

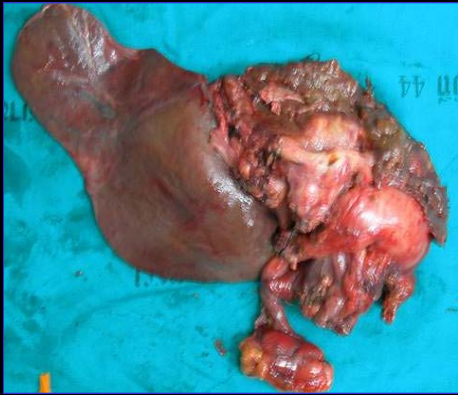
# Genetic Approaches to Cancers

Kawin Leelawat MD, PhD

Department of Surgery

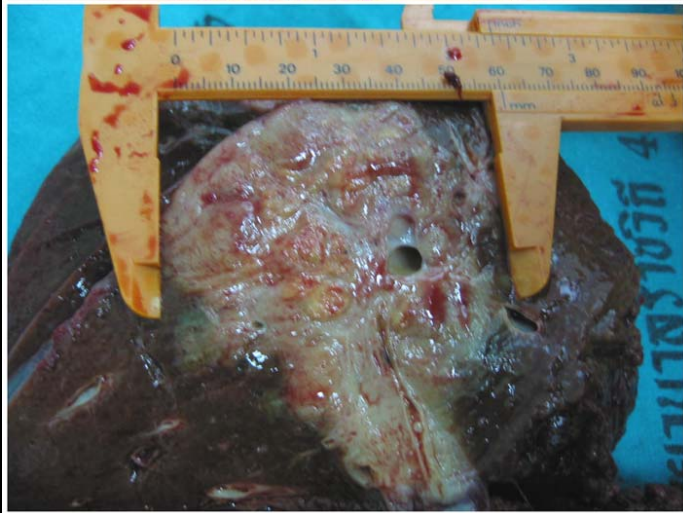
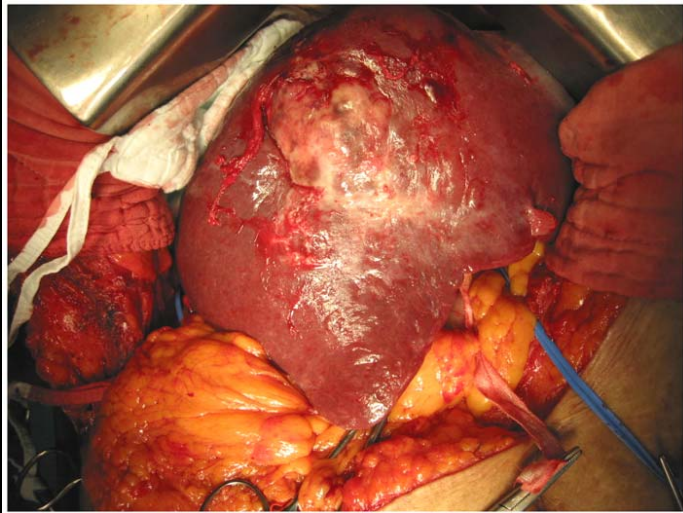
Rajavithi Hospital

# Liver Cancer



- Surgery is the treatment of choice
- < 30% surgical candidates
- High rate of recurrent
- Chemo-Resistant Cancer

