Metody studia epigenetických jevů

- analýza genové exprese:
  Northern, *in situ* hybridizace,
  RT-PCR, *microarrays*
- analýza metylace DNA:
  restrikční enzymy, nukleázy, Southern,
  genomové sekvenování
- analýza modifikací histonů:
  Western, imunobarvení,
  chromatinová imunoprecipitace
- aplikace inhibitorů modifikujících enzymů

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Originální práce o Northern-blottingu :

Dle WoS práce citována 1.771-krát

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5350–5354, December 1977
Biochemistry

Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes

(single-stranded nucleic acids/methyl mercuric hydroxide-agarose gels/*Drosophila melanogaster* RNA/hybrid plasmids)

JAMES C. ALWINE*, DAVID J. KEMP, AND GEORGE R. STARK

Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Communicated by David S. Hogness, September 12, 1977

ABSTRACT **We describe a technique for transferring electrophoretically separated bands of RNA from an agarose gel to paper strips. The RNA is coupled covalently to diazobenzyloxymethyl groups on the paper. After transfer and appropriate treatment of the paper to destroy remaining diazo groups, specific RNA bands can be detected by hybridization with ^{32}P-labeled DNA probes followed by autoradiography. This procedure allows detection of specific RNA bands with high sensitivity and low background.**

was allowed to crystallize. It was collected on a sintered glass filter, washed with pyridine, then washed thoroughly with petroleum ether and dried under reduced pressure. The NBPC (267 g) was stored at −20° in a desiccator.

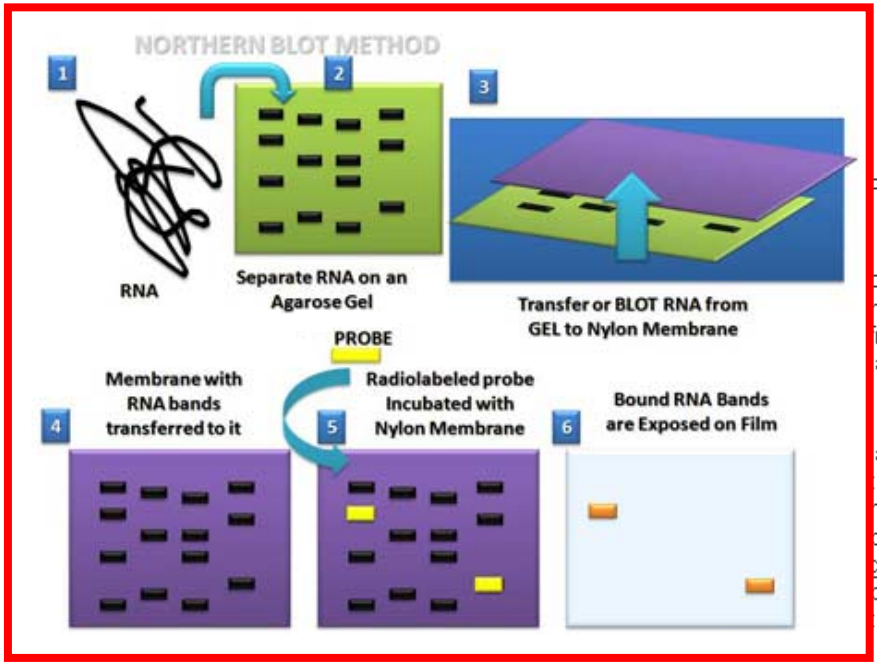
Preparation of Aminobenzyloxymethyl-Paper. The method for making aminobenzyloxymethyl (ABM)-paper and its subsequent conversion to the diazobenzyloxymethyl form (DBM-paper) by diazotization is outlined in Fig. 1. The

Background Information - Northern Blot Technique

Northern analysis despite its age in the high tech world of Real Time PCR, nuclease protection assays (RPAs) and microarrays, is still the gold-standard for the detection and quantitation of mRNA levels. This is because northern blot analysis allows a direct comparison of the messenger RNA abundance between samples on a single membrane.

In northern blot the main difference between the other blotting techniques is that RNA is the factor being detected. Also, due to the fact that RNA is usually single-stranded, it creates complex secondary structures which affect its migration and hence denaturing conditions are used to run the gels (unlike Southern).

RNA is separated out by **RNA gel electrophoresis** (usually agarose gel electrophoresis), subsequent transfer to membrane, hybridization with probe, and finally detection.



Similarly to Southern blotting, the hybridization probes may be DNA or RNA in northern blotting.

A variant of the procedure known as the reverse northern blot was occasionally (although, infrequently) used. In this procedure, the substrate nucleic acid (that is affixed to the membrane) is a collection of isolated DNA fragments, and the probe is RNA extracted from a tissue and radioactively labelled.

The use of DNA microarrays that have come into widespread use in the late 1990s and early 2000s is more akin to the reverse procedure, in that they involve the use of isolated DNA fragments affixed to a substrate, and hybridization with a probe made from cellular RNA. Thus the reverse procedure, though originally uncommon, enabled the one-at-a-time study of gene expression using northern analysis to evolve into gene expression profiling, in which many (possibly all) of the genes in an organism may have their expression monitored

Applications of the Northern Blot

Northern blots have been superceded in most areas by Real Time PCR and microarray approaches. It is not often used for clinical or diagnostic purposes.

The northern blot protocol and its variations are used however in molecular biology research to:

- a gold-standard for the direct study of gene expression at the level of mRNA (messenger RNA transcripts).
- detection of mRNA transcript size
- study RNA degradation
- study RNA splicing - can detect alternatively spliced transcripts
- study RNA half-life
- study IRES (internal ribosomal entry site) - to remove possibility of RNA digestion vs 2nd cistron translation.
- often used to confirm and check transgenic / knockout mice (animals)

Real-time polymerase chain reaction

From Wikipedia, the free encyclopedia

In molecular biology, **real-time polymerase chain reaction**, also called **quantitative real time polymerase chain reaction** (qPCR) or **kinetic polymerase chain reaction**, is a laboratory technique based on the polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification (as absolute number of copies or relative amount when normalized to DNA input or additional normalizing genes) of a specific sequence in a DNA sample.

