Product Specification

SRY, X & Y
Genemer™

Genotyping Primer Pair for Gene, Mutation or Fragment Specific Amplification

Store at -20°C

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

❑	40-2020-10	SRY Genemer™ SRY gene specific amplification primer pair	10 nmole
❑	40-2021-10	X Genemer™ X chromosome alphoid repeat region amplification primer pair	10 nmole
❑	40-2022-10	Y Genemer™ Y chromosome alphoid repeat region amplification primer pair	10 nmole

Background

The human sex determining region on the Y chromosome has been identified and the gene has been termed as *SRY*. Mutations in the *SRY* gene have been found in XY females. Sex reversal in XY females results from the failure of the testis determination or differentiation pathways. Some XY females with gonadal dysgenesis have lost the *SRY* gene from the Y chromosome by terminal exchange between the sex chromosome or by other deletions or mutations affecting activity (1,2).

DNA analysis for a specific region of *SRY* together with alphoid repeat regions of the X and Y chromosome is used for accurate sex determination (in the absence of mutations involving *SRY),* and in the characterization of X-linked genetic diseases, Y chromosome anomalies such as XY females with gonadal dysgenesis, and for XO/XY mosaicism in patients with Turner syndrome. The DNA test involves the amplification of specific regions of X, Y and *SRY*. The presence of amplified product directly indicates the presence of the cognate DNA fragments on the chromosome. Normal XX females will amplify only X chromosome specific fragment showing double intensity as compared with amplification from normal XY male. *SRY* and Y fragments will only be amplified from individuals with a Y chromosome.

Material Supplied

One tube containing lyophilized forward and reverse primers are supplied. Please refer to item number on the top of this sheet (*SRY*, X or Y Genemer™). Each tube contains 10 nmole of the primer pair. The quantity supplied is sufficient for 400 regular 50µl PCR reaction.

A. Reconstitution

Stock Primer Mix: Dissolve the supplied lyophilized Genemer™ in 100µl sterile TE. The 10 nmole of primer when dissolved in 100µl will give a solution of 100 µMolar i.e. 100 pmoles/µl.

Primer Mix: Prepare a 10 pmoles/µl Primer Mix solution by a 10 fold dilution of the stock primer mix. Example; add 180 µl sterile TE to a new tube, to this tube add 20 µl of the primer stock solution. Label this tube as Primer Mix 10 pmoles/µl.

B. PCR* reaction (see next page for details)

Protocol for SRY, X and Y DNA Genotyping

The following PCR* profile has been optimized for *SRY*, X and Y specific product amplification using the supplied Genemer™

PCR Profile		
Denaturation	94°C	30 sec.
Annealing	55°C	30 sec
Elongation	72°C	1 min.
30 cycles, 7 min. 72°C extension, 4°C soak.		

C. Electrophoresis

Load samples to 1.5% agarose gel. Run at 90 mAmps for 2.5 hrs.

D. Results

-Normal female DNA should only amplify X specific fragment.
-Normal male DNA should amplify all fragments (SRY, X & Y)

Normal PCR amplified fragment size		
SRY	**X chrmosome**	**Y chromosome**
422 bp	**130**	**170**

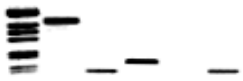

Figure 1. SRY, X and Y PCR amplification gel profile. Lane 1, molecular weight marker. Lanes 2-4 male DNA, lanes 5-7 female DNA. Lanes 2 and 5 SRY amplification, lanes 3 and 6 X amplification, lanes 4 and 7 Y amplification. Note the absence of amplification of SRY and Y from female DNA (lanes 5 & 7).

References

1. Berta et al. (1990) Genetic evidence equating *SRY* and the testis-determining factor. Nature 348:448-451.
2. Jager et al. (1990) A human XY female with frame shift mutation in the candidate testis-determining gene *SRY*. Nature 348:452-453.
Witt, M. & Erickson, R.P. (1989) A rapid method for detection of Y-chromosome DNA from dried blood specimens by the polymerase chain reaction. Hum. Genet. 82:271-274.

