

# Synoptic Reporting for Molecular Diagnostics and FISH

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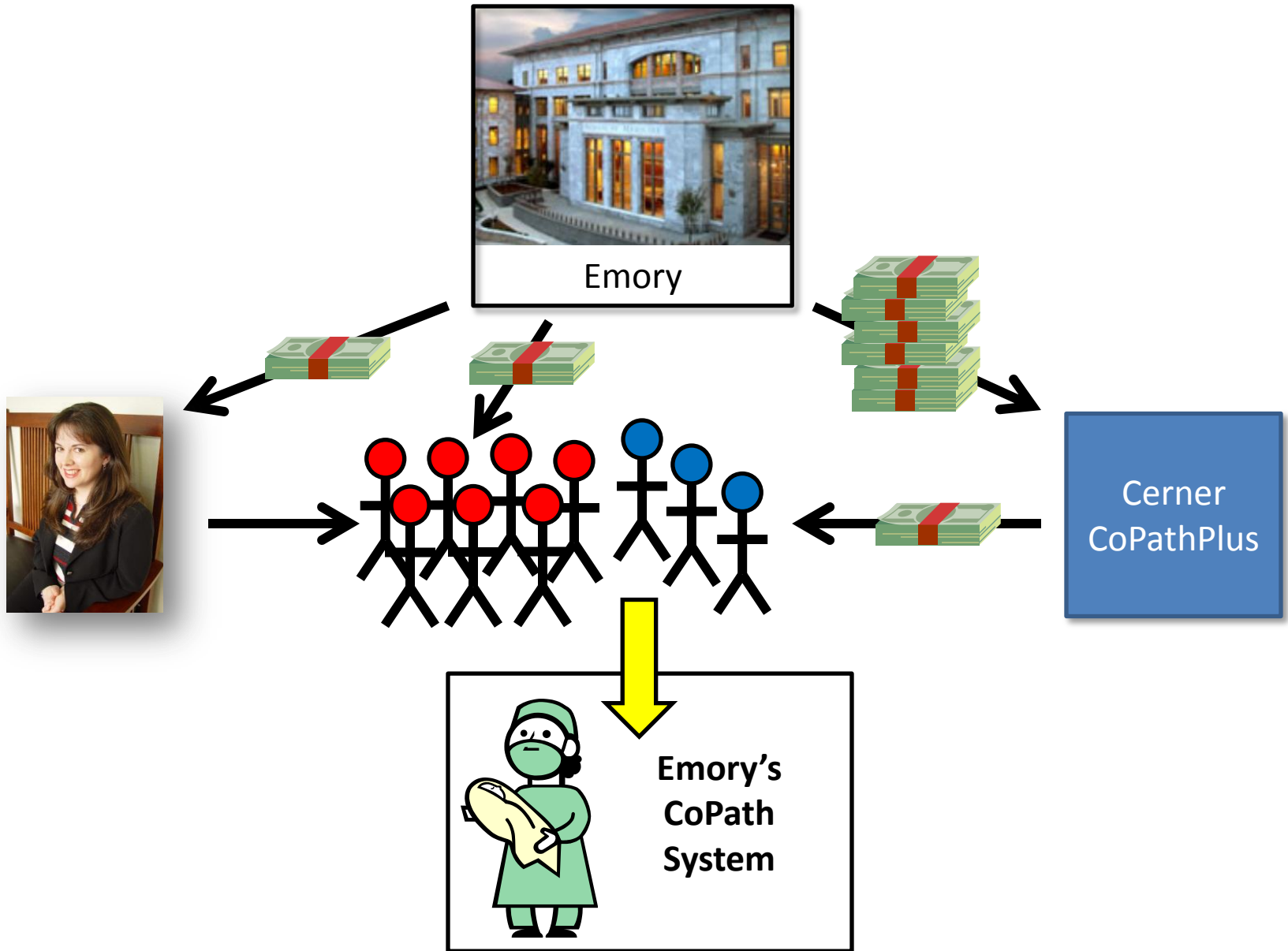
Emory University School of Medicine



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# Disclosures

- No *official* financial disclosures to report



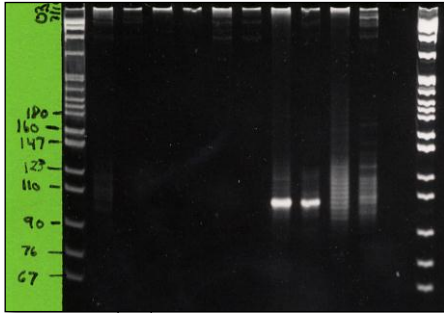
# Goals and Objectives

- By attending this presentation, you should be able to:
  - Describe why molecular and FISH labs need discrete data
  - Understand how to develop and implement custom synoptics for your lab
  - See examples of synoptic data retrieval