# Cancer Research



Incidence

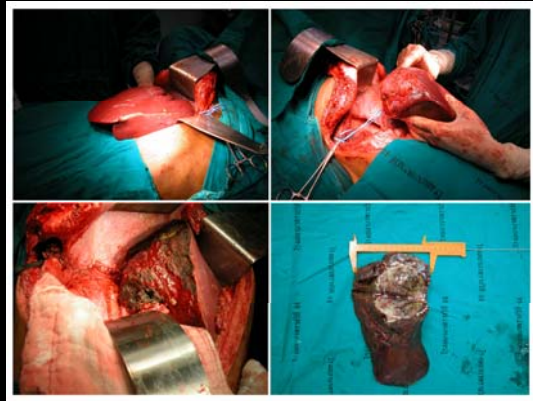
Pathogenesis

Early Diagnosis

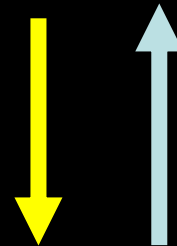
Treatment

Prognosis

# Research Projects

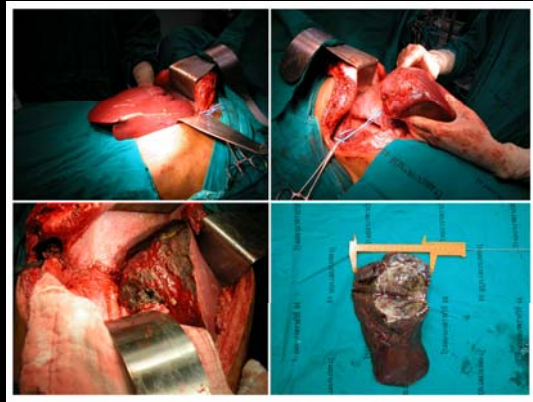


From Bed

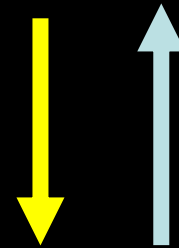


To Bench

# Research Projects



From Bed



To Bench



1. Understand Cancer
2. Identify high risk
3. Early Diagnosis
4. Novel treatment
5. Appropriate treatment
6. Prognosis





**Pathogenesis**

**Tumor Markers**

**Glypican3**

**hTERT**

**Free circulating DNA**



**Pathogenesis**

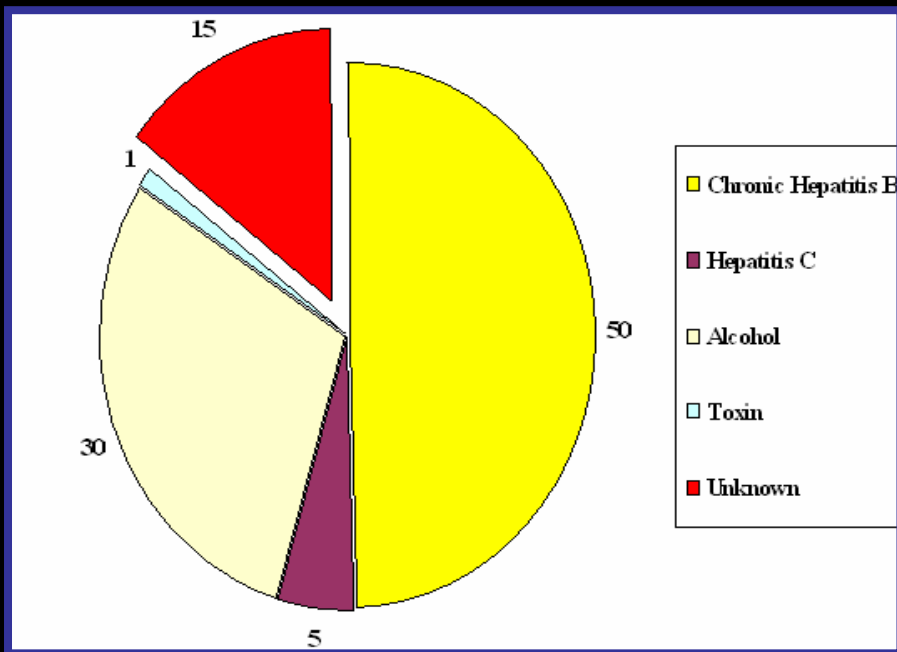
**Tumor Markers**

**Glypican3**

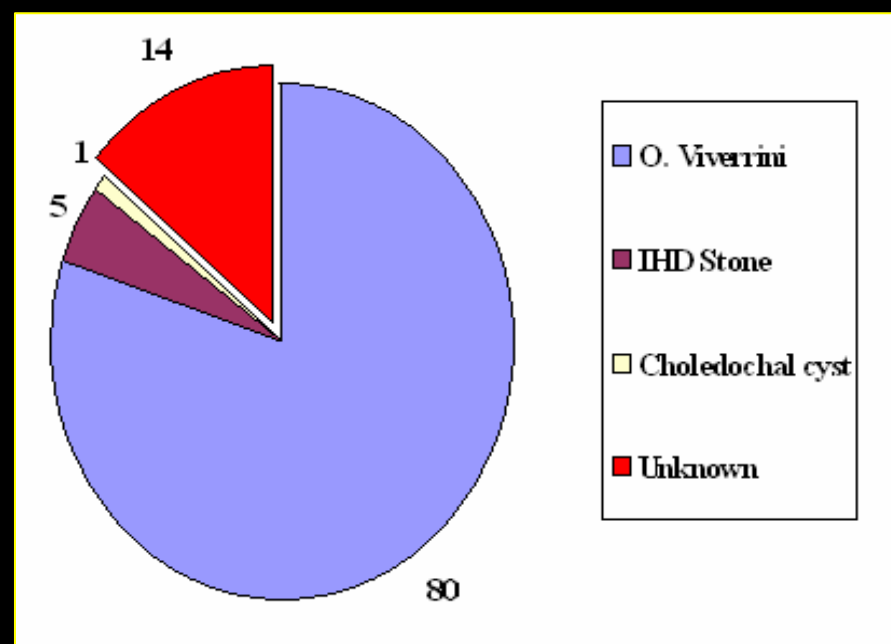
**hTERT**

**Free circulating DNA**

# Risk Factors



HCC



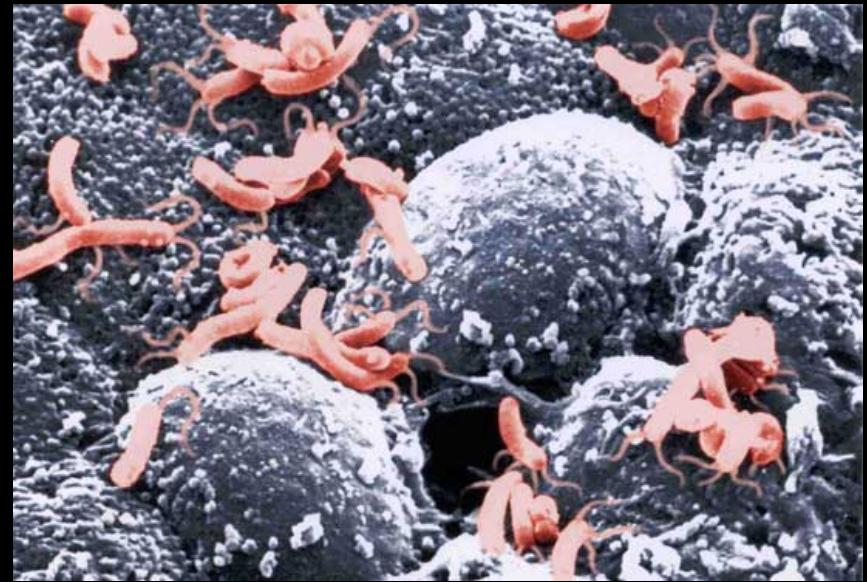
CCA

# Risk Factors for liver cancer

- Chronic Hepatitis B
- Cirrhosis
  - Hepatitis C viral infection
  - Alcohol
  - Drug
- Toxin
- Parasitic infection
  - *Opisthochis viverrini*

# *Helicobacter pylori*

- Chronic gastritis and peptic ulcer disease
- Type I carcinogen for
  - gastric carcinoma
  - gastric mucosa associated lymphoid tissue lymphoma



<http://www.rmutphysics.com/charud/oldnews/64/helicobacterpylori4.jpg>

# Genotypes of Thai *H. pylori* strains and clinical presentation

		Gastritis	Pepticulcer disease	Gastric cancer
Total		n = 36	n = 34	n = 28
	East Asian	8 (22%)	16 (47%)*	20 (71%)**
	South/Central	16 (44%)	7 (21%)	2 (7%)*
	Western	0 (0%)	1 (3%)	0 (0%)
	Mixed (recombinant)	12 (33%)	10 (29%)	6 (21%)
Chinese (n = 20)		n = 5	n = 5	n = 10
	East Asian type	2 (40%)	5 (100%)	10 (100%)*
	South/Asian type	3 (60%)	0 (0%)	0 (0%)
	Mixed type	0 (0%)	0 (0%)	0 (0%)
Thai-Chinese (n = 40)		n = 13	n = 14	n = 13
	East Asian type	6 (46%)	9 (64%)	7 (54%)
	South/Asian type	2 (15%)	2 (14%)	2 (15%)
	Mixed type	5 (38%)	3 (21%)	4 (31%)
Thai (n = 38)		n = 18	n = 15	n = 5
	East Asian type	0 (0%)	2 (13%)	3 (60%)**
	South/Asian type	11 (61%)	5 (33%)	0 (0%)
	Mixed type	7 (39%)	7 (47%)	2 (40%)
	Western type	0 (0%)	1 (7%)	0 (0%)

\*p < .05, \*\*p < .01 and \*\*\*p < .001 compared with gastritis.

# *Helicobacter spp.*

- Bile and gallbladder tissue from patients with chronic cholecystitis
- Liver tissue from patient with HCC
- It is possible that *Helicobacter pylori* may also a risk factor for liver cancers



The real risk factors for these liver cancers may be far more than the known causes



# Detection of *H. pylori* -specific VacA



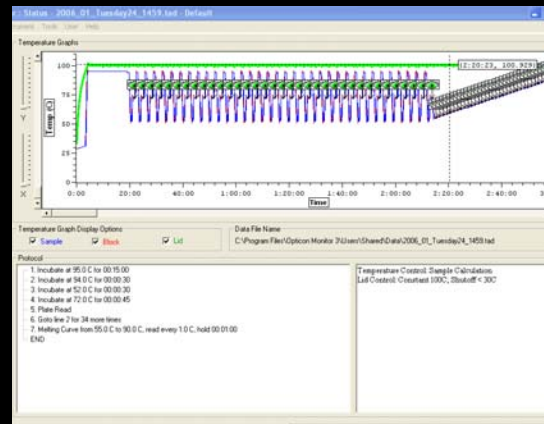
*H. pylori* -specific VacA:

forward primer

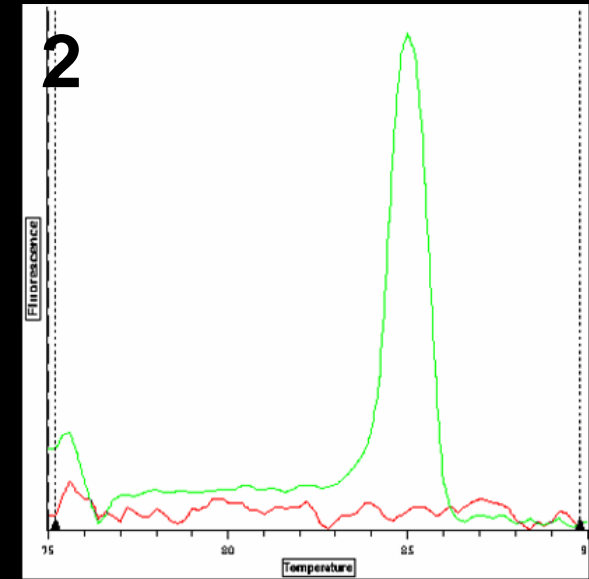
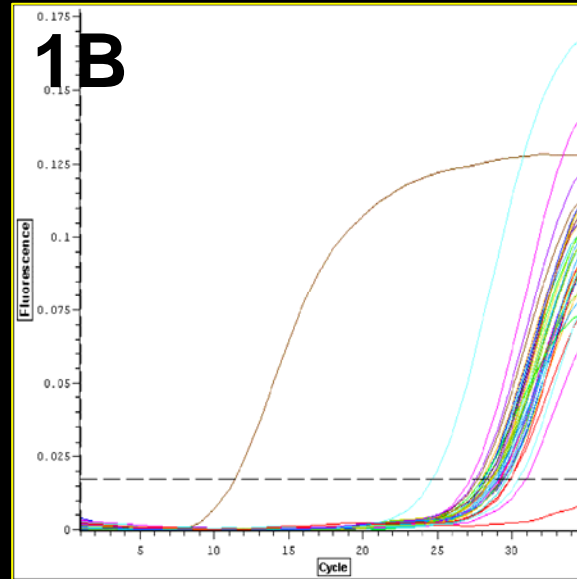
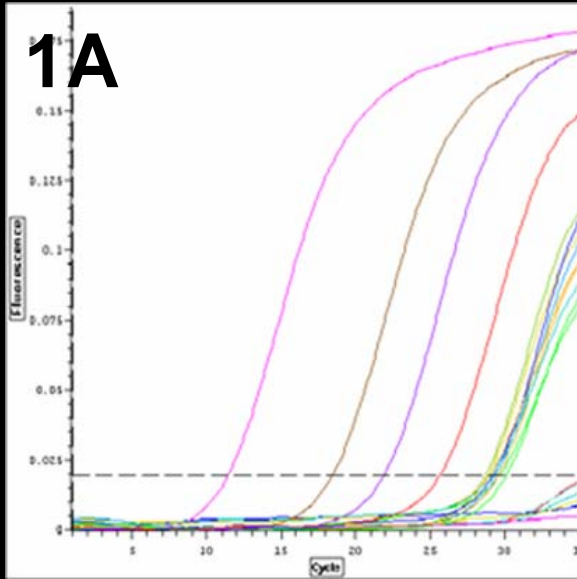
5' ATGGAAATACAACAAACACAC 3'

reverse primer

5' CTGCTTGAATGCGCCAAAC 3'



# Detection of *H. pylori* -specific VacA



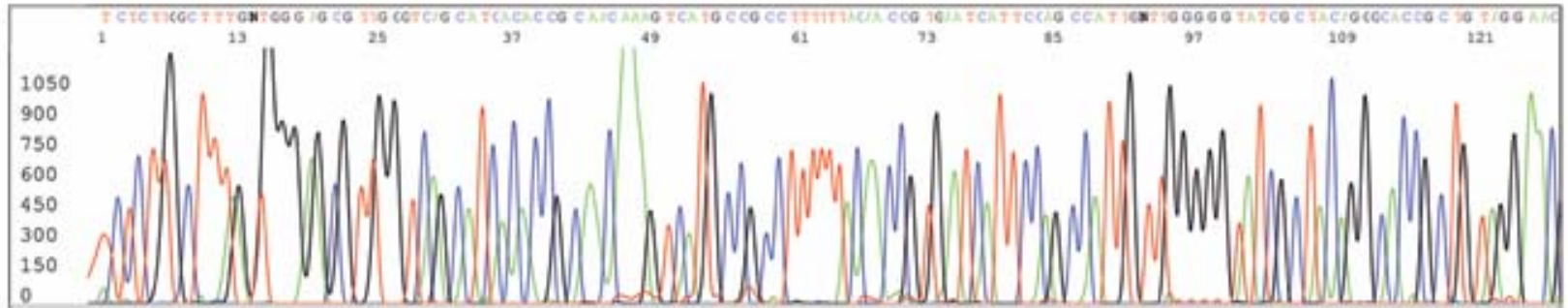
[1] Amplification curves of *H. pylori* Vac A from

