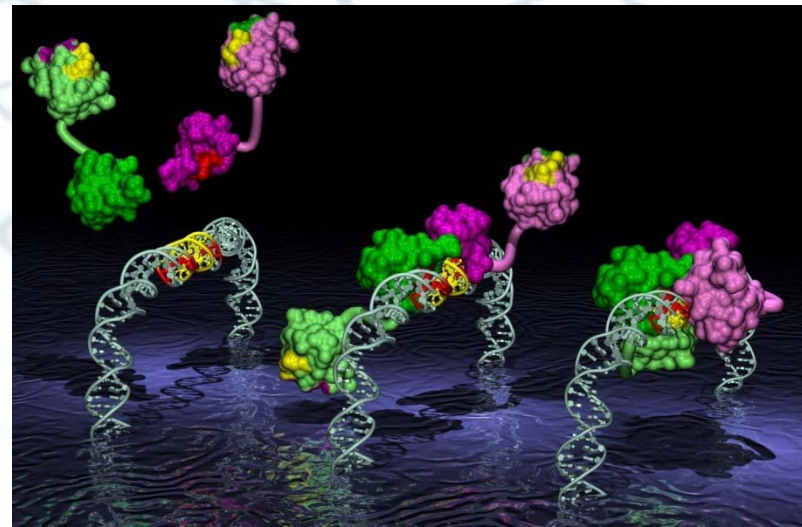


Brief Case presentation

Idit Maya, MD
Inbal Kedar, MsC
Recanati Genetics Institute
Beilinson Hospital
Petah Tikva, Israel.

S.T.

- 31 Yrs male.
- 9 Yrs – T-ALL (chemo+radiotherapy).
- 27 Yrs- CRC (hemicolectomy).
- Brain meningioma.



Family history

Ashkenazi Jewish descent

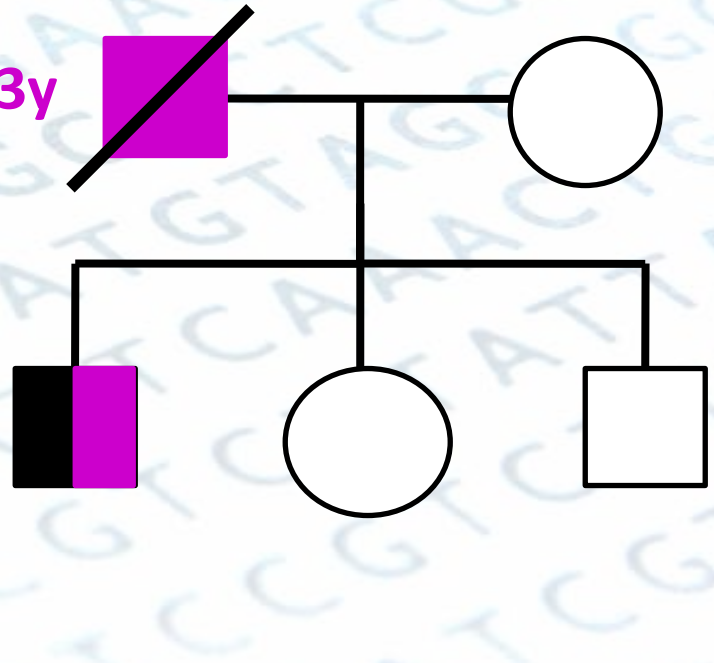
CA Location – Sigma
Pathology – mucin
producing.

Additional 1 small
(0.5cm) colon polyp

CRC-63y

ALL-9y

CRC-27y



Genetic testing



Genetic testing

Li-Fraumeni
Syndrome



P53



Sequencing

MLPA



Genetic testing



Immunohistochemistry



Lack of protein expression

MSH2

&

MSH6

Genetic testing



3 Founder mutation

MSH2

1906G>C ; A636P



MSH6

**c.3984_3987 dup GTCA
c.3959_3962 del CAAG**



Genetic testing



Full Gene Sanger Sequencing

MSH2

MSH6



Genetic testing



MLPA

MSH2
(+EPCAM)



MSH6



Genetic testing

Immunohistochemistry

3 founder mutations

3 genes full sequencing
P53, MSH2, MSH6

2 MLPA
MSH2 (+EPCAM), MSH6

1

P53 MLPA

~ 70% cases
with classic LFS
+ *P53* mutation

< 1% cases with
classic LFS
P53 deletion

2

Other genes ?

