جامعة بغداد

كلية العلوم

قسم التقنيات الاحيائية

الامراض الوراثية و التشخيص الجزيئي العملي

المرحلة الرابعة / الفصل الثاني

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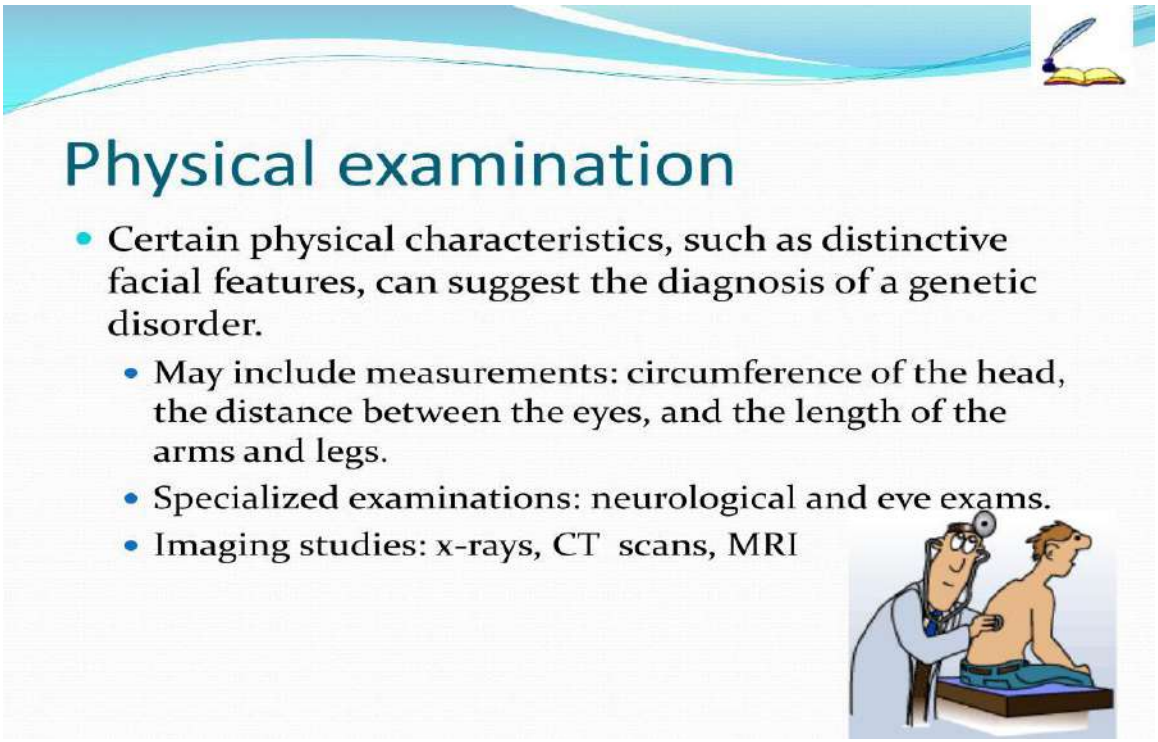
Diagnosis of a Genetic Disease

a diagnosis of a genetic condition on the basis of a person's physical characteristics and family history, or on the results of a screening test.

1- History and Physical Examination

The diagnosis of a genetic disease requires a comprehensive clinical examination



Types of Genetic Testing

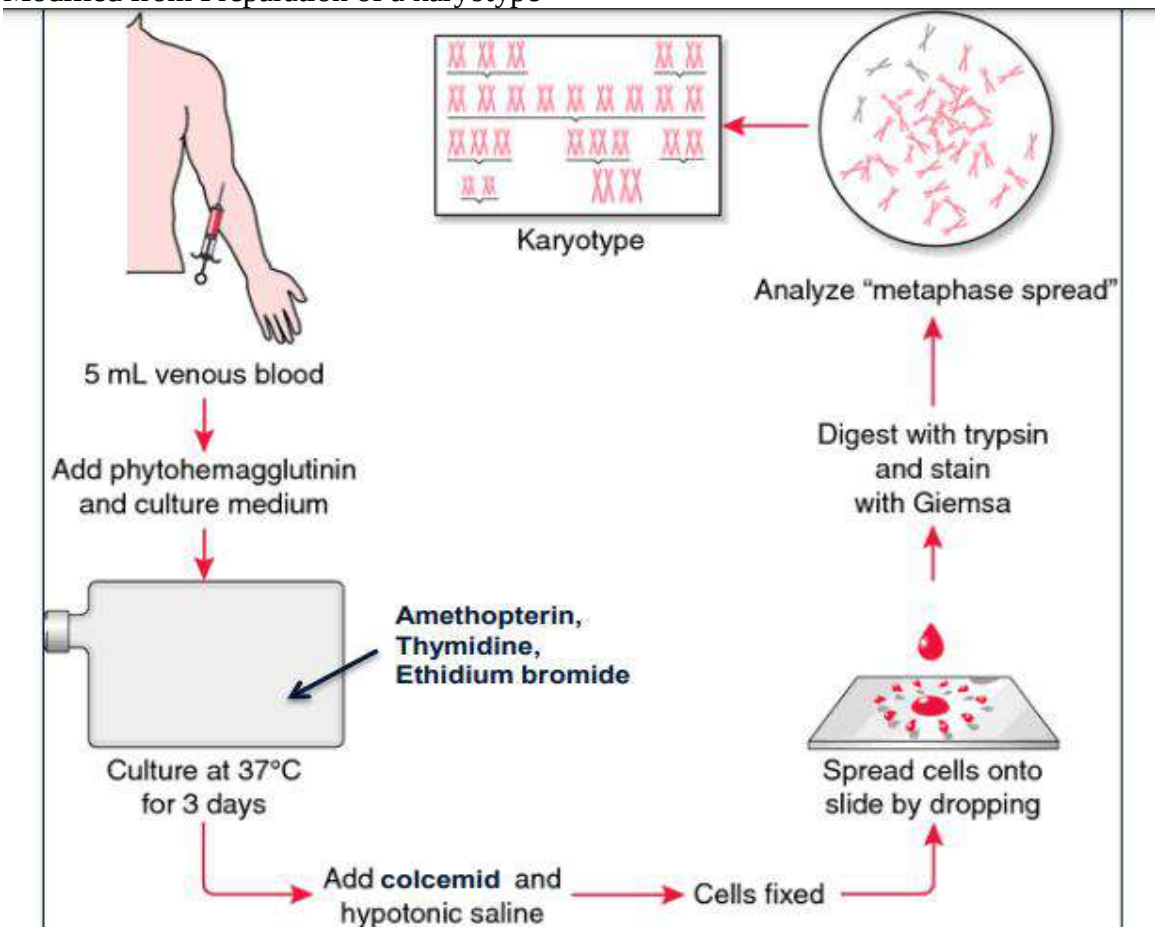
Several different methods are currently used in genetic testing laboratories. The type of test will depend on the type of abnormality that is being measured. In general, three major types of genetic testing are available—cytogenetic, biochemical, and molecular testing to detect abnormalities in chromosome structure, protein function, or DNA sequence, respectively.

1. Cytogenetic Testing

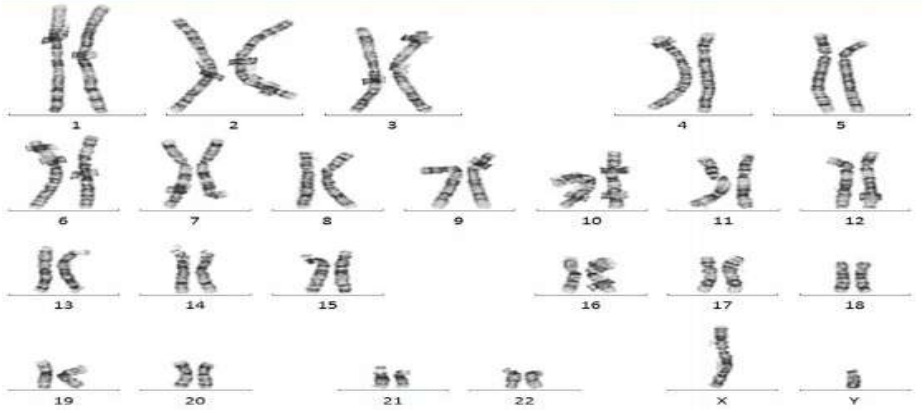
Cytogenetics involves the examination of whole chromosomes for abnormalities. Chromosomes of a dividing human cell can be clearly analyzed under a microscope. White blood cells, specifically T lymphocytes, are the most readily accessible cells for cytogenetic analysis since they are easily collected from blood and are capable of rapid division in cell culture. Cells from other tissues such as bone marrow (for leukemia), amniotic fluid (prenatal diagnosis), and other tissue biopsies can also be cultured for cytogenetic analysis.

Following several days of cell culture, chromosomes are fixed, spread on microscope slides and then stained. The staining methods for routine analysis allow each of the chromosomes to be individually identified. The distinct bands of each chromosome revealed by staining allow for analysis of chromosome structure. Such as

Modified from Preparation of a karyotype



Karyotyping



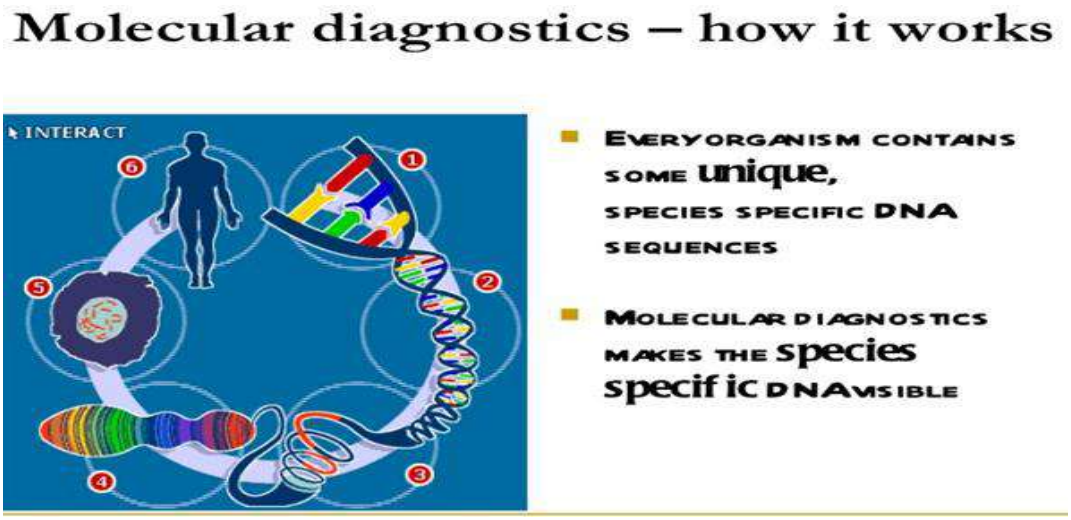
2. Biochemical Testing

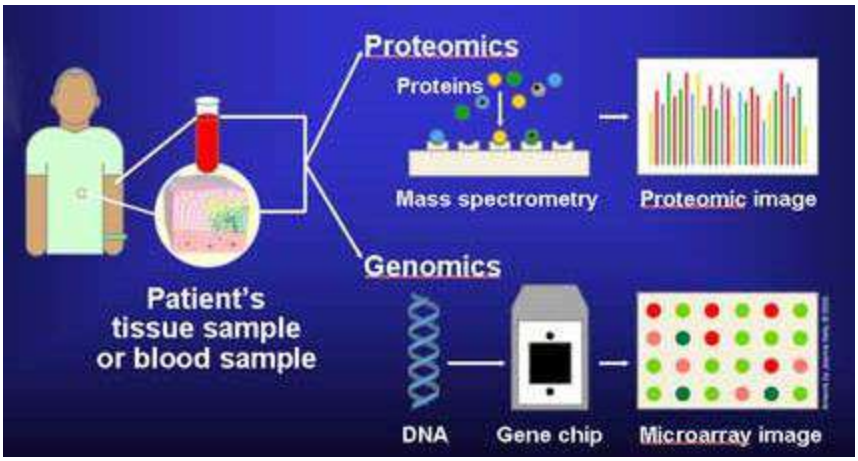
The enormous numbers of biochemical reactions that routinely occur in cells require different types of proteins. Several classes of proteins exist to fulfill the multiple functions, such as enzymes, transporters, structural proteins, regulatory proteins, receptors, and hormones. A mutation in any type of protein can result in disease if the mutation ultimately results in failure of the protein to correctly function.

