Roadmap to Strategic Molecular Testing Integration

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- Founded by members, physicians, pathologists, Ph.D.s, mangers and technologists, who are committed to assisting laboratories in providing the highest quality diagnostic laboratory services.
- Our members are experts in the field of Clinical Laboratory Science and have a record of accomplishment for developing some of the most successful clinical laboratories in the country.
- CLC provides consulting in all laboratory services, well known and specialized in molecular diagnostics
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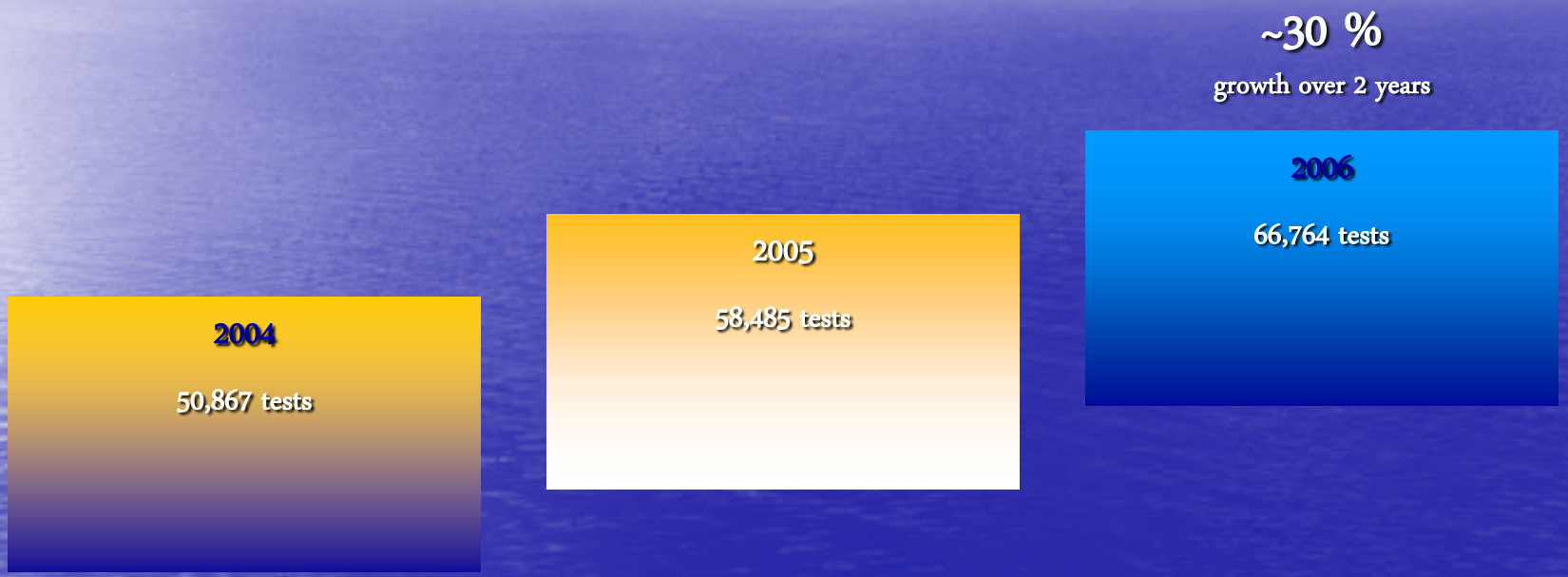
MDx Represents the Fastest Growing and Most Profitable Segment of Lab Services

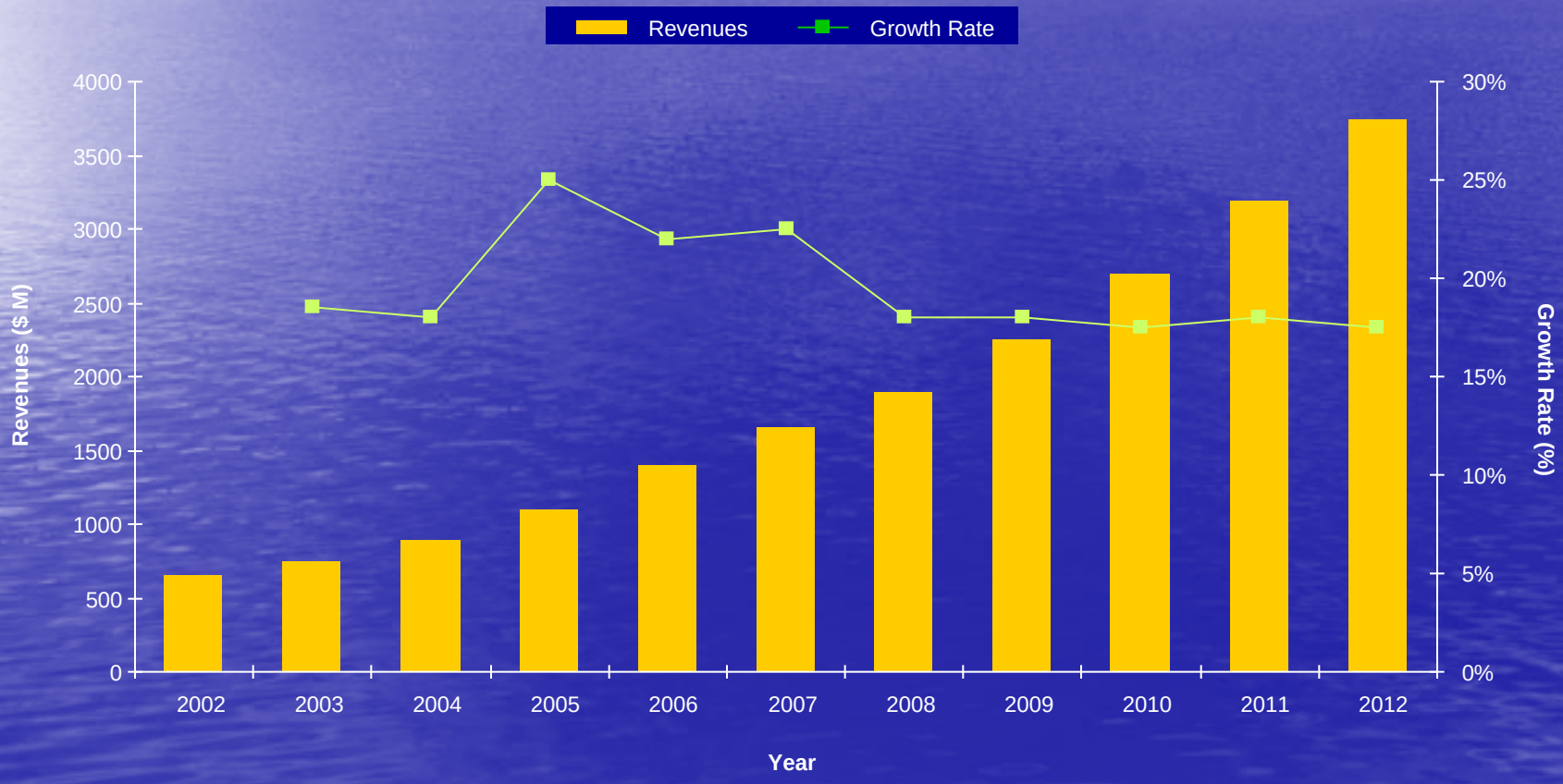


Averaged annual billable molecular tests / laboratory

Source: Molecular Diagnostics State of the market 2007, Washington G-2 Reports

US Molecular Clinical Diagnostic Market

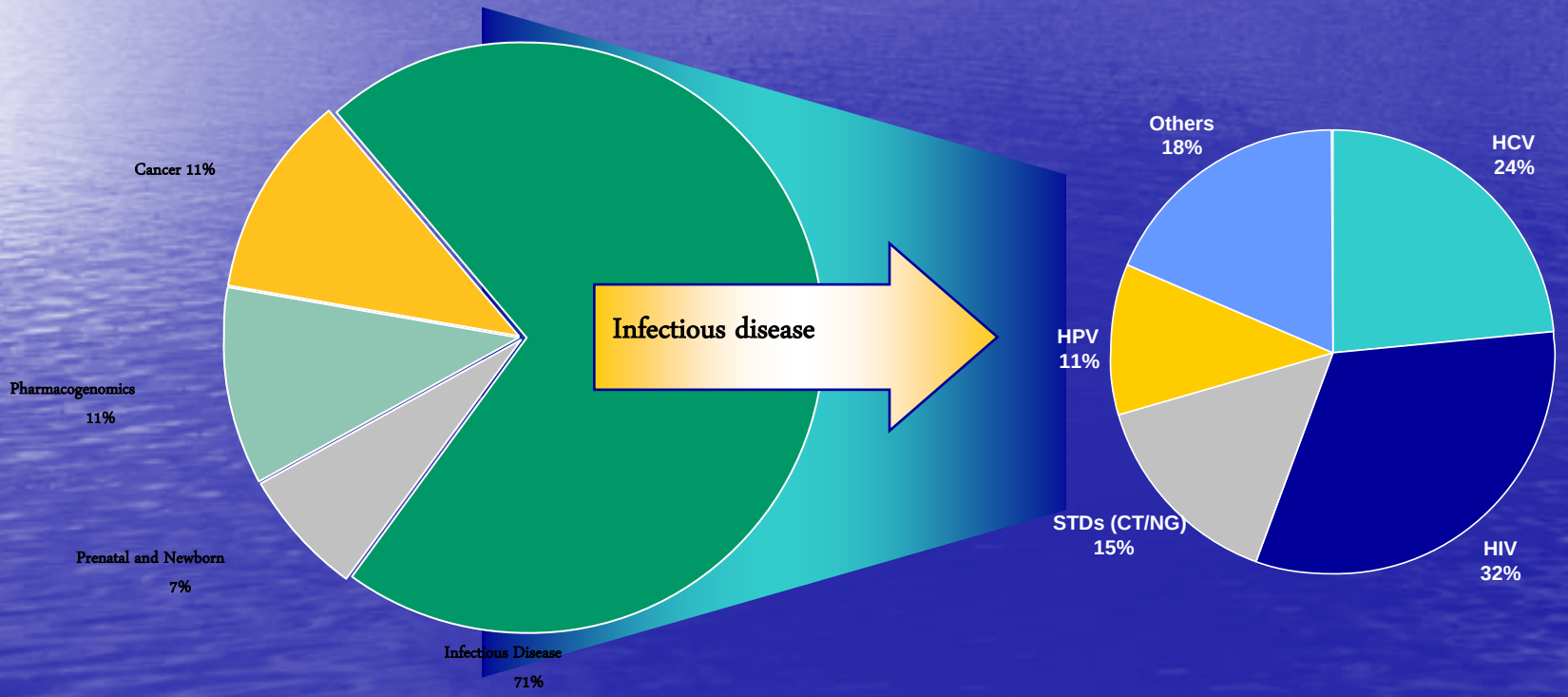
Molecular Diagnostics Revenue Forecasts 2002–2012



Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan

US Molecular Clinical Diagnostic Market

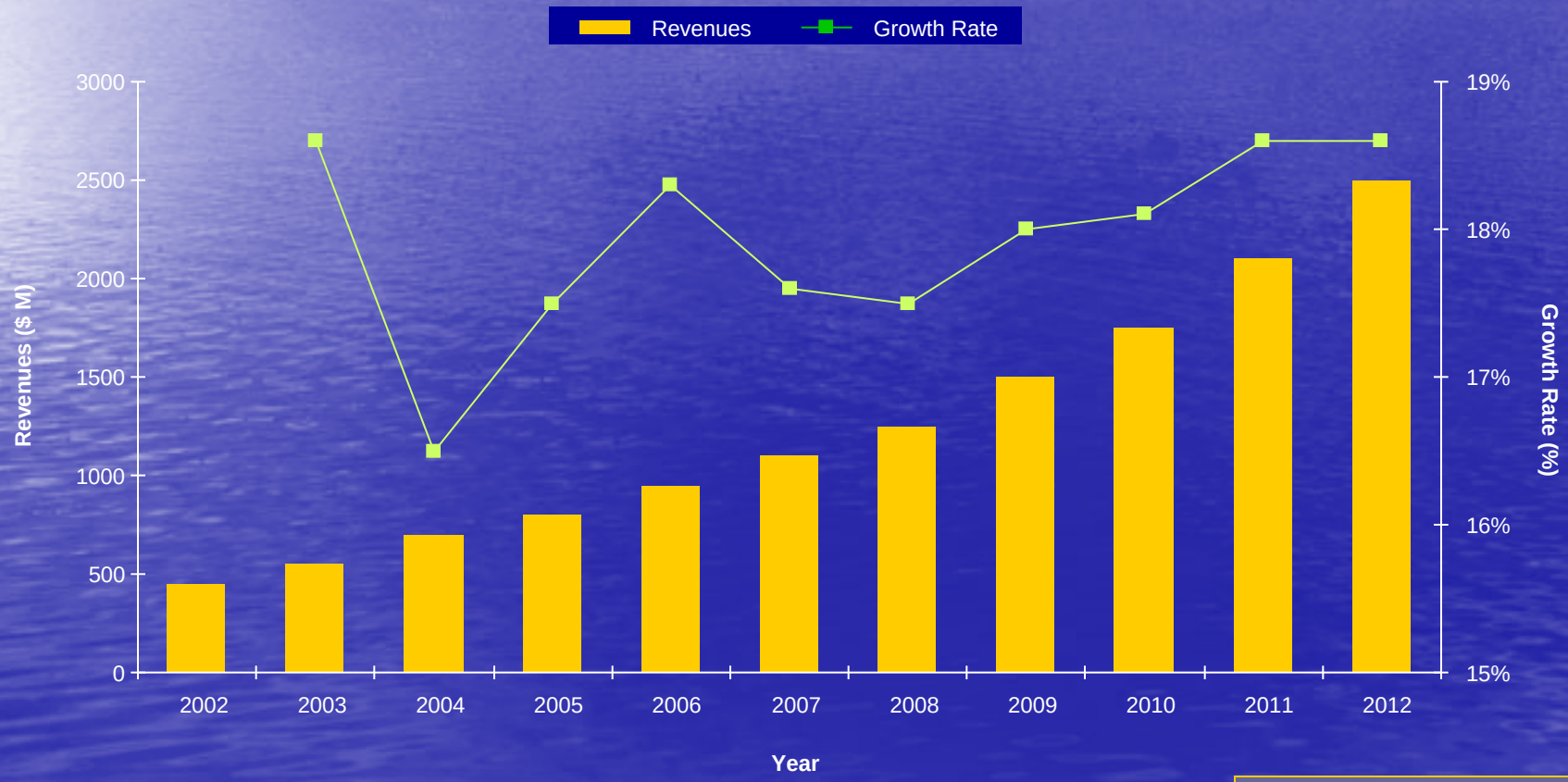
Infectious Disease is currently a high proportion of MDx Testing today



