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

C9orf72 ALS hexanucleotide expansion Genotyping GeneProber™ ALS-GL577

C9orf72 hexanucleotide (GGGGCC) expansion chemiluminescent Southern blot genotyping

Catalog No. 40-2057-41

Storage Condition: -20°C

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

C9orf72 ALS hexanucleotide expansion Genotyping GeneProber™ ALS-GL577

C9orf72 hexanucleotide (GGGGCC) expansion detection probe GL557 digoxigenin labeled probe for Southern blot genotyping

REF	Catalog No.	Description	Size
	40-2057-41	C9orf72 ALS hexanucleotide expansion Genotyping GeneProber™ ALS-GL577	110 µL

Certificate of Analysis & Product Specifications

One tube containing 110 µL of C9orf72 ALS hexanucleotide expansion Genotyping GeneProber™ ALS-GL577 digoxigenin labeled probe at a concentration of ~40ng/uL. This probe is digoxigenin labeled for non-radioactive detection. The quantity supplied is sufficient for at least 5 20x20 cm blots using 20µL for each blot as probe.

Appropriate nuclease free handling, dispensing and storage conditions required.

Product Label Information

RUO Research Use Only	-10°C / -20°C	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 ^{32}P 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.
C9orf72 ALS hexanucleotide expansion GeneProber™ ALS-GL577 Digoxigenin labeled	110 µL	40-2057-41
Mitochondrial DNA deletion GeneProber™ GL557 Digoxigenin labeled	110 µL	40-2055-41
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™ 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. 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/**

Genotyping Amyotrophic lateral sclerosis (ALS) C9orf72 hexanucleotide expansion

Background

Amyotrophic lateral sclerosis (ALS) is a progressive disease that affects motor neurons, which are specialized nerve cells that are important for controlling muscle movement and strength. These nerve cells are found in the spinal cord and the brain. In ALS, motor neurons die over time, leading to problems with muscle control and movement.

There are many different types of ALS; these types are distinguished by their signs and symptoms and their genetic cause or lack of clear genetic association. Most people with ALS have a form of the condition that is described as sporadic, which means it occurs in people with no apparent history of the disorder in their family. People with sporadic ALS usually first develop features of the condition in their late fifties or early sixties. A small proportion of people with ALS, estimated at 5 to 10 percent, have a family history of the condition. The signs and symptoms of familial ALS typically first appear in one's late forties or early fifties. Rarely, people with familial ALS develop symptoms in childhood or their teenage years. These individuals have a form of the disorder known as juvenile ALS.

The first signs and symptoms of ALS may be so subtle that they are overlooked. The earliest symptoms include muscle twitching, cramping, stiffness, or weakness. Affected individuals may develop slurred speech and, later, difficulty chewing or swallowing (dysphagia). Many people with ALS experience malnutrition because of reduced food intake due to dysphagia and an increase in their body's energy demands (metabolism) due to prolonged illness. Muscles become weaker as the disease progresses, and arms and legs begin to look thinner as muscle tissue wastes away (atrophies). Individuals with ALS lose their strength and the ability to walk. Affected individuals eventually become wheelchair-dependent. Over time, muscle weakness causes affected individuals to lose the use of their hands and arms. Breathing becomes difficult because the muscles of the respiratory system weaken. Most people with ALS die from respiratory failure within 2 to 10 years after the signs and symptoms of ALS first appear; however, disease progression varies widely among affected individuals.

Approximately 20 percent of individuals with ALS also develop a condition called frontotemporal dementia (FTD), which is a progressive brain disorder that affects personality, behavior, and language. Changes in personality and behavior may make it difficult for affected individuals to interact with others in a socially appropriate manner. People with FTD increasingly require help with personal care and other activities of daily living. Communication skills worsen as the disease progresses. It is unclear how the development of ALS and FTD are related. Individuals who develop both conditions are diagnosed as having ALS-FTD.

How common is amyotrophic lateral sclerosis?