(A) Serial dilution of positive controls (*H. pylori* DNA)

(B) Samples from liver specimens

[2] The melting curve analysis

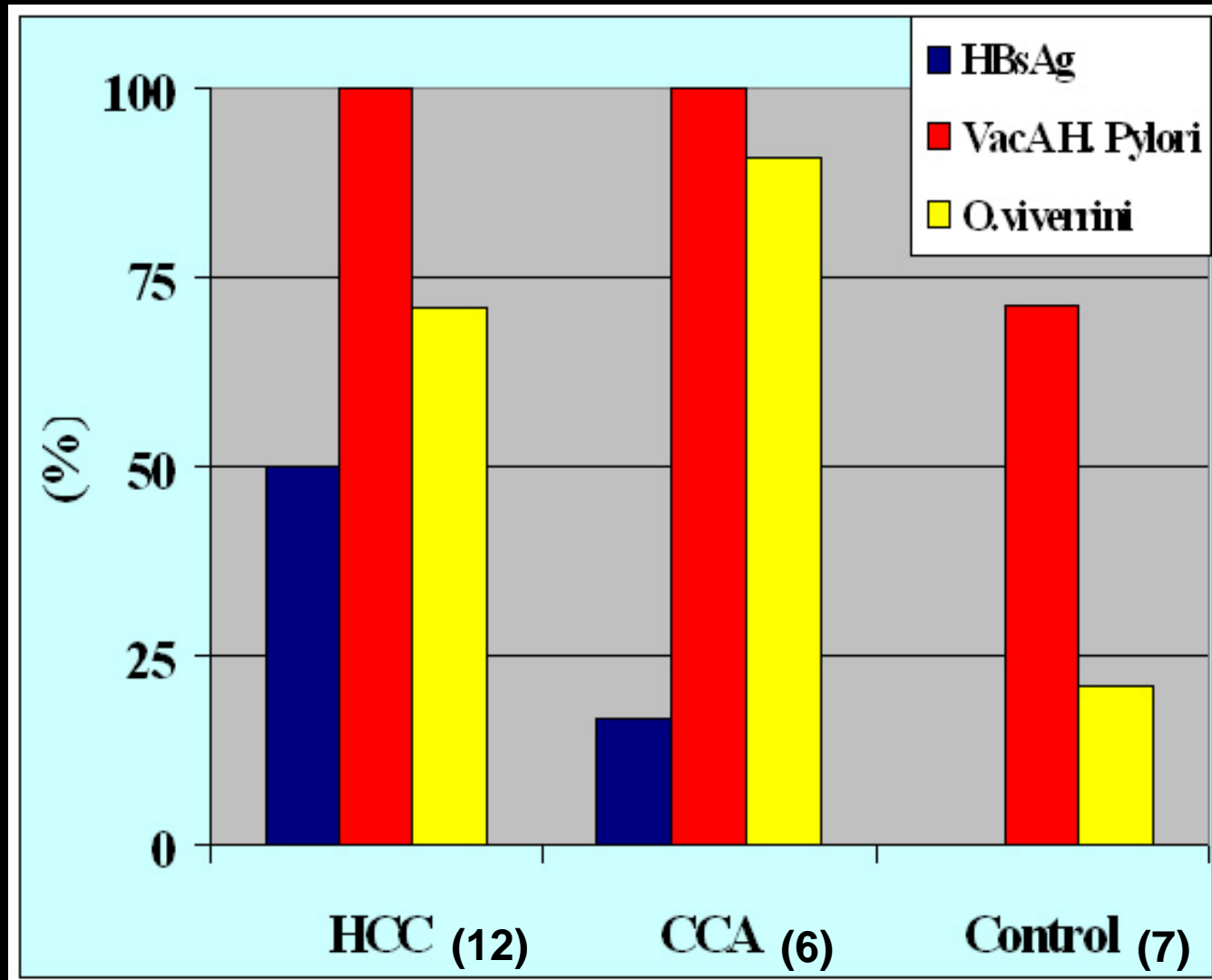
# Detection of *H. pylori* -specific VacA



sample	1	TCTCTTGCTTTAGTGGGAGCGTTGGTCAGCATCACACCGCAACAAAGTCATGCCGCCTTT	60
vacA	11	TCTCTTGCTTTAGTAGGAGCATTGGTCAGCATCACACCGCAACAAAGTCATGCCGCCTTT	70
sample	61	TTTACAACCGTGATCATTCCAGCCATTGTTGGGGGTATCGCTACAGGCACCGCTGTAGGA	120
vacA	71	TTTACAACCGTGATCATTCCAGCCATTGTTGGGGGCATCGCTACAGGCACCGCTGTAGGA	130
sample	121	ACGGTCTCAGGGCTTCTTGGTTGGGGGCTCAAACAAGCCGAAGAAGCTAATAAAACCCCG	180
vacA	131	ACGGTCTCAGGGCTTCTTGGCTGGGGGCTCAAACAAGCCGAAGAAGCCAATAAAACCCCG	190
sample	181	GATAAACCCGATAA	194
vacA	191	GATAAACCCGATAA	204

PCR product of 204 bp was sequenced and its homology was compared with the VacA gene specific for *H. pylori* sequence databases present at [NCBI NC\\_000915](https://www.ncbi.nlm.nih.gov/nuccore/NC_000915)

# Detection of *H. pylori*-specific VacA



*H. pylori* Causes  
Liver Cancer ???





**Pathogenesis**

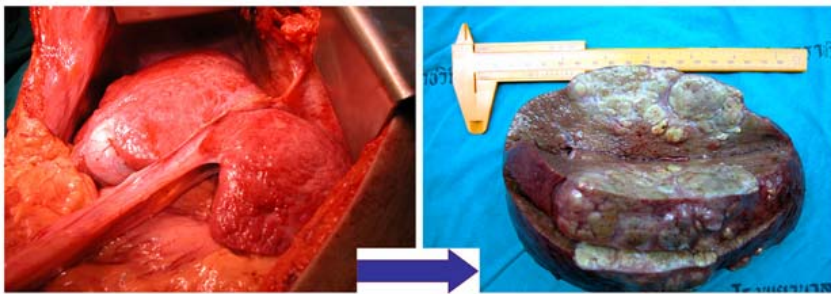
**Tumor Markers**

**Glypican3**

**hTERT**

**Free circulating DNA**

# Tumor Marker Projects



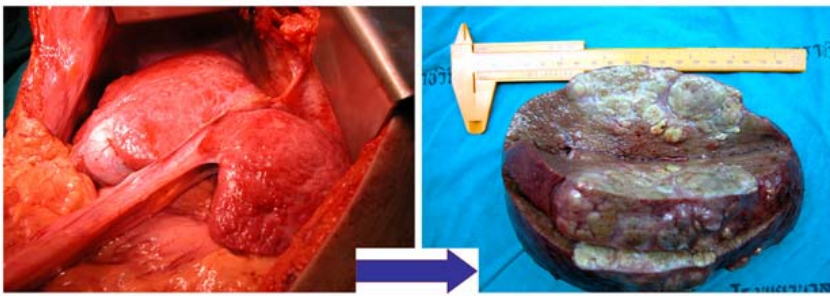
Codelink Human Whole Genome



Isolate RNA

60530	fructose-1,6-bisphosphatase 1 (FBP1)	DOWN	37.70
56348	SEC31-like 2 (S cerevisiae) (SEC31L2), transcript variant 2	DOWN	43.60
764531	metallothionein 1G (MT1G)	DOWN	47.07
80066	glycine N-methyltransferase (GNMT)	DOWN	47.42
85896	602551626F1 NIH_MGC_61 cDNA clone IMAGE:4664023 5'	DOWN	61.45
79099	glutathione S-transferase A1 (GSTA1)	DOWN	63.07
32353	BC039371.1	clone IMAGE:5271214	DOWN 112.14
35497	BC039538.1	clone IMAGE:5745053, mRNA	UP 40.58
43438	BG333416.1	602431267F1 NIH_MGC_18 cDNA clone IMAGE:4548881 5'	UP 34.92
49274	AF108138.1	DNA helicase homolog (PIF1) mRNA	UP 29.95
13231	NM_018136.2	asp (abnormal spindle)-like, microcephaly associated (Drosophila) (AS)	UP 25.61
47944	NM_032261.3	chromosome 21 open reading frame 56 (C21orf56)	UP 19.27
15133	NM_018098.4	epithelial cell transforming sequence 2 oncogene (ECT2)	UP 18.26
18034	NM_004484.2	glypican 3 (GPC3)	UP 16.76
11936	BC033086.1	transcription factor 19 (SC1), mRNA (cDNA clone MGC:45652 IMAGE:	UP 16.10
45577	BQ187081.1	UI-E-EJ1-ajy-n-20-0-Ulr1 UI-E-EJ1 cDNA clone UI-E-EJ1-ajy-n-20-0-UI	UP 15.64
49262	NM_174936.2	proprotein convertase subtilisin/kexin type 9 (PCSK9)	UP 15.52
40178	NM_007057.2	ZW10 interactor (ZWINT), transcript variant 1	UP 15.20
16510	NM_003258.1	thymidine kinase 1, soluble (TK1)	UP 14.76
11358	NM_006547.2	IGF-II mRNA-binding protein 3 (IMP-3)	UP 14.73
19899	NM_001168.1	baculoviral IAP repeat-containing 5 (survivin) (BIRC5)	UP 14.16
37987	NM_018687.3	hepatocellular carcinoma-associated gene TD26 (LOC55908)	UP 14.03
38069	NM_031934.3	RAB34, member RAS oncogene family (RAB34)	UP 13.42
48032	R31367.1	yh75c03r1 Soares placenta Nb2HP cDNA clone IMAGE:135556 5'	UP 13.25
49887	NM_031934.3	RAB34, member RAS oncogene family (RAB34)	UP 11.79
12787	NM_017899.1	hypothetical protein FLJ20607 (TSC)	UP 11.77

