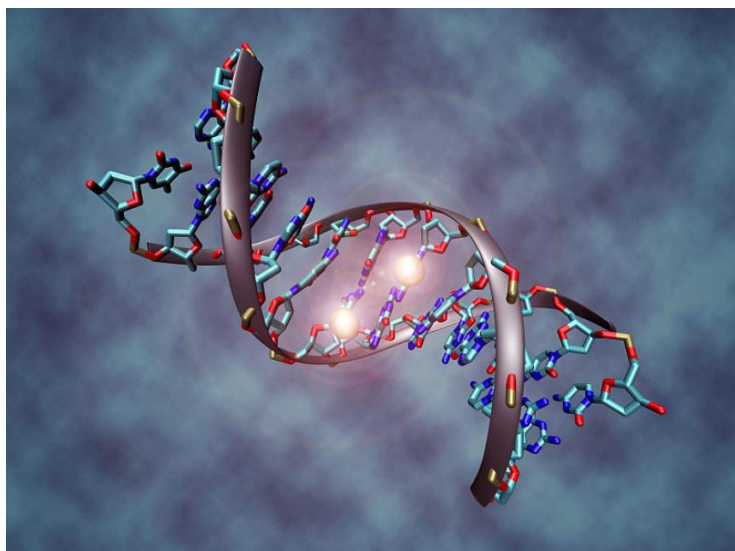
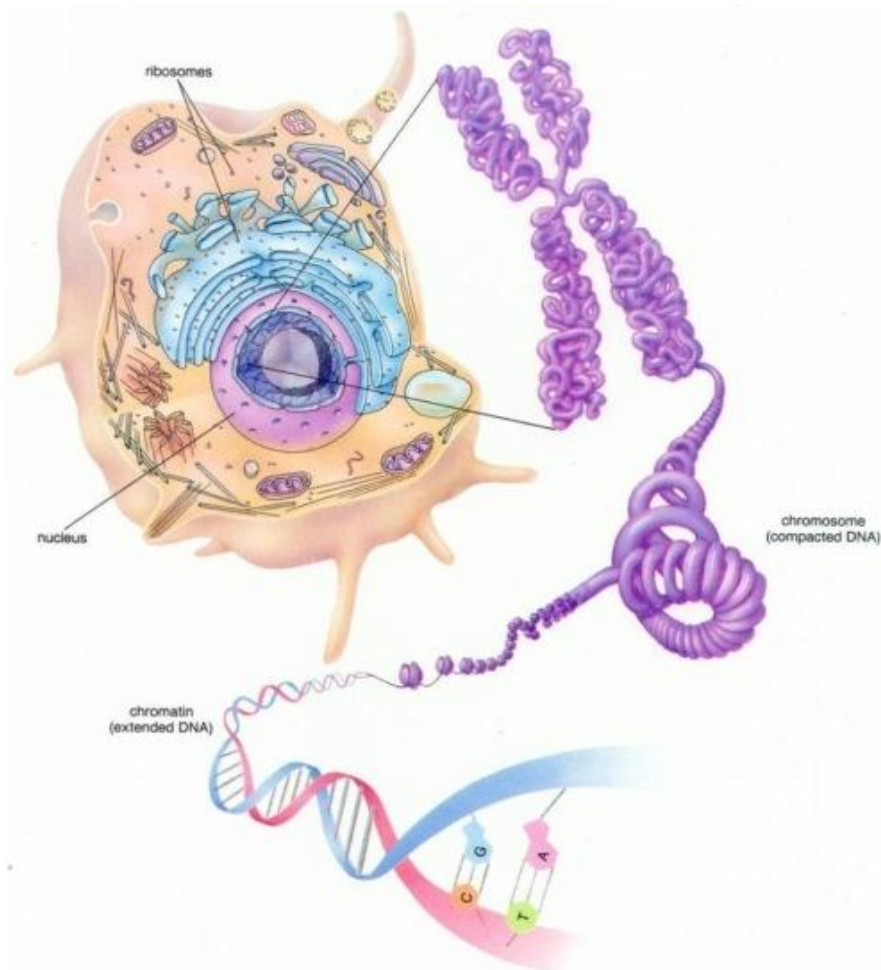


# EPIGENETIKA

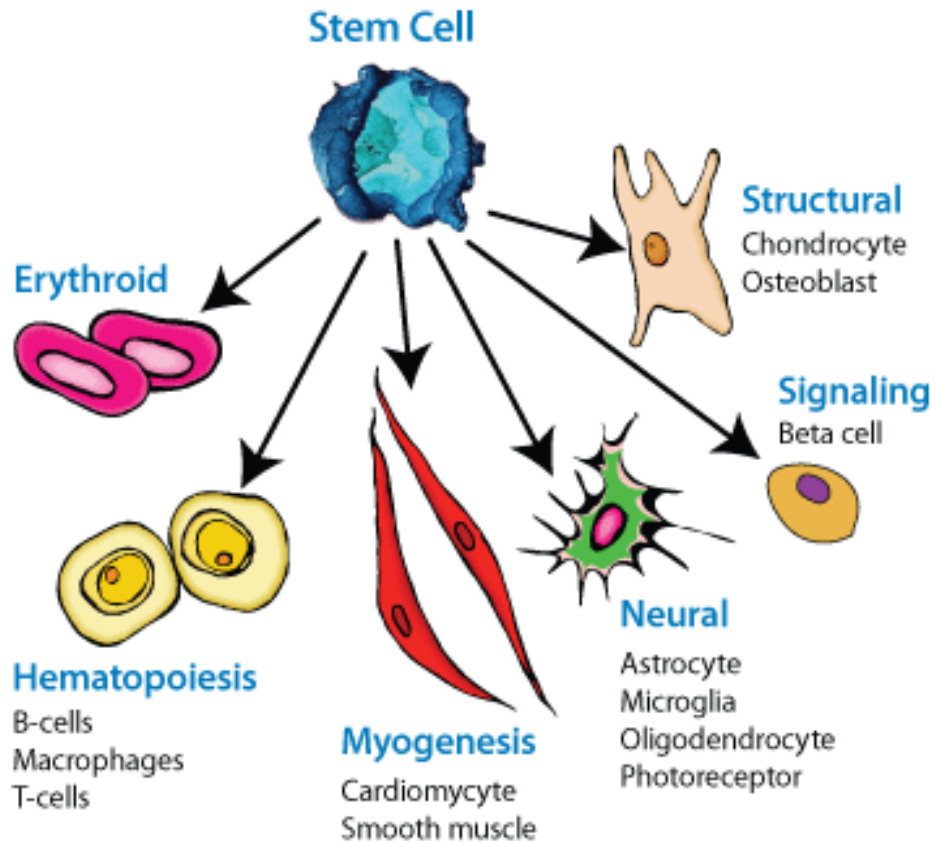
Metilacija kot označevalec  
pri diagnostiki raka





“ČE BI MOLEKULE DNA RAZTEGNILI V RAVNO LINIJO, BI 46 KROMOSOMOV, KI SESTAVLJAJO GENOM ČLOVEŠKE CELICE MERIL BLIZU DVA METRA, VSEH CELIC (100 TRILIJONOV) PA PRIBLIŽNO 182 BILIJONOV KM (610 krat do sonca in nazaj) (*Centre for Integrated Genomics*)

# Diferenciacija celic



# EPIGENETIKA

- grško ( $\epsilon\pi\iota$ ), epi = nad
- **epigenetske modifikacije** so del genomske regulacije
- povzročijo spremembo v izražanju genov
- niso neposredno zapisane v DNA zaporedju
- so dedne (prenos iz celice v celico)

DEFINICIJA: epigenetika obravnava spremembe v izražanju genov, ki niso posledica sprememb v DNA zaporedju (npr. mutacij), so pa dedne (mitoza).

# Epigenetski procesi so ključni za:

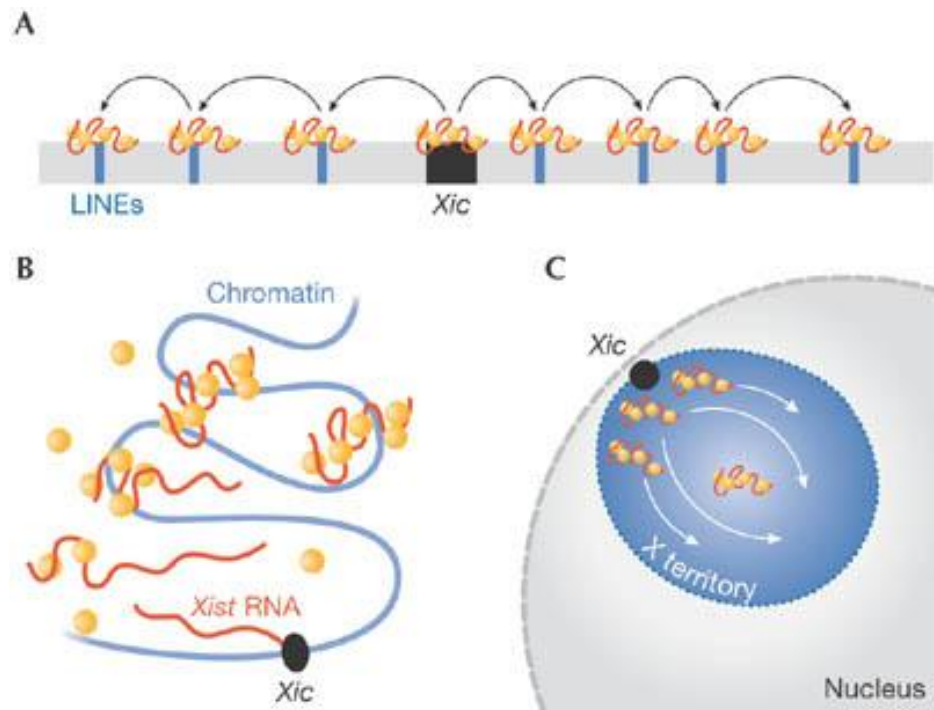
- Diferenciacijo in razvoj
  - Dozacijska kompenzacija (inaktivacija X)
  - Vtisenjenje (imprinting)
- Dolgotrajne odgovore na spremembe okolja (dedovanje, evolucija)
- Delovanje možganov
- Nastanek bolezni (RAK)

# DOZACIJSKA KOMPENZACIJA

Dozacijska kompenzacija (“dosage compensation”) je mehanizem, ki ureja izražanje spolno vezanih genov, ki se razlikujejo v pogledu doze pri samcih in samicah, pri katerih je spol določen z XX in XY kromosomi.

Hipotezo je postavila Mary Lyon (zato tudi hipoteza Lyonove), ki pravi, da je pri sesalcih dosežena dozacijska kompenzacija z inaktiviranjem enega od X kromosomov (naključno izbranem) v somatskih celicah samic. Inaktivirani X daje Barrovo telesce ali spolni kromatin. V primerih polisomije X kromosoma (večje število X kromosomov) so vsi, razen enega, inaktivirani.

# *XIST* lncRNA



*Cis*-Inaktivacija genov na kromosomu X, s tem, da ***XIST RNA*** vezana na represorski protein Polycomb fizično prekrije gene in jih tako inaktivira.

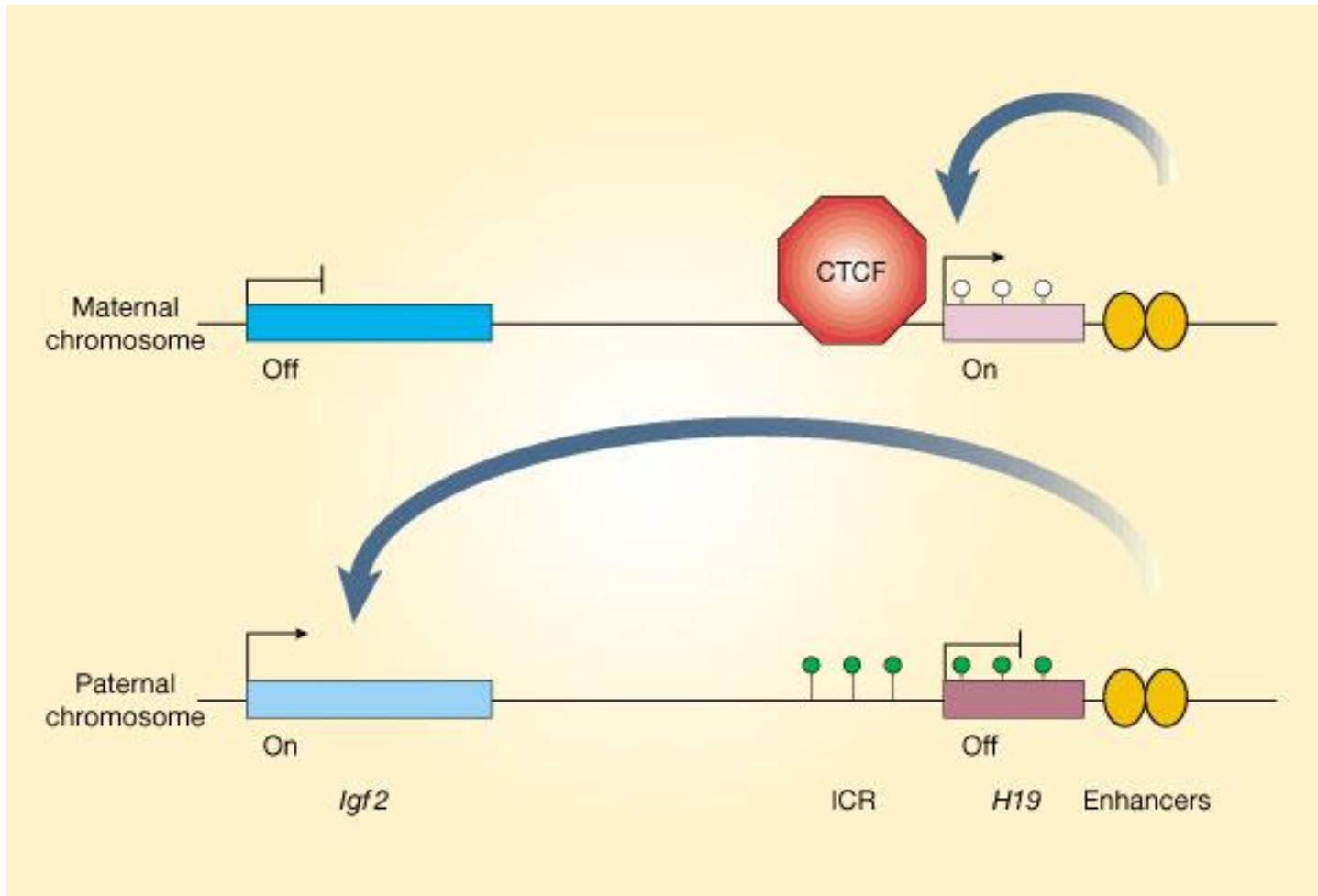
***Xic***, X-inaktivacijski center

# **VTISNJENJE (“IMPRINTING”)**

VTISNJENJE JE PROCES, PRI KATEREM JE KOPIJA GENA  
PODEDOVNA OD ENEGA OD STARŠEV, EPIGENETSKO UTIŠANA



# *H19* lncRNA (2.3kb – 11p15.5)



**CTCF** - je visoko ohranjen protein – (CCCTC-binding factor) cinkov prst, vključen v različne regulatorne vloge: aktivacijo/represijo transkripcije, vtisnjenje, inaktivacijo kromosoma X

**IGF2** – Insuline-Like Growth Factor 2

ICR – “Imprinting Control Region”

# EPIGENETIKA IN DEDOVANJE

- Predstavlja nov nivo v “zakonih” Mendlovega dedovanja, ki ga imenujemo mehki-”Lamarck-izem”
  - Pogoji okolja v zgodnjem življenjskem obdobju lahko povzročijo epigenetske spremembe pri ljudeh, ki ostanejo celo življenje in so lahko posledica modifikacije metilacije DNA ali modifikacije histonskih oznak
  - **Epigenetska sprememba v času nosečnosti se lahko prenese v naslednjo generacijo.**

# Epigenetski regulatorni mehanizem

- Epigenetski spomin se vgradi:
  - v metilacijo in hidroksi-metilacijo citozina v DNA
  - Z modifikacijo kromatina (preko histonskih oznak)

To se dogaja s pomočjo izredne mreže

**ENCIMOV/ KOMPLEKSOV/ MOLEKULSKIH GRADNIKOV**

~ 60

ki vključujejo skupine proteinov **Polycomb** in **Trithorax**, ki so ključna za večino (če ne vse) razvojne procese in programe v organizmu