About 5,000 people in the United States are diagnosed with ALS each year. Worldwide, this disorder occurs in 4 to 8 per 100,000 individuals. Only a small percentage of cases have a known genetic cause.

What genes are related to amyotrophic lateral sclerosis?

Mutations in the *C9orf72* gene are responsible for 30 to 40 percent of familial ALS in the United States and Europe. Mutations in several genes, including the *C9orf72*, *SOD1*, *TARDBP*, *FUS*, *ANG*, *ALS2*, *SETX*, and *VAPB* genes, cause familial ALS and contribute to the development of sporadic ALS. Worldwide, *SOD1* gene mutations cause about 20

percent of familial ALS, *TARDBP* gene mutations account for about 5 percent, *FUS* gene mutations cause about 5 percent, and *ANG* gene mutations account for around 1 percent. The other genes that have been associated with familial ALS each account for a small proportion of cases. It is estimated that 60 percent of individuals with familial ALS have an identified genetic mutation. The cause of the condition in the remaining individuals remains unknown.

The genes associated with ALS play a role in the functioning of neurons or are involved in regulating the production of various proteins, although the specific role of the *C9orf72* gene is not known. It is unclear how mutations in the genes associated with ALS contribute to the death of motor neurons. Most motor neurons affected by ALS have a buildup of protein clumps (aggregates); however, it is unknown whether these aggregates are involved in causing ALS or are a byproduct of the dying cell. Mutations in the *SOD1*, *TARDBP*, or *FUS* gene lead to the production of misfolded proteins that form protein aggregates in motor neurons. When ALS is caused by mutations in other genes, the composition of the protein aggregates is usually a mix of different proteins, although the aggregates are primarily made up of the protein produced from the *TARDBP* gene. It is unclear how the *TARDBP* gene's protein product plays a role in ALS when the gene is not mutated. *C9orf72* gene mutations do not appear to cause protein aggregates. Mutations in this gene likely result in a decrease in normal C9orf72 protein and possibly the production of an altered protein that interferes with cell function.

Studies have identified multiple mechanisms by which gene mutations associated with ALS cause the disorder. Some mutations lead to a disruption in the development of axons, the specialized extensions of nerve cells (such as motor neurons) that transmit nerve impulses. The altered axons may impair transmission of impulses from nerves to muscles, leading to muscle weakness and atrophy. Other mutations lead to a slowing in the transport of materials needed for the proper function of axons in motor neurons, eventually causing the motor neurons to die. Additional gene mutations prevent the breakdown of toxic substances, leading to their buildup in nerve cells. The accumulation of toxic substances can damage motor neurons and eventually cause cell death. In some cases of ALS, it is unknown how the gene mutation causes the condition.

The cause of sporadic ALS is largely unknown but probably involves a combination of genetic and environmental factors. Variations in many genes that are involved in transmission of nerve impulses and transporting materials within neurons, including the *DCTN1*, *NEFH*, *PRPH*, and *SMN1* genes, increase the risk of developing ALS. Gene mutations that are risk factors for ALS may add, delete, or change DNA building blocks (nucleotides), resulting in the production of a protein with an altered or reduced function. A genetic variation can cause an increase in the number of copies of the whole *SMN1* gene. This type of mutation leads to increased production of protein from the *SMN1* gene, which is thought to raise the risk of developing ALS. While variations in these genes have been associated with ALS, it is unclear how these changes influence the development of the disease. People with a gene variation that increases their risk for ALS likely require additional genetic and environmental triggers to develop the disorder.

How do people inherit amyotrophic lateral sclerosis?

About 90 to 95 percent of ALS cases are sporadic and are not inherited. An estimated 5 to 10 percent of ALS is familial and caused by mutations in one of several genes. The pattern of inheritance varies depending on the gene involved. Most cases are inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is sufficient to cause the disorder. In most cases, an affected person has one parent with the condition.

