluorescent genotyping

Catalog No. 40-2025-15

Storage Condition: See Material Supplied List

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

Important Information

HD GScan™ Version 3 kit components are not compatible with earlier version kit components.

All Gene Link products are for research use only.

Not for use in diagnostic procedures for clinical purposes.

Genotyping using this kit requires use of the appropriate fluorescent genetic analyzer instrument and software capable of detection of fluorescently labeled fragments of varying lengths.

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

Huntington's Disease CAG Repeat Genotyping GScan™ Ver3 Kits

HD GScan™ Version 3 kit components are not compatible with earlier version kit components.

Huntington Disease CAG Repeat Genotyping GScan™ V2 Kits			
Kit	Catalog No.	Description	Size
□	40-2025-15FM	Huntington Disease Genotyping GScan™ V3 6-Fam Kit; 100 X 25 µL rxns.	100 rxns
		HD GScan™ V2 Kits 20 X 25 µL Reactions	
□	40-2025-15FMS	Huntington Disease Genotyping GScan™ V3 6-Fam Kit; 20 X 25 µL rxns.	20 rxns

Huntington Disease CAG Repeat Genotyping GScan™ V2 Kit 100 Reactions Components			
Content	Catalog No.	HD GScan™ V2 Kits 100 X 25 µL Reactions	Size
□	40-2025-15FFM	HD GScan™ V3 6-Fam Component F	600 µL
□	40-2025-15E	HD GScan™ V2 Component E	800 µL
□	40-2025-15M	HD GScan™ V2 Component M	450 µL
□	40-2025-15W	HD GScan™ 6-Fam amplified 17/48 CAG Repeats control	10 µL

Huntington Disease Genotyping GScan™ V2 Kit 20 Reactions Components			
Content	Catalog No.	HD GScan™ V2 Kits 20 X 25 µL Reactions	Size
□	40-2025-15FFMS	HD GScan™ V3 6-Fam Component F; 20 X 25 µL Reactions	150 µL
□	40-2025-15ES	HD GScan™ V2 Component E	200 µL
□	40-2025-15MS	HD GScan™ V2 Component M	100 µL
□	40-2025-15WS	HD GScan™ 6-Fam amplified 17/48 CAG Repeats control	5 µL

Storage Condition

Store at -20°C

Certificate of Analysis & Product Specifications

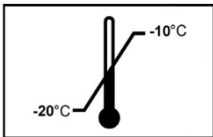
The Huntington's disease GScan™ V2 kit components supplied has been validated to amplify the CAG triple repeat spanning region in the first exon of the *IT15* gene. The length of CAG triple repeat amplification routinely obtained by using standard Taq polymerase is greater than 50 CAG repeats.




Appropriate nuclease free handling, dispensing and storage conditions required.

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

Product Label Information

REF	Catalog No.	Description	Size
	40-2025-15FM	Huntington Disease CAG Repeat Genotyping GScan™ V3 6-Fam Kit; 100 X 25 µL rxns.	100 rxns
	40-2025-15FMS	Huntington Disease CAG Repeat Genotyping GScan™ V3 6-Fam Kit; 20 X 25 µL rxns.	20 rxns

RUO Research Use Only		LOT
Research Use Only	Storage Store at -20°C to -10°C	Lot Number Stated on product tube and packing slip

		
Expiry One year from Date of Shipment	Instructions Consult product manual	QR Code Visit Gene Link website for product details

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
Mitochondrial DNA deletion GeneProber™ GL557 Digoxigenin labeled	110 µL	40-2055-41

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	40-2004-15XX
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/>

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 IT15 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.