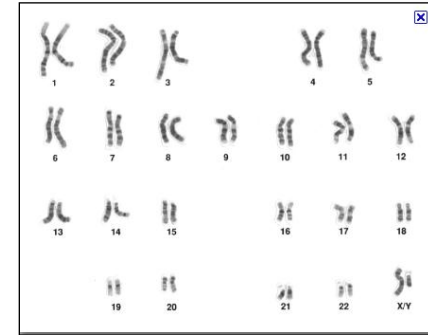
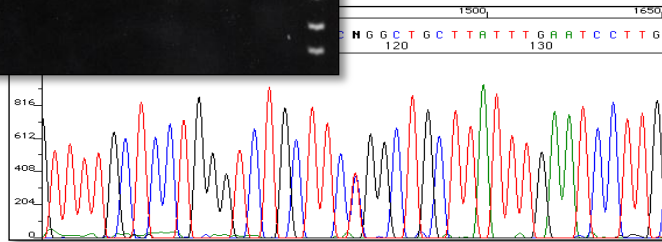
# Caveat and Disclaimers

- Synoptics in this presentation
  - Could be built by **ANY** LIS with the ability to customize synoptics onto ancillary procedure reports
  - Have no endorsement from any agency
  - Developed at Emory
  - Should **not** be construed as required
  - Are **not** the CAP cancer checklists

# Molecular Testing

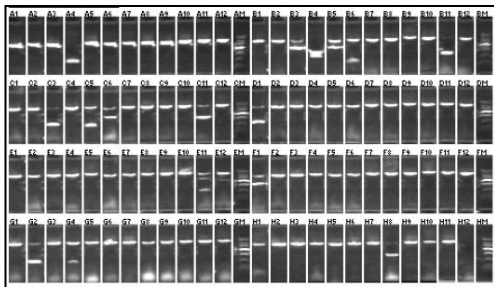


Nucleic acid techniques (PCR, sequencing, arrays, etc.)

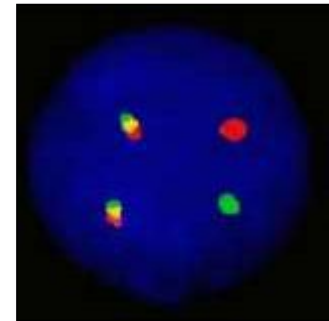


Chromosome analysis (Karyotyping)

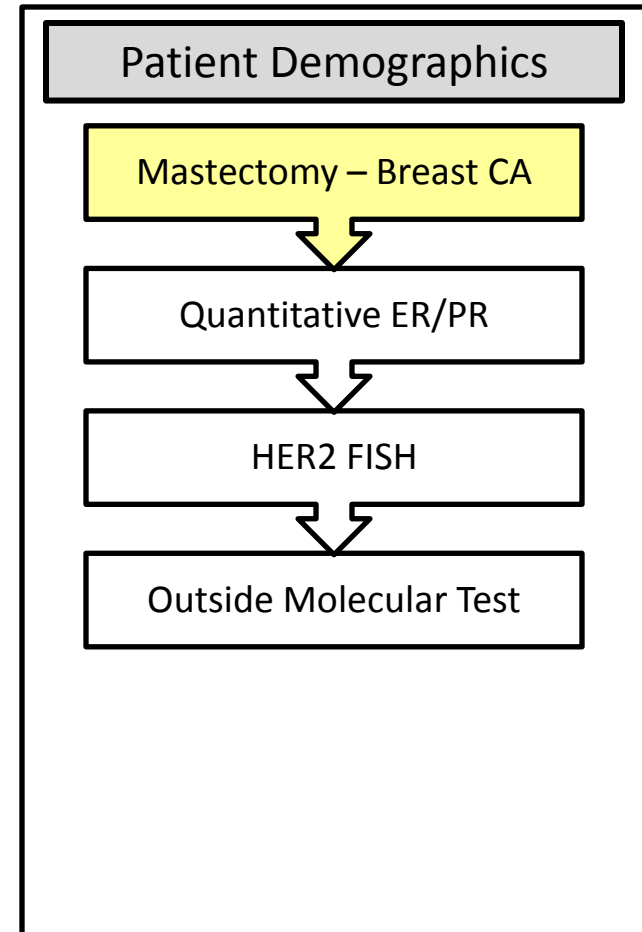
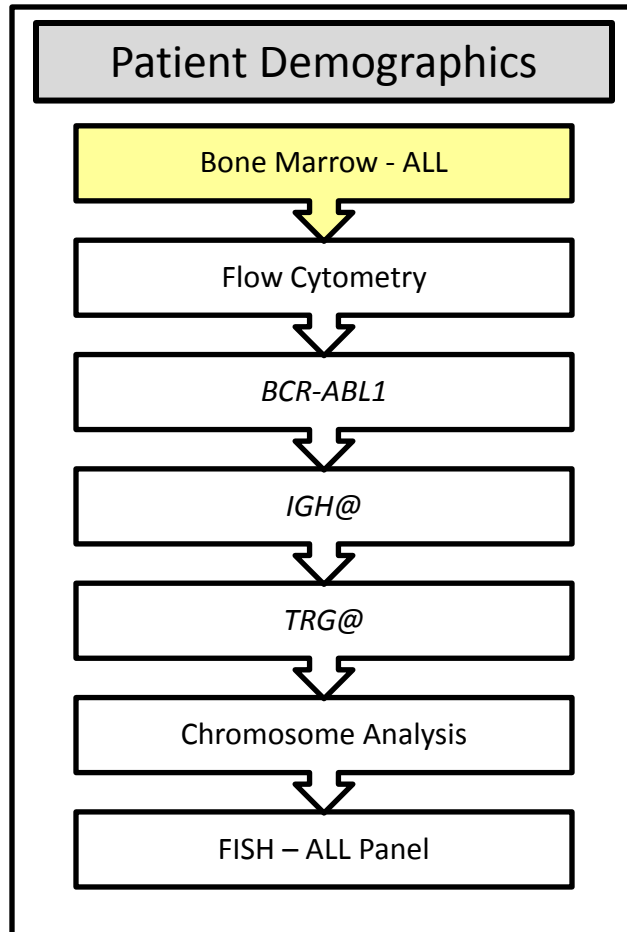
Human Leukocyte Antigen (HLA) molecular testing



