

## Molecular Diagnosis

Nucleic acid based testing in  
Oncology

## Nucleic Acid Testing - Oncology

- Gross alterations in DNA content of tumors (ploidy)
- Gain/Loss of nucleic acids
- Markers of Clonality
- Oncogene/Tumor Suppressor gene mutations
- Tumor Specific Translocations
- mRNA “molecular staging”
- Gene Expression profiling

## Objectives

- Describe uses of NAT in
  - Oncology
    - Diagnosis, Prediction, monitoring.
  - Genetics
    - Screening, presymptomatic testing, diagnostic testing, family studies.
- Identify correct test approaches
- Evaluate clinical significance of tests
- Evaluate Impact of genetic test results on individuals/families (ethical considerations)

## Gain/Loss of Nucleic Acid

- Diagnosis of specific entities:
  - 1p/19q in oligodendroglioma; CLL, trisomy 12; 5q- refractory anemia; 3p- in renal clear cell carcinoma
- Prognostic/Predictive changes
  - *MYCN* amplification in neuroblastoma
  - *HER-2/neu* amplification in Breast CA
- Screening/monitoring for neoplasms
  - “Urovysion” .

## Molecular Diagnosis

- “Nucleic Acid Testing”
  - Use of specific sequence information in nucleic acids - DNA and RNA - for clinical management

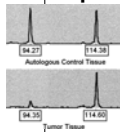
## *MYCN* and Neuroblastoma

Feature	Type 1	Type 2	Type 3
<i>MYCN</i>	Normal	Normal	Amplified
DNA ploidy	Hyperdiploid or near triploid	Near diploid or near tetraploid	Near diploid or near tetraploid
1p LOH	<5%	25–50%	80–90%
14q LOH	<5%	25–50%	<5%
<i>TRK-A</i> exp.	High	Low	Low or absent
<i>TRK-B</i> exp.	Low or truncated	Low or absent	High (full length)
<i>TRK-C</i> exp.	High	Low or absent	Low or absent
Age	Usu. <1 year	Usu. >1 year	Usu. 1–5 years
Stage	Usu. 1, 2, 4S	Usu. 3, 4	Usu. 3, 4
3-Year survival	95%	25–50%	<5%

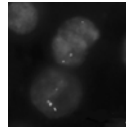
Exp., expression; Usu., usually.

## Chromosome 1p, 19q and Gliomas

- 1p & 19q deletions define Oligodendroglioma.
- Detected either by: FISH or PCR –based Techniques



PCR amplification of microsatellites  
– normal vs tumor tissue.



FISH: green = centromere  
red = 1p.

## Antigen Receptor Gene Rearrangement

- Somatic Ig & TCR gene rearrangement:
  - V-D-J recombination
  - B- & T-cell repertoire diversity
  - Combinatorial Diversity
    - Use of different V-D- and J segments
  - Junctional Diversity
    - Addition or removal of nucleotides between D-J, V-D, or V-J segments.
    - Greatest contribution.

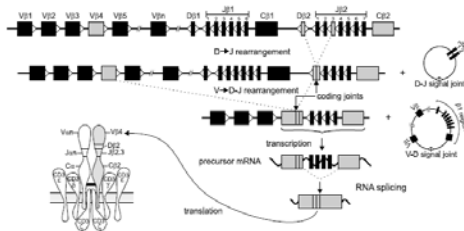
## Molecular Markers of Clonality

- Antigen receptor gene rearrangements.
  - Southern Blotting: IgH, TCR; EBV termini.
  - PCR: Ig and TCR gene rearrangement.
- X-inactivation.
  - Human androgen receptor assay.
- Microsatellite allelotyping.
  - Altered microsatellites in lesional tissue vs germline (PCR amplification)
- Clonal Viral Integration
  - EBV termini, by Southern Blotting

## Antigen Receptor Gene Rearrangement

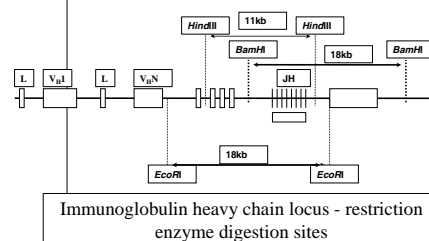
- Clonality Testing
  - Each lymphoma arises from a single cell w/ a unique Antigen receptor gene rearrangement.
  - Inflammatory infiltrates
    - Lymphocytes w/ different rearrangements.
  - Tests for clonality
    - Southern Blotting
    - PCR-based tests

## Antigen Receptor Gene Rearrangement



Unique V-D-J rearrangements for each event.