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PCR Premix preparation
Typical Premix

	/50 µl rxn	/1ml
10 x PCR Buffer	4.5 µl	100µl
dNTP mix (2.5mM each)	4 µl	100µl
Primer Mix (10 pmol/µl each) (25 pmol of each primer/50µl)	2.5 µl	63µl
Sterile water	34 µl	737µl
Total	45 µl	1ml

Taq Premix ***(per 50µl reaction, scale up as required)***

10 x PCR Buffer	0.5µl
Taq polymerase	0.25µl
Sterile water	4.25µl
	5µl/rxn.

PCR reaction (50µl)

Diluted DNA(100ng/µl)	1 µl
PCR premix	45 µl
Taq premix	5 µl

PCR products post-processing

1. For oil layered PCR only. Add 200µl of $CHCl_3$ to each tube, vortex and spin.
2. Transfer the upper aqueous layer to a fresh eppendorf tube, add 1/10 volume of 3M NaAc (pH 5.2), and 2 volumes of absolute ethanol, precipitate DNA at -80^{o}C for 10 minutes.
3. Spin, rinse the DNA pellet with 700µl of 75% ethanol and dry the pellet in the speedvac.
4. Dissolve the pellet in 10 µl TE.
5. Load 5 µl to a 1.5% agarose gel. Perform electrophoretic evaluation

Ordering Information

Genemer™ (Selected List) Primer pair for gene or mutation specific amplification. Special optimized conditions may be required for certain amplifications

Product	Size	Catalog No.	Price, $
Fragile X (spanning CGG triple repeat region)	10 nmole	40-2004-10	100.00
Huntington Disease (spanning CAG triple repeat region)	10 nmole	40-2025-10	100.00
Myotonic Dystrophy (spanning CTG triple repeat region)	10 nmole	40-2026-10	100.00
Friedreich's Ataxia (spanning GAA triple repeat region)	10 nmole	40-2027-10	100.00
Factor V	10 nmole	40-2035-10	100.00
Factor VIII (Hemophilia)	10 nmole	40-2036-10	100.00
STS (Steroid Sulfatase)	10 nmole	40-2023-10	100.00
HGH (Human Growth Hormone)	10 nmole	40-2024-10	100.00
Sickle Cell	10 nmole	40-2001-10	100.00
RhD (Rh D gene exon 10 specific)	10 nmole	40-2002-10	100.00
Rh EeCc (Rh Ee and Cc exon 7 specific)	10 nmole	40-2003-10	100.00
Gaucher (various mutations)	10 nmole	40-2047-10	100.00
Cystic Fibrosis (various mutations)	10 nmole	40-2029-10	100.00
SRY (sex determining region on Y)	10 nmole	40-2020-10	100.00
X alphoid repeat	10 nmole	40-2021-10	100.00
Y alphoid repeat	10 nmole	40-2022-10	100.00

***Please visit www.genelink.com for other Genemer™ not listed here**

Genemer™ Control DNA (Selected List) Control DNA for use with gene or mutation specific Genemer™

Product	Size	Catalog No.	Price, $
Fragile X, various CGG triple repeat region control DNA	500 ng	40-2004-XX	175.00
Huntington Disease various CAG triple repeat region control DNA	500 ng	40-2025-XX	175.00
Myotonic Dystrophy various CTG triple repeat region control DNA	500 ng	40-2026-XX	175.00
Friedreich's Ataxia, various GAA triple repeat region control DNA	500 ng	40-2027-XX	175.00

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GeneProber™ Product Ordering Information

Product	Size	Catalog No.	Price, $
Fragile X GeneProber™ Products Products and kits for non-radioactive genotyping the CGG triple repeat spanning region	Various	40-2004-XX	Check website
Huntington Disease GeneProber™ Products Products and kits for non-radioactive genotyping the CAG triple repeat spanning region	Various	40-2025-XX	Check website
Myotonic Dystrophy GeneProber™ Products Products and kits for non-radioactive genotyping the CTG triple repeat spanning region	Various	40-2026-XX	Check website
Friedreich's Ataxia GeneProber™ Products Products and kits for non-radioactive genotyping the GAA triple repeat spanning region	Various	40-2027-XX	Check website

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

Prices subject to change without notice

All Gene Link products are for research use only

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