Source: US Molecular Diagnostics Market, Frost and Sullivan 2006

US Molecular Clinical Diagnostic Market

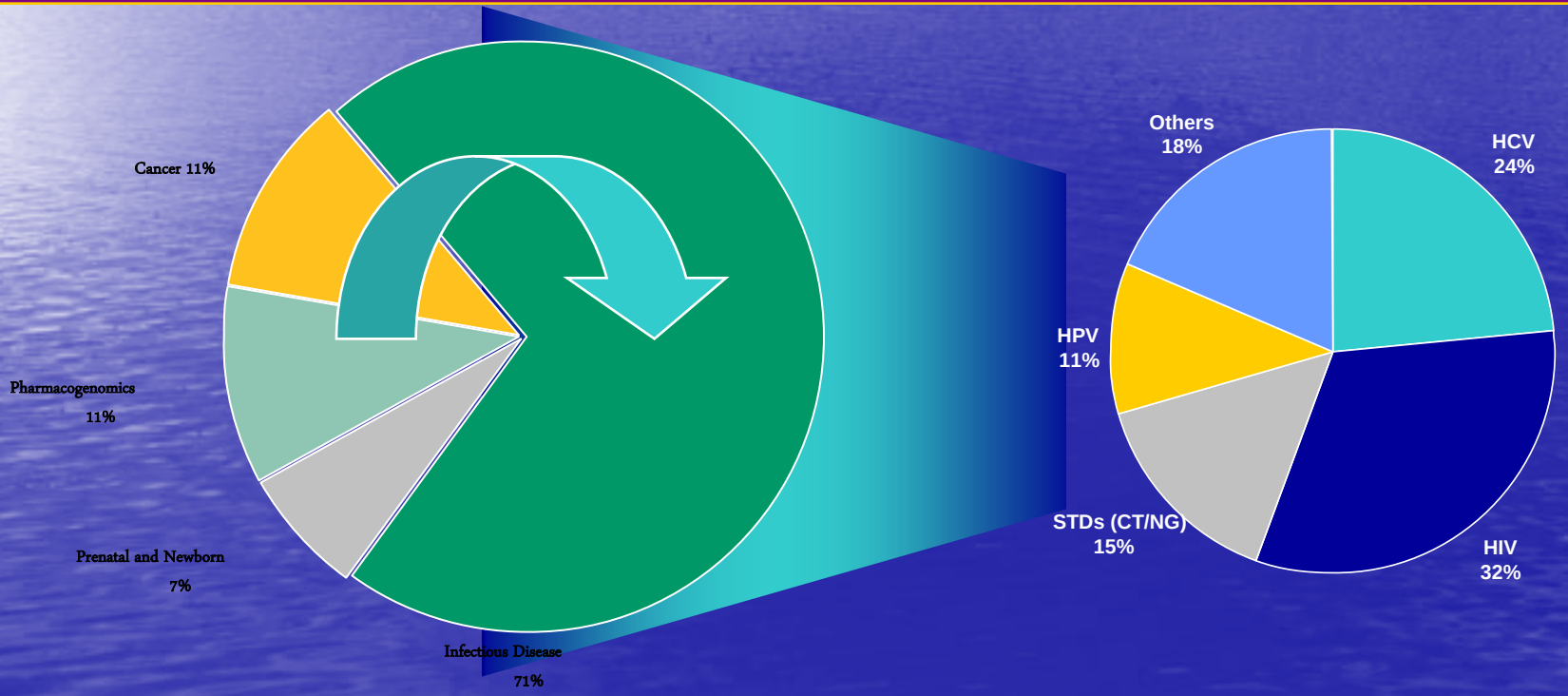
Revenue Forecasts for Infectious Disease Segment



Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan

US Molecular Clinical Diagnostic Market

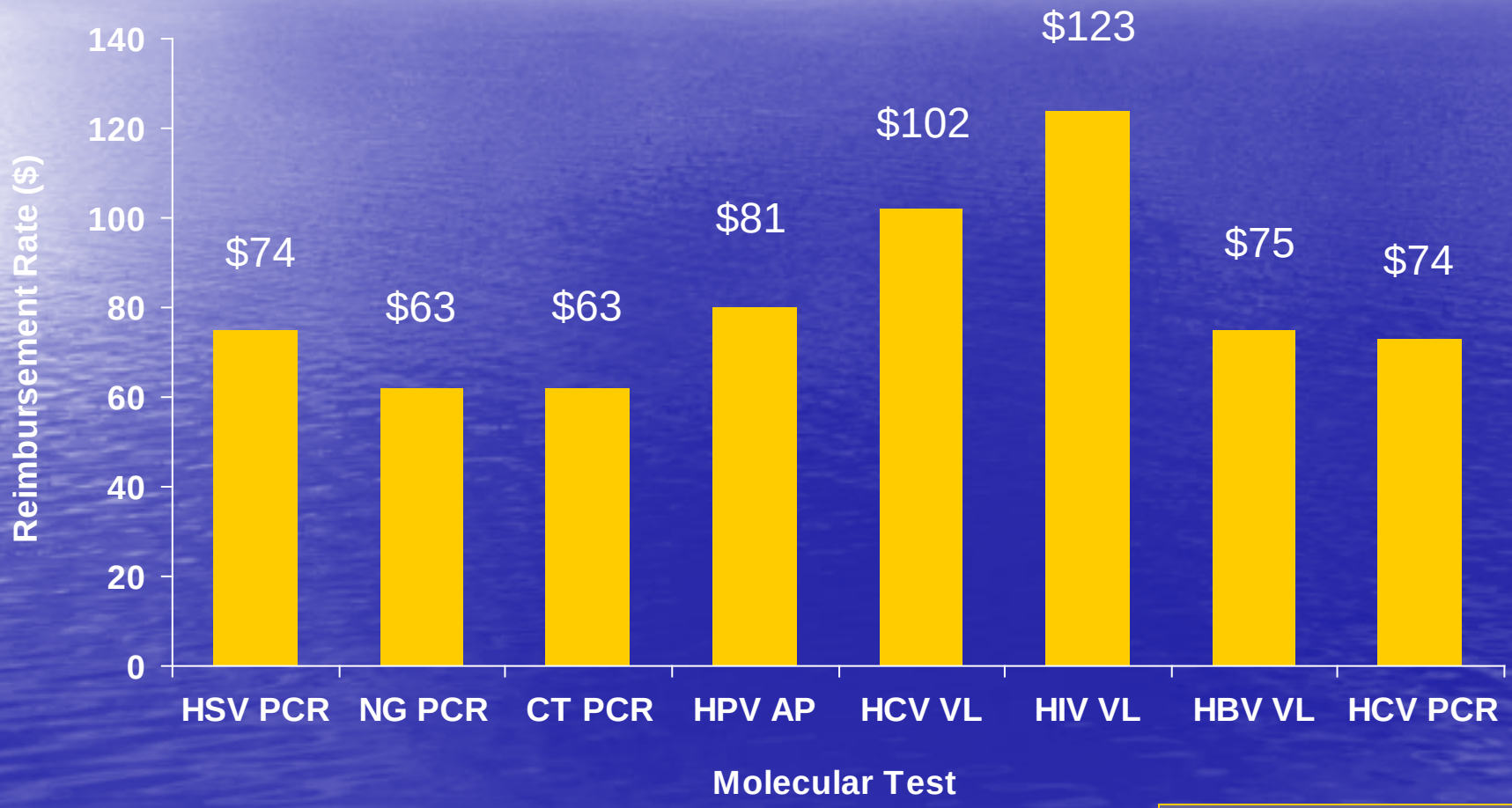
Oncology and Pharmacogenomic tests will increase in contribution to both test and revenue numbers in the future.



Source: US Molecular Diagnostics Market, Frost and Sullivan 2006

US Molecular Clinical Diagnostic Market

Approximate Average Reimbursement Rate 2005



Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan

Molecular Diagnostics Today

- Molecular Genetic
- Infectious Disease
- Molecular pathology (solid tumors and hematopathology)
- Cytogenetics
- Flow cytometry
- Forensic molecular medicine
- HLA and typing
- Personal Medicine and theranostics

Advantages of Molecular Testing

- Rapid and Robust
- Sensitive
- Specific
- Can be performed from all specimen types
- Independent of specimen viability
- Well controlled
- Open to news and updates
- Diversity

Disadvantages of Molecular Testing

- Highly complex technology

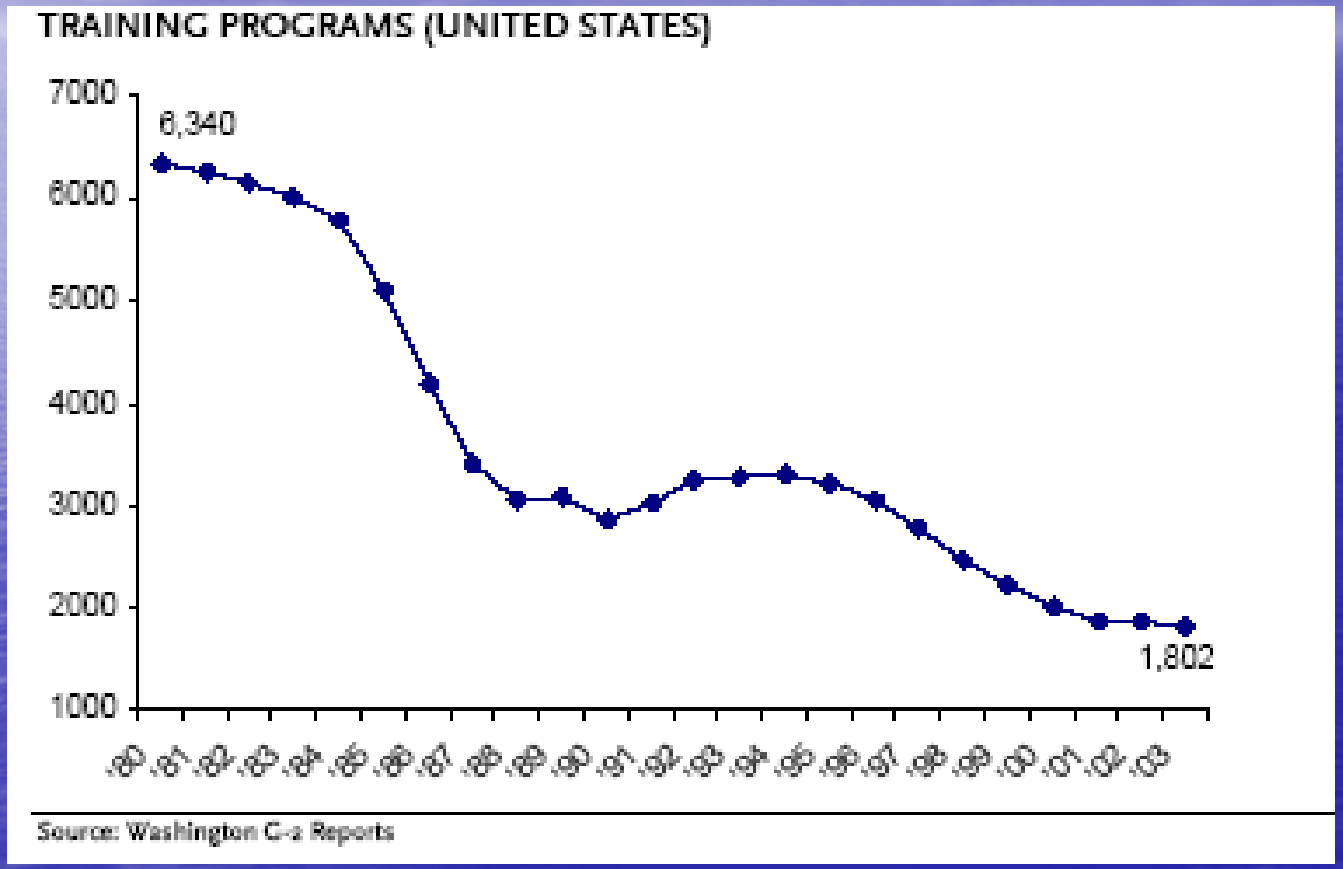
Myths About the Costs of Molecular Diagnostics

Barriers to Molecular	Opportunities
• Myth 1: Molecular requires 3 separate rooms • Myth 2: Don't have the expertise • Myth 3: Molecular costs too much • Myth 4: I don't have the volumes to bring MDx in-house.	• Contamination control and automation has eliminated 3 room concept • Coast lower as competition increase • Training or retraining personnel for molecular testing available • Start with IVDs • Start with most marginal and higher volume tests • Reimbursement per test enables lower volumes than other laboratory disciplines • Physician education

How can I afford Molecular?