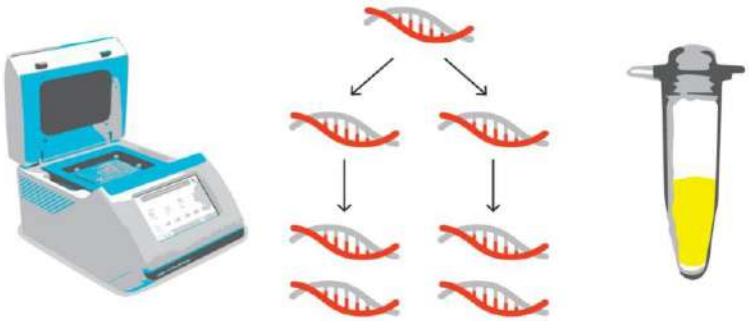
Clinical testing for a biochemical disease utilizes techniques that examine the protein instead of the gene. Depending on the function, tests can be developed to directly measure protein activity (enzymes), level of metabolites (indirect measurement of protein activity), and the size or quantity of protein (structural proteins). These tests require a tissue sample in which the protein is present, typically blood, urine, amniotic fluid, or cerebrospinal fluid. Because proteins are more unstable than DNA and can degrade quickly, the sample must be collected and stored properly and shipped promptly according to the laboratory's specifications.

3. Molecular Testing

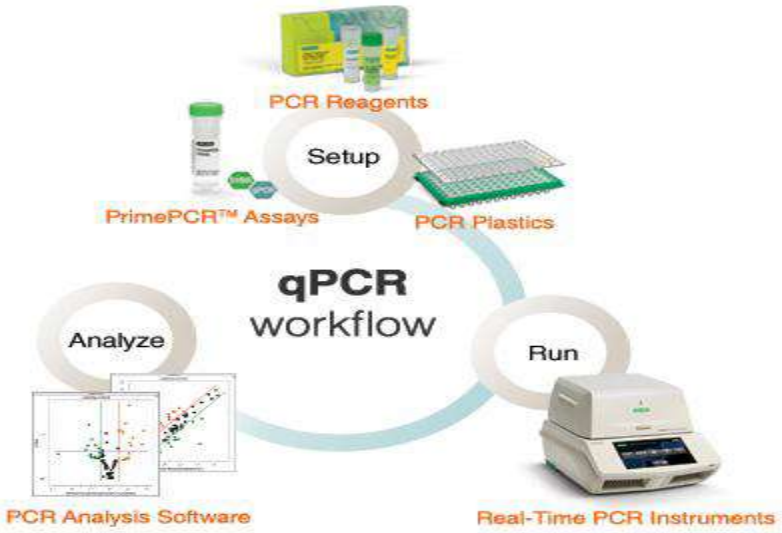
direct DNA testing may be the most effective method, particularly if the function of the protein is not known and a biochemical test cannot be developed. A DNA test can be performed on any tissue sample and require very small amounts of sample. For some genetic diseases, many different mutations can occur in the same gene and result in the disease, making molecular testing challenging.

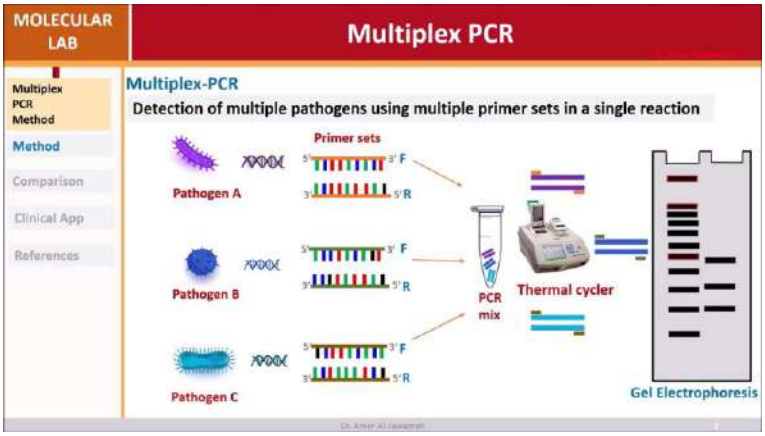






polymerase chain reaction





Uses of Genetic Testing

Genetic tests can be used for many different purposes like

- Newborn Screening
- Carrier Testing
- Prenatal Diagnosis
- Diagnostic/Prognostic
- Predictive/Predispositional

newborn screening : is screened for several genetic diseases. Early detection of these diseases can lead to interventions to prevent the onset of symptoms or minimize disease severity.

Carrier testing :can be used to help couples to learn if they carry—and thus risk passing to their children—a recessive allele for genetic diseases. If both parents are tested, the test can provide information about a couple's risk of having a child with a genetic condition.

Prenatal diagnostic testing: is used to detect changes in a fetus's genes or chromosomes. This type of testing is offered to couples with an increased risk of having a baby with a genetic or chromosomal disorder.

Diagnostic/PrognosticGenetic: tests may be used to confirm a diagnosis in a symptomatic individual or used to monitor prognosis of a disease or response to treatment.

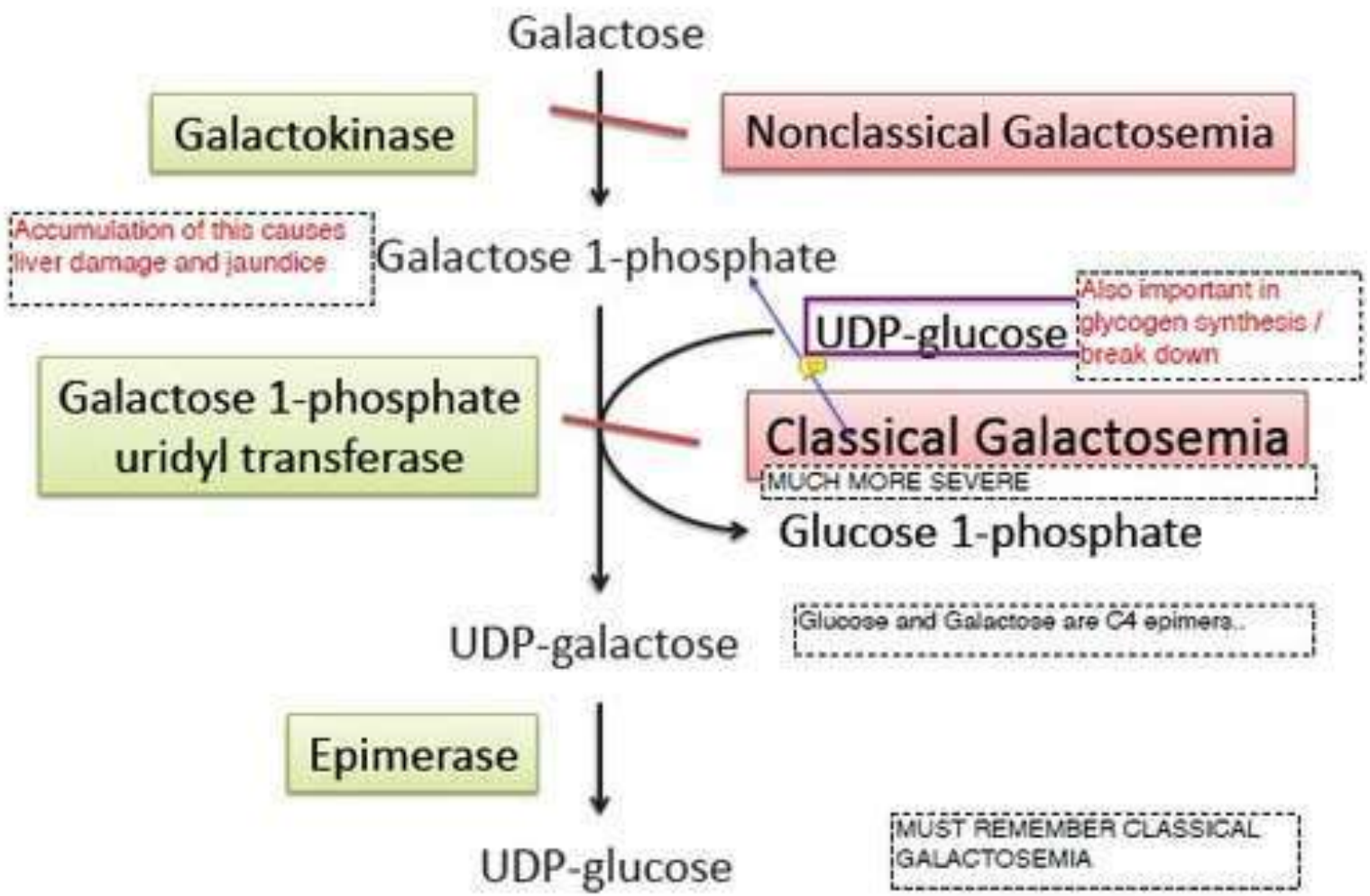
Predictive or predispositional :genetic testing can identify individuals at risk of getting a disease prior to the onset of symptoms . Predictive testing can identify mutations that increase a person's risk of developing disorders with a genetic basis, such as certain types of cancer.

