Genetics of Familial Colorectal Cancer Syndromes

Wade S. Samowitz, M.D.
Professor, Dept. of Pathology
University of Utah

Familial Colorectal Cancer

- Lynch syndrome
- Familial adenomatous polyposis (FAP)
- MYH-associated polyposis (MAP)
- Hamartomatous polyposis

Lynch syndrome (HNPCC)

- Early onset colon cancer
- Right-sided
- Extra-colonic cancers: endometrium, ovary, renal pelvis, ureter, small intestine, stomach, hepatobiliary tract
- Muir-Torre: Lynch + sebaceous neoplasms
- Turcot's: Lynch + brain tumor (GBM) (Hamilton, NEJM, 1995)

Lynch syndrome

- Germline mutations in mismatch repair genes: *MLH1*, *MSH2*, *MSH6* or *PMS2*
- Autosomal dominant
- Phenotype not so obvious (unlike FAP, for example)
- Family history not always obvious or available
- Fortunately, we can use the molecular features of the tumor (Microsatellite instability) to help in work-up

Microsatellite repeats

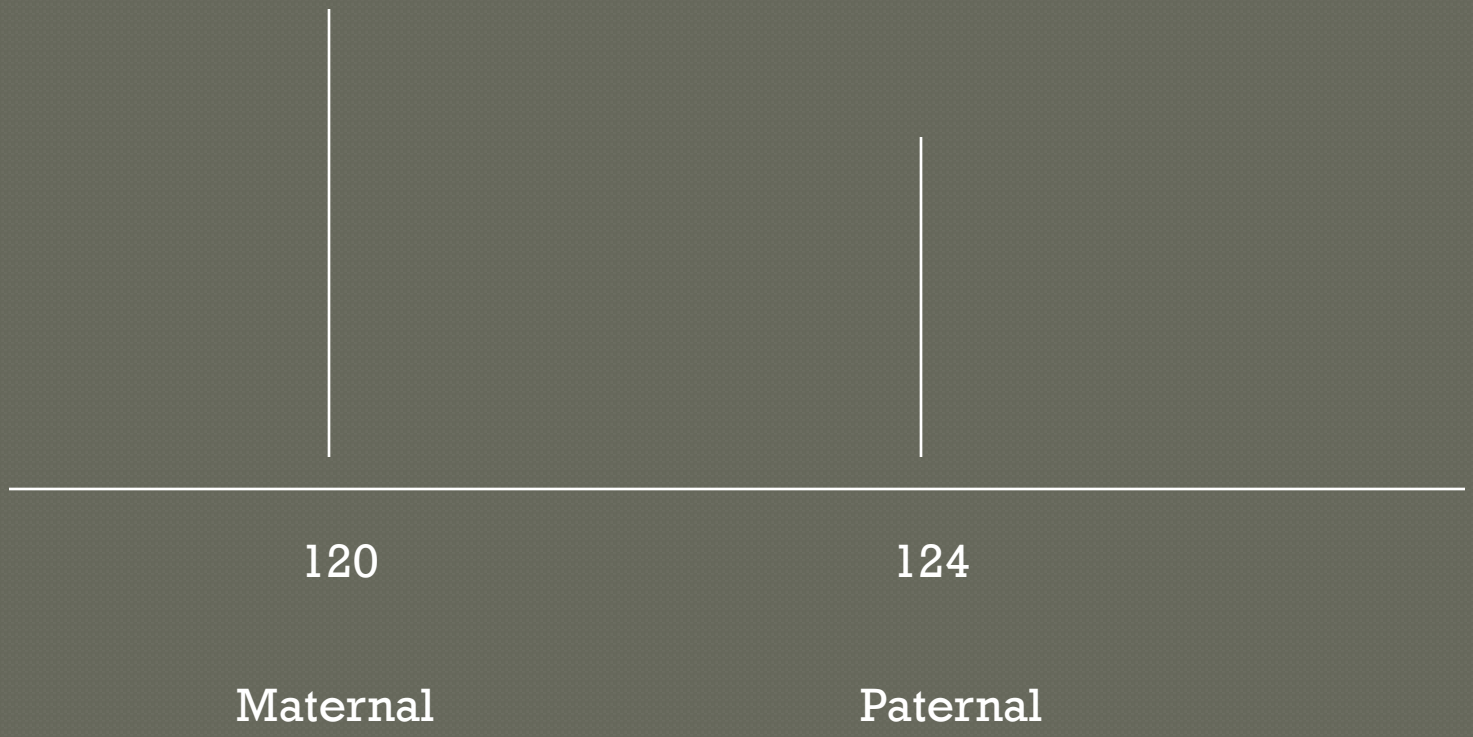
- Type of repetitive DNA in which repeat unit is short (1-6 nucleotides, aka STR)
  - Mononucleotide: AAAAAAAAAAA
  - Dinucleotide: CACACACACACA
- Most in non-coding regions
  - Some exceptions: Mononucleotide repeats such as in TGFBRII
- Often slippage during DNA replication of these repeats
  - Leads to changes in number of repeats
- Usually fixed by mismatch repair apparatus

Microsatellite instability

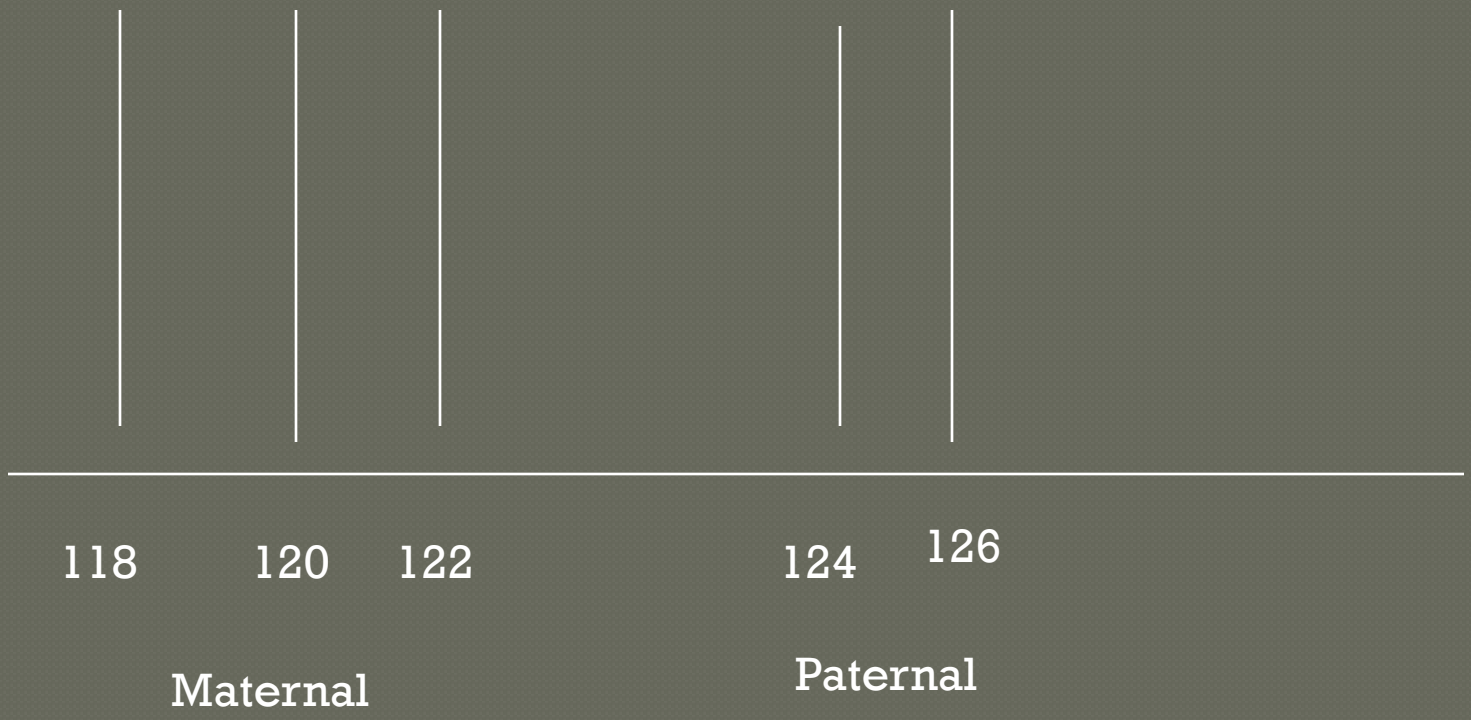
- Expansion or contraction of microsatellite repeats
  - For example, 10 CA's to 14 CA's
- Requires a mistake in replication plus deficiency in mismatch repair