Fluorescence in situ hybridization (FISH) (and other ISH)



# Background – Integrated Reports



# What this means...

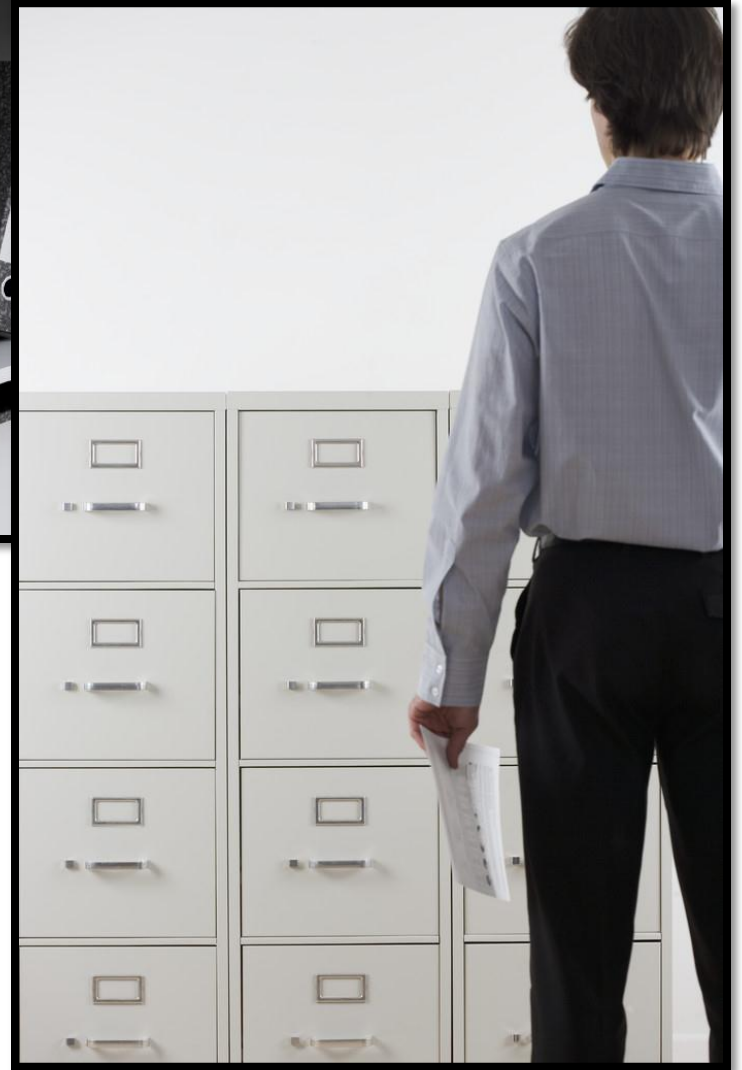
- Molecular and FISH labs may have to use an AP system for reporting

a.k.a. CFTB

(Conglomerate of Free Text Blobs)

# Why do we care?

- AP systems not equipped to handle discrete molecular data including FISH
- How does data get recorded and stored?



