DETECTION OF gr/gr DELETION in AZFc REGION OF Y CHROMOSOME

AMPLI-SET Y Chromosome gr/gr Cat. n.1.501/gr

The AZFc region of Ychromosome consists almost entirely of repetitive sequence blocks , amplicons.Due to its repetitive structure , tha AZFc region is particuraly susceptible to homologous intrachromosomal recombinations events, which may lead to deletions. Different rearrangements in AZFc region have been identified ,and some of them have been reported to be a direct cause or genetic risk factor for male infertility .
The complete deletion of AZFc (deletion b2/b4) is the most common molecular genetic cause of spermatogeneic failure.Recently , new types of AZFc deletions, called partial deletion, have been reported. They remove about half of AZFc content , including two DAZ genes, one copy of CDY1 and one copy of BPY2 gene. They arise by the same molecular mechanism of the complete microdeletion of AZFc region.
Gr/gr partial deletions is considered a genetic risk factor for spermatogenic impairment by many research groups .The deletions gr/gr are detected by PCR (Polymerase Chain Reaction)of some "sequence tagged sites" (STSs): sY1291-sY1191-sY1206-sY1201-sY142-sY1197.The absence of sY1291and the presence of the other STSs characterizes the gr/gr partial deletion .
In a recent study of italian population gr/gr carriers have a 7.9-fold increased risk of having impaired spermatogenesis compared with men without such a deletion. The studies recently published demonstrate that ,without a drastic phenotypic effect , the partial deletions are a cofactor for impaired sperm production.Moreover, as this genetic risk factor will be trasmitted to the male offspring , it is relevant also for genetic counselling, to inform the couple about the transmission of a predisposition to impaired sperm production. .
The AMPLI CHROMOSOME Y gr/gr allows to detect the presence of gr/gr partial deletions by a PCR with specific primers for sY1291 and sY1191 and for β-globina, as internal control of multiplex PCR . The presence of gr/gr partial deletions is characterized by the absence of amplification of sY1291 .

Principle of method : A) extraction of genomic DNA B) amplification C) detection on agarose gel.
Applicability: On extracted and purified DNA from samples of whole blood and biological liquids .
Number of Test: 24.

REAGENTS and STORAGE

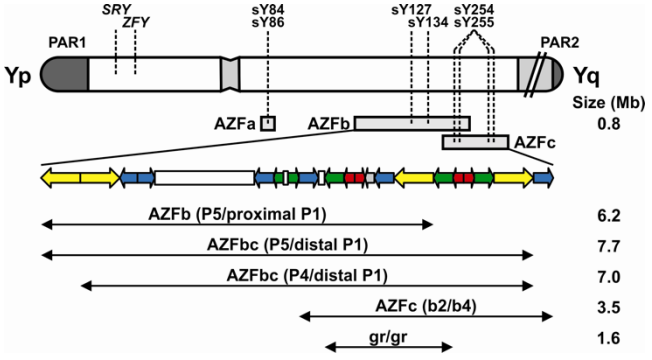
AMPLIFICATION	
M-PCR mix	-20°C
H_2O sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
MALE GENOMIC DNA negative CONTROL	-20°C

Stability: over 12 months if correctly stored (Agarose gels, if protected by light, can be stored 1 year at room temperature).

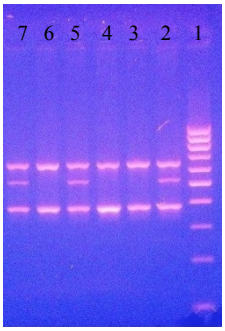
References:
1. Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T,Kuroda-Kawaguchi T, de Vries JW, Oates RD, Silber S, van der Veen F, Page DC,Rozen S. Nat Genet. 2003 Nov;35(3):247-51.
2. Giachini C, Laface I, Guarducci E, Balercia G, Forti G, Krausz C. Partial AZFc 2008 Nov;124(4):399-410. doi: 10.1007/s00439-008-0561-1. Epub 2008 Sep 21.
3. Krausz C, Chianese C, Giachini C, Guarducci E, Laface I, Forti G. J Endocrinol Invest. 2011 May;34(5):376-82. doi: 10.3275/7612. Epub 2011 Mar 21. Review. PubMed PMID: 21422806
4. Skaletsky H Kuroda-Kawaguchi Y, Minx PJ, Cordum Hs et Nature 2003 423:825-837.

ANALYSIS OF RESULTS

Schematic representation of the Y chromosome and the current microdeletion model. (Repping et al. 2002). Repetitive sequences (colour coded palindromes) explain the origin od deletions in AZFb region by hologous recombination between identical sequences .The location of primers suggested by the EAA/EMQN 2013 is indicated by dashed lines . Since four copies of DAZ gene are usually present on the Y chromosome, the STS primers sY254, sY255 amplifie 4 loci in AZFc. The AZFc(b2b4)deletion is the most frequent type (~80%) of Y-chromosomal microdetetions found in men with severe oligi/azoospermia.



Pattern of amplification:
SY1191= 385bp SY1291= 527bp, internal control multiplex PCR β-globina 641bp.



1) ladder 100 bp;
2) male genomic DNA negative Control
3) deleted sample for sY 1291;
4) deleted sample for sY 1291;
5) male genomic DNA negative Control
6) deleted sample for sY 129
7) male genomic DNA negative Control