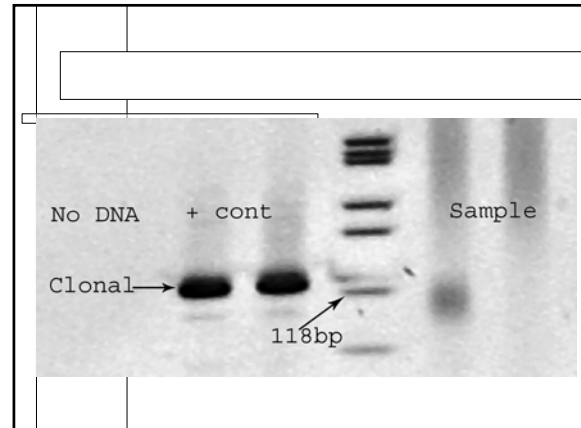
## Southern Blotting for Clonality



Immunoglobulin heavy chain locus - restriction enzyme digestion sites

## Southern Blotting for Clonality

- Test based on combinatorial diversity
- Digest high DNA w/ restriction enzyme
- Separate resulting fragments by size
  - Electrophoresis (agarose gel)
- Transfer to membrane, immobilize, denature, hybridize with a probe.

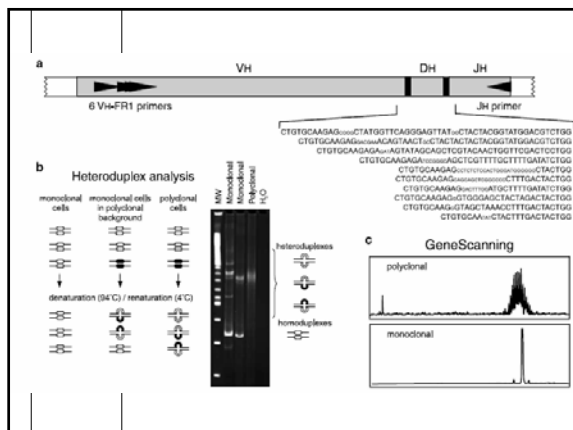


## PCR-based Tests for Clonality

- Primers w/c bind to relatively invariant sites flanking sites of recombination
  - No amplification w/o recombination (primer binding sites too far)
- Polyclonal lymphoid population
  - Mixture of PCR products (combinatorial and junctional diversity)
- Clonal lymphoid population
  - Single Predominant PCR product
  - Differentiate by
    - Plain Electrophoresis
    - Capillary Electrophoresis
    - "heteroduplex analysis"
    - Denaturation - either on gel, or w/ "real-time" PCR

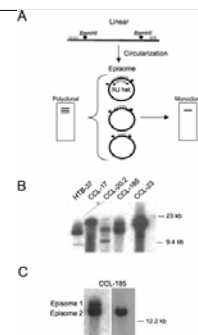
## Southern Blotting vs PCR-based Tests

- |   |   |
|---|---|
| <ul style="list-style-type: none"> <li>• Southern                             <ul style="list-style-type: none"> <li>- Needs High MW DNA</li> <li>- 5-10 µg DNA</li> <li>- Labor intensive</li> <li>- Tech. demanding</li> <li>- At least 5% neoplastic cells</li> <li>- Detects most recombinations</li> </ul> </li> <li>- Does not detect junctional diversity</li> </ul> | <ul style="list-style-type: none"> <li>• PCR                             <ul style="list-style-type: none"> <li>- Can use partially degraded DNA</li> <li>- 0.1-1 µg DNA</li> <li>- Quick</li> <li>- Simple</li> <li>- May go down to 1%</li> <li>- Up to 30% of rearrangements not detected due to primer binding.</li> <li>- Junctional diversity may be used to follow clone.</li> </ul> </li> </ul> |
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## Clonal EBV-Integration

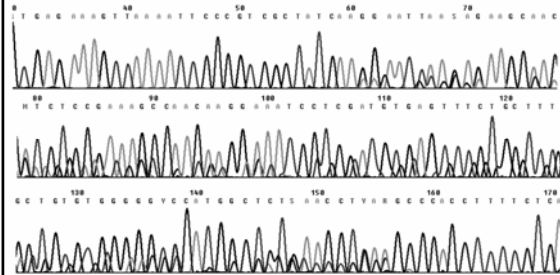
- EBV-associated neoplasms:
  - Post-transplant lymphoproliferative disorder (PTLD)
  - Nasopharyngeal CA
  - AIDS-associated "leiomyosarcoma" (pediatric)



## Oncogene/Tumor suppressor mutations

- **KIT/PDGFR** mutations in gastrointestinal stromal tumors
  - Target for treatment w/ Imatinib
- **EGFR** mutations in Lung CA
  - Adenocarcinomas in non-smokers
  - Response to treatment w/ Iressa
- **PIK3CA** and **PTEN** mutations
  - Activation of AKT pathway
  - Susceptibility to Rapamycin-type agents.