# Tumor Marker Projects



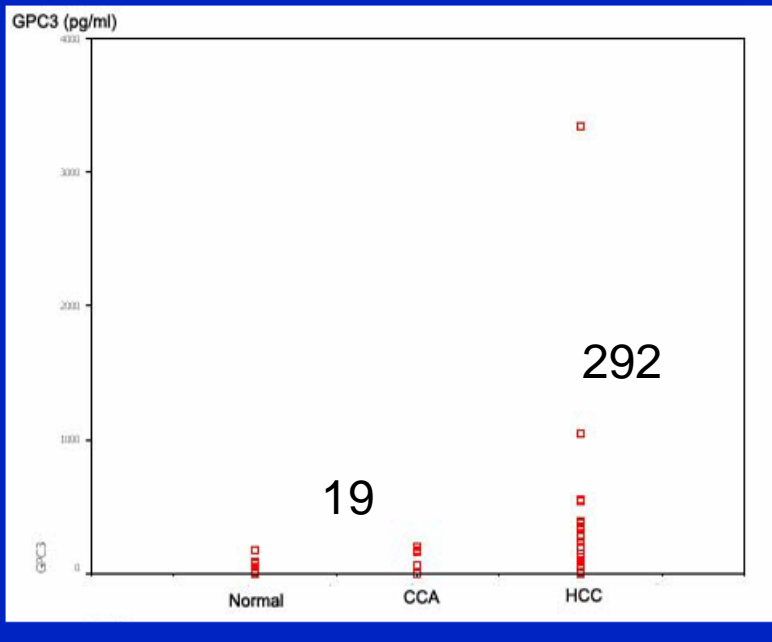
60530	fructose-1,6-bisphosphatase 1 (FBP1)	DOWN	37.70
56348	SEC31-like 2 (S cerevisiae) (SEC31L2), transcript variant 2	DOWN	43.60
764531	metallothionein 1G (MT1G)	DOWN	47.07
80066	glycine N-methyltransferase (GNMT)	DOWN	47.42

Code

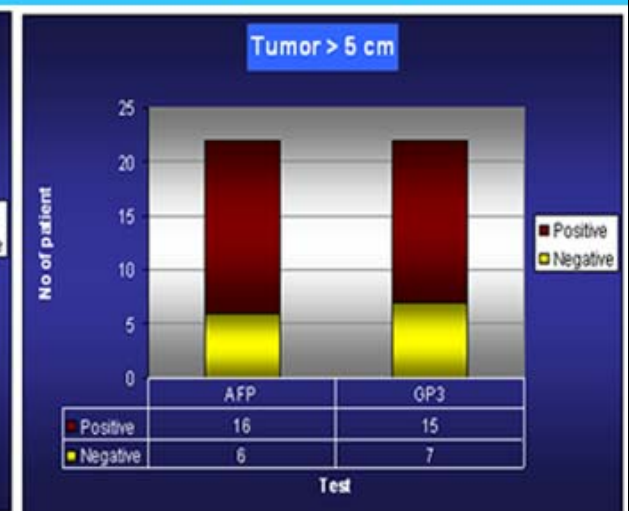
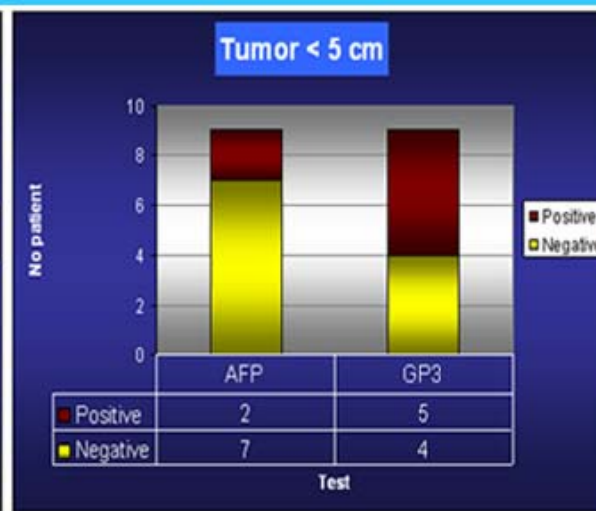
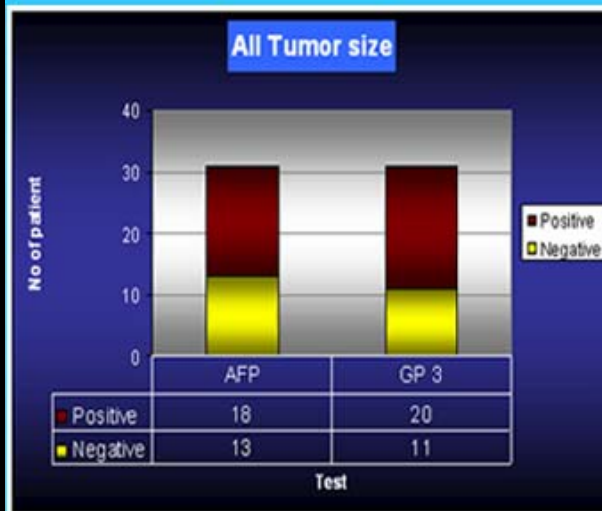
# Fast Track Marker Validation

49262	NM_174936.2	GE87076	proprotein convertase subtilisin/kexin type 9 (PCSK9)	UP	15.52
40178	NM_007057.2	GE81721	ZW10 interactor (ZWINT), transcript variant 1	UP	15.20
16510	NM_003258.1	GE57538	thymidine kinase 1, soluble (TK1)	UP	14.76
11358	NM_006547.2	GE54824	IGF-II mRNA-binding protein 3 (IMP-3)	UP	14.73
19899	NM_001168.1	GE59394	baculoviral IAP repeat-containing 5 (surivin) (BIRC5)	UP	14.16
37987	NM_018687.3	GE80387	hepatocellular carcinoma-associated gene TD26 (LOC55908)	UP	14.03
38069	NM_031934.3	GE80438	RAB34, member RAS oncogene family (RAB34)	UP	13.42
48032	R31367.1	GE86340	yh75c03r1 Soares placenta Nb2HP cDNA clone IMAGE:135556 5'	UP	13.25
49887	NM_031934.3	GE87430	RAB34, member RAS oncogene family (RAB34)	UP	11.79
12787	NM_017899.1	GE55572	hypothetical protein FLJ20607 (TSC)	UP	11.77

