

Table of Contents

<u>Chapter</u>		<u>Page</u>
1	Introduction	1
1.1	Historical background	2
1.2	CFTR Structure and Function	3
1.3	Cystic Fibrosis Mutations	5
1.4	CF in the Arabic world	8
2	Objectives	10
3	Materials and Methods	11
3.1	Patients and Sampling	11
3.1.1	Patients Selection Criteria	11
3.1.2	Sample Collection and Processing	12
3.2	DNA Extraction	12
3.2.1	AUTOPURE L S “Large Sample Nucleic Acid Purification”	13
3.2.2	QIAamp DNA Mini Kit	14
3.3	Determination of DNA Quality	14

3.3.1	DNA Concentration	14
3.3.2	DNA Purity	15
3.3.3	DNA Quality Test by PCR	15
3.4	Polymerase Chain Reaction (PCR) of the CFTR Gene Coding Region	16
3.5	INNO-LIPA CFTR	18
3.6	ΔF508 Mutation Detection by Heteroduplex Analysis	21
3.6.1	Heterozygote Mutation Analysis	22
3.6.2	Homozygote Mutation Analysis	23
3.7	Next Generation Sequencing (NGS) of the PCR Products	23
3.8	Confirmation of Identified Mutations by Sanger Sequencing	25
3.9	Allele Specific Mutation Analysis (ASMA)	27
3.10	Multiple Ligation-dependent Probe Amplification (MLPA)	29
4	Results	31
4.1	DNA Quantity and Quality	31
4.2	INNO-LIPA CFTR	32
4.3	ΔF508 Mutation Detection by Heteroduplex Analysis	33
4.4	Polymerase Chain Reaction (PCR) of the CFTR Gene Coding Region	34

4.5	Next Generation Sequencing (NGS) of the PCR Products	34
4.6	Confirmation of Identified Mutations by Sanger Sequencing	37
4.7	Multiple Ligation-dependent Probe Amplification (MLPA)	37
4.8	Mutational Spectrum of Cystic Fibrosis	38
4.9	Allele Specific Mutation Analysis (ASMA)	39
5	Discussion	40
6	Recommendations	45
7	References	46