# EPIGENETSKI MEHANIZMI

- **MODIFIKACIJE HISTONOV**

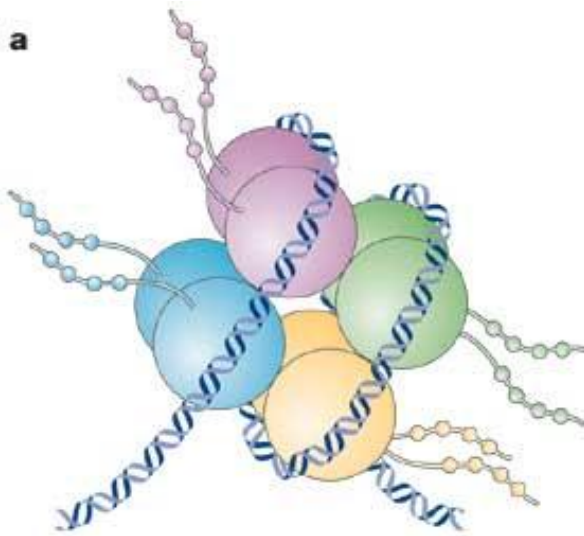
1. acetilacija
2. metilacija
3. fosforilacija
4. ubikvitinacija
5. sumoilacija

- **MODIFIKACIJA DNA**

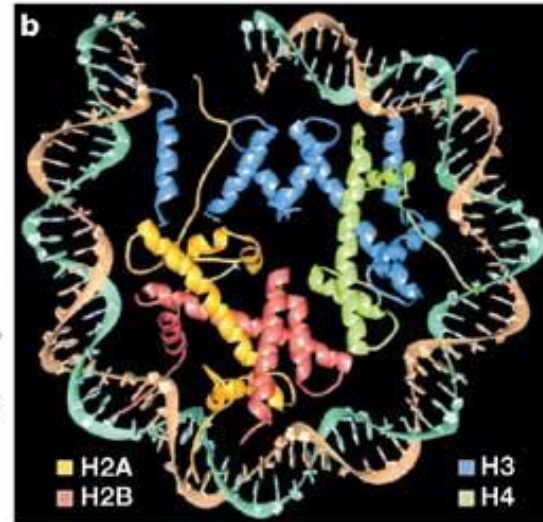
1. metilacija DNA

# EPIGENETIKA

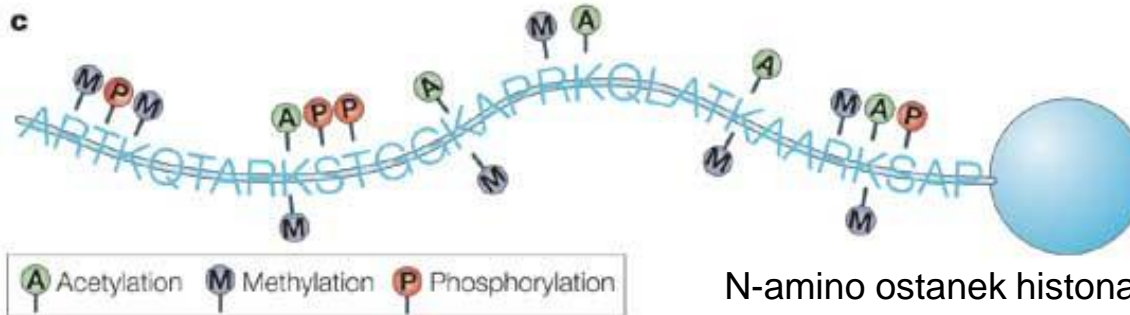
## Modifikacija histonov



Nukleosom

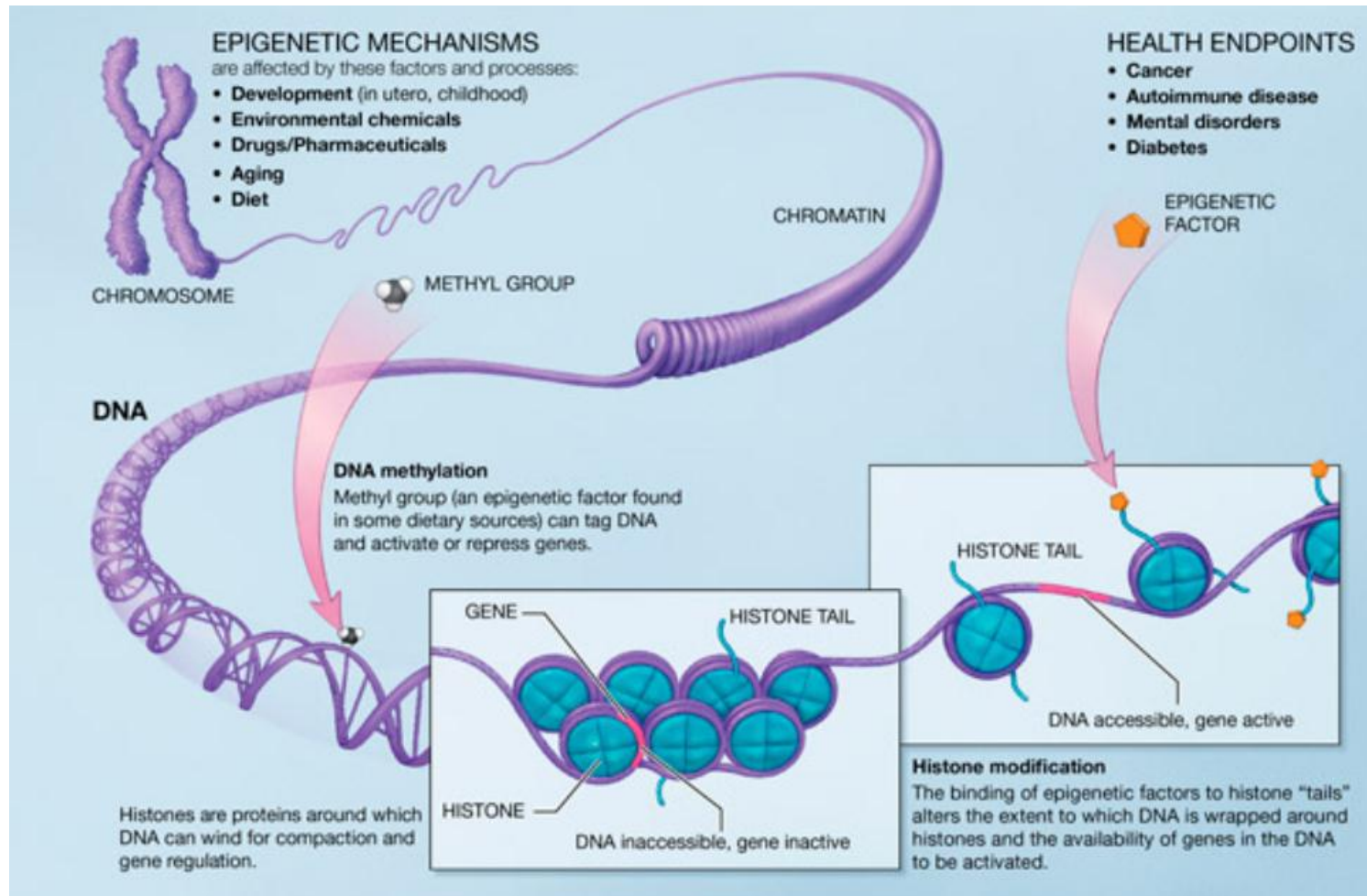


Kristalna struktura nukleosoma in DNA

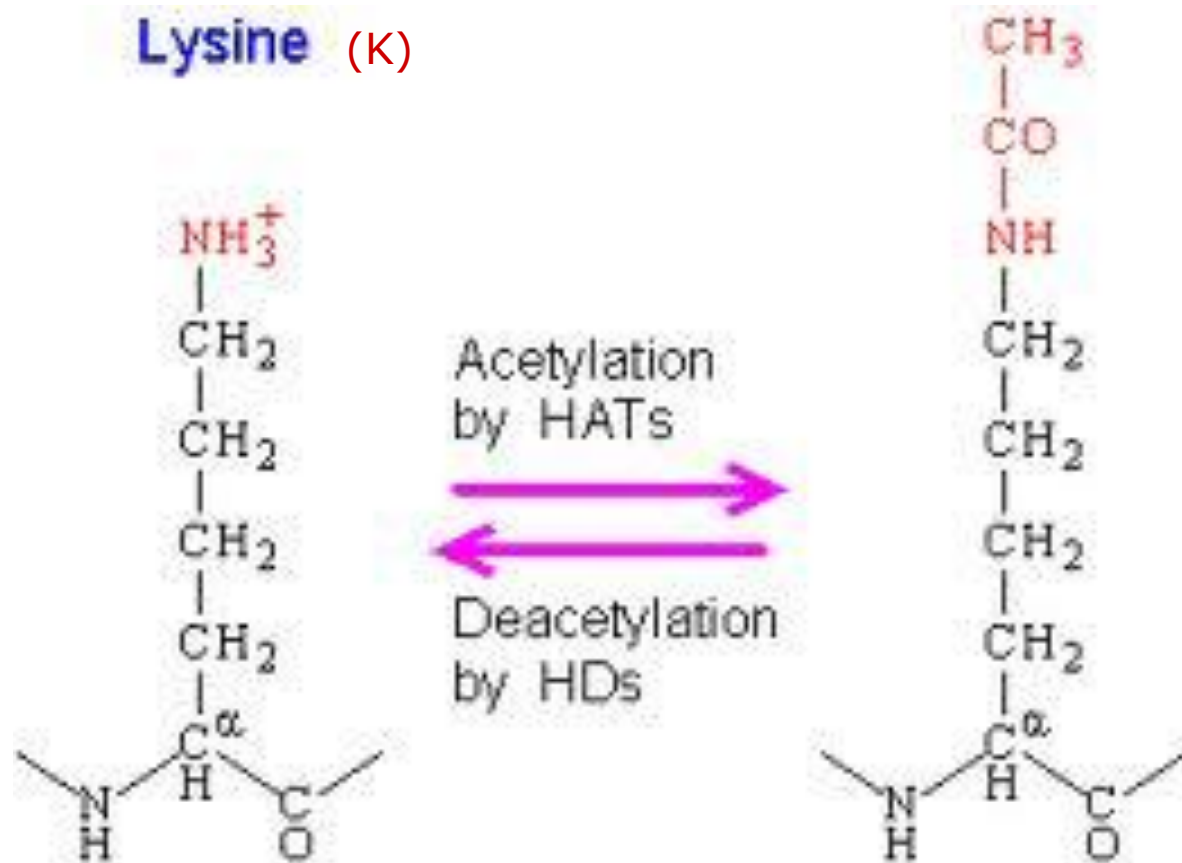


N-amino ostanek histona

# GENETIKA/EPIGENETIKA

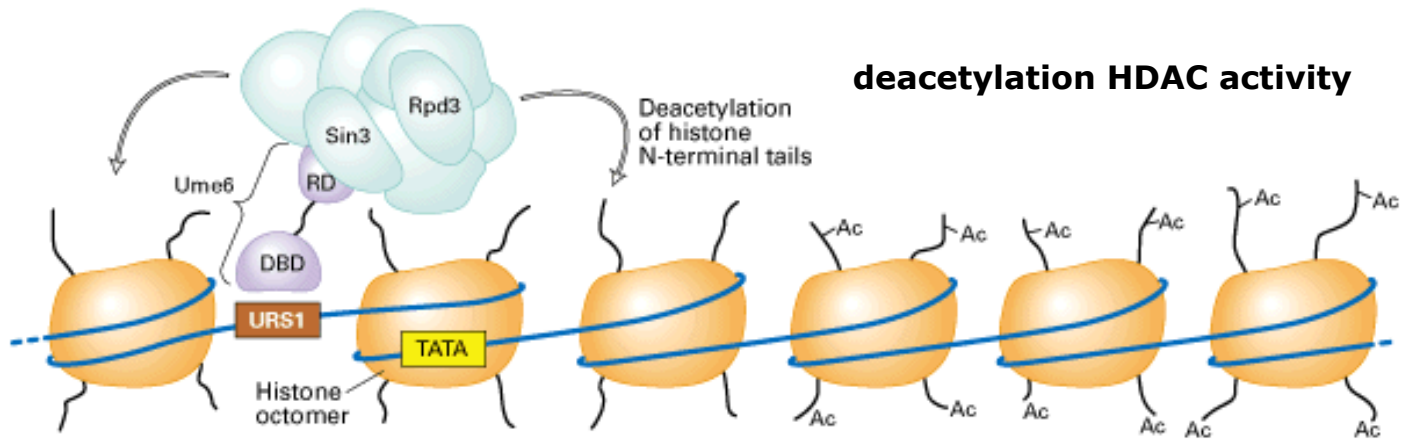


## Lysine (K)

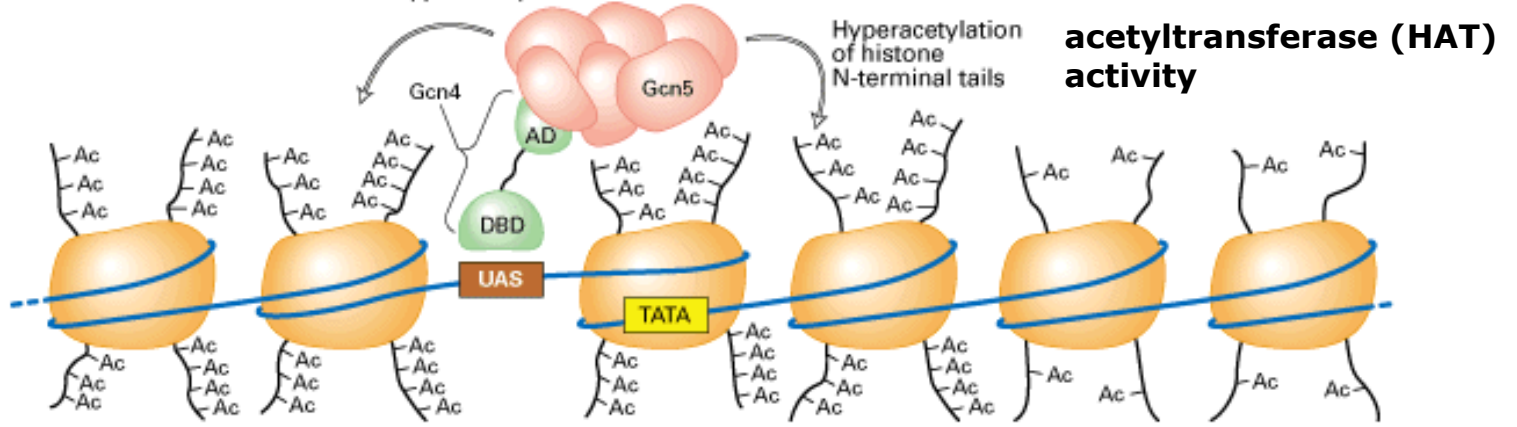


# Acetilacija histonov

(a) Repressor-directed histone deacetylation

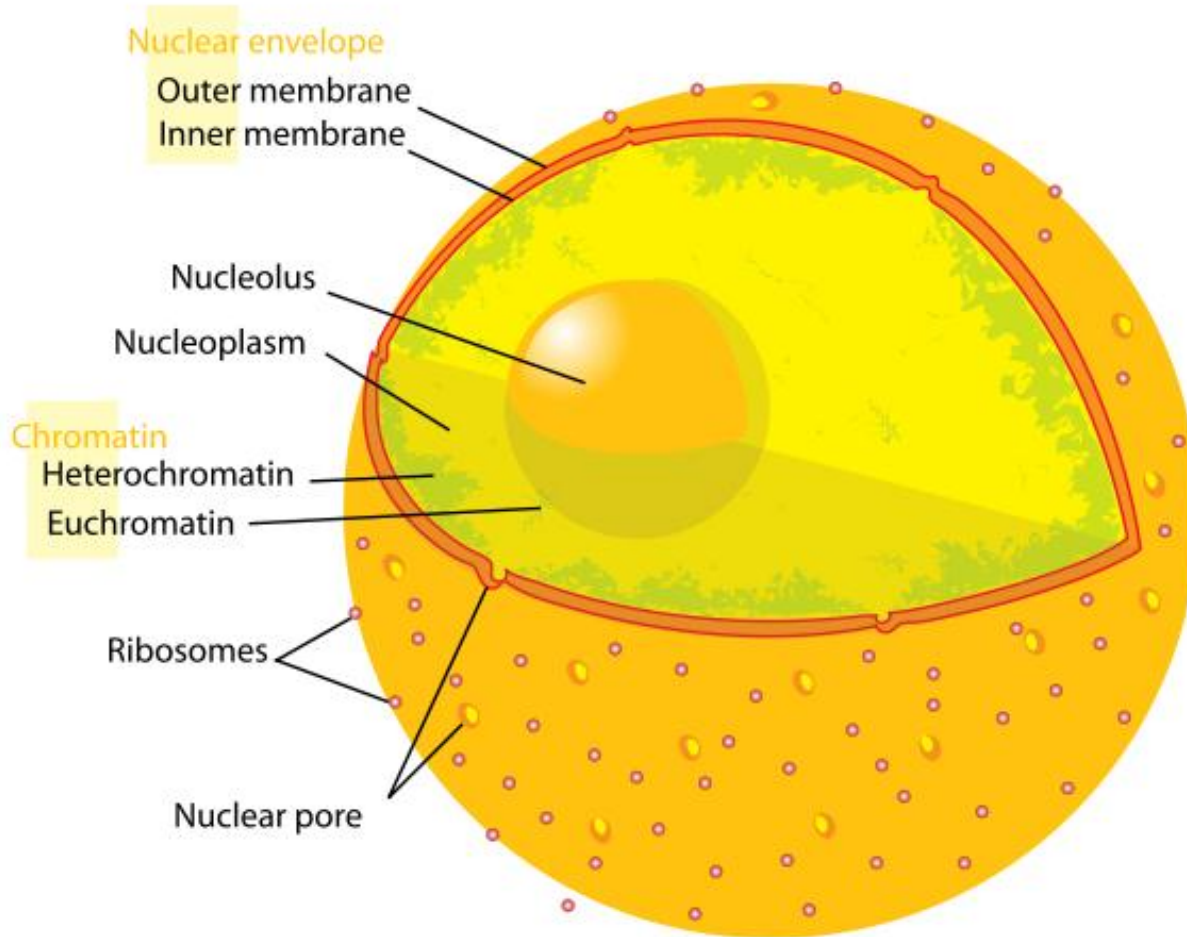


(b) Activator-directed histone hyperacetylation

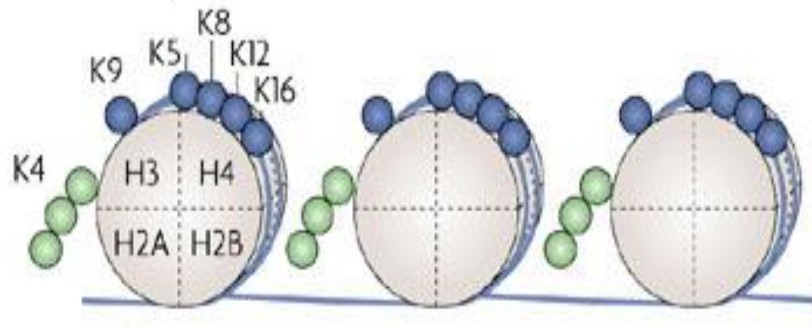


