Genetics of cancer in EGAT study

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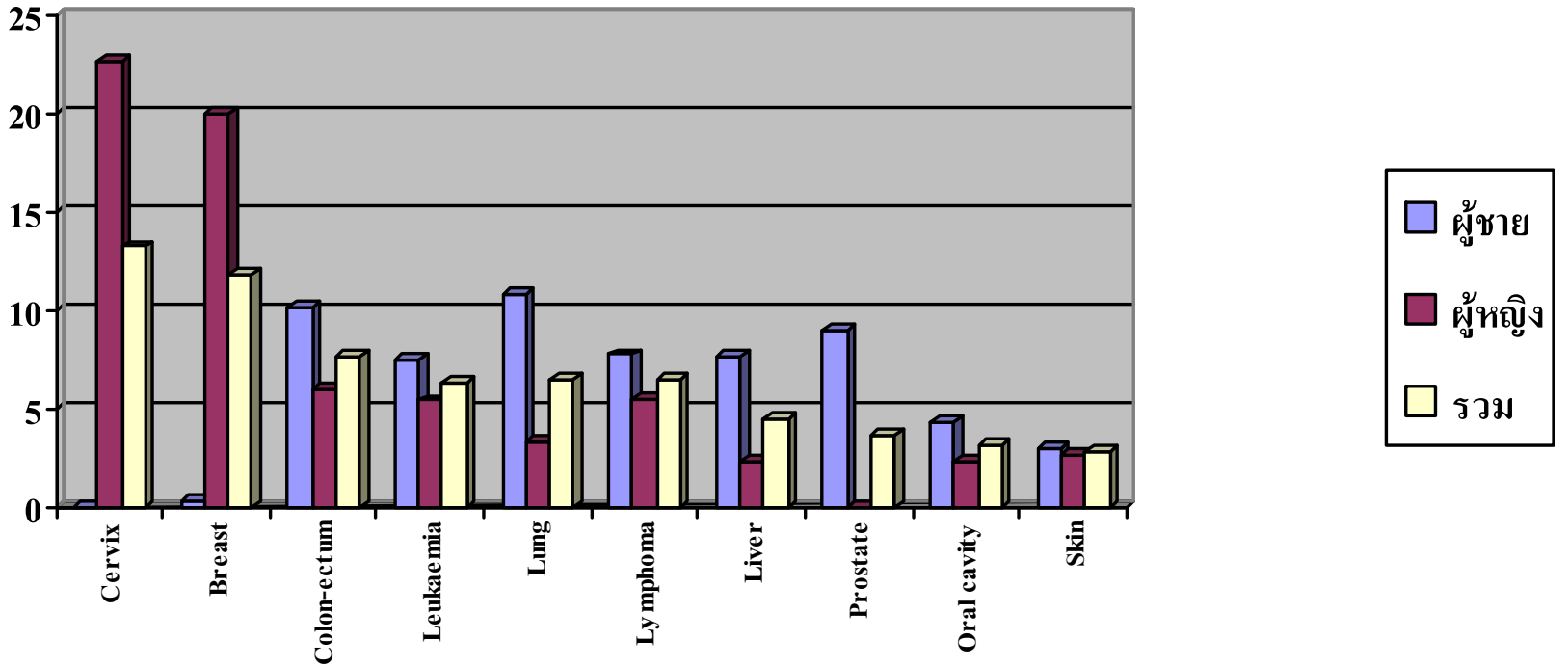
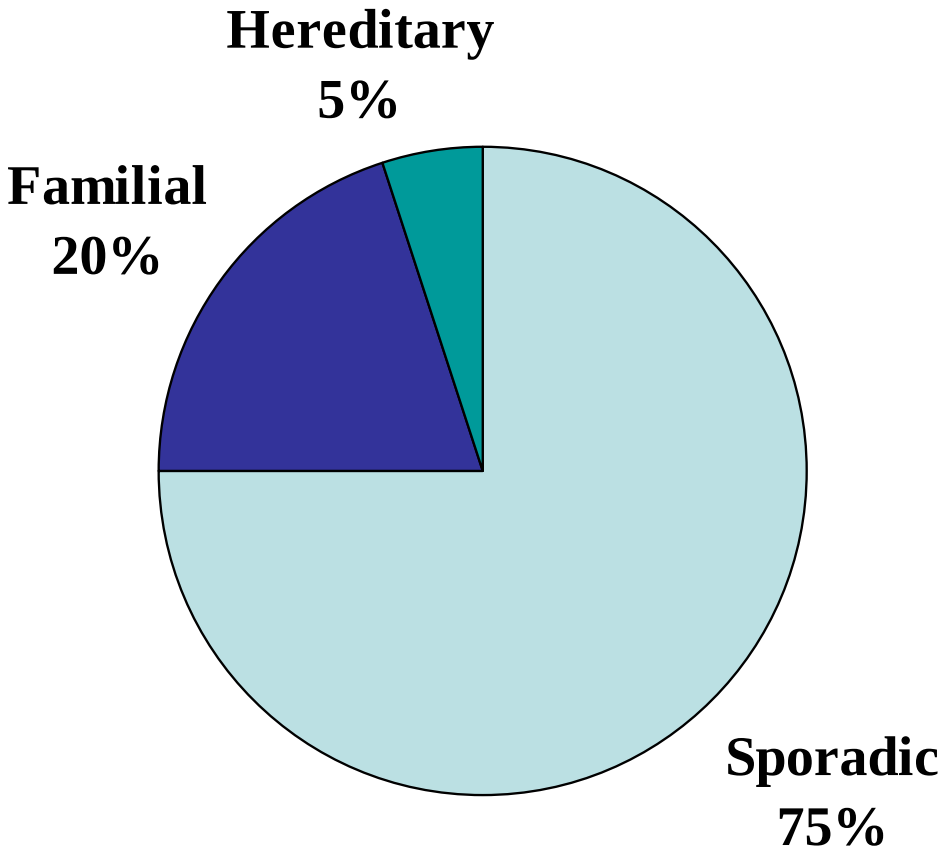
- **Cancer develops because of a complex mix of environmental and genetic factors.**
- **For some**:-
  - Environmental factors pose the greatest risk
- **For others**:-
  - It is inherited susceptibility
- **For most**:-
  - Combination of all the above.

The leading sites of cancer in Thailand

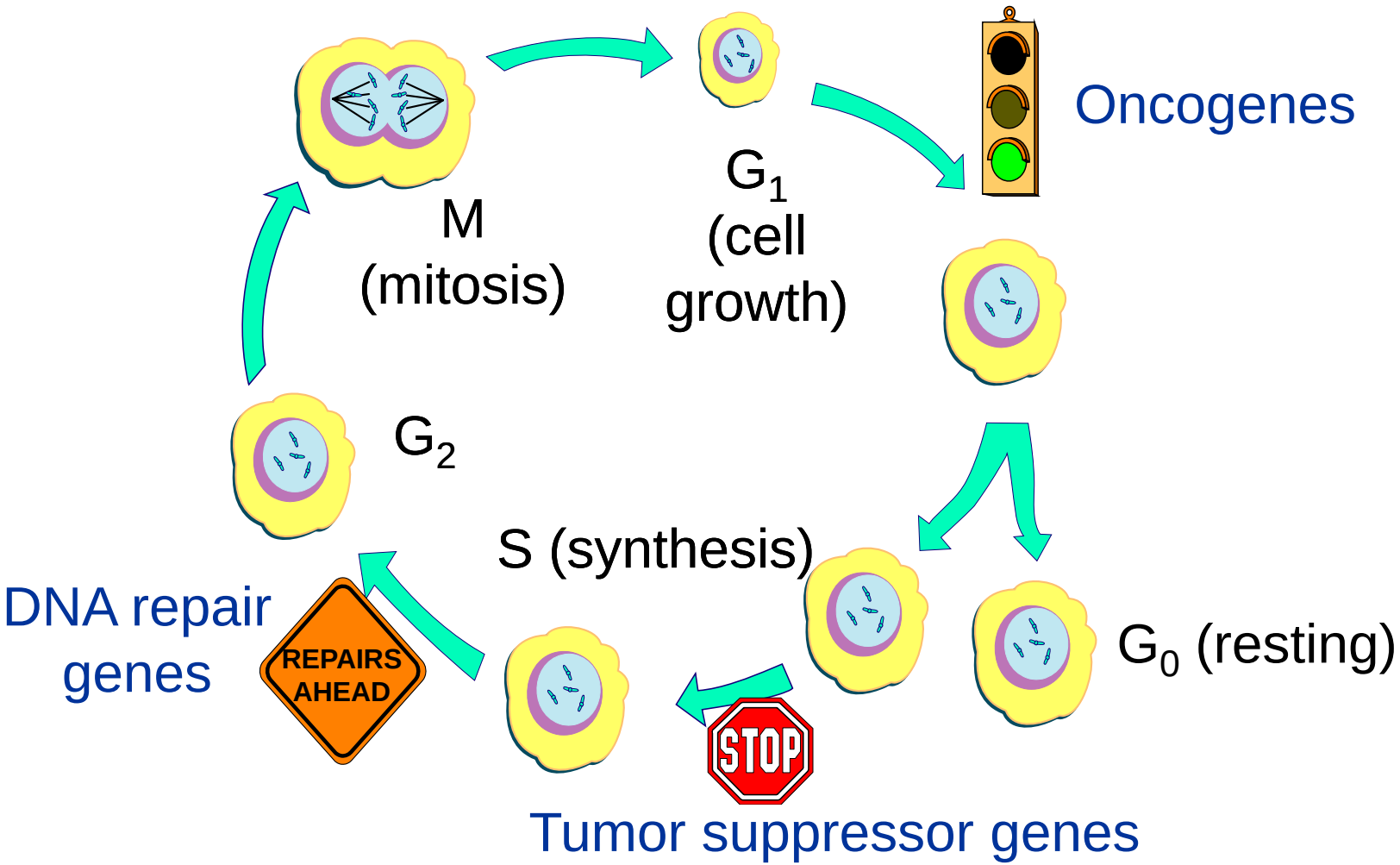
Ministry of Health 2005



Etiology of Cancers

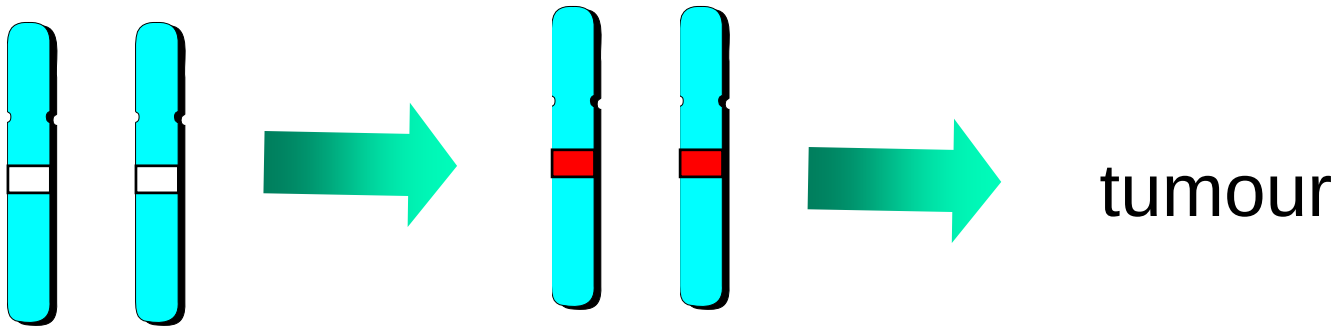


The Cell Cycle



Cancers Arise From Gene Mutations

in genes protecting **against** cancer

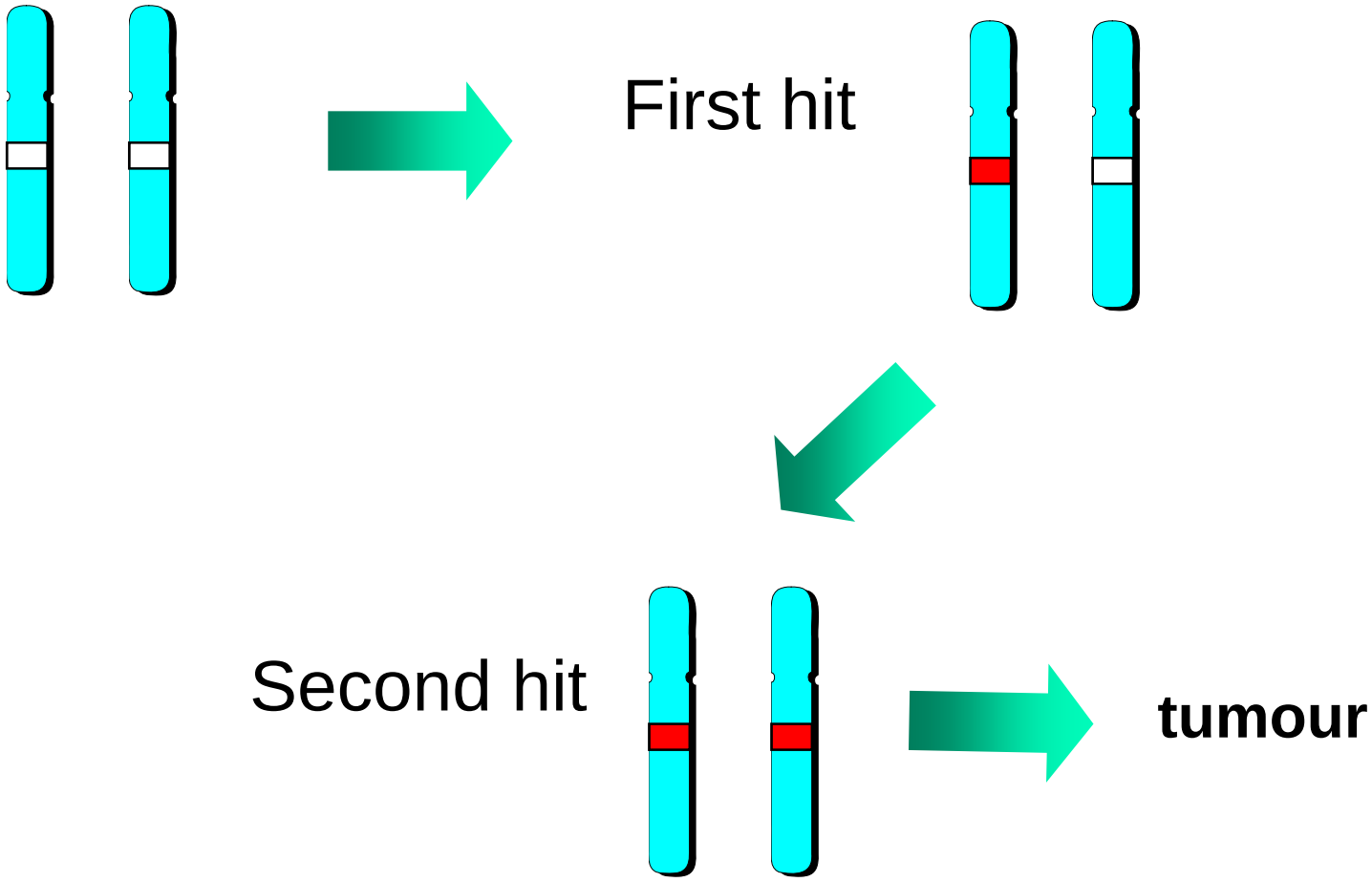


All cancer is genetic

BUT

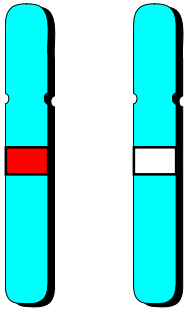
not all cancer is inherited!

