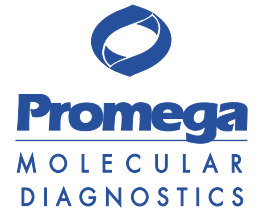


The Y Chromosome Deletion Detection System, Version 1.1



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The identification and analysis of pathology associated with Y chromosome deletions is an important tool for the study of male infertility. Screening for Y chromosome deletions has been hampered by a lack of standardized primer sets for sequence tagged sites (STSs). The Y Chromosome Deletion Detection System, Version 1.1^(a,b), offers the opportunity for standardization using four Multiplex Master Mixes that screen for a total of eighteen previously identified and mapped STSs. Y chromosome deletions in the regions amplified by these primers are associated with male infertility.

INTRODUCTION

The cause, diagnosis and treatment of infertility are increasingly important areas of scientific investigation. There have been various estimates of the frequency of infertility in the population (1–8). Approximately 15% of all couples experience some form of infertility (2,5). It is generally accepted that approximately half of those cases are attributable, at least in part, to male infertility. The initial evaluation for suspected male factor infertility focuses on semen analysis and hormone testing. Semen analysis may reveal azoospermia (defined as the absence of motile sperm in the ejaculate), oligozoospermia (less than 20 million sperm per milliliter) or normal sperm counts with abnormal morphology. More complex testing may include karyotyping. In most cases, if initial hormone replacement therapy fails to improve sperm parameters, the patient is referred for Assisted Reproductive Technology (ART).

Y CHROMOSOME DELETIONS

The causes of male infertility include infectious agents, chemical exposure, trauma, immune or endocrine disorders and genetic disorders. Presently, much work is directed toward the understanding of genetic causes of male infertility. In particular, there has been interest in understanding the association of Y chromosome deletions with male infertility. The importance of the long arm of the Y chromosome (Yq) for spermatogenesis was first shown by comparative cytogenetic studies of men with azoospermia and their fertile fathers (9). Subsequent cloning efforts led to the discovery of several Y-linked genes (the *RBM* (10) and *DAZ* (11) gene families, *DFFRY* (12), *DBY* (13) and others (14,15), which seem to be required for the production of normal sperm cells and whose expression is limited to the testis. PCR amplification of STSs, nested in and around the spermatogenesis-required genes, has allowed for the definition of several large regions that are associated with the production of sperm cells, namely AZFa, AZFb and AZFc (16). Subintervals, defined by naturally occurring (in infertile patients) deletion intervals have further led to the identification of regions that may be thought of as additional AZF regions or subintervals of previously defined AZF regions, namely proximal and distal (contains *DFFRY*

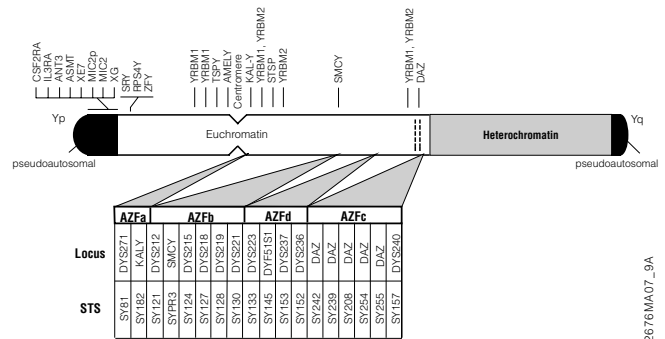


Figure 1. Y chromosome map.

and *DBY*) AZFa, proximal (contains *SMCY*), middle and distal (contains *RBM1*) AZFb, and proximal (provisionally called AZFd) middle (contains *DAZ*) and distal AZFc (17,18; Figure 1). Deletions in these four regions, AZFa, AZFb, AZFc and proximal AZFc (AZFd), are associated with male infertility (11, 17–37). In most cases there is no clear association between the location of the deletion and a distinct phenotype; however, some generalizations can be made with regard to the relationship of Y chromosome deletions to infertility. In men with deletions in AZFa, 75% have Sertoli cell only and 25% have partial spermatogenic arrest associated with severe oligozoospermia. Individuals with large deletions involving multiple AZF regions generally exhibit equally severe phenotypes. Deletions in AZFb and AZFc have been associated with azoospermia or oligozoospermia. Finally, patients with proximal AZFc (AZFd) deletions may have sperm counts ranging from azoospermia to normal sperm with abnormal sperm morphology.