Molecular diagnosis of Galactosemia

Galactosemia

- Classic galactosemia is an autosomal recessive disorder caused by the deficiency of galactose 1-phosphate uridyltransferase, GALT
- It presents with vomiting and diarrhea in neonates within a few days of milk intake. Most patients develop jaundice and hepatic failure. If untreated, it is potentially lethal.
- Newborn screening for galactosemia is routine in all states in the USA and in many other countries.
- Elimination of dietary galactose is the main treatment. However, this does not prevent secondary complications such as growth retardation, mental retardation, dyspraxia, cataracts, ataxia, and ovarian failure later in life

Galactose metabolism



GALT enzyme activity assay

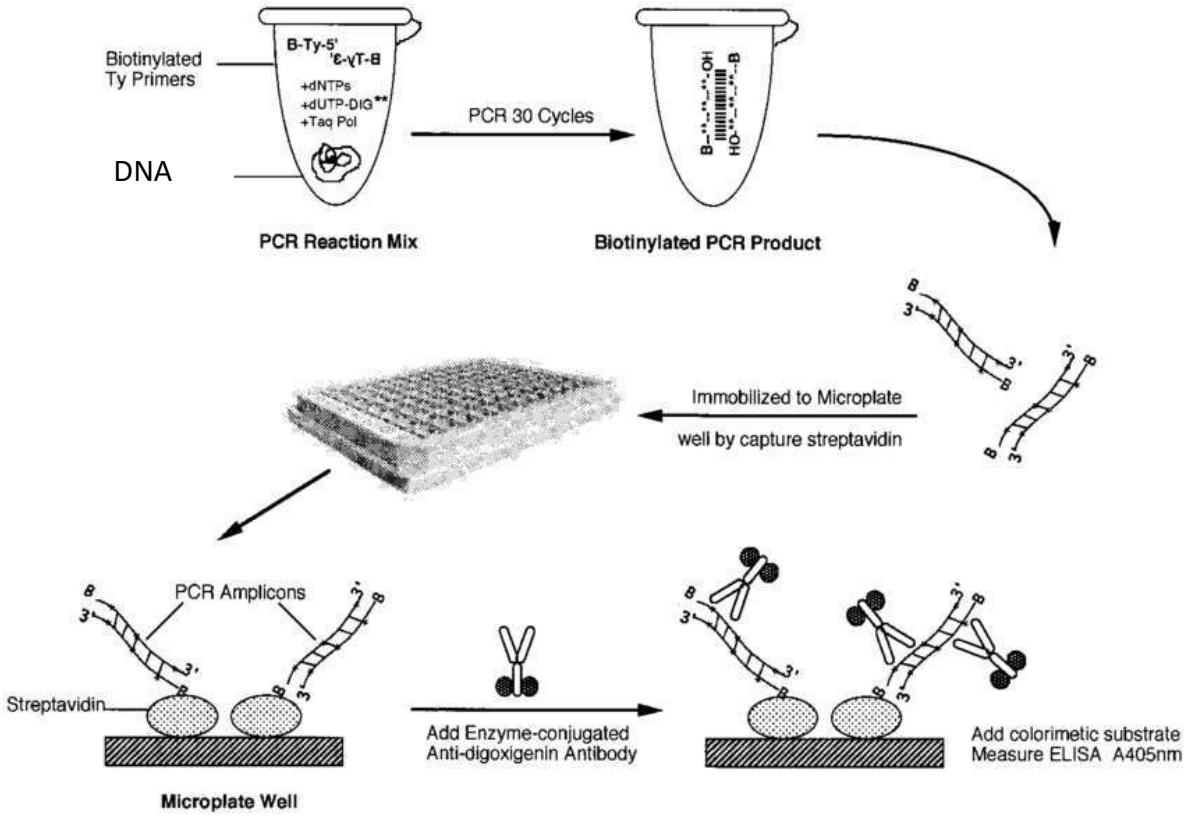
GALT enzyme activity in red blood cells (RBC's) is assayed by measuring the conversion of [14C]-galactose-1-phosphate ([14C]-Gal-1-P) and UPD-glucose (UDPG) to glucose-1-phosphate (Glu-1-P) and 14C-UDP-galactose at 37°C for 1 hour by erythrocyte hemolysate normalized to hemoglobin content.

Result

- The proband presented in the neonatal period with classical galactosemia confirmed by high levels of Gal-1-P [25.1 mg/dL (normal 1.0 mg%)]
- Absent activity of erythrocyte GALT [normal 36.3 1.7 moles/gHbg/(Hr)]

PCR-ELISA test

- PCR-ELISA is containing of PCR and ELISA techniques that allows to detection of nucleic acid instead of protein.
- With this assay, we can determine quantity the PCR product directly after immobilization with biotinylated DNA on a microplate.



- **PCR-ELISA**
- Principle: is based on interaction of between DIG-labeled DNA sequencing and anti-DIG antibody.
- By using of probe in this technique, if target DNA is wrong copied,probe cannot connect to it and the answer will be negative
- **Method**:
- 1- A specific part of the gene is selected, and primers designed for it.
- 2-Amplification was carried out by:
- digoxigenin-11-dUTP (DIG-dUTP) nucleotide.
- Probe is labelled with biotin at its 5'end (Preferred probe complementary in the middle of the gene because it can increase the specificity).
- 3- streptavidin which has high affinity to biotin is coated in microplate.
- 4- Double strand DNA is singled by heat shock and is added to microplate. Complementary sequence is connected to probe and extra material is washed with PBST.
- 5- Anti-DIG antibody is added, and optimal density is measured by spectrophotometry.
- This manner is quick, easy and can be getting the exact result at maximum 4 hours, without needed to advance laboratory and professional person. This method is safe and its color is non- mutagenic.

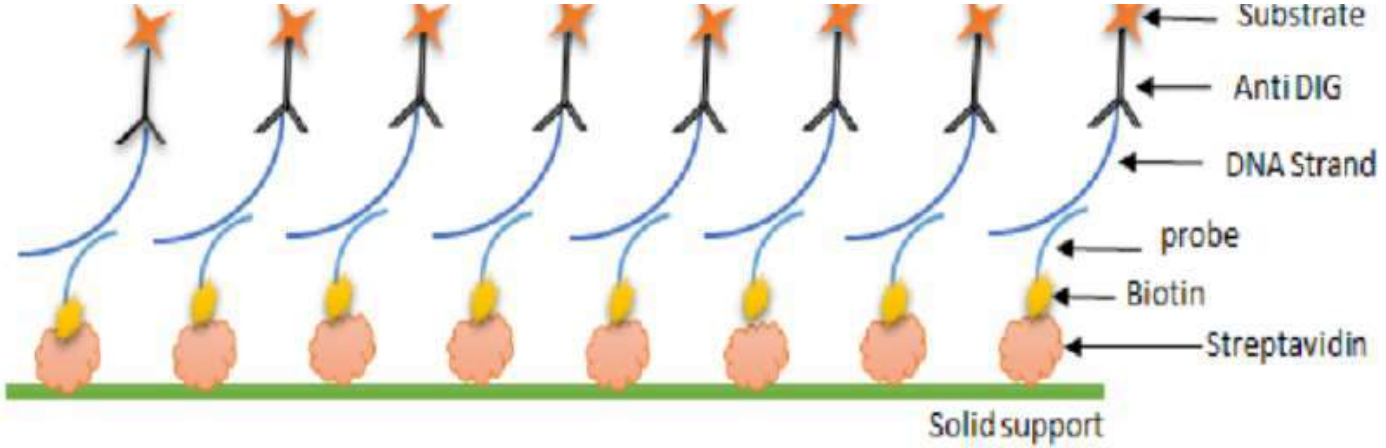


Figure 1. Detection of biotinylated DNA using an anti-DIG-peroxidase conjugate with substrate ABTS to forma blue-green color reaction that is both visible and measured using a spectro-photometer

Advantage of PCR-ELISA :

- This method a more sensitive than agarose gel electrophoresis analysis. Because in this way products are analyzed by colorimetric method,
- The risk of toxicity of color materials and DNA pollution is reduced.
- The specificity of the assay is too high because the detection uses gene-specific probes
- Another advantage of this method is largescale screening with standard laboratory
- The compared with conventional PCR methods, the analytical time of PCR-ELISA is shorter
- Due to its low cost, if study does not require very high sensitivity, PCR-ELISA can be a good option instead of quantitative PCR (qPCR)

Primer used in this test

- The GALT alleles can amplify using three sets of primers:
  - IVS2-F, S135L-R,
  - GALT 6-5, GSINT7R,
  - GALT 9-5, and INTJR.

Table 1

sed to amply the GALT gene for specific mutation ana

Primer sequence (5′ ≥ 3′)
GGGTGGGCCTTCCCTACTCC
GGACCGACATGAGTGGCAGCGTTA
AGGAGGGAGTTGACTTGGTGT
GGGGACACAGGGCTTGGCTCTCTC
GGTCAGCATCTGGACCCCAGG
GCCTGCACATACTGCATGTGA
CACATACTGCATGTGAGAGTC

- **Result**
- Hybridized products developed a green color after incubation with peroxidase substrate.
- This method enabled us to detect the presence of the seven most common mutations of the GALT gene–Q188R, N314D, S135L, L195P, Y209C, IVS2 and K285N.2,4

Interpretation of result

- The GALT genotypes should be compared among mother, father and proband following multiplex PCR of their GALT DNA (Fig. 1).
- Note that from this initial PCR-based analysis, the proband has mutation in the K285N,
- The mother has mutation for the N314D
- Father has mutation for both the K285N and the N314D.

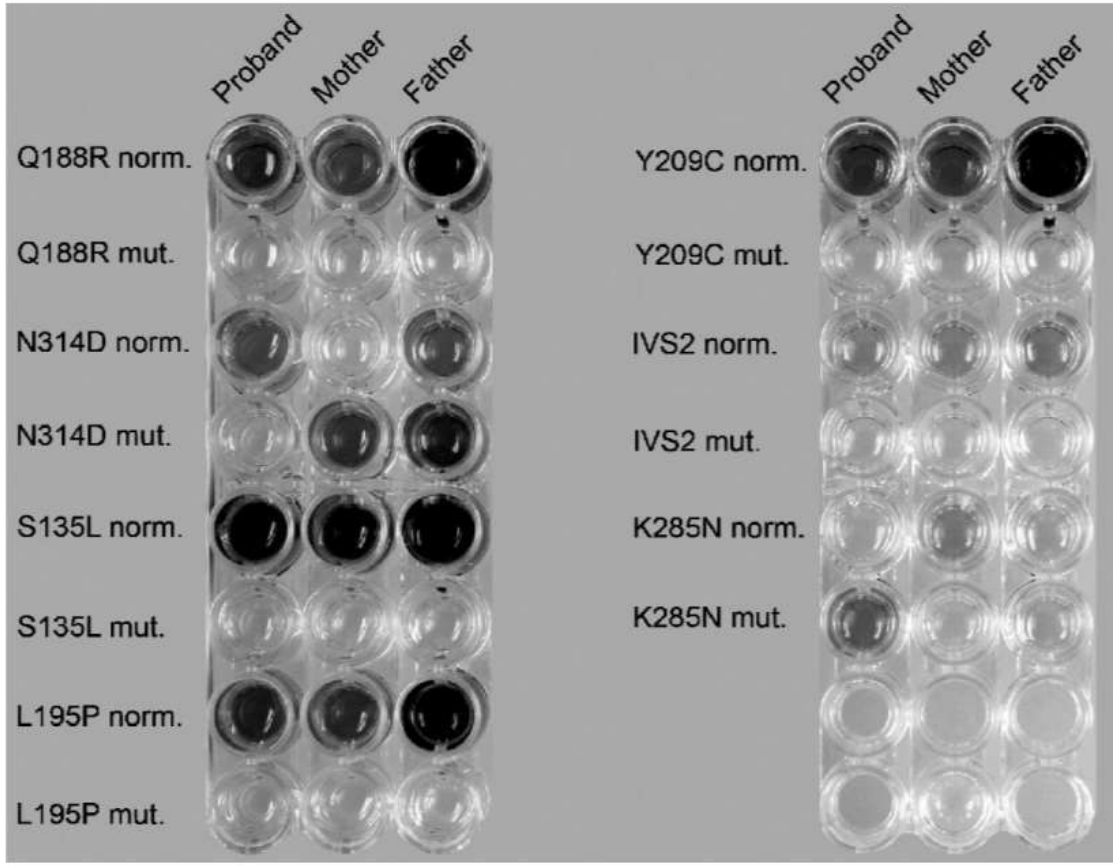


Fig. 1. Allele-specific oligonucleotide (ASO) hybridization for seven most commor mutations of GALT gene. PCR amplified DNA fragments from proband, her mother an father were hybridized with biotinylated oligonucleotides corresponding to either norma (norm.) or mutated (mut.) sequence of each mutation.