Knudson’s ‘Two-Hit Hypothesis’ (Somatic Mutation)

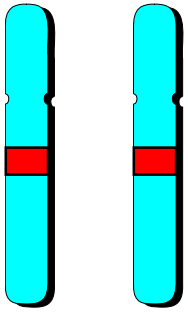


Knudson's 'Two-Hit Hypothesis' (Germline Mutation)

First hit is in germline

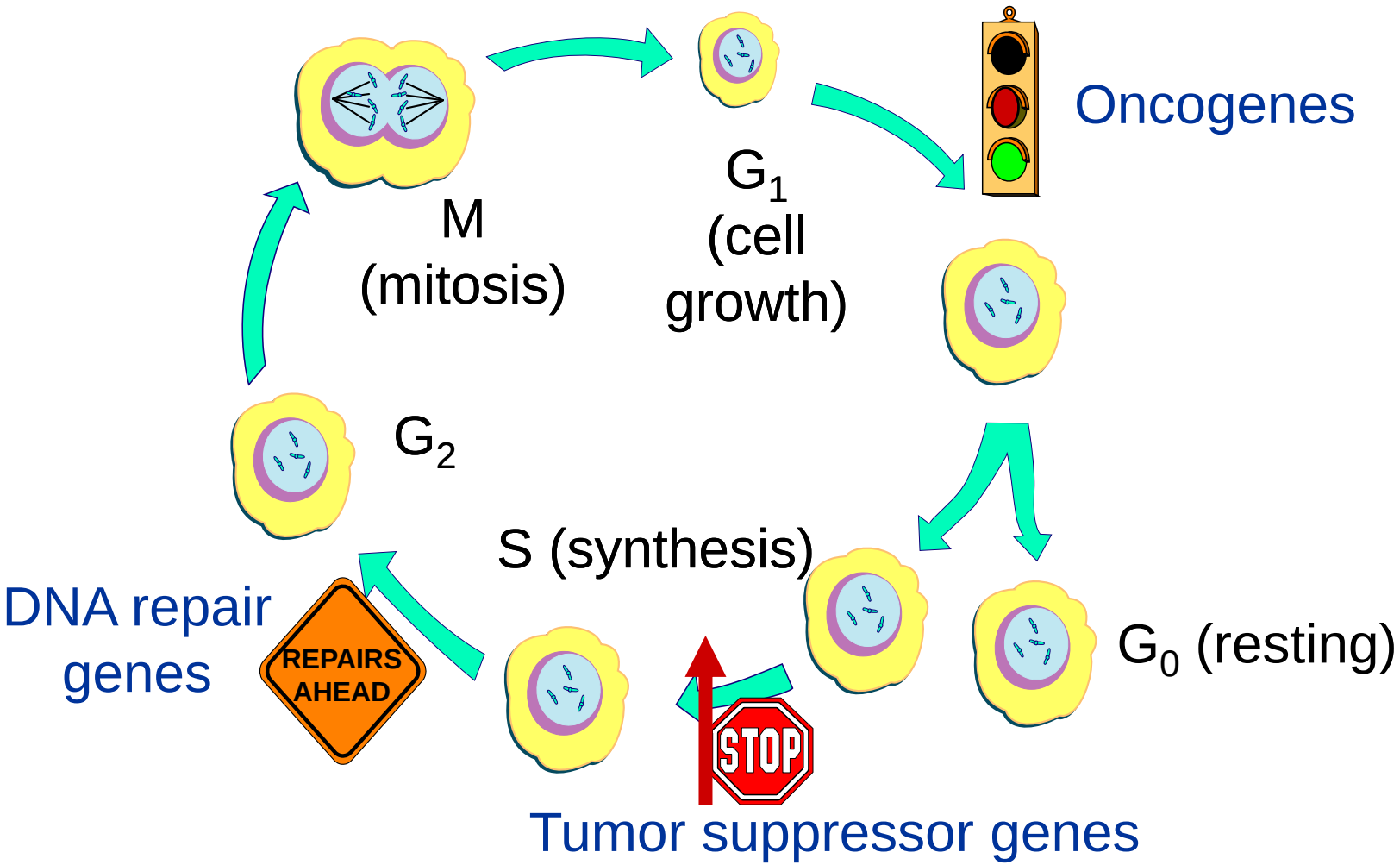


Second hit is somatic

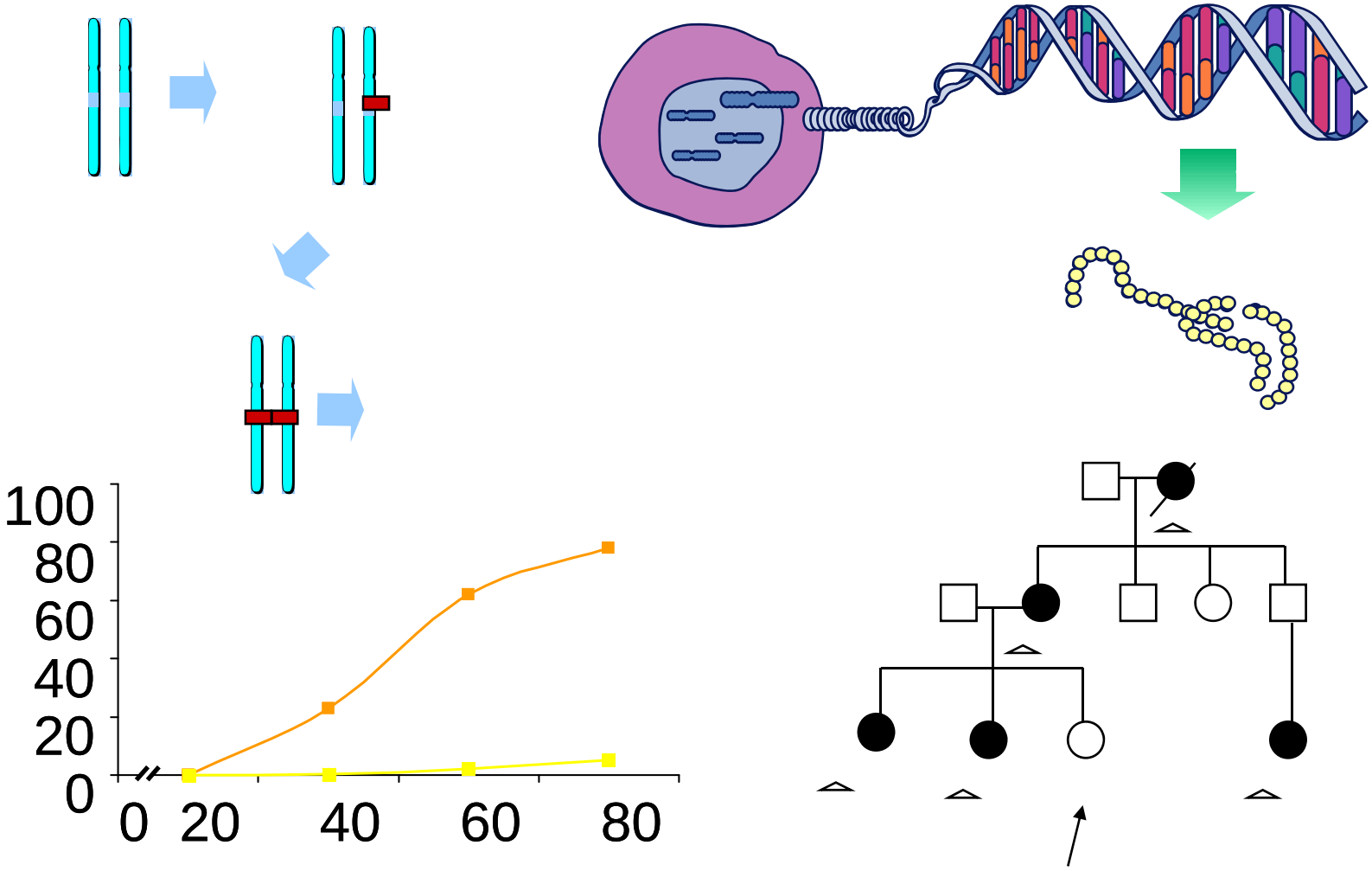


tumour

The Cell Cycle



In summary…



Pattern of cancer

	Skin	GI tract	Malegenital tract	Female genital tract	Endocrine	Nervous system	Musculoskeletal	Urinary tract	Haematological
BRCA1	●			●					
BRCA2	●		●	●					
HNPCC	●	●		●		●		●	●
FAP (APC)	●	●			●	●	●		
PJS		●	●	●	●			●	
Hereditary gastric (ECAD)		●							
LFS	●	●			●	●	●	●	
VHL					●	●		●	
Cowden	●	●		●	●	●	●	●	
Gorlin	●					●			
RB	●					●	●		
MEN1		●			●	●			
MEN2					●				
NF2	●					●			
Fumarase carrier				●					

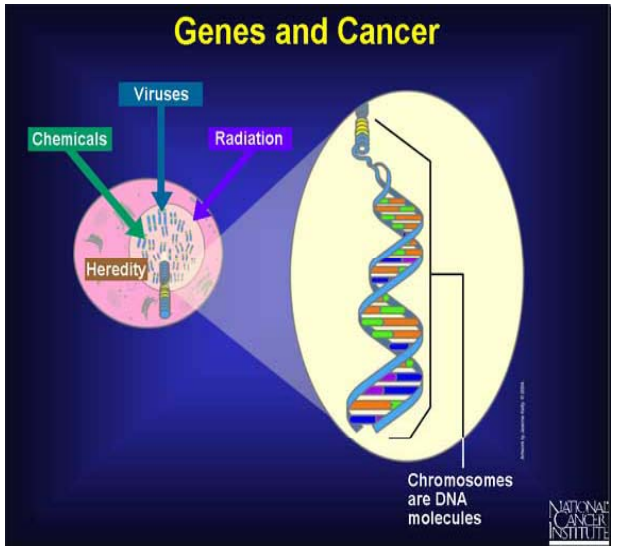
Population of EGAT study

- EGAT 1 ~ 2,800
- EGAT 2 ~ 2,200
- EGAT 3 ~ 2,000

Cancers ~ 680

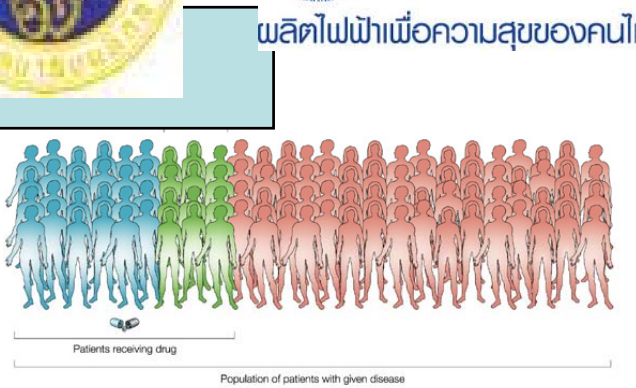
Cancers ~ 572

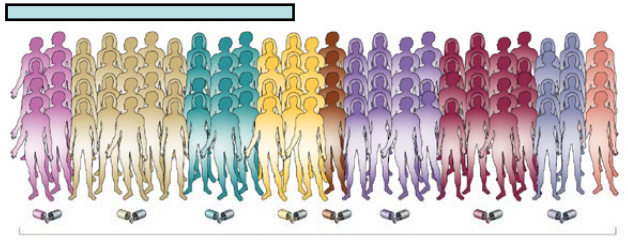
Breast, Colon, others

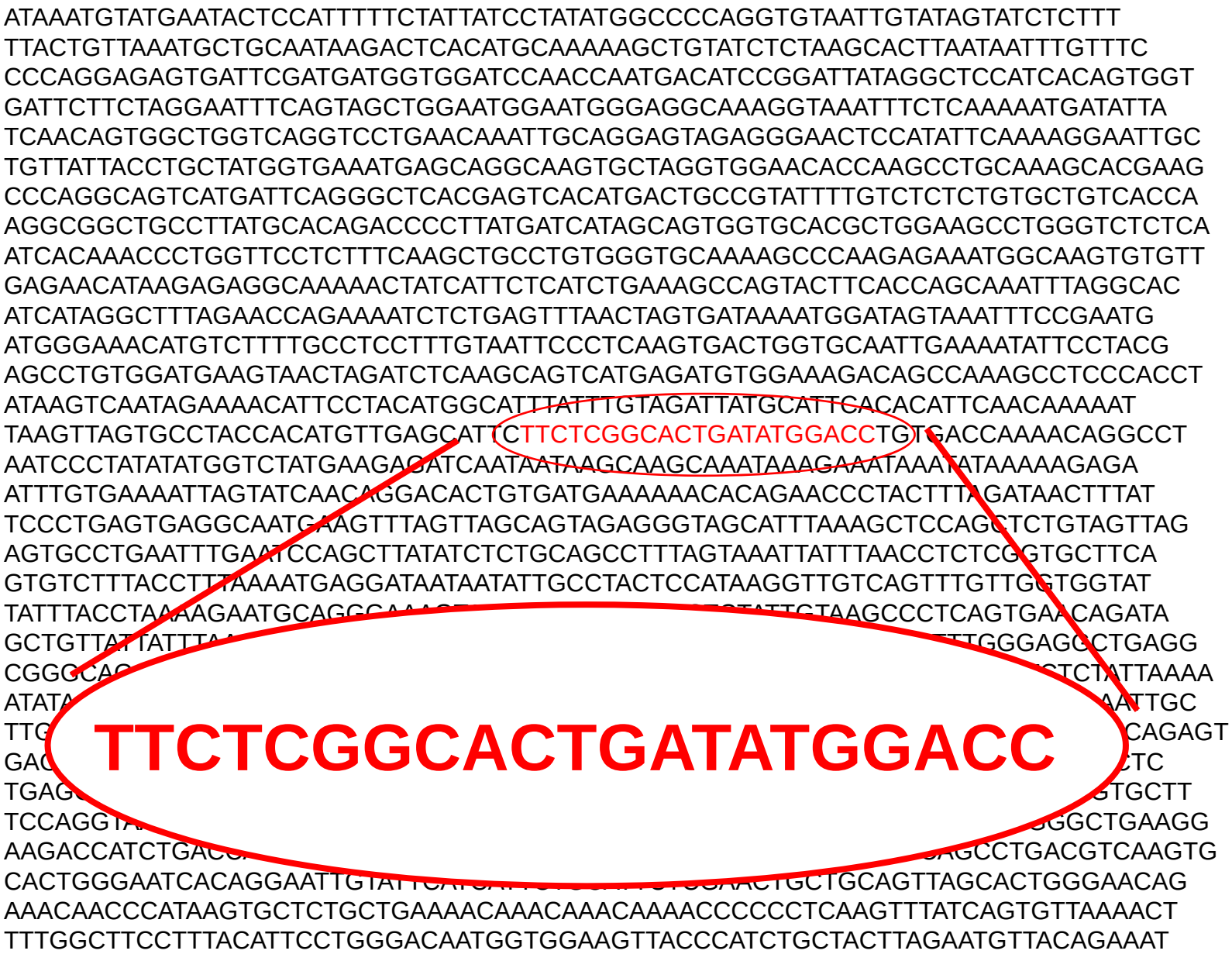


Outcome Prediction

1. Identified group of cancer genes in EGAT population
2. Identified individual ‘s risk for cancer prevention
3. Apply these genetic markers to Thai population
4. Appropriate surveilance screening
5. Compromise health economics

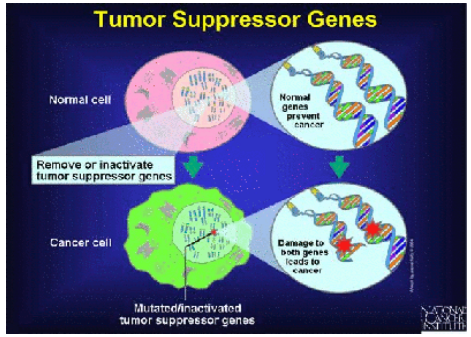


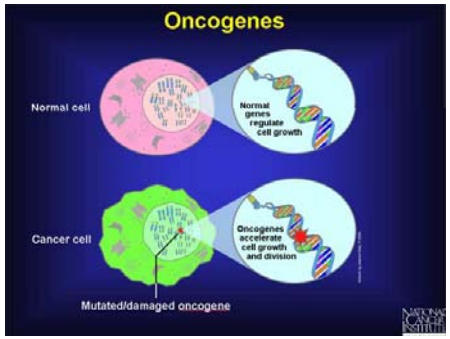


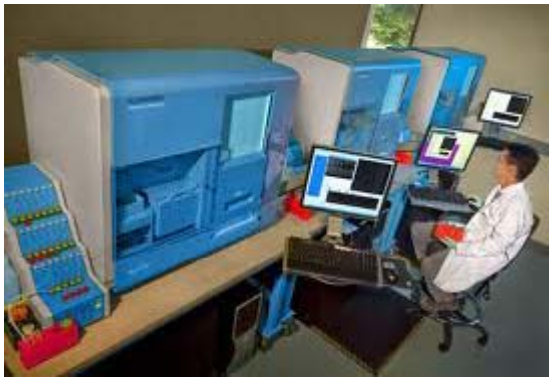

TTCTCGGCACTGATATGGACC

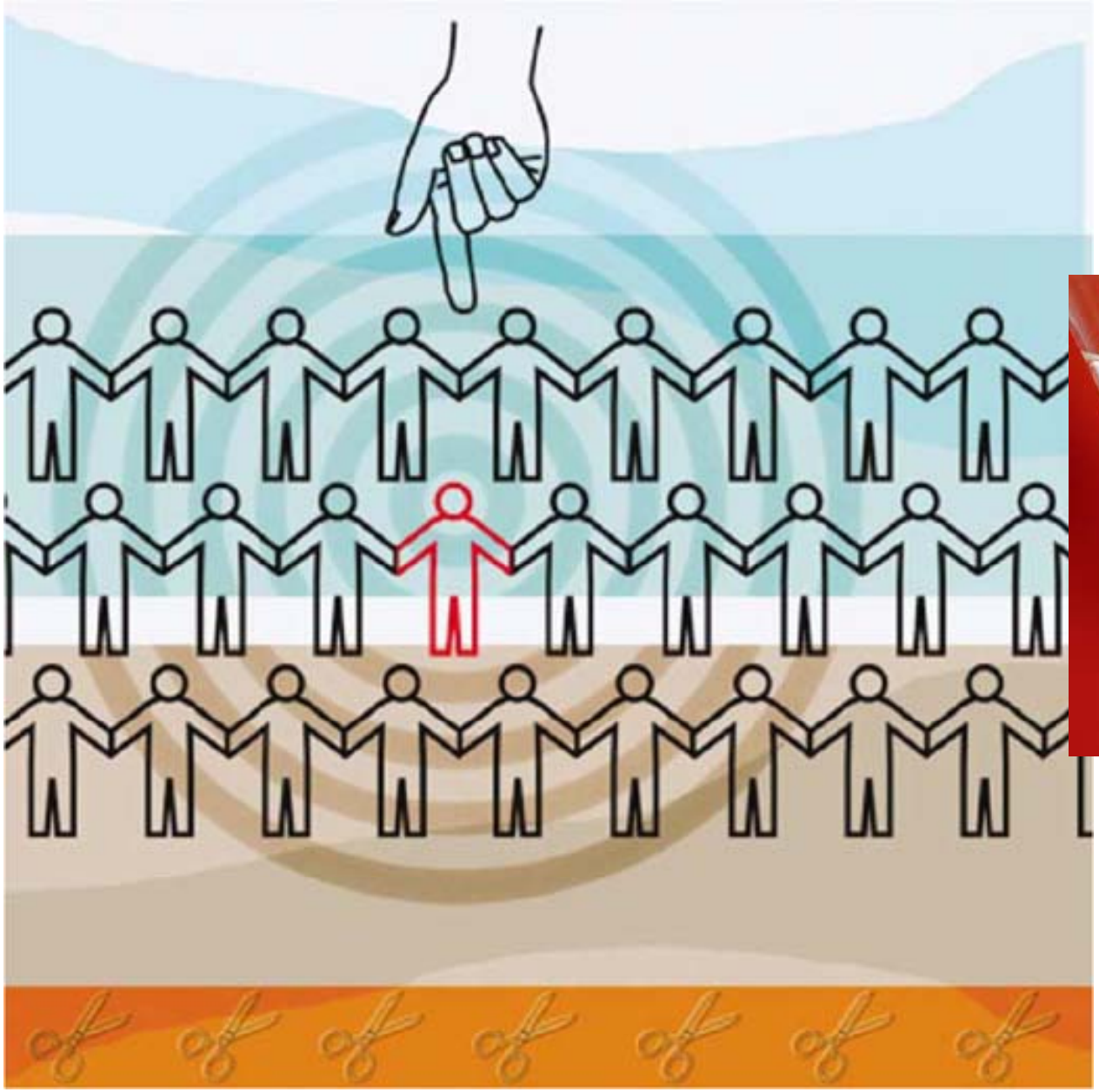
- cIllumina's Cancer SNP Panel candidate gene-based genotyping platform
- GoldenGate genotyping assay
- Illumina SNPs 1,421 ตำแหน่งที่อยู่SNP500 Cancer database[2] ซึ่งเป็นส่วนหนึ่งของ
- Cancer Genome Anatomy Project
- National Cancer Institute