The identification and analysis of Y chromosome deletions is an important research tool for studying male infertility. Other areas of Y chromosome study include the identification of specific gene products required for spermatogenesis and male infertility, the association of specific genotypes with the corresponding phenotype and the epidemiological parameters of male infertility. The goal of this research is the complete understanding of the genetic processes involved in male infertility. This understanding will aid in the development of accurate diagnostic testing and treatment. The use of Intra-Cytoplasmic Sperm Injection (ICSI) to treat severe male factor infertility has provided one example of the need to identify genetic mutations associated with male infertility. ICSI involves direct injection of sperm or spermatids into the egg. Patients with a Y chromosome deletion who undergo ICSI can transmit that deletion to male offspring (22,25,38). Clearly, this is an area where the ability to provide information on an individual's genotype could impact treatment decisions.

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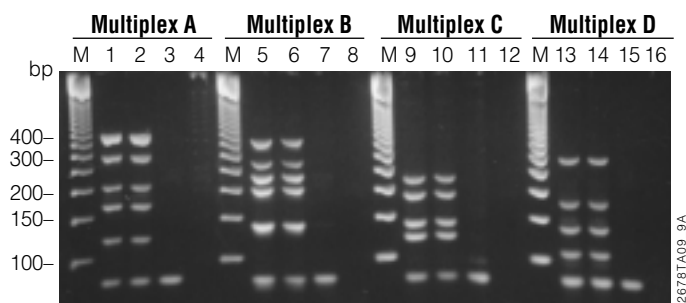


Figure 2. Multiplex analysis of wildtype male and female genomic DNA. Lanes 1–4 show the amplification products from reactions using Multiplex A Master Mix with male DNA (lanes 1 and 2), female DNA (lane 3) and no DNA (lane 4). Lanes 5–8 show the amplification products from reactions using Multiplex B Master Mix with male DNA (lanes 5 and 6), female DNA (lane 7) and no DNA (lane 8). Lanes 9–12 show the amplification products from reactions using Multiplex C Master Mix with male DNA (lanes 9 and 10), female DNA (lane 11) and no DNA (lane 12). Lanes 13–16 show the amplification products from reactions using Multiplex D Master Mix with male DNA (lanes 13 and 14), female DNA (lane 15) and no DNA (lane 16). Lanes M: 50bp DNA Step Ladder (Cat.# G4521). The gel is a 4% NuSieve® 3:1 plus Reliant® gel.

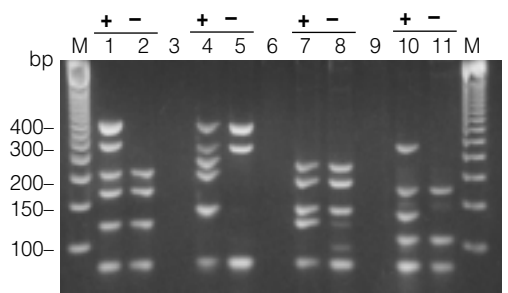


Figure 3. Y chromosome deletion analysis. The amplification products from Multiplex A Master Mix (lanes 1 and 2), Multiplex B Master Mix (lanes 4 and 5), Multiplex C Master Mix (lanes 7 and 8) and Multiplex D Master Mix (lanes 10 and 11) reactions are shown. Control human male genomic DNA (+) from Promega (lanes 1, 4, 7 and 10) is compared to a test genomic DNA (-) from a male with a Y chromosome deletion (lanes 2, 5, 8 and 11; note the deleted bands in these lanes). Markers (M) are the 50bp DNA Step Ladder (Cat.# G4521). No samples were loaded in lanes 3, 6 and 9. The gel is a 4% NuSieve® 3:1 plus Reliant® gel.