HD L34202 digested with: BamHI, PstI

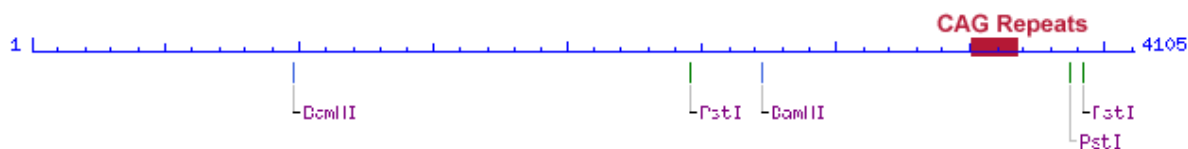


Table 1. Trinucleotide Repeats in Human Genetic Disease

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

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

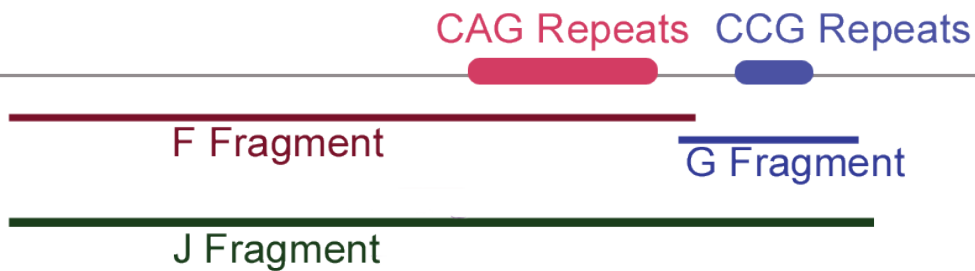
Amplification of CCG and CAG + CCG Regions

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.



Huntington Disease CAG & CCG Amplification Fragments

*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 $308 + 3(\text{CAG})_n + 3(\text{CCG})_n$, where n is the number of trinucleotide repeats

Genemer™ Kit Agarose Gel Analysis

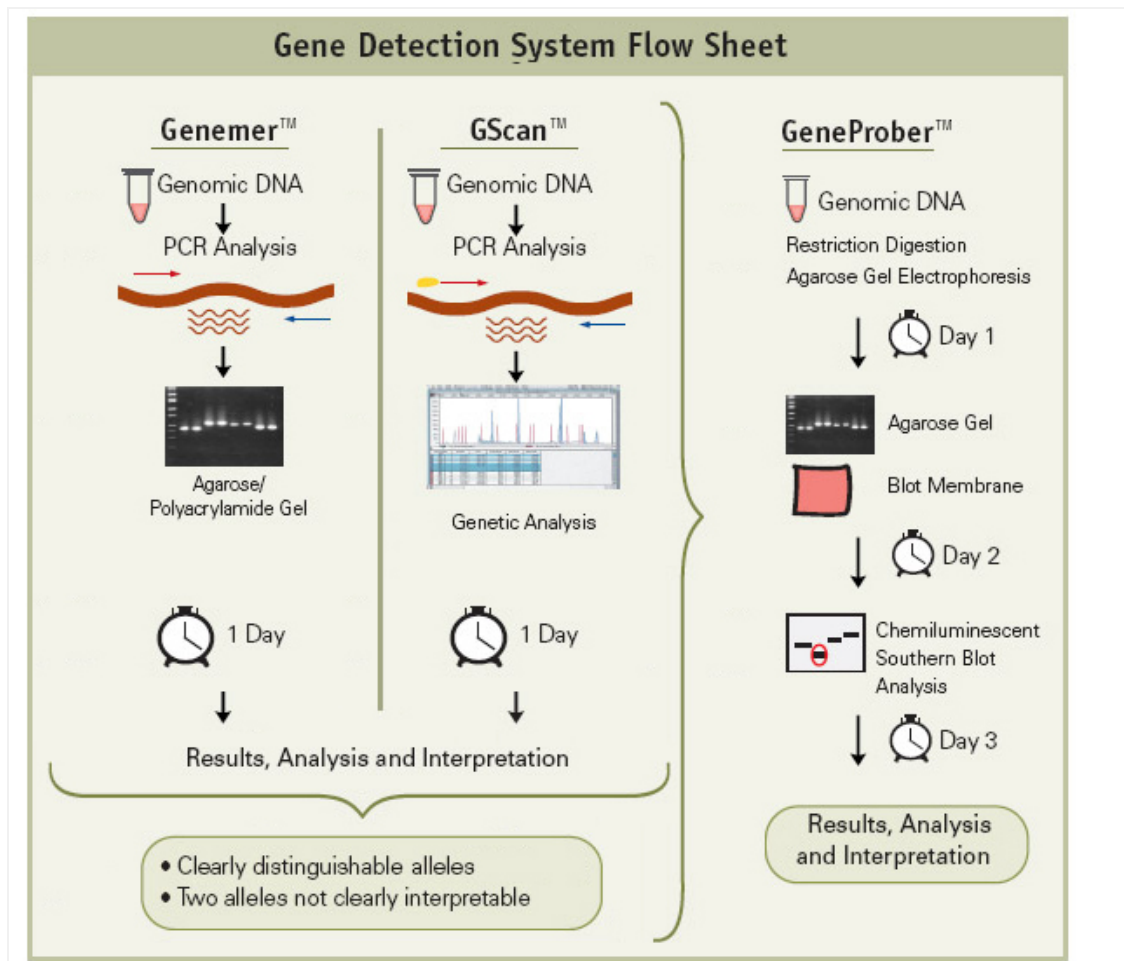
Genemer™ kit contains optimized components for PCR amplification of greater than 100 triple repeats using standard Taq polymerase. Amplified samples are resolved by agarose gel electrophoresis. This Genemer™ method or GScan™ fluorescent detection is recommended for initial screening of all samples.