Less frequently, ALS is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. The parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene, but they typically do not show signs and symptoms of the condition. Because an affected person's parents are not affected, autosomal recessive ALS is often mistaken for sporadic ALS even though it is caused by a familial genetic mutation.

Very rarely, ALS is inherited in an X-linked dominant pattern. X-linked conditions occur when the gene associated with the condition is located on the X chromosome, which is one of the two sex chromosomes. In females (who have two X chromosomes), a mutation in one of the two copies of the gene in each cell is sufficient to cause the disorder. In males (who have only one X chromosome), a mutation in the only copy of the gene in each cell causes the disorder. In most cases, males experience more severe symptoms of the disorder than females. A characteristic of X-linked inheritance is that fathers cannot pass X-linked traits to their sons.

Some people who inherit a gene mutation known to cause ALS never develop features of the condition. (This situation is known as reduced penetrance.) It is unclear why some people with a mutated gene develop the disease and other people with a mutated gene do not.

Changes in these genes are associated with amyotrophic lateral sclerosis. Genetics home reference, NML, NIH.

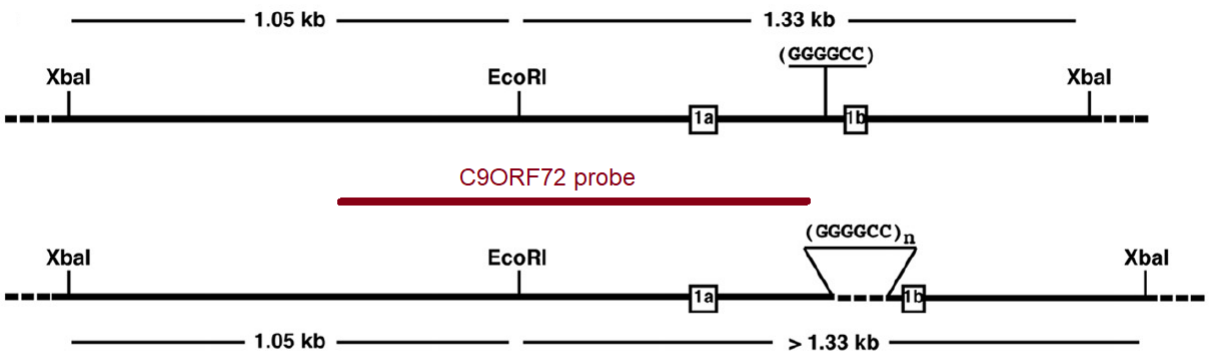
- ALS2
- ANG
- ATXN2
- C9orf72
- DCTN1
- FIG4
- FUS
- NEFH
- OPTN
- PRPH
- SETX
- SIGMAR1
- SMN1
- SOD1
- SPG11
- TARDBP
- UBQLN2
- VAPB
- VCP

Reference

Accessed Jan 08, 2016 http://ghr.nlm.nih.gov/condition/amyotrophic-lateral-sclerosis

C9ORF72 Probe and Southern Blot Analysis

The probe for C9orf72 is similar to that described by Buchman et al. Simultaneous and independent detection of C9ORF72 alleles with low and high number of GGGGCC repeats using an optimized protocol of Southern blot hybridization. Molecular Neurodegeneration 2013, 8:12. The probe supplied by Gene Link is labelled with digoxigenin and is composed of smaller fragments that are preferred for hybridization. These fragments cover the segment shown in the diagram below.