The procedure follows the general principle of polymerase chain reaction; its key feature is that the amplified DNA is quantified as it accumulates in the reaction in *real time* after each amplification cycle. Two common methods of quantification are the use of fluorescent dyes that intercalate with double-stranded DNA, and modified DNA oligonucleotide probes that fluoresce when hybridized with a complementary DNA.

Frequently, real-time polymerase chain reaction is combined with reverse transcription polymerase chain reaction to quantify low abundance messenger RNA (mRNA), enabling a researcher to quantify relative gene expression at a particular time, or in a particular cell or tissue type.

Although real-time quantitative polymerase chain reaction is often marketed as RT-PCR, it should not be confused with reverse transcription polymerase chain reaction, also known as RT-PCR.

Contents [hide]

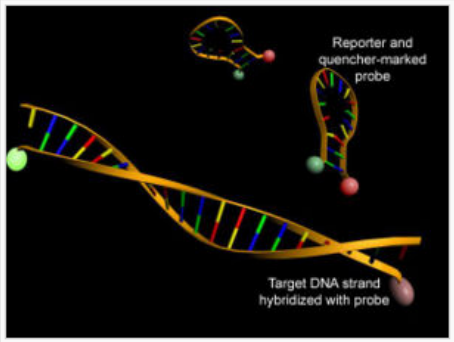
Background [edit]

Cells in all organisms regulate their cellular activities by activating or deactivating the expression of their genes. Gene expression is usually directly proportional to the number of copies of messenger RNA (mRNA) of a particular gene in a cell or tissue.

Traditionally, the expression level of a gene has been estimated by visualizing the abundance of its mRNA transcript in a sample with a technique called northern blotting. In this method, purified RNA is separated by agarose gel electrophoresis, transferred to a solid matrix (such as a nylon membrane), and probed with a specific DNA probe that is complementary to the gene of interest. Although this technique is still used to assess gene expression, it requires relatively large amounts of RNA and provides only qualitative or semiquantitative information of mRNA levels.

In order to robustly detect and quantify gene expression from small amounts of RNA, amplification of the gene transcript is necessary. The polymerase chain reaction is a common method for amplifying DNA; for mRNA-based PCR the RNA sample is first reverse transcribed to cDNA with reverse transcriptase.

Development of PCR technologies based on reverse transcription and *fluorophores* permits measurement of DNA amplification during PCR in real time, i.e., the amplified product is measured at each PCR cycle. The data thus generated can be analysed by computer software to calculate *relative gene expression* in several samples, or *mRNA copy number*. Real-time PCR can also be applied to the detection and quantification of DNA in samples to determine the presence and abundance of a particular DNA sequence in these samples.



Real time quantitative PCR uses fluorophores in order to detect levels of gene expression.

Real-time PCR using double-stranded DNA dyes [edit]

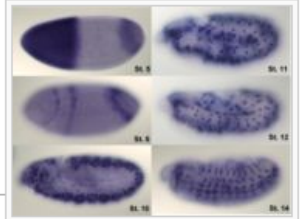
A DNA-binding dye binds to all double-stranded (ds)DNA in a PCR reaction, causing fluorescence of the dye. An increase in DNA product during PCR therefore leads to an increase in fluorescence intensity and is measured at each cycle, thus allowing DNA concentrations to be quantified. However, dsDNA dyes such as SYBR Green will bind to all dsDNA PCR products, including nonspecific PCR products (such as "primer dimers"). This can potentially interfere with or prevent accurate quantification of the intended target sequence.

1. The reaction is prepared as usual, with the addition of fluorescent dsDNA dye.
2. The reaction is run in a thermocycler, and after each cycle, the levels of fluorescence are measured with a detector; the dye only fluoresces when bound to the dsDNA (i.e., the PCR product). With reference to a standard dilution, the dsDNA concentration in the PCR can be determined.

In situ hybridization

From Wikipedia, the free encyclopedia

In situ hybridization (ISH) is a type of hybridization that uses a labeled complementary DNA or RNA strand (i.e., probe) to localize a specific DNA or RNA sequence in a portion or section of tissue (*in situ*), or, if the tissue is small enough (e.g. plant seeds, Drosophila embryos), in the entire tissue (whole mount ISH). This is distinct from immunohistochemistry, which localizes proteins in tissue sections. DNA ISH can be used to determine the structure of chromosomes. Fluorescent DNA ISH (FISH) can, for example, be used in medical diagnostics to assess chromosomal integrity. RNA ISH (hybridization histochemistry) is used to measure and localize mRNAs and other transcripts within tissue sections or whole mounts.



In situ-Hybridization of wild type Drosophila embryos at different developmental stages for the RNA from a gene called hunchback.

Process [edit]

For hybridization histochemistry, sample cells and tissues are usually treated to fix the target transcripts in place and to increase access of the probe. As noted above, the probe is either a labeled complementary DNA or, now most commonly, a complementary RNA (riboprobe). The probe hybridizes to the target sequence at elevated temperature, and then the excess probe is washed away (after prior hydrolysis using RNase in the case of unhybridized, excess RNA probe). Solution parameters such as temperature, salt and/or detergent concentration can be manipulated to remove any non-identical interactions (i.e. only exact sequence matches will remain bound). Then, the probe that was labeled with either radio-, fluorescent- or antigen-labeled bases (e.g., digoxigenin) is localized and quantitated in the tissue using either autoradiography, fluorescence microscopy or immunohistochemistry, respectively. ISH can also use two or more probes, labeled with radioactivity or the other non-radioactive labels, to simultaneously detect two or more transcripts.

