Hematology Case Conference

9/24/02

Clinical History

- 46 year-old female admitted on 5/24/02 to Memorial City Hospital with severe asthma, subsequently developed pulmonary embolism
- Patient has a history of coronary artery disease; no prior history of thrombophilia; also no history of thrombophilia in her family.
- Specimen was collected and submitted for thrombophilia work-up before patient was put on anticoagulant.

Laboratory Results

Tests	Results	Reference Range
PT	9.1	8.8 – 11.3 sec
aPTT	20.6	24.5 – 35.6 sec
Fibrinogen	311	221-430 mg/dl
Thrombin Time	15.5	15.0-21.2 sec
StaClot LA (Hexagonal neutralization)	Negative for lupus anticoagulant	Negative
ATIII, functional	171	80-120%
Factor II	173	83-117%
Factor VIII	208	55-145%
Protein C, functional	98	73-147%
Protein C, Antigen	304	65-145%
Protein S, functional	82	54-137%
Protein S, total	144	58-146%
Lipo (a)	14	< 30 mg/dL
Plasminogen	145	75-124%
APC Resistance	Positive (ratio=0.72)	Negative (ratio>0.81)
Factor V Leiden, R506Q	Positive, heterozygote	Negative

Laboratory Results (cont'd)

Tests	Results	Reference Range
MTHFR, C677T	Negative	Negative
Prothrombin Gene Mutation, G20210A	Negative	Negative
Anti-Cardiolipin Antibodies	-	-
IgG	7.0	< 15 GPL
IgM	2.0	< 12.5 MPL
IgA	7.0	< 22 APL
Anti-Phosphatidylserine Antibodies	-	-
IgG	1.0	< 16 GPS
IgM	6.0	< 22 MPS
IgA	3.0	< 20 APS
Anti-Beta 2 Glycoprotein I Antibodies	-	-
IgG	3	< 18 SGU
IgM	3	< 18 SMU