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

Important Information

Genotyping using this kit requires use of the appropriate fluorescent genetic analyzer instrument(s) and software capable of detection of fluorescently labeled fragments of varying lengths. This kit has been optimized for an ABI3100 genetic analyzer.

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.

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

HD Amplification File		
Step	Time and Temperature	Cycles
Initial Denaturation	2 minutes at 95°C	1
Denaturation	20 seconds at 94°C	42
Annealing	20 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 µL rxns.*
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
Taq Polymerase*	1 µL	10 µL
Total	26 µL	260 µL
*The kit is optimized for regular Taq Polymerase. Any good quality Taq polymerase that is regularly used in the laboratory can be used.		

B. PCR Setup

PCR Setup	
Component	Volume
PCR premix (from above)	25 μ L
Template DNA (~100ng chromosomal DNA)	1 μ L

Transfer all sample tubes to thermal cycler and start amplification.

C. Post PCR Processing

Process sample for [Omni-Clean](#) purification of amplified products. This purification method is preferred over Sephadex G-50 spin column chromatography or ethanol precipitation as it is the most effective way to reduce the fluorescent primers and shorter primer dimers if present. [Omni-Clean Kit](#) is available from Gene Link, catalog number 40-4130-10 or 40-4140-10.

OR

Process sample for Sephadex G-50 spin column chromatography. G-50 columns should be equilibrated with sterile water.

OR

Process sample for ethanol precipitation.

Dissolve purified fragments in 20 μ L water. Ready for fragment analysis.

D. Fragment Analysis

Follow genetic analyzer manufacturer's instructions. The amplified fragments are labelled with 6-Fam as the fluorophore. Use appropriate filter set for detection of 6-Fam emission. Molecular weight markers preferable should be 500 Liz or other compatible dye set for particular instrument type.

Run two aliquots of sample at different concentrations. A brief protocol is given below for ABI3100 instrument.

1. Add 0.5 µL of appropriate size standards.
2. Add 1 & 2 µL of sample in duplicate tubes.
3. Add 12 µL of formamide loading buffer.
4. Mix and heat denature at 95°C for 5 minutes.
5. Transfer to ice.
6. Samples ready to load on instrument.