References [edit]

1. Jin L, Lloyd RV. In situ hybridization: methods and applications. J Clin Lab Anal. 11(1):2-9, 1997. PMID 9021518
2. Comprehensive and annotated in situ hybridization histochemistry

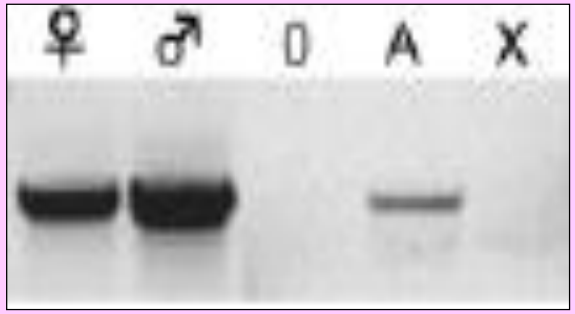
External links [edit]

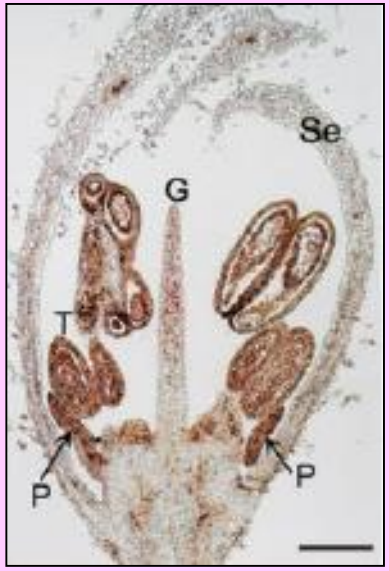
- MeSH *In+Situ+Hybridization*
- Whole-Mount In Situ Hybridization of RNA Probes to Plant Tissues
- Preparation of Complex DNA Probe Sets for 3D FISH with up to Six Different Fluorochromes
- Transcript In Situ Hybridization of Whole-Mount Embryos for Phenotype Analysis of RNAi-Treated Drosophila