# Serum Glypican-3 for diagnosis of HCC



- Cut-off point was set at 76 pg/ml
- Sensitivity 64.5%
- Specificity 87.5%

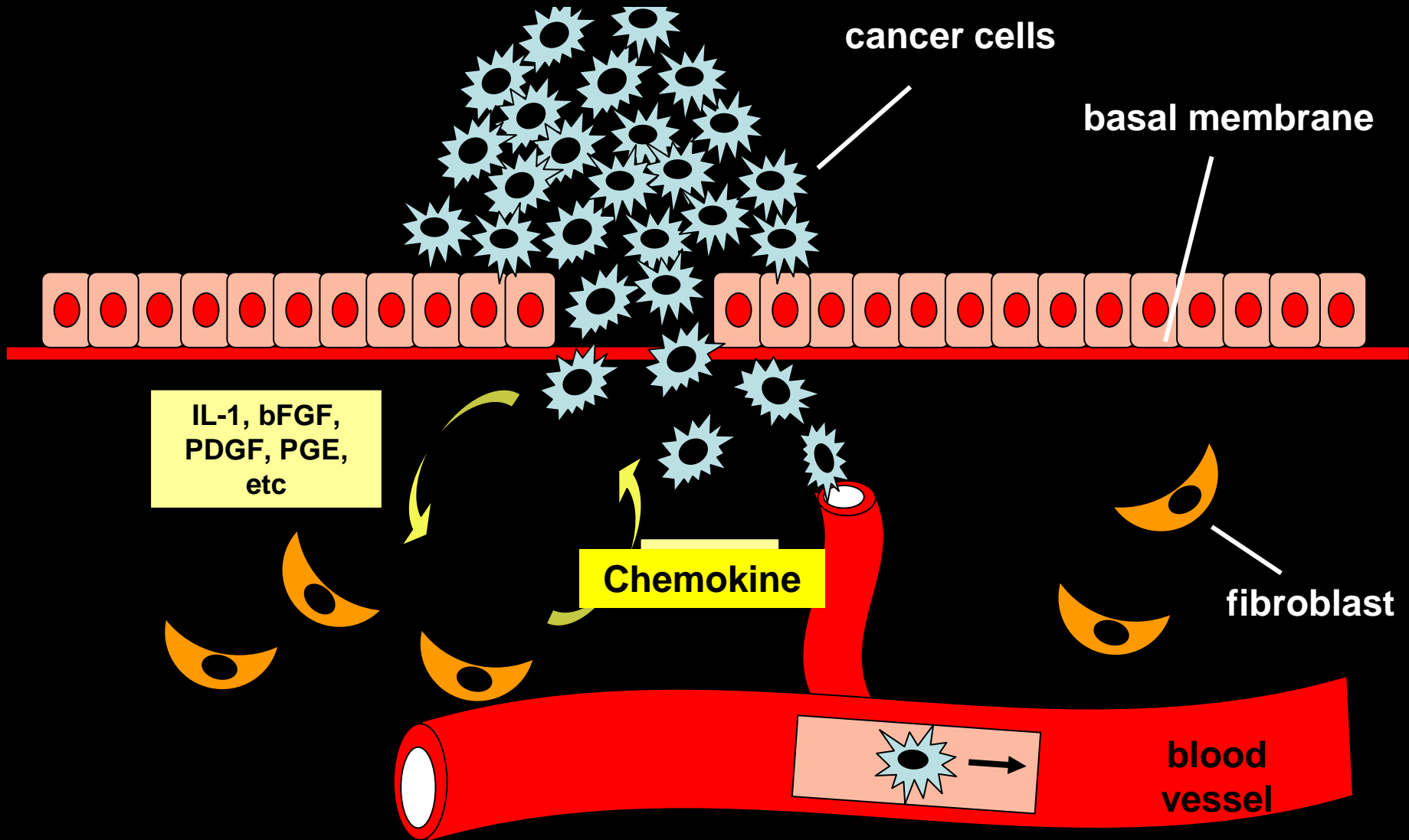


# Tumor Marker Projects

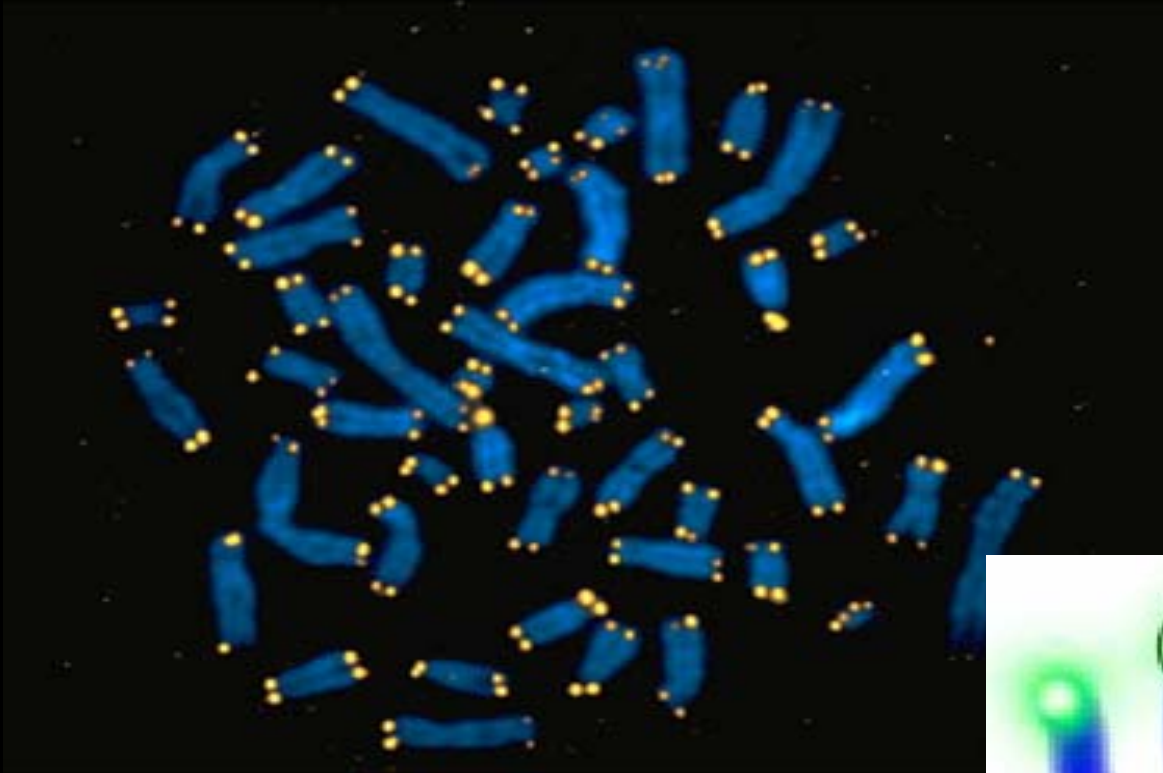
## PCR for detection of Circulating nucleic acids



# Cancer metastasis

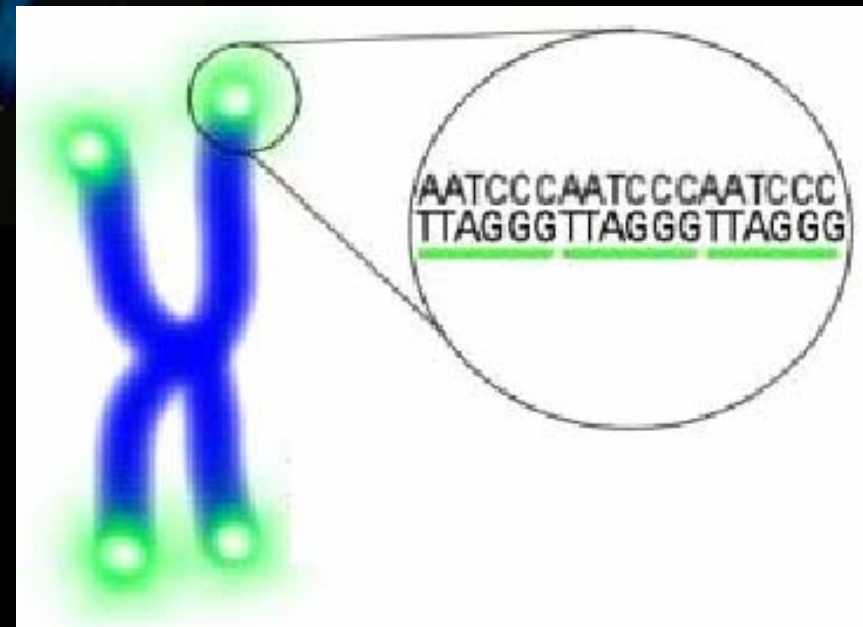


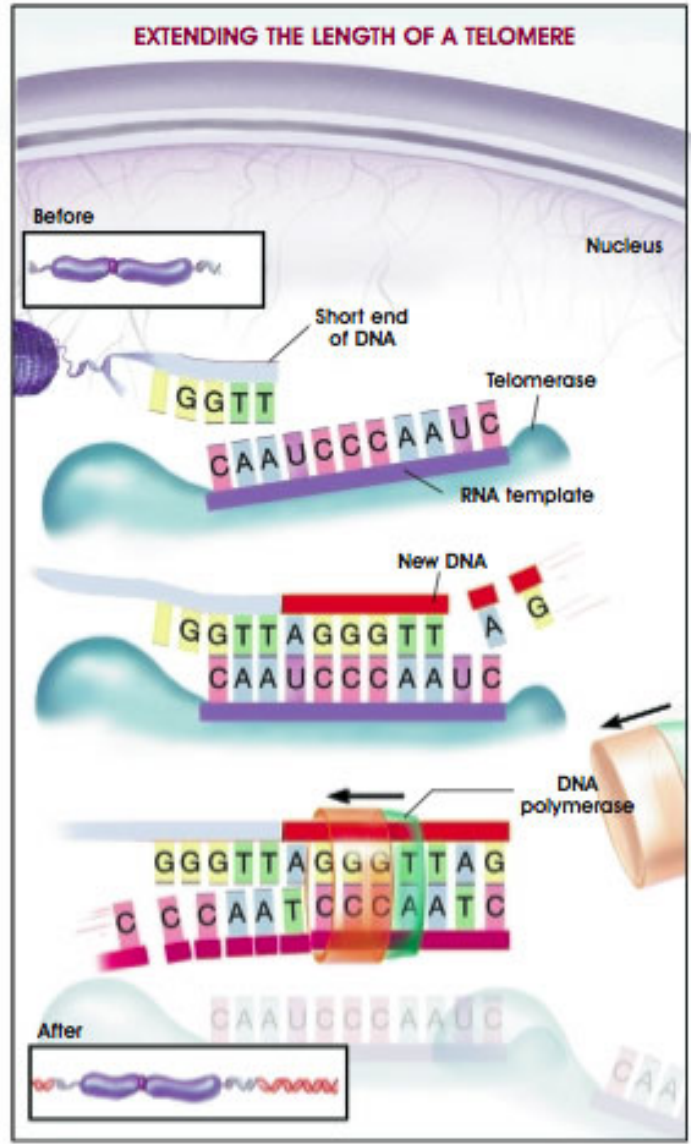
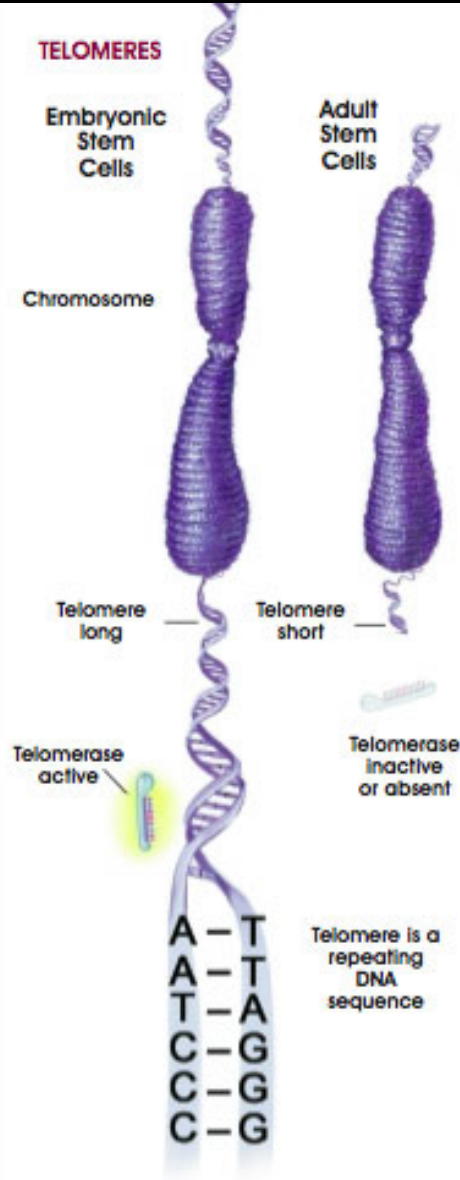
# Human Telomere TTAGGG