Can you afford not be in Molecular?

Personnel Shortage



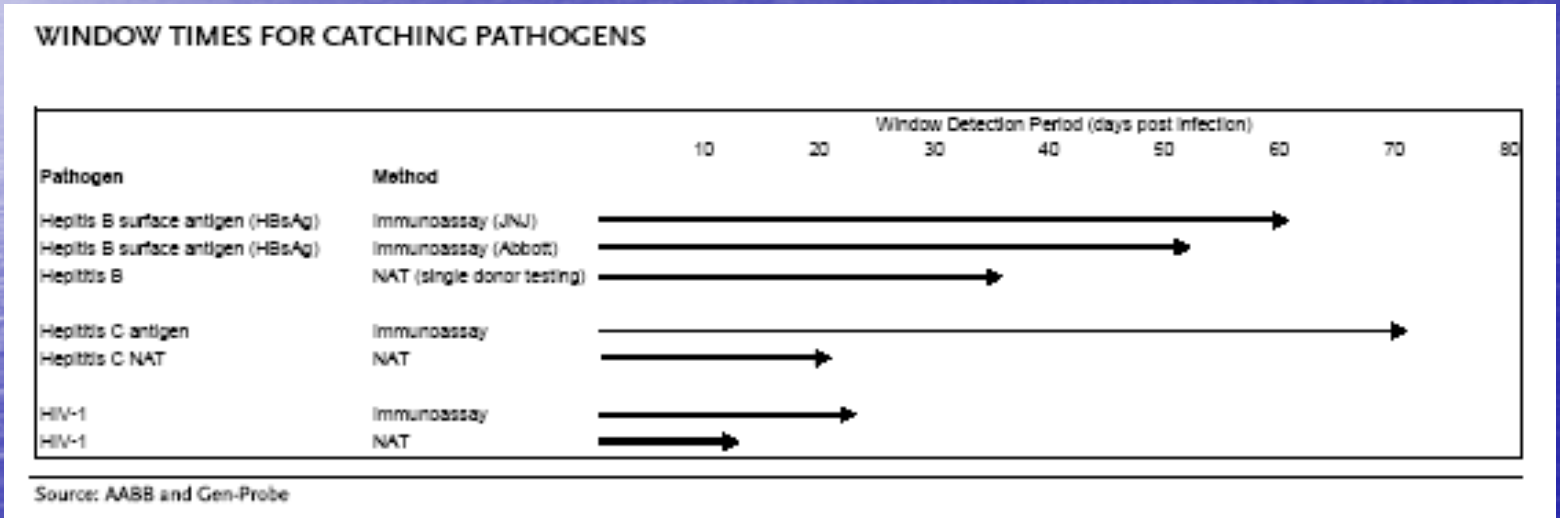
Current Molecular Applications

- Blood Banking
- Infectious Disease
- Sexually Transmitted Disease (STD)
- Genetic Testing
- Molecular Oncology
- Pharmacogenomics

Blood Bank and Tissue Screening

- In addition to 7 immuno-chemistry assays and 1 serology assay, 3 MDx tests are used
- HIV, HCV and WNV (qualitative)
- HBV is also used in some countries
- HIV and HCV used for tissue screening
- In transplants, CMV and EBV viral load

Importance of MDx in Blood Bank



Molecular Infectious Disease

- HIV viral load and genotyping (Currently available as IVD)
- HCV viral load and genotyping (Currently available as IVD and ASR)
- HBV viral load and genotyping (Currently available as ASR)
- Mycobacterium (ASR, some probes IVD)
- Other infectious pathogens (IVD and ASR)

Sexually Transmitted Disease

- *C. trachomatis* (TMA, PCR, HC2, BD)
- *N. gonorrhoeae* (TMA, PCR, HC2, BD)
- HPV (HC2 only IVD available, ASR)
- HPV genotyping (ASR)
- HSV 1&2 by PCR (ASR)
- GBS by PCR (IVD and ASR)
- Other STD infectious agents

Genetic Testing/Screening

- Diagnostic (mutation detectionz)
  - Factor V Leiden, F2, MTHFR,
  - FRAXA, HD, DMD,
  - Hemochromatosis
  - Cystic Fibrosis (diagnostic)
- Prognostic/genetic risk screening
  - Cystic Fibrosis carrier screening
  - Thrombotic risk screening (FV, F2)
  - Familial breast and ovarian cancer risk screening BRCA1, BRCA2)
  - Screening for DNA MMR gene in Colorectal Cancer

Molecular Oncology

- Oncology or solid tumor markers
- Hematopathology or leukemia and lymphoma

1. Diagnostic
2. Prognostic and therapeutic monitoring

Molecular Oncology

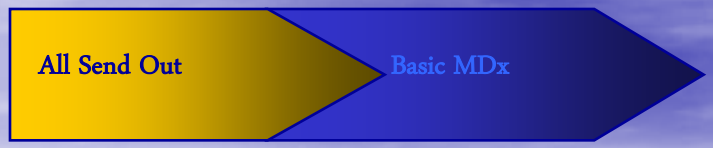
1. Diagnostic
   - K-*ras,* and *p53* in pancreatic and lung cancer
   - Leukemia and Lymphoma (BCR/ABL, JAK2, flt3)
   - Other chromosomal abnormalities

2. Prognostic and therapeutic monitoring
   - Her2*neu* in breast cancer
   - BRCA1 and BRCA2 in breast and ovarian cancer
   - Microsatellite instability in HNPCC

Pharmacogenomics

- Screening for genetic polymorphism of the enzyme responsible for drug metabolism
- Drug response and adverse drug reactions
- CYP2D6, poor, intermediate, extensive and ultrametabolizer
- TPMT polymorphism in leukemia and autoimmune disease dosage treatment
- Other drugs methabolized by CYP2C9*2 & CYP2C9*3
- VKORC1 for coumadin dosage

Bringing Molecular Dx in house



Send Out ***In-house***

Barriers to overcome
- Test volumes too low
- Space
- Expertise
- Costs

Reasons to bring MDx in-house
- Increased in send outs
- ↑Demand for new MDx tests
- ↓Decreased MDx cost
- Technical and clinical support
- Improve profitability
- Foundation for growth
- Capital/Financing Available

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A Laboratory Advocate

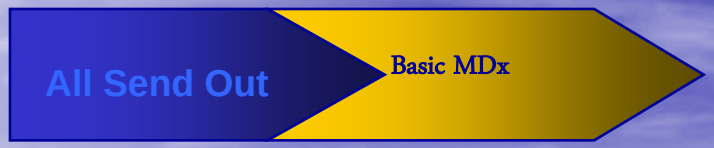
Source: US Molecular Diagnostics Market, Frost and Sullivan 2006

Financial Goals for New to Molecular Labs

Losing less money is a legitimate financial goal

- All institutions are paying for some molecular tests today
- Bringing in Molecular testing will contribute to improving the profitability of the institution
- Molecular Dx will continue to grow in percentage of total laboratory tests
- Infectious disease (CT/GC, HPV) will remain a foundation for many molecular laboratories.

Start with Basic Molecular Dx



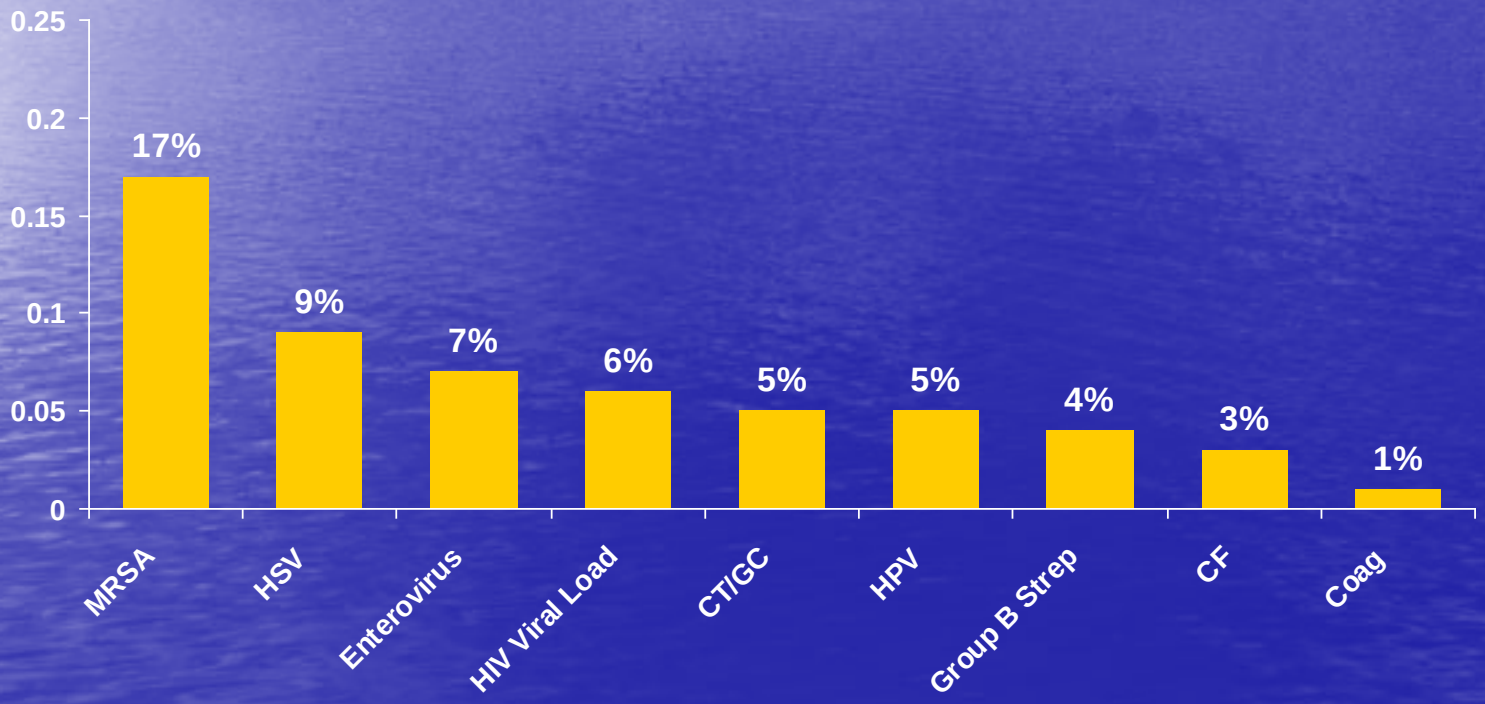
- Start with FDA approved tests
- Offer high volume (CT/GC, HPV) tests
- Chosen an initial MDx vendor (go with reagent rental)
- Negotiate the price (consult experienced experts)
- Go with one platform with a brought menu (closed system)
- Higher consultant to training staff in MDx techniques (cheaper then to enroll then in the MDx course)
- Collaborate with other labs (consulting until you establish your team
- Use hired vendor to train your sale reps on upsaling MDx

Increasing Your Molecular Dx Menu

- Continue expanding the menu
  - Plasma: Viral Load (HIV, HCV, CMV)
  - Other OBGYN tests: HSV, GBS
  - MRSA
- Genetics: Factor II Prothrombin, Factor V Leiden , MTHFR, CF
- Again go with IVDs first, if not use ASR (no RUO or LDT)

What Tests to Start With

Demand and market will dictate what test to bring or expand



Source: 2006 / 2007 Survey of 600 US Molecular Laboratories

The Evolution of a Molecular Diagnostic Laboratory

- More Automation with brought menu (closed systems less space)
- Less labor and errors (interfacing with LIS)
- Lower volume, high impact tests
  - Molecular oncology (BCR/ABL, JAK2)
  - CYP450 2C9 and VKOR
- Reimbursement based on methods
- Grouped CTP codes
- ASR and home brew methods are added
- Report formatting

Increasing Volumes through Marketing and Education

- Increased marketing and sales efforts to attract new clinicians (use vendors to help)
- Use available brochures and literature
- Use guidelines and recommendations (ACOG, AMCG, AAP, AMP)
- Provide clinicians with the specialized training for the interpretation (education using brochures and pamphlets)
- Set up presentations for clients