Adapted from Buchman et al. Molecular Neurodegeneration 2013, 8:12 ALS-GL577 [40-2057-41]

Scheme of a region around exons 1a and 1b of human C9ORF72 gene with a single copy of GGGGCC sequence (top) or expansion of this repeat (bottom). The probe segment is shown that was used for Southern blot hybridization. The sizes of fragments produced by double EcoRI and XbaI digestion and detected by hybridization with this probe are shown. (Adapted from Buchman et al. Simultaneous and independent detection of C9ORF72 alleles with low and high number of GGGGCC repeats using an optimized protocol of Southern blot hybridization. Molecular Neurodegeneration 2013, 8:12)

Procedure: Chemiluminescent Southern Protocol

Material Supplied

One tube containing 110 µL of C9orf72 ALS hexanucleotide expansion genotyping GeneProber™ ALS-GL577 probe at a concentration of ~40ng/µL. This probe is digoxigenin labeled for non-radioactive detection. The quantity supplied is sufficient for at least 5 20x20 cm blots using 20µL for each blot as probe. Experienced users can optimize hybridization conditions for use of 2-5 µL GeneProber™ probe and/or save and reuse the hybridization solution 2-3 times.

A. Chromosomal DNA digestion: Double digestion with EcoRI and XbaI

Restriction Digestion	
Component	Volume|Quantity
Genomic DNA	5 to 10µg
10x Restriction enzyme buffer	10 µL
EcoRI (~40 u/µL)	4 µL
XbaI (~40 u/µL)	4 µL
H_2O to	**100 µL**

♦ **Incubate over night at 37^{O}C**

♦ **Ethanol Precipitate the digests**

-To 100 µL DNA add 10 µL of 3M Na Acetate pH 5.2
-Add 2 volumes (250 µL) of 100% ethanol
-Put in the freezer (-20 °C) for 20-30 minutes
-Spin at -10 °C for 5 minutes
-Discard the supernatant
-Add 100 µL of 70% ethanol, vortex.
-Spin again at -10 °C for 5 minutes
-Dry samples

♦ **Dissolve the pellets in 10 µL of 1x loading buffer**

B. Electrophoresis and Transfer

1. Load samples to a 0.8% agarose gel. Electrophorese over night at 45mA for 20-24 hours. (1.6 kb fragment on the bottom of the gel).
2. Depurinate with 0.25N HCl (add 10 mL HCl to 500 ml H_2O) for 10 minutes.
3. Denature the DNA with 0.4N NaOH/0.6M NaCl for 30 min. at room temperature (RT).
4. Neutralize with 1.5M NaCl/0.5M Tris (pH 7.5) for 30 min. at RT.
5. Transfer overnight by Southern blot procedure to positively charged nylon membrane using 10xSSC.
6. Wash the membrane with 2x SSC and then bake at 80^{O}C for 2 hours.

C. Hybridization

1. Perform prehybridization at 55°C for 3 hours in 10 mL of Easy Hyb buffer (Roche Biochemicals).
2. Boil 20µL GeneProber™ probe in 500µL of Easy Hyb for 10 minutes. Chill directly on ice.
3. Add the above probe to 10mL of Easy Hyb.
4. Discard the prehybridization buffer and replace it with the hybridization buffer containing the boiled probe. Hybridize overnight at 55°C.
5. Washing. Wash the membrane in 2xSSC/0.1% SDS at RT twice (5 min/wash), 0.5xSSC, 0.1%SDS twice at 65°C to 70°C (15 min/wash).
6. Warm the blocking reagent at this point. Prepare fresh 100 mL of Buffer MB by adding 10 mL of 10% blocking reagent [Gene Link Catalog no: 40-5026-10; Blocking solution for hybridization (10%)] and 10 mL of Maleic acid buffer 10X (Buffer M 10X) [Gene Link Catalog no: 40-5025-20; Maleic acid buffer 10X (Buffer M 10X)] to 80 mL of sterile water. Use 80 mL for blocking and the rest of 20 mL for making Anti-DIG-AP conjugate.

D. Anti-Dig Alkaline Phosphatase Binding

1. Equilibrate the membrane in 100 mL of 1x washing buffer M for 1 minute.
2. Incubate the membrane in 80 mL of Buffer MB (prepared in step 6 above) blocking solution at RT for 30 min.
3. Prepare 1:10000 Anti-DIG-AP conjugate at this point. Example, add 2 µL to 20 ml Buffer MB (prepared in step 6 above).
4. Incubate the membrane in 20 mL of Anti-DIG-AP conjugate solution at RT for 30 min.
5. Wash the membrane twice, 15 min/wash in 200 mL of 1x washing buffer M at RT.
6. Equilibrate the membrane in 50 mL of 1x Detection buffer for 2 min. Repeat 2 times. Total of three washes.