[http://www.laskerfoundation.org/awards/images/2006\\_telomere.jpg](http://www.laskerfoundation.org/awards/images/2006_telomere.jpg)

<http://docinthemachine.com/wordpress/wp-content/uploads/2006/11/telomere.bmp>





# Telomerase

- 2 subunits
  1. telomerase RNA template (**hTR**)
  2. telomerase transcriptase protein (**hTERT**)

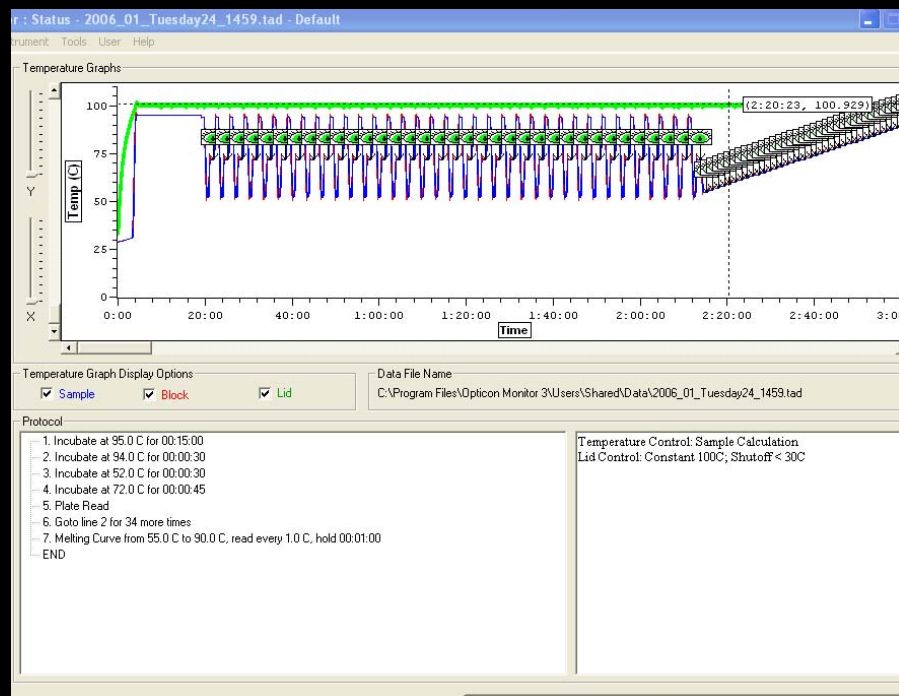
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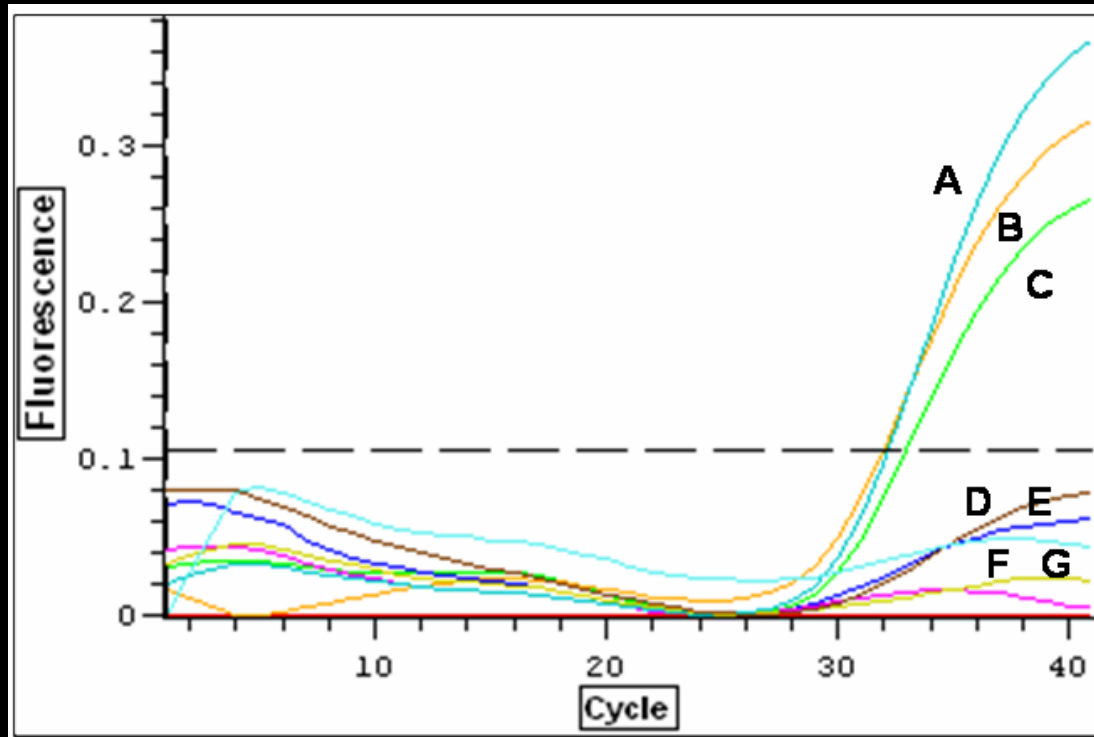
tcctcaggaccctggtccgaggtgtccctgagtatggct

gcgtggtgaacttgcggaagacagtggtaacttcct 3'

# Detection of hTERT By Quantitative Real-Time PCR



# Analysis of hTERT expression by real time RT-PCR



A, B and C : Cholangiocarcinoma

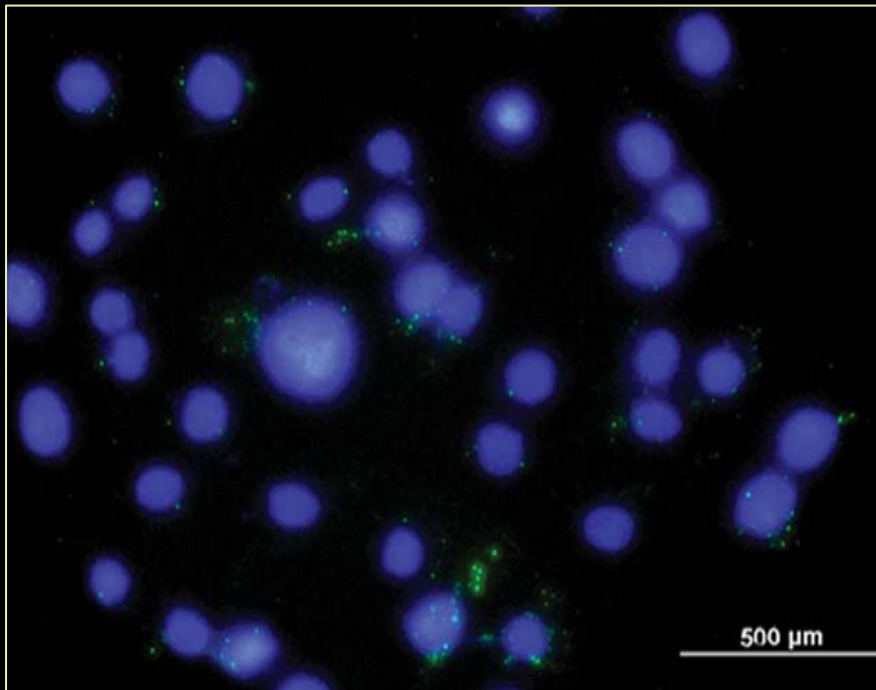
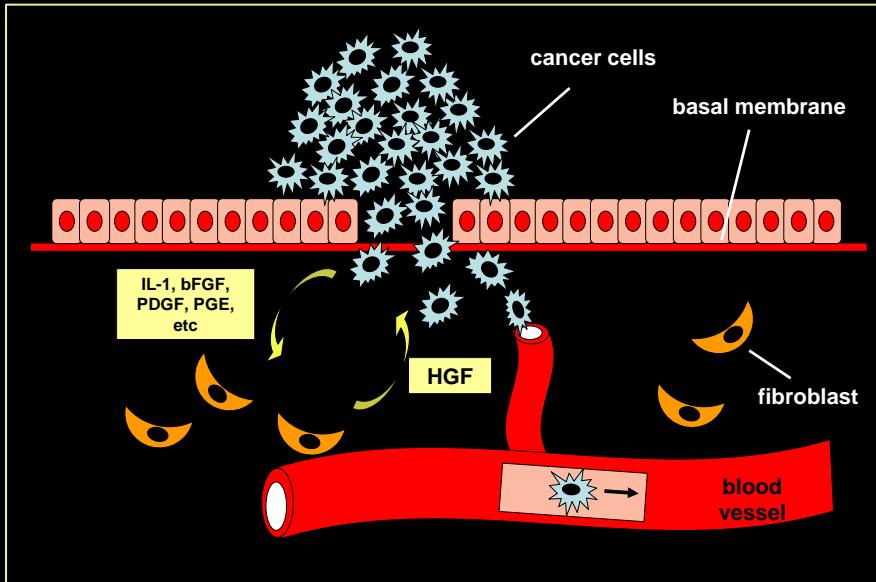
D and E : Corresponding non-cancerous tissues