Duplikativní přenos MADS-boxového genu z autozomu na chromozom Y

Detekce exprese (mRNA) genu na histologickém preparátu

autozomální kopie exprimovaná v petalech *SlAP3*

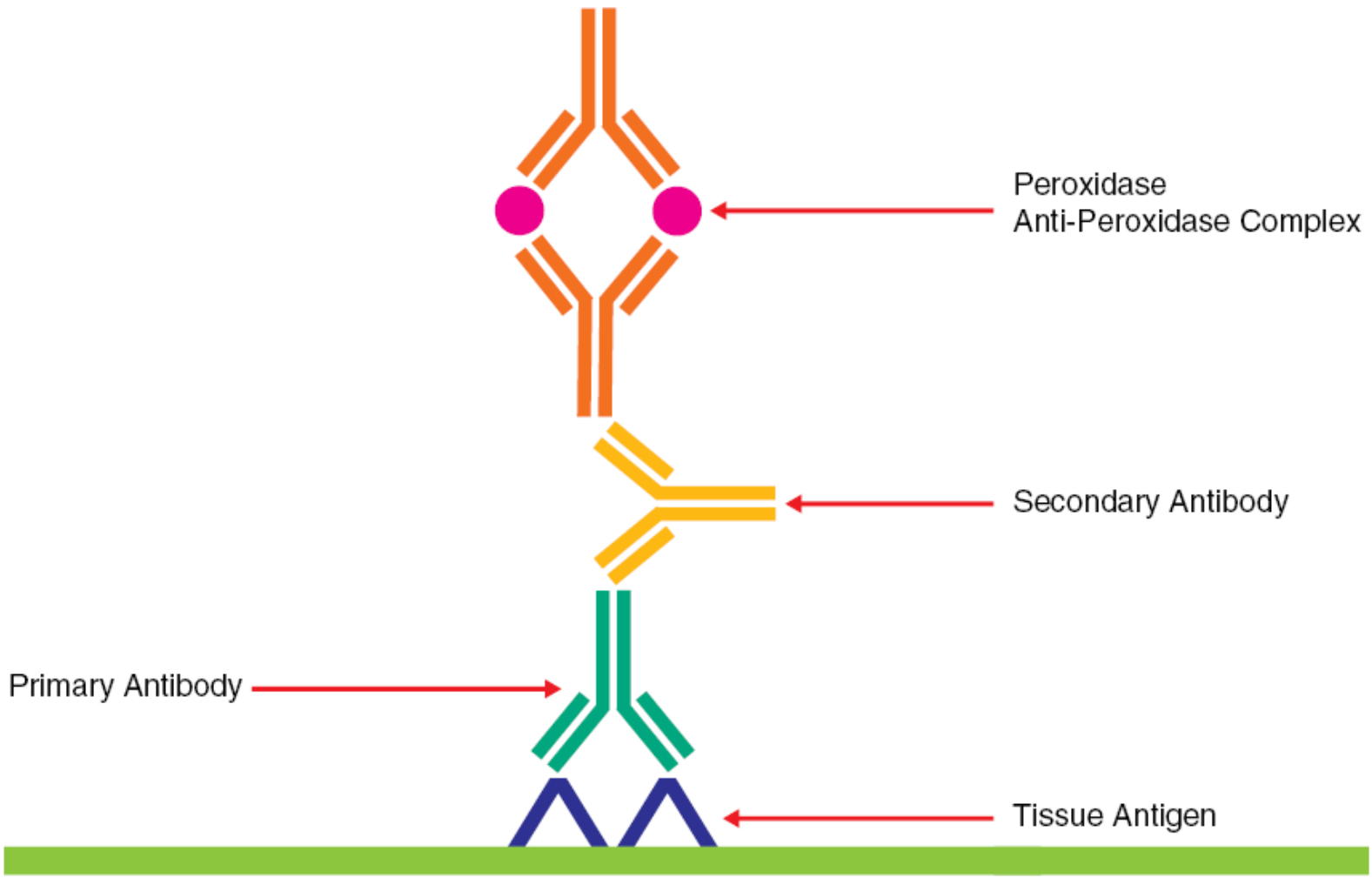




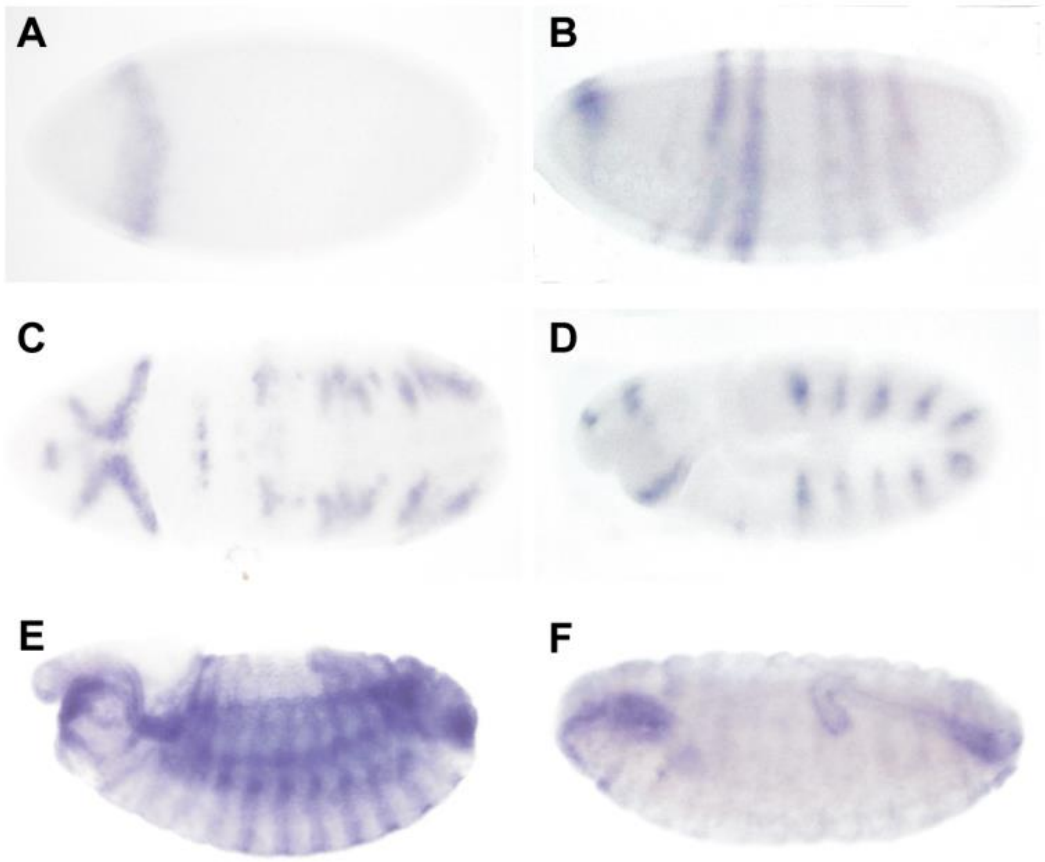
Y-vázaná kopie exprimovaná v tyčinkách

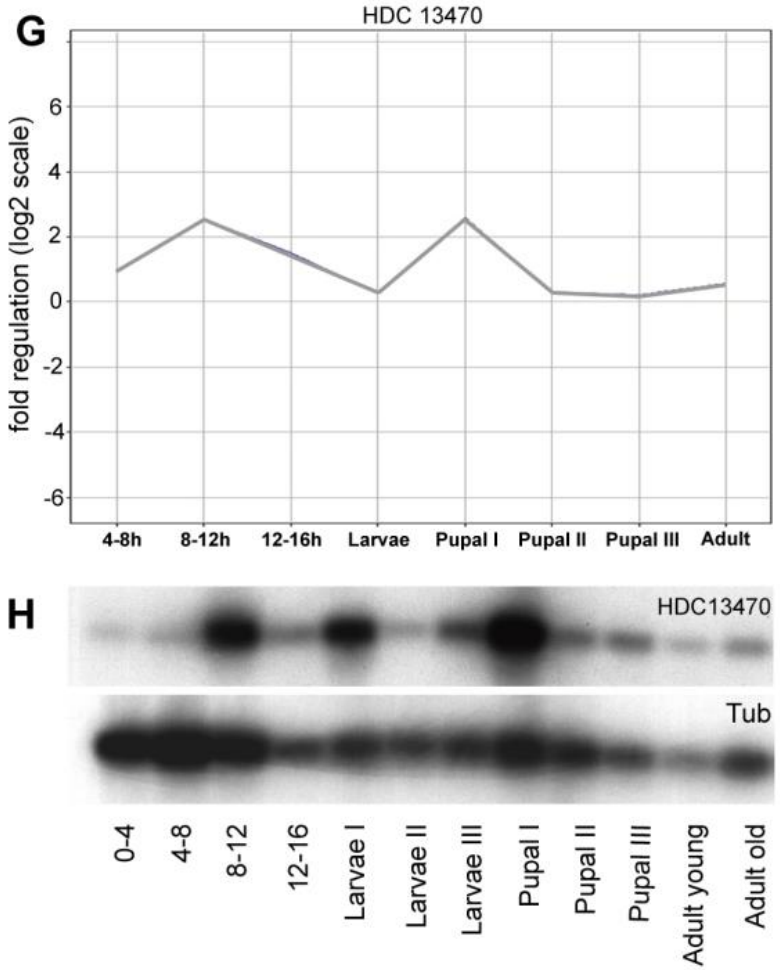
žádný homolog genu *SlAP3* se nevyskytuje na X-chromozomu