E. Detection

Detection with CDP star (Tropix) as substrate will yield reliable result by exposing to Kodak X-OMAT or XAR X-ray film for 1 hour to overnight at room temperature.

Transfer blot to a plastic sheet, (sheet protector cut from two sides to open up) and drain off excess buffer. Wipe off edges with paper towel. Blot should not be allowed to dry.

Spray CDP-star ready-to-use substrate evenly to cover the blot. DO NOT OVER SPRAY. Cover the blot with plastic sheet and wipe entire surface of the covered blot to expel any excess substrate and air bubbles. Expose the film at room temperature for 1 hr. or for shorter or longer time as required.

Luminescence continues for at least 24 hours and signal intensity remains almost constant during the first few hours. Multiple exposures can be taken to achieve the desired signal strength.

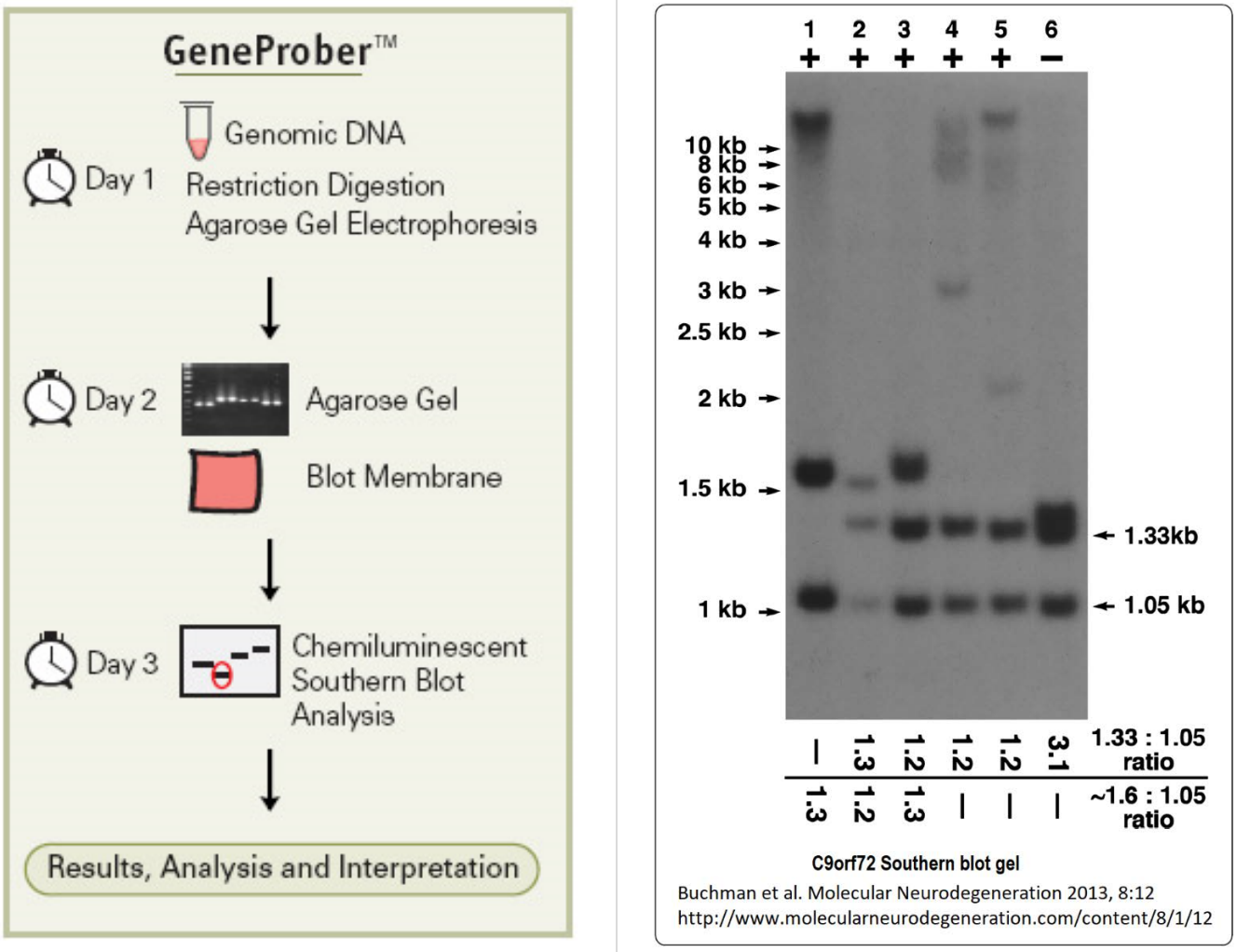
F. Stripping

Wash the membrane in water to remove the substrate. Then wash the membrane in 0.2N NaOH/0.1% SDS at 37°C for 30 minutes. Rinse the membrane in 2XSSC. Air dry.

Results and Analysis

Gene Link's C9orf72 Southern blot genotyping GeneProber™ GL577 is a derived from a ~1 kb fragment and labelled with digoxigenin for chemiluminescent Southern blot analysis.

Southern blot analysis for C9orf72 expansion genotyping involves the cleavage of DNA with restriction enzyme EcoRI and XbaI to generate fragments of 1.05 and 1.33 kb hybridizing the probe.