# Data Retrieval

- Why is data retrieval important?
  - Research and quality studies
  - Validation of new methods
  - Accreditation of laboratory

# CAP Accreditation

- MOL.20550 Test Result Statistics

When appropriate, statistics on molecular pathology test results (e.g. percentages of normal and abnormal findings, allele frequencies) are maintained, and appropriate comparative studies performed.

# CAP Accreditation

- Validation studies
  - MOL.30900 Specimen Selection

Validation studies include specimens representing each of the possible reportable results (genotypes).

- MOL.36110 Report Criteria

In genetic testing for complex disease genes with multiple possible mutations, the report includes (where appropriate) an estimate of mutation detection rate and the residual risk of being a carrier for one of the mutations not tested for.

# FISH

- Preclinical validation requires determination of the “normal cutoff” value
  - a.k.a. false positive cutoff percentage
  - Positive signal patterns  $< X\%$   $\rightarrow$  normal
  - X depends on probe types used
- **Periodic reevaluation of established cutoffs against clinical results**

# Cancer Registry Reporting

- State of Georgia
  - All cancer cases and patients have to be reported to the state
  - Patient's actual location is irrelevant
- Our molecular and FISH labs do a lot of testing for malignancies
  - May be the only state presentation of that patient for a malignancy

# Data Retrieval

- How are labs managing this now?

# Data Retrieval – Retro Style

- Data retrieval – three options
  1. Pay \$\$\$ to have someone read reports
    - Double data entry
    - HIPAA issues
  2. Keep separate (shadow) database
  3. Natural language search on electronic reports