**Acetyl-Coenzyme A**

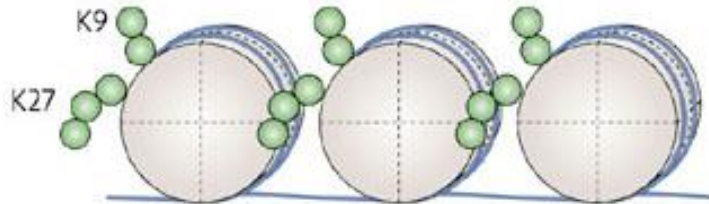




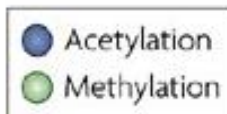
# Histonske oznake (kode)



**Transkripcija**

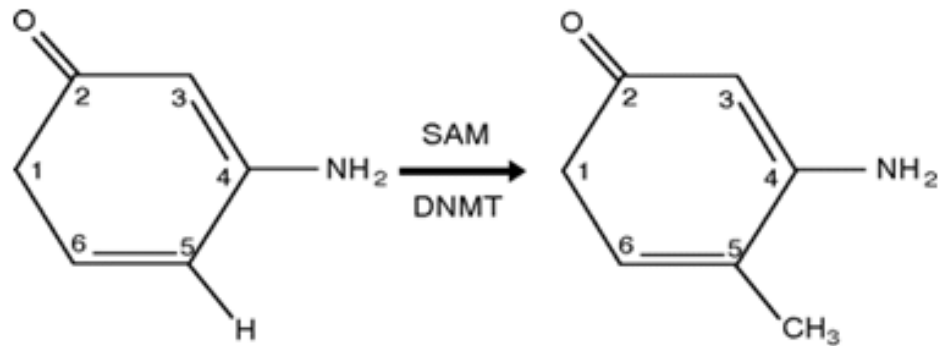
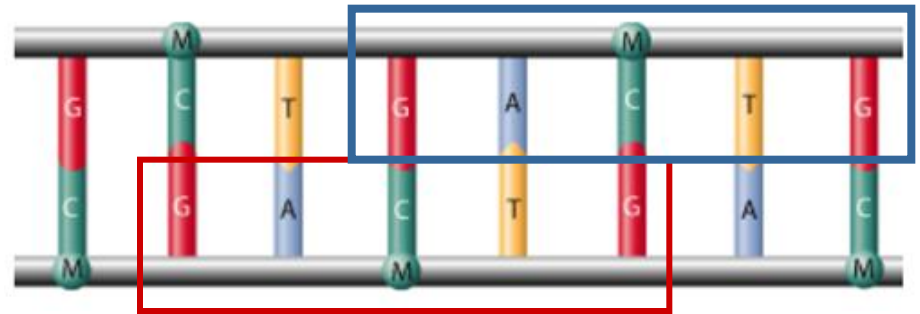


**Inhibicija transkripcije**



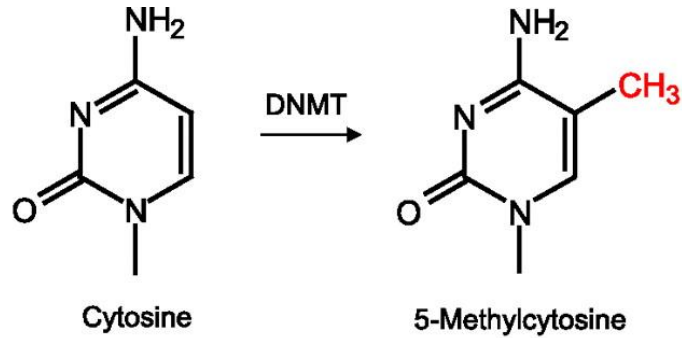
# METILACIJA DNA

- kovalentna vezava metilne skupine na citozin
- palindromna struktura
- reakcijo katalizirajo encimi iz družine metiltransferaz DNA
- metiliranih 60-90% CpG mest v humanem genomu, izjema CpG otoki v promotorskih delih aktivnih genov

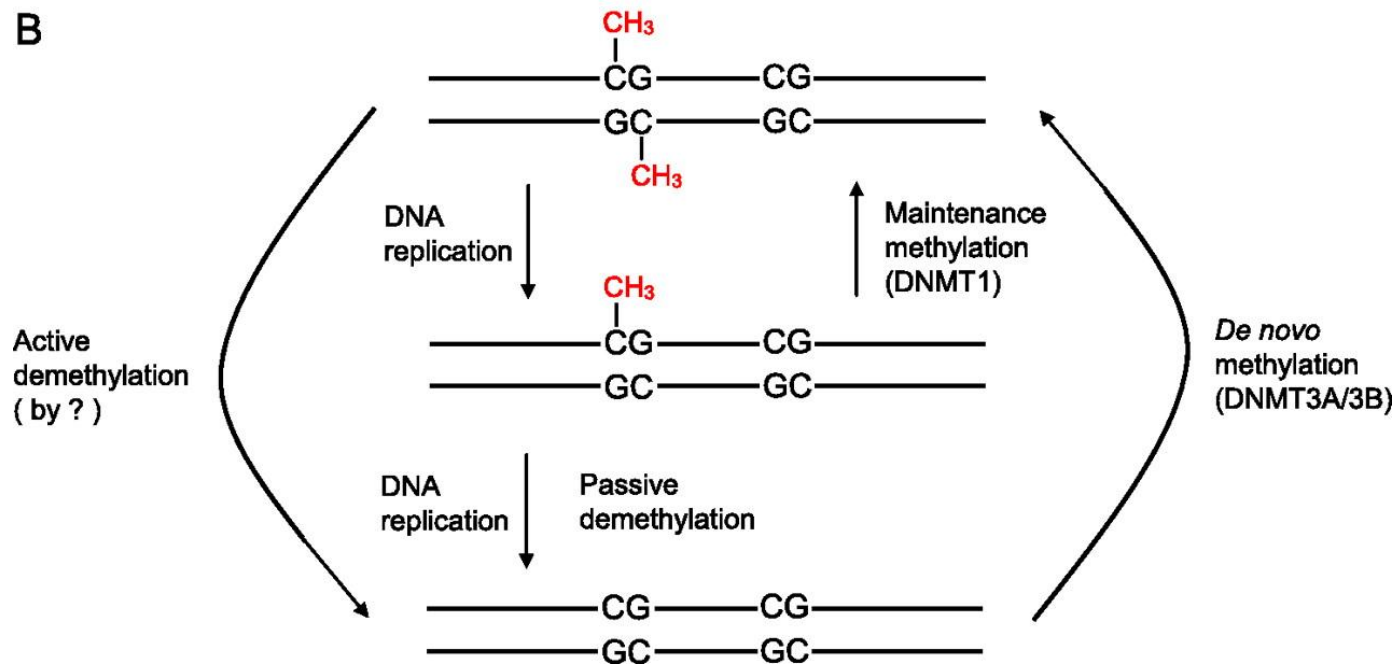


# Pregled mehanizmov vpletenih v DNA metilacijo in de-metilacijo pri sesalcih

A

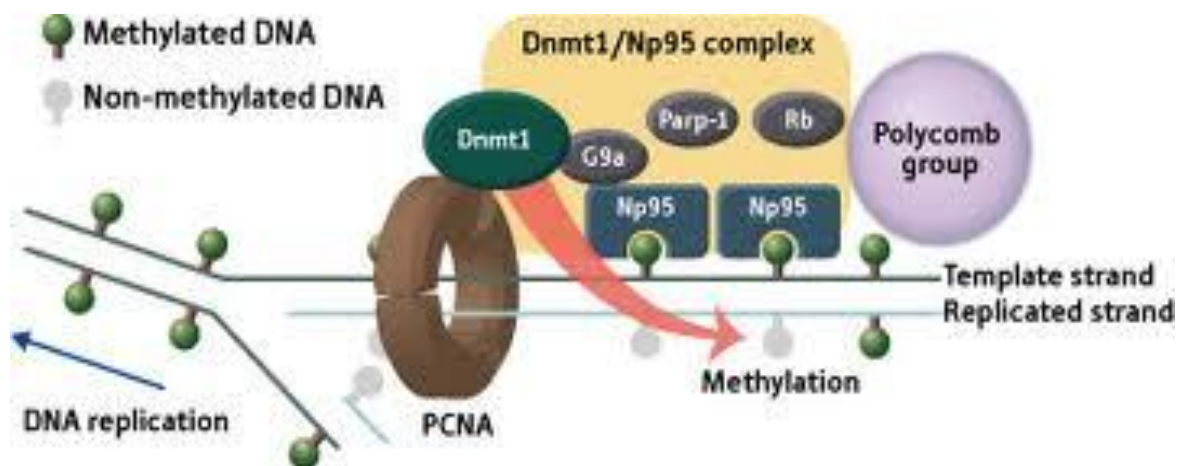


B



Chen Z , Riggs A D J. Biol. Chem. 2011;286:18347-18353

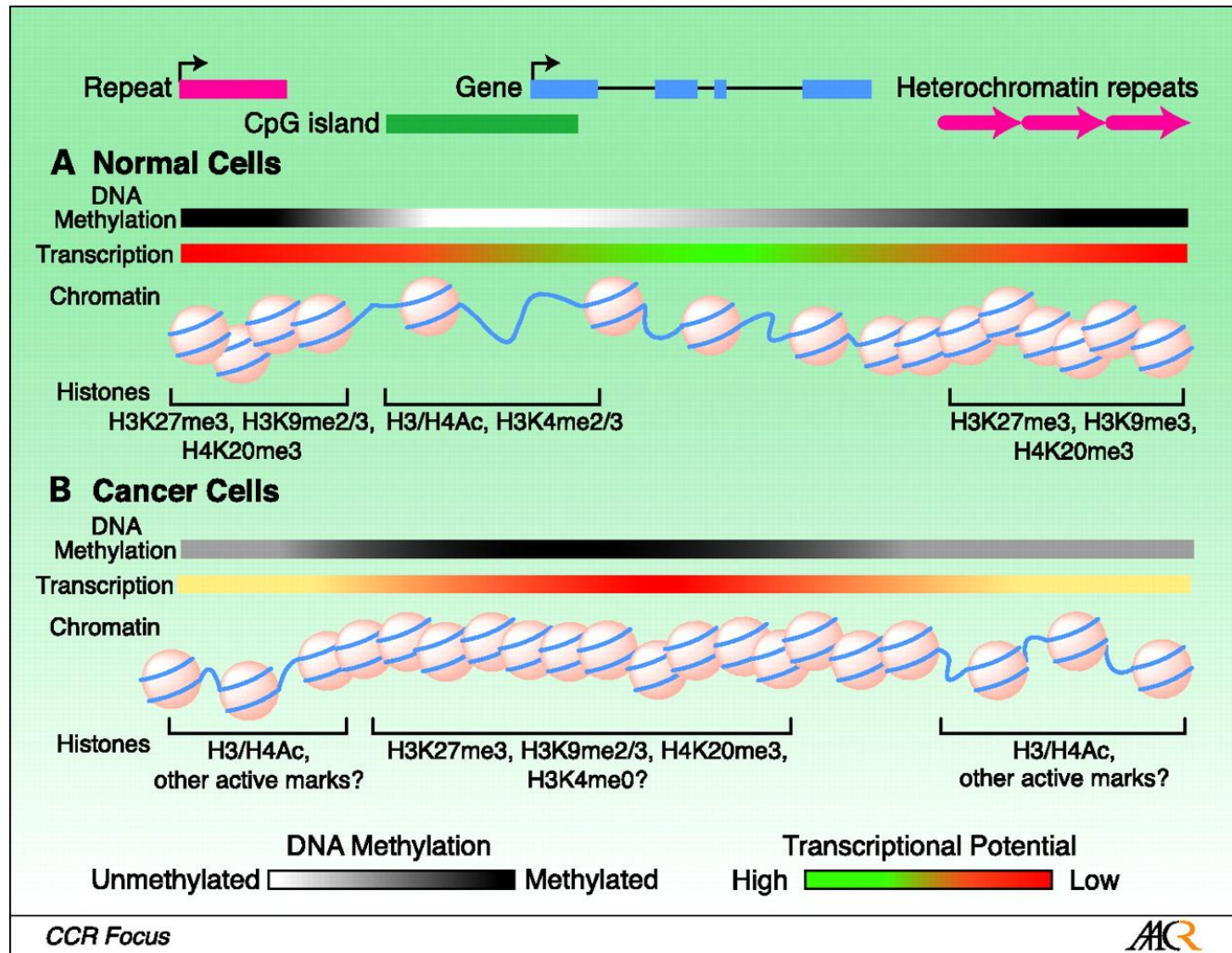
# Podvojevanje DNA-metilacijskega vzorca