- case-control association study
  (proto-oncogenes) และยีนที่เพิ่มความเสี่ยงต่อโรคมะเร็ง
- (tumour-suppressor genes หรือ cancer-susceptibility genes อื่นๆ)
- [2] ข้อดีของ SNPs ในฐานข้อมูลนี้คือเป็น SNP ที่พยายามเลือกมาโดยให้อยู่ภายในยีนหรือถ้าไม่เช่นนั้นก็อยู่ใกล้กับยีนมาก
- และโดยเฉพาะอย่างยิ่งพยายามเลือก SNPs ในยีนที่มีลักษณะ non-synonymous
- ซึ่งน่าจะส่งผลต่อการเกิดโรคโดยตรงมากกว่าจะเป็นเพียง linked polymorphic markers เท่านั้น







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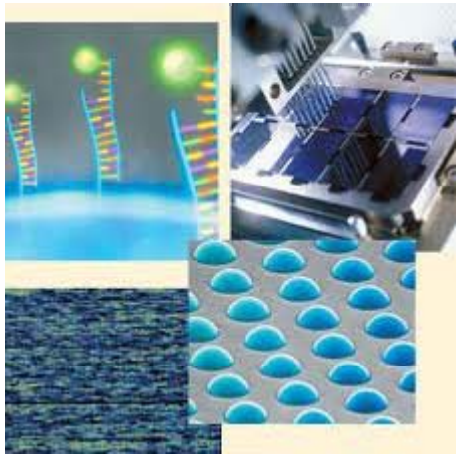
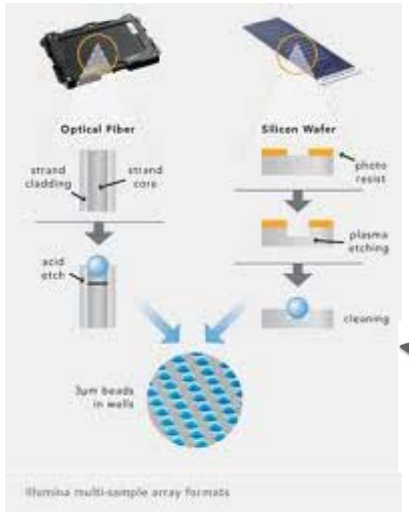
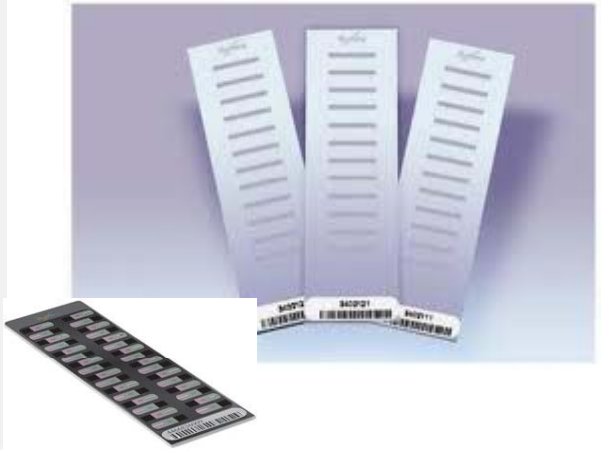
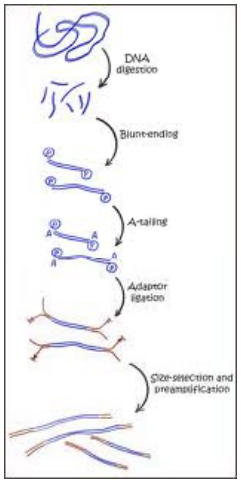
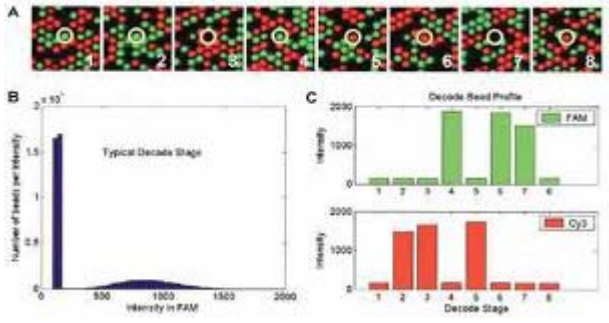
Illumina technique

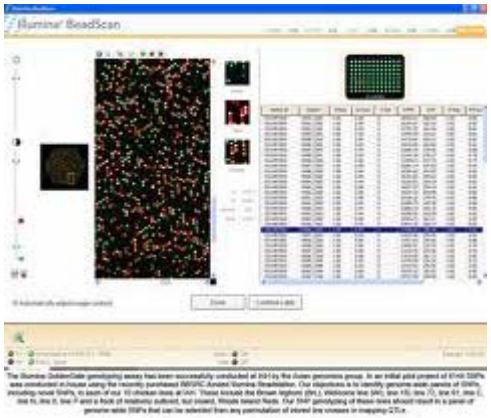






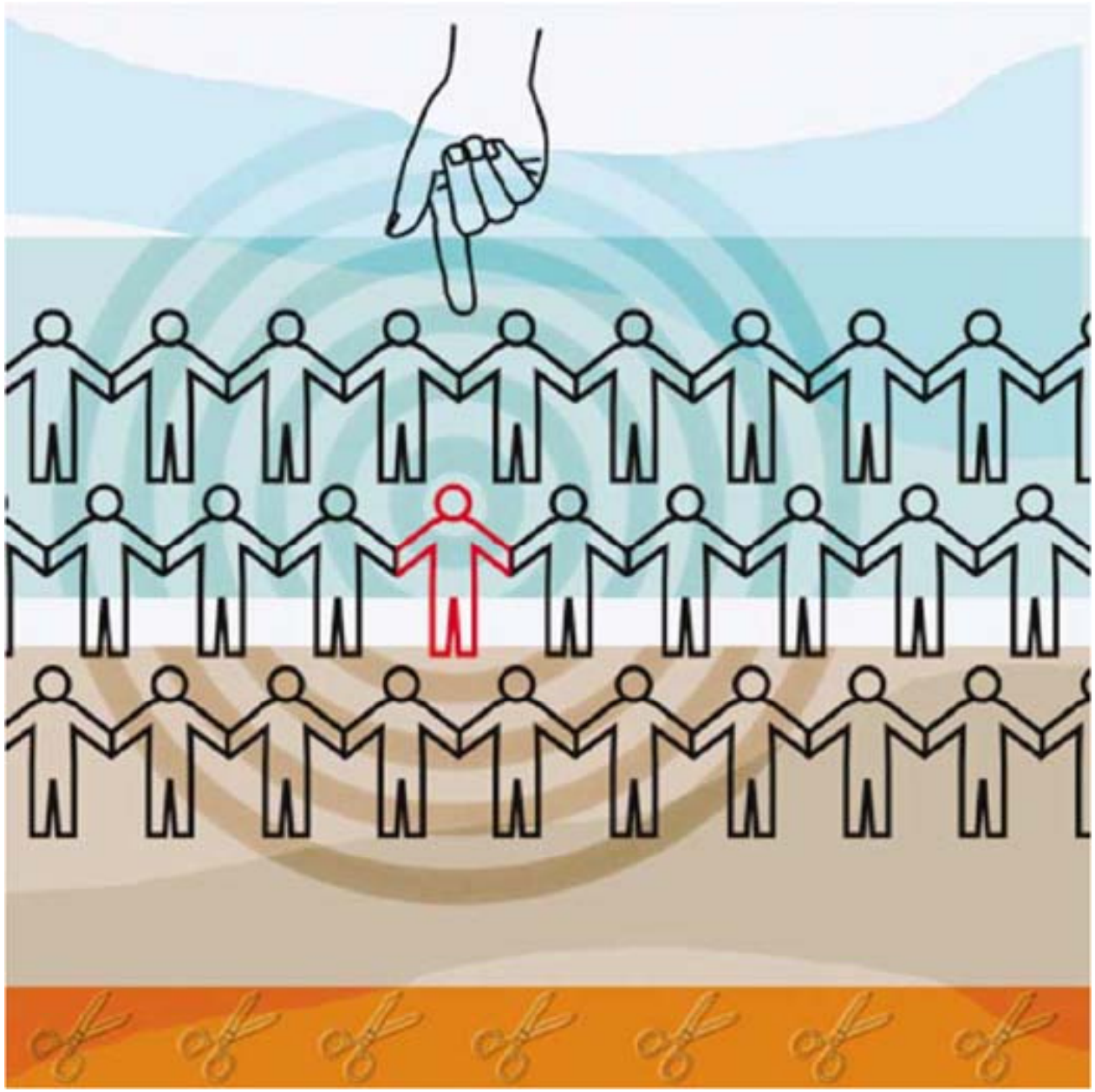






Colon cancer

Informative SNPs	*= 178*
1st correction	*=93*
2nd rearrangement	*=23*
3rd validation	*= 19*



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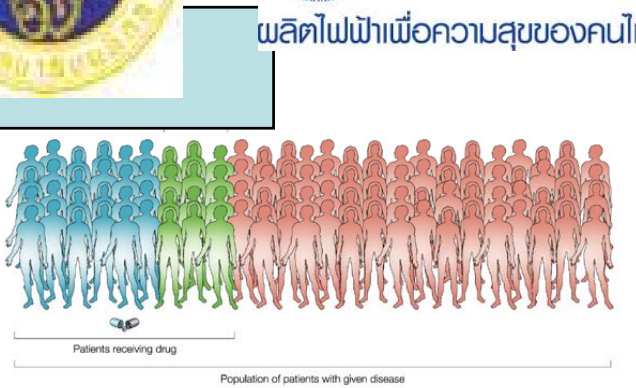

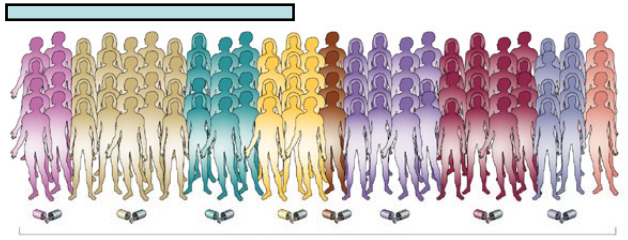

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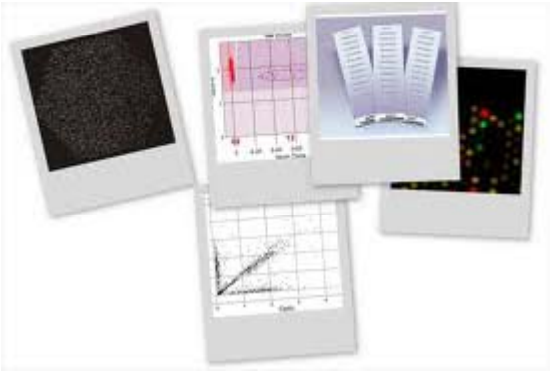
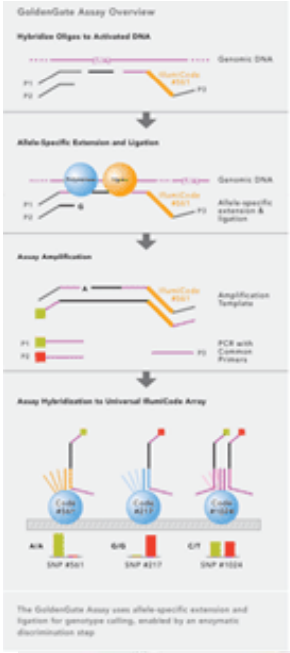
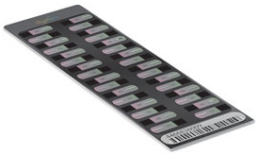
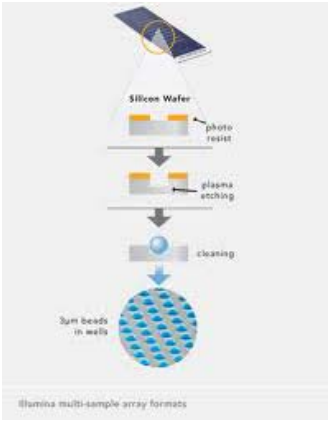


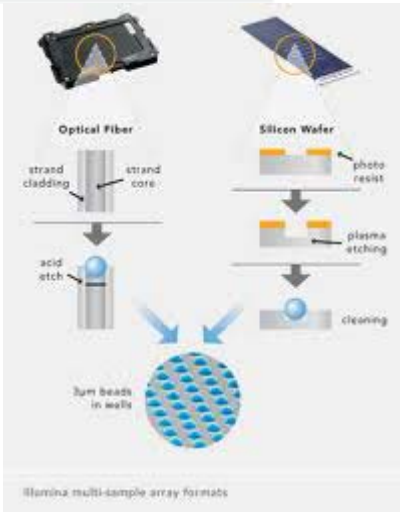


The GoldenGate Assay uses allele-specific extension and ligation for genotype calling, enabled by an enzymatic discrimination step





Illumina multi-sample array formats



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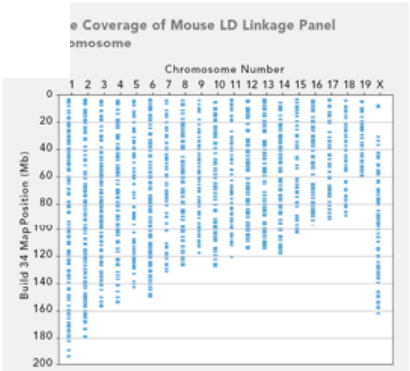
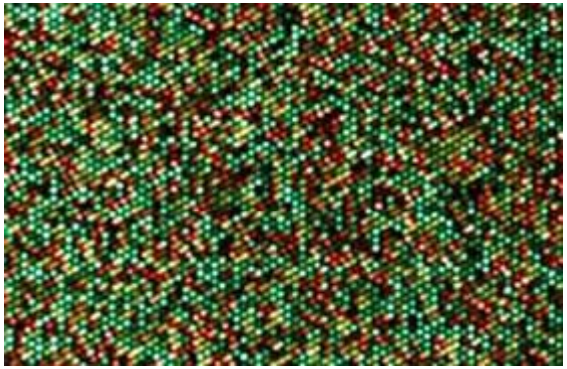
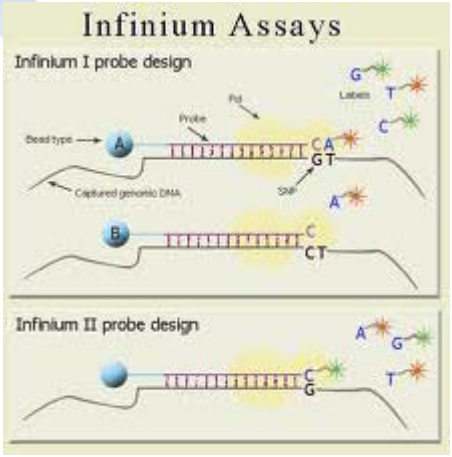
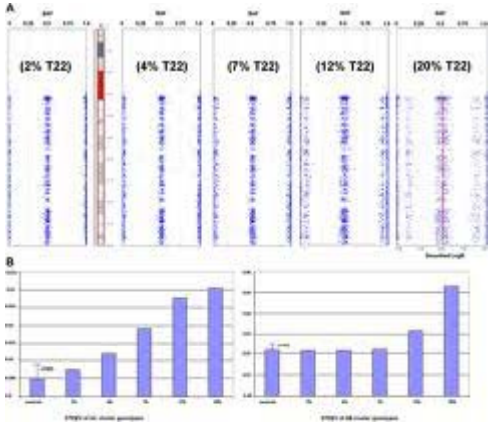


Approximately four SNPs per 27 Mb interval across the genome were chosen for the panel.




Infinium Assays
Infinium I probe design
Infinium II probe design


(2% T22)
(4% T22)
(7% T22)
(12% T22)
(20% T22)

What is that Stringy Stuff?

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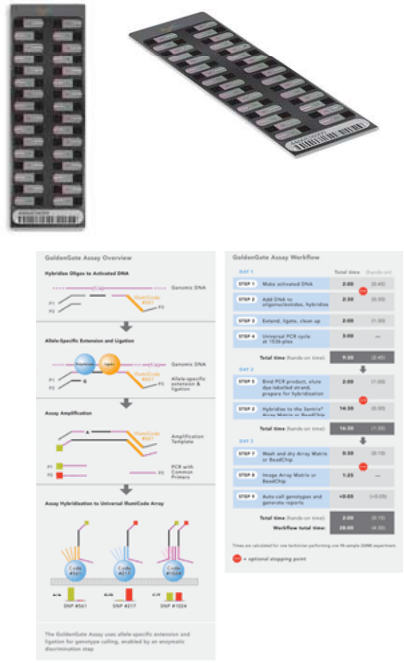
กฟผ.