THE Y CHROMOSOME DELETION DETECTION SYSTEM, VERSION 1.1

Published studies have included more than 6,000 individuals who have been screened for Y chromosome deletions using amplification of Y-linked STSs. Despite this large cumulative population, it has been difficult to interpret the results because of the lack of standardization across screening protocols. In particular, the number and location of STSs employed for screening has varied greatly, with published studies using anywhere from 1 to 131 STSs. This problem has been cited numerous times as an impediment to progress in this field (11,17,18,25,39,40). The Y Chromosome Deletion Detection System, Version 1.1 (Cat.# MD1101), provides a standardized method for identifying Y chromosome deletions.

The Y Chromosome Deletion Detection System provides reagents and protocols to screen for the presence or absence of 18 different loci on the Y chromosome (11,14). Combined data from many studies were used to select STSs for inclusion in the system (17,18,20,21,24,26–28,41–46). Table 1 shows the STSs included in this system. Table 2 shows a comparison of the STSs included in the Y Chromosome Deletion Detection System with those used in several other studies (17,21,26,28,41–43). (An expanded version of Table 2 with extensive references is available at: www.promega.com/moldx/). The Y Chromosome Deletion Detection System detects deletions in nonpolymorphic, pathology-related STSs from all AZF regions. The eighteen primer pairs have been combined into four sets for multiplex PCR amplification, making it possible to determine the presence or absence of all eighteen loci by performing four PCR amplifications.

Furthermore, the Y Chromosome Deletion Detection System, Version 1.1 enhances the standardization and reproducibility of Y chromosome

screening protocols. The reagents have been combined into four Multiplex Master Mixes, containing everything necessary for amplification except the *Taq* DNA Polymerase^(c) and the DNA sample. This design minimizes the number of steps required to set up PCR thereby minimizing the possibility for pipetting error or contamination.

REPRODUCIBILITY

The Y Chromosome Deletion Detection System has been extensively tested for reproducibility and robustness, and quality controlled to ensure optimum performance. The system incorporates controls to ensure performance of the amplification reagents and to identify problems with genomic DNA samples. A positive control Male Genomic DNA is included for use in evaluating the amplification reactions. The presence of the appropriate amplification products in the positive control reactions indicates that the reagents are performing as expected.

INTERNAL CONTROLS

Each Multiplex Master Mix contains a control primer pair that amplifies a region of the X-linked *SMCX* locus. This primer pair is an internal control for the amplification reaction and the integrity of the DNA sample. Figure 2 shows the amplification of male and female genomic DNA, as well as the negative no-DNA control for each of the four Multiplex Master Mixes. Note that the internal control is amplified even in the absence of a Y chromosome, indicating the quality of the reaction components and the DNA sample. Figure 3 shows a comparison of test DNA carrying a Y chromosome deletion and the positive control Male Genomic DNA and indicates a deletion in the regions AZFd and AZFc.

Table 1. Primer Sets in the Y Chromosome Deletion Detection System, Version 1.1.

Multiplex Master Mix	Locus/STS 1	Internal Locus/STS 2	Locus/STS 3	Locus/STS 4	Locus/STS 5	Control
A	DAZ/SY254	DYS240/SY157	DYS271/SY81	DYS221/SY130	KAL-Y/SY182	SMCX
B	SMCY/SYPR3	DYS218/SY127	DAZ/SY242	DAZ/SY239	DAZ/SY208	SMCX
C	DYS219/SY128	DYS212/SY121	DYF51S1/SY145	DAZ/SY255		SMCX
D	DYS236/SY152	DYS223/SY133	DYS237/SY153	DYS215/SY124		SMCX

Table 2. Comparison of STSs Included in the Y Chromosome Deletion Detection System, Version 1.1, to Those Used in Other Studies.