E. CAG Size Standards

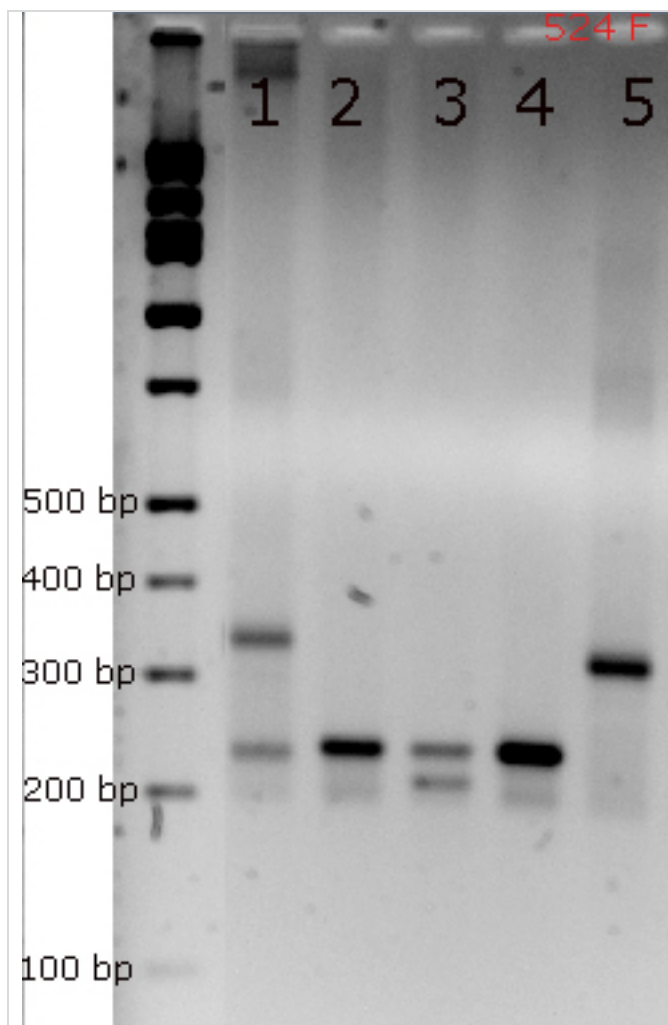
Due to their high GC content and the increased probability of secondary structure, fragments containing the CAG expansion tend to migrate faster during gel electrophoresis than corresponding molecular weight standards that usually have random GC contents. Furthermore, electrophoretic mobility increases with the size of the CAG expansion. A ready to run HD gene GScan™ sample is included in this kit. Please refer to page 2 of this manual for exact specifications. We recommend that 2 µL of this sample be loaded with appropriate molecular weight markers. The sample is provided in water. Follow genetic analyzer manufacturer's instructions relating to running samples, data recovery, and interpretation.

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

The results obtained from the genetic analyzer will approximately show the fragment size amplified, based on these results an interpretation can be made about the genotype of the sample. It is known that there is an overlap between the normal and HD allele sizes. The repeat sizes obtained falling in the overlap region should be preferably repeated and possibly run with more samples from other family members. Individuals with 36 repeats can be affected, and individuals with 36-39 repeats can reach old age without developing HD. There is evidence that repeats in the 30-35 repeat range are prone to expansions at meiosis, so it may be wise to suggest prenatal diagnosis, where appropriate, for individuals carrying such expansions.

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.