ผลิตไฟฟ้าเพื่อความสุขของคนไทย

DNA Test Panel Support



Genome Analyzer IIx Instrument

GoldenGate assay highlights

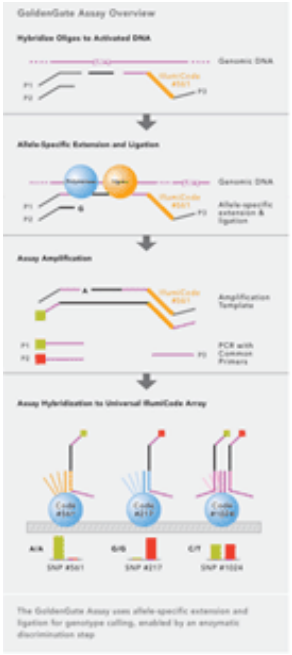
Proven technology: Used in genotyping centers worldwide

High-quality data: Average call rates > 99%

Scalable solution: 96, or from 384 to 3,072 SNPs per sample, 12 to 96 samples in parallel

Streamlined workflow: Manual or automated processing with multiple stopping points

Flexible content: An expanding selection of standard panels or custom panels with the SNP loci of your choice



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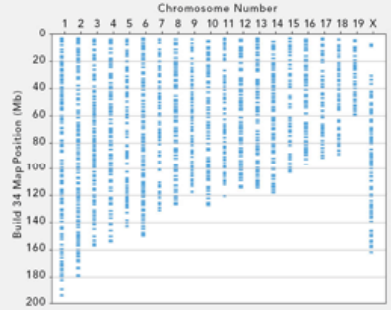
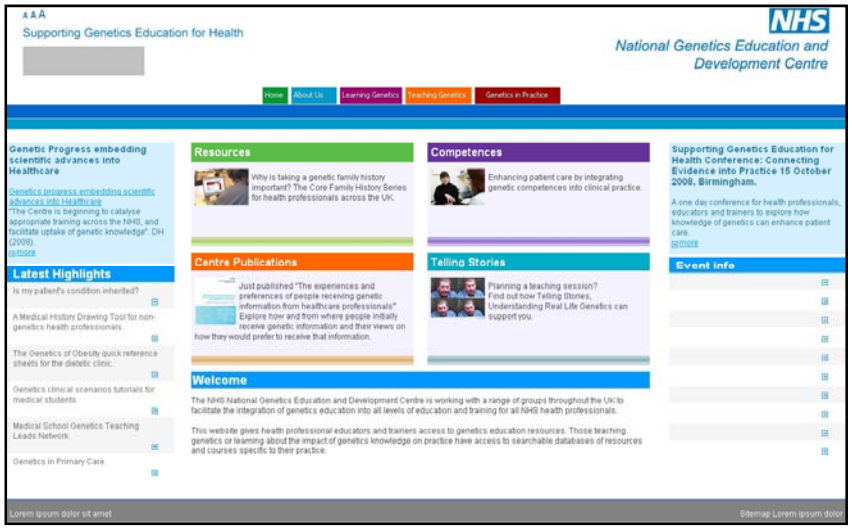


Approximately four SNPs per 27 Mb interval across the genome were chosen for the panel.

www.geneticseducation.nhs.uk

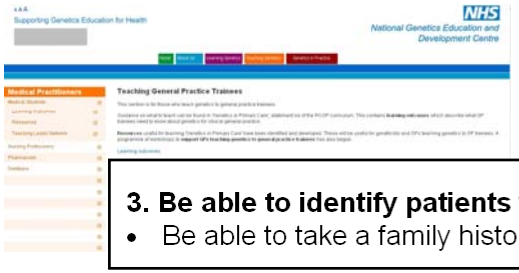
Resource database

- Existing resources
- Resources developed by the Centre



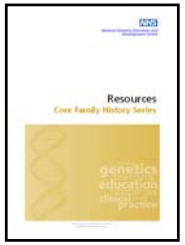
Searchable

- Search all
- Linked to educational outcomes



3. Be able to identify patients with, or at risk of, a genetic condition

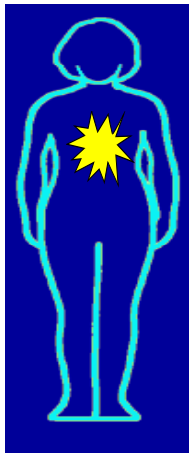
- Be able to take a family history and construct and interpret a pedigree



Evaluated

Most Cancers Arise From Somatic Mutations

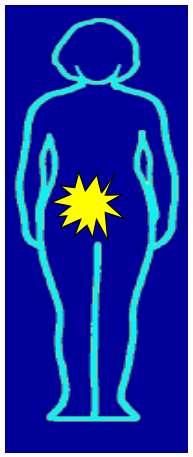
- Somatic mutation
  - Localised to a specific tissue



breast

or

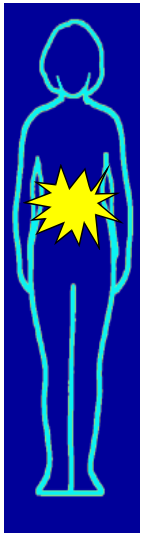
bowel



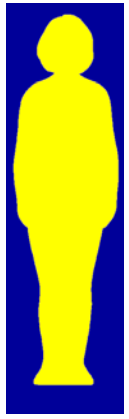
  - Not in germline tissues
  - Not inherited

5-10% of Cancers Arise From Germline Mutations

Parent

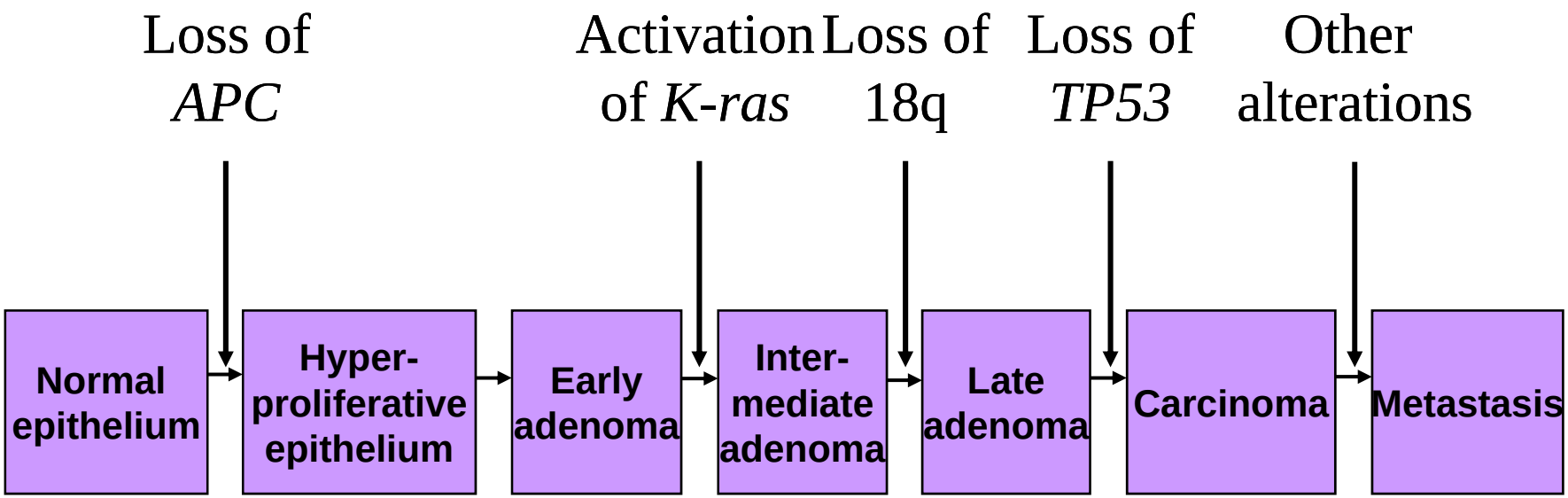


Child



- Germline mutation
  - In egg or sperm
  - May be passed on (inherited)
  - All cells in offspring carry the mutation

Multi-Step Carcinogenesis (eg, Colon Cancer)

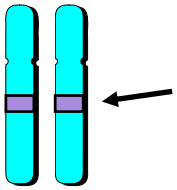


Adapted from Fearon ER. *Cell* 61:759, 1990

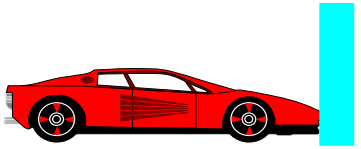
Genes Associated With Cancer

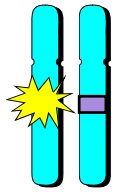
1. **Tumor suppressor genes**:

the cell's brakes for tumor growth

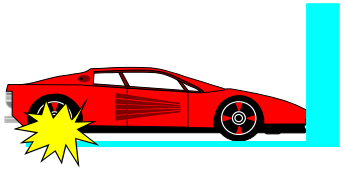


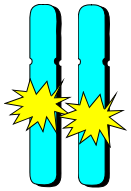
Normal genes
(prevent cancer)



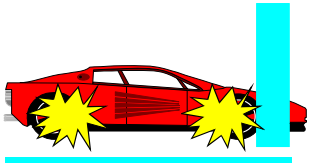


1st mutation
(susceptible carrier)





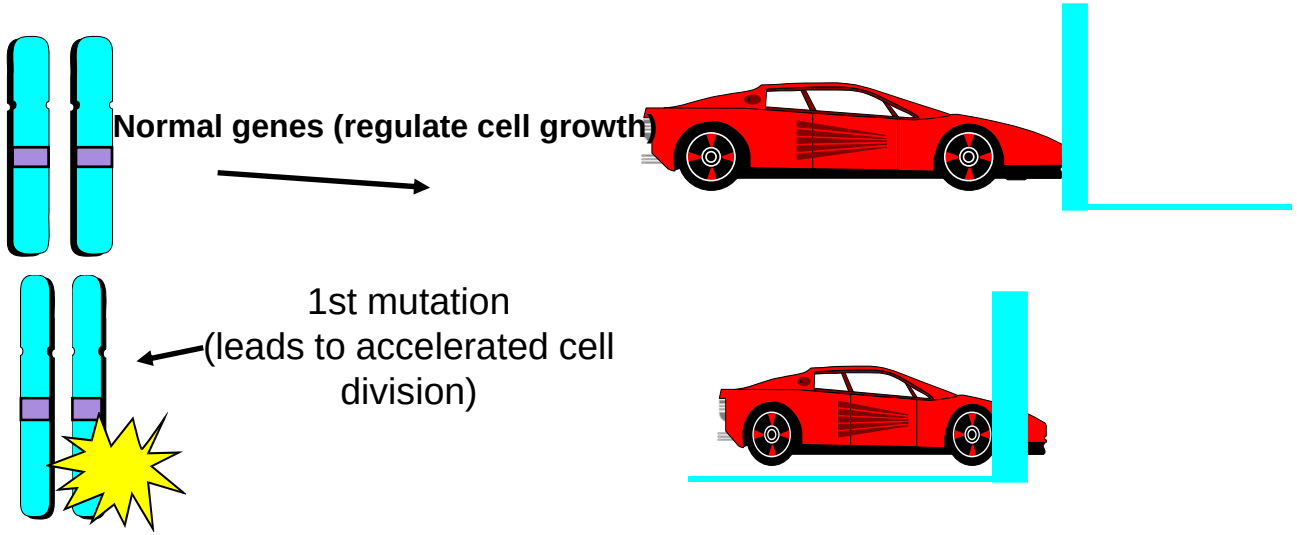
2nd mutation or loss
(leads to cancer)



Genes Associated With Cancer

2. Oncogenes:

accelerate cell division

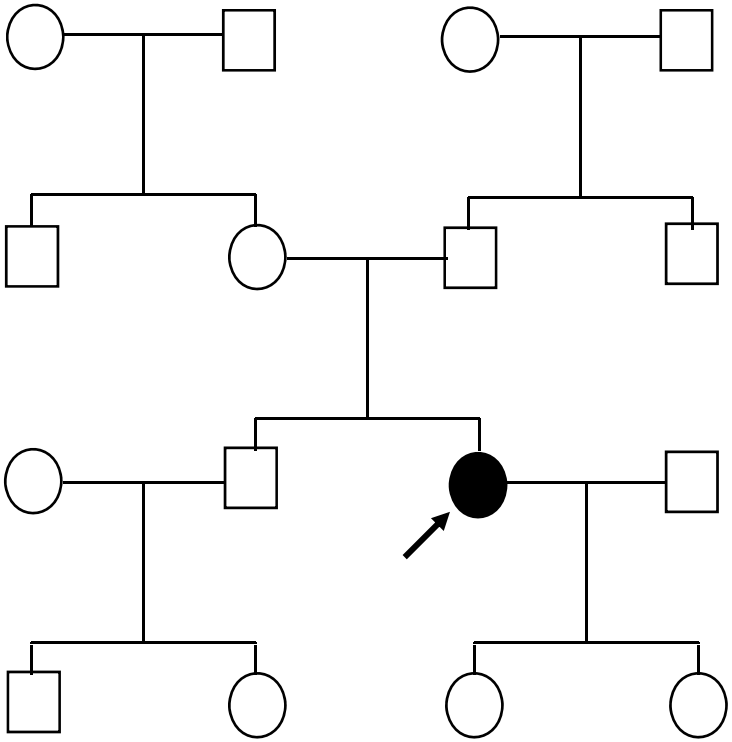


1 mutation sufficient for role in cancer development

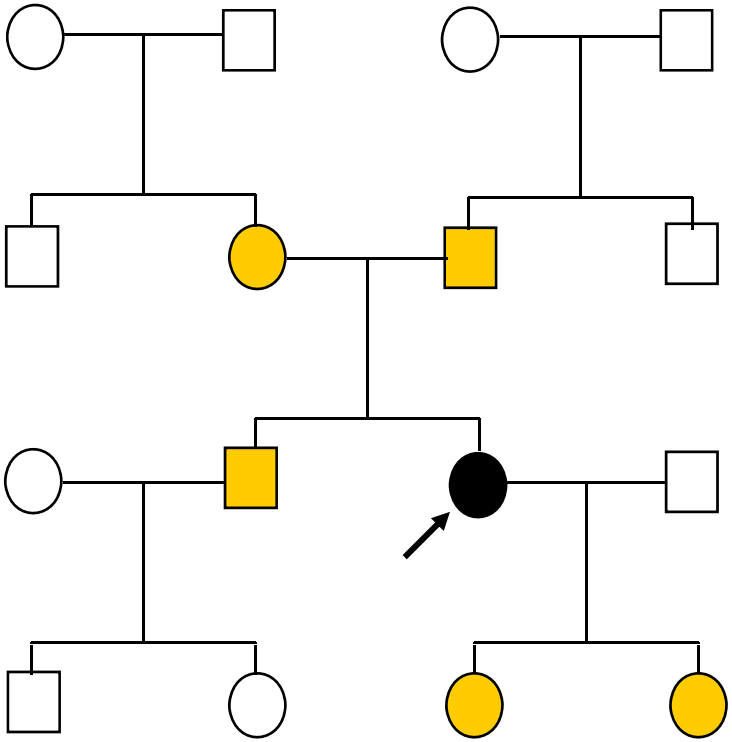
Examples of hereditary cancer genes

1p35	SDHA/B/C	Phaechromocytoma/Paraganglioma
2p16	MSH2	HNPCC
2p16	MSH6	HNPCC
3p21	MLH1	HNPCC
3p25	VHL	von Hippel-Lindau
5q21	APC	Colon polyposis
7p22	PMS2	HNPCC
9p21	P16 (CDKN2)	Melanoma/pancreatic
10q22	PTEN	Cowdens syndrome
10q11	RET	MEN2
11q13	MEN1	MEN1
13q12	BRCA2	HBOC
13q14	RB1	Retinoblastoma
16q22.1	CDH1	Gastric cancer
17p13	TP53	Li-Fraumeni
17q21	BRCA1	HBOC

Half of the population have had a 1st or 2nd degree relative diagnosed with cancer

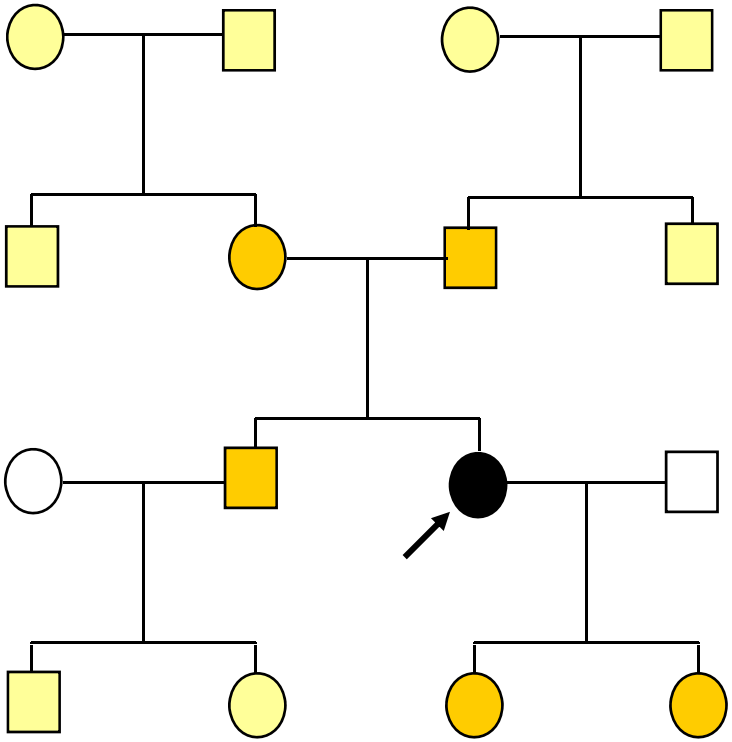


Half of the population have had a 1st or 2nd degree relative diagnosed with cancer