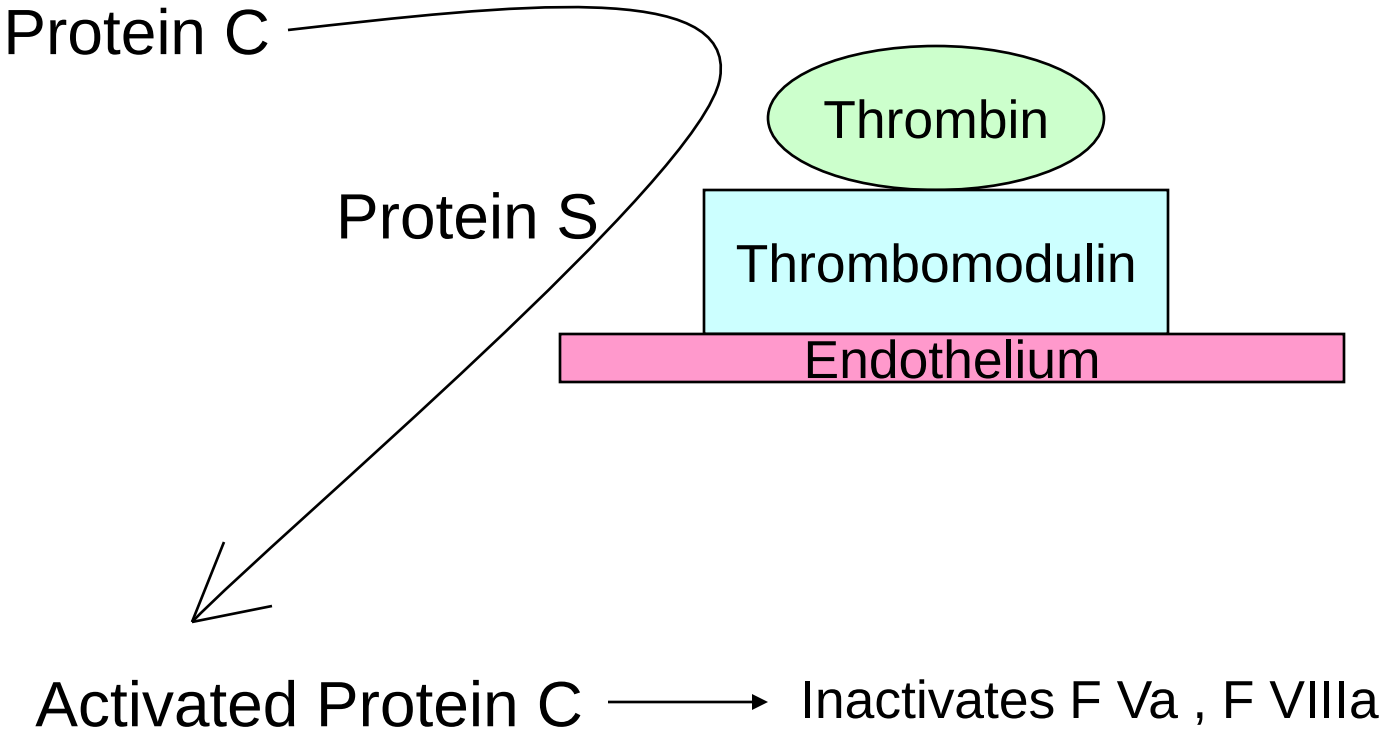
Factor V Leiden

- Dahlback described an inherited (autosomal dominant) disorder associated with venous hypercoagulation (1993).
- This disorder is due to a mutation in Factor V gene on chromosome 1 (the mutated gene is called Factor V Leiden). Mutation at nucleotide 1691:
Guanine-> Adenine, causing substitution at position 506:
Arginine-> Glycine

Note: FV HR2 haplotype (A4070G, His199Arg) has unknown risk

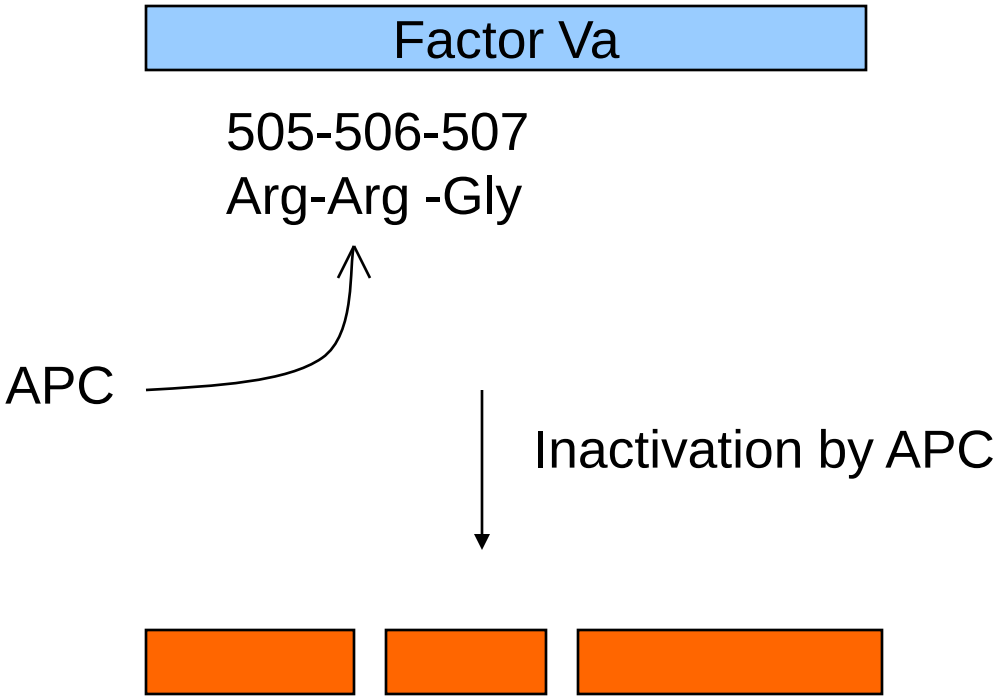
- Review of Protein C pathway: down-regulation of coagulation with activated protein C complex (APC)
- Emphasis on laboratory testing