Region	Version 1.1	References						Region	Version 1.1	References										
		17,26	41	42	21	43	28			17,26	41	42	21	43	28					
Yp=tdf		SRY	SRY	SRY		SRY		AZFb												
			ZFY					AZFb		SY136										
								AZFb												SY164
centromere		SY75						AZFb												SY138
				SY78				AZFb		SY139										SY139
				SY79				AZFb		SY142										
AZFa	SY81	SY81		SY81				AZFb		SY141										
AZFa		SY82						AZFb												SY143
AZFa				SY83				AZFb			RBM1		RBM1	RBM1	RBM1					
AZFa		DBY						AZFb					RBM2							
AZFa			SY86	SY86				AZFd**	SY145	SY145										
AZFa		SY85						AZFd**	SY153	SY153		SY153	SY153	SY153						
AZFa		SY84	SY84	SY84				AZFd**	SY152	SY152		SY152	SY152							
AZFa		<i>DFFRY</i>		<i>DFFRY</i>				AZFd**						SY220						
AZFa		SY87		SY87				AZFd**		SY221										
AZFa		SY165						AZFd**		SY150				SY150						
AZFa		DBY*						AZFc												FR15-II
AZFa		UTY*						AZFc		SY155		SY155	SY155	SY155						
AZFa		TB4Y*						AZFc												
				SY88				AZFc					SY147	SY147	SY147					
				SY89				AZFc					SY148							
		SY182 (<i>KAL-Y</i>)	SY182 (<i>KAL-Y</i>)					AZFc	SY242	SY242										
								AZFc					SY146							
				SY151				AZFc	SY239	SY239										
								AZFc												
								AZFc												
				SY95				AZFc												
								AZFc												
				SY97				AZFc												
								AZFc		SY149				SY149	SY149					
				SY98				AZFc	SY208	SY208										
AZFb		SY100						AZFc					SY277							
AZFb		SY101						AZFc	SY254	SY254	SY254	SY254	SY254	SY254						SY254
AZFb		SY102						AZFc					SY279							
AZFb		SY105						AZFc					SY283							
AZFb		SY109						AZFc	SY255	SY255	SY255	SY255	SY255	SY255						SY255
AZFb		SY113						AZFc												SPGY1
AZFb								AZFc		SY248										
AZFb		SY117		SY117				AZFc												SY272
AZFb		SY118						AZFc												SY273
AZFb		SY119						AZFc					SY269	SY269						
AZFb	SY121	SY121						AZFc	SY157	SY157										SY157
AZFb	<i>SMCY</i>	<i>SMCY</i>						AZFc												SY243
AZFb	SY124	SY124		SY124				AZFc												SY167
AZFb				SY125				AZFc		SY202										
AZFb								AZFc		SY158		SY158	SY158							
AZFb		SY126						AZFc				SY159								SY159
AZFb	SY127	SY127	SY127	SY127						OX7										
AZFb	SY128	SY128									SY160		SY160	SY160						
AZFb				SY129	SY129															
AZFb		SY131		SY131	SY131															SY131
AZFb	SY130	SY130			SY130															
AZFb		SY132			SY132															
AZFb	SY133	SY133																		
AZFb		SY134	SY134		SY134	SY134														

*The order of these STSs has not been determined.

**AZFd may also be described as proximal AZFc in some references. AZFd excludes *DAZ*.

COMPREHENSIVE TECHNICAL LITERATURE

The Y Chromosome Deletion Detection System, Version 1.1, is provided with a detailed Technical Manual (TM234) containing comprehensive instructions for reaction set-up, thermal cycling and gel electrophoresis. Thermal cycling profiles are available for the Perkin Elmer 480 (requires oil) and the Perkin Elmer GeneAmp® System 9600 (does not require oil) thermal cyclers. Worksheets for use in data analysis and deletion mapping are also provided. For help in identifying and solving problems that may arise in the use of this system, the Technical Manual includes a troubleshooting guide. The Y Chromosome Deletion Detection System is also supported by the Promega Technical Services Department (e-mail: techserv@promega.com).

SUMMARY

The Y Chromosome Deletion Detection System, Version 1.1, provides a standardized, reliable method for the identification of Y chromosome deletions for use by investigators in the area of male infertility. Our intent is to provide an easy-to-use system that will allow investigators to focus on experimental design and interpretation rather than the technical aspects associated with multiplex PCR amplification.

For more information about the Y Chromosome Deletion Detection System, Version 1.1, please visit: www.promega.com/moldx/.

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Ordering Information

Product	Size	Cat.#
Y Chromosome Deletion Detection System, Version 1.1	25 reactions	MD1101

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Related Products

Product	Size	Cat.#
Blue/Orange 6X Loading Dye	3 × 1ml (3ml)	G1881
50bp DNA Step Ladder	90µg	G4521
Wizard® Genomic DNA Purification Kit	100 × 300µl reactions	A1120
	500 × 300µl reactions	A1125
	100 × 10ml reactions	A1620

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