Immunohistochemical Staining Methods



Peroxidase Anti-Peroxidase (PAP) Complex Method



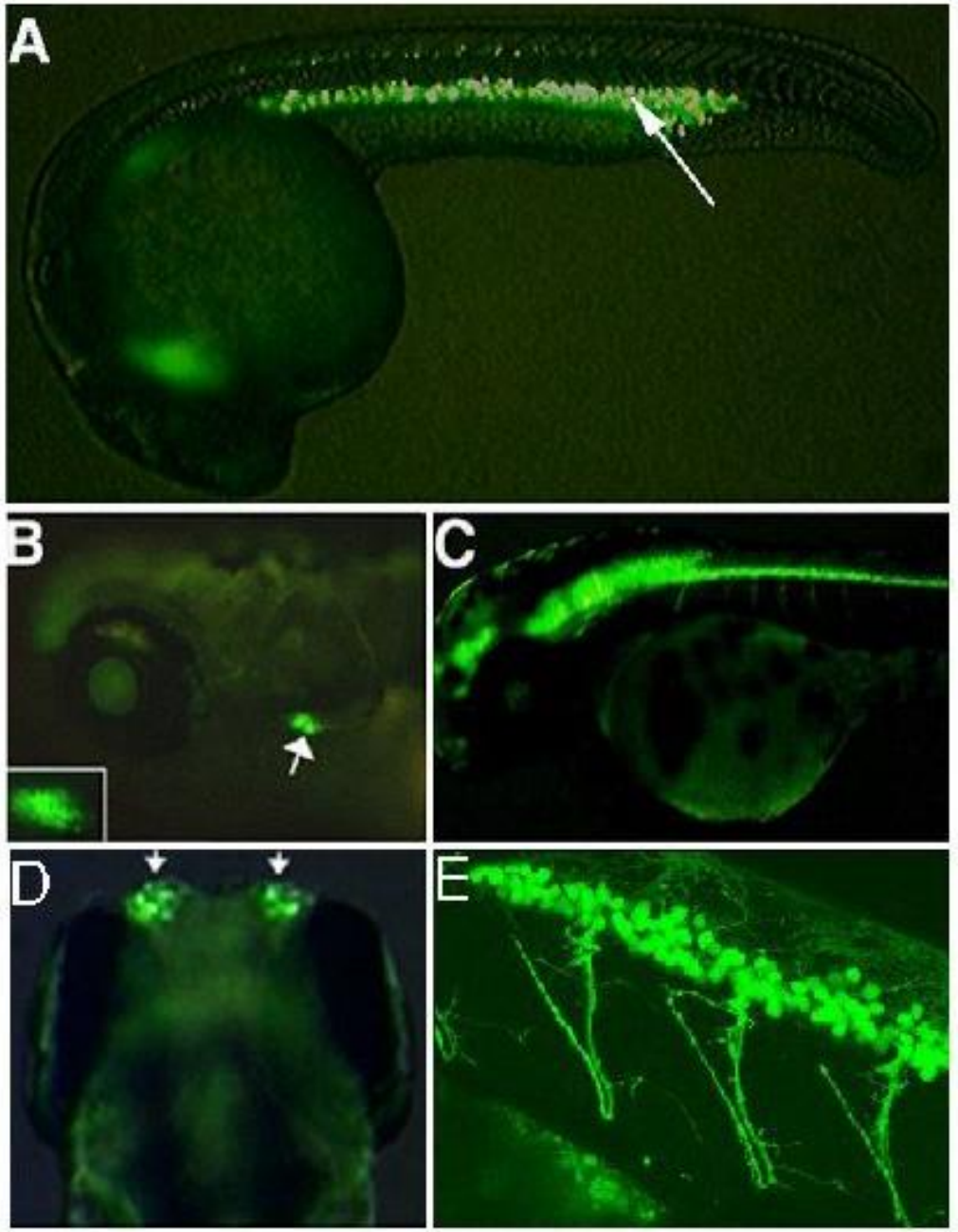


Imunodetekce morfogenů (proteinů) kódovaných larválními vývojovými geny v embryogenezi drosofily (whole-mount approach)

transgenní ryba *(Danio rerio* **zebřička)**

Reportérový gen z medúzy

- „green fluorescence protein“



A. Blood **B**. Thymus **C**. Central Nervous System
D. Olfactory Neurons **E**. Motor Neurons

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METYLACE DNA lze studovat na základě :

(a) metylačně sensitivních restrikčních endonukleáz,

(b) siřičitanového působení na DNA, které deaminuje cytosin na uracil,

(c) chromatinové imunoprecipitace s využitím protilátky vůči metyl-cytosinu

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TABLE A4-1 Isoschizomer Pairs That Differ in Their Sensitivity to Sequence-specific Methylation

Methylated Sequence[b]	Isoschizomer Pairs[a]	
	Cut by	Not Cut by
T^{m5}CCGGA	*Acc*III	*Bsp*MII
TC^{m5}CGGA	*Acc*III	*Bsp*MII
GGWC^{m5}C	*Afl*I	*Ava*II (*Eco*47I)
TCCGG^{m6}A	*Bsp*MII	*Acc*III
C^{m5}CWGG	*Bst*NI (*Mva*I)	*Eco*RII
GGTAC^{m5}C	*Kpn*I	*Asp*718I
C^{m5}CGG	*Msp*I	*Hpa*II (*Hap*II)
C^{m4}CGG	*Msp*I	*Hpa*II
G^{m6}ATC	*Sau*3AI (*Fnu*EI)	*Mbo*I (*Nde*II)
TCGCG^{m6}A	*Sbo*13I	*Nru*I
RG^{m6}ATCY	*Xho*II	*Mfl*I
CC^{m5}CGGG	*Xma*I (*Cfr*9I)	*Sma*I

In each row, the first column lists a methylated sequence, the second column lists an isoschizomer that cuts this sequence, and the third column lists an isoschizomer that does not cut this sequence. For references, please see McClelland and Nelson (1988). (Reprinted, with permission, from McClelland and Nelson 1988.)