Protein C Pathway



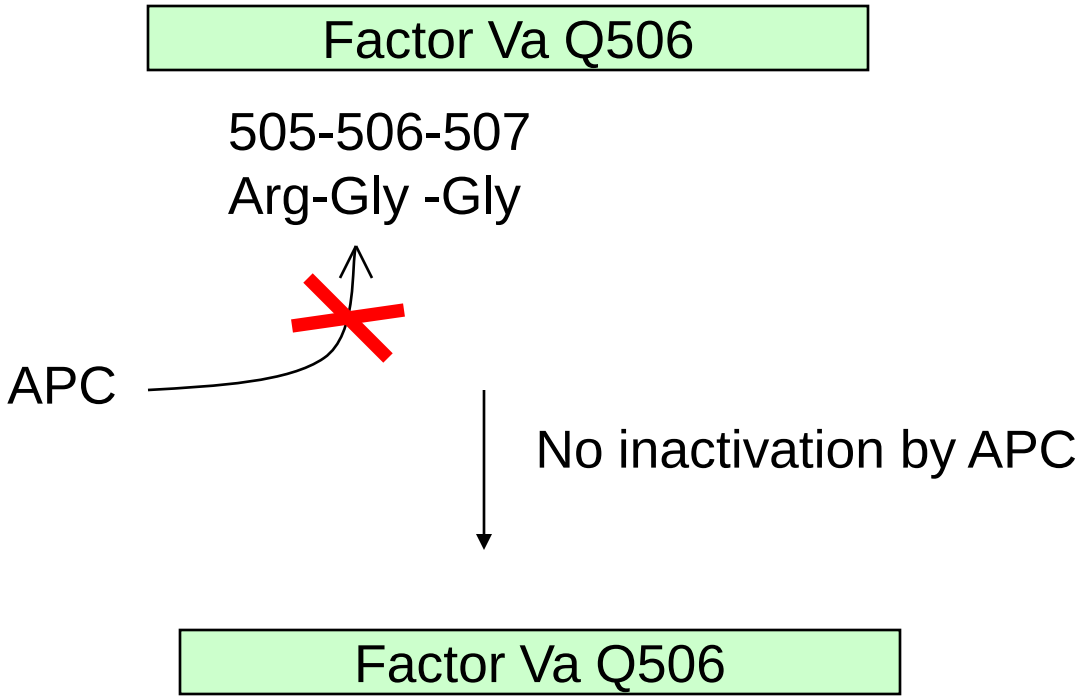
Cleavage Site on Factor V by APC:

Inactivation of Factor V in normal patient



Cleavage Site on Factor V by APC:

No inactivation of Factor V in patient with
Factor V Leiden (95% of APC resistance cases)



Prevalence of Inherited Disorders in Hypercoagulation

- Protein C deficiency: 5-6%
- Protein S deficiency: 5-6%
- Antithrombin III deficiency: 1-4%
- *Factor V Leiden*: 20-60%

Hypercoagulation incidence: 1/1,000/year

Two Forms of Factor V Leiden

- Heterozygous: 3-7% of Caucasian population, 3-5 fold increase in risk of deep vein thrombosis, 20% have thrombosis by 33 y/o (mean age of first thrombotic episode)
- Homozygous: 0.06-0.25% of Caucasian population, 50-100 fold increase in risk of deep vein thrombosis, 40% have thrombosis by 33 y/o

Other Relevant Information on Factor V Leiden

- Some patients do not have thrombosis unless exposed to hemostatic challenge
- Increased risk for hypercoagulation in combination with other risk factors (such as Protein C or S deficiency)
- Factor V procoagulant activity is normal
- Treatment: heparin, coumadin

Testing for Factor V Leiden

- Clot-based testing (blue top tube)
- Polymerase chain reaction (PCR) testing (purple top tube)

Clot-based Testing

- Determines the resistance to APC, using platelet-poor plasma
- Principle of test: in patient with APC resistance (APCR), Factor V is not inactivated by APC, hence (PTT with APC) is not prolonged. This will shorten the APCR Ratio

Clot-based Testing (cont'd)

- APCR Ratio = (PTT with APC)/
  (PTT without APC)

APCR > 2 -> negative for APC resistance
APCR < 2 -> positive for APC resistance

- Considerable overlap between FV Leiden heterozygous and normal

Note: cut-off value is dependent on particular test kits

Clot-based Testing (cont'd)

- Inaccurate result with:intrinsic factor deficiency, lupus anticoagulant, anticoagulant (need to get pre-treatment sample)
- New generation test (COATEST by Chromogenix)

1. Predilution of patient sample with FV deficient plasma before testing: makes assay more sensitive and alleviates coumadin interference
2. Polybrene: alleviates heparin intereference