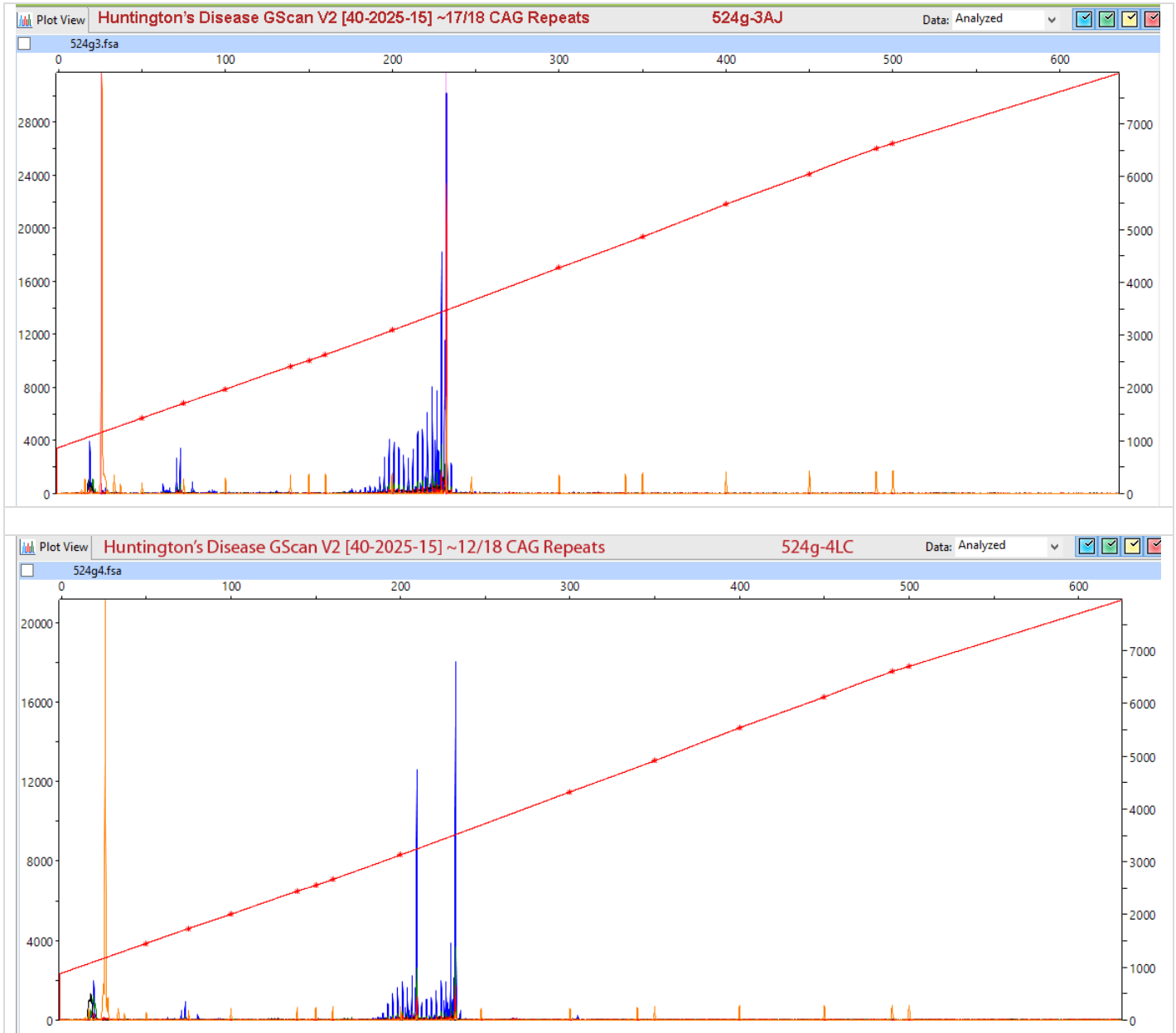
Lane 2 & 3. Normal genomic DNA sample, ~ 220 and 240 bp fragments.

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.