F and G : GIST

# Comparison analysis of serum hTERT RNA and serum CA 19-9 in diagnosis of CCA

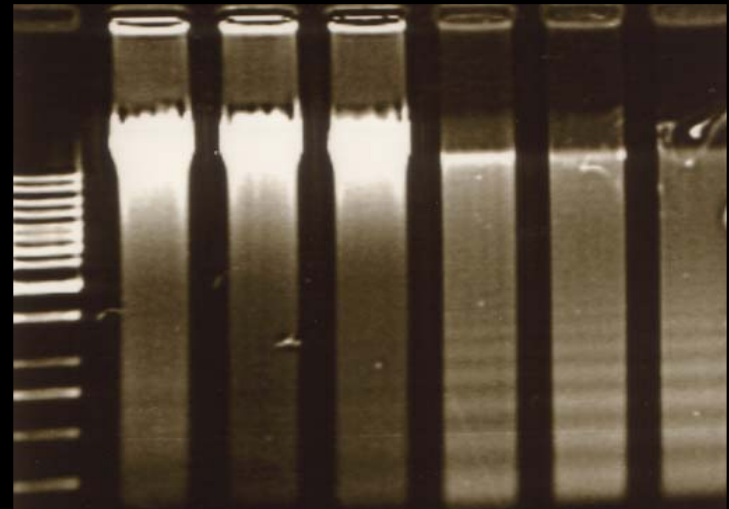
	Serum hTERT (%)	Serum CA19-9 (%)
Sensitivity	84.85	60.6
Specificity	78.05	80.49
Positive predictive value	75.68	71.43
Negative predictive value	86.49	71.74
False Negative	13.51	28.26
False Positive	24.32	28.6

# Source of Circulating DNA

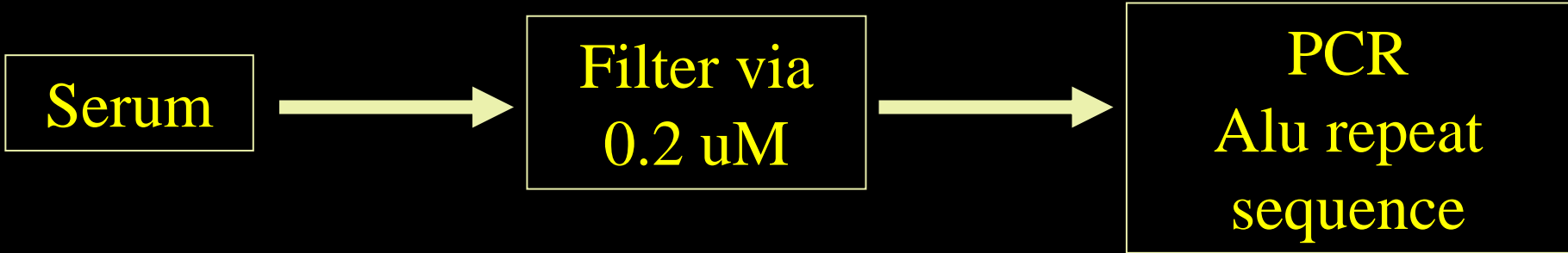
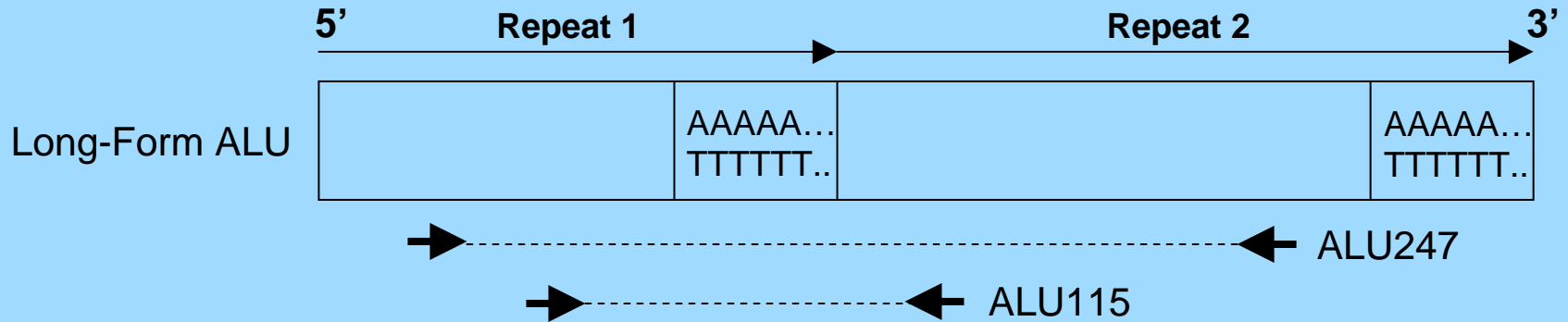


Necrosis

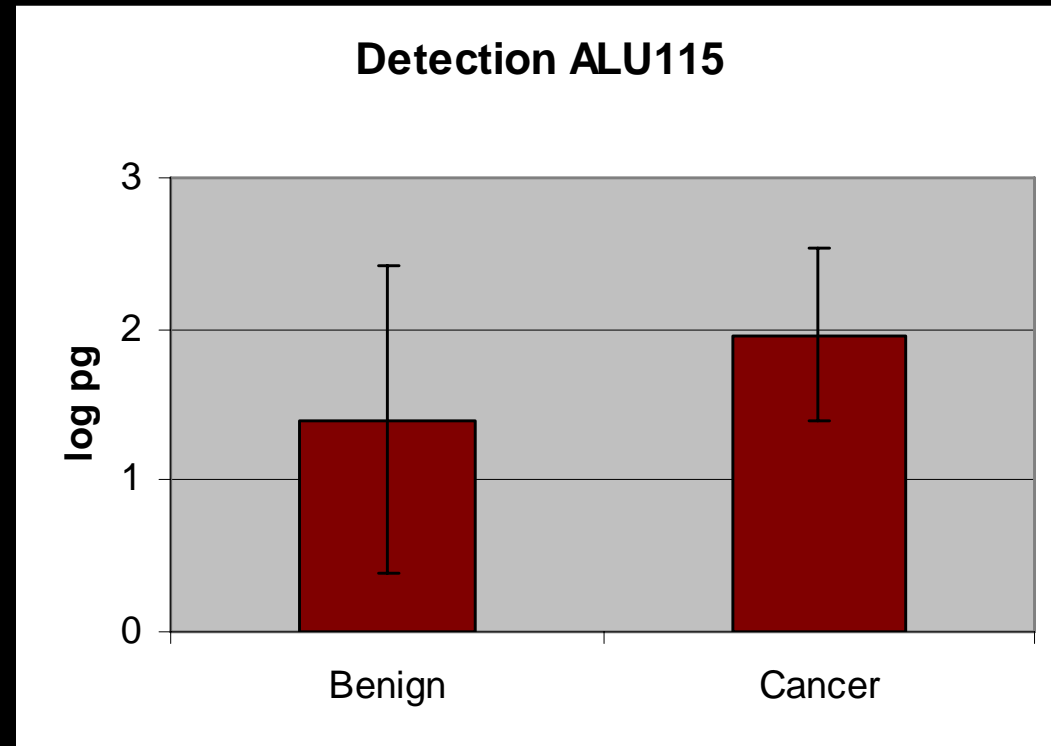
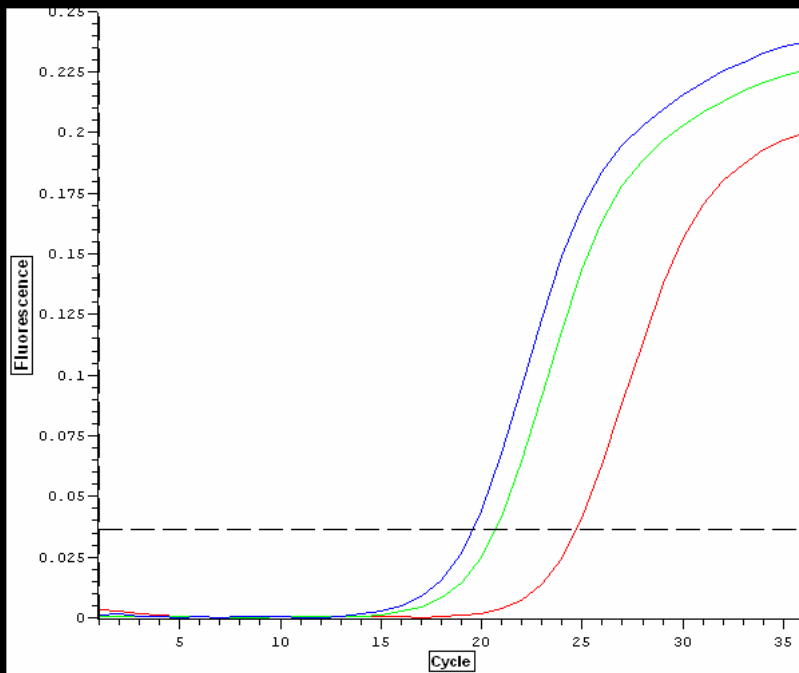
Apoptosis