PCNA-”proliferating cell nuclear antigen” ki objame DNA

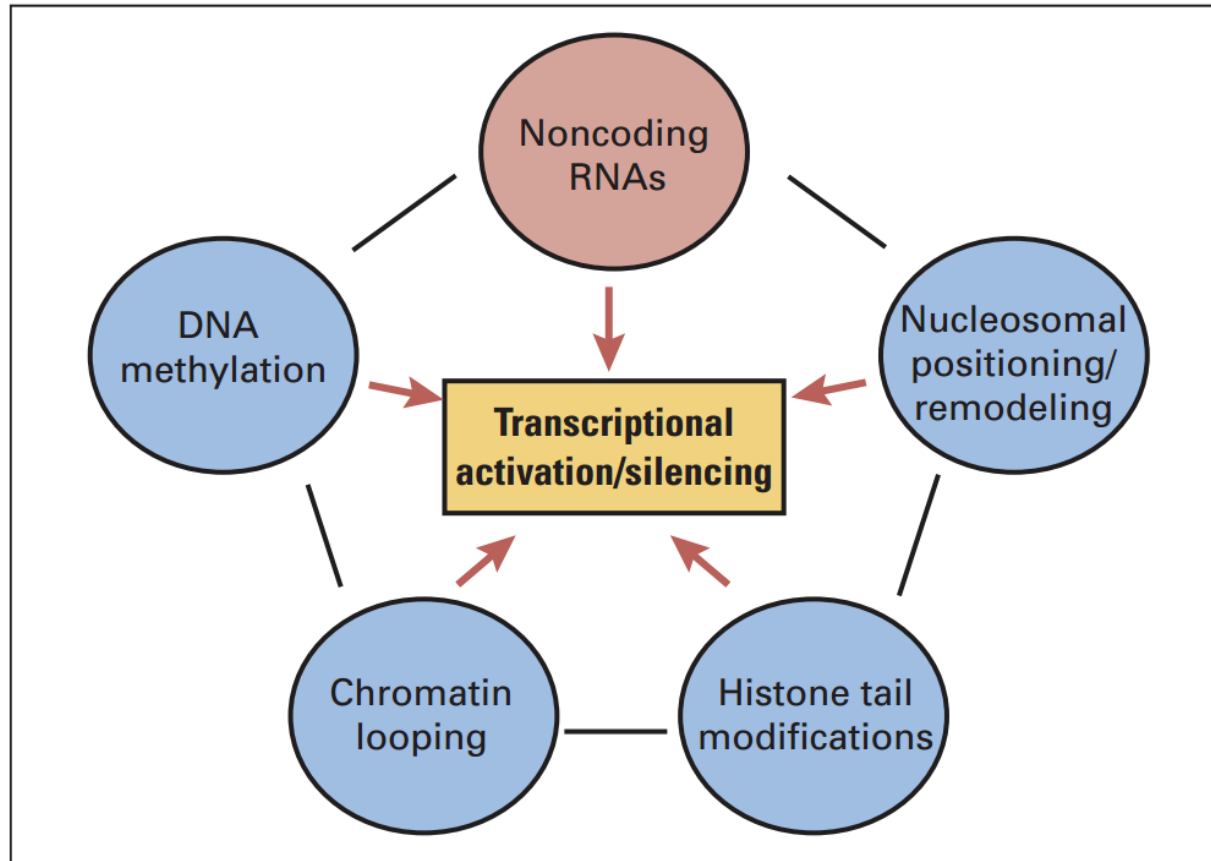
**Np95 odkrije mesto metilacije v matrični verigi, sledi vezanje encima Dnmt1 (DNA-metil-transferaza) na Np95 in metilacija podvojene verige**

# Metilacija DNA in modifikacije histonov se pri raku spremenijo

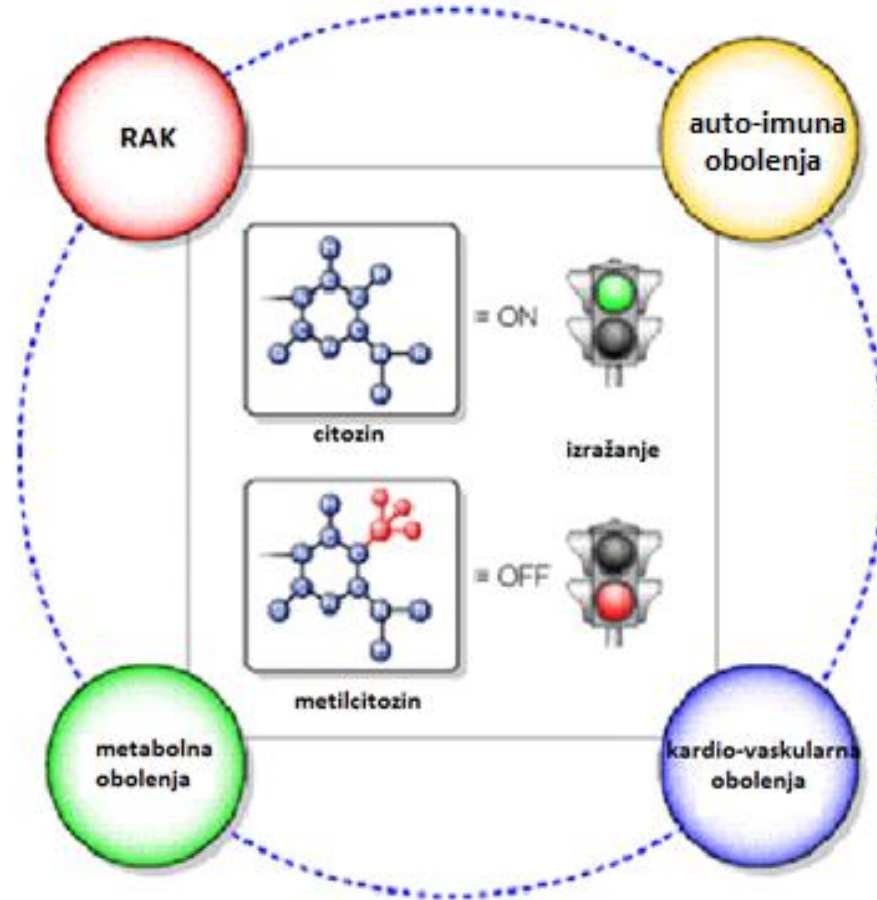


McCabe M T et al. Clin Cancer Res 2009;15:3927-3937

# EPIGENETIKA RAKA

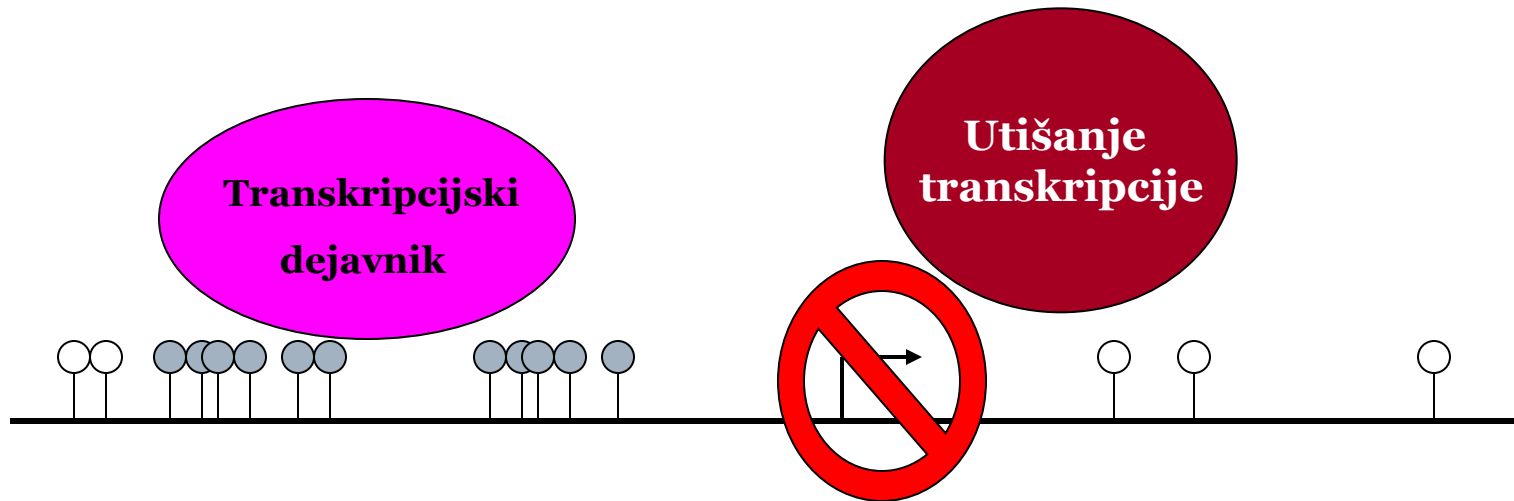


# METILACIJA DNA in bolezni





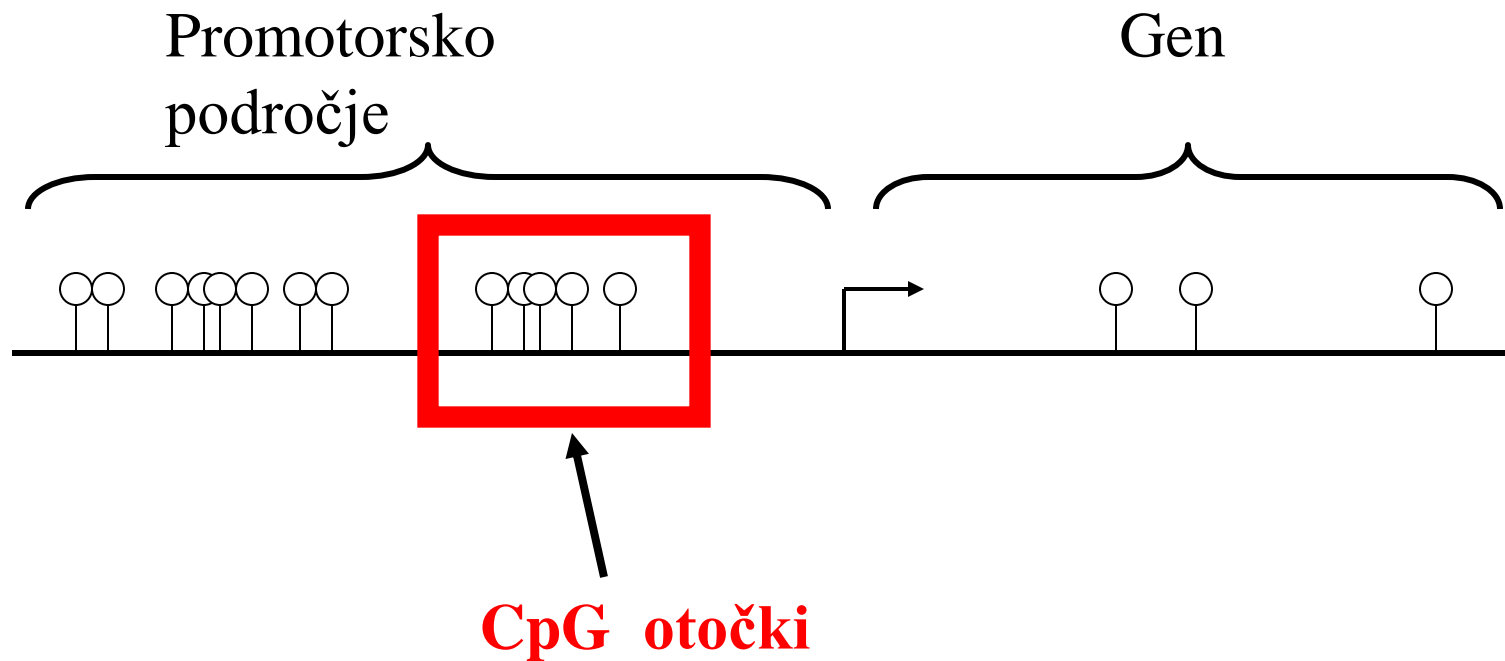
# Posledica hipermetilacije promotorjev genov



○ = **CpG**

● = **metil-CpG**

# Kako določiti metilacijo ?



○ = CpG

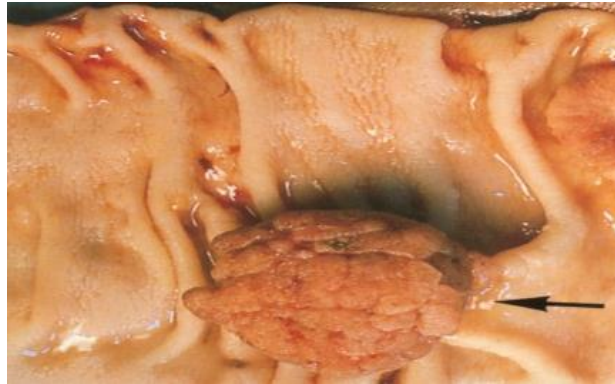
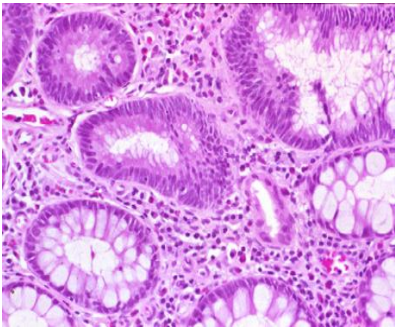
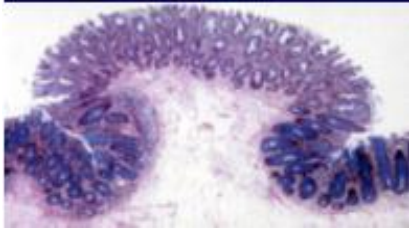
Metilacijo DNA določamo v **promotorski regiji** določenega gena, ki je **CpG bogata** in se nahaja na **5' koncu** gena.

# Metode za detekciju DNA metilacije

- Bisulfitno sekveniranje
- MS-SSCP; MS-DHPLC;
- Metilacijsko specifičen PCR (MSP)
- MS-HRM
- MLPA
- MethyLight
- MALDI-TOF masna spektrometrija
- Pirosekveniranje
- imunoprecipitacija (MeDIP)
- .....in mnogo drugih !!!!

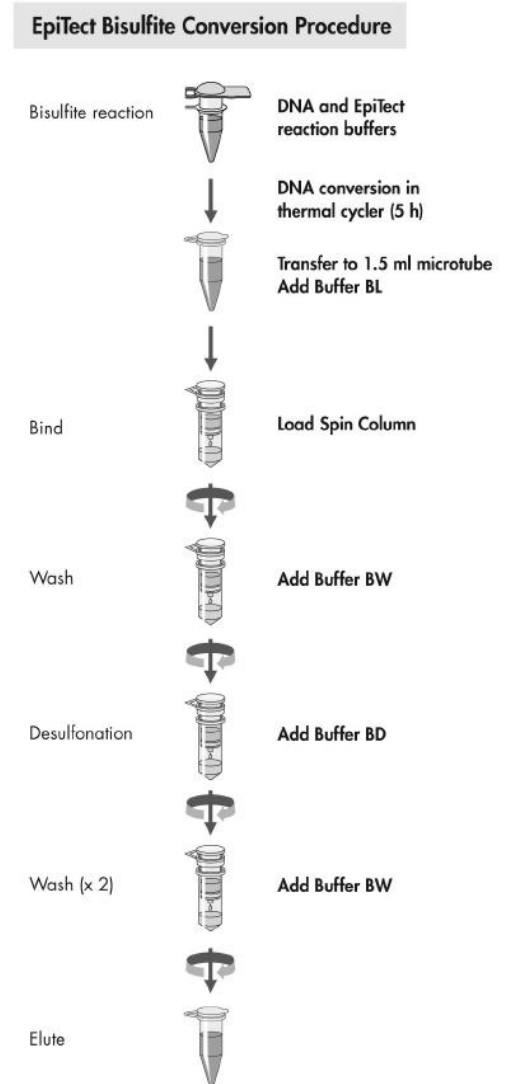
# Korak 1 - izolacija DNA

(iz svežega biopsijskega vzorca, zamrznjenega tkiva, tkiva shranjenega v stabilizacijske raztopine npr. RNAlater, tkiva vključenega v parafin, iz celic pridobljenih z mikrodisekcijo, krvi,...)