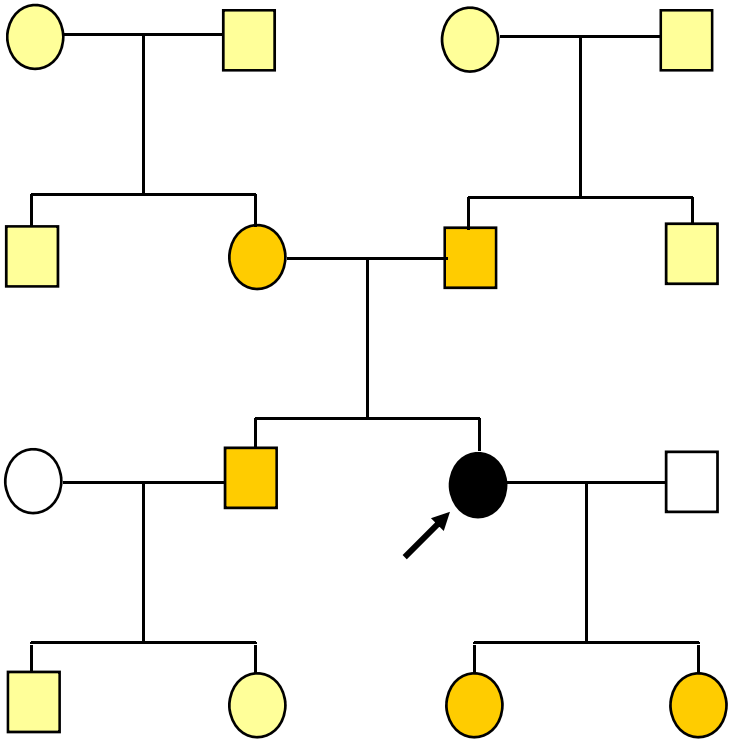
1st degree (parents, siblings, children)

Half of the population have had a 1st or 2nd degree relative diagnosed with cancer



2nd degree (grandparents, aunts, uncles, nieces, nephews)

Half of the population have had a 1st or 2nd degree relative diagnosed with cancer



2nd degree (grandparents, aunts, uncles, nieces, nephews)

Only 5% - 10% will have an inherited genetic factor

Gene mutations and inherited cancer

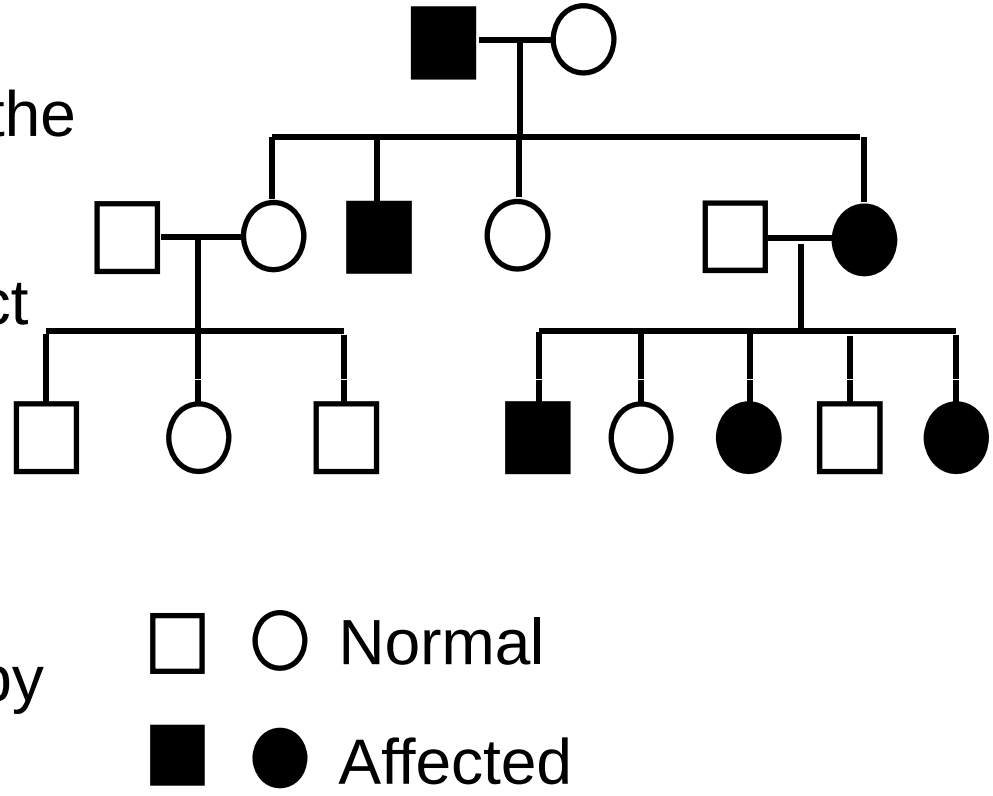
How are cancer predispositions inherited?

Hereditary Breast Cancer (high risk)

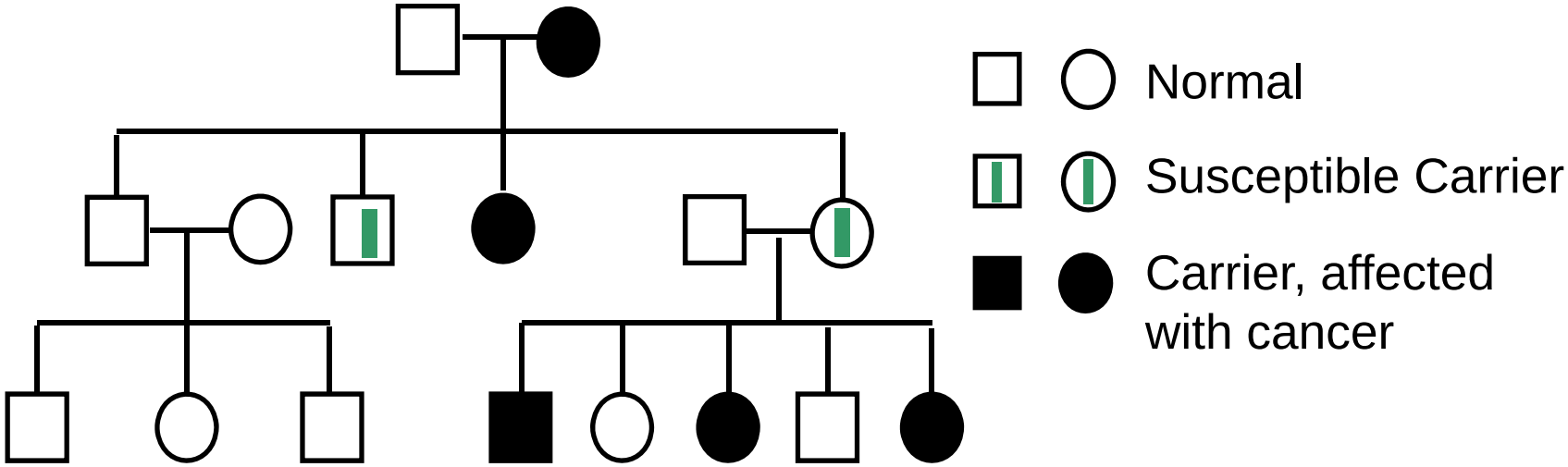
- ~5% of all Breast Cancers
- Monogenetic disorder
- Autosomal dominant inheritance
- High penetrance
- Early onset
- Cancer syndrome (other cancers also)
- BRCA1+2 only known genes of major importance

Autosomal Dominant Inheritance

- Each child has 50% chance of inheriting the mutation
- Equally likely to affect males and females
- No “skipped generations”
- Equally transmitted by men and women

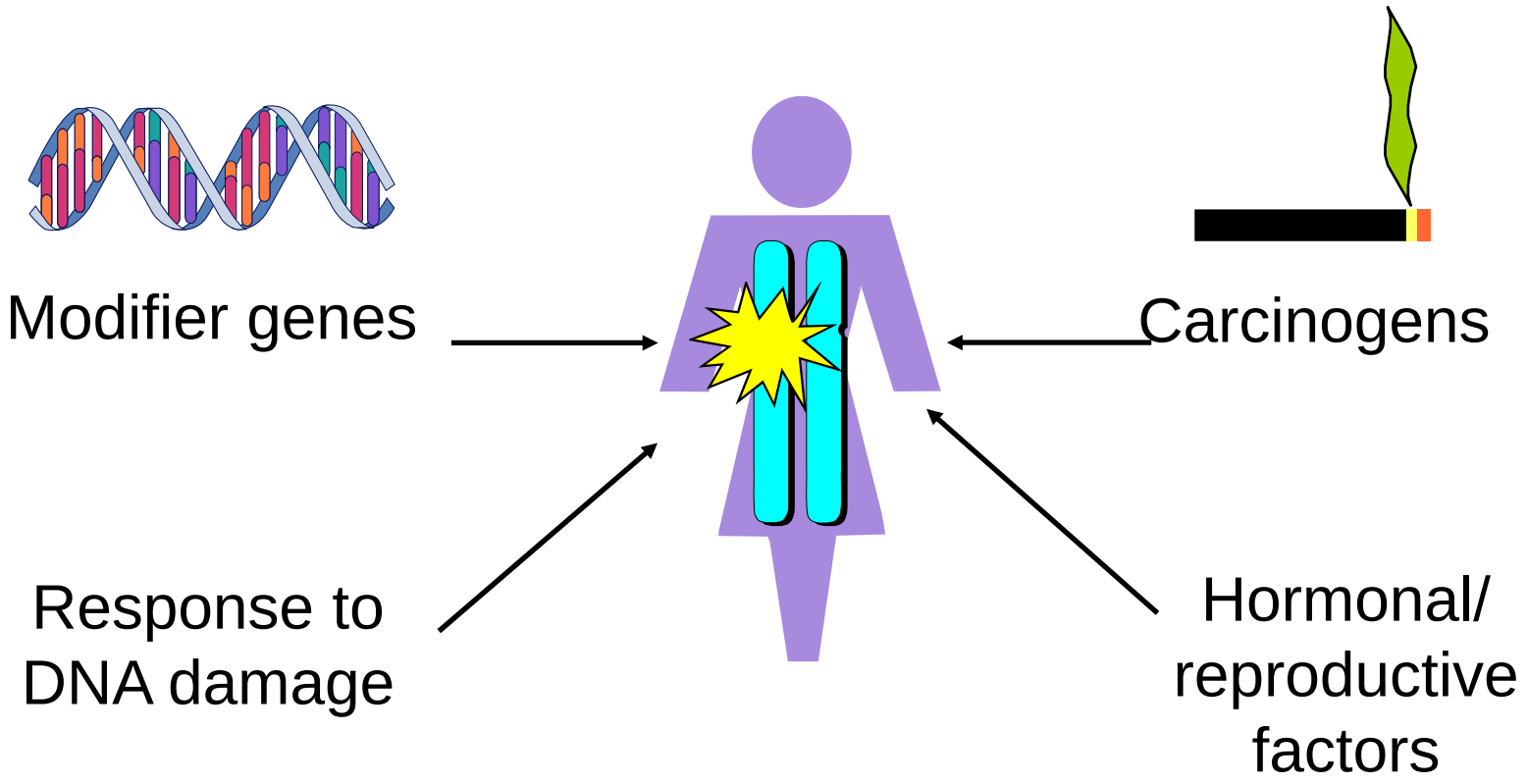


Penetrance



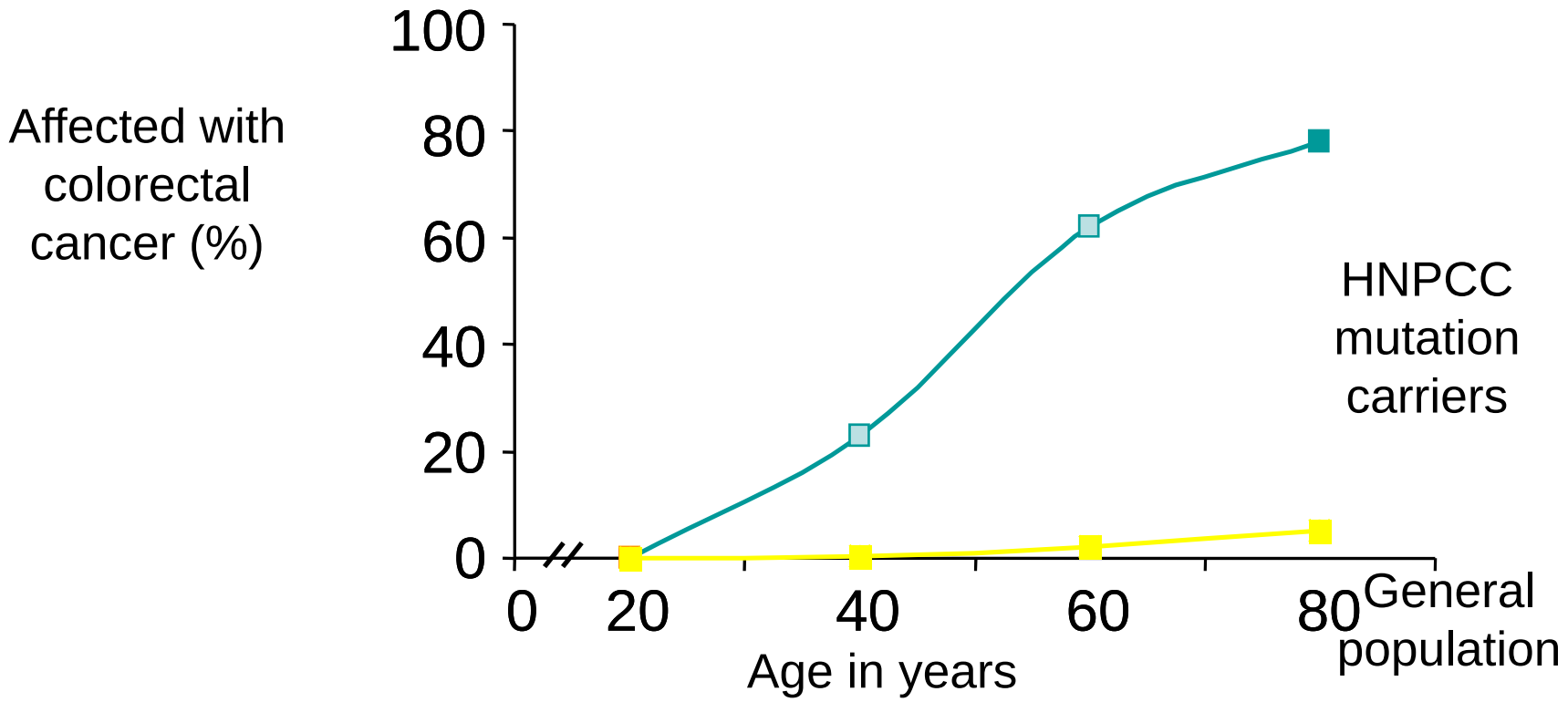
- May appear to “skip” generations
- Individuals inherit altered cancer susceptibility gene - not cancer

Factors Affecting Penetrance



Not everyone with an altered gene develops cancer

Age-Specific Penetrance

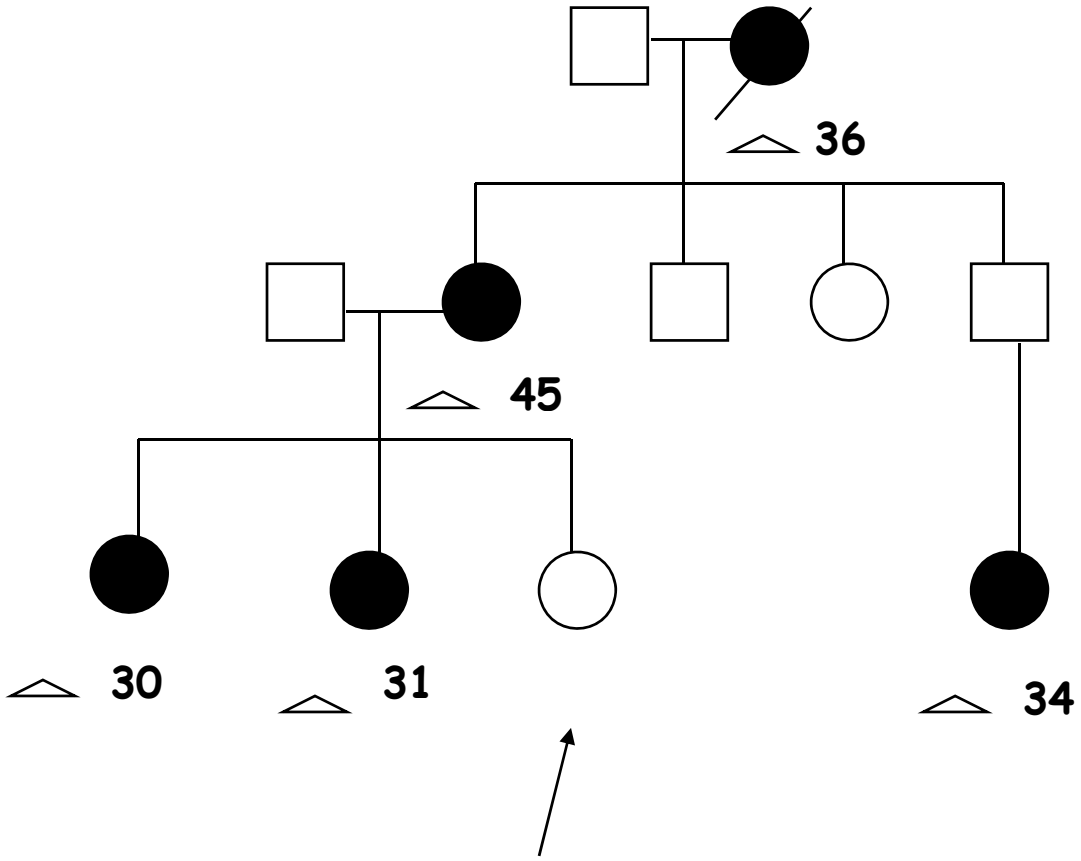


Modified from Aarnio M et al. *Int J Cancer* 64:430, 1995

High Risk Indicators

- Multiple family members with tumours at same site
- Early age of onset
- History of individuals with multiple primary tumours
- Recognised associations:
  - Breast/ovary
  - Bowel/Endometrium
  - etc.

