# Molecular Diagnosis of Male Factor Infertility using APEX microarrays

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## AIM OF THE STUDY

Male factor infertility (MFI) issues are causative in approximately 15-20% of infertile couples. The unfavourable genetic background is thought to cause for 15-30% of male factor infertility cases. Therefore, the correct determination of genetic basis of infertility is very important for further treatment of patients. Here, we demonstrate a single-step Arrayed Primer Extension (APEX) based microarray assay for the MFI diagnosis.

## SAMPLING

A total of 62 DNA samples were used for validation MFI APEX assay. Ten DNA samples were obtained from men without infertility issues and 52 DNA samples obtained from MFI patients provided by the Andrology Unit of Tartu University. Seven out of 52 DNA samples have microdeletions in the AZFc region, 2 DNA samples have microdeletions in the AZFb+c region, 14 DNA samples have Klinefelter syndrome (47,XXY) and 29 DNA samples obtained from idiopathic infertile men.

## MATERIAL and METHODS

The MFI APEX assay includes:

1. A set of SNPs in regions Xp11-Xq13 and in pseudoautosomal (PAR1) region of X and Y chromosome for diagnosis Klinefelter syndrome;
2. A set of SNPs for detection AZFa, AZFb, AZFc- microdeletions;
3. Six haplotype- tagging SNPs in androgen receptor (AR) gene (Saare *et al*, 2007);
4. Forty two mutations/SNPs from 18 genes (*PRDM9*, *PRM1*, *PRM2*, *DDX25*, *TEKT2*, *ESR2*, *FSHB*, *GNRHR*, *RBMXL2*, *NLRP14*, *SYCP3*, *USP26*, *UTP14c*, *DNAI1*, *DNAH5*, *DNAH11*, *INSL3*, *RXFP2*) associated with idiopathic infertility, hypogonadism or cryptorchidism;
5. Two hundred and seventy mutations and variations in cystic fibrosis transmembrane conductance regulator (CFTR) gene.

Analysis of DNA samples were performed with PicDBAutoScan 5.0 program of the Genorama Genotyping Software™ (Asper Biotech Ltd).

## RESULTS

MFI APEX assay validation was performed in blinded study using 62 DNA samples.

1. AZFc microdeletion was correctly identified in 7 DNA samples;
2. AZFb+c microdeletion was correctly identified in 2 DNA samples;
3. Klinefelter syndrome (47,XXY) was correctly identified in 13 DNA samples. Detection of 47,XXY is based on counting heterozygous SNPs in regions Xp11-Xq13 and copy number analysis of heterozygous SNPs in the PAR1 region of the X/Y chromosome. The threshold for annotating DNA samples as 46,XY or 47,XXY was determined (Oitmaa *et al*, 2010). Analysis of the allelic ratio of heterozygous SNPs from PAR1 region resulted 2 false positives (▲) and one false negative (■) (Figure 1);
4. Genetic variations were found in 25 DNA samples (Table 1).

   Genotyping quality parameters of MFI APEX assay are given in Table 2.

## REFERENCES

Saare, M *et al.* Androgen receptor gene haplotype is associated with male infertility. Int J Androl. 2008 Aug;31(4):395-402

Oitmaa *et al.* Molecular diagnosis of Down syndrome using quantitative APEX-2 microarrays. Prenat Diagn. 2010 Dec;30(12-13):1170-7.

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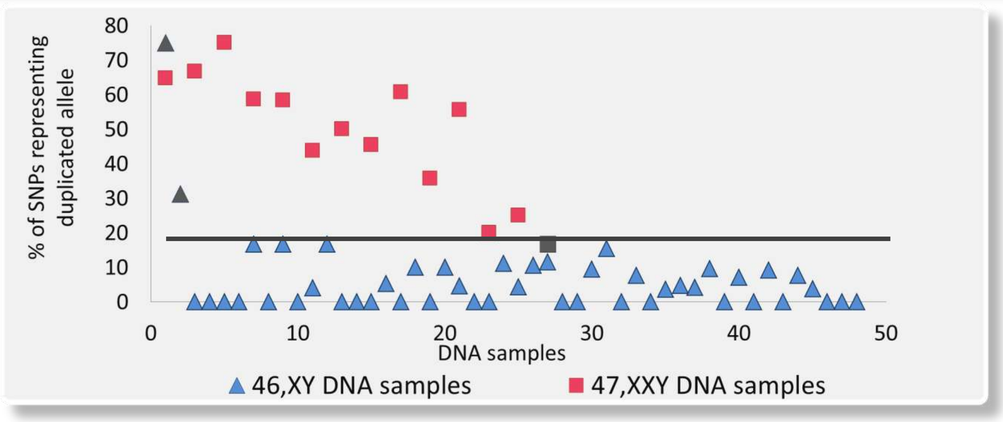
## FIGURE 1. ANALYSIS OF THE ALLELIC RATIO OF HETEROZYGOUS SNPs FROM PAR1 REGION




## TABLE 1. GENETIC VARIATIONS IN STUDIED DNA SAMPLES

| Study Group | Gene | Variation | No of Cases |
|---|---|---|---|
| Healthy controls [# 10] | *CFTR* | heterozygous p.Leu88IlefsX22 (394delTT) | 1 |
| | *CFTR* | heterozygous c.1210-12T[5T] | 3 |
| | *RXFP2* | heterozygous p.Ile604Val | 1 |
| | *ESR2* | heterozygous c.984G>A (p.= ) | 1 |
| | *AR* | haplogroup 4 | 1 |
| Infertile men (AZF microdeletion or Klinefelter syndrome) [# 23] | *CFTR* | heterozygous p.Glu217Gly | 1 |
| | *CFTR* | heterozygous c.1210-12T[5T] | 1 |
| | *RXFP2* | heterozygous p.Ile604Val | 6 |
| | *ESR2* | heterozygous c.984G>A (p.= ) | 3 |
| Infertile men (idiopathic infertility) [# 29] | *CFTR* | heterozygous p.Ile148Thr | 1 |
| | *CFTR* | heterozygous p.Arg668Cys | 1 |
| | *CFTR* | heterozygous c.1210-12T[5T] | 1 |
| | *RXFP2* | heterozygous p.Ile604Val | 6 |
| | *RXFP2* | homozygous p.Ile604Val | 1 |
| | *ESR2* | heterozygous c.984G>A (p.= ) | 5 |
| | *USP26* | homozygous p.Thr121ins (c.364_365ins3) | 1 |
| | *AR* | haplogroup 4 | 2 |
| **TOTAL (# 62)** | | | **36** |

## TABLE 2. GENOTYPING QUALITY PARAMETERS OF MFI APEX ASSAY

| Parameter | Value (%) |
|---|---|
| Call rate (22492 out of 22630 counts) | 99 |
| Accuracy | 95 |
| Specificity | 93 |
| Sensitivity | 97 |

## CONCLUSIONS

Our experiments demonstrated that MFI-APEX assay is suitable for the rapid single-step, robust, reliable and cost-effective detection of possible genetic cause for male infertility problem. Furthermore, MFI-APEX testing is recommended before the couple undergoes assisted reproduction in order to prevent the possible inheritance of the genetic lesion to the next generation.

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