[a]An enzyme is classified as insensitive to methylation if it cuts the methylated sequence at a rate that is at least one tenth the rate at which it cuts the unmethylated sequence. An enzyme is classified as sensitive to methylation if it is inhibited at least 20-fold by methylation relative to the unmethylated sequence.

[b]Sequences are in 5′→3′ order. R = G or A; Y = C or T; W = A or T; ^{m4}C = 4-methylcytosine, ^{m5}C = 5-methylcytosine, and ^{m6}A = 6-methyladenine.

MspI

Catalog #	Size	Concentration	Price	Qty
R0106L	25,000 units	20,000 units/ml	$224.00	1
R0106M	25,000 units	100,000 units/ml	$224.00	1
R0106S	5,000 units	20,000 units/ml	$56.00	1
R0106T	5,000 units	100,000 units/ml	$56.00	1

Prices are in US dollars and valid only for US orders.

Download: MSDS PDF

Recognition Site:

5′...C▼CGG...3′
3′...GGC▲C...5′

isoschizomers | compatible ends | single letter code

Source:
A *E. coli* strain that carries the MspI gene from *Moraxella* species (ATCC 49670).

Reagents Supplied:
NEBuffer 2

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1: 75%
NEBuffer 2: 100%
NEBuffer 3: 50%
NEBuffer 4: 50%

When using a buffer other than the optimal (supplied) NEBuffer, it may be nec
more enzyme to achieve complete digestion.

Methylation Sensitivity:
dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Not sensitive

štípe
C/mCGG

Heat Inactivation:
65°C for 20 minutes

HpaII

Catalog #	Size	Concentration	Price	Qty
R0171L	10,000 units	10,000 units/ml	$224.00	1
R0171M	10,000 units	50,000 units/ml	$224.00	1
R0171S	2,000 units	10,000 units/ml	$56.00	1

Prices are in US dollars and valid only for US orders.

Download: MSDS PDF

Recognition Site:

5′...C▼CGG...3′
3′...GGC▲C...5′

isoschizomers | compatible ends | single letter code

Source:
A *E. coli* strain that carries the HpaII gene from *Haemophilus parainfluenzae* (ATC

Reagents Supplied:
NEBuffer 1

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1: 100%
NEBuffer 2: 50%
NEBuffer 3: 10%
NEBuffer 4: 100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be nece
more enzyme to achieve complete digestion.

Methylation Sensitivity:
dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Blocked