10 CA
12 CA
Chromosome
5 Maternal
Chromosome
5 Paternal

PCR of Normal



PCR of MSI



Nl

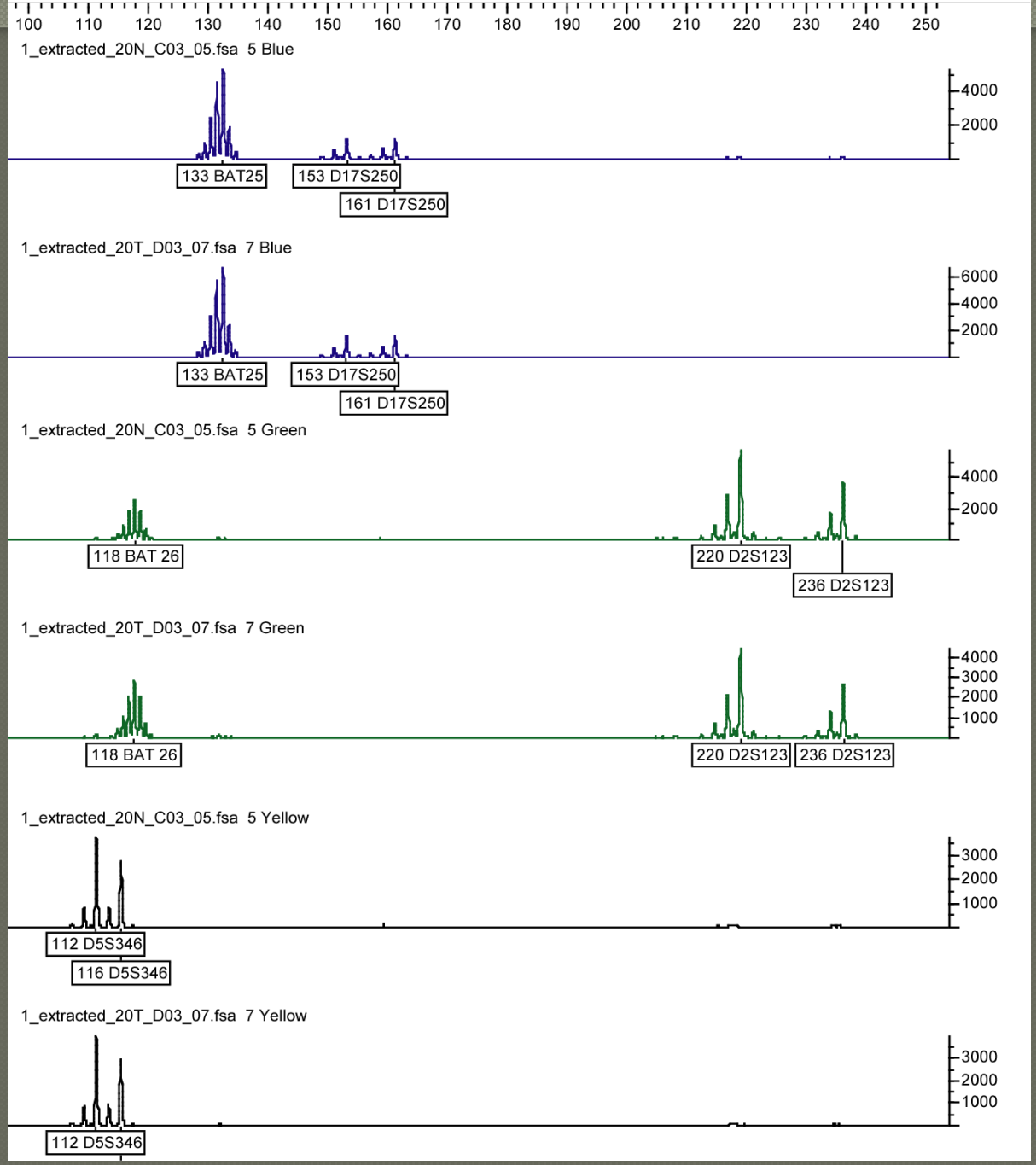
T

Nl

T

Nl

T


100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250
1_extracted_20N_C03_05.fsa 5 Blue
4000
2000
133 BAT25
153 D17S250
161 D17S250
1_extracted_20T_D03_07.fsa 7 Blue
6000
4000
2000
133 BAT25
153 D17S250
161 D17S250
1_extracted_20N_C03_05.fsa 5 Green
4000
2000
118 BAT 26
220 D2S123
236 D2S123
1_extracted_20T_D03_07.fsa 7 Green
4000
3000
2000
1000
118 BAT 26
220 D2S123
236 D2S123
1_extracted_20N_C03_05.fsa 5 Yellow
3000
2000
1000
112 D5S346
116 D5S346
1_extracted_20T_D03_07.fsa 7 Yellow
3000
2000
1000
112 D5S346