MLH3

MSH3

EXO1

PMS1

3



*New
sequencing
technologies*

Should we change our serial testing strategy?
Current approaches for molecular genetic testing
are often stepwise, taking the best candidate gene
approach with testing of additional genes if initial
results are negative.

Expanding DNA diagnostic panel testing: is more better?

Klee EW, Hoppman-Chaney NL, Ferber MJ.

Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA.

Abstract

During the last 25 years, a small number of meaningful DNA-based diagnostic tests have been available to aid in the diagnosis and subsequent treatment of heritable disorders. These tests have targeted a limited number of genes and are often ordered in serial testing strategies in which results from one preliminary test dictate the subsequent test orders. This approach can be both time and resource intensive when a patient requires several genes to be sequenced. Recently, there has been much discussion regarding how 'massively parallel' or 'next-generation' DNA sequencing will impact clinical care. While the technology promises to reduce the cost of sequencing an entire human genome to less than US\$1000, one must question the diagnostic utility of complete genome sequencing for routine clinical testing, given the many interpretive challenges posed by this approach. At present, it appears next-generation DNA sequencing may provide the greatest benefit to routine clinical testing by enabling comprehensive multigene panel sequencing. This should provide an advantage over traditional Sanger-based sequencing strategies while limiting the total test output to sets to genes with known diagnostic value. This article will discuss the current and near future state of clinical testing approaches and explore what challenges must be addressed in order to extract diagnostic value from whole-exome sequencing and whole-genome sequencing, using hereditary colon cancer as an example.

Recently, there has been much discussion regarding how “massively parallel” or “next generation” DNA sequencing will impact clinical care?
At present , it appears NGS may provide multigene panel sequencing

Whole cancer genome sequencing by next-generation methods.

Ross JS, Cronin M.

Department of Pathology, Albany Medical College, 47 New Scotland Ave., Albany, NY 12208, USA.

Abstract

Traditional approaches to sequence analysis are widely used to guide therapy for patients with lung and colorectal cancer and for patients with melanoma, sarcomas (eg, gastrointestinal stromal tumor), and subtypes of leukemia and lymphoma. The next-generation sequencing (NGS) approach holds a number of potential advantages over traditional methods, including the ability to fully sequence large numbers of genes (hundreds to thousands) in a single test and simultaneously detect deletions, insertions, copy number alterations, translocations, and exome-wide base substitutions (including known "hot-spot mutations") in all known cancer-related genes. Adoption of clinical NGS testing will place significant demands on laboratory infrastructure and will require extensive computational expertise and a deep knowledge of cancer medicine and biology to generate truly useful "clinically actionable" reports. It is anticipated that continuing advances in NGS technology will lower the overall cost, speed the turnaround time, increase the breadth of genome sequencing, detect epigenetic markers and other important genomic parameters, and become applicable to smaller and smaller specimens, including circulating tumor cells and circulating free DNA in plasma.



Bioinformatics

ColoSeq Provides Comprehensive Lynch and Polyposis Syndrome Mutational Analysis Using Massively Parallel Sequencing.

Pritchard CC, Smith C, Salipante SJ, Lee MK, Thornton AM, Nord AS, Gulden C, Kupfer SS, Swisher EM, Bennett RL, Novetsky AP, Jarvik GP, Olopade OI, Goodfellow PJ, King MC, Tait JF, Walsh T.

Department of Laboratory Medicine, University of Washington, Seattle, Washington.