PCR Testing

- Amplifies the mutated gene fragment. Results: negative, heterozygous, homozygous.
- Results not effected by factor deficiency, lupus anticoagulant, anticoagulant
- PCR testing cannot detect APC resistance not due to FV Leiden

PCR Testing (cont'd)

- Genomic DNA from lymphocytes
- DNA sequence flanking the mutation site is amplified by PCR, resultant product is analyzed by restriction enzyme digestion
- Normal (wild type): two normal FV alleles

  Heterozygous: one abnormal allelle

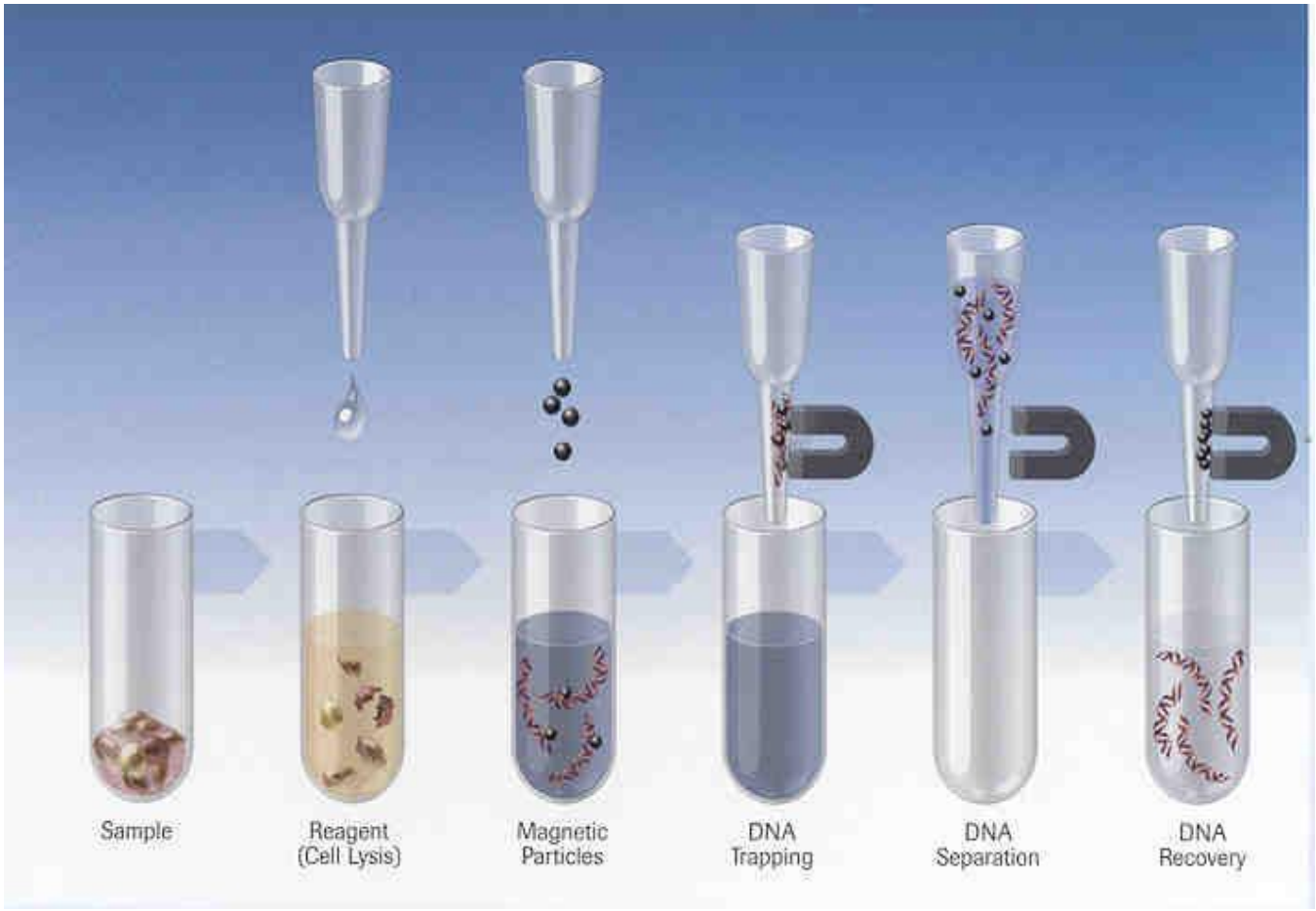
  Homozygous: two abnormal allelles

New PCR Testing: LightCycler (Roche)