Nl

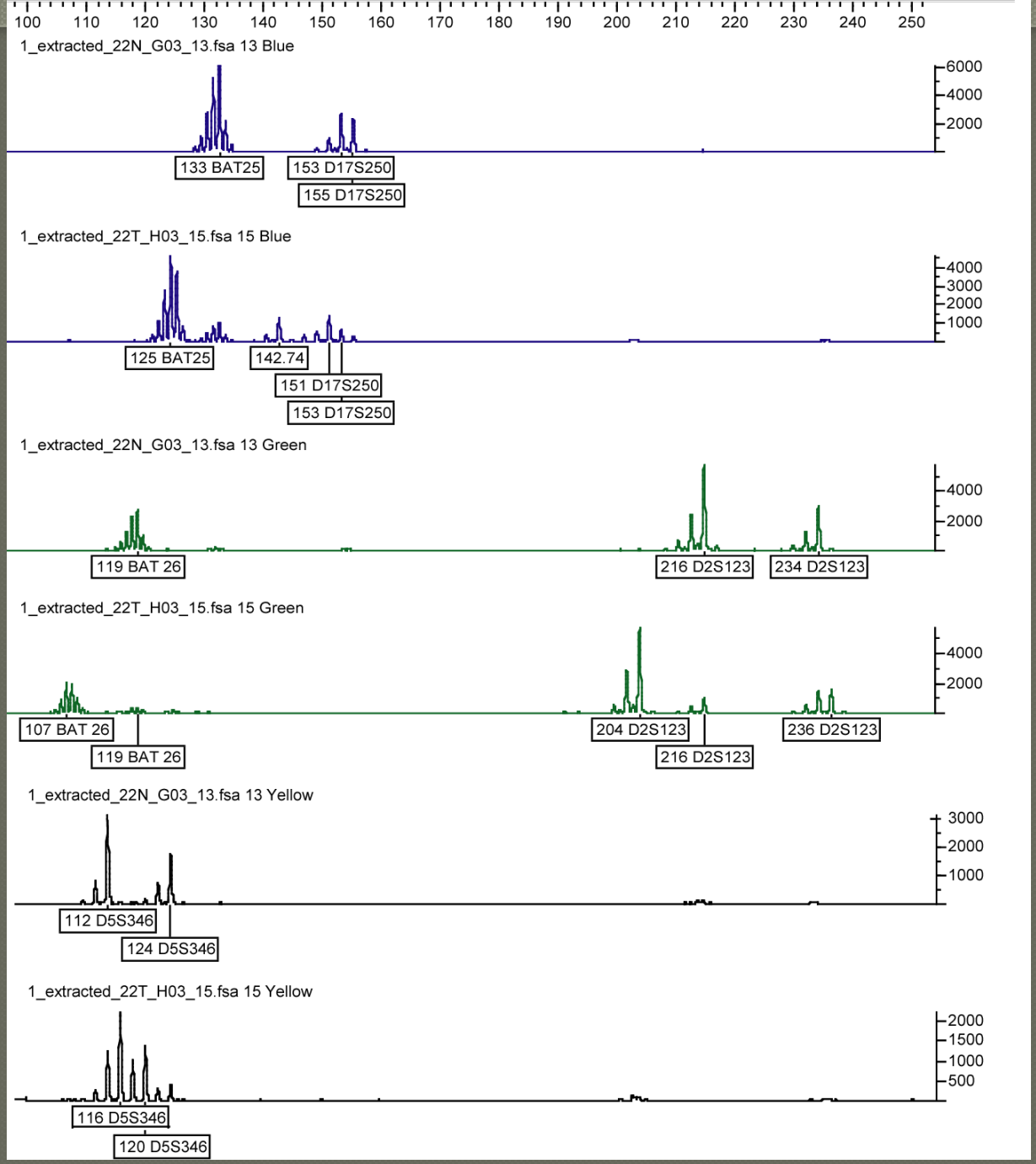
T

Nl

T

Nl

T

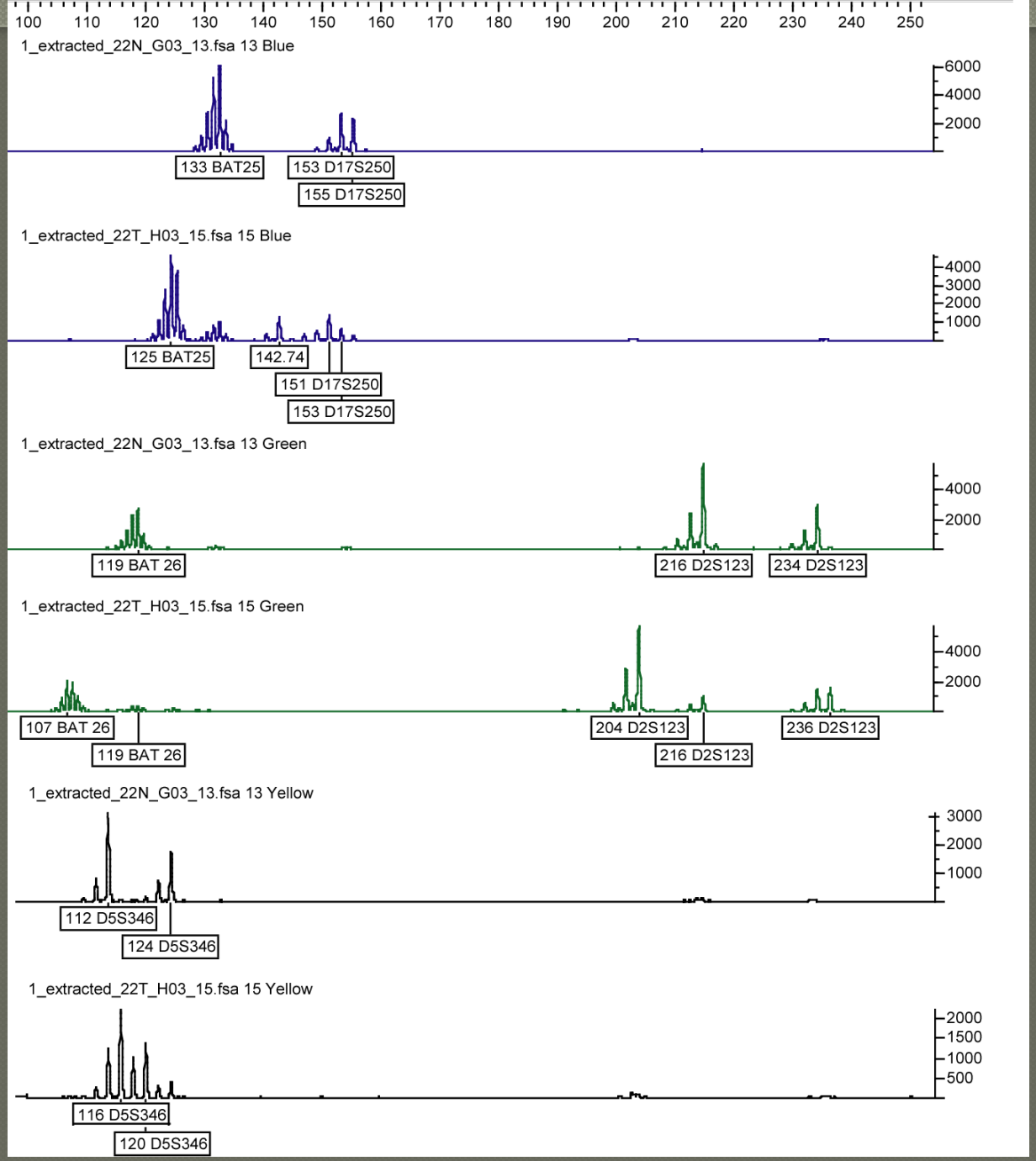

100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250
1_extracted_22N_G03_13.fsa 13 Blue
6000
4000
2000
133 BAT25
153 D17S250
155 D17S250
1_extracted_22T_H03_15.fsa 15 Blue
4000
3000
2000
1000
125 BAT25
142.74
151 D17S250
153 D17S250
1_extracted_22N_G03_13.fsa 13 Green
4000
2000
119 BAT 26
216 D2S123
234 D2S123
1_extracted_22T_H03_15.fsa 15 Green
4000
2000
107 BAT 26
119 BAT 26
204 D2S123
216 D2S123
236 D2S123
1_extracted_22N_G03_13.fsa 13 Yellow
3000
2000
1000
112 D5S346
124 D5S346
1_extracted_22T_H03_15.fsa 15 Yellow
2000
1500
1000
500
116 D5S346
120 D5S346

MSI in Colorectal Cancer: Clinical Context

- Lynch/HNPCC
  - Germline mutation in one of the mismatch repair genes (MLH1, MSH2, MSH6, or PMS2)
- 10-15% Sporadic colorectal cancer
  - Acquired hypermethylation of MLH1 promoter

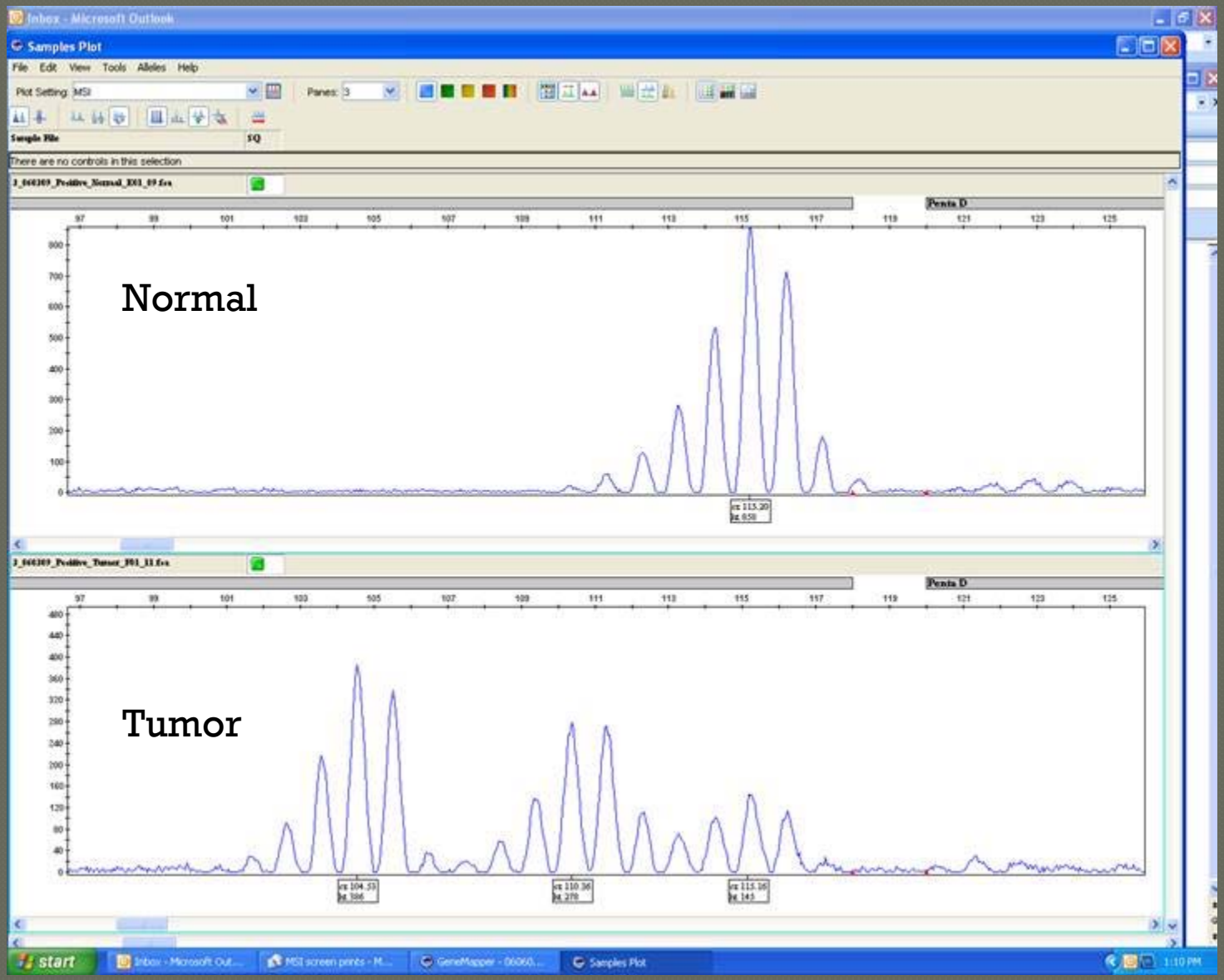
Bethesda Consensus Panel

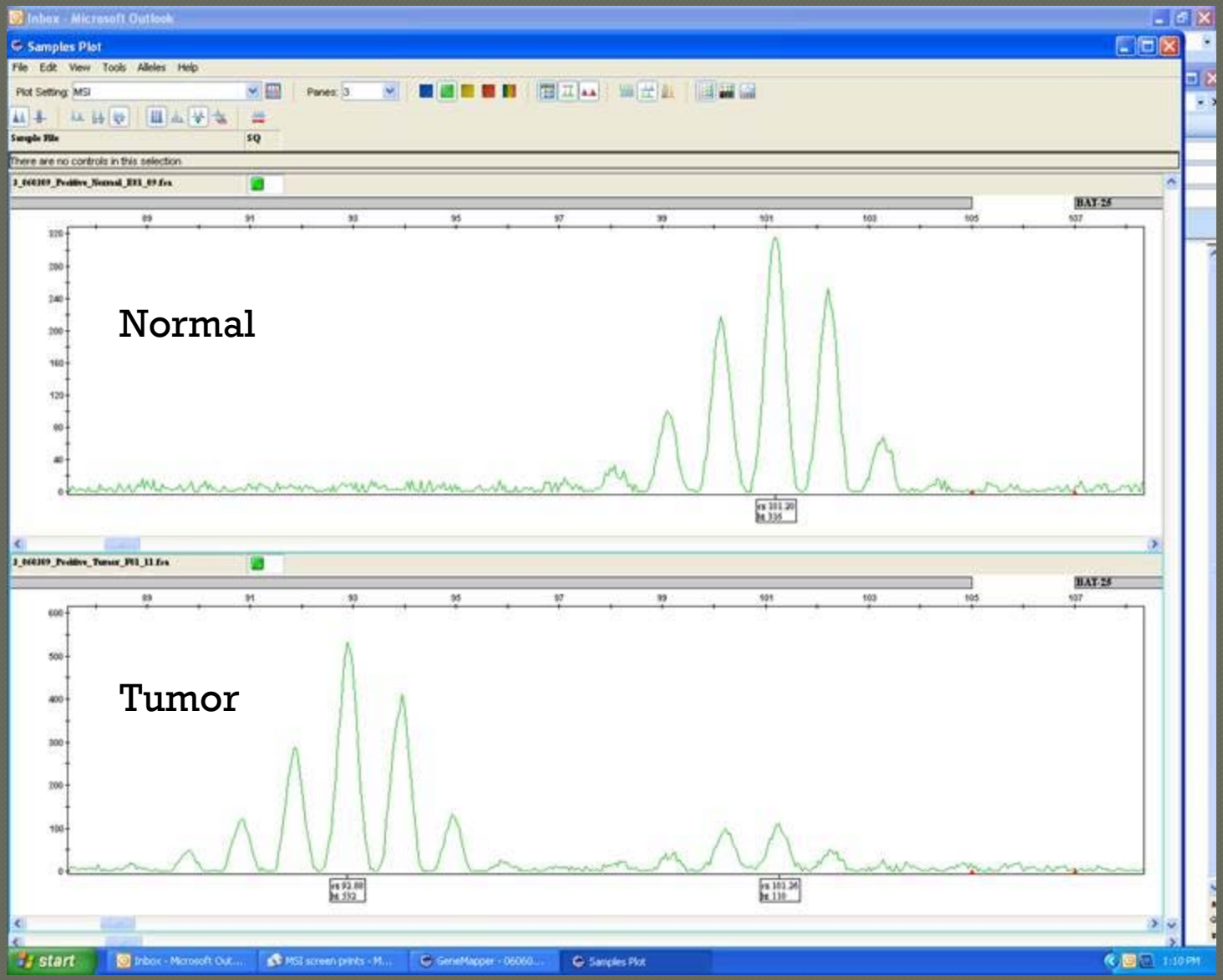
- Two mononucleotide repeats, three dinucleotide repeats
- MSI high: Instability in two or more repeats
- Microsatellite stable (MSS): No instability
- MSI low: Instability in one repeat
  - Controversial
  - Lynch-associated cancers show MSI high, not low