Breast Cancer



NICE - familial breast cancer

Mammographic surveillance

High risk:

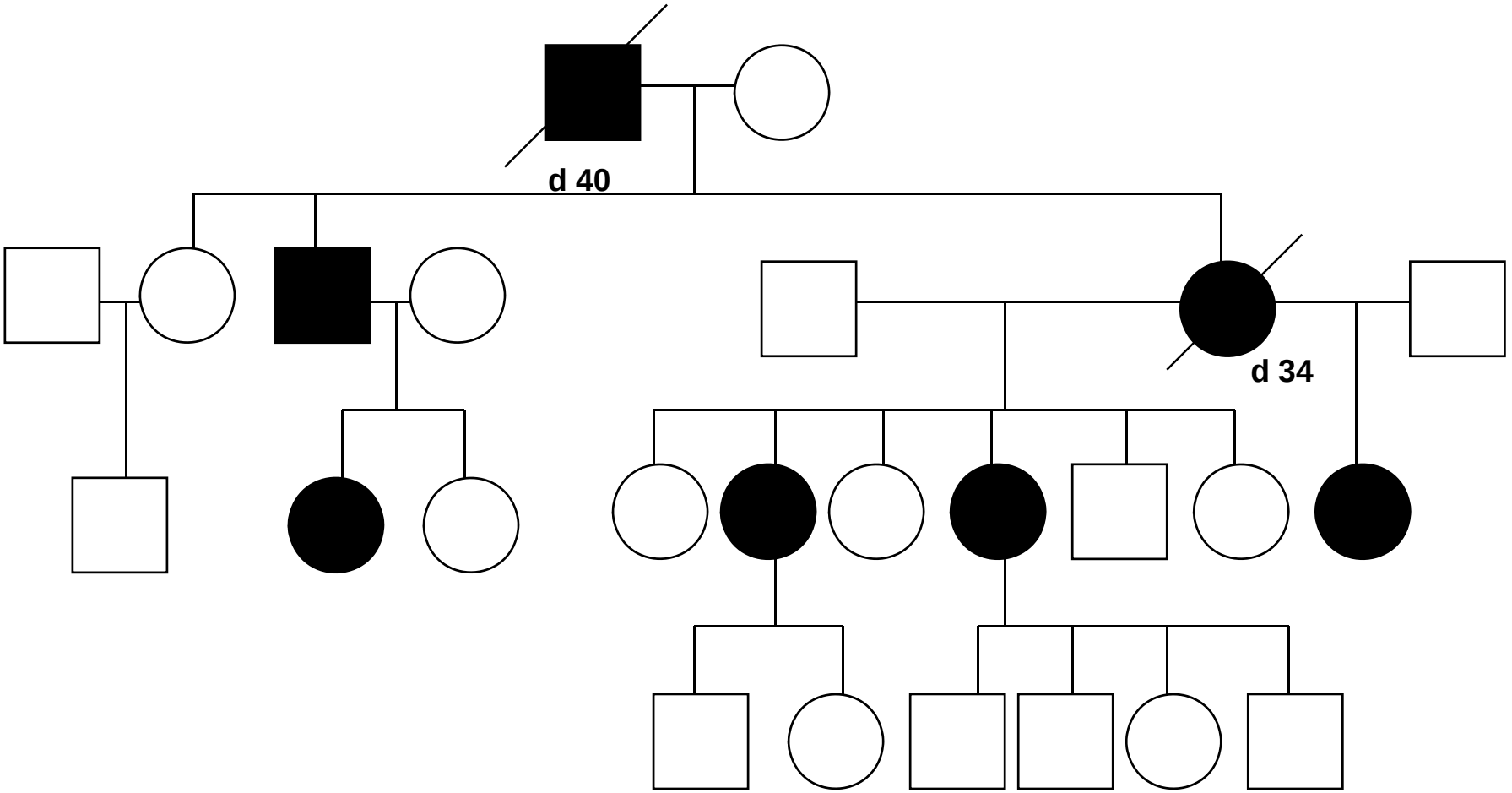
30-40 *individualised strategies*

– Mammography or MRI?

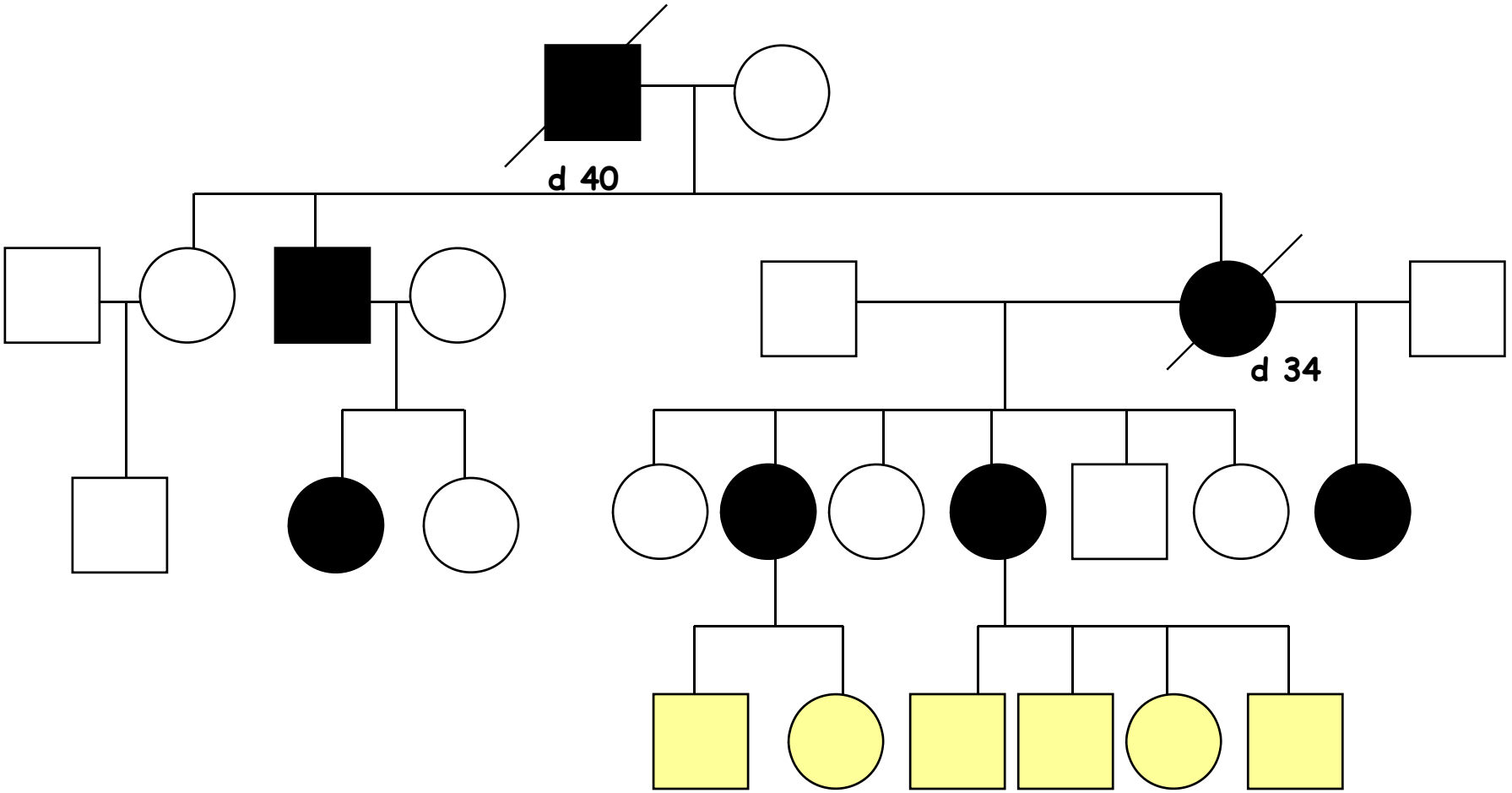
40-50 annual

50+ *individualised strategies*

FAP



FAP



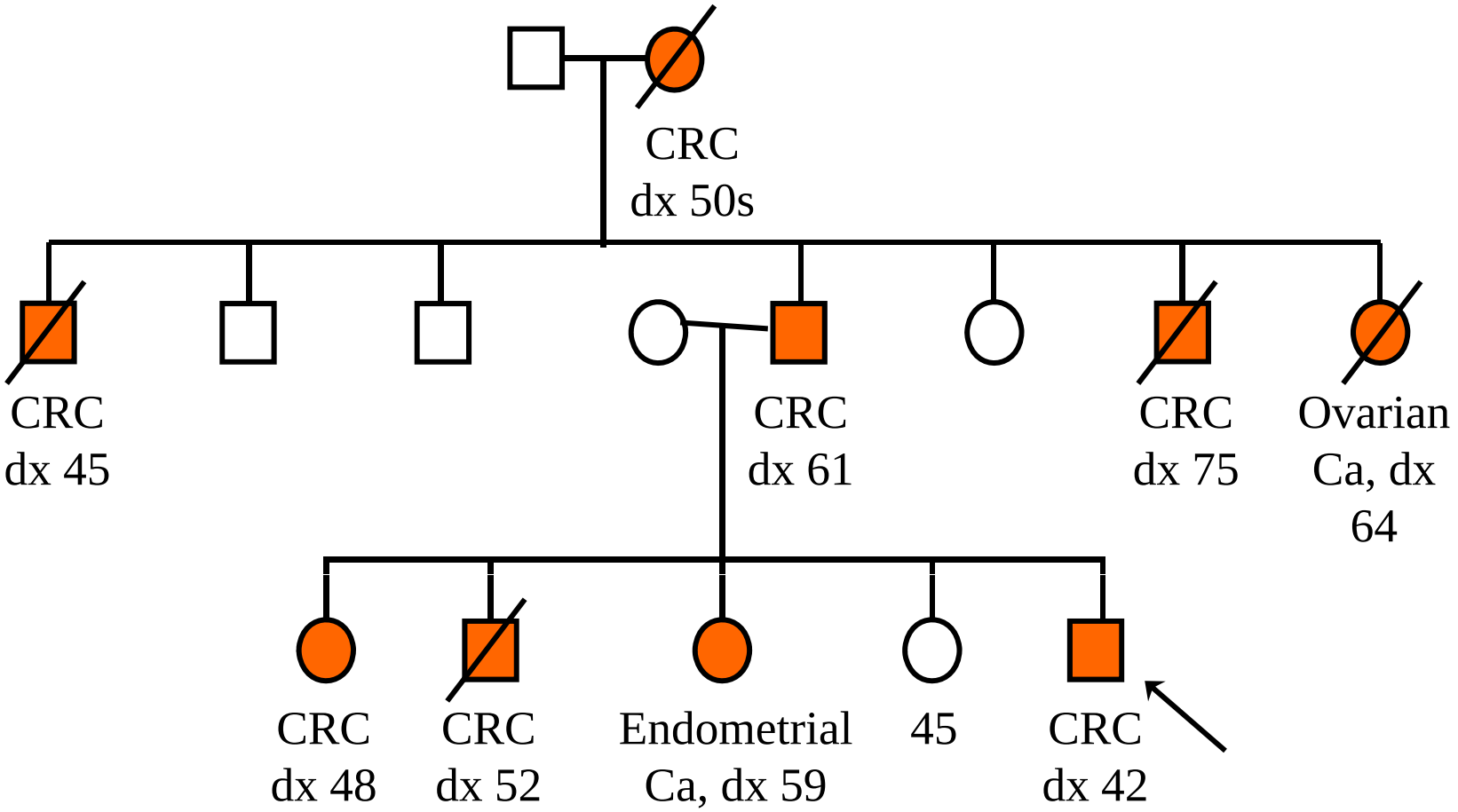
Genetics of FAP

- Caused by mutations in APC gene (found on chromosome 5)
- ~30% occur as the result of new mutations
- Correlation between position of mutation &:
  - Severity of effect
  - Presence of CHRPEs and desmoids

FAP: Key Points

- CRC risk is 100% in untreated FAP patients
- Genetic testing identifies most *APC* mutation carriers
- Endoscopic surveillance and prophylactic colectomy can improve survival in at-risk patients
- Non-carriers can be spared anxiety and the need for increased surveillance

Family History is the key to diagnosing HNPCC



Genetic Features of HNPCC

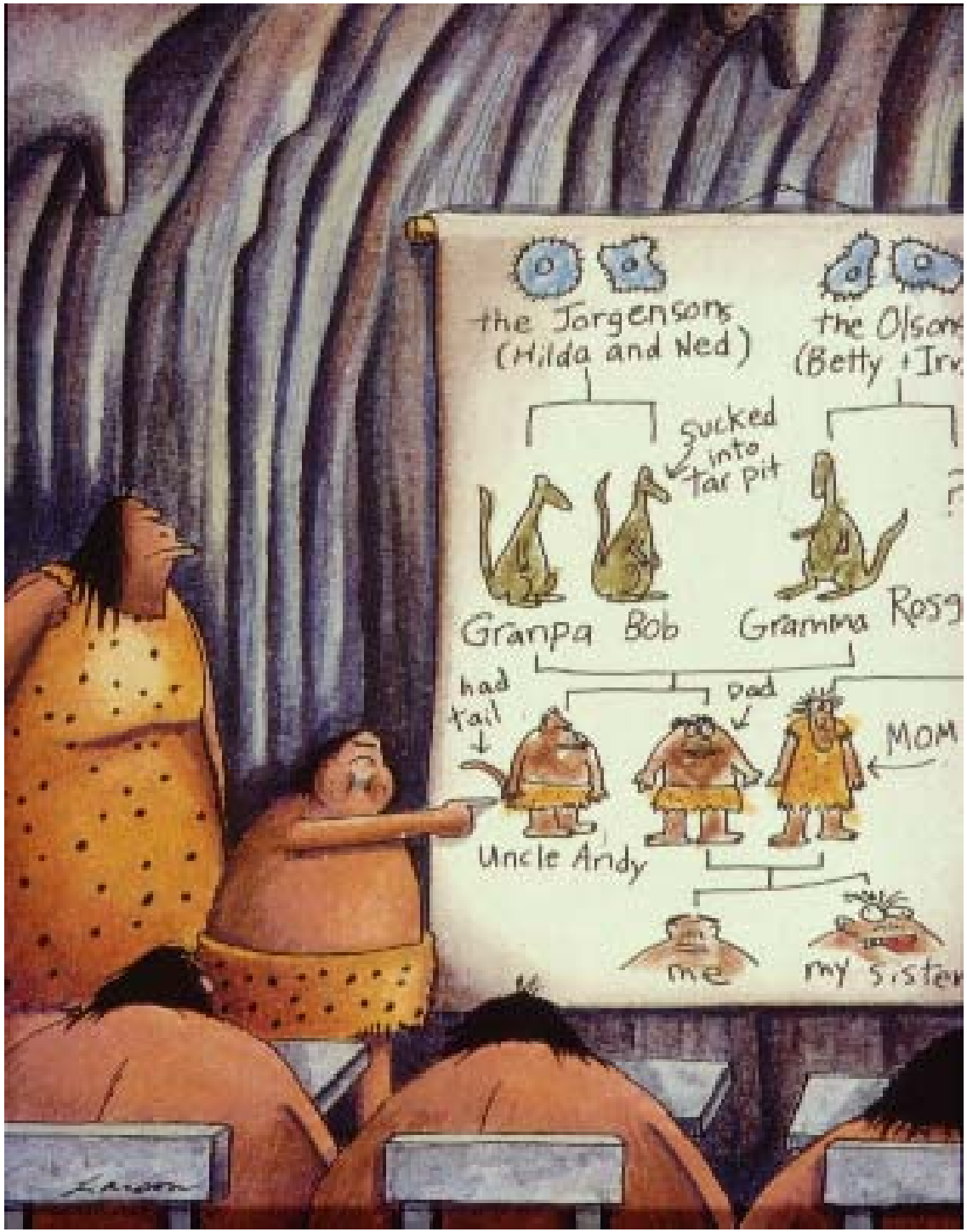
- Autosomal dominant inheritance
- Penetrance ~80%
- Genes belong to DNA mismatch repair family
- A number of genes involved
  (*MLH1, MSH2, MSH6, PMS1, PMS2*)

Amsterdam Criteria

- 3 or more relatives with CRC
- One case a 1º degree relative of the others
- Two or more generations
- One CRC by age 50
- FAP excluded

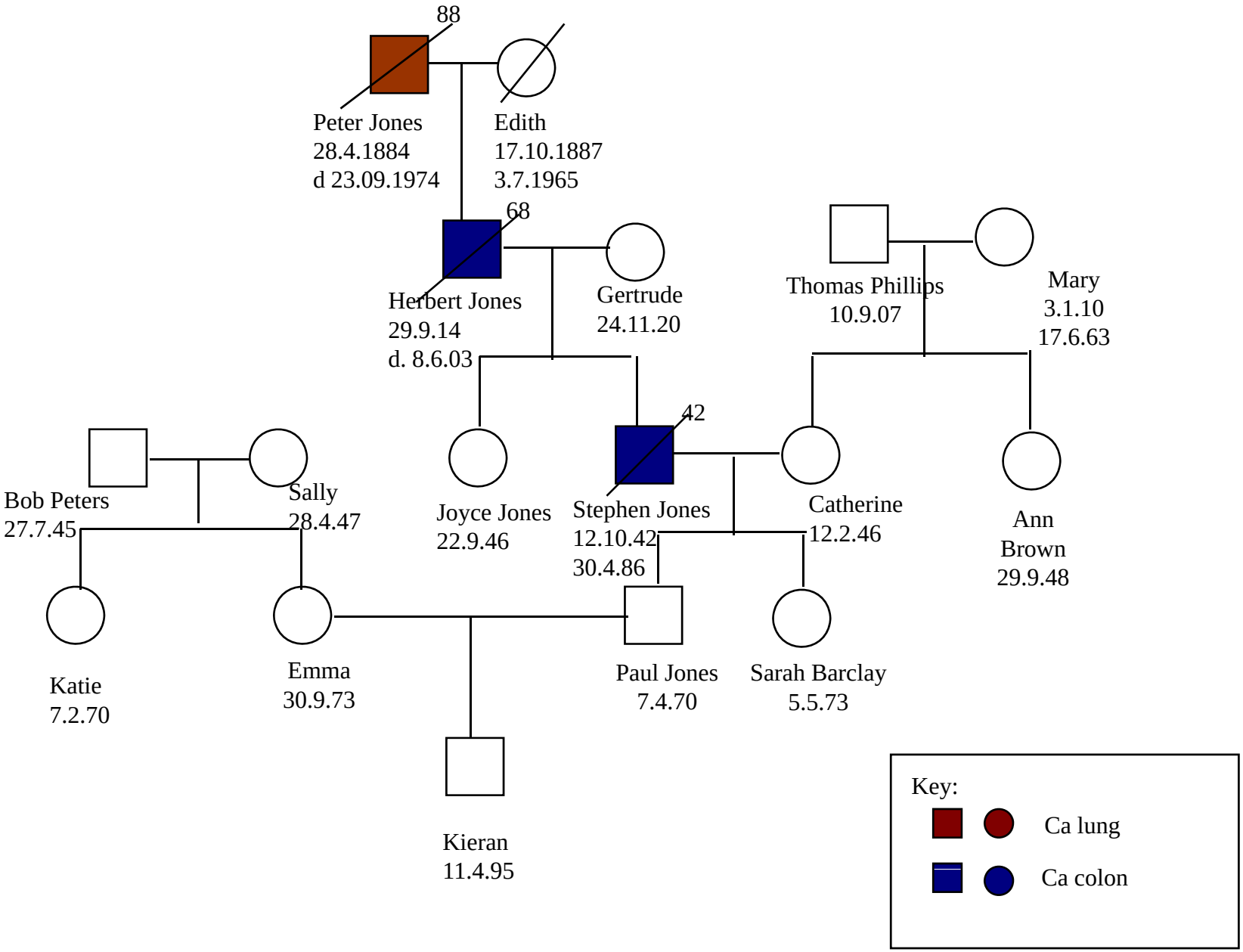
- Modified Amsterdam criteria: An endometrial cancer can be substituted for one of the CRC