Abstract

Lynch syndrome (hereditary nonpolyposis colon cancer) and adenomatous polyposis syndromes frequently have overlapping clinical features. Current approaches for molecular genetic testing are often stepwise, taking a best-candidate gene approach with testing of additional genes if initial results are negative. We report a comprehensive assay called ColoSeq that detects all classes of mutations in Lynch and polyposis syndrome genes using targeted capture and massively parallel next-generation sequencing on the Illumina HiSeq 2000 instrument. In blinded specimens and colon cancer cell lines with defined mutations, ColoSeq correctly identified 28/28 (100%) pathogenic mutations in MLH1, MSH2, MSH6, PMS2, EPCAM, APC, and MUTYH, including single nucleotide variants (SNVs), small insertions and deletions, and large copy number variants. There was 100% reproducibility of detection mutation between independent runs. The assay correctly identified 222 of 224 heterozygous SNVs (99.4%) in HapMap samples, demonstrating high sensitivity of calling all variants across each captured gene. Average coverage was greater than 320 reads per base pair when the maximum of 96 index samples with barcodes were pooled. In a specificity study of 19 control patients without cancer from different ethnic backgrounds, we did not find any pathogenic mutations but detected two variants of uncertain significance. ColoSeq offers a powerful, cost-effective means of genetic testing for Lynch and polyposis syndromes that eliminates the need for stepwise testing and multiple follow-up clinical visits.

Mutations types:

- Single nucleotide
- Indel
- Deletion/Duplication

**NEXT GENERATION
SEQUENCING**

TUMOR CELL LINES

*MLH1
MSH2
MSH6
PMS2
EPCAM
APC
MUTYH.*

ColoSeq Workflow

Prepare genomic DNA in libraries

↓ Paired-end, 200 bp inserts

Capture 7 genes of interest (SureSelect)

↓ 96-index barcoding, pooling

96 samples on 1 lane of HiSeq2000

↓ Millions of 2 x 101 bp reads

~0.3 Gb of sequence data per sample

↓ Align to Hg19 reference genome

Filter common variants (>5%), low reads

↓ Internal database of >1,000 patients

dbSNP, EVS, mutation databases

↓

Pathogenic mutations

Variants of uncertain significance

PolyPhen2, conservation

Mutation(s) confirmation

↓

Clinical Report Issued

UW Academic • LMG 968 Sequencing • Pathological Blood UW-Denver 7/20/11

100%
pathogenic
mutation
detection
rate

99.4%
SNP
detection
rate

ColoSeq Gene Panel

ColoSeq™ is a comprehensive genetic test for hereditary colon cancer that uses next-generation sequencing to detect mutations in multiple genes associated with Lynch syndrome (HNPCC, hereditary non-polyposis colorectal cancer, HNPCC), familial adenomatous polyposis (FAP), MUTYH-associated polyposis (MAP), hereditary diffuse gastric cancer (HDGC), Cowden syndrome, Li-Fraumeni syndrome, Peutz-Jeghers syndrome, Muir-Torre syndrome, and Turcot syndrome. The assay sequences all exons, introns, and flanking sequences of the 11 genes listed in the table below. Large deletions and duplications are **also** detected by the assay and reported. **In June 2012, the panel was expanded from 7 to 11 genes** to include *CDH1*, *PTEN*, *TP53*, and *STK11*. There is no change to pricing, ordering, or specimen requirements with the expanded panel.

Gene	RefSeq	Disease Association	#Exons	Complete Sequencing	Del/Dup	Added
<i>MLH1</i>	NM_000249.3	Lynch, Muir-Torre	19	Yes	Yes	November 2011
<i>MSH2</i>	NM_000251.1	Lynch, Muir-Torre	16	Yes	Yes	November 2011
<i>MSH6</i>	NM_000179.2	Lynch	10	Yes	Yes	November 2011
<i>PMS2</i>	NM_000535.5	Lynch	15	Yes	Yes	November 2011
<i>EPCAM</i>	NM_002354.2	Lynch	9	Yes	Yes	November 2011
<i>APC</i>	NM_000038.5	FAP, Turcot	16	Yes	Yes	November 2011
<i>MUTYH</i>	NM_001128425.1	MAP	16	Yes	Yes	November 2011
<i>CDH1</i>	NM_004360.3	HDGC	16	Yes	Yes	NEW June 2012
<i>PTEN</i>	NM_000314.4	Cowden	9	Yes	Yes	NEW June 2012
<i>STK11</i>	NM_000455.4	Peutz-Jeghers	10	Yes	Yes	NEW June 2012
<i>TP53</i>	NM_000546.5	Li-Fraumeni	11	Yes	Yes	NEW June 2012