- Melting curve analysis method
- “Real time” analysis
- 35 thermal cycles in 25 min, followed by melting curve analysis in 5 min -> results in 30 min
- Batch of 32 samples
- Designed for clinical lab setting
- Optional module for automated DNA extraction (60 min for 32 sample extraction)

MagNA Pure LC and LightCycler

Magnetic Bead Technology for DNA Extraction



LightCycler Schematics

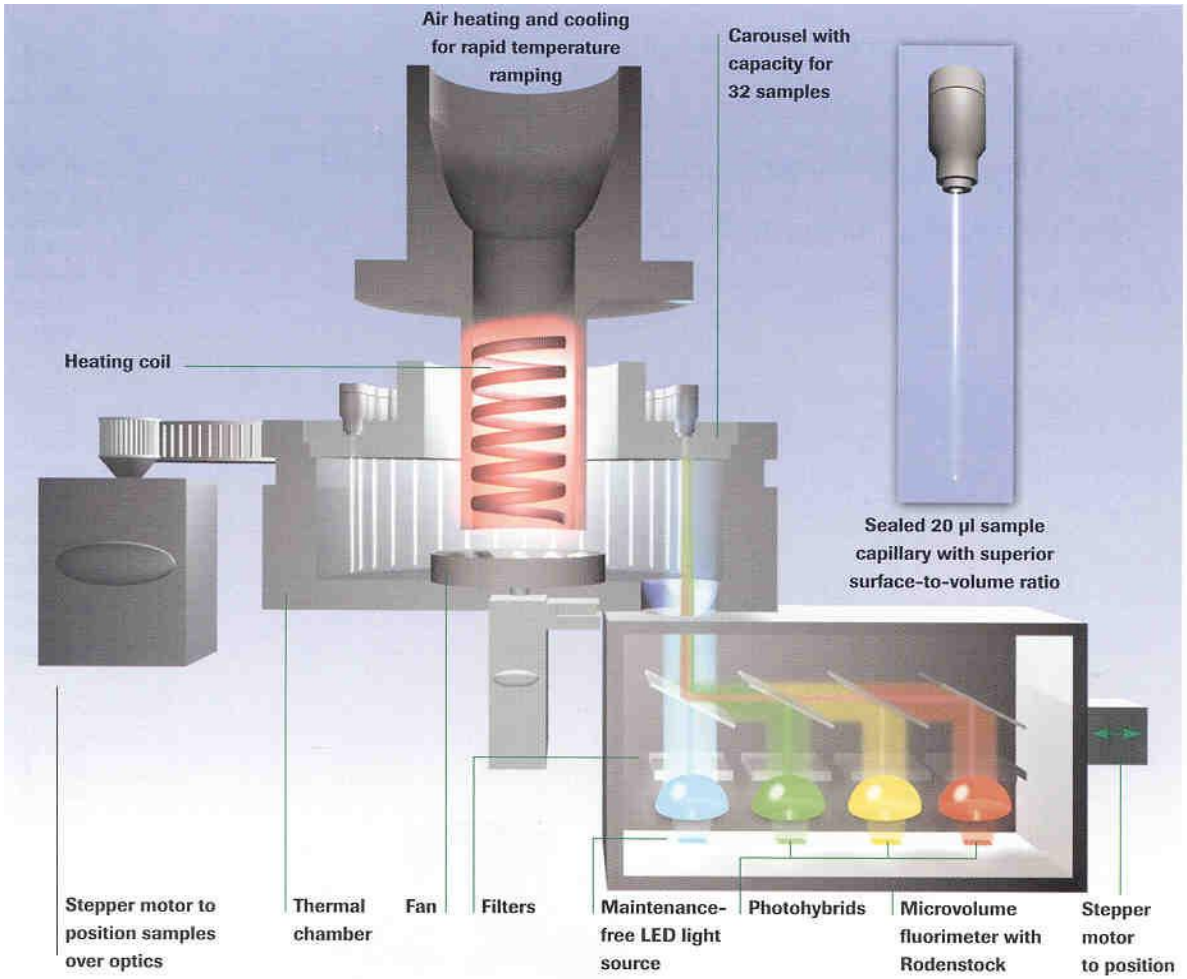
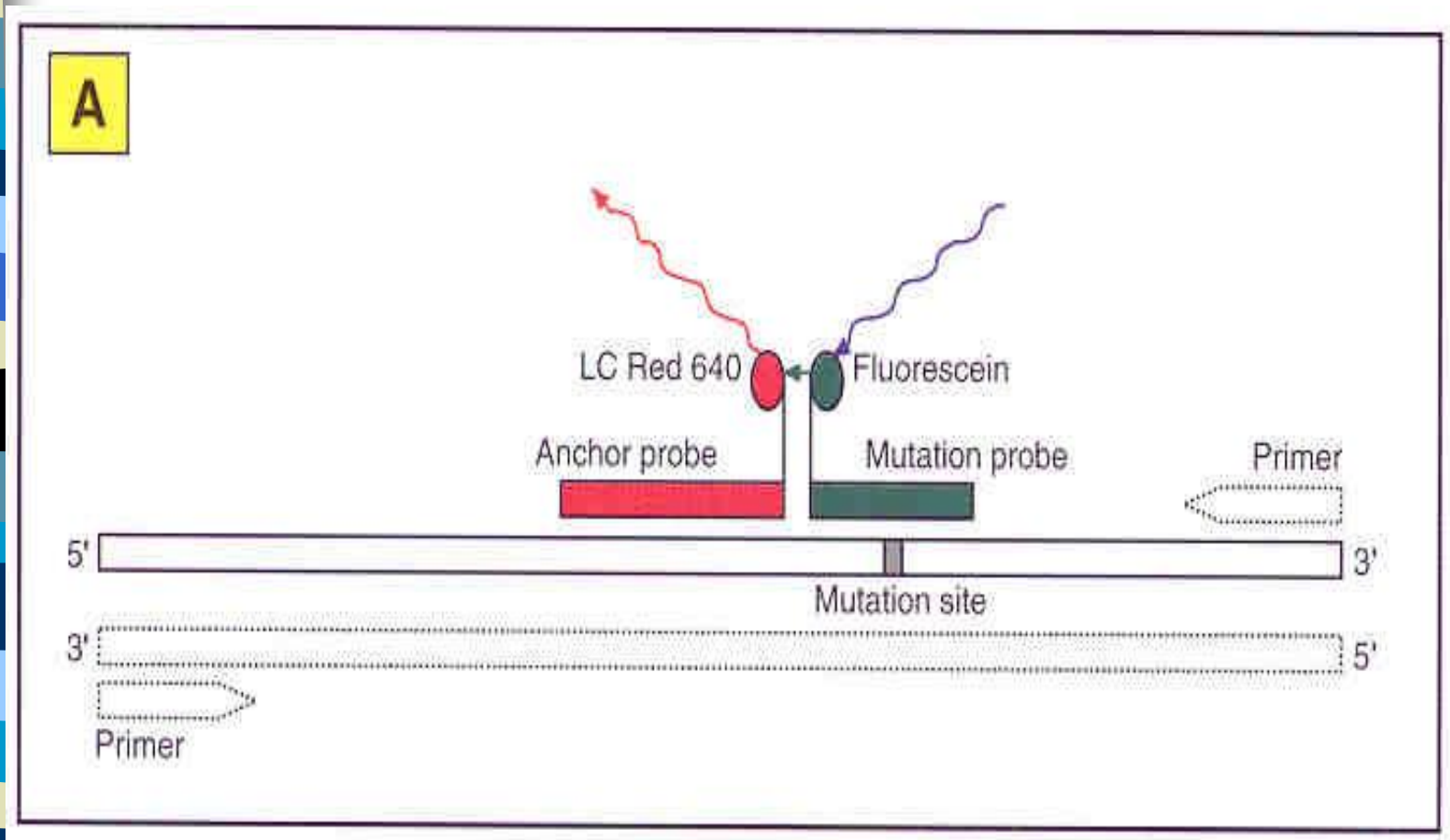


Figure 1. Schematic of the LightCycler System.

FV Leiden Mutation: Hybridization Probe with Fluoresence Resonance Energy Transfer (FRET)



FV Leiden Mutation: Melting Curve Analysis