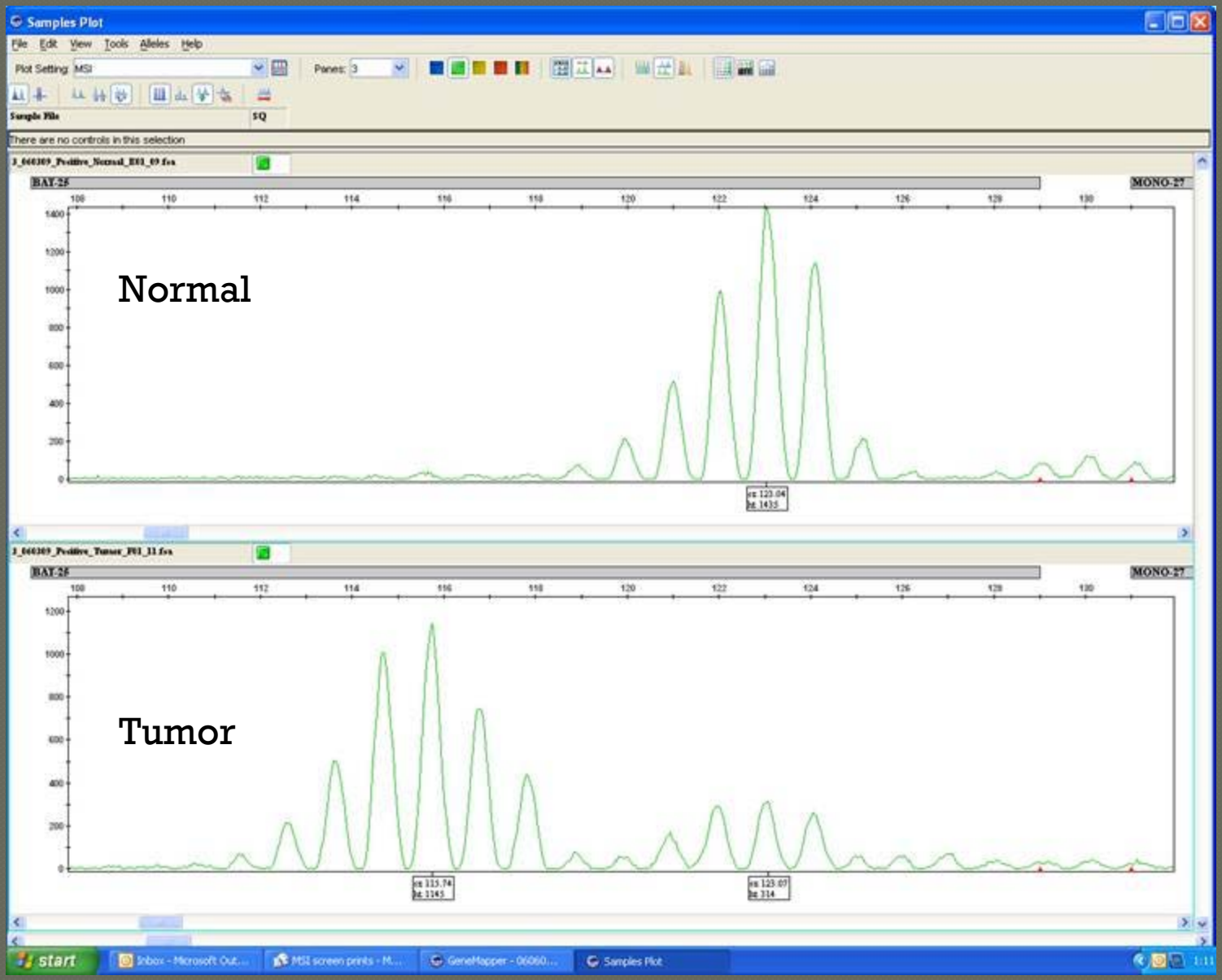

100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250
1_extracted_22N_G03_13.fsa 13 Blue
6000
4000
2000
133 BAT25
153 D17S250
155 D17S250
1_extracted_22T_H03_15.fsa 15 Blue
4000
3000
2000
1000
125 BAT25
142.74
151 D17S250
153 D17S250
1_extracted_22N_G03_13.fsa 13 Green
4000
2000
119 BAT 26
216 D2S123
234 D2S123
1_extracted_22T_H03_15.fsa 15 Green
4000
2000
107 BAT 26
119 BAT 26
204 D2S123
216 D2S123
236 D2S123
1_extracted_22N_G03_13.fsa 13 Yellow
3000
2000
1000
112 D5S346
124 D5S346
1_extracted_22T_H03_15.fsa 15 Yellow
2000
1500
1000
500
116 D5S346
120 D5S346