## EGFR: in-frame 15bp Exon19 deletion



Direct sequencing: mixture of tumor and normal – lower mutant peaks

## Oncogene/TSP mutations

- **Oncogene mutations:**
  - Activating mutations
  - Limited number of mutations/gene
  - Tests to detect specific mutations.
- **Tumor Suppressor Gene mutations**
  - Inactivating mutations
  - Many possible mutations
    - Occasionally hot-spots, related to gene structure and/or mutagen.

## Tumor-specific translocations

- **Translocations w/o fusion gene products**
  - Dysregulation of gene expression, no fusion mRNA
  - Detect translocation by:
    - Classical cytogenetics, FISH, Southern Blotting or DNA PCR.
  - DNA PCR for translocations
    - Requirement for recurrent breakpoints OR
    - Long range PCR.

## Somatic mutation tests

- **Recurrent/Known mutations:**
  - Tests to detect specific sequences
    - Probes, restriction enzymes, sequence-specific primers, etc.
    - Can detect mutation in minority of cells.
    - Use of sequence-specific primers can enrich for mutant sequence.
- **Tests for unknown mutations**
  - Mutation scanning techniques
  - Direct Sequencing.

## Translocations w/o Fusion RNA

Tumor	Translocation	Activated Gene	Mechanism of Activation
B-All/Burkitt	t(8;14)(q24;q32)	MYC	Relocation to IgH locus
Large Cell Lymphoma	t(3;14)(q27;q32)	BCL6	Relocation to IgH locus
Mantle Cell Lymphoma	t(11;14)(q13;q32)	Cyclin D1	Relocation to IgH locus
Follicular B-cell lymphoma	t(14;18)(q32;q21)	BCL2	Relocation to IgH locus
T-cell ALL	t(8;14)(q24;q11)	MYC	Relocation to TCR $\alpha/\delta$ locus
T-cell ALL	t(1;14)(p32;q11)	TAL1	Relocation to TCR $\alpha/\delta$ locus

## DNA PCR for translocations: BCL2

### • t(14;18)(q32;q21)

- BCL2 placed under immunoglobulin enhancer – overexpressed – loss of apoptosis – follicular lymphoma .
- 14q32 breakpoints constant.
- 18q21 breakpoints variable:



## Translocations w/chimeric products: solid tumors

## DNA PCR for translocations

### • Variable breakpoints:

- Not all translocations will be amplified by a limited primers sets.
  - E.g., BCL2 – 60%, BCL1 – 50%
- Need for “long range” PCR to increase detection
  - Technically difficult.
  - Needs high quality DNA – will not work on paraffin.

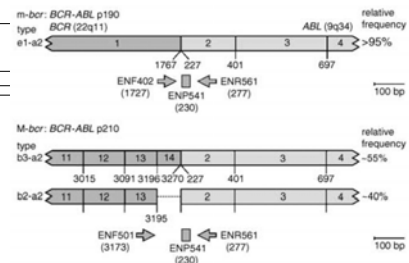


Figure 12 Schematic diagram of the BCR-ABL FG transcript covered by the RQ-PCR primer and probe set. For m-bcr, set: ENF402-ENP541-ENR561; and for M-bcr, set: ENF501-ENP541-ENR561. The number under the primers and probe refers to their 5' nucleotide position in the normal gene transcript (see Tables 14 and 17). Relative frequency refers to the proportion of e1-a2 transcript among m-bcr variants and the proportion of b3-a2 and b2-a2 transcripts among M-bcr variants.

## Translocations w/ fusion RNA - Heme

TUMOR	Translocation	Gene fusion
Chronic myelogenous leukemia	t(9;22)	BCR-ABL(p210)
Acute promyelocytic leukemia	t(15;17); t(11;17)(q23;q21); t(5;17)(q35;q21); t(11;17)(q13;q21) der(17)	PML-RAR PLZF-RAR NPM-RAR NUMA-RAR STAT5b-RAR
AML	t(8;21)(q22;q22)	AML1-ETO
AML and ALL (esp. infants and post-Rx)	11q23	MLL(-~40 partners)
Anaplastic large cell lymphoma (pediatric)	t(2;5)(p23;q35)	NPM-ALK
ALL	t(9;22)	BCR-ABL(p190)
MALT lymphoma	t(11;18)	API2-MLT