How to Compete

Patient Requirements

Physician Requirements

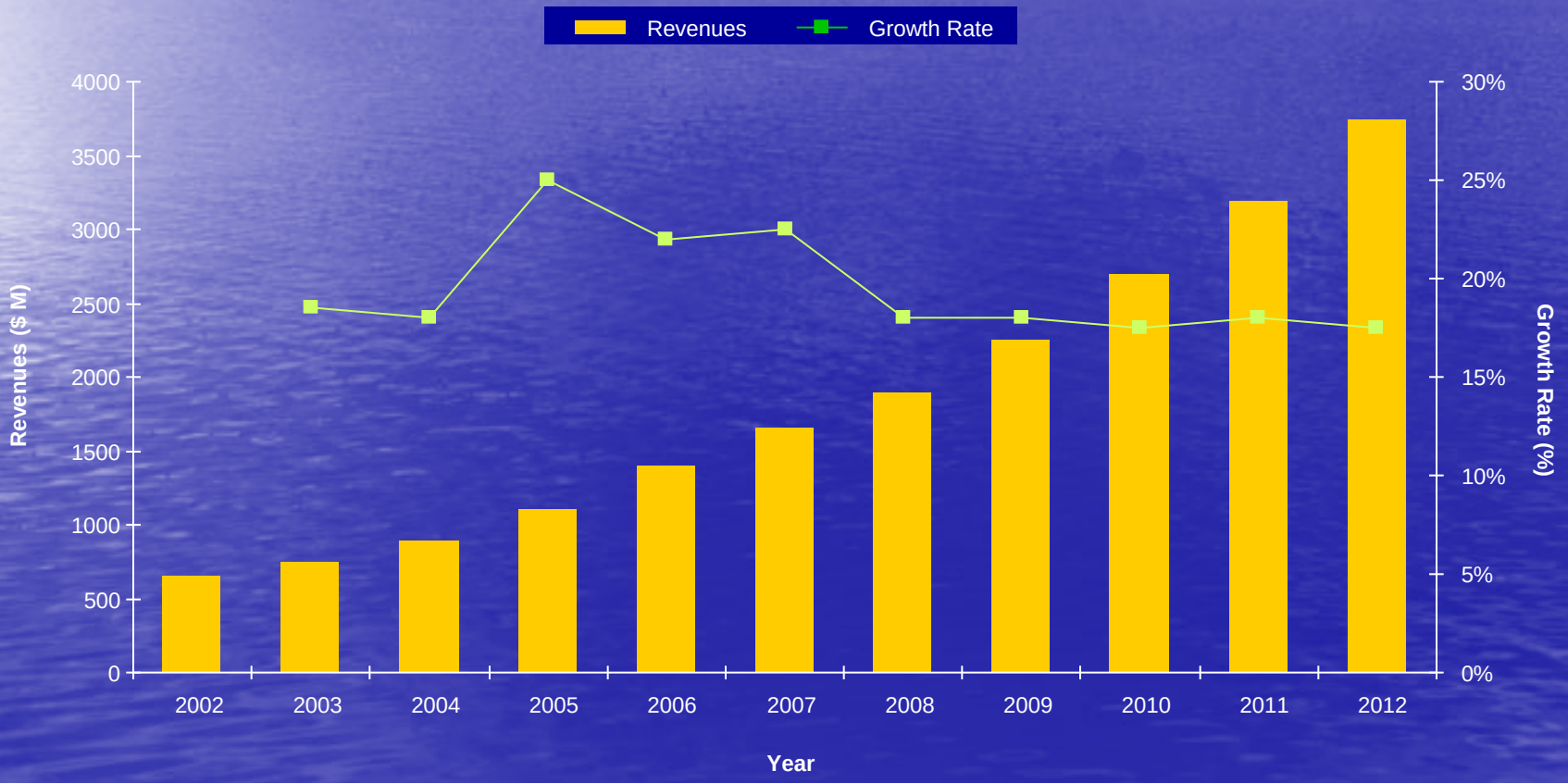
Rapid Identification	Screening	Esoteric Testing	Disease Monitoring

Laboratory Requirements

• Rapid TAT • Small volume • Maximum flexibility	• Medium TAT • Large volume • Low prevalence • Cost effective	• Medium TAT • Cost effective • Various levels of complexity	• Medium TAT • Medium volume • High positivity • Consistency

US Molecular Clinical Diagnostic Market

Revenue Forecasts 2002–2012



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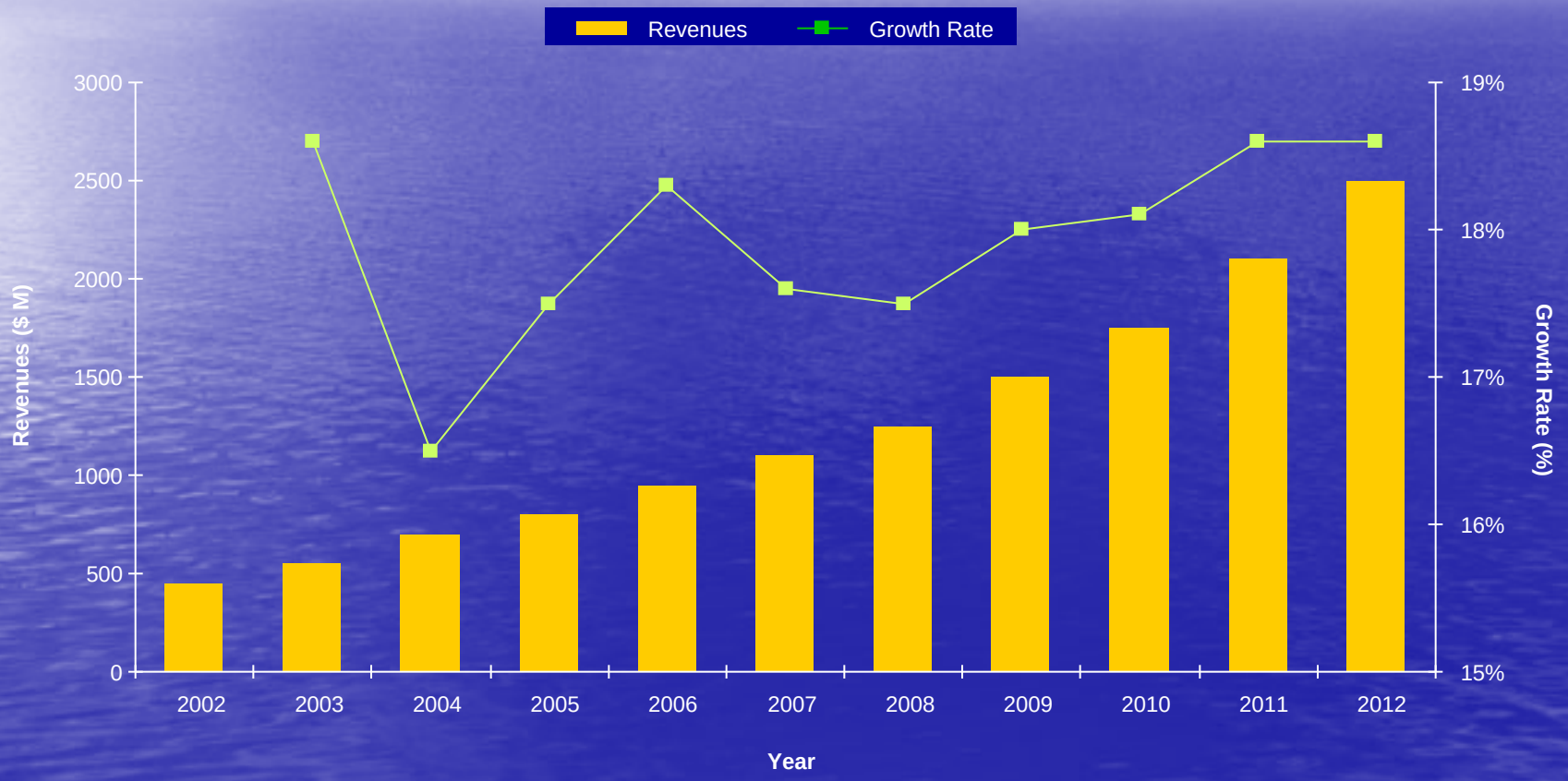
Revenue Forecasts for Infectious Disease Segment



Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan

US Revenues for the Infectious Disease Testing Segment 2005

Segment	2005 (%)
HIV	32.1
HCV	23.6
STDs	14.9
HPV	11.0
Others*	18.5
Total	**100.0**

* Includes CMV, HBV, WNV, GAS, GBS etc.
Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan

Summary

The costs of molecular are real, but so are the Opportunities

1. Molecular Diagnostics is improving patients lives
2. Molecular Diagnostic testing will continue to grow:
   - MDx delivers actionable healthcare information to answer unique clinical questions
   - More FDA approvals and more options
3. Get in the Game!
   - Infectious disease testing will continue to be the foundation for most molecular labs because of established reimbursement and clinical utility
   - Oncology and pharmacogenomic tests requests will grow however physicians will require more local support for these complex methods.
   - Molecular labs today can and will provide clinical, operational, and financial benefits to their institutions.

Molecular Diagnostic Testing

- Molecular biology is the study of nucleic acids
  - DNA or RNA
- Molecular diagnostics is the study of nucleic acids for diagnostic purposes
  - Identification of whole genes
  - Identification of a specific sequence
  - Identification of a single mutation

Molecular Diagnostic Testing

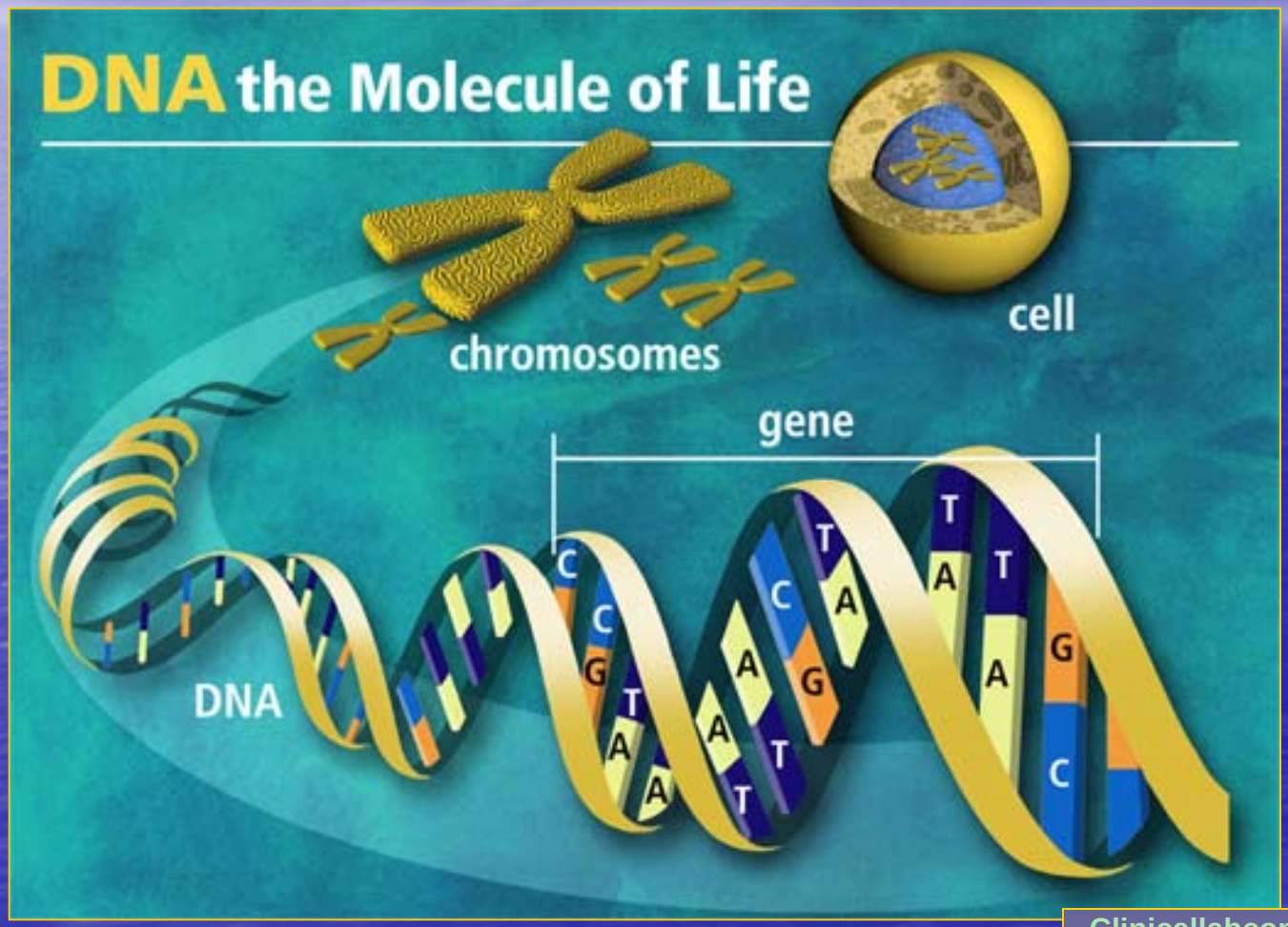
- Nucleic acid sequences are unique
  - A single specific sequence can be detected from the massive amounts present in a whole genome
  - Humans have ~ 6 billion bp
    - 4- base 4^4= 256 bp
    - 8- base 4^8= 65,536 bp
    - 20-base 4^{20}= 4,398,046,511,104 bp

Molecular Diagnostic Technologies

Molecular testing typically consists of three parts:

1. Extraction and Purification
2. Amplification
3. Detection

Extraction:
Releasing Nucleic Acid from the Cell

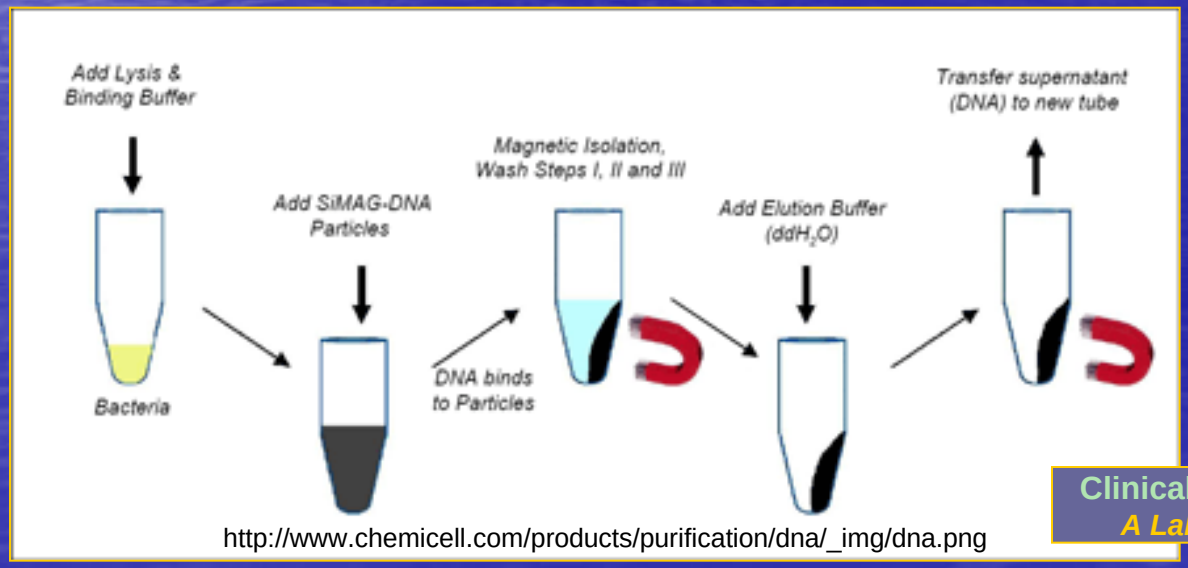


http://www.paternityexperts.com/img/DNA-of-life.jpg

NA Extraction

All protocols use 4 basic steps:

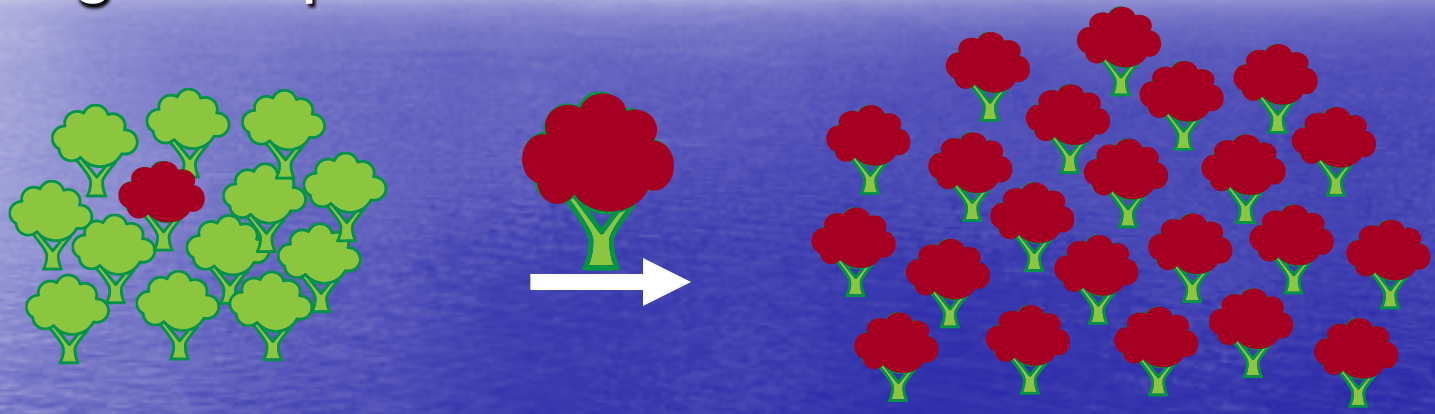
Step 1: Lyse	**Step 2:** Bind NA	**Step 3:** Remove contaminants	**Step 4:** Release purified NA
• Boiling • Homogenization • Sonication • Pressure • Detergents • Enzymes	• Silica-based particles • Magnetic beads	• Ethanol-based buffer	• Water • Buffer



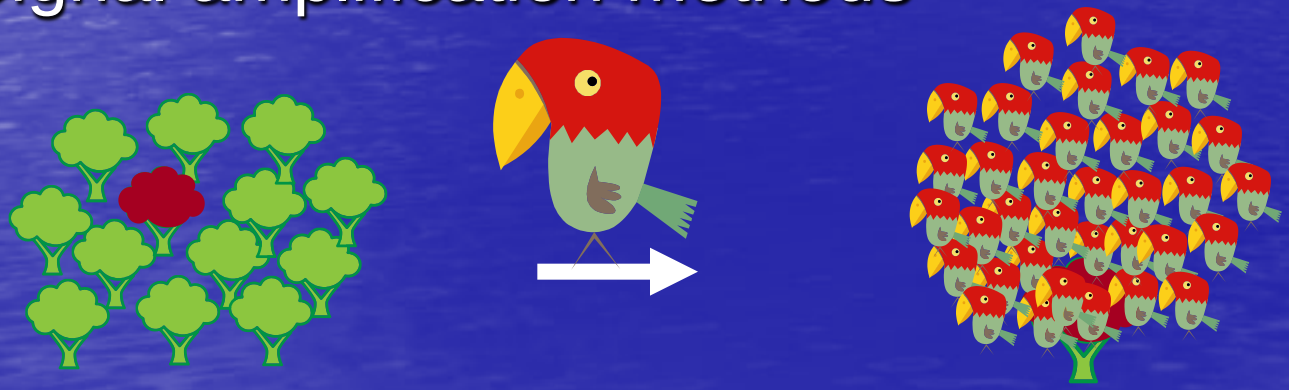
http://www.chemicell.com/products/purification/dna/_img/dna.png

Amplification

1. Target amplification methods



2. Signal amplification methods

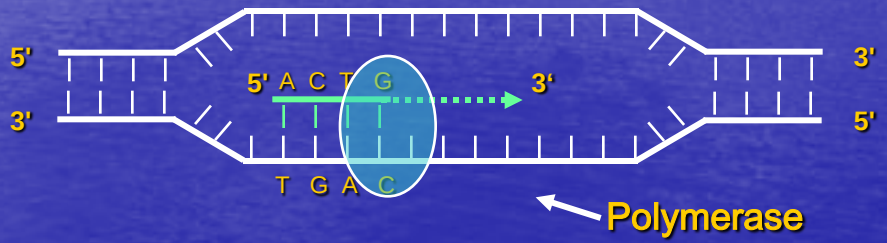


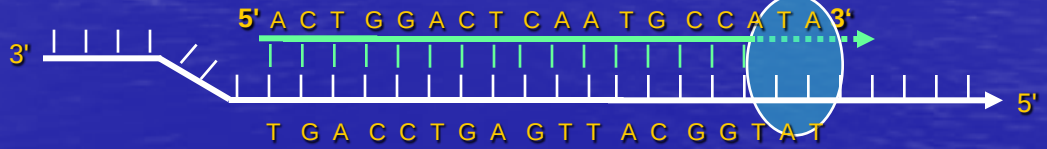
Molecular Diagnostics Target Amplification Technologies

- Molecular biologists manipulate normal DNA replication and other normal cellular processes in the laboratory to make more of the nucleic acid for subsequent analysis

DNA Replication: The Basics

- Double stranded DNA
- Strand denaturation
- Primer annealing
- Polymerase extension





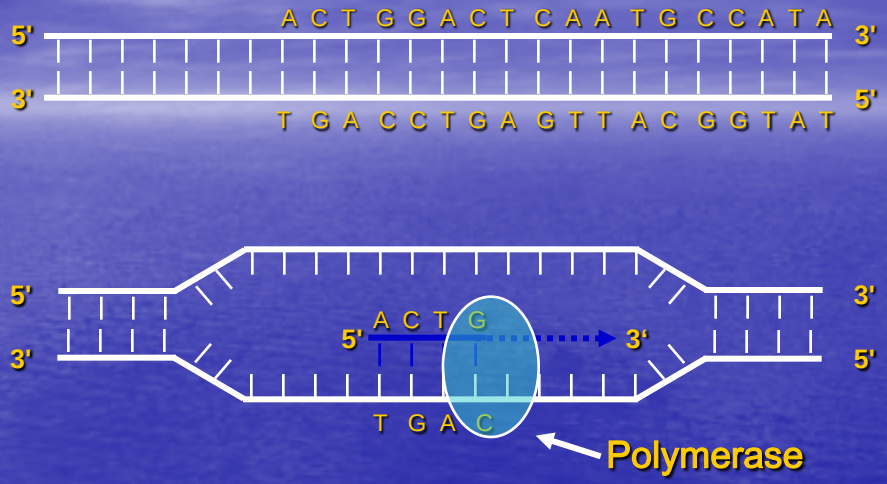
Replication is bi-directional, resulting in two new strands of DNA

Target Amplification Methods

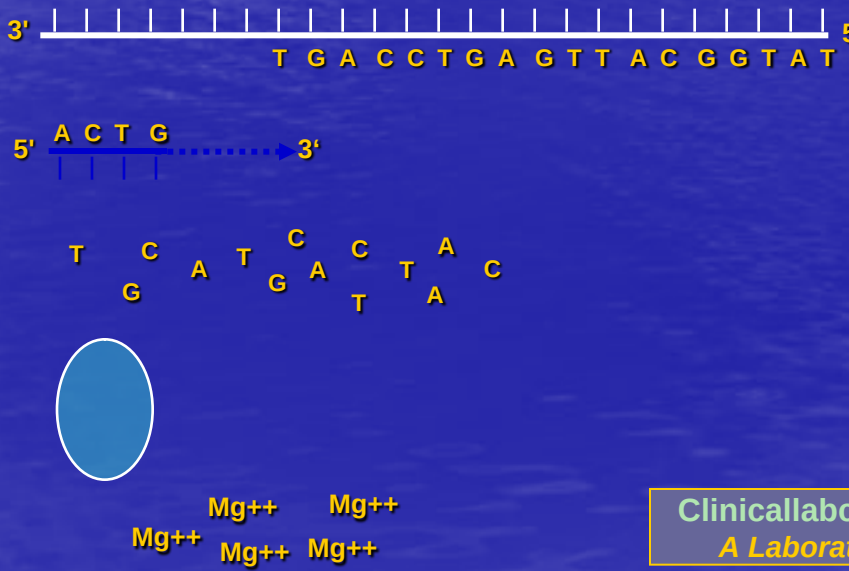
- Polymerase Chain Reaction (PCR)
- Real-time PCR
- Transcription mediated amplification
- Nucleic acid sequence based amplification

DNA Replication

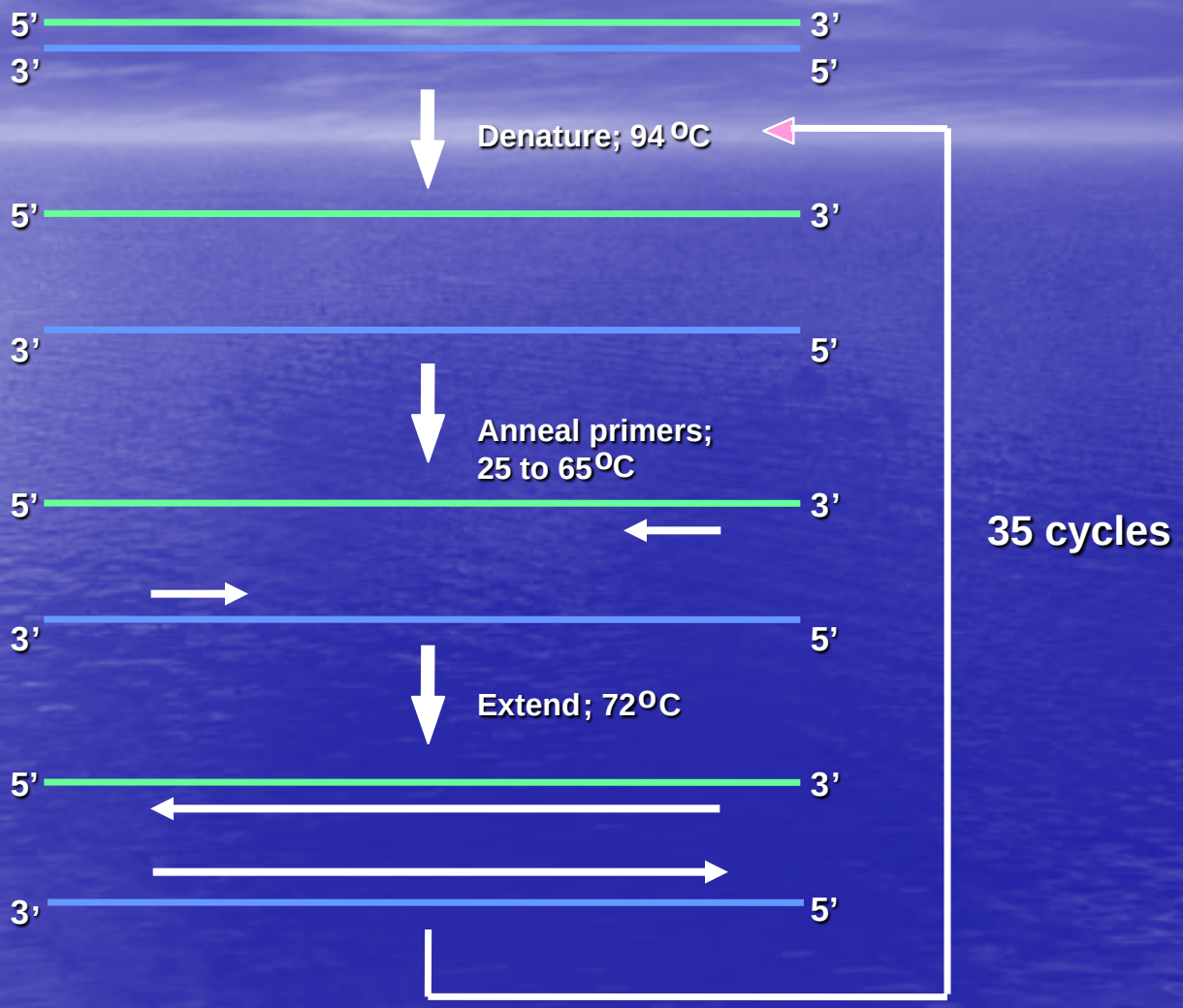
- Double stranded DNA
- Strand denaturation
- Primer annealing
- Polymerase extension