Southern blot genotyping analysis of GGGGCC repeat expansion in both alleles of the C9ORF72 locus. Hybridization of EcoRI and XbaI digested DNA extracted from the cultured lymphoblastoid cell lines of patients positive (+) or negative (−) for GGGGCC repeat expansion according to results of the repeat-PCR analysis. Note the presence of ~1.6 kb band representing an allele with relatively small, although what is considered pathological, i.e. >30, number of repeats in one of the C9ORF72 loci in the genome of three patients (lanes 1 – 3). In contrast to patients carrying the second allele without pathological repeat expansion (lanes 2–6), one patient (lane 1) has the second allele with very high number of repeats. Where relevant, the ratios of the 1.33 kb to 1.05 kb and/or ~1.6 kb to 1.05 kb bands intensities are shown below the image. Note that for sample 6 the intensity used for calculation is combined intensities of two close ~1.33 kb size bands.

(Adapted from Buchman et al. Simultaneous and independent detection of C9ORF72 alleles with low and high number of GGGGCC repeats using an optimized protocol of Southern blot hybridization. Molecular Neurodegeneration 2013, 8:12).

Required reagents with recommended suppliers

Roche Applied Science http://www.roche-applied-science.com

Product Description	Catalog Number
Nylon Membranes, positively charged ; 20 sheets 10 x 15 cm	11209272001
DNA Molecular Weight Marker III, DIG-labeled ; 500 µl 10 µg/ml 5 µg	11218603910
DIG Easy Hyb ; 500 mL	11603558001
DIG Wash and Block Buffer Set ; 1 set 30 blots	11585762001
Anti-Digoxigenin-AP, Fab fragments from sheep; 200 µL 150 U	11093274910
CDP Star Ready to use; 2X 50 mL	12041677001