Taking a Pedigree

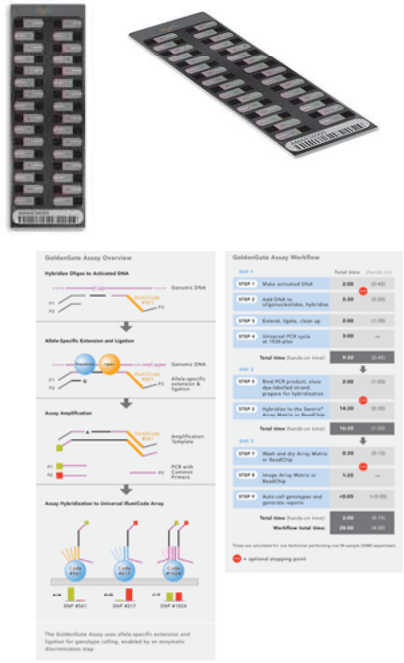


Drawing up the family tree gives information about the relatives and also:

- **helps establish the family agenda and dynamics**
- **may reveal individuals interpretation and beliefs about what is happening in the family**
- **Has the potential to raise issues of paternity**


88
Peter Jones
28.4.1884
d 23.09.1974
Edith
17.10.1887
3.7.1965
68
Herbert Jones
29.9.14
d. 8.6.03
Gertrude
24.11.20
Thomas Phillips
10.9.07
Mary
3.1.10
17.6.63
42
Bob Peters
27.7.45
Sally
28.4.47
Joyce Jones
22.9.46
Stephen Jones
12.10.42
30.4.86
Catherine
12.2.46
Ann
Brown
29.9.48
Katie
7.2.70
Emma
30.9.73
Paul Jones
7.4.70
Sarah Barclay
5.5.73
Kieran
11.4.95
Key:
Ca lung
Ca colon

DNA Test Panel Support



Genome Analyzer IIx Instrument

GoldenGate assay highlights

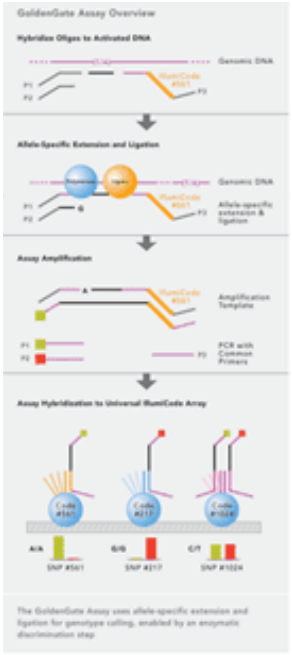
Proven technology: Used in genotyping centers worldwide

High-quality data: Average call rates > 99%

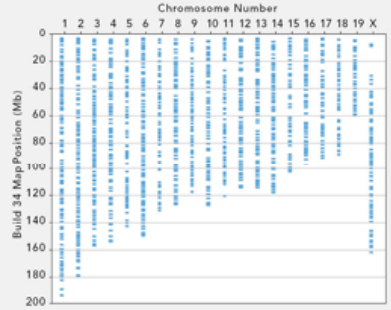
Scalable solution: 96, or from 384 to 3,072 SNPs per sample, 12 to 96 samples in parallel

Streamlined workflow: Manual or automated processing with multiple stopping points

Flexible content: An expanding selection of standard panels or custom panels with the SNP loci of your choice



The GoldenGate Assay uses allele-specific extension and ligation for genotype calling, enabled by an enzymatic discrimination step

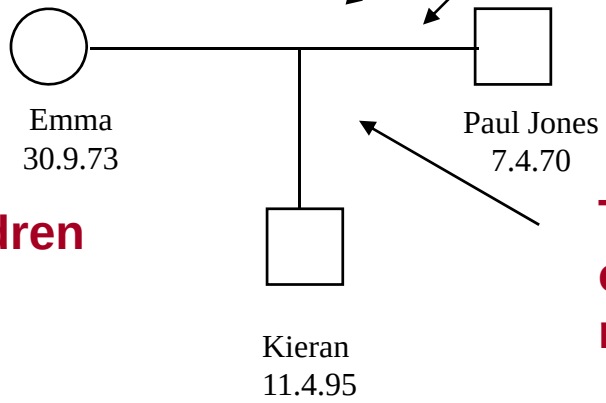


Approximately four SNPs per 27 Mb interval across the genome were chosen for the panel.

Start with the couple being seen

Use clear symbols: circles for females, squares for males

The horizontal line denotes a relationship (males usually on the left, females on right)

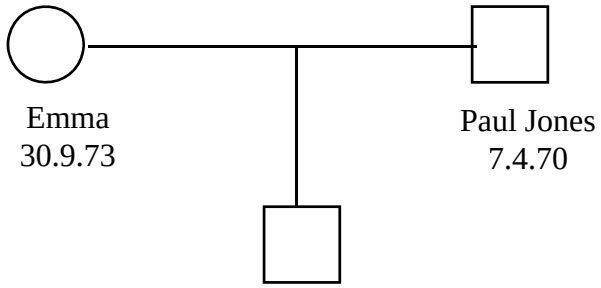


Add in their children

The vertical line denotes offspring of the relationship

“Have you had any children with other partners?”

Record names, dates of birth

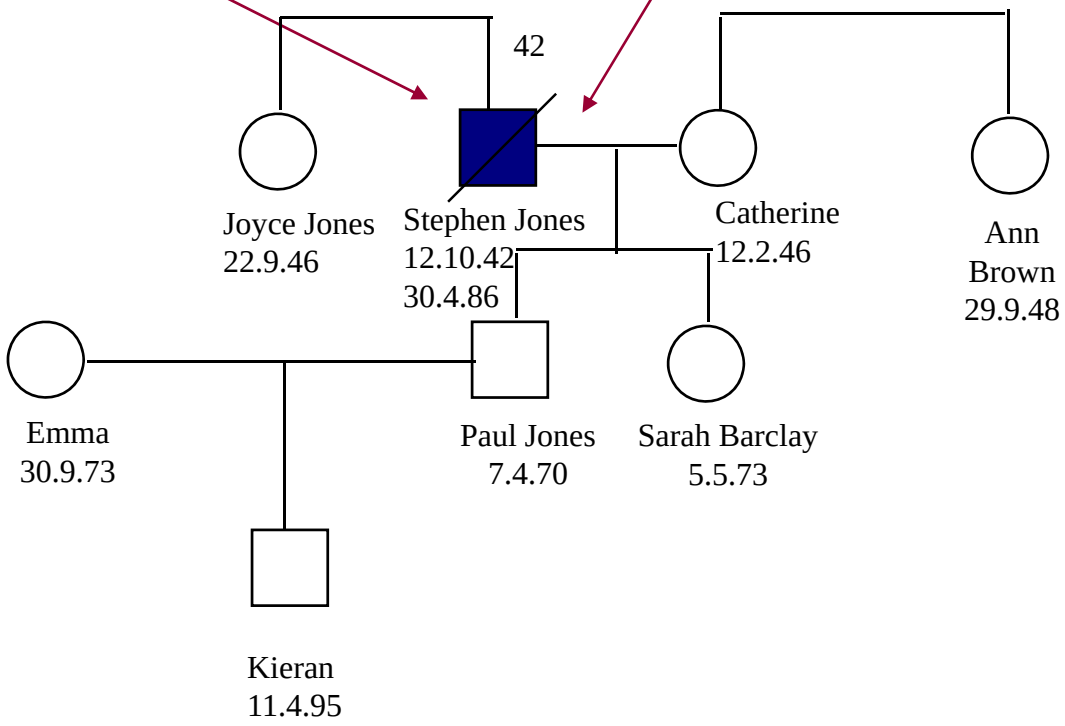


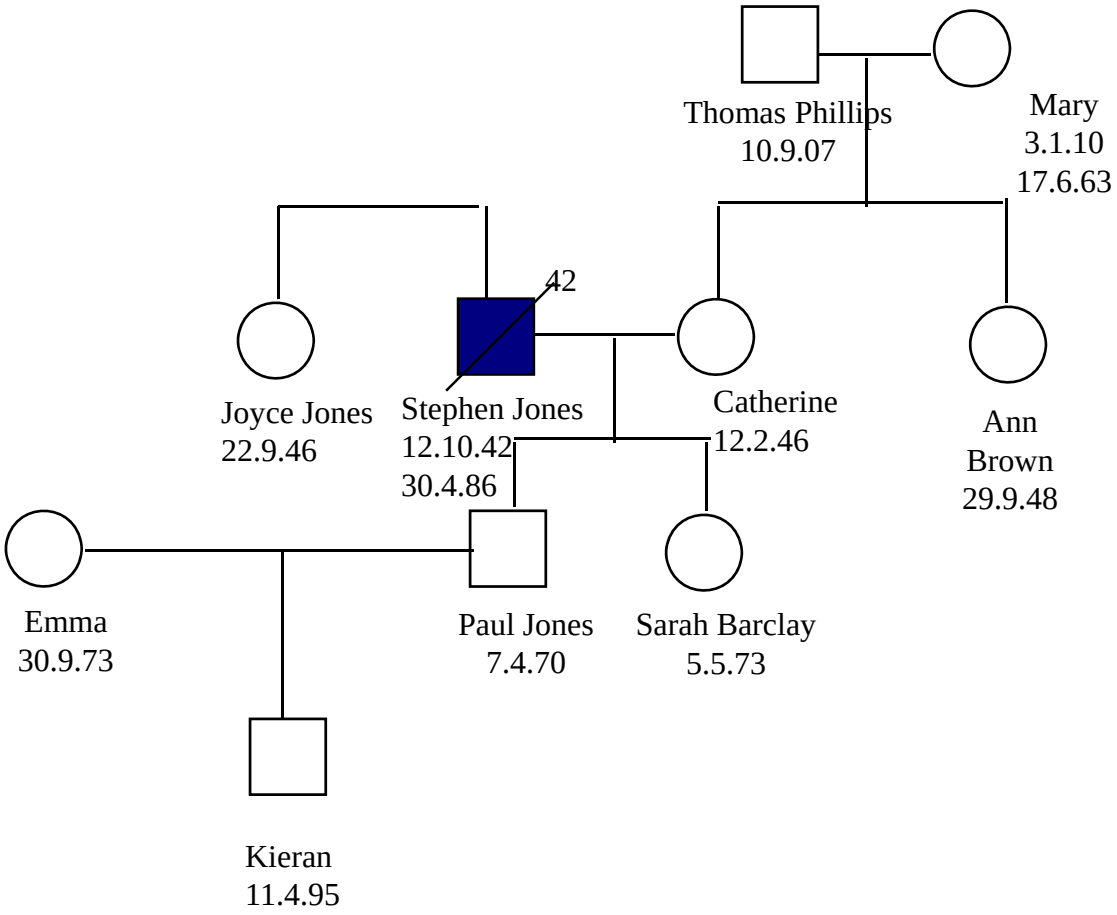
Choose one parent and ask about:
brothers and sister and their children
parents and
grandparents
Make sure you ask about ethnicity

Colour in the symbol if the person is affected

Add the age at which diagnosis was made

Put a sloping line through the symbol (from the bottom left hand corner) if the person has died