The end

Diagnosis polycystic kidney disease

Autosomal dominant polycystic kidney disease:

(ADPKD) tends to be diagnosed in adults over 30 years of age because symptoms do not usually start before then.

- **a diagnosis, about symptoms and family's medical history.**
- **Urine and blood tests:**

urine tests to check for blood or protein in your urine

blood tests so the rate your kidneys are filtering your blood can be estimated

- Scans

have an ultrasound scan to look for cysts in kidneys or other organs, such as in liver.

In some cases, may need to have a CT scan or MRI scan. These will show both kidneys in more detail. An MRI scan will be recommended if you have a family history of brain aneurysms.

4-**Screening**

There are 2 methods that can be used to confirm a diagnosis of ADPKD.

They are:

- using an ultrasound, CT or MRI scan to check for kidney abnormalities
- in special circumstances, using genetic blood tests to determine whether person have inherited one of the genetic faults known to cause ADPKD in his family.

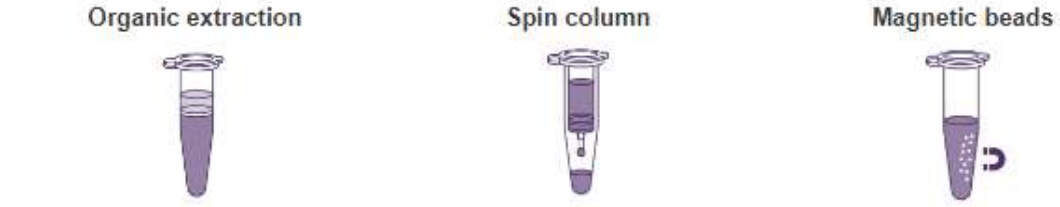
Molecular Genetic Testing in ADPKD

The identification of *PKD1* and *PKD2* followed by detailed characterization of their genomic structures has provided all of the essential reagents that are required for molecular diagnosis of ADPKD..

Genetic diagnosis of autosomal dominant polycystic kidney disease using multiplex-PCR

Multiplex PCR is the simultaneous detection of multiple targets in a single reaction well, with a different pair of primers for each target.

1- nucleic acid isolation methods include:



2- Estimation of the DNA concentration and purity

3- Multiplex PCR Reaction

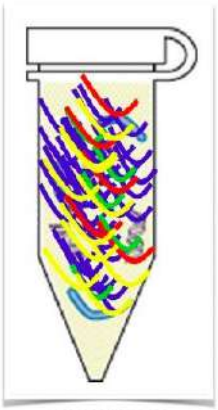
- **Gene primers of ADPKD.**

PKD1 primer	
F	**5′-CGCAGCCTTACCATCCACCT-3′**
R	**5′-TCATCGCCCCTTCCTAAGCA-3′**
PKD2 primer	
F	**5′-GTGGAGACAGAAGCCAACCAAAGAG-3′**
R	**5′-GGATGCGAGATGGAGCCCG-3′**

- The reaction mixture contained 5 µl of DNA template, 1 µl of forward primers (10 mM), 1 µl of reverse primer (10 mM), and 12.5 PCR Master mix and 5.5 µl nuclease-free distilled water. this reaction for each gene.

- total volume of 25 µl. Thermal cycling programs consisted of a denaturation step for 4 min at 94^{0}C followed by 32 cycles of 30 sec at 94^{0}C, 45 sec at 68^{0}C, and 45 sec at 72^{0}C, with a final extension step for 10 min at 72^{0}C.

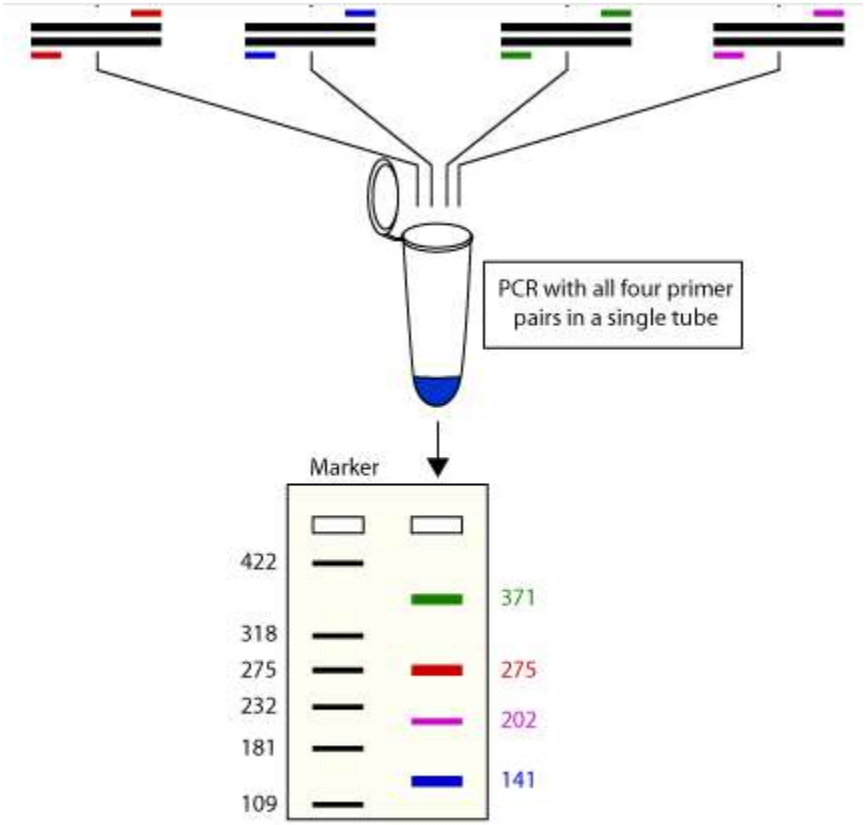
4- Sequencing



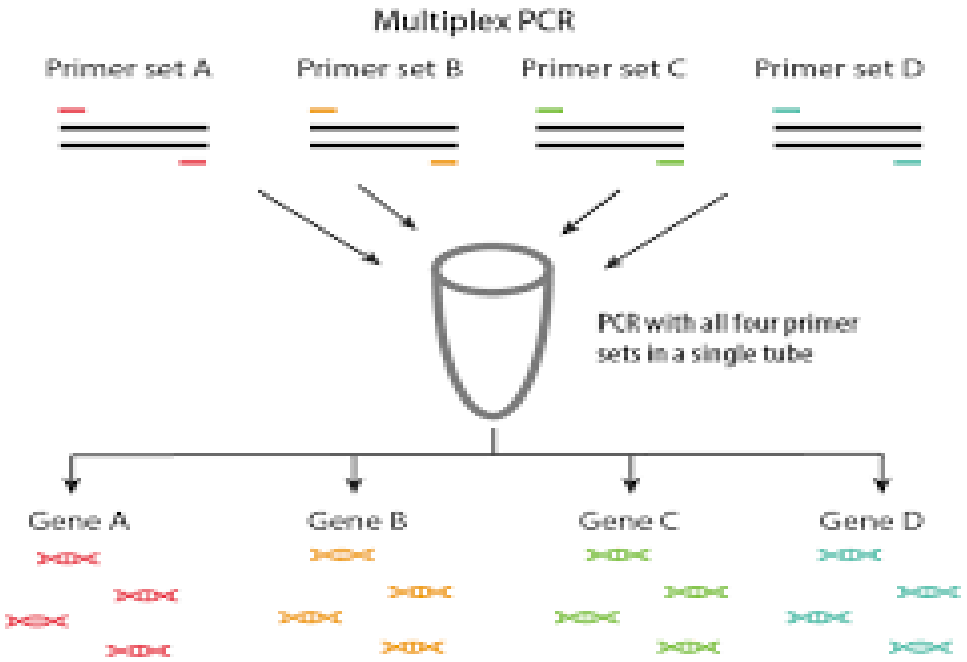
REACTION MIXTURE

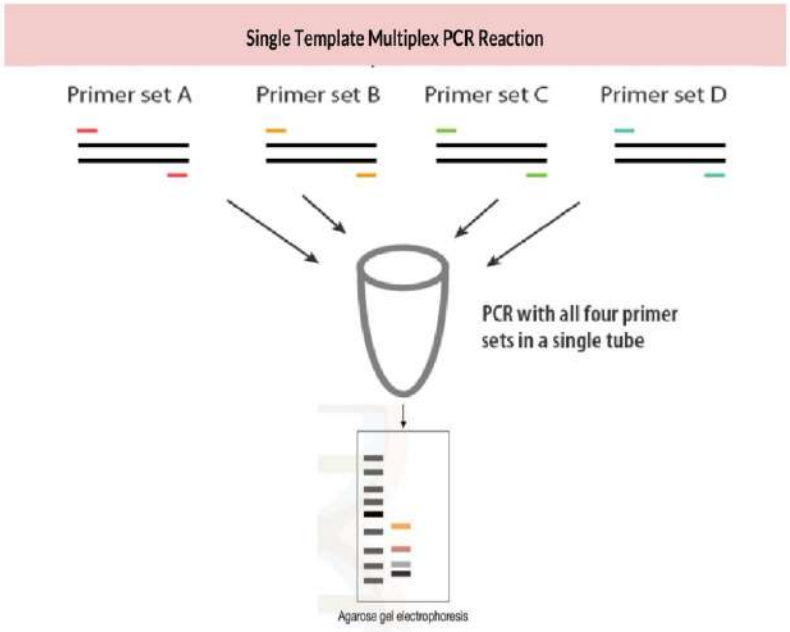
OPTIMIZATION OF MULTIPLEX REACTION COMPONENTS (REACTION MIX):

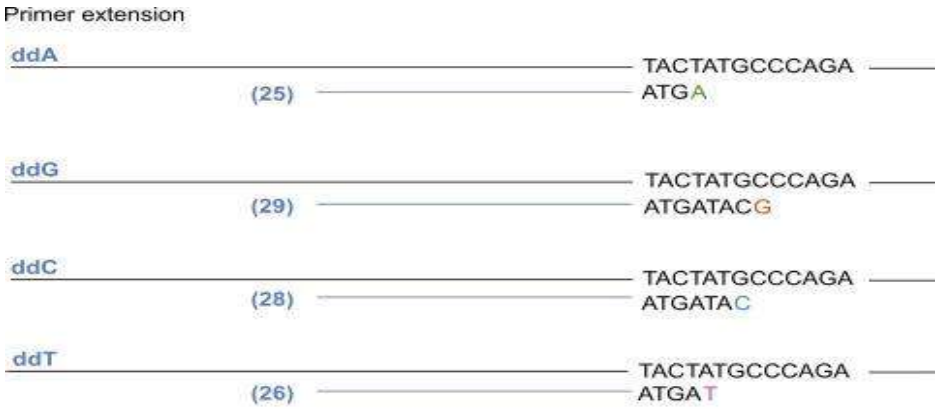
- Amount of Primer
- dNTP and $MgCl_2$ Concentrations
- dNTP/$MgCl_2$ Balance
- PCR Buffer Concentration
- Amount of Template DNA and Taq DNA Polymerase
- Use of Adjuvants: DMSO, Glycerol, BSA