- Template
- Primer(s)
- Nucleotides (A,C,T,G)
- Polymerase
- Buffer



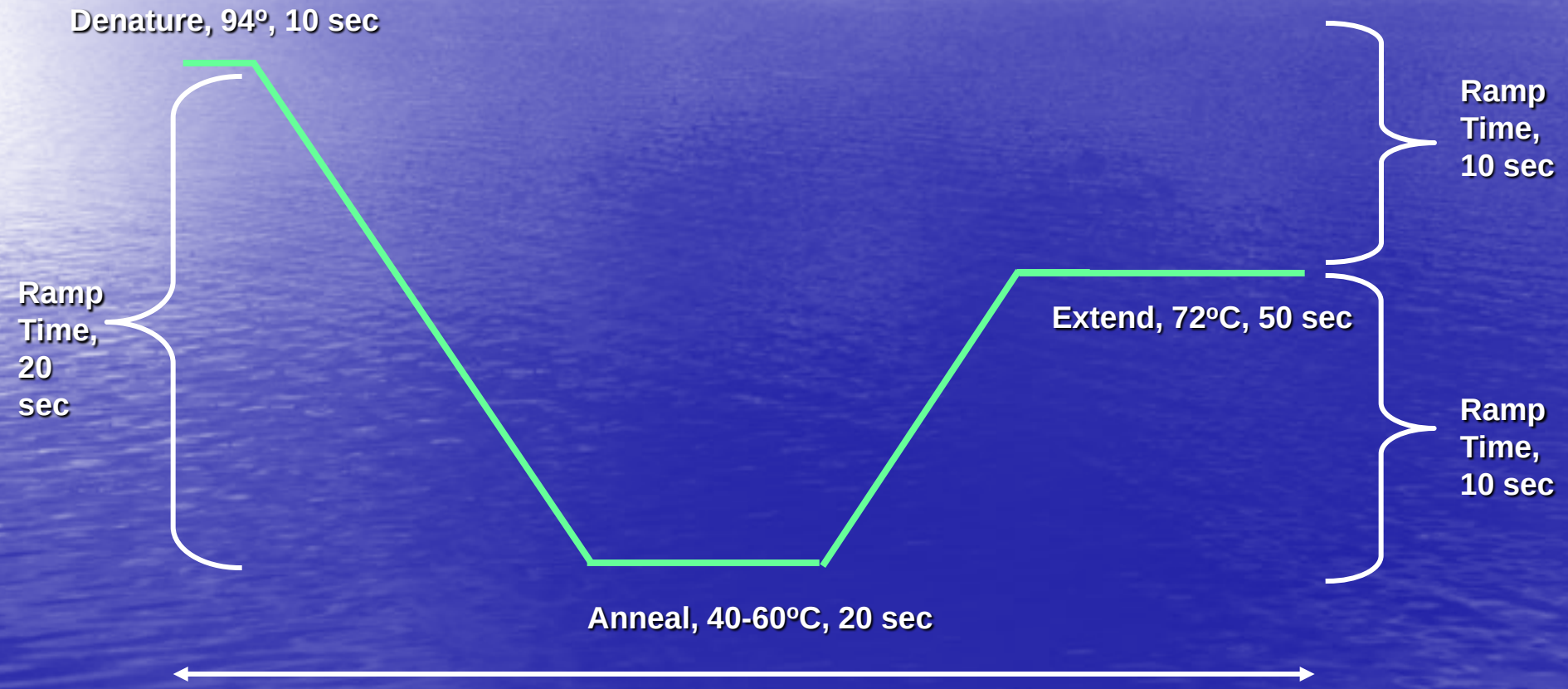
Polymerase Chain Reaction (PCR)



The primers determine the boarders of the piece of DN

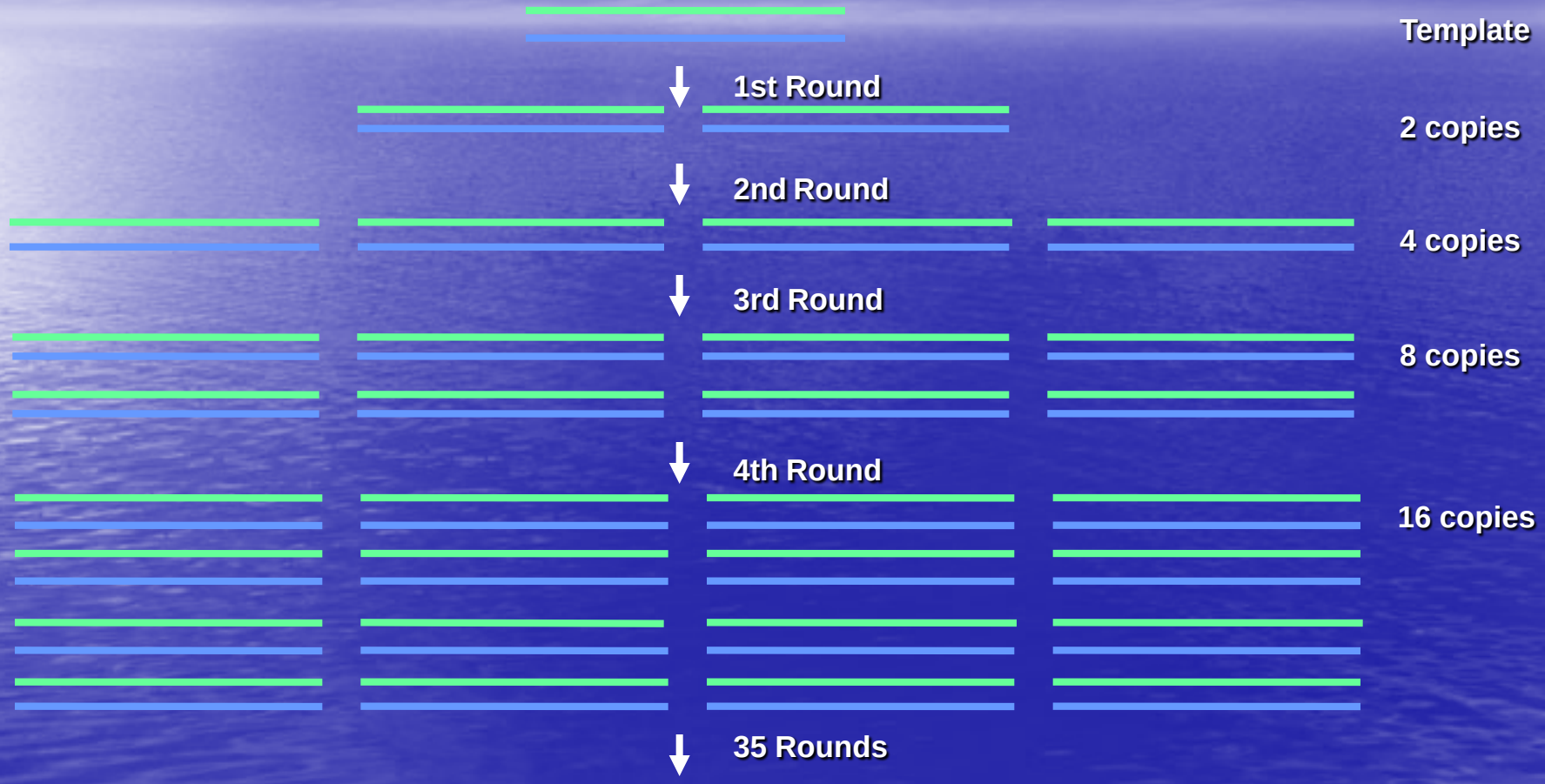
Traditional PCR Cycle

One cycle of ‘traditional’ PCR



One cycle = 2 minutes

The Power of “2”

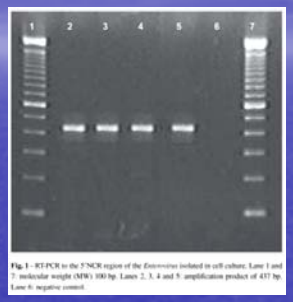


Over a million copies

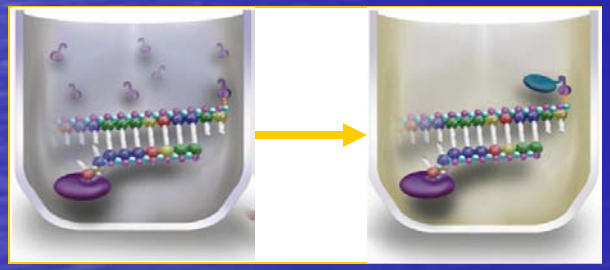
Detection Methods

- Detection occurs after the reaction is performed
  - End-point detection
- Methods include:
  - Agarose gel electrophoresis
  - Colorimetric reactions

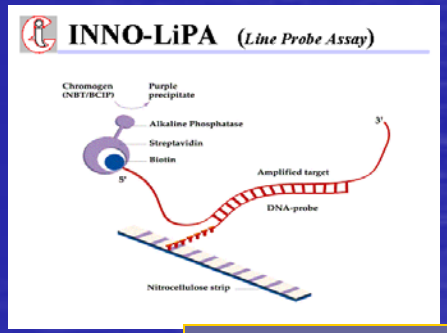
http://oceanexplorer.noaa.gov/explorations/03bio/background/molecular/media/gel_plate_600.jpg



http://www.scielo.br/img/revistas/rimtsp/v48n4/a04fig01.gif



www.roche-molecular.com



www.inn

Real-Time PCR

- Real-time PCR detects the amplification product as it occurs, or in “real-time”
- Three technologies were needed to accomplished this:
  - instrumentation, detection chemistries, analysis
- What are some of the differences between traditional PCR and real-time PCR?

Traditional and Real-Time PCR

Heating blocks for heat transfer.

High velocity air for heat transfer

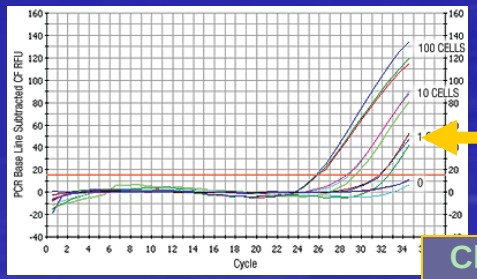
Thin-walled PCR reaction tubes

Glass capillary reaction chambers

Tubes are placed in heating blocks

Capillaries are placed in holder and act as cuvettes

Detection is separate

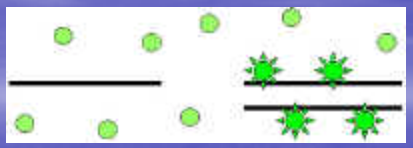


Detection occurs simultaneously

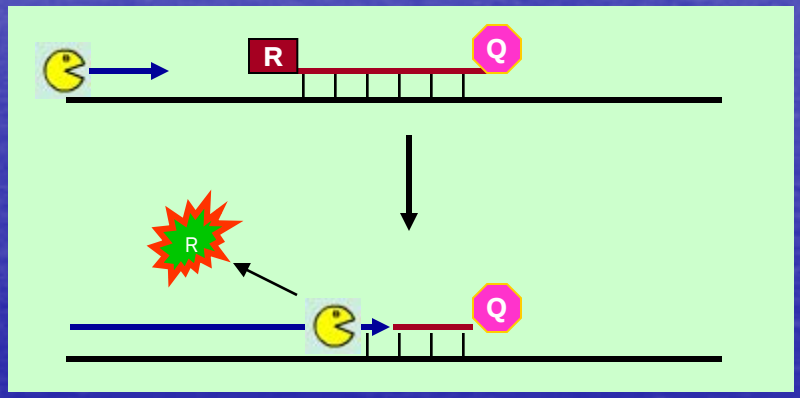
www. applied.biosystems.com, www.biologyreference.com, www.roche-applied-science.com