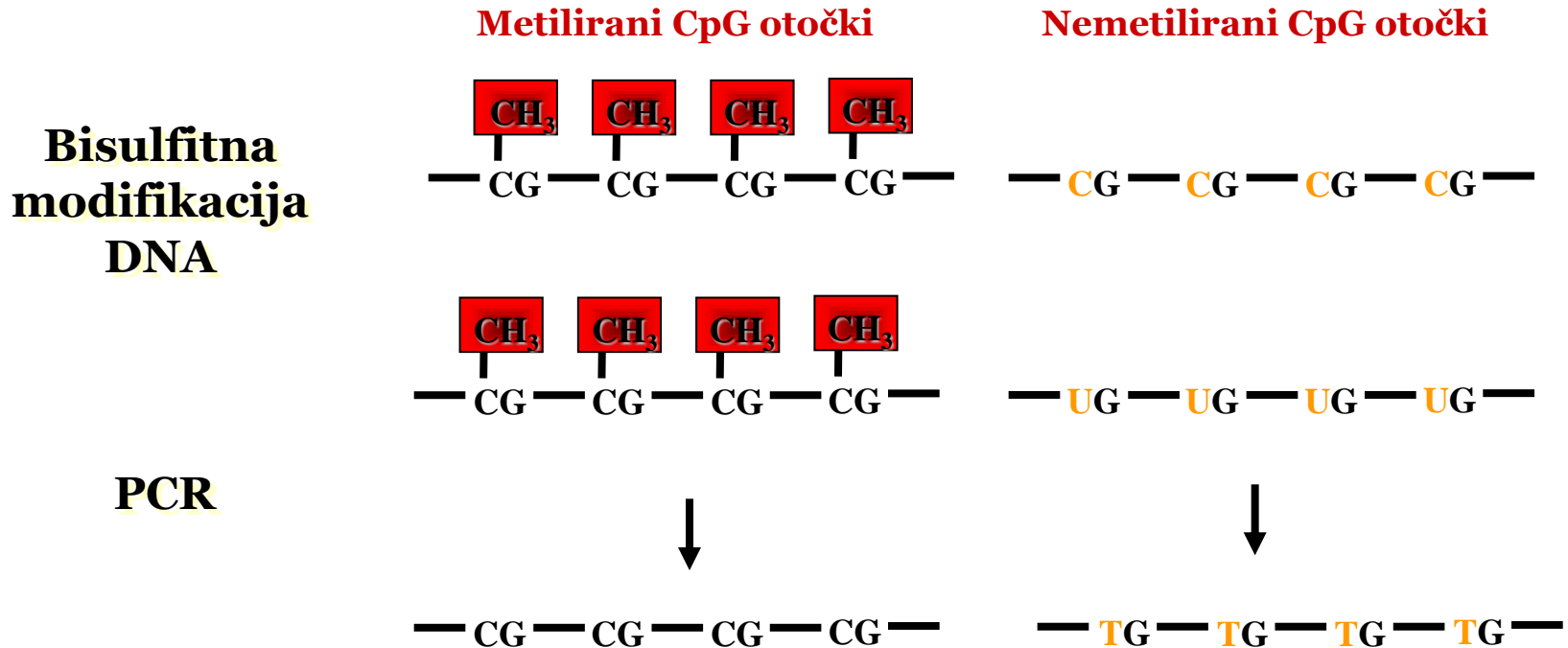
# Korak 2 - bisulfitna reakcija

- pripravimo ustrezno redčitev DNA
- dodamo bisulfitni koktejl
- s pomočjo ustreznega aparata izvedemo 5-urni proces več zaporednih inkubacij in denaturacij
- sledita čiščenje in elucija bisulfitno pretvorjene DNA
- pomagamo si z uporabo komercialnih kitov



Component	Volume per 25 $\mu$ l reaction*	Volume per 10 $\mu$ l reaction*	Final concentration	Step	Time	Temperature
<b>Reaction mix</b>				Denaturation	5 min	95°C
2x EpiTect HRM PCR Master Mix	12.5 $\mu$ l	5 $\mu$ l	1x	Incubation	25 min	60°C
10 $\mu$ M (each) primer mix <sup>†</sup>	1.9 $\mu$ l	0.75 $\mu$ l	0.75 $\mu$ M forward primer 0.75 $\mu$ M reverse primer	Denaturation	5 min	95°C
				Incubation	85 min (1 h 25 min)	60°C
RNase-free water	Variable	Variable	-	Denaturation	5 min	95°C
<b>Template DNA<sup>‡</sup></b> (added at step 4)	Variable	Variable	5–10 ng/reaction <sup>§</sup>	Incubation	175 min (2 h 55 min)	60°C
				Hold	Indefinite <sup>†</sup>	20°C
<b>Total volume per reaction</b>	<b>25 <math>\mu</math>l*</b>	<b>10 <math>\mu</math>l*</b>	-			

# Bisulfitna reakcija



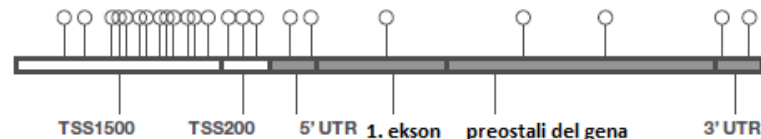
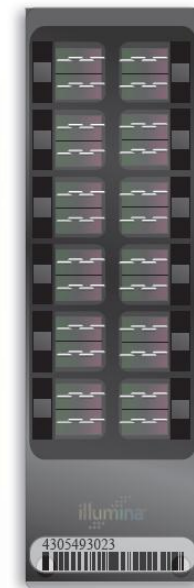
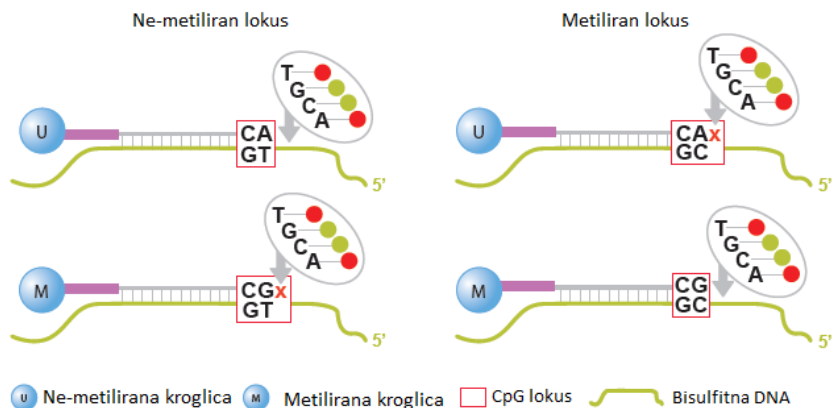
Ob prisotnosti bisulfitna se nemetilirani citozini hidrolitično deaminirajo v uracile, metilirani citozini pa ostanejo nespremenjeni. V kasnejši verižni reakciji s polimerazo se namesto uracilov v verigo vključujejo timini. Spremenjeno (T) oz. nespremenjeno (C) zaporedje v DNA sekvenci nadalje dokazujemo.

# Korak 3:

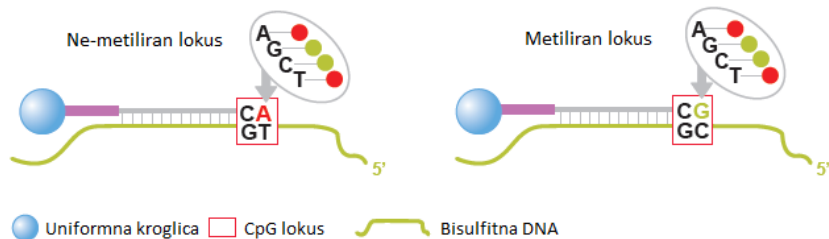
# Vse-genomska analiza metilacije

450.000 CpG otkov

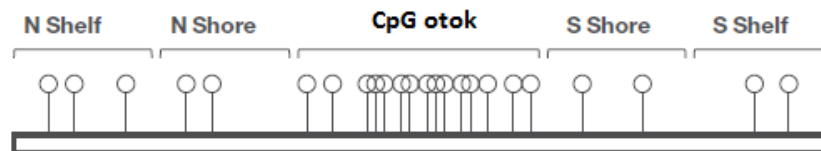
## A. Infinium I



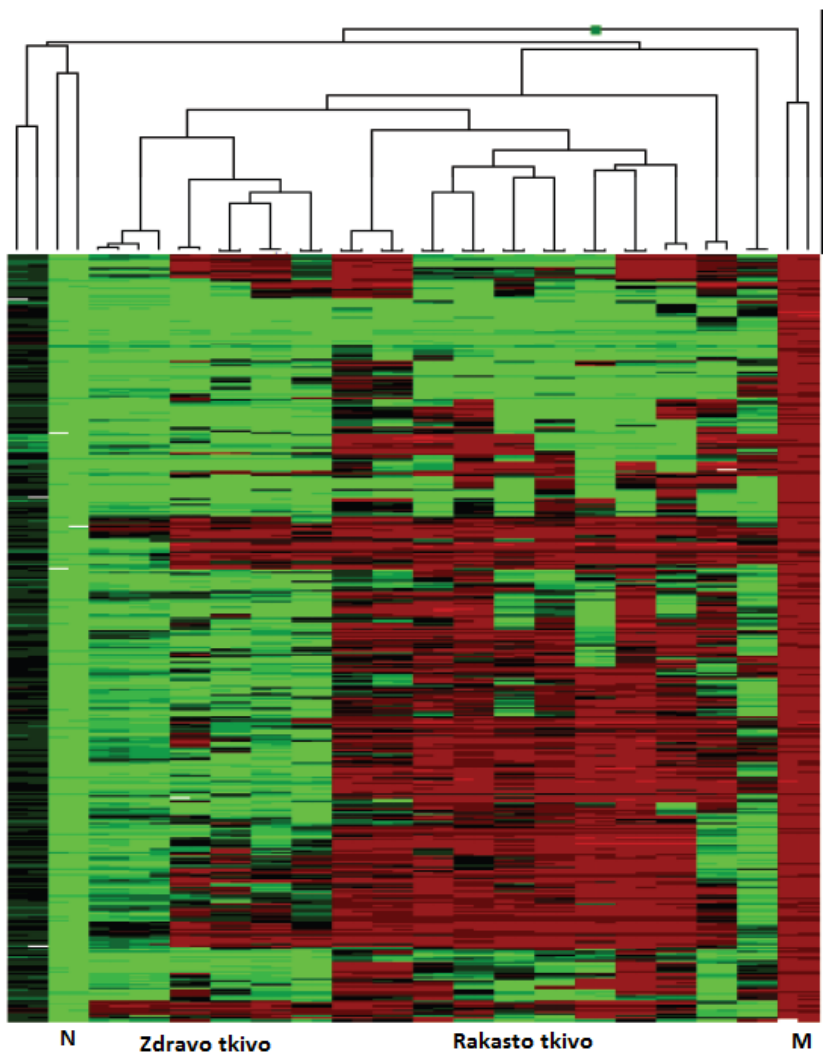
## B. Infinium II



Feature Type	Genes Mapped	Percent Genes Covered	Number of Loci on Array
NM_TSS200	14895	0.79	2.56
NM_TS1500	17820	0.94	3.41
NM_5'UTR	13865	0.78	3.34
NM_1stExon	15127	0.80	1.62
NM_3'UTR	13042	0.72	1.02
NM_GeneBody	17071	0.97	8.97
NR_TSS200	1967	0.65	1.84
NR_TSS1500	2672	0.88	2.92
NR_GeneBody	2345	0.77	5.34



# Rezultat vse-genomske analize:

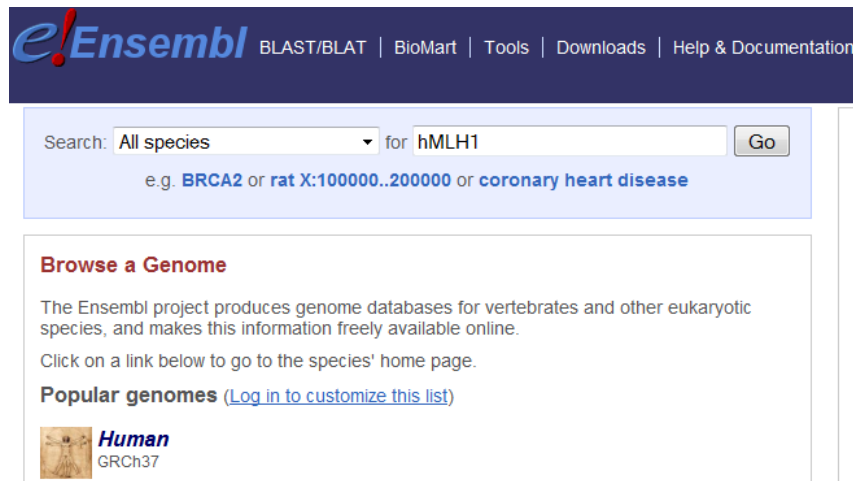


gen	NORMAL 1	NORMAL 2	TUMOR 1	TUMOR 2	TUMOR 3
BLCAP	0.770115	0.7235704	0.9442897	0.9615089	0.9511719
<b>TMEM25</b>	<b>0.2673522</b>	<b>0.3224146</b>	<b>0.9048566</b>	<b>0.9523582</b>	<b>0.6932387</b>
<b>CDO1</b>	<b>0.5809312</b>	<b>0.4483464</b>	<b>0.9053926</b>	<b>0.9148936</b>	<b>0.9036032</b>
CXXC5	0.84	0.62	0.933	0.936	0.931
SERPINB5	0.8287671	0.8100899	0.9481865	0.939976	0.9665664



# Korak 4:

## Izbranemu genu določimo promotorsko regijo



**MLH1** [ Ensembl/Havana merge gene: ENSG0000076242 ]

**Description** mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli) [Source:HGNC Symbol;Acc:7127] [Type: protein coding Ensembl/Havana merge gene]

**Location** [3:37034823-37107380:1](#)

**Source:** e62; **Feature type:** Gene; **Species:** Homo sapiens;

Izberemo si želeni gen, npr. *MLH1*. Stran Ensembl nam pokaže gen, kot ga poznamo (introni, eksoni).



5' upstream sequence

.....gtatgggtcgtggtcgaatcccaaccattctctgcaagetaa  
gcctgtctgtcgaaggactcaggattgcgacatgagcgcaccaactgaaatgat  
gagtcagggttgattatggtcagaagatctcttgcacctccaactcagggctcaacggp  
ggataaagaccagagaggttagttctcatagggcccaaaagcctggctgcacaagcgaag  
aataggcttaaaagtcctcgtcgtttaaanaagctgggtgcgtagattcctgtcaatgc  
tcaggatcctctgcttgtgatctctggagataagtcgaagcctgcgaagcagcttaact  
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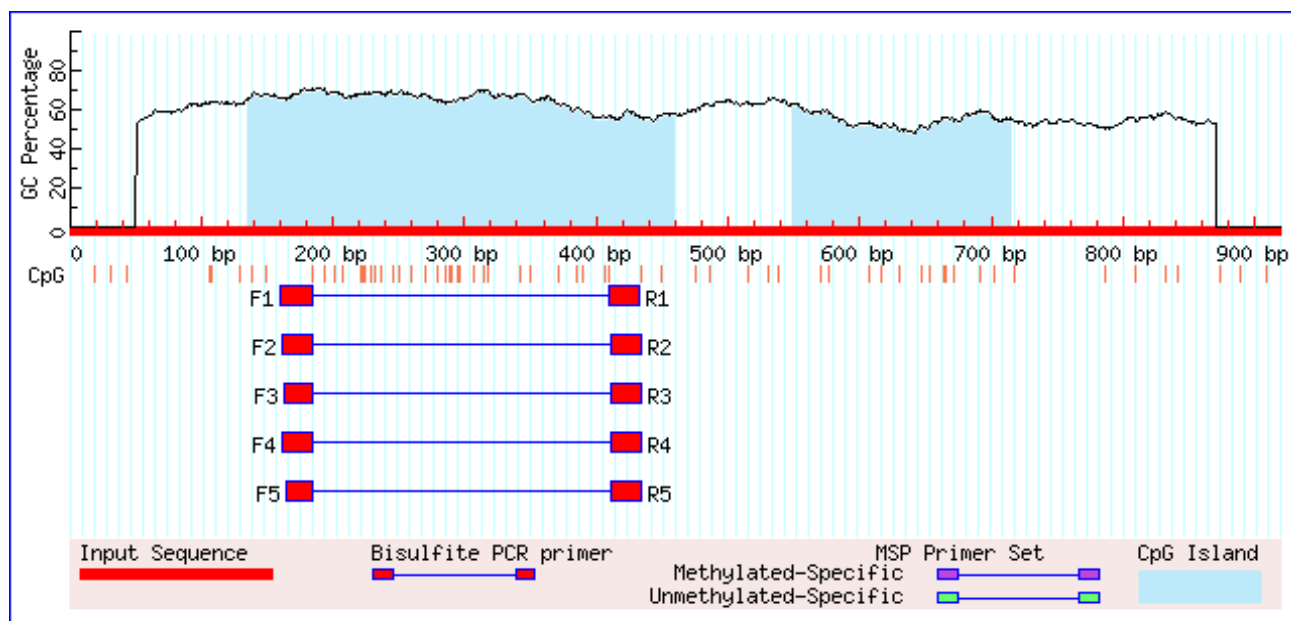
1	<a href="#">ENSE00001943203</a>	37,034,823	37,035,154	-	2	332
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Intron 1-2 37,035,155 37,038,109 2,955

gtacggagggagtcgagccgggctcaactaaaggcctacgactaaaggccggcgtcactc  
aatggcggcagacagcctcttggccgggagagggatgtaacagcgaatgccacaaggg  
cgagggccgggggttcctcagcgtgccagtcagcctctctcctttccgagacagcgtgt  
gtttctttacgcctccccggagacctttaaagggttggttggaggtgtaagtgaggaa

# Določitev promotorja gena in CpG otoka v njegovem promotorju

Različni on-line programi, kot je npr. MethPrimer lahko izvedejo predikcijo CpG otoka (osnovni kriterij je vsaj 55% prisotnost GC parov). V program vnesemo naš odsek preiskovane sekvence in program nam izpiše stanje za npr. gen *MLH1*. Vidimo dva velika CpG otoka. Hkrati nam program ponudi tudi ustrezne oligonukleotidne začetnike za pomnoževanje želenega odseka.