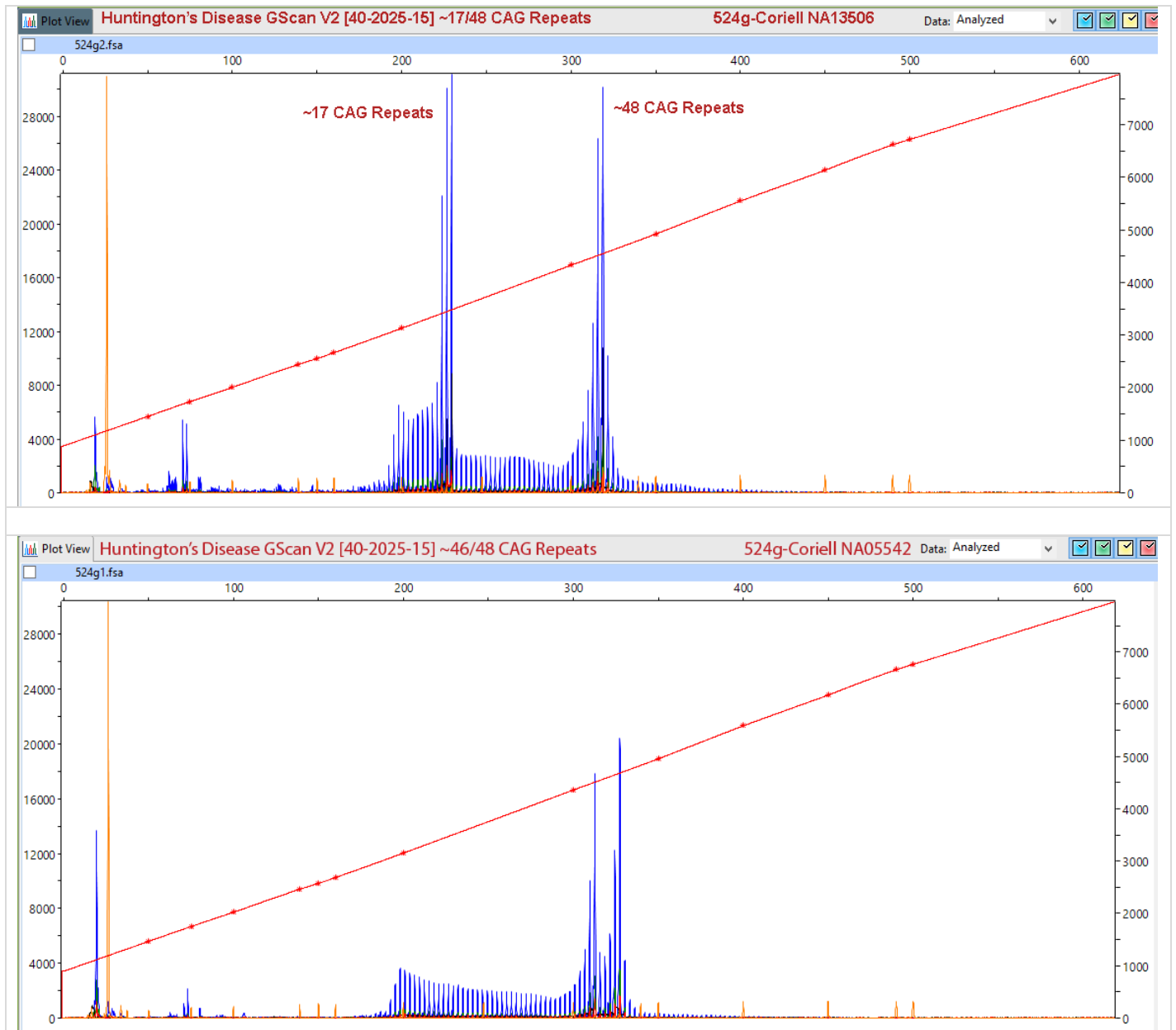
Huntington's Disease GScan V2™. Huntington Disease CAG triple repeat fluorescent genotyping

For research use only. Not for use in diagnostic procedures for clinical purposes.



Huntington's Disease GScan V2™. Huntington Disease CAG triple repeat fluorescent genotyping

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References

1. Kremer, B et al. (1993) N. ENG. J. Med. 330: 1401-1406.
2. The American College of Medical Genetics/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.

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

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

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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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