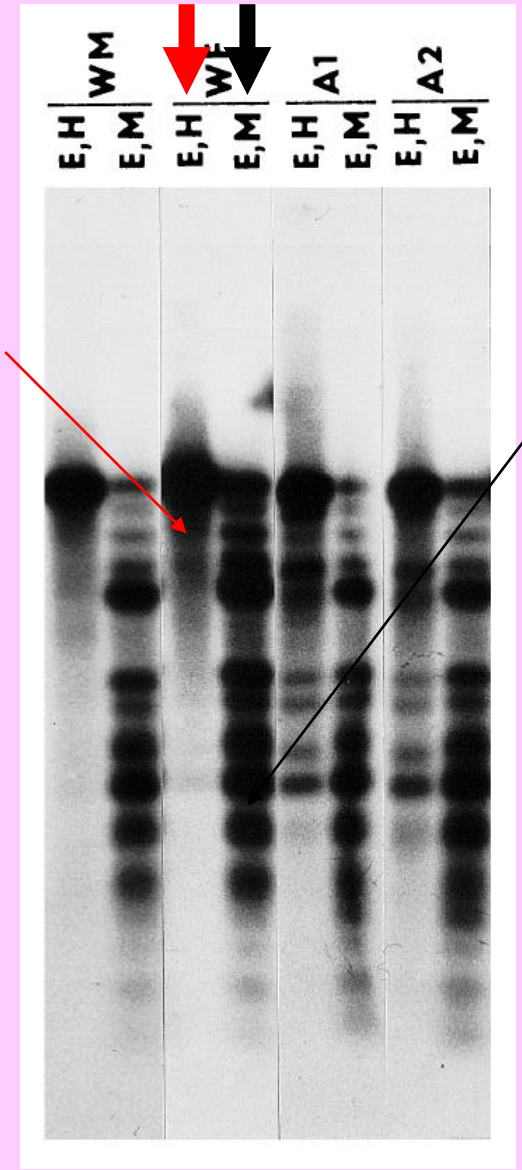
neštípe !
CmCGG

Heat Inactivation:
65°C for 20 minutes

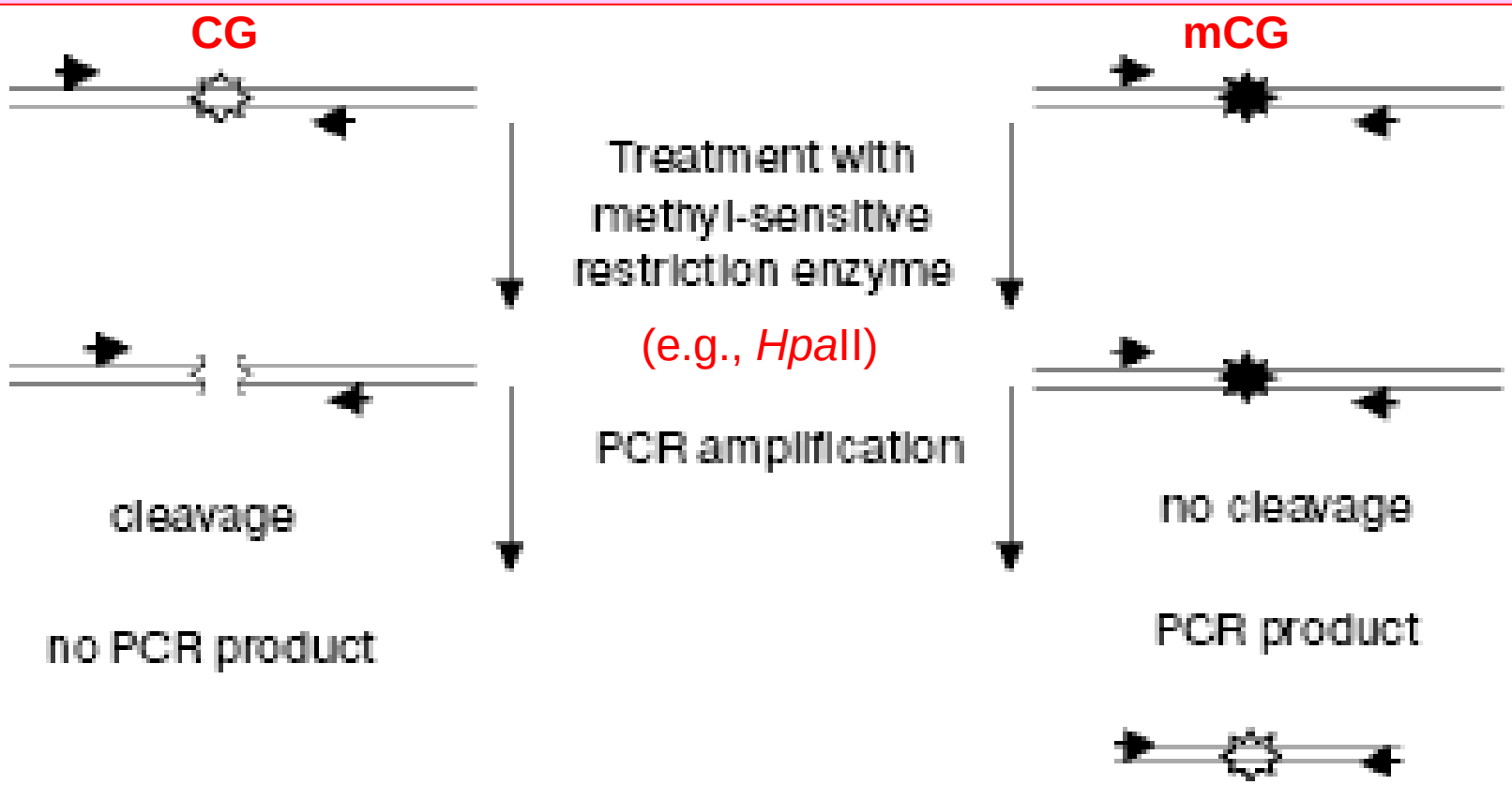
DNA izolovaná z hypometylované rostliny štěpená enzymem *Hpa*II citlivým na CmCGG metylaci (Southern blot)