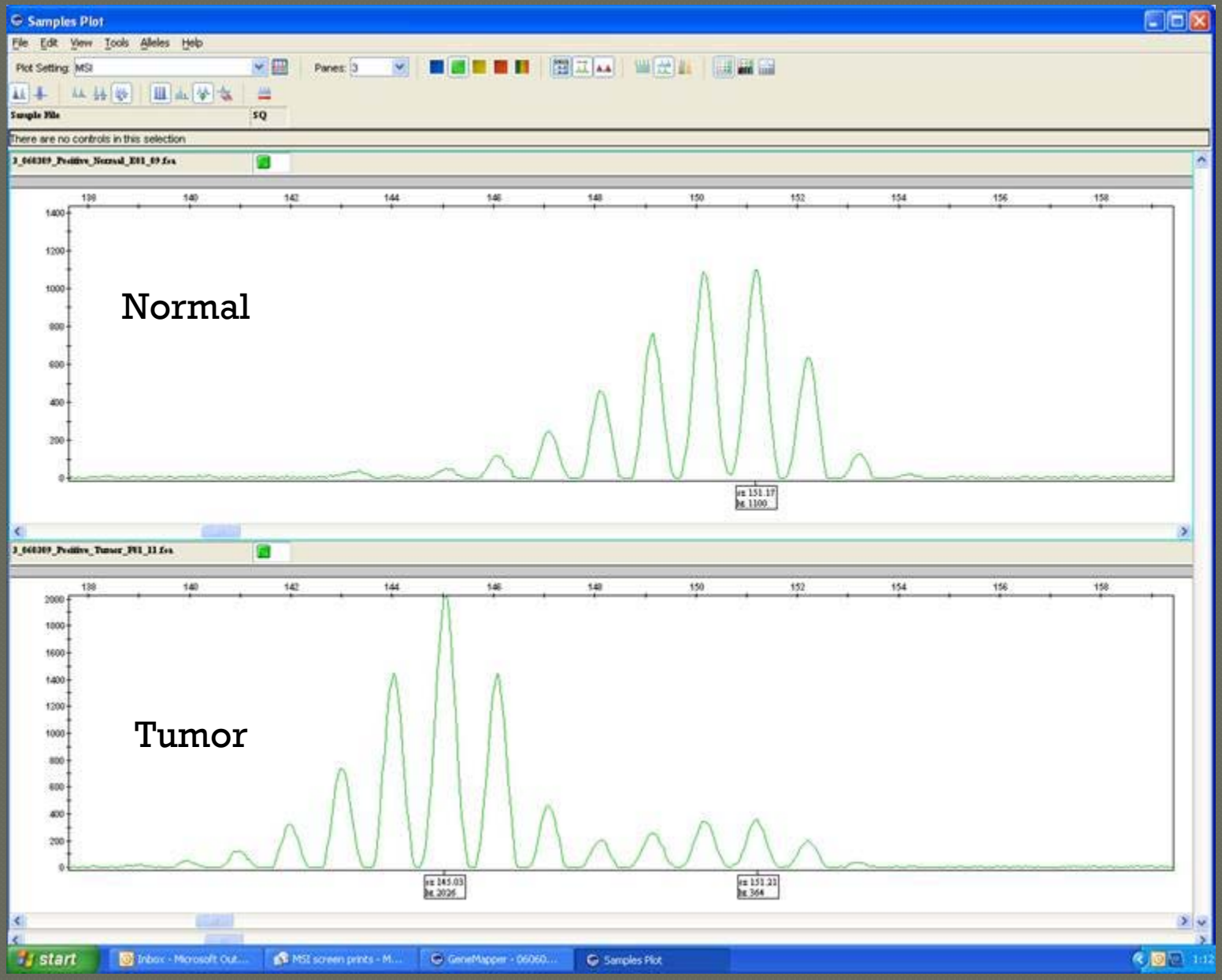
Mononucleotide repeat panel

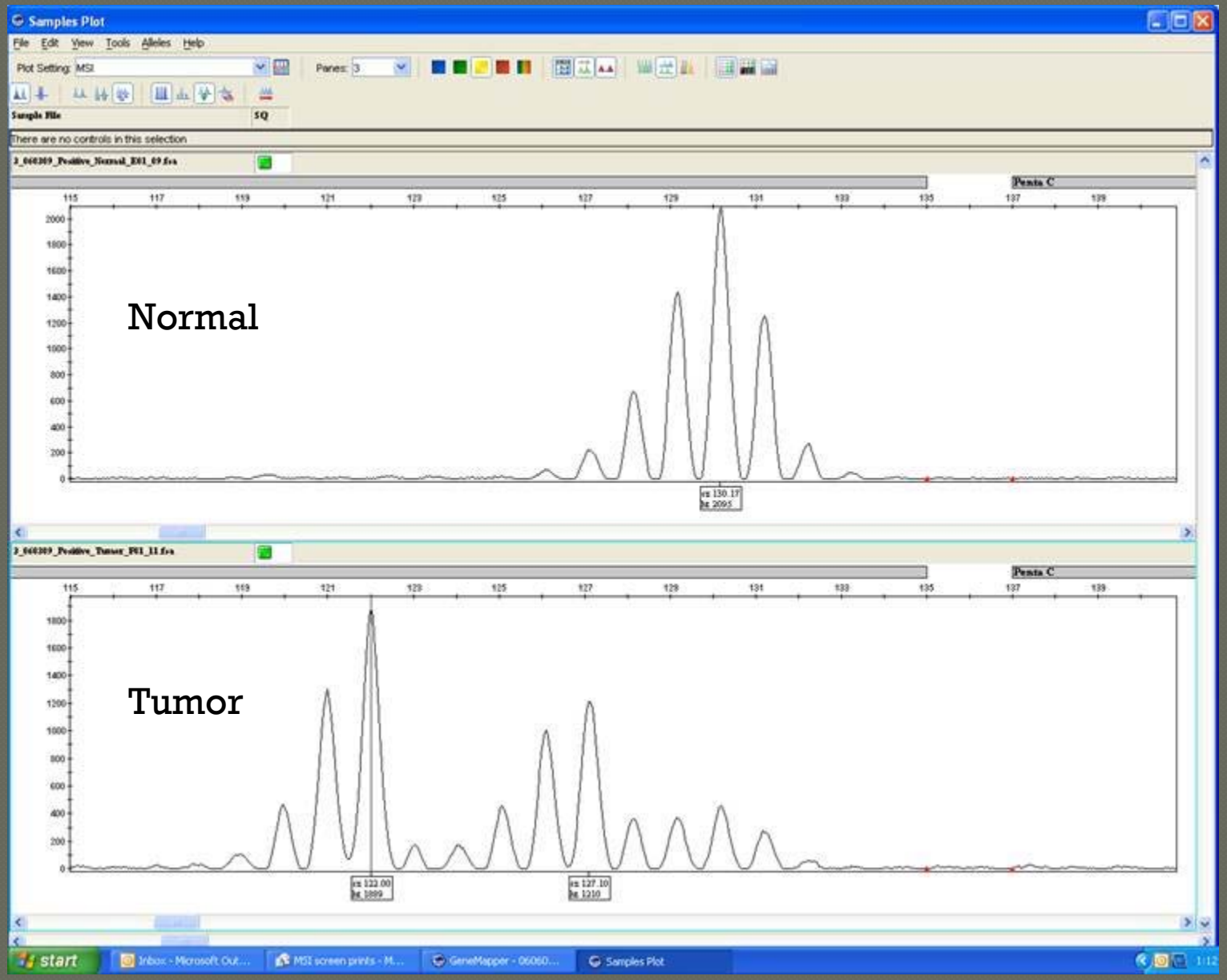
- Mononucleotide repeats are probably more sensitive and specific for MMR deficiency
- New panel(s) of 5 mononucleotide repeats
  - MSI high: two or more unstable, although typically all (or almost all) repeats are unstable
  - Since instability in even one mononucleotide repeat may indicate MMR deficiency, instability in one repeat is termed "indeterminate" rather than MSI low


Samples Plot
File Edit View Tools Alleles Help
There are no controls in this selection
Penta D
Normal
Penta D
Tumor


Inbox - Microsoft Outlook
Samples Plot
File Edit View Tools Alleles Help
Plot Setting: MSI
Panes: 3
There are no controls in this selection
BAT-25
Normal
BAT-25
Tumor
start
Inbox - Microsoft Out...
MSI screen prints - M...
GeneMapper - 06060...
Samples Plot
1:10 PM

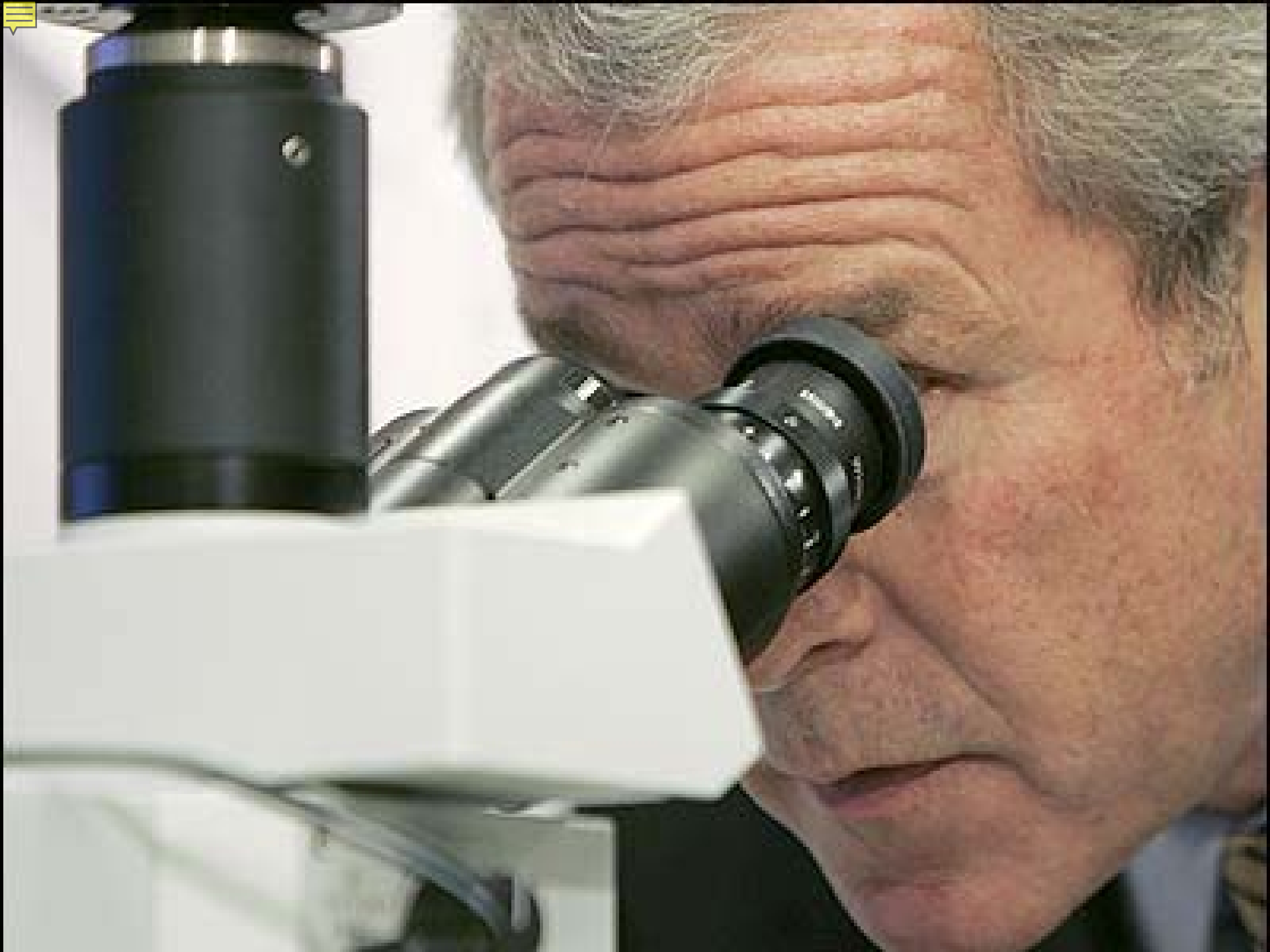

Samples Plot
File Edit View Tools Alleles Help
Plot Setting: MSI
Panes: 3
There are no controls in this selection
BAT-25
MONO-27
Normal
BAT-25
MONO-27
Tumor