Gene Link **http://www.genelink.com/geneprodsite/category.asp?c=44**

Non-radioactive Southern Blot Reagents

Product Description	Catalog No.	Unit Size
Agarose LE Molecular Biology Grade 100 gms	40-3010-10	100 gms
TAE Buffer 50 X Concentrate 1000 mL	40-3007-10	1 L
TBE Buffer 5 X Concentrate; 1L	40-3008-10	1 L
Loading buffer 10X BPB/XC non-denaturing; 1mL	40-3003-10	1 mL
Loading buffer 10X BPB/XC non-denaturing ; 15 mL	40-3003-15	15 mL
Lumisol II, Hybridization Solution; 200 mL	40-5023-20	200 mL
Depurination Solution (2X) for Southern Blotting; 1 L	40-5034-10	1 L
Denaturation Solution (2X) for Southern Blotting; 1L	40-5035-10	1 L
Neutralization Solution (2X) for Southern Blotting; 1L	40-5036-10	1 L
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC); 250 mL	40-5020-25	250 mL
Hybwash B, Hybridization Wash Solution (10% SDS) ; 100 mL	40-5021-10	100 mL
Maleic acid buffer 10X (Buffer M 10X); 200 mL	40-5025-20	200 mL
10% Blocking Reagent; 100 mL	40-5026-10	200 mL
Detection Buffer 10X; Alkaline Phosphatase detection buffer; 100 mL	40-5031-10	100 mL
CDP-Star® Substrate; Ready-to-Use 0.25 mM in spray bottle; 10 mL	40-5010-10	10 mL

Reagent Preparation

Most reagents with composition listed below are available in a molecular biology laboratory or these can be prepared in house. Gene Link catalog numbers are also listed if you like to purchase these common reagents.

Depurination Solution (0.25M HCl)		
Product Description	**Catalog No.**	**Volume**
Depurination Solution (2X) for Southern Blotting	40-5034-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Denaturation Solution (0.5M NaOH, 1.5M NaCl)		
Product Description	**Catalog No.**	**Volume**
Denaturation Solution (2X) for Southern Blotting	40-5035-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Neutralization Solution (0.5M Tris-HCl pH 7.5, 1.5M NaCl)		
Product Description	**Catalog No.**	**Volume**
Neutralization Solution (2X) for Southern Blotting	40-5036-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Hybwash I (2xSSC, 0.1% SDS)		
Product Description	**Catalog No.**	**Volume**
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC)	40-5020-25	35 mL
Sterile water		311 mL*
Hybwash B, Hybridization Wash Solution Concentrate (10% SDS)	40-5021-10	4 mL*
Total Volume		**350 mL**
*** Volumes adjusted to whole numbers**		

Hybwash II (0.5xSSC, 0.1%SDS)		
Product Description	**Catalog No.**	**Volume**
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC)	40-5020-25	9 mL*
Sterile water		337 mL
Hybwash B, Hybridization Wash Solution Concentrate (10% SDS)	40-5021-10	4 mL*
Total Volume		**351 mL**
*** Volumes adjusted to whole numbers**		

1X Maleic Acid Buffer (Buffer M 1X) (100 mM Maleic acid, 150 mM NaCl pH7.5)		
Product Description	**Catalog No.**	**Volume**
Maleic acid buffer 10X (Buffer M 10X)	40-5025-20	10 mL
Sterile water		90 mL
Total Volume		**100 mL**

Buffer MB (1 x Maleic acid buffer (Buffer M) with Blocking Reagent) Always prepare fresh!		
Product Description	**Catalog No.**	**Volume**
Maleic acid buffer 10X (Buffer M 10X)	40-5025-20	10 mL
Sterile water		80 mL
10% Blocking Reagent*	40-5026-10	10 mL
Total Volume		**100 mL**
The prepared reagent will be turbid yellow in color * The 10% Blocking Reagent is turbid yellow in color and will form precipitates on storage. Warm to 50°C and shake well before aliquoting. DO NOT SHAKE VIGOROUSLY		

1X Detection Buffer, Alkaline phosphatase detection buffer (100mM Tris-HCl pH 9.5, 100mM NaCl)		
Product Description	**Catalog No.**	**Volume**
Detection Buffer 10X; Alkaline phosphatase detection buffer	40-5031-10	10 mL
Sterile water		90 mL
Total Volume		**100 mL**

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.

Southern Blot Buffers& Reagents

Product	Catalog No.	Unit Size
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

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

*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.

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