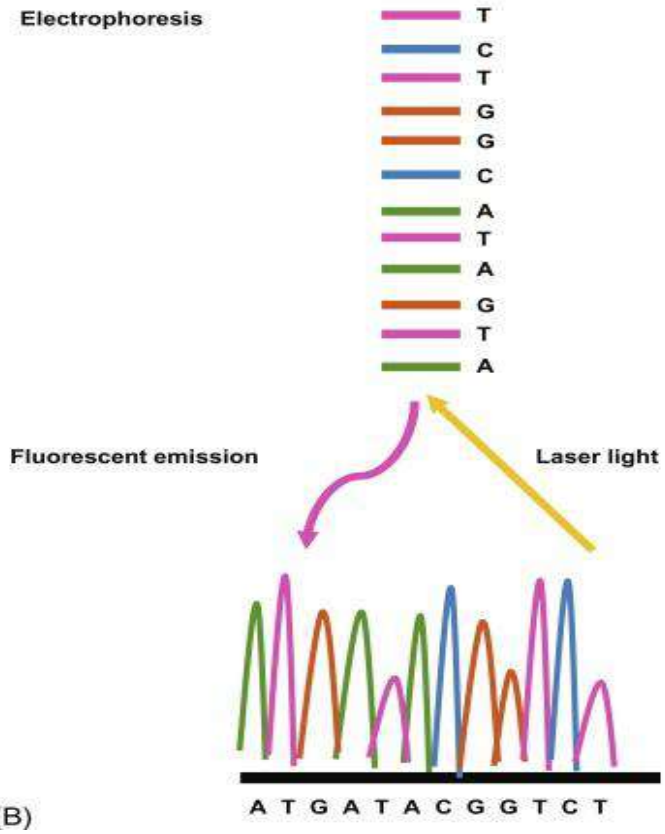
show multiplex PCR reaction


Multiplex PCR
Primer set A
Primer set B
Primer set C
Primer set D
PCR with all four primer sets in a single tube
Gene A
Gene B
Gene C
Gene D


Single Template Multiplex PCR Reaction
Primer set A
Primer set B
Primer set C
Primer set D
PCR with all four primer sets in a single tube
Agarose gel electrophoresis

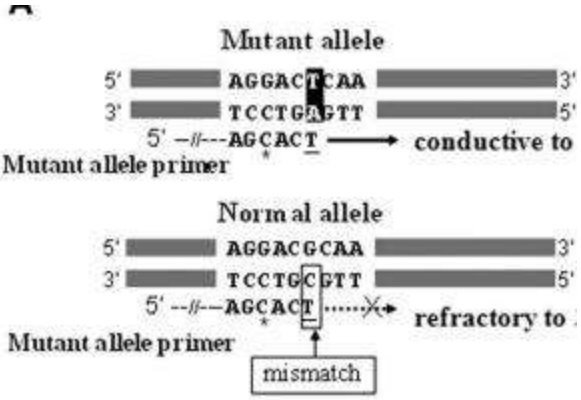


(A)



(B)

Automated Sequencing


A
Mutant allele
5'
AGGACTCAA
3'
3'
TCCTGAGTT
5'
5' -//---AGCACT
conductive to
Mutant allele primer
Normal allele
5'
AGGACGCAA
3'
3'
TCCTGCGTT
5'
5' --//--AGCACT
refractory to
Mutant allele primer
mismatch

Lab 4 diagnosis of DMD and BMD

DNA Diagnosis in BMD/DMD Patients

❖ **Method**

multiplex PCR deletion analysis

60% of the patients have a deletion of one or more exons.

❖ **Diagnosis**

To identify a deletion in the patient's DNA, **two multiplex PCRs** should be preformed in which 9 exons can be amplified simultaneously, and 98% of all deletions could be identified After PCR and agarose gel electrophoresis

❖ **Result**

the absence of amplicons in the gel can simply be scored as a deletion of the corresponding exon

Table 1
Primer Sequences for the Multiplex PCR Deletion Screening, Kit 1 *(8)*

Exon	F/R	Sequence 5′–3′	Size
45	F	AAACATGGAACATCCTTGTGGGGAC	547
	R	CATTCCTATTAGATCTGTCGCCCTAC	
48	F	TTGAATACATTGGTTAAATCCCAACATG	506
	R	CCTGAATAAAGTCTTCCTTACCACAC	
19	F	TTCTACCACATCCCATTTTCTTCCA	459
	R	GATGGCAAAAGTGTTGAGAAAAAGTC	
17	F	GACTTTCGATGTTGAGATTACTTTCCC	416
	R	AAGCTTGAGATGCTCTCACCTTTTCC	
51	F	GAAATTGGCTCTTTAGCTTGTGTTTC	388
	R	GGAGAGTAAAGTGATTGGTGGAAAATC	
8	F	GTCCTTTACACACTTTACCTGTTGAG	360
	R	GGCCTCATTCTCATGTTCTAATTAG	
12	F	GATAGTGGGCTTTACTTACATCCTTC	331
	R	GAAAGCACGCAACATAAGATACACCT	
44	F	CTTGATCCATATGCTTTTACCTGCA	268
	R	TCCATCACCCTTCAGAACCTGATCT	
4	F	TTGTCGGTCTCCTGCTGGTCAGTG	196
	R	CAAAGCCCTCACTCAAACATGAAGC	

Table 2
Primer Sequences for the Multiplex PCR Deletion Screening, Kit 2 *(7)*

Exon	F/R	Sequence 5′–3′	Size
Pm	F	GAAGATCTAGACAGTGGATACATAACAAATGCATG	535
	R	TTCTCCGAAGGTAATTGCCTCCCAGATCTGAGTCC	
3	F	TCATCCATCATCTTCGGCAGATTAA	410
	R	CAGGCGGTAGAGTATGCCAAATGAAAATCA	
43	F	GAACATGTCAAAGTCACTGGACTTCATGGA	357
	R	ATATATGTGTTACCTACCCTTGTCGGTC	
50	F	CACCAAATGGATTAAGATGTTCATGAAT	271
	R	TCTCTCTCACCCAGTCATCACTTCATAG	
13	F	AATAGGAGTACCTGAGATGTAGCAGAAAT	238
	R	CTGACCTTAAGTTGTTCTTCCAAAGCAG	
6	F	CCACATGTAGGTCAAAAATGTAATGAA	202
	R	GTCTCAGTAATCTTCTTACCTATGACTATGG	
47	F	CGTTGTTGCATTTGTCTGTTTCAGTTAC	181
	R	GTCTAACCTTTATCCACTGGAGATTTG	
60	F	AGGAGAAATTGCGCCTCTGAAAGAGAACG	139
	R	CTGCAGAAGCTTCCATCTGGTGTTCAGG	
52	F	AATGCAGGATTTGGAACAGAGGCGTCC	113
	R	TTCGATCCGTAATGATTGTTCTAGCCTC	

Preparation in Batches of Multiplex PCR Kits

1. Prepare the master mix for each kit. Final concentrations of the mix for the deletion detection kits are:

- 1X multiplex PCR buffer
- 0.5 μ*M* of each primer.
- H2O free Dnase Rnase water
- Template

2. Template:

DNA of patients (test), positive controls (with different deletions) , **normal controls (healthy)**, and negative control/to check contamination (no-**DNA)** .

3.Place the PCR tray in a preheated thermal cycler,

set at 95°C (hot start). an initial denaturation of 5 min at 95°C followed by 25 cycles of 94°C for 30 s, 53°C for 30 s, and 65°C for 4 min, with a final extension period of 5 min at 65°C. Cool down to 4°C after the PCR has finished.

	1n	xn
Master mix	12.5 µl	
Fv primer	0.5µl*9 exons (4.5)	
Rv primer	0.5µl*9 exons(4.5)	
H2O	1.5µl	
template	2µl(100 ng/µl)	
Total volume	25µl	

Example: N=7

- **test sample(3)**
- **Positive unhealthy control (1)**
- **Normal healthy control(2)**
- **Negative control (1)**

The reaction prepared for two panels:

- **First (kit one)(primer for exons:44,48,19,17,51,8,12,44 and 4)**
- **Second (kit two)(primer for exons:PM,3,43,13,50,6,47,60,52**

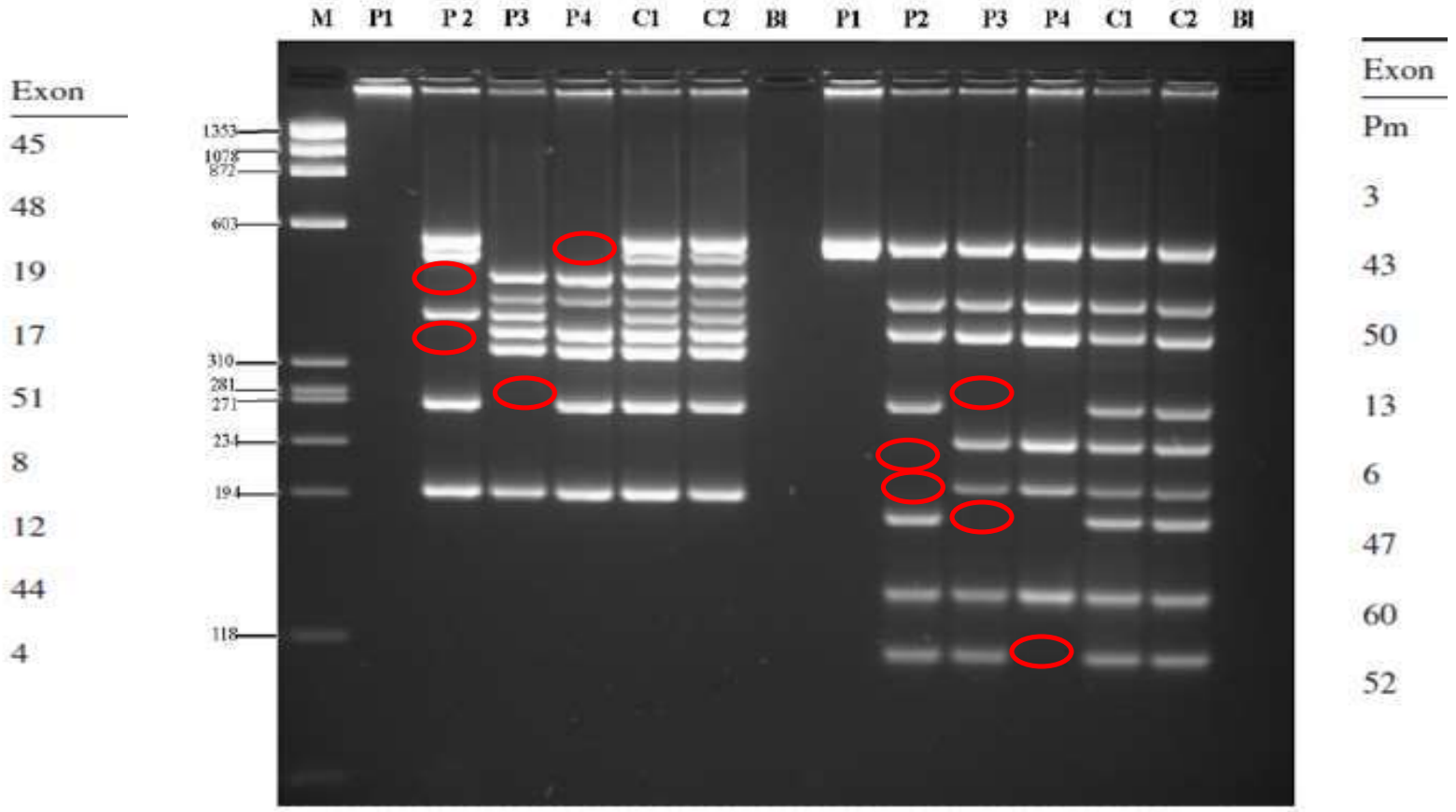
4.Place the gel in an electrophoresis tank containing 1X TBE running buffer with 0.2 μg/mL ethidium bromide.