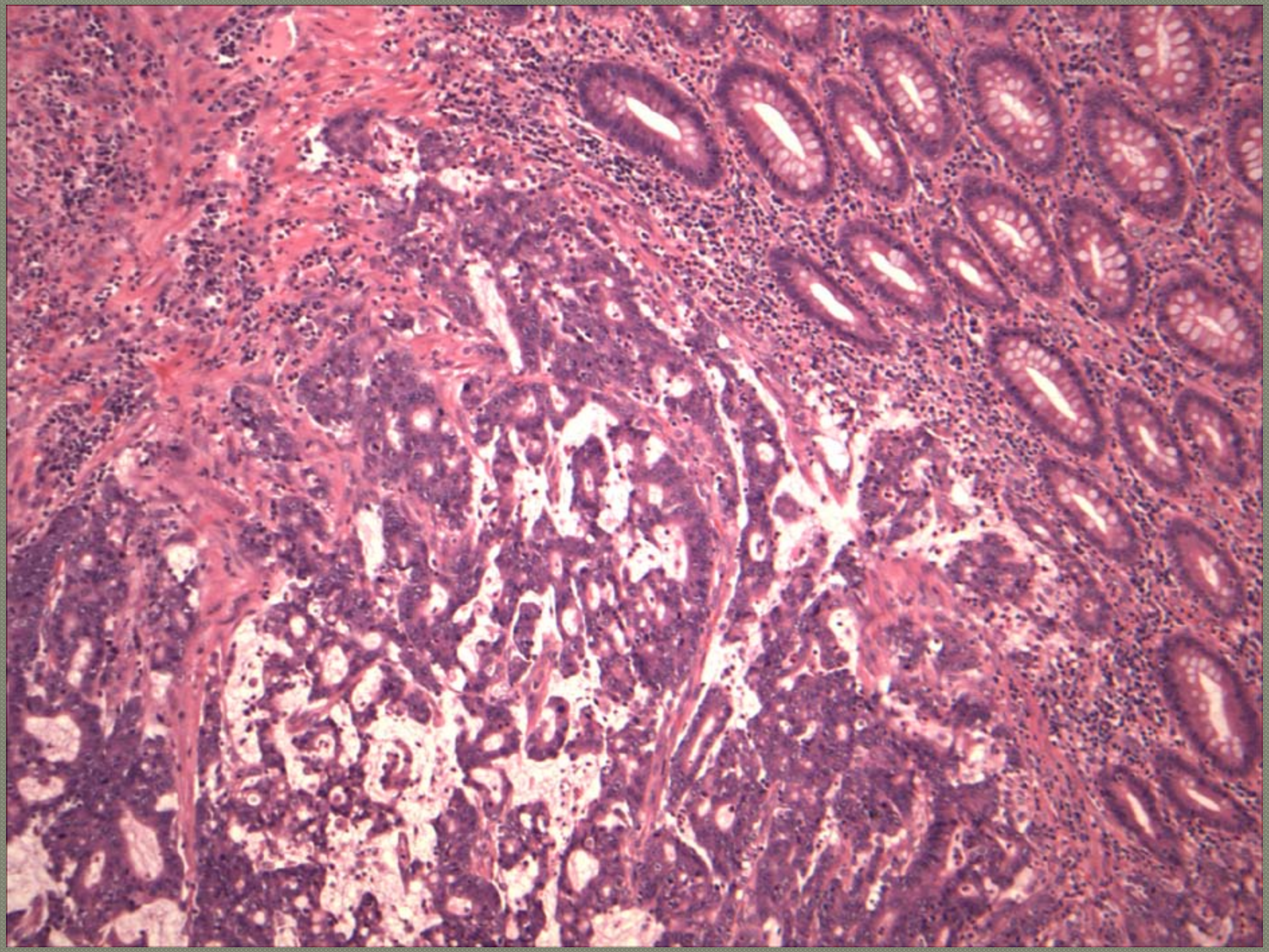

Samples Plot
File Edit View Tools Alleles Help
Plot Setting: MSI
Panes: 3
There are no controls in this selection
Normal
Tumor
start
Inbox - Microsoft Out...
MSI screen prints - M...
GeneMapper - 06060...
Samples Plot

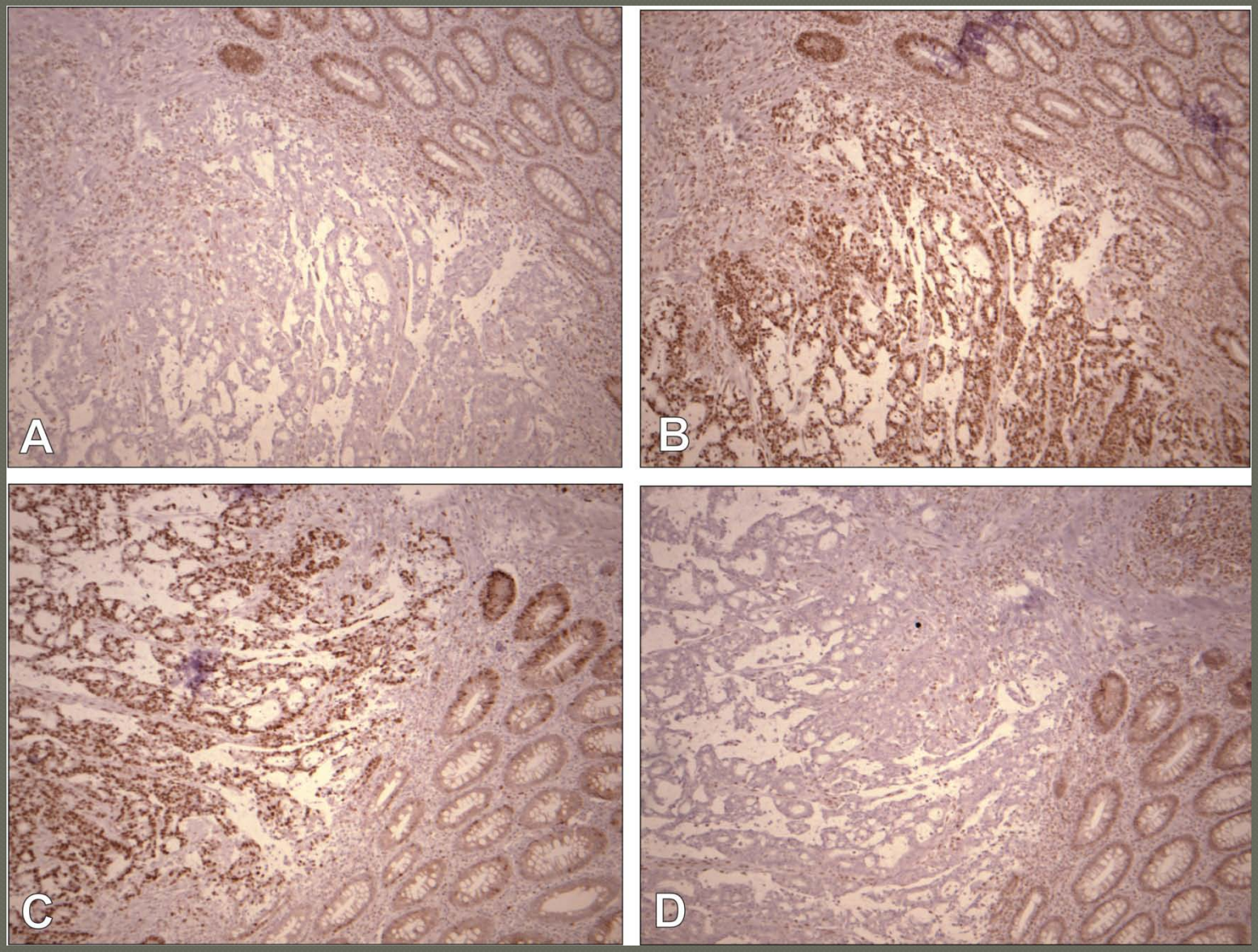

Samples Plot
File Edit View Tools Alleles Help
Plot Setting: MSI
Panes: 3
There are no controls in this selection
Penta C
Normal
Penta C
Tumor

Immunohistochemistry (IHC) Mismatch Repair Proteins

- MMR proteins are expressed in proliferating cells (like cancer)
- Lack of MMR expression of one or more proteins is a good surrogate test for MSI
- IHC can also guide subsequent germline MMR evaluation






A
B
C
D

IHC interpretation

- MLH1 complexes with PMS2
- MSH2 complexes with MSH6
- The stability of PMS2 and MSH6 depends upon these complexes
- Therefore, if MLH1 is negative, PMS2 is usually negative; if MSH2 is negative, MHS6 is negative.
- Corollary usually not true (MLH1 and MSH2 bind to other proteins as well)

IHC Result	Likely Defective Gene
Loss of MLH1, PMS2	MLH1
Loss of MSH2, MSH6	MSH2
Isolated Loss of MSH6	MSH6
Isolated Loss of PMS2	PMS2

IHC vs. PCR

- Complementary
- MSH6 negative tumors can be stable by PCR
  - Mononucleotide repeat panels may help identify these
- Missense mutations may be normal by IHC
- IHC can be hard to interpret
- IHC can guide subsequent mismatch repair gene testing

Sporadic MSI

- 10-15% of all colon cancer
- Acquired hypermethylation of *MLH1* promoter
- More common than Lynch/HNPCC
- Leads to IHC profile: MLH1/PMS2 negative
- Lynch due to MLH1 germline mutation can have the same IHC profile

Unstable, MLH1/PMS2 negative: sporadic or Lynch?

- BRAF V600E mutation in about 50% of sporadic unstable colorectal cancers, only rarely occurs in Lynch/HNPCC (so far, minority of those with PMS2 germline mutation; Senter, Gastroenterology, 2008)
- **BRAF V600E not useful for extra-colonic tumors like endometrium
- MLH1 methylation in most sporadic unstable tumors, only rarely in Lynch/HNPCC
- Rare reports of germline MLH1 methylation

Requirements for MSI (PCR and IHC), BRAF and methylation

- All work on formalin-fixed, paraffin-embedded tissue
- MSI by PCR requires both normal and tumor
- All require only one 5 micron slice (maybe more if scant tissue, e.g. biopsy)
- Cancers better than adenomas, resections better than biopsies (exception: rectal cancer obliterated by preop chemorads)