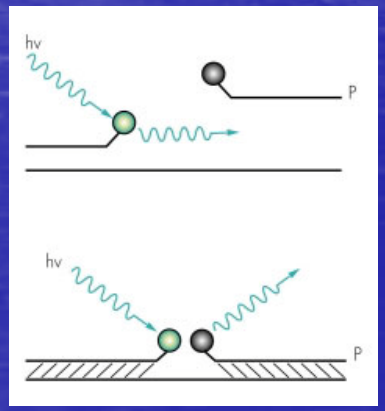
Real-Time PCR Chemistries

- SYBR Green I
- TaqMan
- FRET Probes
- Molecular Beacons
- + others

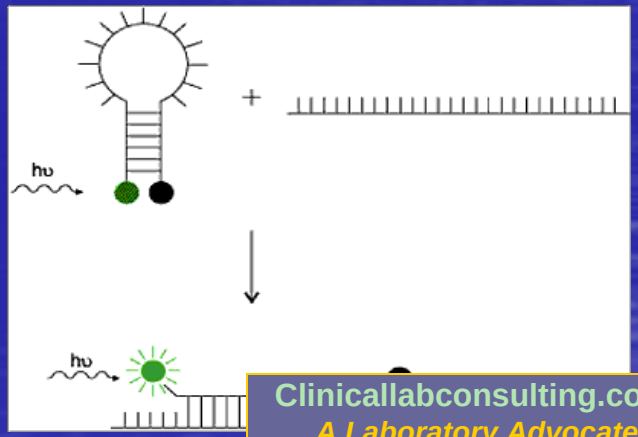


http://pathmicro.med.sc.edu/pcr/SYBRGreen_small.jpg





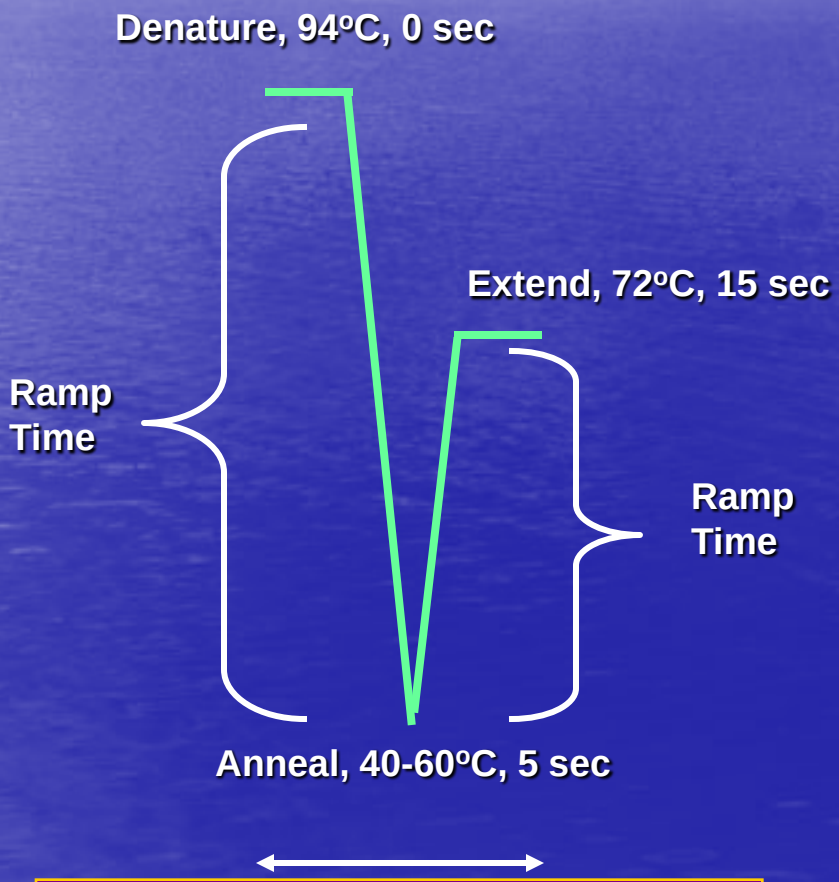
www.iba-go.com/naps/naps_p_rg_fret.html



http://www.clinical-virology.org/graphic/pcr_mb.gif

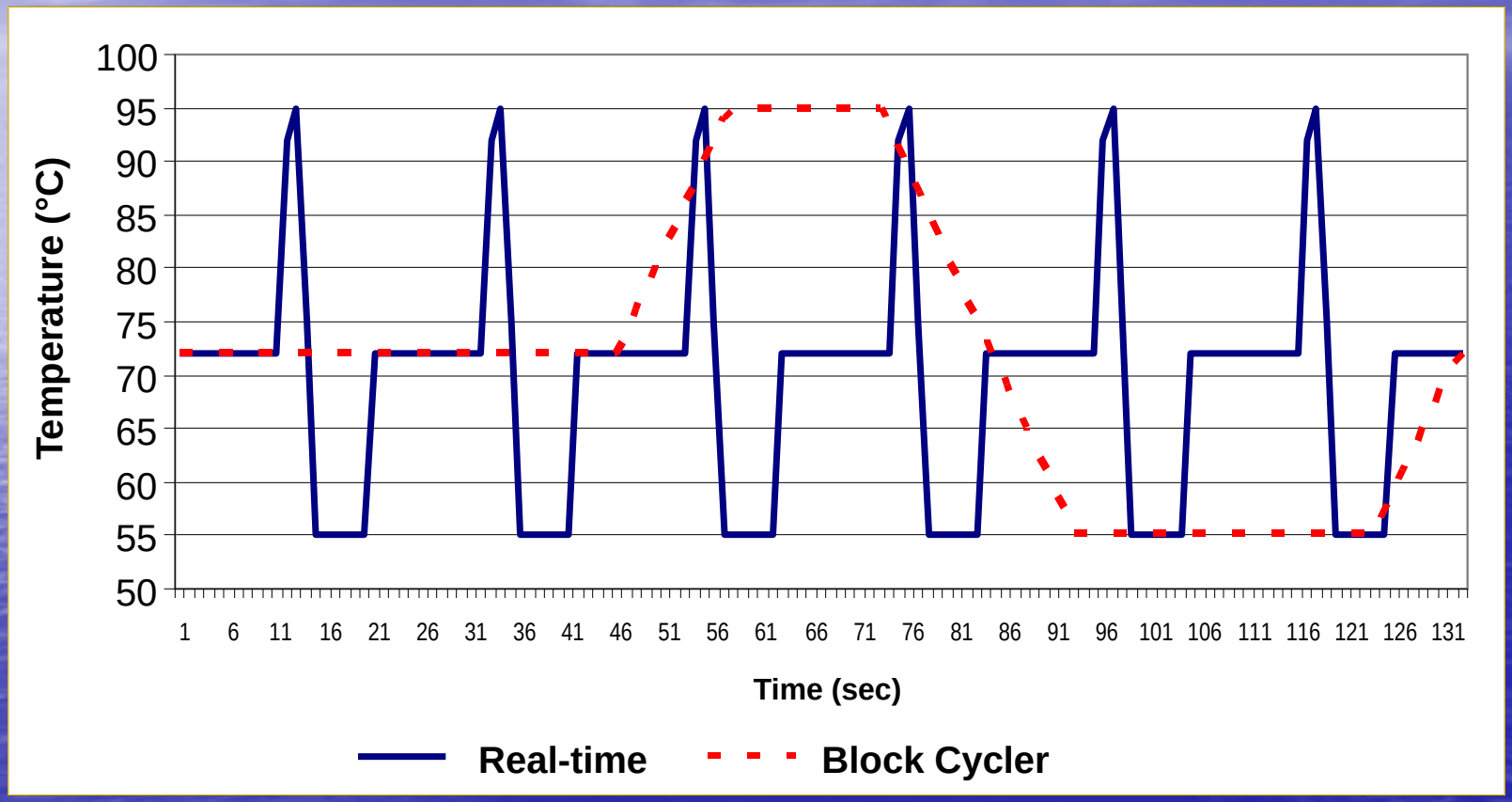
Real-Time Cycle Time

One cycle of real-time PCR



One cycle = 20 seconds

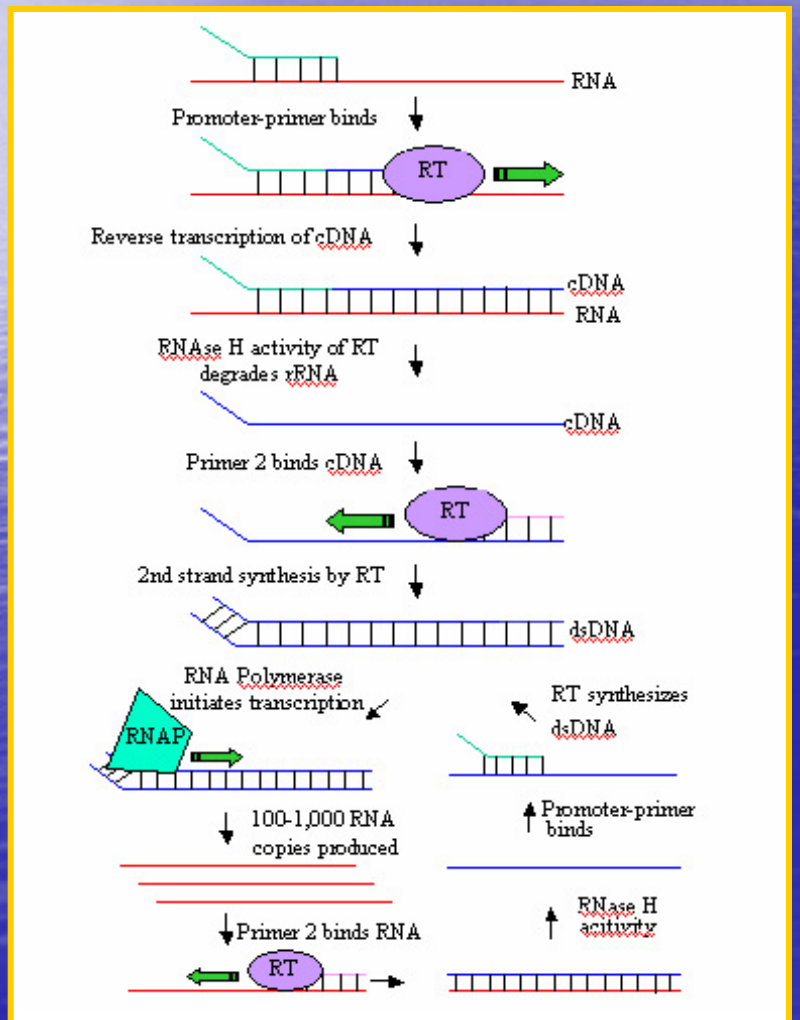
PCR and Real-Time PCR

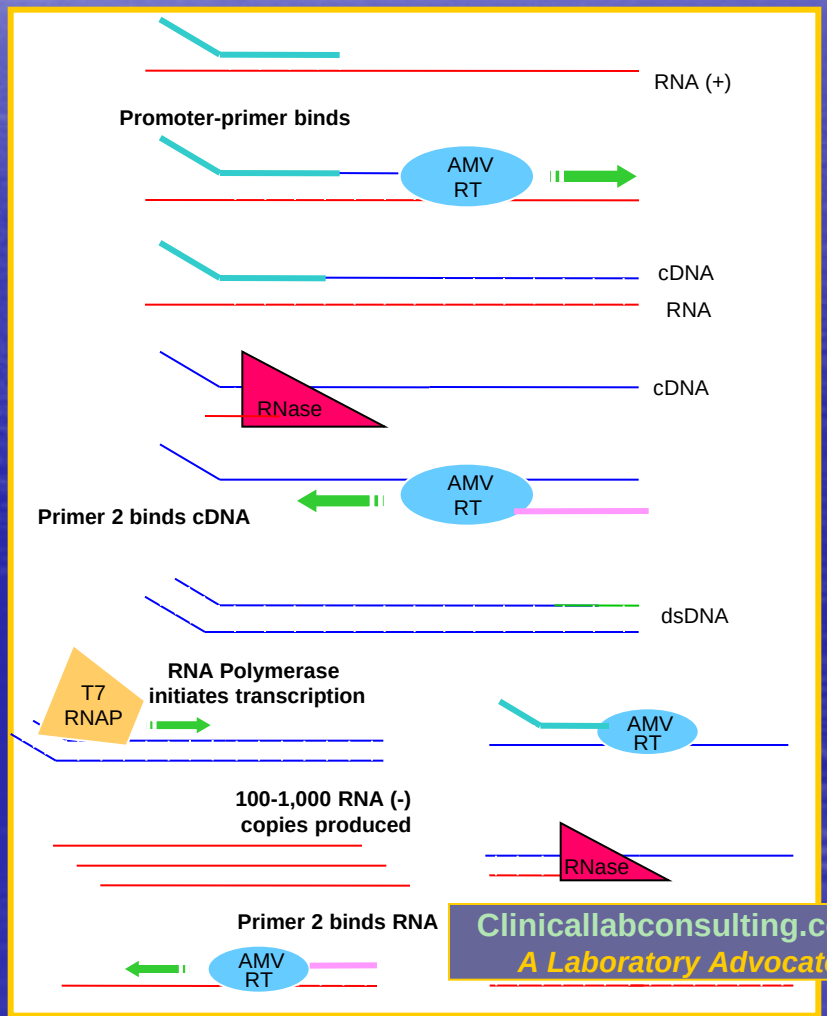


Traditional PCR = ~ 2 minutes/ 1 complete cycle

Real-time PCR = ~ 2 minutes/ 6 comple

Transcription Mediated Amplification (TMA) and Nucleic Acid Sequence Based Amplification (NASBA)