**All have significant potential for error**

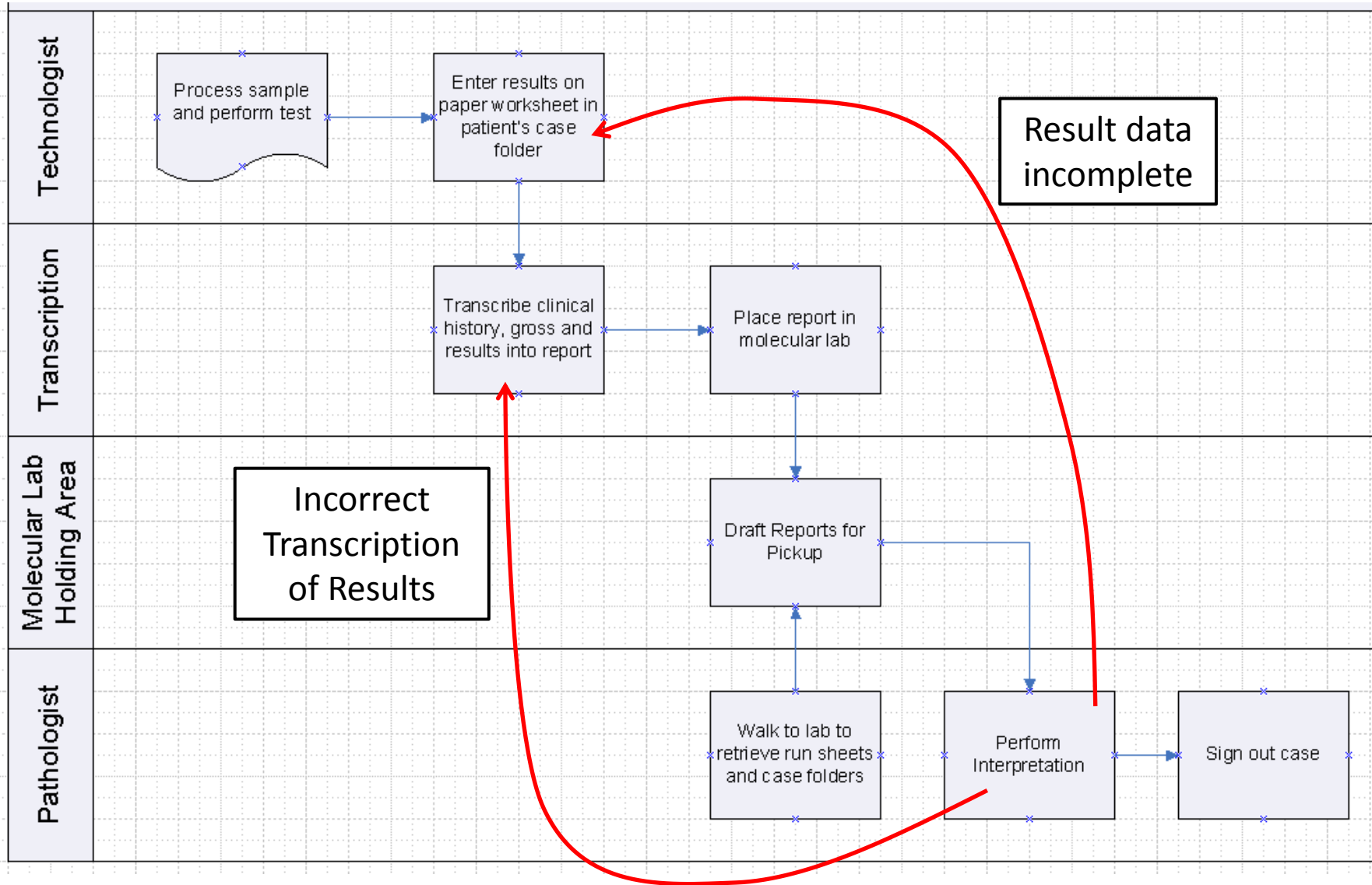
# History

- Migration to CoPath from PathNet Classic
- Synoptic worksheets implemented
  - CAP Cancer Protocols
  - Synoptics could be added to procedure reports
  - Synoptics could be custom-designed
  - Synoptic data is stored as discrete data elements, including for custom synoptics
    - They can be queried

# Is anyone else doing this?

- PubMed search → zilch
- Sent queries to two listservs
  - AMP → zilch
  - API (even after the promise of beer) →

zilch (sort of)



# Goals

- **Molecular and FISH will use synoptics because:**
  - Facilitates direct result entry by technologists
  - Reduces dependence on paper worksheets
  - Eliminates transcription errors for results
  - Markedly improve data retrieval
    - Quality projects
    - Research
    - Cancer Registry

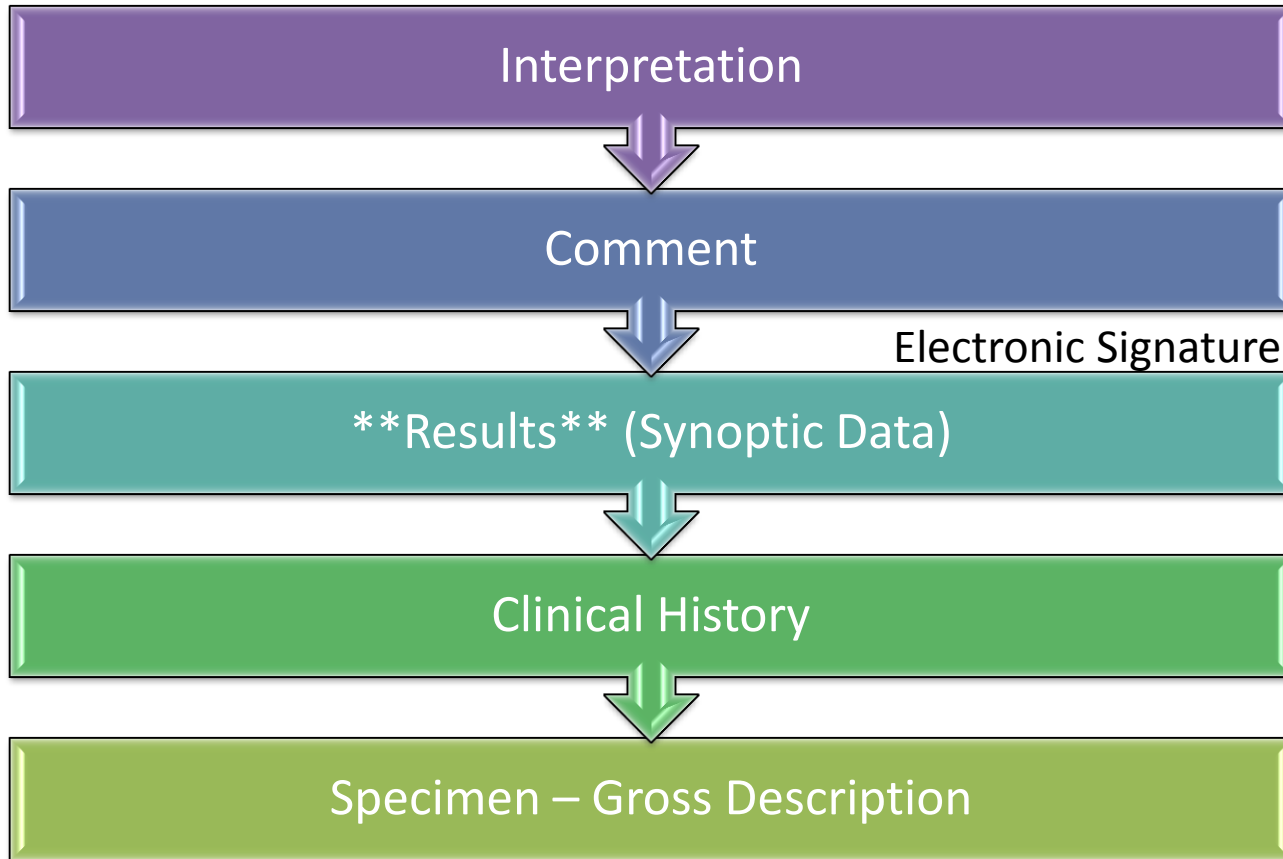
# Out of Scope

- Extraction parameters
  - Amount of DNA/RNA
  - A260/280 Ratios, etc.
- Reagent lot tracking
- Elimination of paper run sheets