Strategies for Lynch work-up

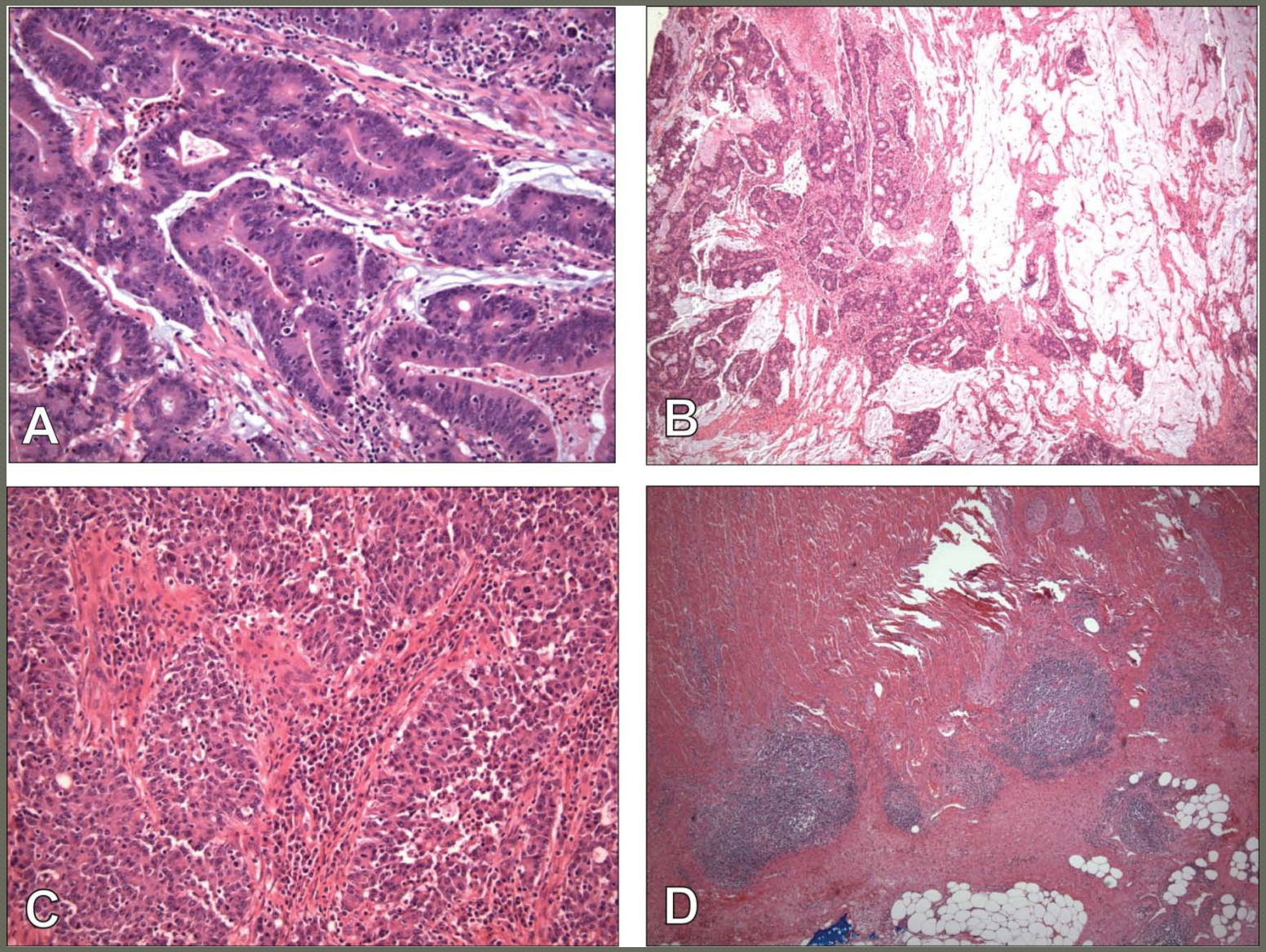
- Revised Bethesda criteria
- General screening

Table 2. The Revised Bethesda Guidelines for testing colorectal tumors for microsatellite instability (MSI)

Tumors from individuals should be tested for MSI in the following situations:

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors, regardless of age.
3. Colorectal cancer with the MSI-H histology (TIL's, Crohn's-like lymphoid reaction, mucinous/signet ring, medullary) diagnosed in a patient who is less than 60 years of age.
4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.

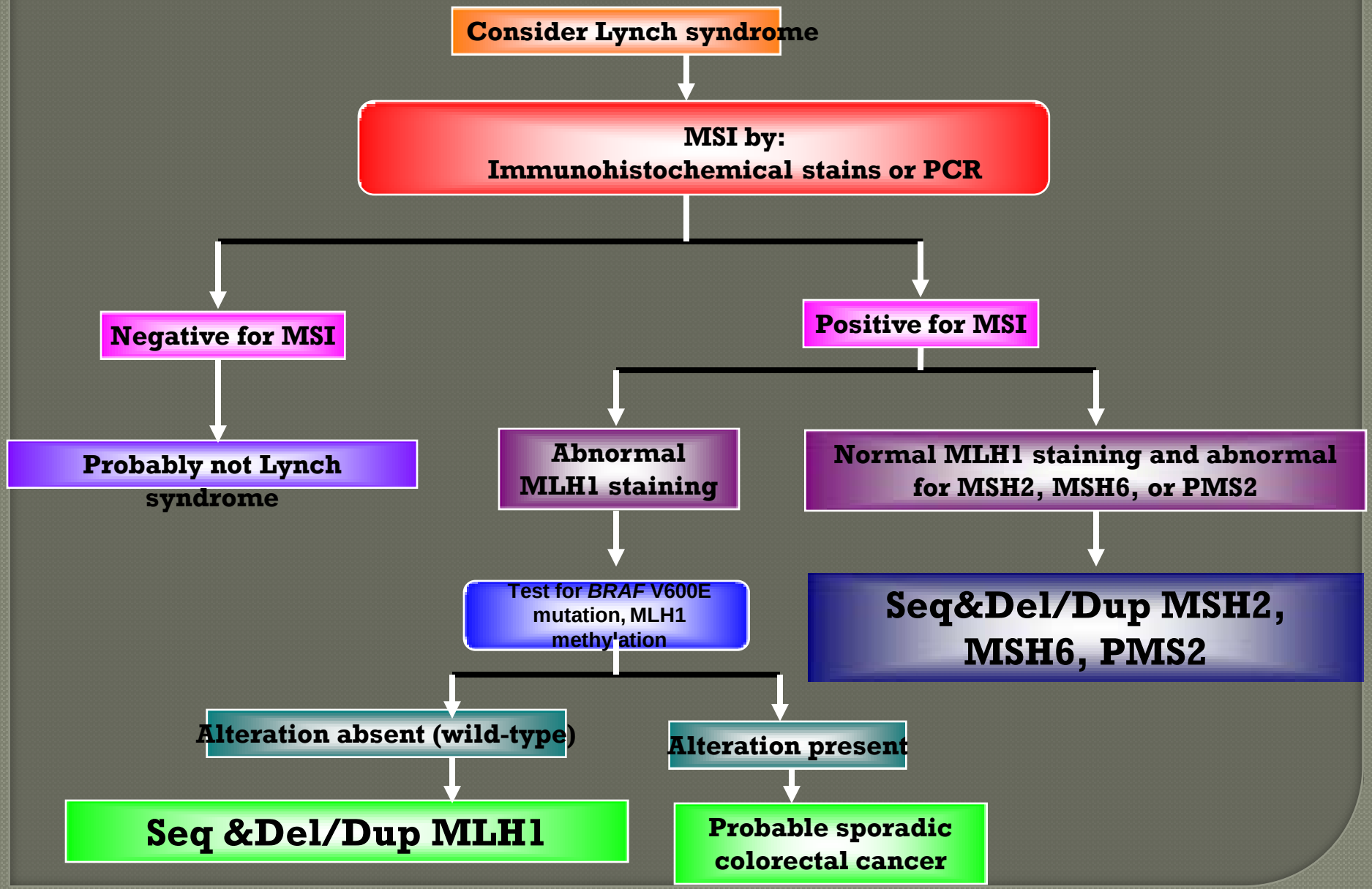
Umar, JNCI, 2004


A
B
C
D

General Screening

- Individuals can present with Lynch syndrome at older ages (Hampel, NEJM, 2005)
- Family history not always available
- Histologic features are not 100% predictive for MSI
- IHC is good, fairly cheap screening test