5. Load 25 μL of the PCR reaction on gel

6. Electrophorese the gel for 2 h at 90 V. The bromophenol blue dye must migrate at least 10 cm into the gel for a good separation.

7. Prepare an image of the gel using an imaging system. Check for absence of product formation in the no-DNA control.

Result



Gel Image of the two multiplex PCR assays for deletion detection in patients. For Patient 1, a deletion of all DMD exons with the exception of exon 1 is detected (amplicon Pm is present). Patient 2 shows a minimal deletion of exons 8 to 19. In Patient 3, the exact deletion of exons 44 to 50 is characterized using this multiplex because exons 43 and 51 are present. Patient 4 has a minimal deletion of exons 45 to 52, the deletion may extend further at the 3′ end (exons 53 and beyond). Note that for all four patients, the observed deletion confirms the results of both multiplex PCRs.

Diagnosis of Burkitt Lymphoma

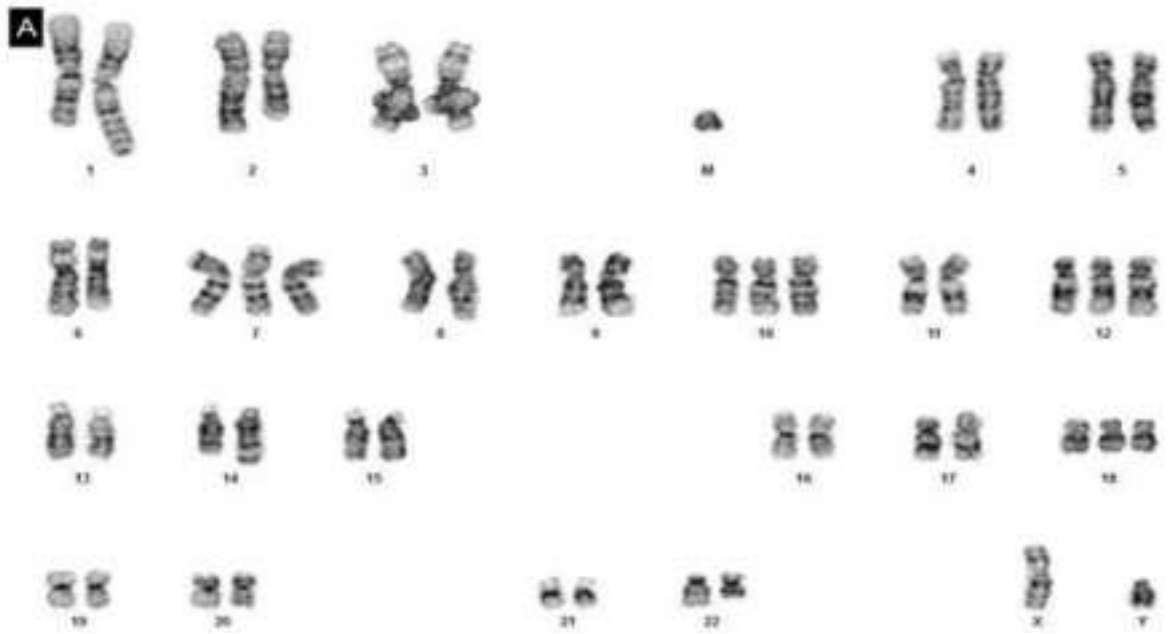
A- If Burkitt lymphoma is suspected, all or part of an enlarged lymph node or other suspicious disease site will be biopsied. In a biopsy, a sample of tissue is examined under a microscope. This will confirm or rule out Burkitt lymphoma.

B- Molecular diagnosis:

Tumor-biopsy specimens from patients with the diagnosis of diffuse large-B cell lymphoma

1- cytogenetic findings (including the presence or absence of a c *myc* translocation

Karyotyping is valuable in assessing the gross genomic changes of lymphoma cells, such as ploidy, deletions, translocations and marker chromosomes. The advantage of using karyotyping is its ability to determine the partner chromosome of the *c-MYC* translocation.



the derivative (18) t(14;18)

Lab 5 Genetic disease and molecular diagnosis Dr.Aseel shakir

2- gene expression:

the molecular alterations that lead to *c-MYC* dysregulation, and their effect on prognosis and diagnosis in specific types of B-cell lymphoma.

The detection of *c-MYC* gene translocation and c-MYC gene expression has become essential in the clinical diagnosis and prognosis of aggressive B-cell lymphomas

RNA targeting the c-*myc* gene from patients and healthy control

-RNA isolation.

Isolation of total RNA using TRIzol™ reagent was conducted according to the manufacturer's recommendations

The resulting RNA pellet was air-dried, resuspended in nuclease-free water

RNA was quantified using a nanodrop $A_{260/280}$ readings between 1.8 and 2.0 were used to ensure purity.

-Reverse Transcription-Quantitative PCR (RTq-PCR)

The GoTaq®1-Step RT-qPCR System kit It is a reagent system for quantitative analysis of RNA using a one-step RT-qPCR protocol. Reverse Transcriptase and GoTaq®qPCR Master Mix for efficient, sensitive and linear one-step RT-qPCR quantification over a wide range of RNA template inputs. The system contains the proprietary fluorescent DNA-binding dye SYBR®Green I dye that exhibits greater fluorescence enhancement, upon binding to double-stranded DNA (dsDNA).

-Synthesis of complementary DNA (cDNA) from mRNA and expression The isolated RNA was reversely transcribed to cDNAusing the GoTaq® 1- Step RT-qPCR System,which included the following components: • GoTaq®qPCR Master Mix

- GoScript™ RT Mix (cDNA)KIT for 1-Step RT-qPCR
- Nuclease-Free Water

Lab 5 Genetic disease and molecular diagnosis Dr.Aseel shakir Reaction total vol. 20 µl

• GoTaq®qPCR Master Mix (1X): 10 µl

• GoScript™ RT Mix for 1-Step RT-qPCR (1X): 0.5µl

• Forward primer (10µM): 2µl

• Reverse primer (10µM):2µl

• RNA (62 - 110ng/µl): 5 µl

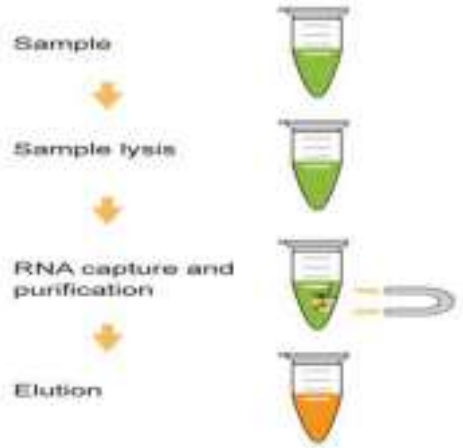
• Nuclease-free water: 0.5 µl

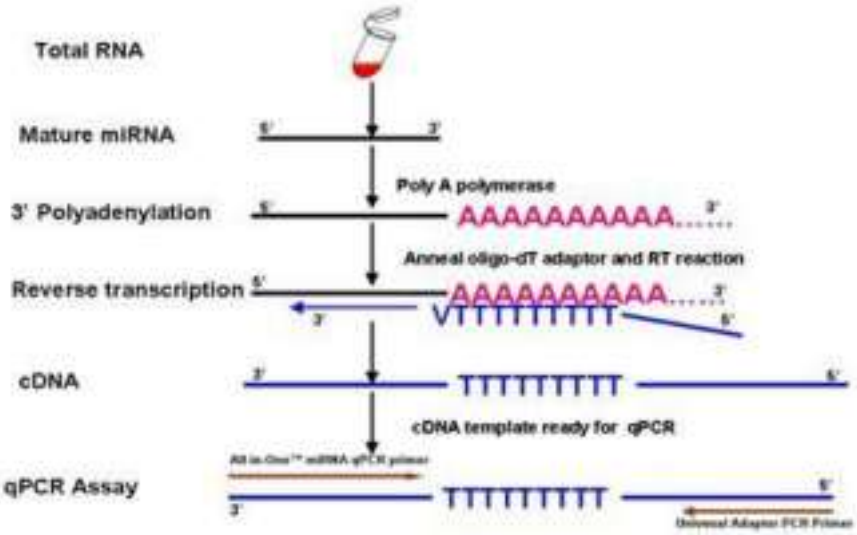
Primers target gene and control gene
c-*myc* forward primer: 5′-TAC CCT CTC AAC GAC AGC AG-3′;
c-*myc* reverse primer: 5′-TCT TGA CAT TCT CCT CGG TG-3′;
β-actin forward primer: 5′-TCA CCC ACA CTG TGC CCA TCT ACG A-3′;
β-actin reverse primer: 5′CAG CGG AAC CGC TCA TTG CCA ATG G-3′(control gene).