Vir: <http://www.urogene.org/methprimer/index1.html>

# Korak 5 - izbira oligonukleotidnih začetnikov

The screenshot displays the 'BSP Primers' software window. At the top, a menu bar includes 'File', 'Edit', 'Sequence Features', 'Design Primers', 'Reports', and 'Help'. Below the menu, a horizontal line represents the CpG representation of a DNA sequence, with a region of interest highlighted in green. Directional arrows indicate the positions of forward and reverse primers. A callout box states: 'Selected primer pairs (as represented by directional arrow pairs) turn white'. The interface shows 'Forward primers' on the left (position 1332) and 'Reverse primers' on the right (position 1850). Below the CpG representation, a text box displays the 'Forward Primer-Reverse Primer Selected Sequences' for a 22 bp forward primer: 5' AGATTGGCTGCTTAATTTAGAG 3'. A legend identifies symbols for 'Region of Interest' (green line), 'Forward Primers' (red arrow), 'Reverse Primers' (orange arrow), 'Forward Primer Selected' (white arrow), 'Reverse Primer Selected' (white arrow), 'Translation Start Codon' (red bar), 'Transcription Bases' (blue bar), and 'CpG Sites' (purple tick). The 'gDNA Sequence' is shown at the bottom, with a callout box pointing to a bisulfite-modified sequence: 'The sequence, after bisulfite treatment, includes Ts where Cs once were'. Navigation buttons '< Back' and '> BSP Primer Report' are at the bottom right.

Methyl Primer Express®  
Software

Version 1.0

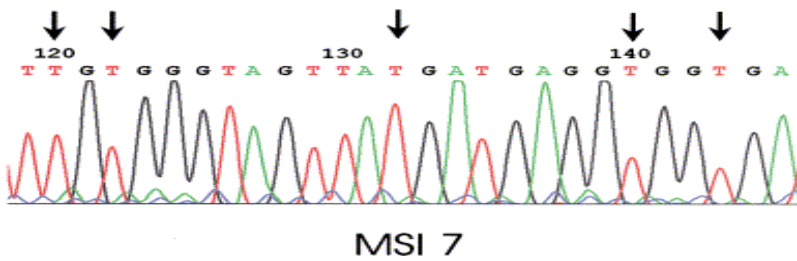
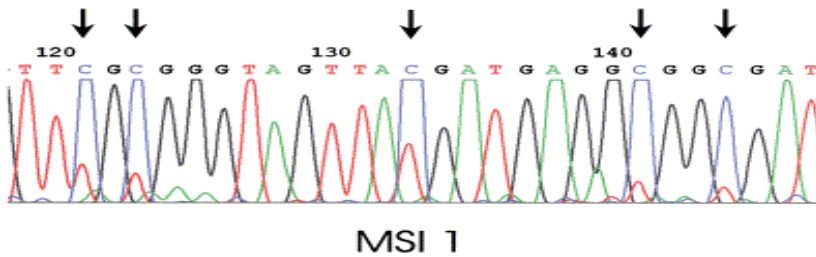
Za določanje oligonukleotidnih začetnikov priporočajo program MethylPrimerExpress.

Želene začetnike nam nato izdelajo po naročilu – npr. Eurofins.



<http://www.eurofins.com/en.aspx>

# Korak 6: 1. Sekveniranje bisulfitno obdelane DNA



5'– GAGT**CGC**.....**CG**.....<sup>m</sup>**CG**.....**GCTTTTA** – 3'  
 3'– **CTCAGCG**.....**GC**.....**G<sup>m</sup>C**.....**CGAAAAT** – 5'

Denaturation  
 Bisulphite Reaction

5'– GAGT**UGU**.....**UG**.....<sup>m</sup>**CG**.....**GU**TTTTA – 3'  
 3'– **UTUAGUG**.....**GU**.....**G<sup>m</sup>C**.....**UGAAAAT** – 5'

PCR amplification (strand specific)

5' – GAGT**TTGT**.....**TG**.....**CG**.....**GTTTTTA** – 3'  
 3' – CTCAACA.....AC.....GC.....CAAAAAT – 5'



# 3. MS-HRM – METILACIJSKO SPECIFIČNA ANALIZA TALILNE KRIVULJE DNA PRI VISOKI LOČLJIVOSTI (Methyl-Specific High Resolution Melting)



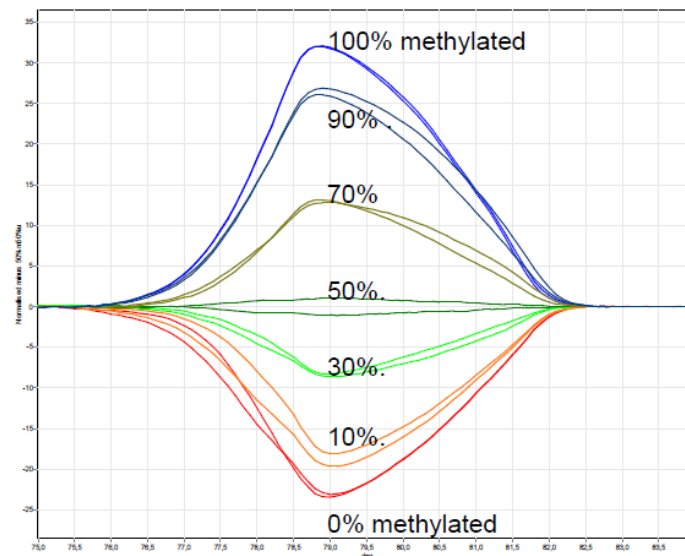
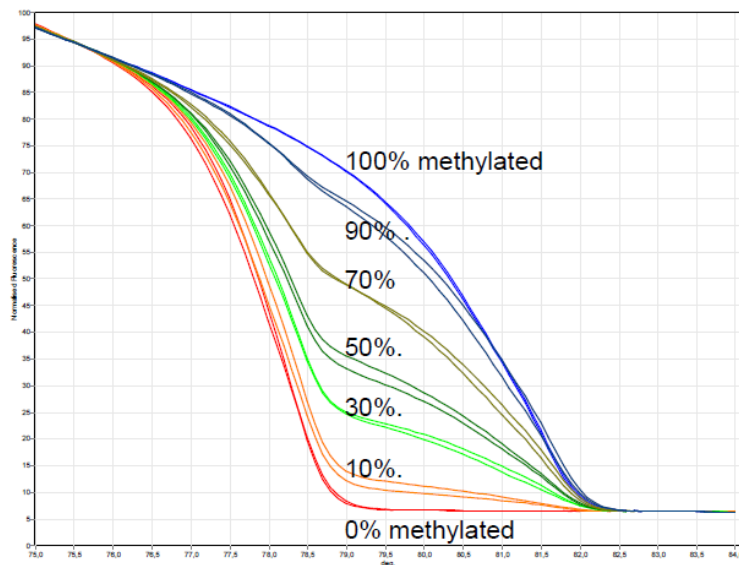
Component	Volume per 25 $\mu$ l reaction*	Volume per 10 $\mu$ l reaction*	Final concentration
<b>Reaction mix</b>			
2x EpiTect HRM PCR Master Mix	12.5 $\mu$ l	5 $\mu$ l	1x
10 $\mu$ M (each) primer mix <sup>†</sup>	1.9 $\mu$ l	0.75 $\mu$ l	0.75 $\mu$ M forward primer 0.75 $\mu$ M reverse primer
RNase-free water	Variable	Variable	–
<b>Template DNA<sup>‡</sup></b> (added at step 4)	Variable	Variable	5–10 ng/reaction <sup>§</sup>
<b>Total volume per reaction</b>	<b>25 <math>\mu</math>l*</b>	<b>10 <math>\mu</math>l*</b>	–
<b>Additional comments</b>			
<b>Initial PCR activation step</b>	<b>5 min</b>	<b>95°C</b>	HotStarTaq <i>Plus</i> DNA Polymerase is activated by this heating step.
<b>3-step cycling:</b>	<b>Important: Optimal performance is only assured using these cycling conditions</b>		
Denaturation	10 s	95°C	
Annealing	30 s	55°C	
Extension	10 s	72°C	Fluorescence data acquisition on the “Green” channel.  Suitable for PCR products up to 150 bp. For longer PCR products, use 8 s extension time per 100 bp of PCR product length.
<b>Number of cycles</b>	45	5–10 ng template DNA	
	40	11–50 ng template DNA	
<b>HRM analysis for:</b>	2 s	65–95° C	Fluorescence data acquisition on the “HRM” channel, for details see page 9.
<b>Rotor-Gene Q</b>		0.1 °C increments	

Vir: <http://www.qiagen.com>

# MS-HRM

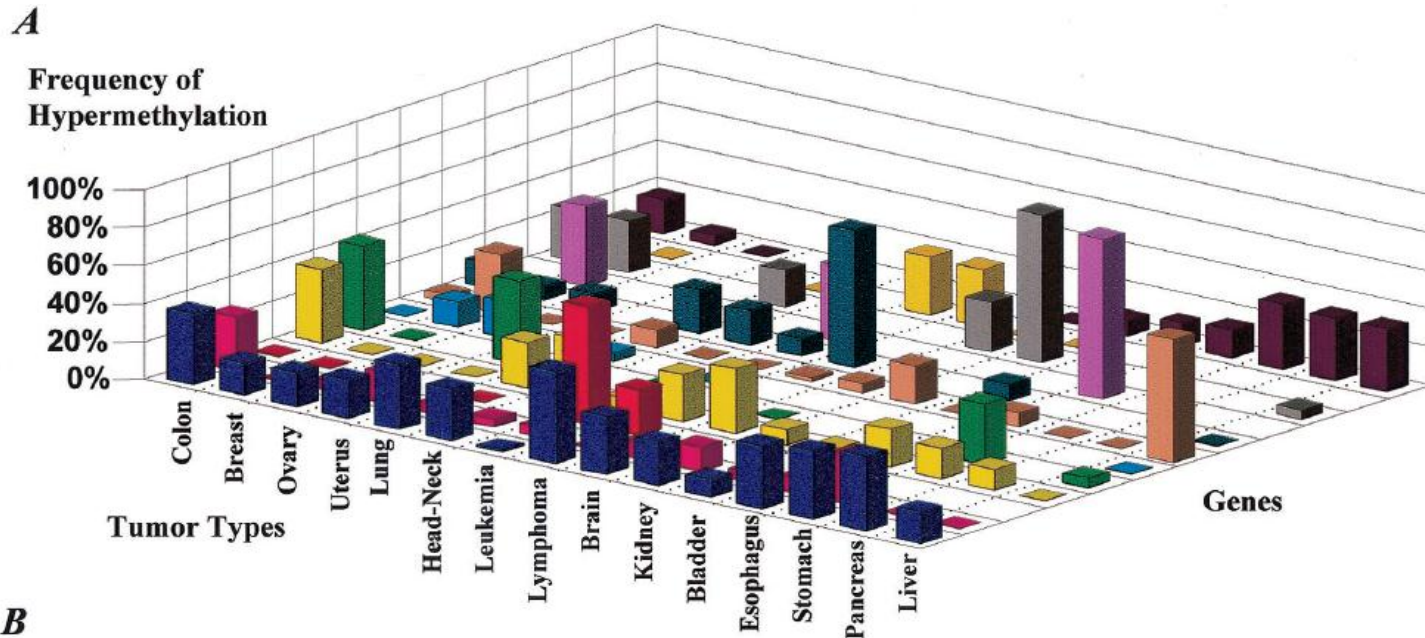
Izkoriščamo možnost detekcije **razklenitve dvojnoverižne DNA zaradi zviševanja temperature.**

Metoda je:  
-hitra, enostavna  
-visoko občutljiva (0,1%)  
-visoko ponovljiva



Prirejeno po: Wojdacz et al ,  
*Nucl. Acid Res.* 2007 35(1):e41

# Hipermetilacijski profil pri raku



**B**



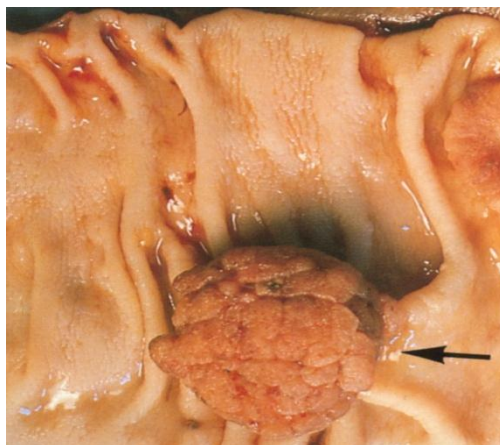
Celični cikel

Popravljanje  
DNA

adhezija/metastaze



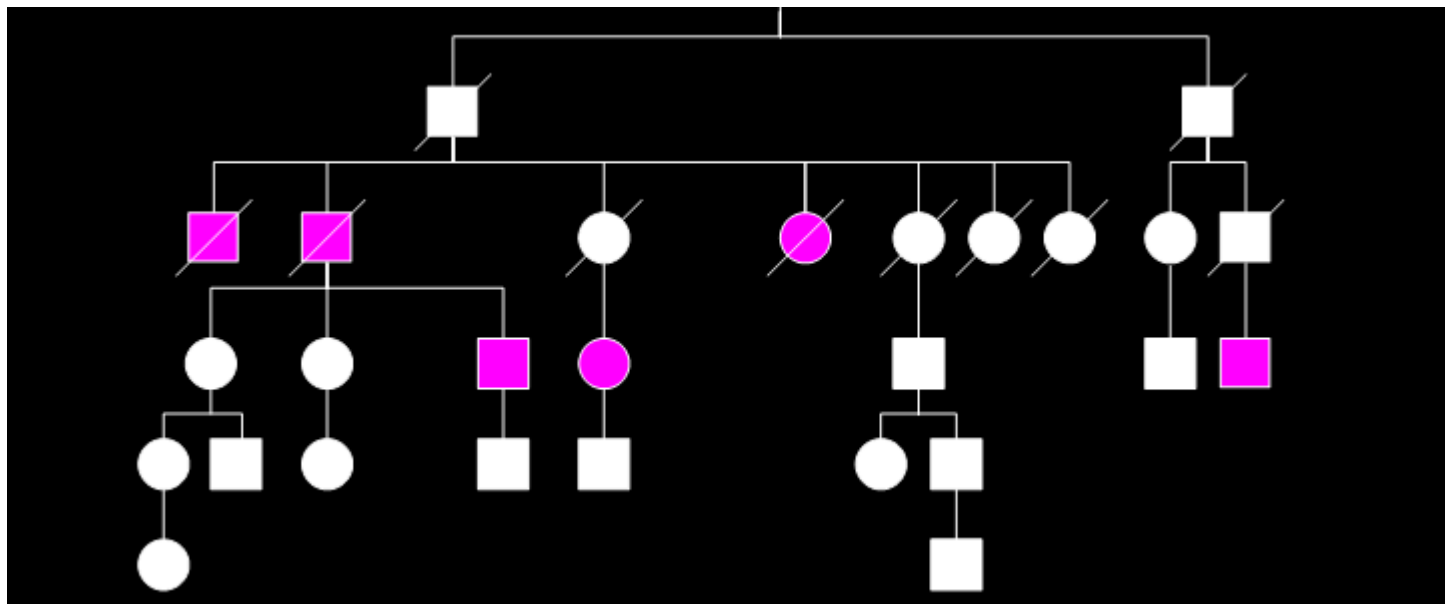
## DEDNI NEPOLIPOZNI KOLOREKTALNI RAK - HNPCC (Sindrom Lynch)



- avtosomno dominantno dedovanje
- pogostost bolezni 1 : 200-1000
- >90% tumorjev bolnikov izraža H-MSI
- Podedovane mutacije v genih popravljalnega mehanizma DNA neujemanja
  - **MLH1, MSH2, MSH6, PMS1, PMS2**