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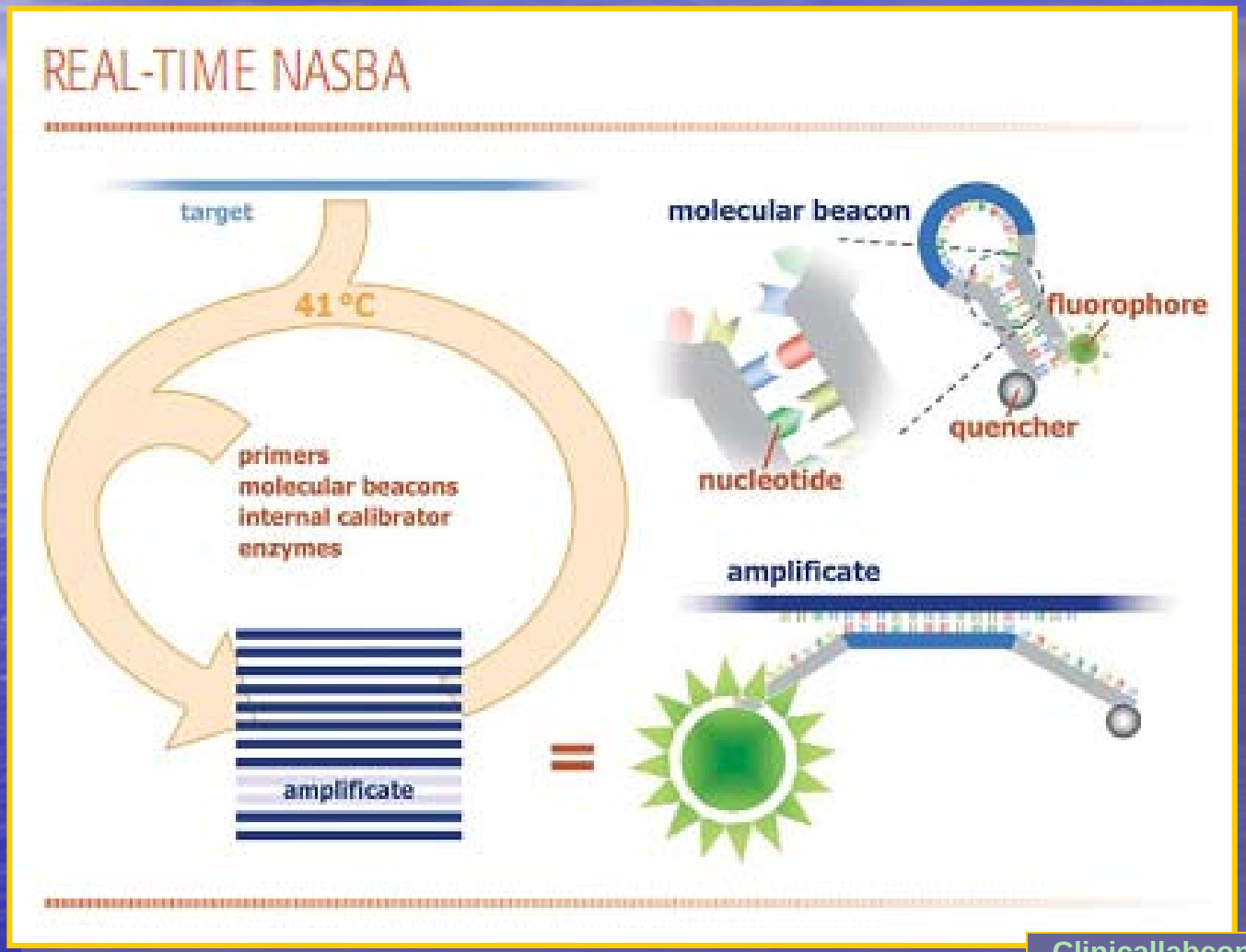
A Laboratory Advocate

Real-Time NASBA

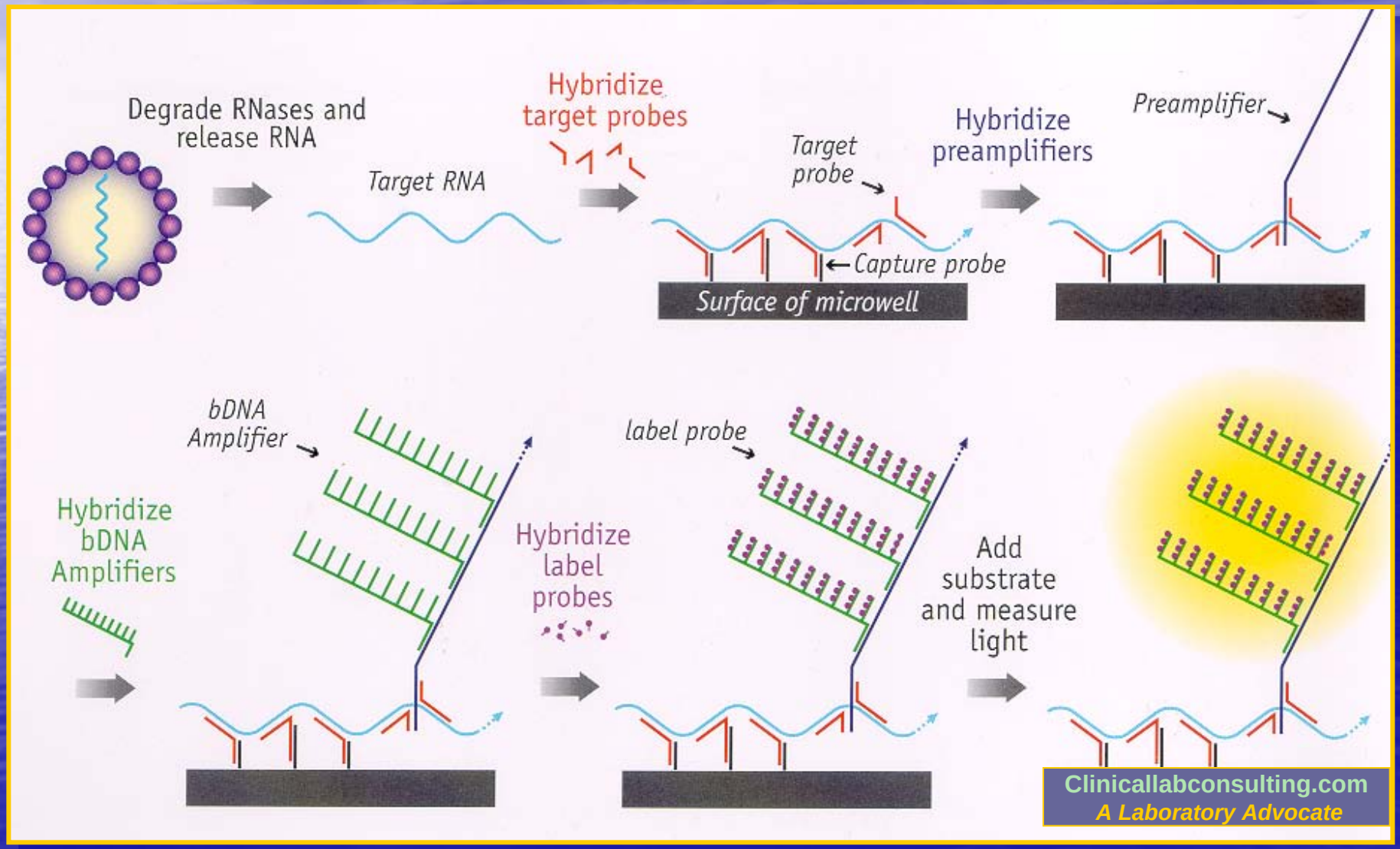


www.biomerieux-diagnostics.com

Signal Amplification Methods

- Branched DNA (bDNA)
- Hybrid Capture
- Invader

Branched DNA (bDNA)



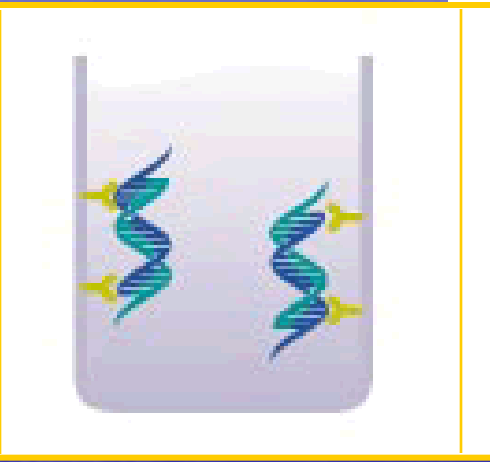
Hybrid Capture

Release Nucleic Acids

Clinical specimens are combined with a base solution which disrupts the virus or bacteria and releases target DNA.

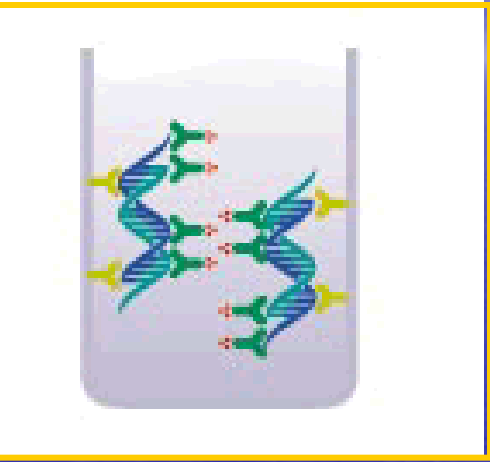
Hybridize RNA Probe with Target DNA

Target DNA combines with specific RNA probes creating RNA:DNA hybrids.



Capture Hybrids

RNA:DNA hybrids are captured onto a microtiter well coated with capture antibodies specific for RNA:DNA hybrids.

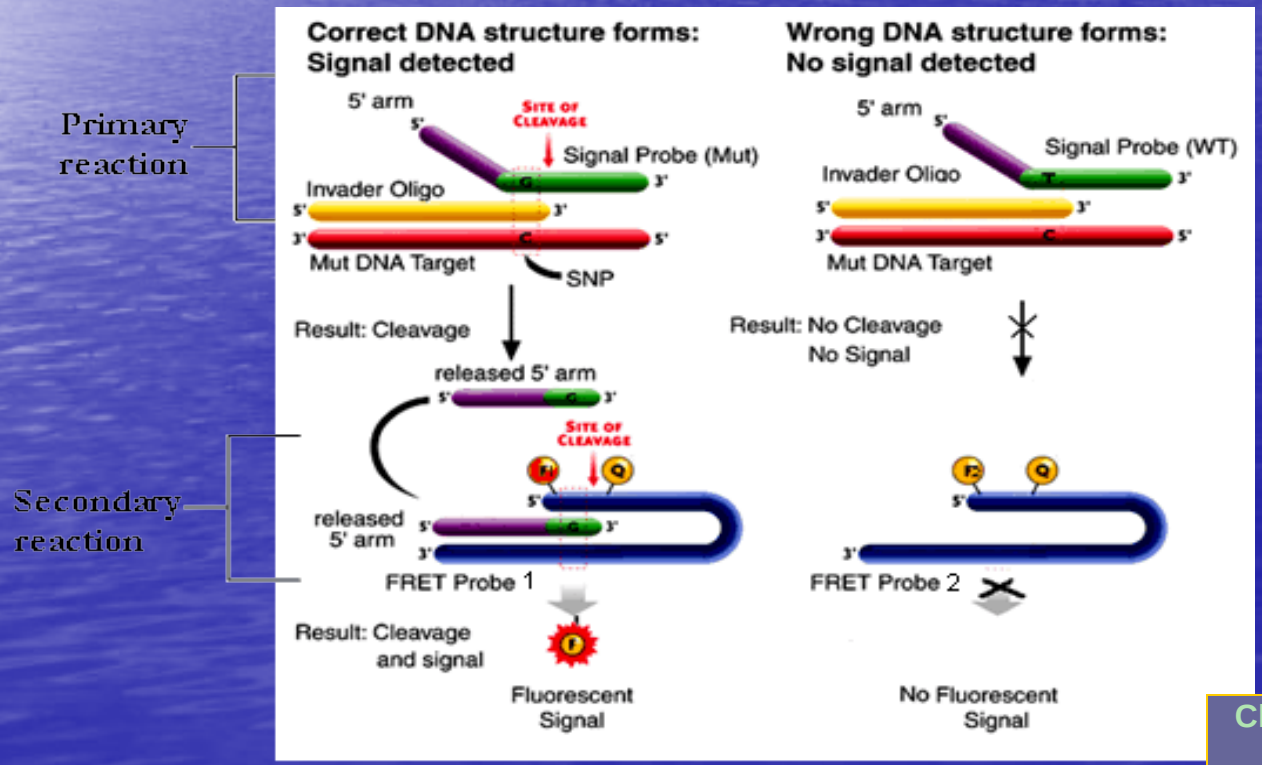


Label for Detection

Captured RNA:DNA hybrids are detected with multiple antibodies conjugated to alkaline phosphatase.

Invader Chemistry

1. Probe oligo and invader oligo bind to specific target sequence
2. Clevase recognizes this structure and cleaves the probe oligo, releasing 5' sequence
3. 5' flap sequence binds second FRET oligo
4. Cleavage releases fluorophore resulting in signal



http://www.ngrl.org.uk/wessex/images/invader.gif

The End