

Technical Datasheet

Sex Test Kit

The use of the Amelogenin gene for determination of sex

Cat No:	CA-1067-02 CA-1067-10	Pack size:	200 tests 1000 tests
Contents:	Standard 1mg/ml (100µg) AMG Forward 4.5mg/ml (20µg) AMG Reverse 4.5mg/ml (20µg)		

The location of X- and Y-linked amelogenin sequences can be determined by means of the polymerase chain reaction (PCR) and two primers (AMELF and AMELR). The forward and reverse primers (identical in sequence of the X and Y) span the first intron, but produce different sized X (542bp) and Y (358bp) fragments because of the differences in the sizes of the introns on X and Y. The Cambio kit contains these two primers and a control DNA of known sex.

The enclosed paper shows the results and analysis of a series of somatic cell hybrids carrying deleted X chromosomes, and the results include DNA from a normal male and Y-only somatic cell hybrid as controls. The region of the X chromosome present in each of the hybrids is indicated schematically in line with each track of the figure. The pattern of deletion maps the X (AMELX) fragment in the interval Xp22.1-p21.2 but excludes it from the region Xpter-p21.2.

Figure (A) DNA is taken from a normal male in and a series of somatic cell hybrids were subjected to PCR analysis with the primers AMELF and AMELR.

1. Normal male DNA – primers gave both products
2. Normal female DNA – primers gave only the X product
3. Y-only somatic cell hybrid – primers gave only the Y product
4. Somatic cell hybrid deleted for Xpter-Xp22.3 – primers gave only the X product
5. Somatic cell hybrid deleted for Xpter-Xp22.1 – primers gave only the X product
6. Somatic cell hybrid deleted for Xpter-Xp21.2 – primers gave no product
7. Somatic cell hybrid deleted for Xp21-qter – primers gave only the X product
8. Mouse DNA – primers gave no product
9. Hamster DNA – primers gave no product

The location of the Y homologue (AMELY) has been determined by analysing DNA from a series of XX males (generated by X-Y interchange) and individuals with aberrant Y chromosomes. The regions of the Y short arm present in these individuals have been determined previously. Figure B shows the PCR results for amelogenin of some individuals. WC presses as monocentric iso-Yp and is positive for AMELY, clearly mapping the Y homologue to Yp. AMELY is present in XX male PW but absent from XX male DR. The presence of AMELY in RW, KS, and the XY female AM (generated by a reciprocal of the X-Y interchange leading to XX males and resulting in a large deletion of distal Xp) confirms the location of the Y-linked gene. ED possesses two normal X chromosomes and two isodicentric Yq chromosomes containing an intact Y long arm and extensive deletion of Yp including the interval containing AMELY. Hence ED excludes AMELY from Yq.

RESEARCH USE ONLY

These primers should prove useful for sexing of not only human samples but other primate species.

Figure (B) PCR analysis of individuals with deleted Y chromosomes. ED and AM contain an intact Y long arm. All other individuals possess only segments of the Y short arm. KS, DR, and PW are XX male individuals generated by X-Y interchange; RW is an XO male with a fragment Y chromosome: AM is an XY female generated by X-Y interchange; WC is a male with monocentric iso-Yp chromosome; ED is a female with dicentric iso-Yq chromosome with a breakpoint in proximal Yp11.1.

Method:

Take 250ng genomic DNA from each sample and amplify under the following conditions:

Prepare PCR buffer containing 25mM TAPS, pH 9.3, 50mM KCl, 1mM DTT, 2mM MgCl₂, 0.05% (v/v) W1 detergent (Sigma), and 0.4U Taq polymerase in a 50µl reaction.

Carry out thermal cycling as follows: 1st cycle: 94°C 5 min; thereafter 30 cycles, 94°C for 30 sec, 60°C for 1 min, 72°C for 1 min, 72°C for 10 min.

Analyse PCR products on a 1% agarose gel and visualise by staining with ethidium bromide.

Note: Use of PCR requires a licensed Taq DNA Polymerase and purchase of this product from Cambio Ltd is accompanied by a limited licence to use it in the PCR process for research in conjunction with an authorized thermocycler. PCR is covered by patents owned by Hoffmann-La Roche.

Storage: Store at -20°C.

Reference paper:

The X-Y Homologous Gene Amelogenin Maps to the Short Arms of Both the X and Y Chromosomes and is Highly Conserved in Primates

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Genomics 14, 203 – 205 (1992)
ISBN 0888-7543/92