# Process

- Developed in conjunction with faculty and staff input
- Iterative process
  - Averaged 2 builds per synoptic
- Design focus
  - Ease of data entry for techs
  - Report display
  - Data retrieval needs

# Procedure Report Format



# Custom Synoptics

- Installed 19 custom synoptic reports
  - Molecular hematopathology testing
  - Molecular solid tumor testing
  - FISH
  - Chromosome Analysis
  - Transplant kidney biopsy

# Custom Synoptics

- **Molecular hematopathologic analysis**
  - *BCL2-IGH@* by PCR
  - *BCR-ABL1* by RQ-PCR
  - *FLT3*-ITD and *FLT3*-D835 Analysis
  - *IGH@* Gene Rearrangement by PCR
  - *JAK2* V617F by RQ-PCR
  - *PML-RARA* by RQ-PCR
  - *TRG@* Gene Rearrangement by PCR
- **Molecular solid tumor analysis**
  - ARMS Translocations by PCR
  - *BRAF* Sequencing
  - *KRAS* Sequencing
  - *SSX1* and *SSX2* Translocations by PCR
- **Chromosome Analysis**
- **FISH**
  - FISH - ALL Panel
  - FISH - AML Panel
  - FISH - CLL Panel
  - FISH - IGH Reflex Panel
  - FISH - MDS Panel
  - FISH - Myeloma Panel
  - Miscellaneous FISH
- **Transplantation pathology**
  - Kidney transplant biopsy

# Worksheet examples

Synoptic Diagnosis Worksheet Entry/Edit

Worksheet # 1 of 1  
Proc/Part X: {source}

Page 1 of 1

**BCL2-IGH Analysis**

**BCL2-IGH (MBR) Result**

A1 DETECTED  
A2 MBR Amplicon length(s) in bp: \_\_\_\_\_  
A3 Indeterminate  
A4 Not Detected

**BCL2-IGH (3'MBR) Result**

B1 DETECTED  
B2 3'MBR Amplicon length(s) in bp: \_\_\_\_\_  
B3 Indeterminate  
B4 Not Detected

**BCL2-IGH (MCS) Result**

C1 DETECTED  
C2 MCS Amplicon length(s) in bp: \_\_\_\_\_  
C3 Indeterminate  
C4 Not Detected

**BCL2-IGH Sample Adequacy**

D1 Adequate  
D2 Quality / quantity of nucleic acid not sufficient for analysis

Beginning  Worksheet Complete OK Cancel Comment Menu Help

Synoptic Diagnosis Worksheet Entry/Edit

Worksheet # 1 of 1  
Proc/Part X: {source}

Page 1 of 1

### BCL2-IGH Analysis

**BCL2-IGH (MBR) Result**

A1 DETECTED  
 A2 MBR Amplicon length(s) in bp: \_\_\_\_\_  
 A3 Indeterminate  
 A4 Not Detected

**BCL2-IGH (3'MBR) Result**

B1 DETECTED  
 B2 3'MBR Amplicon length(s) in bp: \_\_\_\_\_  
 B3 Indeterminate  
 B4 Not Detected

**BCL2-IGH (MCS) Result**

C1 DETECTED  
 C2 MCS Amplicon length(s) in bp: \_\_\_\_\_  
 C3 Indeterminate  
 C4 Not Detected

**BCL2-IGH Sample Adequacy**

D1 Adequate  
**D2 Quality / quantity of nucleic acid not sufficient for analysis**

Beginning  Worksheet Complete OK Cancel Comment Menu Help

Synoptic Diagnosis Worksheet Entry/Edit

Worksheet # 1 of 1  
Proc/Part X: {source}

Page 1 of 1

### BCL2-IGH Analysis

**BCL2-IGH (MBR) Result**

**A1 DETECTED**

**A2 MBR Amplicon length(s) in bp: 300**

A3 Indeterminate

A4 Not Detected

**BCL2-IGH (3'MBR) Result**

B1 DETECTED

B2 3'MBR Amplicon length(s) in bp: \_\_\_\_\_

B3 Indeterminate

**B4 Not Detected**

**BCL2-IGH (MCS) Result**

C1 DETECTED

C2 MCS Amplicon length(s) in bp: \_\_\_\_\_

C3 Indeterminate

**C4 Not Detected**

**BCL2-IGH Sample Adequacy**

**D1 Adequate**

D2 Quality / quantity of nucleic acid not sufficient for analysis

BCL2-IGH (MBR) Result:	DETECTED
MBR Amplicon length(s) in bp:	300
BCL2-IGH (3'MBR) Result:	Not Detected
BCL2-IGH (MCS) Result:	Not Detected
BCL2-IGH Sample Adequacy:	Adequate