# ***DETEKCIJA SINDROMA LYNCH***

***(DRUŽINSKA ANAMNEZA)***



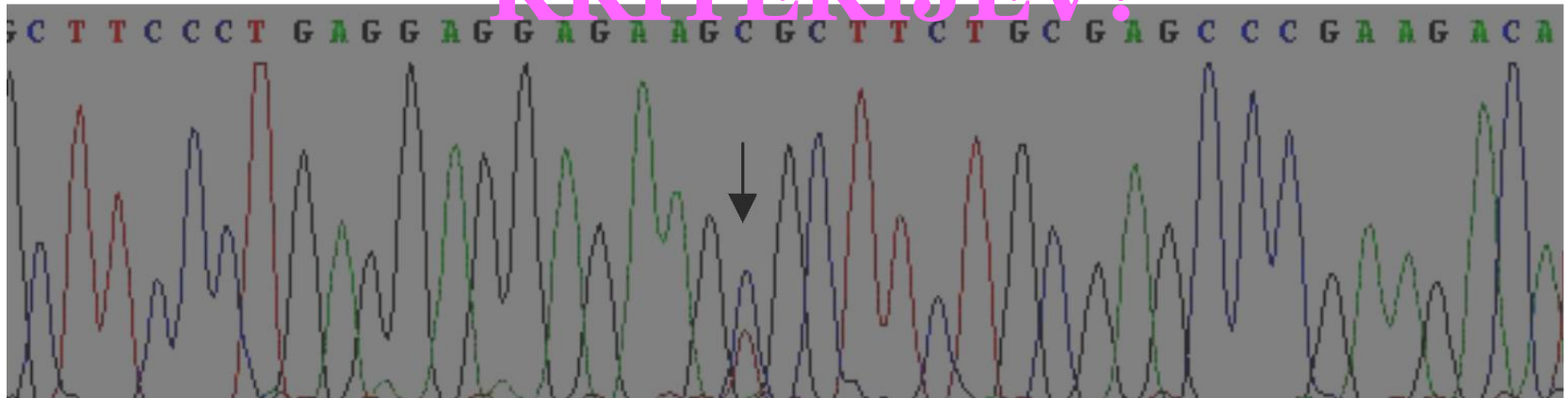
4 0

4 1

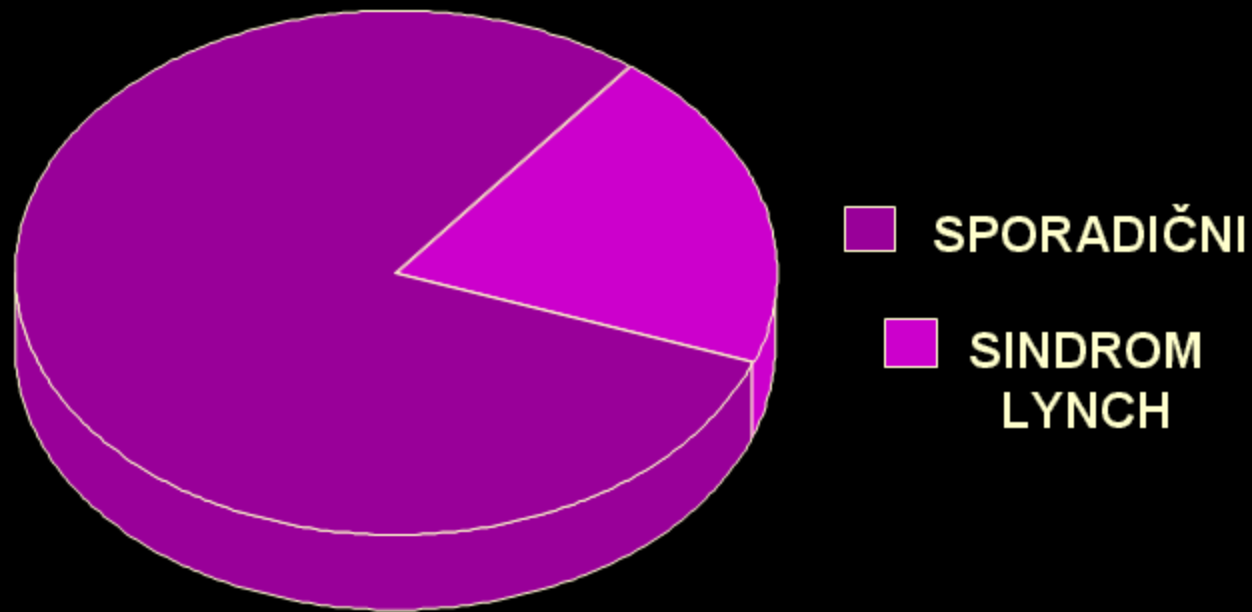
4 2

ALI LAHKO  
PREPOZNAMO DRUŽINE S  
SINDROMOM LYNCH SAMO  
NA OSNOVI  
MOLEKULARNOGENETSKIH  
KRITERIJEV?

T/C



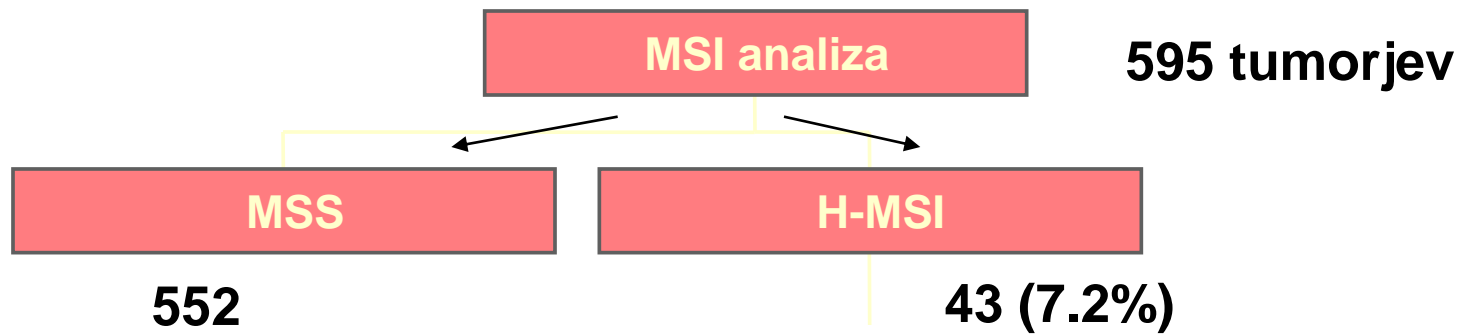
# **PORAZDELITEV VISOKO MSI TUMORJEV**



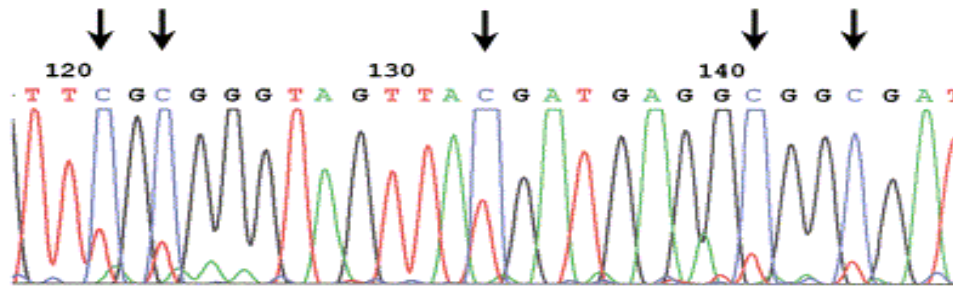
**■ LYNCH- MUTACIJE V GENIH POPRAVLJALNEGA MEHANIZMA  
DNA NEUJEMANJA**

**■ SPORADIČNI- HIPERMETILACIJA PROMOTORJA *MLH1***

# NAKLJUČNO IZBRANI KOLOREKTALNI RAKI

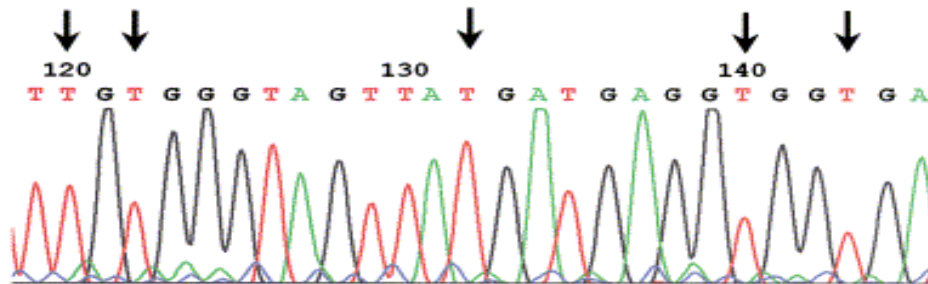


# DOLOČITEV STOPNJE METILACIJE



MSI 1

Hipermetilacija

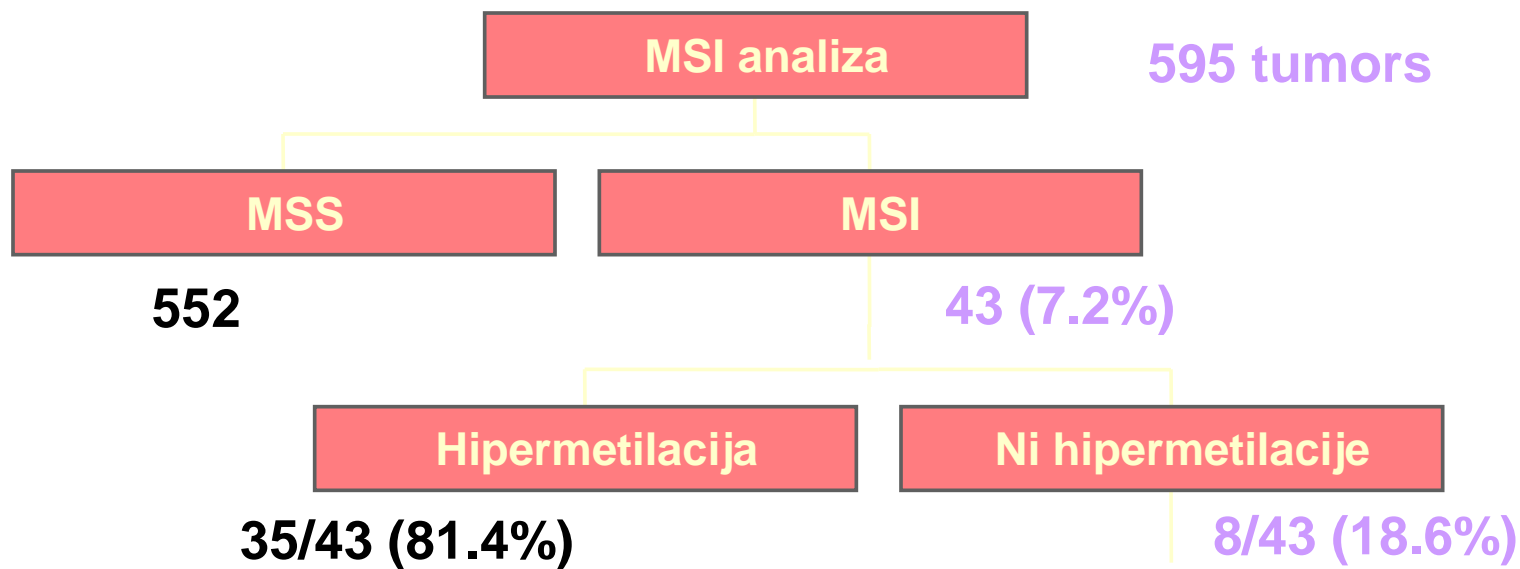


MSI 7

Ni Hipermetilacije

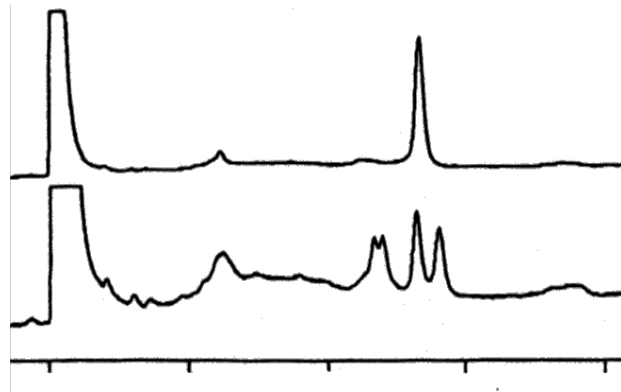
Stopnja metilacije promotorja gena MLH1 je bila določena s sekveniranjem z bisulfitom spremenjene DNA

# NAKLJUČNO IZBRANI KOLOREKTALNI RAKI

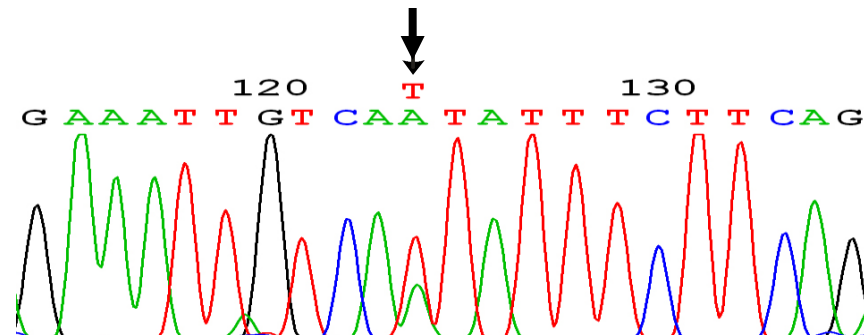
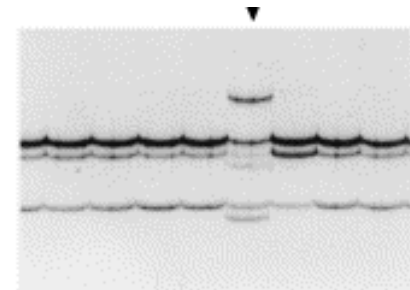