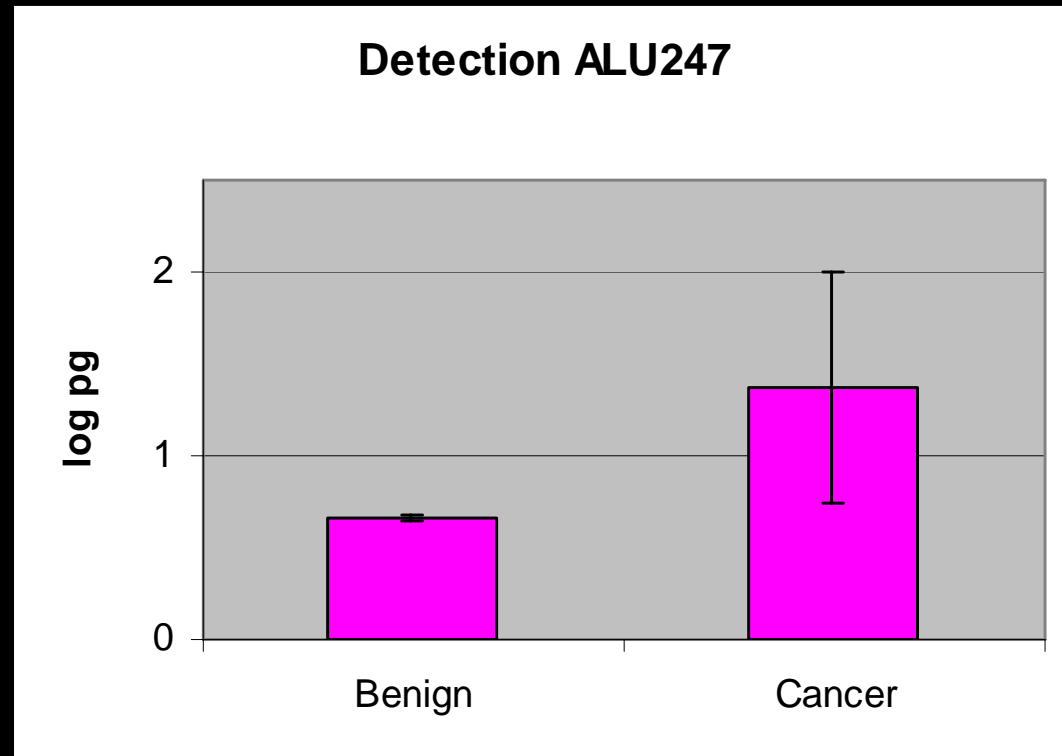
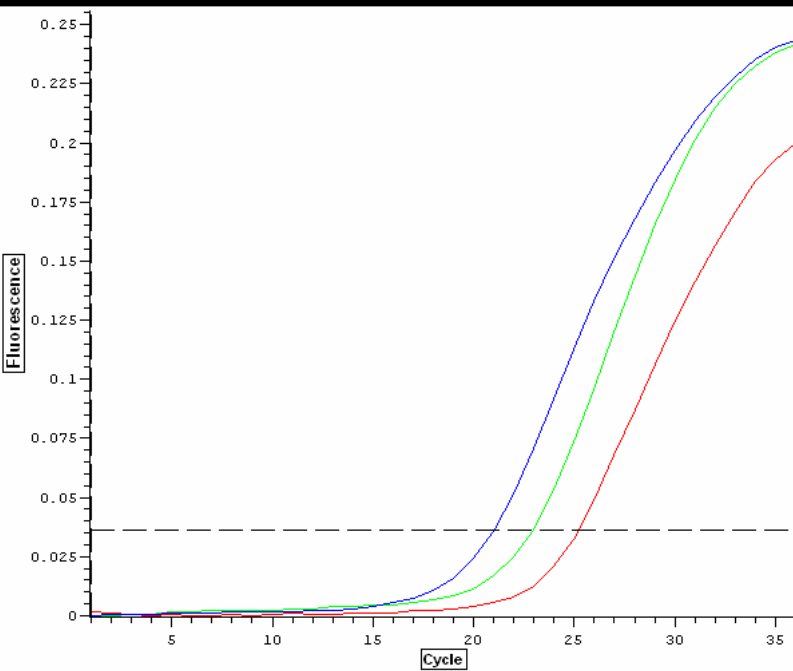
GGCCGGGCGCG**GTGGCTCACGCCTGTAATCC**CAGCACTTTGGGAGGCCGA  
 GGCGGGCGGATCA**CCTGAGGTCAGGAGTTCGAG**ACCAGCCTGGCCAACAT  
 GGTGAAACCCCGTCTCTACTAAAATACAAAATTAGCCGGGCGTGTTGG  
 CGCGCGCC**TGTAATCCAGCTACTCGGG**AGGCTGAGGCAGGAGAATCGCT  
 TGAACCCGGGAGGCGGAGGTTGCAGTGAGCCGAGATCGCG**CCACTGCACT**  
**CCAGCCTG**GGCGACAGAGCGAGACTCCGTCTCAAAAAAAAA



# Alu 115 BP



# Alu 247 BP



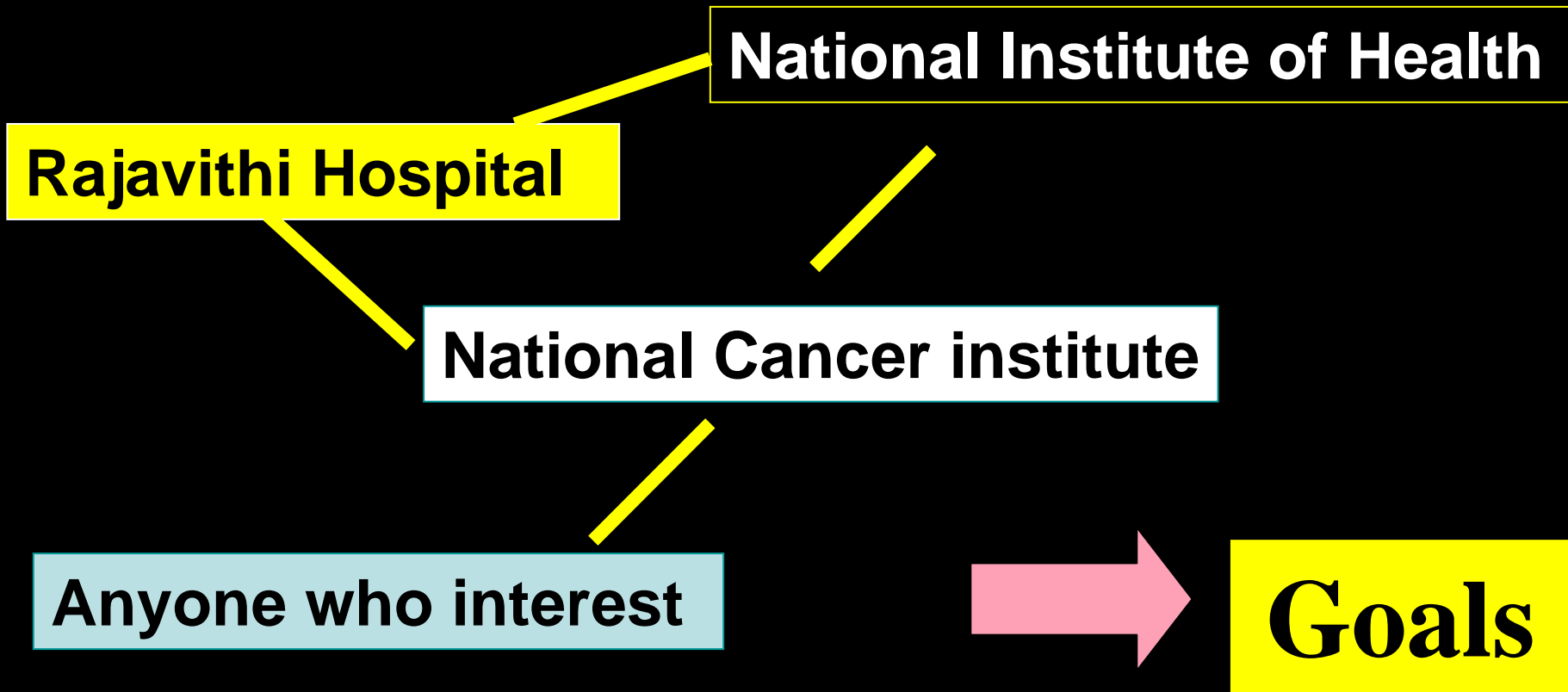
# Circulating DNA

DNA Integrity (Alu 247 / Alu 115)





# Cancer Research Development



# Acknowledgements

**Dr. Surang Leelawat**

**Dr. Panada Tepaksorn**

**Ms. Anicha Leungchaweng**

**Mr. Apichai Prachasupap**

**Ms. Siriluck Narong**

**Ms. Wandee Udomchaiprasertkul**

**Dr. Suchart Chantavibul**

- *Faculty of Pharmacy, Rangsit University*
- *Medical Biotechnology Center, National Institute of Health*
- *Department of Surgery, Rajavithi Hospital*



**Thank You**  
**For your Attention**

**ありがとうございました。**