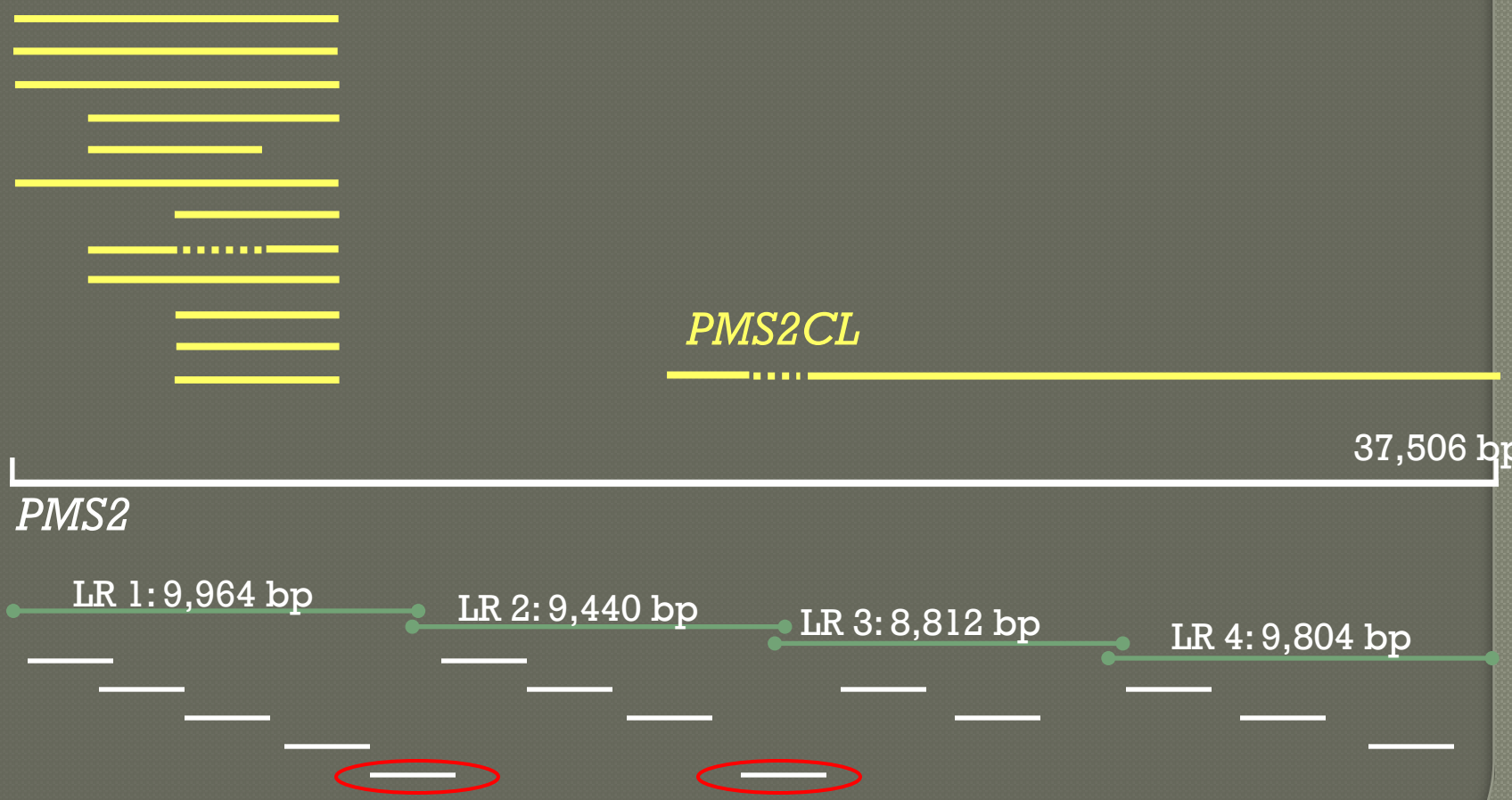
Lynch Syndrome Test Algorithm



Mutation detection

- Sequencing
- Southern blots or multiplex ligation-dependent probe amplification (MLPA) for deletions of an exon or more, especially important for MSH2 and PMS2

PMS2 Pseudogenes



Clendenning, et al. 2006
Vaughn, et al. 2010

PMS2: Detection of 3' deletions

PMS2:

PMS2CL:

New MLPA kit
Sequence both the gene and pseudogene
(Vaughn et al, 2011)

Constitutional mismatch repair deficiency syndrome

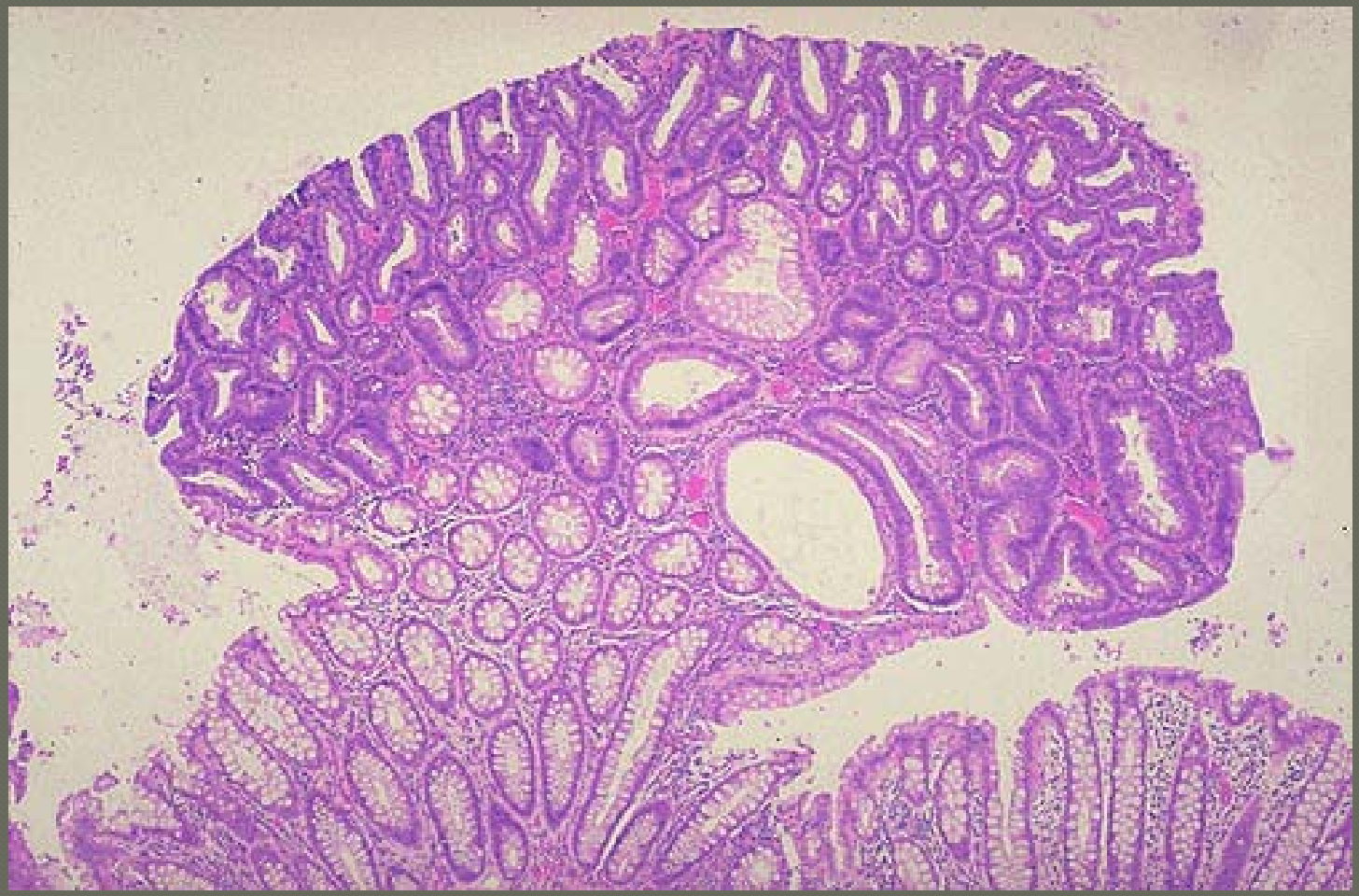
- Bi-allelic mutations in an MMR gene
- Often in setting of consanguinity
- Childhood cancers
  - Hematologic malignancies
  - Brain tumors
  - Colorectal cancers
- NF 1 manifestations (café au lait spots)

Germline methylation

- MLH1 methylation
  - Rare, usually not inherited
- MSH2 methylation
  - Deletion of 3' end of EPCAM (aka TACSTD1)
  - Transcription read through into MSH2 leads to promoter hypermethylation
  - Cancer risk mostly limited to colorectal cancer

Colon with hundreds of polyps

Adenomatous polyp



Familial Adenomatous Polyposis

- Risk of colorectal cancer is basically 100%, average age onset 39 years
- Extra-colonic intestinal manifestations: duodenal (mostly peri-ampullary) and jejunal adenomas and carcinomas, gastric antral adenomas (?cancer), fundic gland polyps (some with superficial adenomatous change)

Attenuated FAP

- Variable number of adenomas, usually less than 100, lower risk and later onset of colon cancer than classic FAP
- Mutations, although knockout type like in classic FAP, are at the extreme five prime and three prime ends of the gene, probably leading to a partially functional APC protein
- Upper GI tract manifestations are variable, may not be attenuated

Named FAP syndromes

- Gardner's: FAP plus various extra-intestinal manifestations, including desmoids, osteomas, dental abnormalities, and epidermal cysts (also APC mutations, probably more extreme end of spectrum)
- Turcot's (recently renamed Crail's): FAP plus brain tumors; if due to APC gene usually medulloblastoma
- Other extra-intestinal manifestations: congenital hypertrophy of the retinal epithelium (CHRPE), hepatoblastoma (in children) and thyroid cancer

Sporadic colorectal cancer

- Acquired APC gene mutations are thought to be the initiating event in adenoma formation
- Adenomas are the probable precursor of most cases of sporadic colorectal cancer
- APC is a tumor suppressor gene: "two hits" are required to inactivate the gene
- In FAP the first mutation is inherited; in sporadic colorectal cancer, both mutations are acquired.

MYH-associated polyposis (MAP)

- Colonic phenotype usually more like attenuated FAP
- Extra-colonic manifestations: duodenal adenomas/carcinomas, cancer of ovary, bladder, skin, sebaceous gland reported, no desmoids or other APC related conditions
- Most mutations are homozygous or compound heterozygotes of Y179C and G396D (formerly known as Y165C and G382D)
- Sometimes referred to as FAP (or AFAP) caused by MYH mutations

MAP mechanism

- Oxidative damage to DNA causes oxidation of guanine leading to 8-oxo-7,8-dihydroxyguanine.
- This mispairs with adenine, leading to G:C to T:A transversion
- MYH performs base excision repair during replication, removing the adenine

Hamartomatous polyposis

- All probably have increased risk of colorectal cancer
- Cowden's syndrome: PTEN gene mutations in 80% (also thyroid, breast, endometrium)
- Peutz-Jegher: STK11/LKB1 mutations in 50-90% (also breast, pancreas, ovarian, lung)
- Juvenile polyposis: BMPR1A mutations in 20%, SMAD4 mutations in 20%
- See Genetests for descriptions

Future

- Next generation sequencing
  - Biggest impact on Lynch syndrome
  - May decrease the need for many tissue tests as costs come down for sequencing
  - PMS2 still likely to be problematic
  - Will facilitate genetic definitions of syndromes, especially hamartomatous polyposes