Reaction real time PCR:

Steps	Temperature	Time	Cycles
• cDNA Synthesis	37°C	15minutes	1
• Initial Denaturation	95°C	5 minutes	1
• Denaturation	95°C	30 seconds	40
• Annealing	60°C	30 seconds	
• Extension	72°C	30 seconds	
• Melt curve	65-90°C		1
• Holding	4°C		

c-MYC overexpression results in B-cell lymphomas


Sample
Sample lysis
RNA capture and purification
Elution


Total RNA
Mature miRNA
Poly A polymerase
3' Polyadenylation
AAAAAAAAAA
Anneal oligo-dT adaptor and RT reaction
Reverse transcription
AAAAAAAAAA
VTTTTTTTTT
cDNA
TTTTTTTTT
cDNA template ready for qPCR
qPCR Assay
TTTTTTTTT

1 a. **RNA**
RNA consist of Start codon AUG and ends with poly A tail

b. **Oligo dT Primer**
Oligo dT Primer is binding to RNA poly A tail

c. **Reverse Transcriptase and dNTPs**

d. **Reverse Transcriptase** is an enzymes binds to oligo dT primer and synthesises the cDNA by adding dNTPs

e. **RNA hybrid formation :**
First – strand cDNA synthesis

- **Lab6: Laboratory Diagnosis of Cystic fibrosis disease**

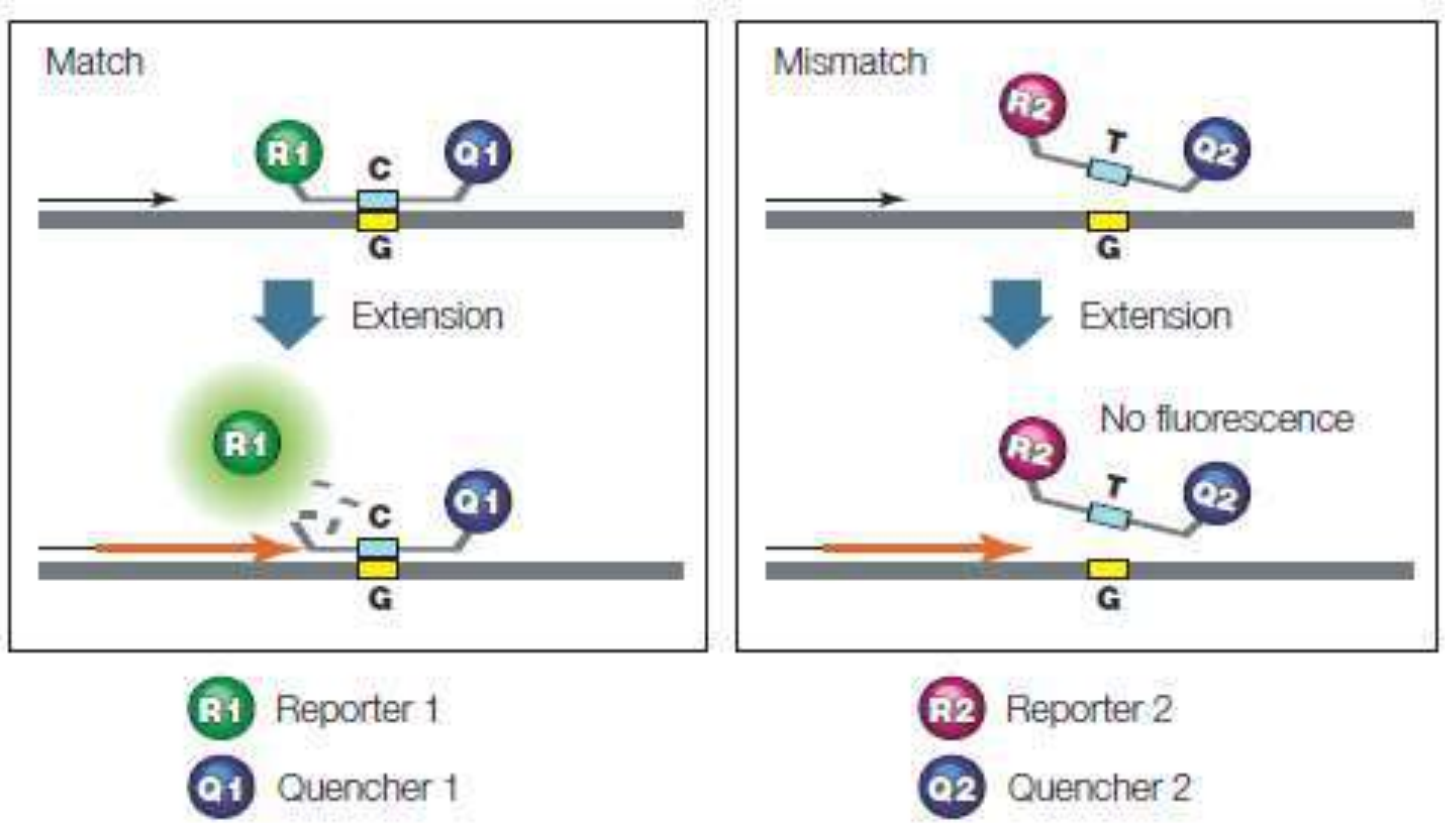
Dr. Rasha Al-khalidi

Q-PCR

- Q-PCR combined with melting curve analysis allows for faster detection
- Carry-over contamination can be prevented in the closed-tube system.
- The quality control of the synthesized PCR products is assured in the determinations by performing the melting curve analysis.
- The whole diagnostic procedure takes 1 h for 32 samples.
- Multiplexing can be assessed by using several primer-probe detection systems to screen for larger numbers of mutations in the same capillary tube at the same time

TaqMan probes used to discriminate between allelic variants

- The TaqMan probes used for allelic discrimination are differentially labeled fluorescent
- probes that are specific for each allele. One probe is specific for the wild-type (WT)
- allele and another probe is specific for an allelic variant.
- The probes are differentially labeled with a 5' fluorescent reporter dye.

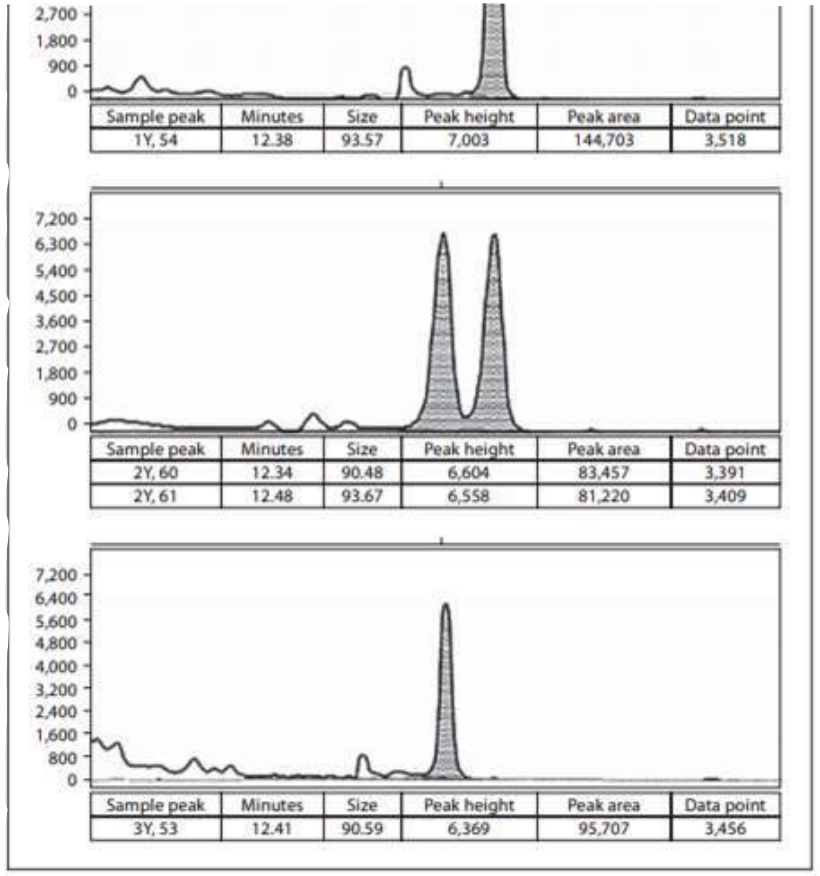


Quantitative Real-Time PCR and Melting Curve Analysis

material	1n	xn
Master mix	10	
Fv primer	1	
Rv primer	1	
Probe 1	1	
probe2	1	
H2O	?	
DNA	40 ng/µl	
Final volume	20µl	

Quantitative real-time PCR	
Q-CF forward	5′-GGA-GGC-AAG-TGA-ATC-CTG-AG-3′
Q-CF reverse	5′-CCT-CTT-CTA-GTT-GGC-ATG-CT-3′
P1	5′-TTT-TCC-TGG-ATT-ATG-CCT-GGC-ACC-ATT-AA-F
P2	LCRed640-GAA-AAT-ATC-AT-CTT-TGG-TGT-TTC-C-P

- **The samples should be tested in duplicates** and in each run there was also a **normal healthy sample**, a **heterozygous sample** and a **D.W** as controls.
- The initial 10 min denaturation at 95 ° C was followed by 35 cycles, at denaturation (95 ° C; 0 s), annealing (63 ° C; 25 s) and extension (72 ° C; 5 s).
- Melting curve analysis was performed following the PCR and Tm were determined.



- **Result**
- 1.Detection of F508del using fluorescent PCR and DNA fragment analysis. The electrophoretograms show a healthy (wild type) sample with a PCR fragment of 93 bp at the top, a heterozygous sample with 90 and 93 bp fragments in the middle, and a homozygous F508del with a 90 bp fragments at the bottom

•Figure 2 shows the melting curves of Q-PCR. The Tm of the F508del PCR product is 49 ° C, while the wild type PCR product has a Tm of 60 ° C.

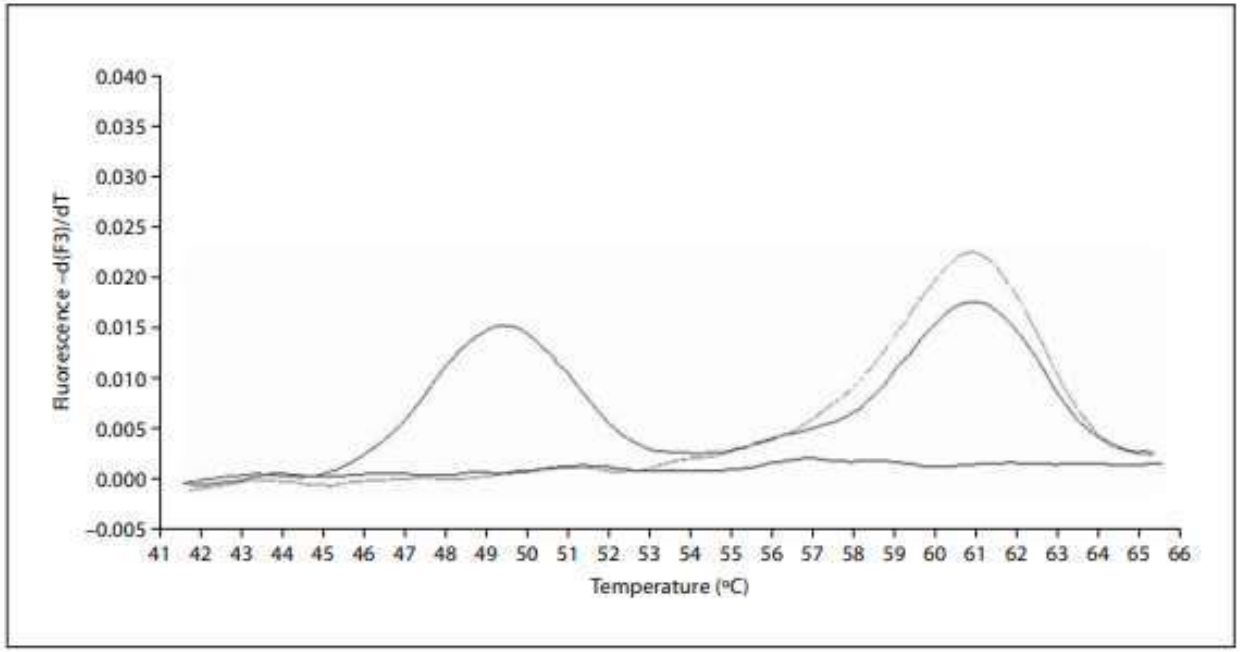
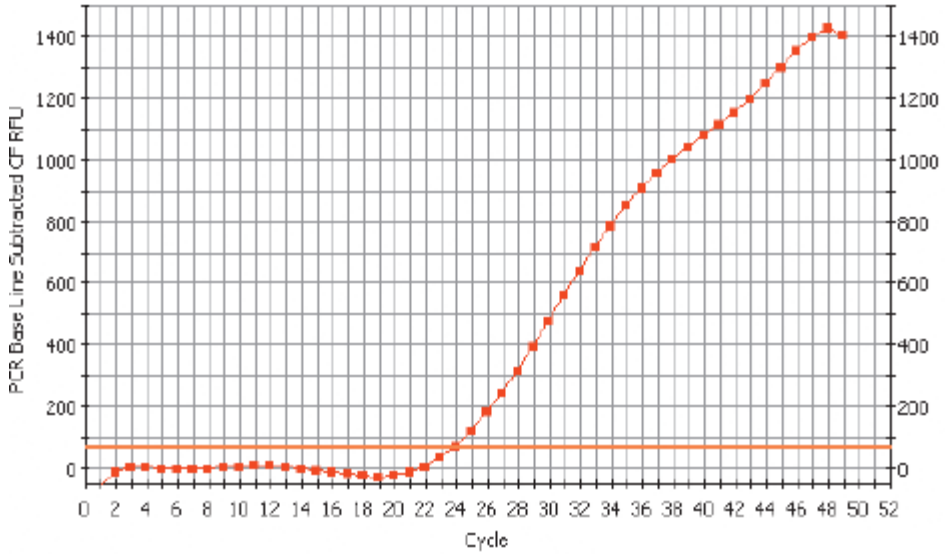