Worksheet Complete

Synoptic Diagnosis Worksheet Entry/Edit X

Worksheet # 1 of 1  
Proc/Part X: {source}

Page 1 of 6

### FISH

NOTE: Sections A and B do not appear on the final report but help make sure that all data is filled out.

**# of Autosomal Probe Sets Analyzed**

A1 Zero autosomal probes - XY probe set ONLY  
 A2 One (1)  
 A3 Two (2)  
 A4 Three (3)

**X/Y Probe Set Performed**

B1 Yes  
 B2 No

===== **FIRST PROBE SET** =====

**Probe Set (1)**

C1	1p36/1q25 and 19p13/19q13 for 1p/19q deletion	C12	EGFR,D7Z1 for EGFR amplification
C2	PBX1,TCF3 for t(1;19)	C13	D7Z1 for gain of 7
C3	MYCN for amplification	C14	D8Z2 for gain of 8
C4	ALK for t(2;5)	C15	MYC for amplification
C5	D3Z1 for gain of 3	C16	MYC for translocation
C6	CEP4 for gain of 4	C17	MYC,IGH@ for t(8;14)
C7	FGFR3,IGH@ for t(4;14)	C18	RUNX1T1,RUNX1 for t(8;21)
C8	SCFD2,LNX1,PDGFRA,KIT for 4q12	C19	CEP9 for gain of 9
C9	D5S23,D5S721,EGR1 for -5/del(5q)	C20	BCR,ABL1 for t(9;22)
C10	MYB for del(6q)	C21	CEP10 for gain of 10
C11	D7S486,D7Z1 for -7/del(7q)	C22	PTEN for del(10)(q23)
		C23	ATM for del(11q22.3)
		C24	MLL for translocation or deletion of 11q23
		C25	CCND1,IGH@ for t(11;14)
		C26	D12Z3 for gain of 12
		C27	ETV6,RUNX1 for t(12;21)
		C28	MDM2 for amplification of 12q15
		C29	D13S25,LAMP for del(13q)
		C30	IGH@ for t(14q)
		C31	MAF,IGH@ for t(14;16)
		C32	BCL2,IGH@ for t(14;18)
		C33	MALT,IGH@ for t(14;18)
		C34	PML,RARA for t(15;17)
		C35	CBFB for rearrangement of 16q
		C36	FUS for translocation of 16p11

*—PROBE SET (1) Continued on next page—*

Worksheet Complete

Synoptic Diagnosis Worksheet Entry/Edit Page 2 of 6

Worksheet # 1 of 1  
Proc/Part X: {source}

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—PROBE SET (1) Continued from previous page—

C37 D17Z1 for gain of 17

C38 HER-2/CEP 17 Pathvision for HER-2 amplification

**C39 TP53,D17Z1 for del(17p)**

C40 RARA for PML variants

C41 SYT for translocation 18q11.2

C42 D20S108 for del(20q)