12 Genes

Turnaround Time
12 weeks

Cost: 2650\$

<http://web.labmed.washington.edu/tests/genetics/COLOSEQ>

G.G.A. OncoGenetic DNA Chips

Gene	Exons	Mutations/SNP	Tiles	Bases
APC	16	745	1506	65007
BRCA1	24	463	949	42988
BRCA2	27	568	1161	55119
CDKN2A	3	134	267	10712
KRAS	5	20	43	2482
MLH1	19	440	897	36273
MSH2	16	401	814	34252
MSH6	10	146	302	15727
MUTYH	19	61	138	7403
PTEN	9	167	343	15556
TP53	12	182	381	16856
Totals	160	3327	6801	302375
Exon-Introns Re-sequencing – 55,000bp			Maximum Capacity	303366
			Use of Capacity	99.67%

**11 Genes
3300 known
mutations**

**Turnaround
Time
3-4 weeks**

Cost: \$ 2500

\$1500, 2-3w

5 Genes:

**APC, MUTYH, MLH1,
MSH2, MSH6**

ABL1	BRCA1	CREBBP	FANCC	IDH1	MLH1	NPM1	PRKAR1A	SMARCB1	WAS
ABL2	BRCA2	CTNNB1	FANCE	IL21R	MLH3	NRAS	PTCH	STK11	WHSC1
AKT1	BRIP1	CYLD	FANCF	IL6ST	MPL	NTRK1	PTEN	STK11IP	WRN
AKT2	BUB1B	EGFR	FBXW7	ITK	MSH2	NTRK3	PTPN11	SUFU	WT1
ALK	CARD11	ENG	FGFR1	JAK2	MSH6	PALB2	RAD51L1	SYK	WTX
APC	CBL	EP300	FGFR2	JAK3	MUTYH	PDGFRA	RARA	TAF15	XPA
ARHH	CDH1	ERBB2	FGFR3	KIT	MYC	PDGFRB	RB1	TCF1	XPC
ATM	CDK4	ERCC2	FLI1	KRAS	MYCN	PHOX2B	RECQL4	TGFBR2	
AXIN2	CDK6	ERCC3	FLT3	LCK	MYH11	PIK3CA	REL	TLX1	
BCL10	CDKN2A	ERCC4	GATA1	MAF	MYH9	PIK3R1	RET	TLX3	
BCL2	CDX2	ERCC5	GATA2	MAFB	NBS1	PIM1	ROS1	TOP1	
BCL9	CEBPA	EWSR1	GPC3	MAP2K4	NF1	PLAG1	RUNX1	TP53	
BLM	CHEK2	EXT1	GRAF	MDM2	NF2	PML	RYR1	TSC1	
BMPR1A	COPEB	EXT2	HIP1	MEN1	NFKB2	PMS1	SH3GL1	TSC2	
BRAF	CREB1	FANCA	HRAS	MET	NOTCH1	PRDM16	SMAD4	VHL	

142

NGS
PCR BASED
RainStorm Technology

94%
COVERAGE

ONLY EXONS

**Turnaround
Time:
16 weeks**

Cost: \$2500

NGS for CRC patients ?