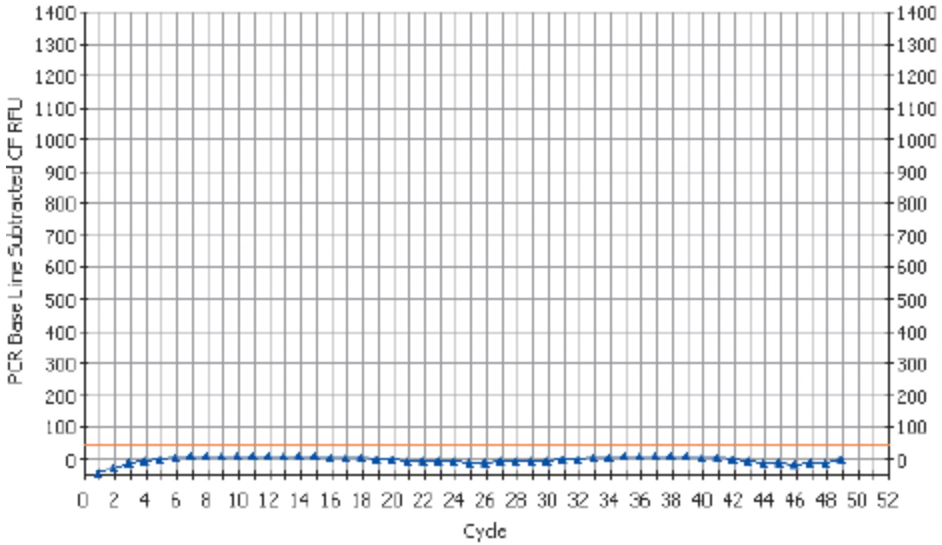
Fig. 2. Detection of *ΔF508del* using Q-RT-PCR and melting curve analysis. The figure shows the melting curve analysis curve of a heterozygous (double peaks), a healthy wild sample (one peak) and a d.water (negative control; no peak). The PCR product of *ΔF508del* has a T_m of 49°C and the wild type 60°C.

CFTR wt probe/FAM

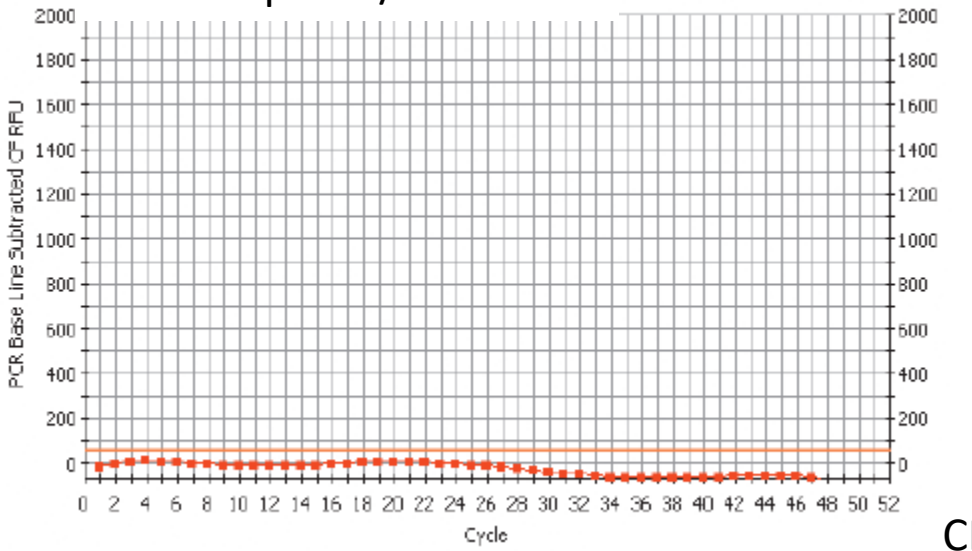


CFTR mut probe/VIC

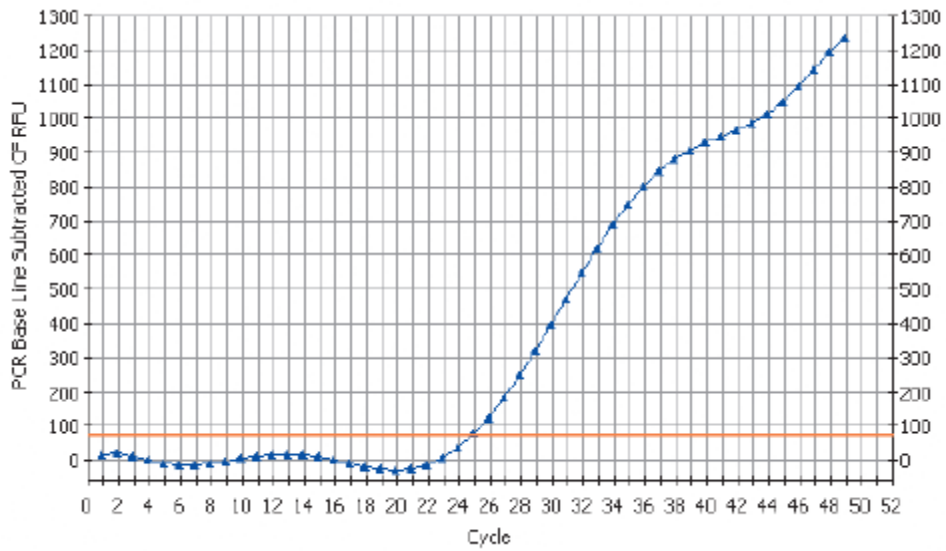
WT



CFTR wt probe/FAM

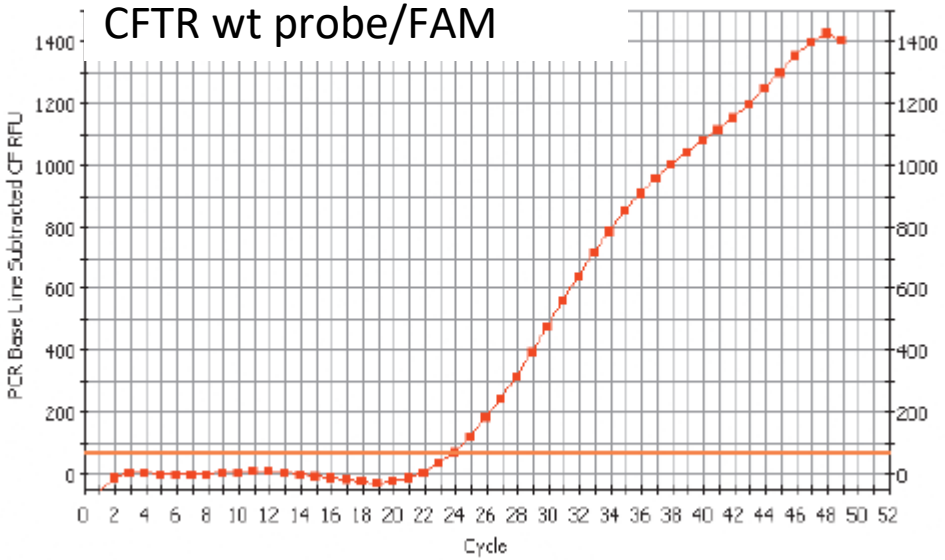


CFTR mut probe/VIC

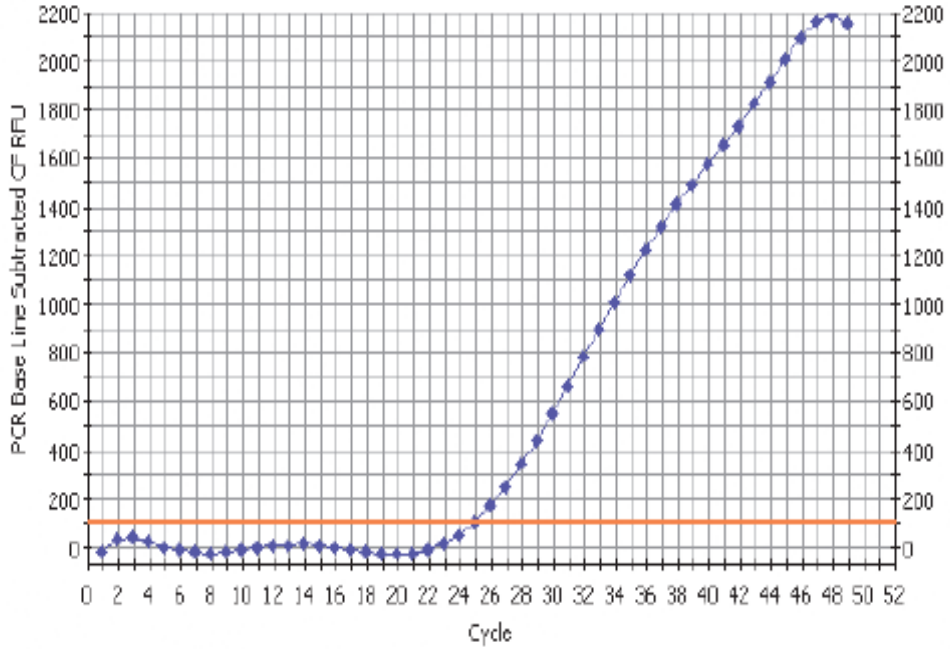


mut

CFTR wt probe/FAM



CFTR mut probe/VIC



Heterozygote

- Thank you