Thomas Phillips
10.9.07
Mary
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Joyce Jones
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Stephen Jones
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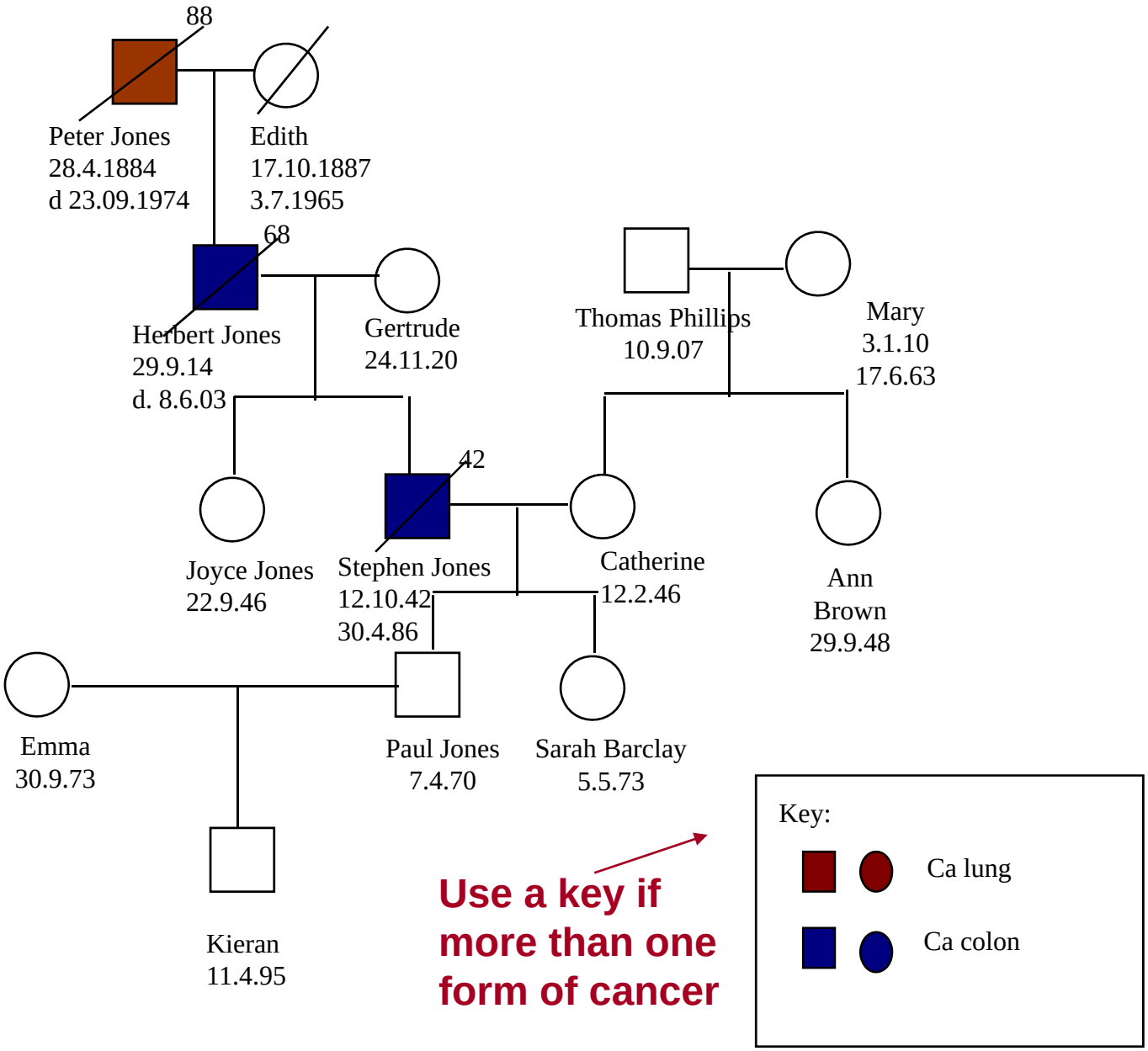
Paul Jones
7.4.70
Sarah Barclay
5.5.73
Kieran
11.4.95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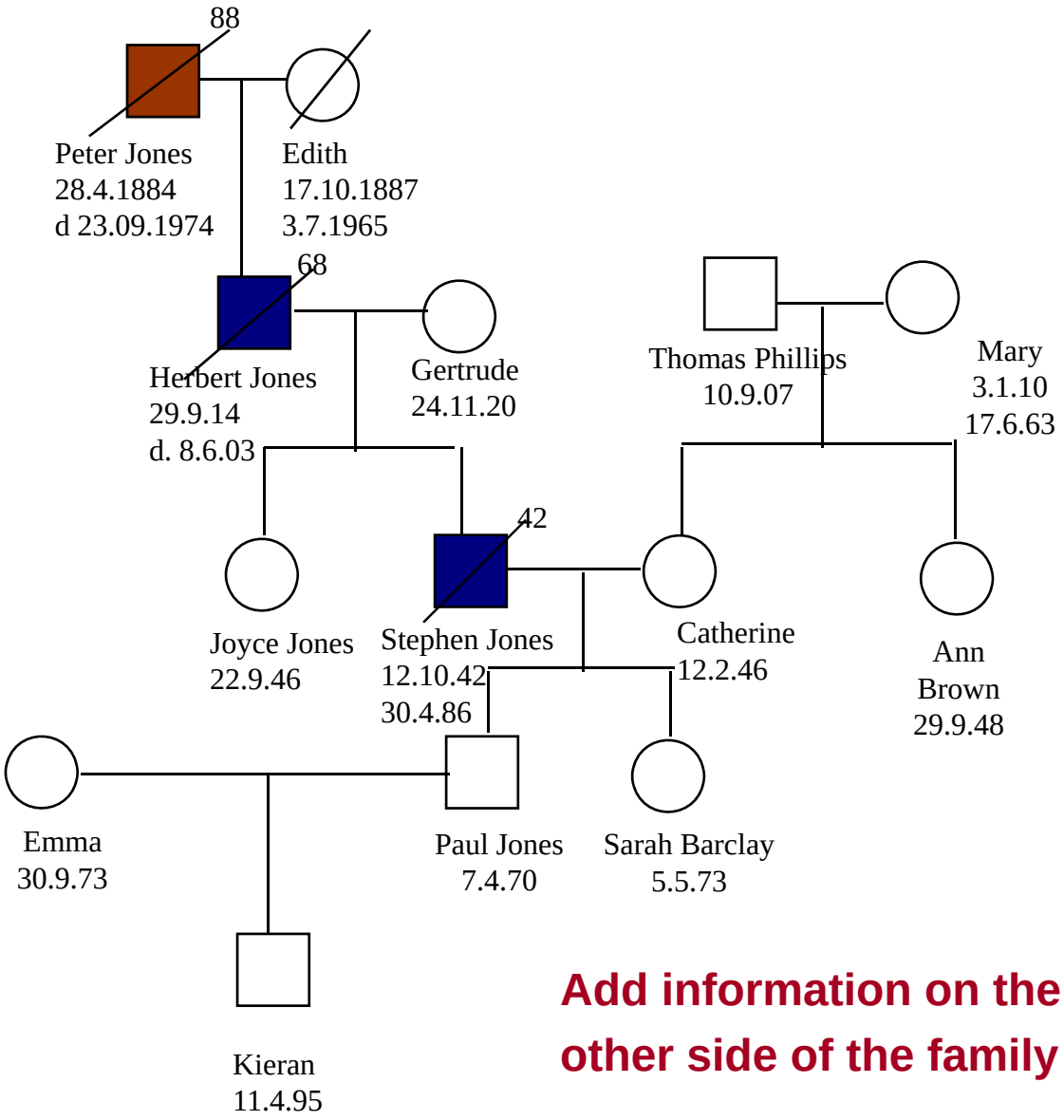
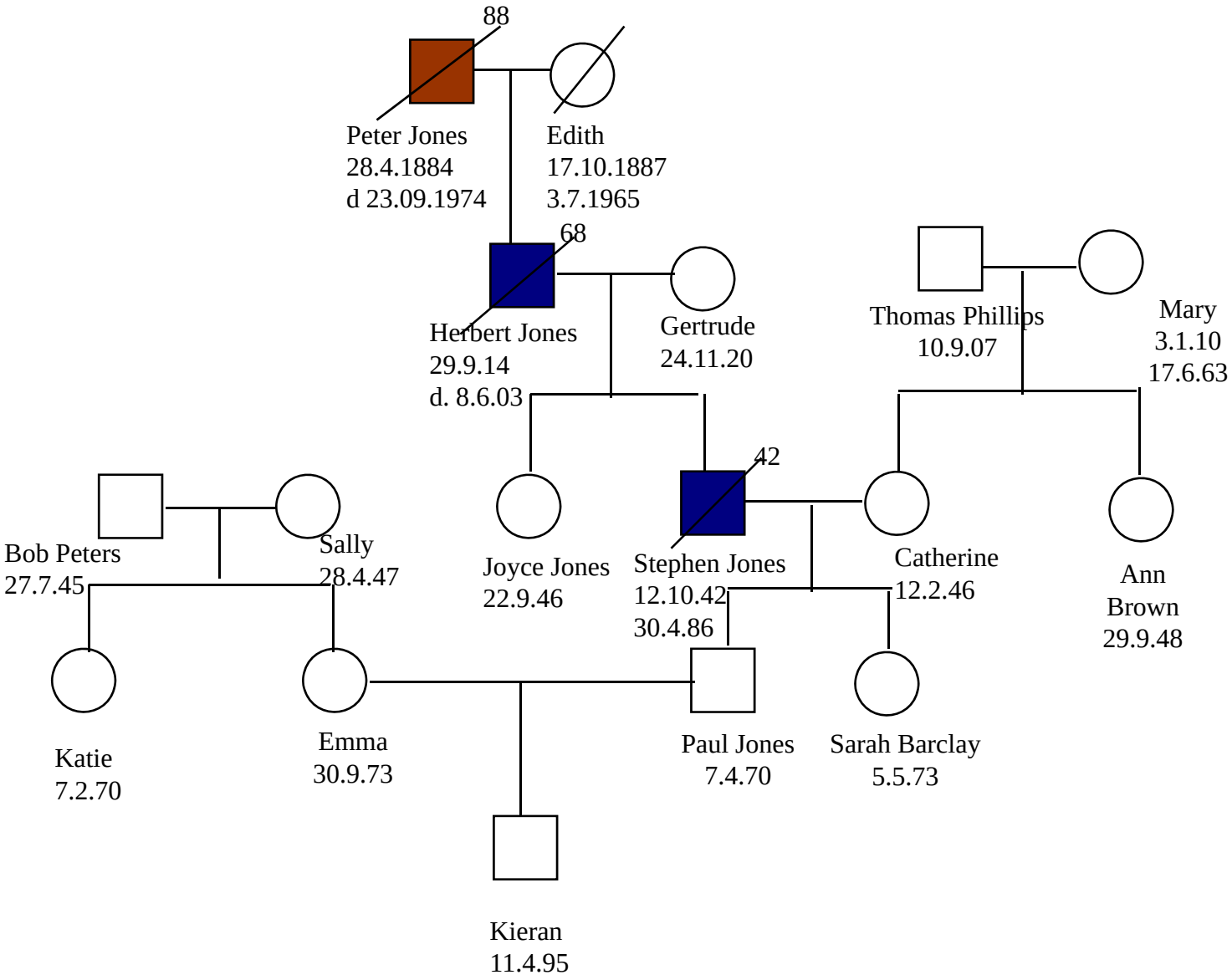
Paul Jones
7.4.70
Sarah Barclay
5.5.73
Kieran
11.4.95
Use a key if more than one form of cancer
Key:
Ca lung
Ca colon



Add information on the other side of the family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Sarah Barclay
5.5.73
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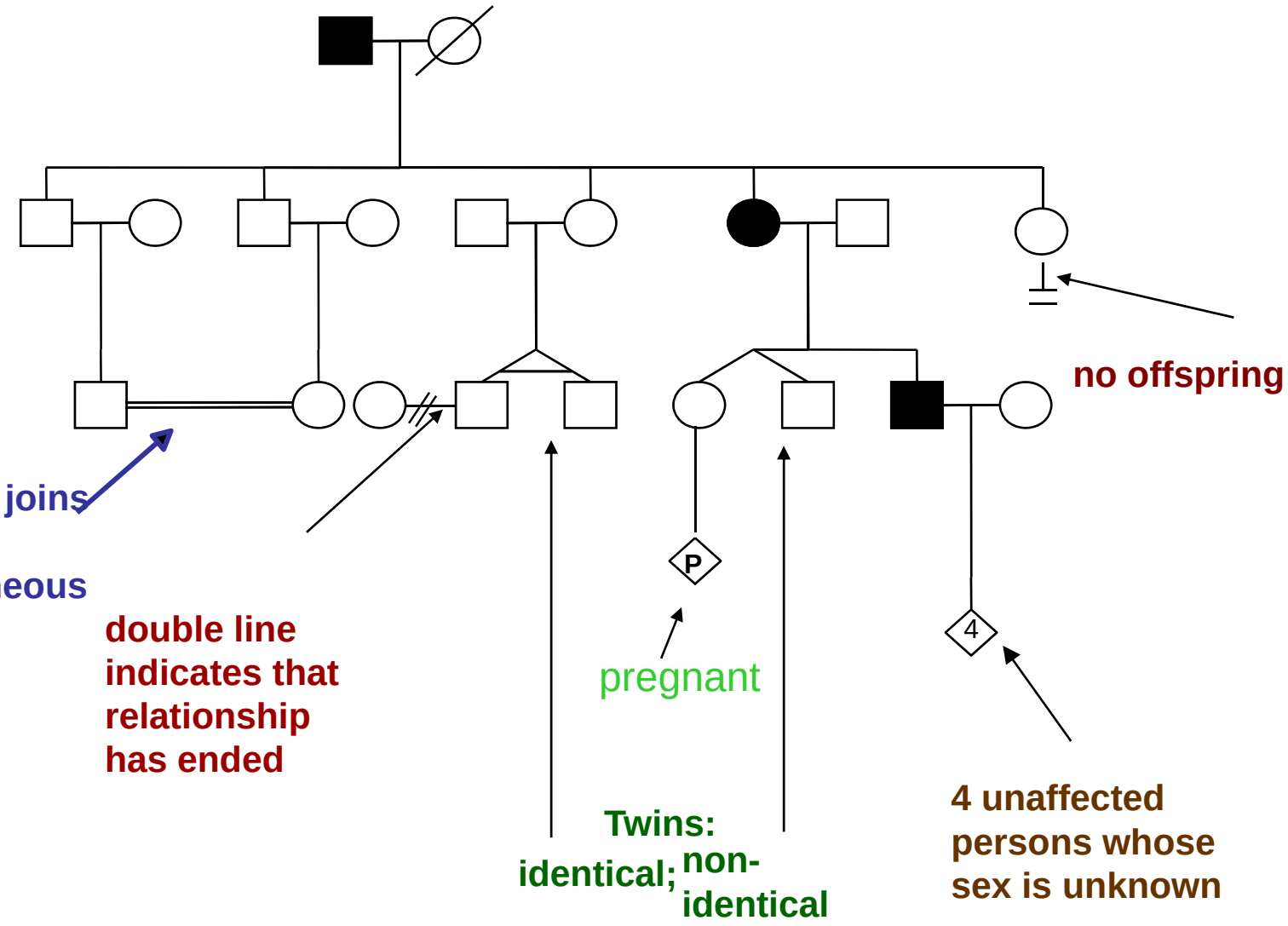
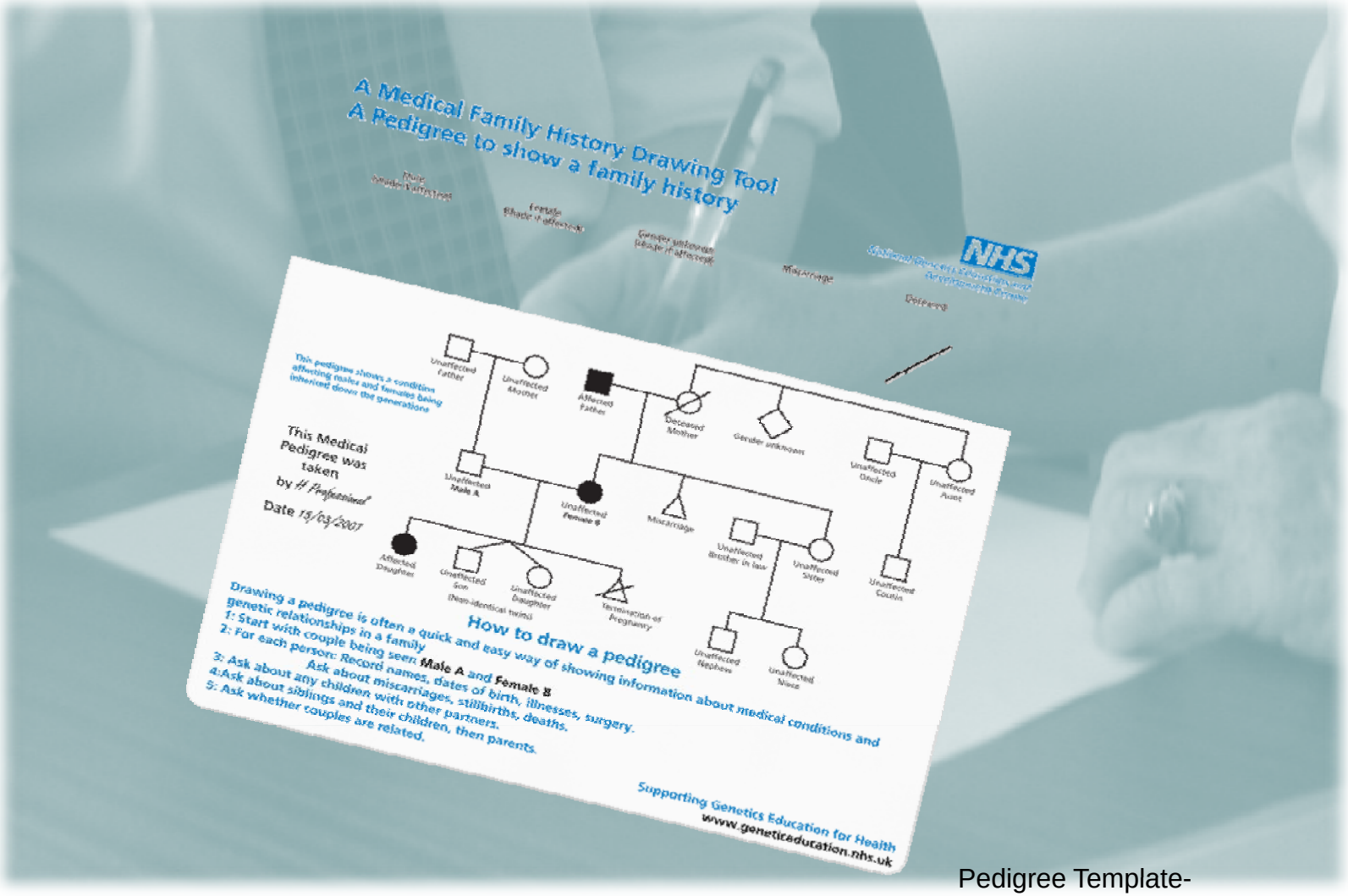
11.4.95

Other pedigree symbols



Drawing a pedigree

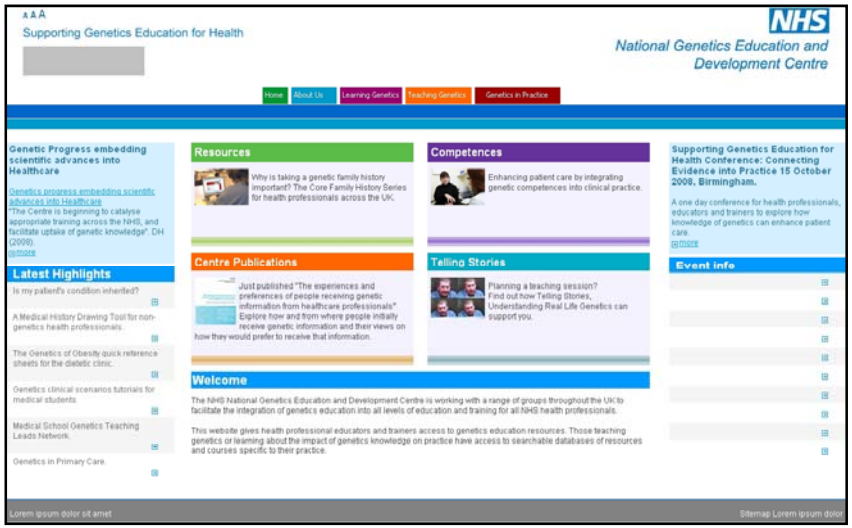


Pedigree Template-
One of the resources available from the NHS National Genetics Education and Development Centre

www.geneticseducation.nhs.uk

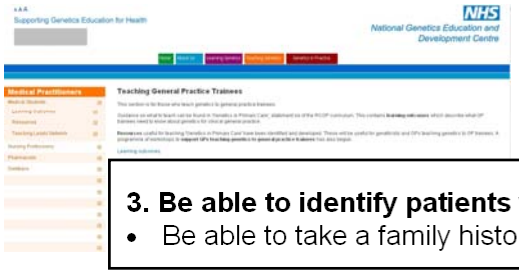
Resource database

- Existing resources
- Resources developed by the Centre



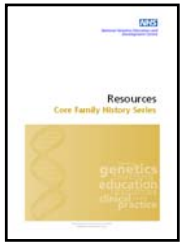
Searchable

- Search all
- Linked to educational outcomes



3. Be able to identify patients with, or at risk of, a genetic condition

- Be able to take a family history and construct and interpret a pedigree



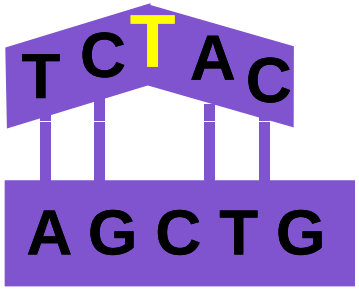
Evaluated

Genes Associated With Cancer

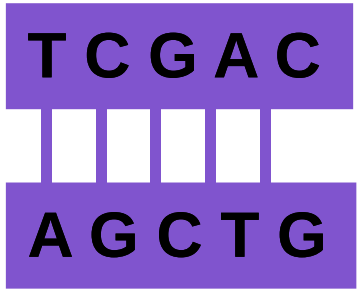
3. DNA damage-response genes:

the repair mechanics for DNA

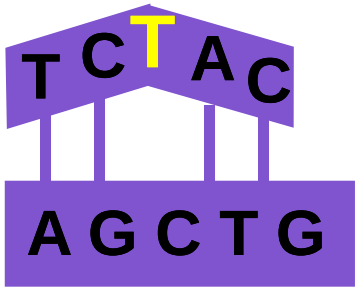
Base pair mismatch



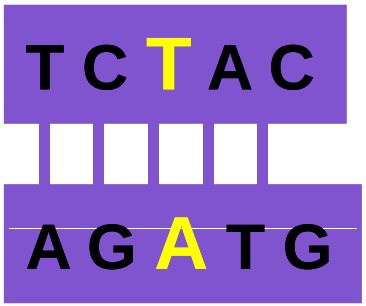
Normal DNA repair



DNA damage-response genes:



Base pair mismatch



Mutation introduced by unrepaired DNA