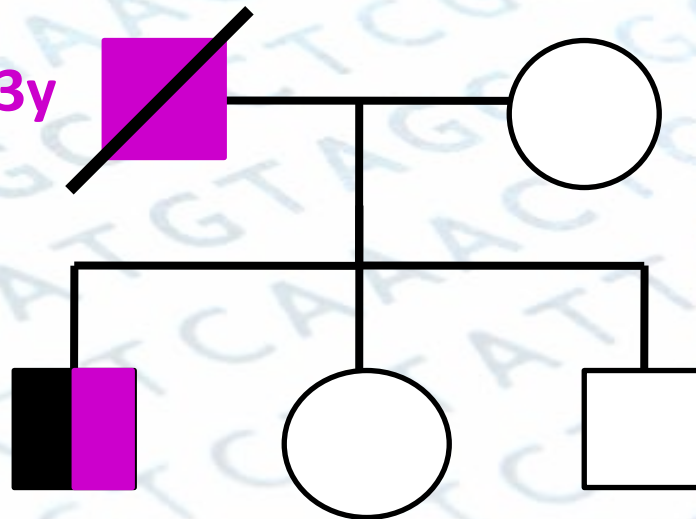
Ashkenazi Jewish descent

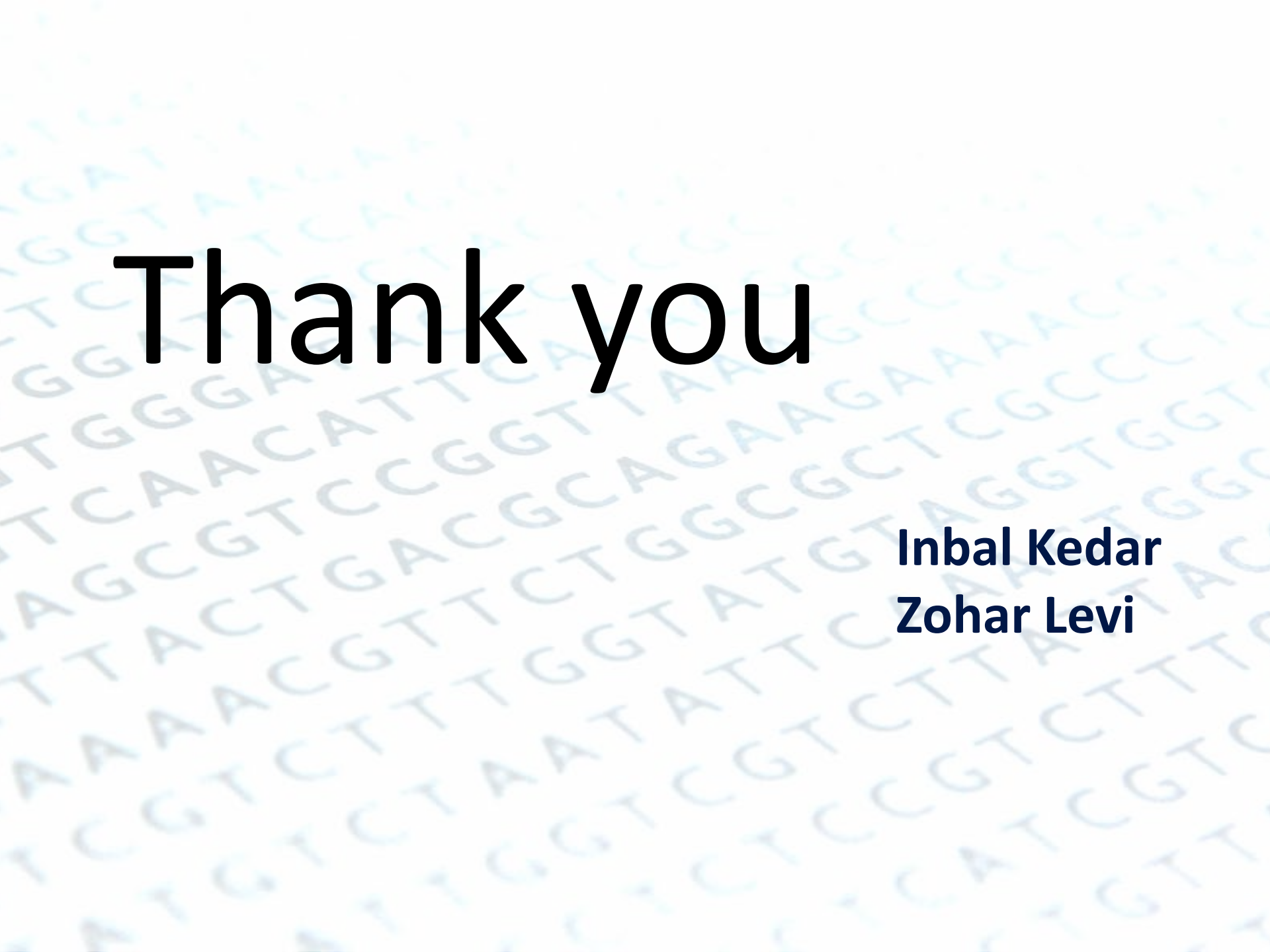
WHEN ?

CRC-63y

ALL-9y

CRC-27y





Thank you

Inbal Kedar
Zohar Levi