# DOLOČANJE MUTACIJ V GENIH *MLH1* in *MSH2*

DHPLC



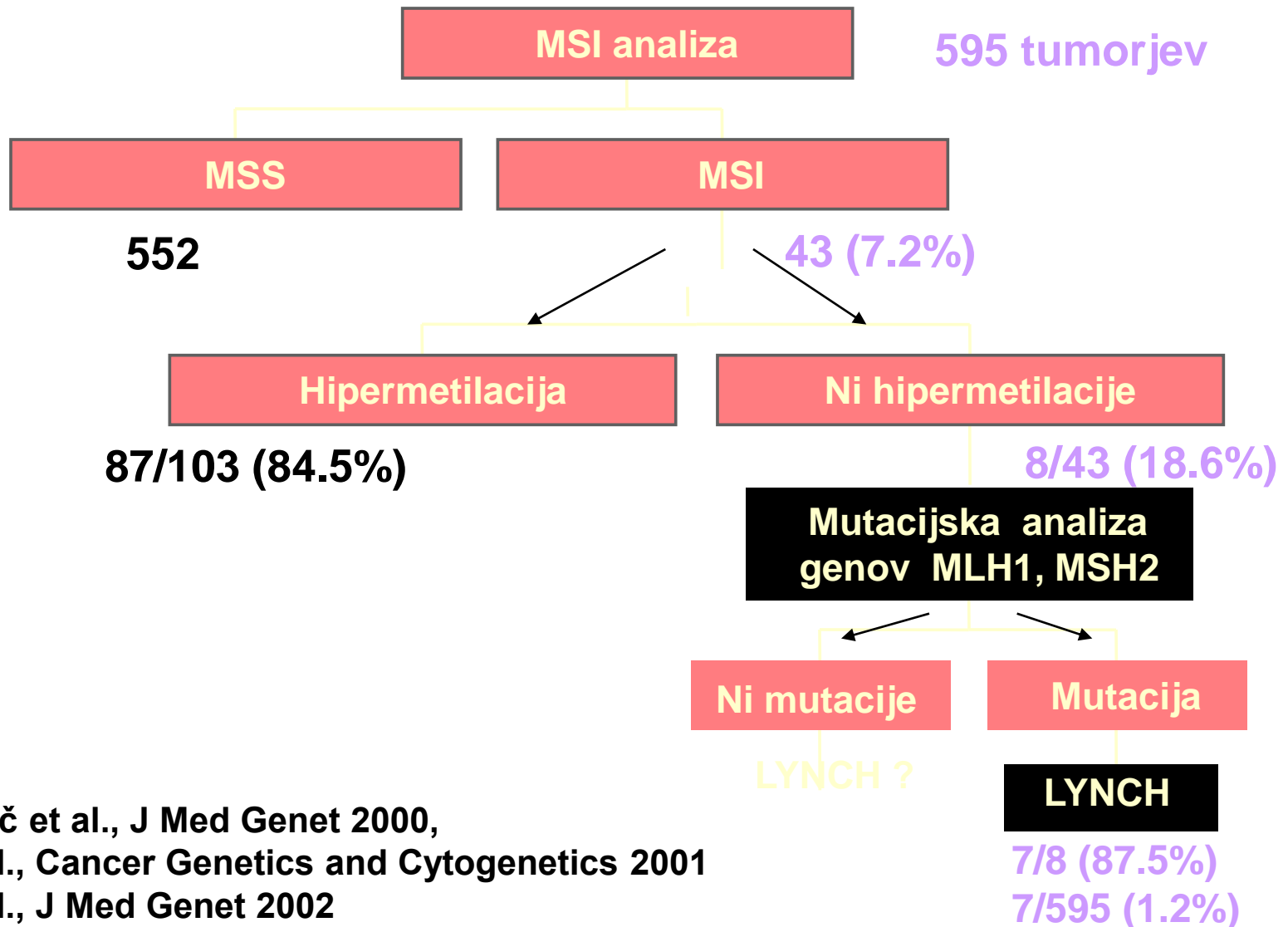
SSCP



SEKVENIRANJE



# NAKLJUČNO IZBRANI KOLOREKTALNI RAKI



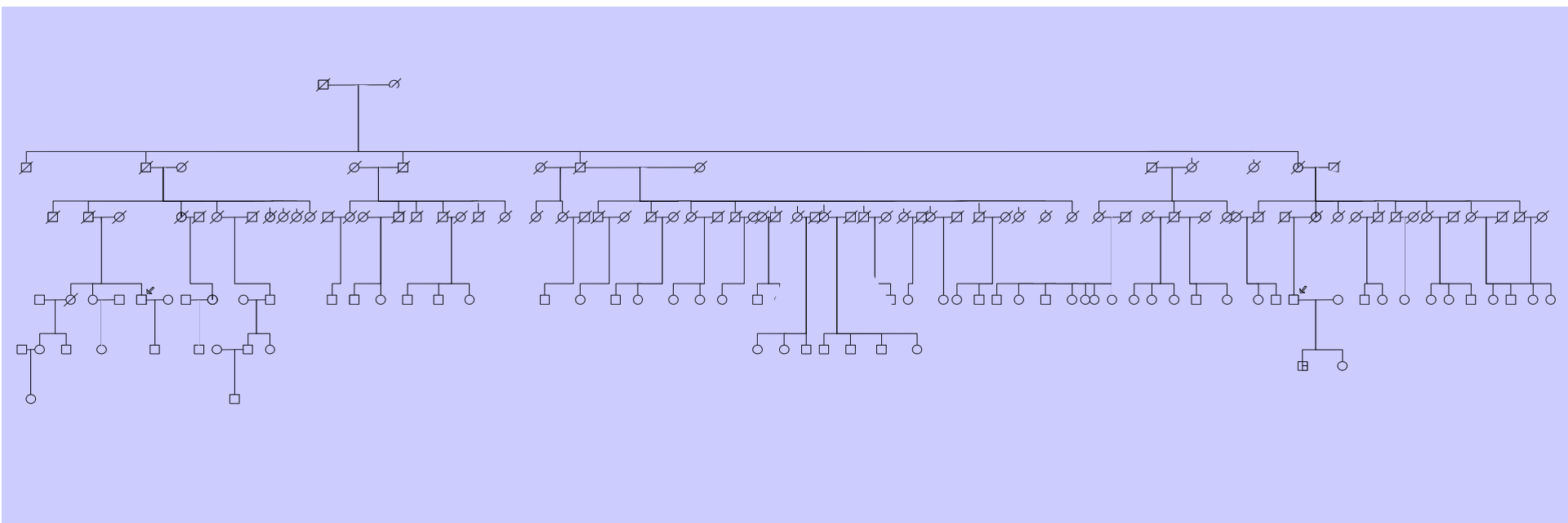
Ravnik-Glavač et al., J Med Genet 2000,  
Potočnik et al., Cancer Genetics and Cytogenetics 2001  
Potočnik et al., J Med Genet 2002

# DETEKCIJA MUTACIJ

- **Amsterdamski kriterij**  
(družinska anamneza)
  - ~ 60% verjetnost za odkritje mutacije
- **Molekularnogenetski kriterij**
  - 87.5% detekcija mutacije

# ODRITJE NOVIH DRUŽIN S SINDROMOM LYNCH

(162 družinskih članov)



**Ravnik-Glavač et al. Hum Hered 1998**

**Potočnik et al. Hum Hered 2000**

**Ravnik-Glavač et al., J Med Genet 2000,**

**Potočnik et al., Cancer Genetics and Cytogenetics 2001**

# SMISELNOST ODKRIVANJA DRUŽIN S SINDROMOM LYNCH

- OKRITJE PODEDOVANE MUTACIJE POMENI DOKONČNO DIAGNOZO O PRISOTNOSTI SINDROMA LYNCH
- OMOGOČENO JE GENETSKO SVETOVANJE IN TESTIRANJE PRED POJAVOM SIMPTOMOV ZA SORODNIKE S TVEGANJEM OBOLENJA
  - Olajšanje skrbi ne-noslicem mutacije
  - Preventivni programi za nosilce mutacij, ki imajo 80 % doživljenjsko tveganje
  - (periodični pregledi)

# EPIGENETSKA TERAPIJA

- aktivirati epigenetsko utišane gene

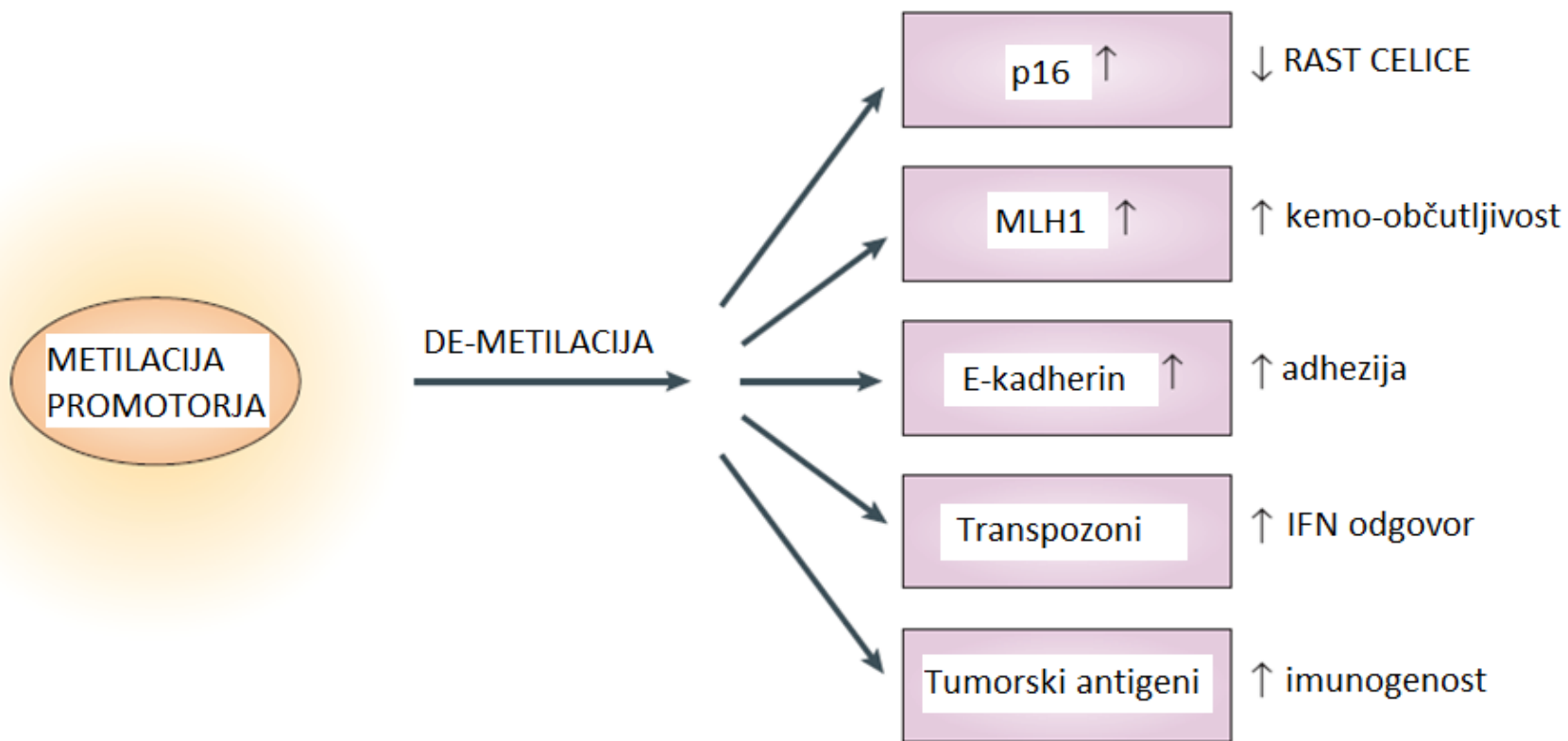
## PREDNOSTI:

- nizek nivo doziranja (nižja toksičnost v primerjavi s kemoterapijo)
- učinek predvsem na celice, ki se hitro delijo

## TEŽAVE:

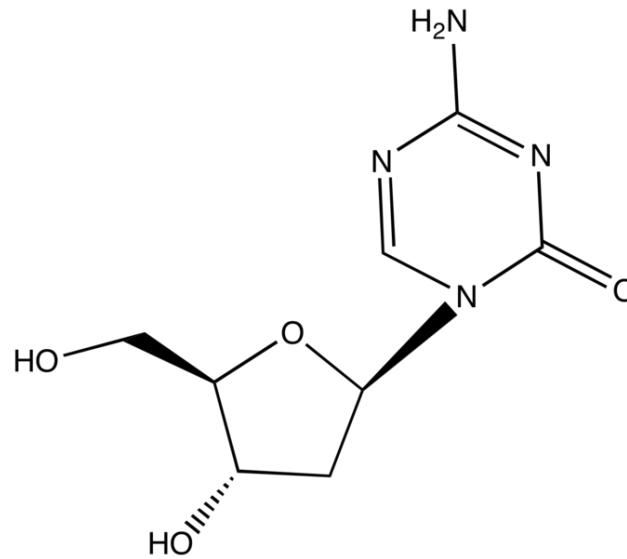
- specifičnost
- selektivnost
- uporaba pri otrocih in nosečnicah
- zaenkrat brez uspehov pri preživetju!!

# Uporaba de-metilacije pri zdravljenju raka



- inhibitorji histonskih deacetilaz (HDAC inhibitorji)
- inhibitorji DNA metiltransferaz

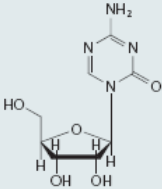
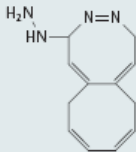
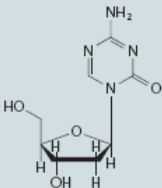
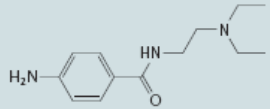
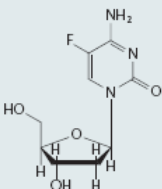
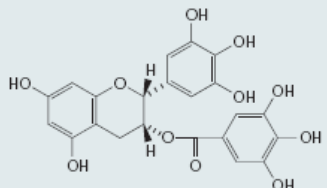
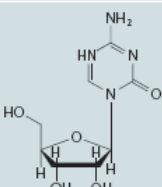
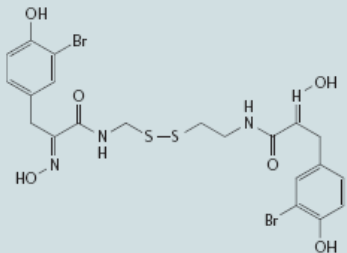
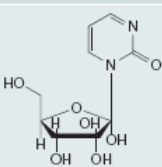
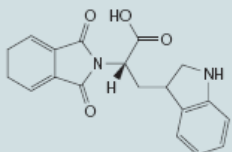
**Decitabine** pri mielodisplastičnem sindromu (MDS)



**5-aza-2'-deoxycytidine**

**Upočasni proces do transformacije v akutno mieloidno levkemija (AML)**

# Inhibitorji DNA metilacije, nukleozidni analogi in ne-nukleozidni analogi

Inhibitor	Structure	Dose range	Clinical trials	References	Inhibitor	Structure	Dose range	Clinical trials	References
5-Azacytidine		μM	Phase I, II, III: haematological malignancies	51	Hydralazine		μM	Phase I: cervical cancer	86
5-Aza-2'-deoxycytidine		μM	Phase I, II, III: haematological malignancies; cervical, non-small-cell lung cancer	52-55,129,130	Procainamide		μM	Preclinical	82-84
5-Fluoro-2'-deoxycytidine		μM	Phase I	47,66	EGCG		μM	Preclinical	80
5,6-Dihydro-5-azacytidine		μM	Phase I, II: ovarian cancer and lymphomas	65	Psammaplin A		nM-μM	Preclinical	79
Zebularine		μM-mM	Preclinical	16,74	MG98	N/A	N/A	Phase I: advanced/metastatic solid tumours	77-78
					RG108		μM	Preclinical	75



Current testing and clinical application of DNA methylation for cancer

**Diagnosis and early detection**

Study	Cancer type	Gene	Tissue	Sensitivity
Belinsky et al (129)	Lung	<i>p16<sup>INK4A</sup>, PAX5B, MGMT, DAPK, GATA5, RASSF1A</i>	Sputum	64%
Gonzalgo et al (130)	Prostate	<i>GSTP1</i>	Urine	58%
Hoque et al (131)	Prostate	<i>GSTP1, p16<sup>INK4A</sup>, p14<sup>ARF</sup>, MGMT</i>	Urine	87%
Chen et al (132)	Colon	<i>VIM</i> exon1	Stool	43%
Lenhard et al (133)	Colon	<i>HIC1</i>	Stool	42%
Krassenstein et al (134)	Breast	<i>DAPK, RARB, p16<sup>INK4A</sup>, p14<sup>ARF</sup>, RASSF1A, GSTP1</i>	Nipple aspirate	82%

<b>Prognosis</b>			
<b>Study</b>	<b>Cancer Type</b>	<b>Gene</b>	<b>Outcome</b>
Lu et al (114)	Lung	<i>DAPK</i>	HR for death (M vs. U) 1.69
Brock et al (117)	Lung	<i>p16, H-cadherin, APC, RASSF1A</i>	HR for death (M vs. U) up to 15.5
Harbeck et al (115)	Breast	<i>PITX2</i>	HR for distant recurrence (M vs. U) 2.35
Alumkal et al (119)	Prostate	<i>ASC, CDH-13</i>	HR for PSA recurrence (M vs. U) 5.64

HR (hazard ratio) = razmerje tveganosti

Prediction of Response				
Study	Cancer Type	Gene	Therapy	Outcome
Esteller et al (121)	Glioma	<i>MGMT</i>	Carmustine	HR for death U vs. M: 9.5
Hegi et al (122)	Glioma	<i>MGMT</i>	Temozolomide	HR for death U vs. M: 2.2
Taniguchi et al (126)	Ovarian	<i>FANCF</i>	Cisplatin	In vitro assays IC50, <1.0 µmol/L (S), >1.0 µmol/L(RS)
Satoh et al (125)	Gastric	<i>CHFR</i>	Taxane	Increased sensitivity in in vitro assays
Agrelo et al (128)	Colon	<i>Werner-1</i>	Irinotecan	OS 39.4 (M) vs. 20.7 (U) months <i>P</i> < 0.05

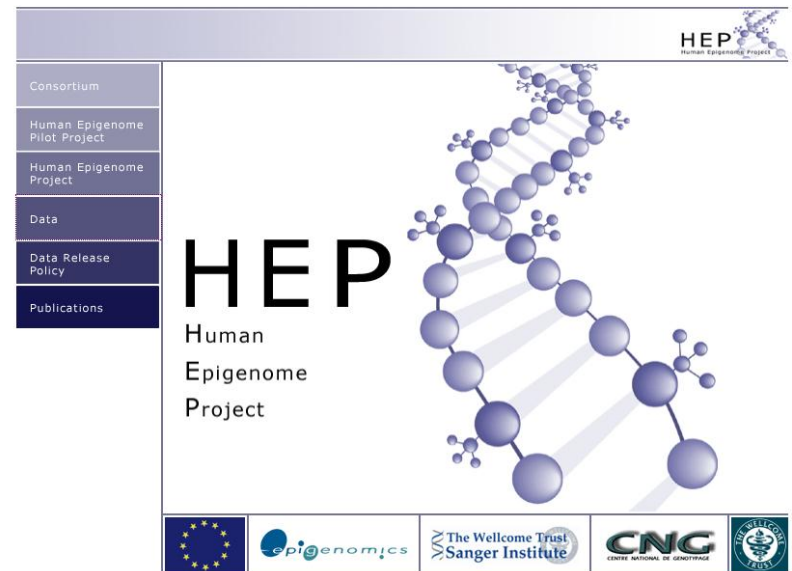
- Abbreviations: HR, hazard ratio; PSA, prostate-specific antigen.

# Koristne e-povezave:

<http://www.epigenome.org/index.php>

<http://www.methdb.de/>

<http://www.protocol-online.org/>



[http://hstalks.com/main/search\\_bar.php?s=dna+methylation&l=252&k=TALK](http://hstalks.com/main/search_bar.php?s=dna+methylation&l=252&k=TALK)

(za ogled predavanj mnogih profesorjev z uglednih univerz je potrebna predhodna registracija)