C43 RUNX1 for gain of 21

C44 EWSR1 for translocation 22q12

**Rearrangement Results (1)**

**D1 POSITIVE FOR STRUCTURAL ABNORMALITY**

**D2 Description: del(17p)**

**D3 in 30 % of cells**

D4 Equivocal / Indeterminate

D5 Negative

**Copy Number Results (1)**

**E1 POSITIVE FOR COPY NUMBER ABNORMALITY**

E2 Description: \_\_\_\_\_

E3 in \_\_\_\_ % of cells

E4 Equivocal / Indeterminate

**E5 Negative**

**FISH**

**Amplification Results (1)**

F1 POSITIVE FOR AMPLIFICATION

F2 Description: \_\_\_\_\_

F3 in \_\_\_\_ % of cells

F4 Equivocal / Indeterminate

**F5 Negative**

F6 Ratio = \_\_\_\_\_

F7 Comment: \_\_\_\_\_

**Other Results (1)**

G1 POSITIVE FOR \_\_\_\_\_

G2 Description: \_\_\_\_\_

G3 in \_\_\_\_ % of cells

G4 Equivocal / Indeterminate

**G5 Negative**

**Variant Present (1)**

H1 Yes

H2 Description: \_\_\_\_\_

**H3 No**

—————NEXT PAGE—————

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Worksheet Complete

# First Report Output

Probe Set (1):	FGFR3,IGH@ for t(4;14)
Rearrangement Results (1):	POSITIVE FOR STRUCTURAL ABNORMALITY, t(4;14), in 30 % of cells
Copy Number Results (1):	Negative
Amplification Results (1):	Negative
Other Results (1):	Negative
Variant Present (1):	No
Probe Set (2):	MYC for amplification
Rearrangement Results (2):	Negative
Copy Number Results (2):	Negative
Amplification Results (2):	Negative
Other Results (2):	Negative
Variant Present (2):	No
X Result:	70% XX Donor
Y Result:	30% XY Patient
Cultures Studied for FISH:	48-hour

# Second Report Output

Probe Set 1:	FGFR3,IGH@ for t(4;14) POSITIVE FOR STRUCTURAL ABNORMALITY, t(4;14), in 30 % of cells
Probe Set 2:	MYC for amplification No abnormality detected
X Result:	70% XX Donor
Y Result:	30% XY Patient
Cultures Studied for FISH:	48-hour

# Results

- Ease of data entry
  - No more duplicate data entry into shadow database
  - Techs had learning curve
- Reduce usage of paper worksheets
  - Minimal effect in both labs
    - still have worksheet but has less data
    - Synoptics don't handle specimen extraction data or cytotechnologist counts

# Results

- Data retrieval
  - Take a look...

# BCL2 example

- Since go-live

<b>Total BCL2 cases</b>	<b>52</b>
MBR-positive	3
3'MBR-positive	0
MCS-positive	1

- Construct query = 5 minutes
- Total query time = 2 seconds

# FISH example

- **Variant t(9;22) query**

<b>Total BCR-ABL1 probes run in MISC FISH Worksheet</b>	<b>112</b>
Variant t(9;22) reported	5
Single fusion only	2
Single fusion with loss of ABL	2
Single fusion with extra non-fused BCR and non-fused <i>ABL</i>	1

- Construct Query = 5 minutes
- Total query time = 2 seconds

# What we learned

- Copath synoptics are not easy to build
  - Learning curve, attention to detail
- CoPath synoptics can be hard to alter
  - Especially if adding values...
    - The week before go-live

# What we learned

- Molecular and FISH data were already part of some of the CAP synoptics
  - Problems for our pathologists
  - Reporting data not evaluated

BREAST: Invasive Carcinoma (Complete Excision and Mastectomy)		age 13 of 16
<u>Fluorescence In Situ Hybridization (FISH) for HER2/neu</u>		<u>*Other Ancillary Studies</u> (results for invasive carcinoma performed on this specimen or a prior core needle biopsy or incisional biopsy)
Aa82 Performed		BB1 *Performed on this specimen
Aa83 Performed on this specimen		BB2 *Name of Test: _____
Aa84 Performed on another specimen		BB3 *Results: _____
Aa85 *Specify Specimen (accession number): _____		BB4 *Performed on another specimen
Aa86 Pending		BB5 *Specify Specimen (accession number): _____
Aa87 Not performed		BB6 *Name of test: _____
Aa88 Other (specify): _____		BB7 *Results: _____
<u>Results</u>		----- <u>STAGE (PTNM)</u> -----
Aa89 Not Amplified (HER2 gene copy < 4.0 or ratio < 1.8)		<u>TNM DESCRIPTORS (check all that apply)</u>
Aa90 Equivocal (HER2 gene copy 4.0 to 6.0 or ratio 1.8 to 2.2)		CC1 Not applicable
Aa91 Amplified (HER2 gene copy > 6.0 or ratio > 2.2)		CC2 m (multiple foci of invasive carcinoma)
Aa92 *Average Number of HER2 Gene Copies per Cell _____		CC3 r (recurrent)
Aa93 *Average Number of Chromosome 17 per Cell _____		CC4 y (post-treatment)
Aa94 *Ratio: _____		
Aa95 Results unknown		<u>PRIMARY TUMOR (INVASIVE CARCINOMA) (pT)</u>
Aa96 Other (specify): _____		# For the purposes of this checklist, these categories should only be used in the setting of preoperative (neoadjuvant) therapy for which a previously diagnosed invasive carcinoma is no longer present after treatment.
Aa97 *Name of Assay: _____		DD1 pTX: Primary tumor cannot be assessed
		DD2 pT0: No evidence of primary tumor#
		DD3 pTis (DCIS): Ductal carcinoma in situ#

# 3 months after go live...

Suddenly, I'm everyone's best friend

# Build it and they will come...

- Custom synoptics in development
  - DNA and RNA extraction
  - Ewing Sarcoma/PNET (*EWS-FLI1*)

# Questions?