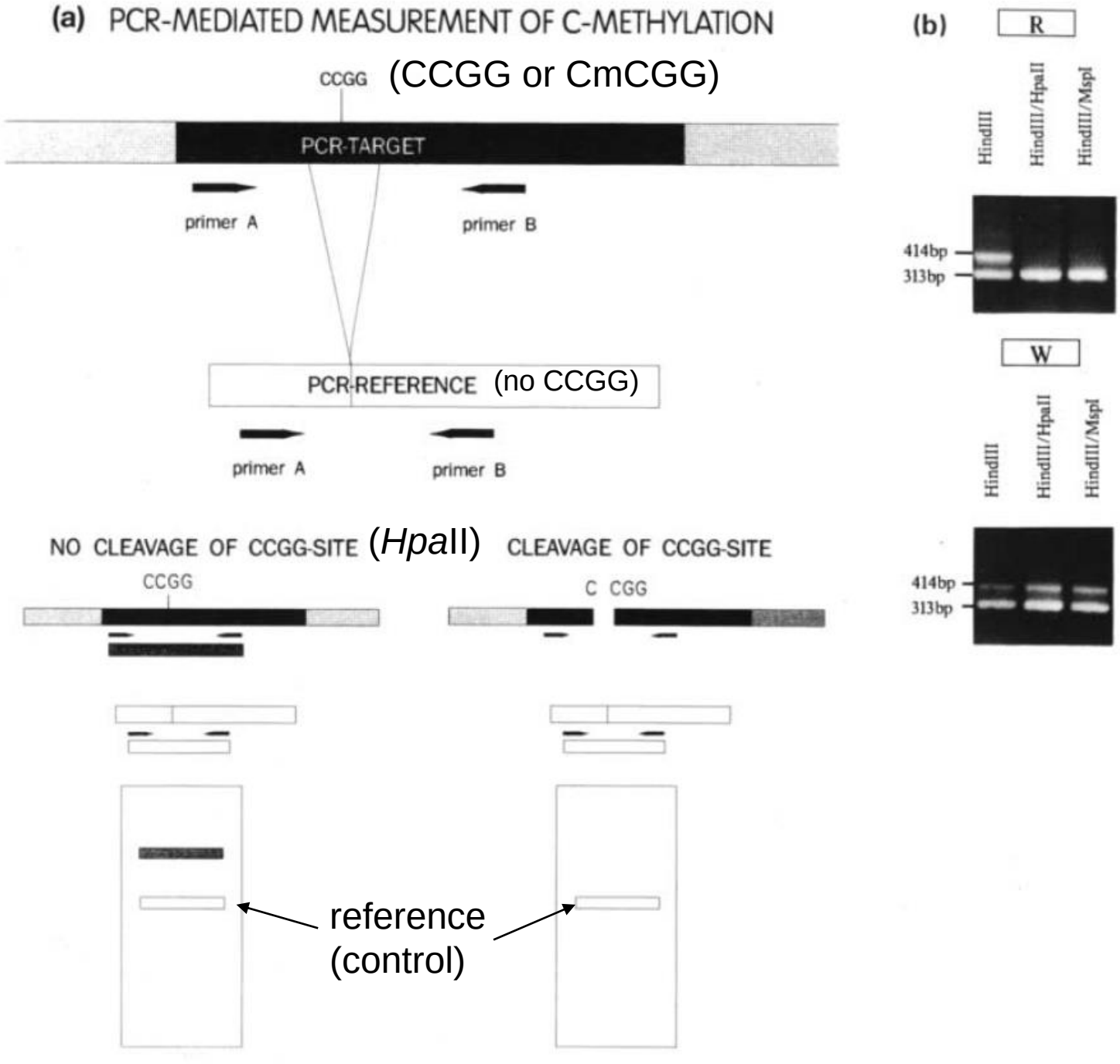
kontrola

Restriktáza *Hpa*II je metylací cytozinu blokována, výsledkem jsou jen velké fragmenty DNA



Restriktáza *Msp*I štěpí jadernou DNA bez ohledu na metylaci cytozinu na menší fragmenty


CG
mCG
Treatment with
methyl-sensitive
restriction enzyme
(e.g., HpaII)
PCR amplification
cleavage
no cleavage
no PCR product
PCR product


(a) PCR-MEDIATED MEASUREMENT OF C-METHYLATION
CCGG (CCGG or CmCGG)
PCR-TARGET
primer A
primer B
PCR-REFERENCE (no CCGG)
primer A
primer B
NO CLEAVAGE OF CCGG-SITE (HpaII)
CLEAVAGE OF CCGG-SITE
CCGG
C CGG
reference
(control)
(b)
R
HindIII
HindIII/HpaII
HindIII/MspI
414bp
313bp
W
HindIII
HindIII/HpaII
HindIII/MspI
414bp
313bp

Journal of Molecular Biology
Volume 98, Issue 3, 5 November 1975, Pages 503-517

Abstract | References | PDF (2669 K)

doi:10.1016/S0022-2836(75)80083-0 Cite or Link Using DOI

Podle WoS práce citována 31.304-krát !

Detection of specific sequences among DNA fragments separated by gel electrophoresis

E.M. Southern

Medical Research Council Mammalian Genome Unit Department of Zoology University of Edinburgh West Mains Road, Edinburgh, Scotland

Received 3 March 1975; revised 26 June 1975. Available online 1 August 2006.

This paper describes a method of transferring fragments of DNA from agarose gels to cellulose nitrate filters. The fragments can then be hybridized to radioactive RNA and hybrids detected by radioautography or fluorography. The method is illustrated by analyses of restriction fragments complementary to ribosomal RNAs from *Escherichia coli* and *Xenopus laevis*, and from several mammals.

Prof. Sir Edwin M. Southern, FRS

(Emeritus Professor, formerly Whitley Professor of Biochemistry, Department of Biochemistry, Oxford)

Professor Sir Edwin Southern

EMS joined Peter Walker in the MRC Mammalian Genome Unit, Edinbu DNA sequencing with an analysis of satellite DNAs from guinea pig (1) and mouse (2,3). These studies led to theories, still accepted, of the structure and e equences and set the scene for the debate on the existence of non-coding sequences in the genomes of the higher eukaryotes (1). The studies of s ethods of analysis. EMS was the first to introduce the use of type II restriction endonucleases to study the structure of sequences from eukaryotic DNA

With Peter Ford, EMS showed that the 5s RNAs of ovaries and somatic 5), an observation which led to the discovery of the internal promoters of RNA polymerase III. An attempt to isolate the genes coding for the two types cific sequences among restriction fragments by molecular hybridization (6), which subsequently became known as the "Southern blot" and found many app . EMS used it in studies of the structure of ribosomal (7,8) and histone genes. With Adrian Bird, he developed a method to characterize methylation pattern show, are important for gene regulation in vertebrates. The method formed the basis of Jeffrey's DNA fingerprinting technique and was used to isolate and to create the first genetic maps of the human genome.

EMS set up the first project to map the human genome using molecular ytogenetics Unit in 1979 (7). Technical innovations he developed in response to the need to analyse sequences on the large scale required by such projects tion fragments by gel electrophoresis (9); automated methods for reading DNA sequences from sequencing gels (10); a new method for the separation lectrophoresis, based on a theory of how DNA fragments move through gels under the influence of an electric field (11). In 1985, he moved to Oxford to take . In 1988, he introduced methods of analysis using oligonucleotide arrays or "DNA chips", which are used to analyse DNA sequences by molecular hybridiz sets of oligonucleotides could be made on a glass surface by in situ synthesis in few steps by efficient combinatorial methods (12,13). The array method has yping, resequencing and expression analysis. In addition to his genome studies his current interests include basic studies of hybridization behaviour of nucleic acids, particularly how the duplex formation is influenced by structure in the single strands (14-16). His group are also involved in the development of more effective antisense reagents for therapeutic applications and for use as knockdown reagents in the study of uncharacterized gene sequences (17,18).

Other research on methods for high throughput analysis of nucleic acids is carried out in Oxford Gene Technology, a company founded by EMS in 1996. EMS is the Chief Scientific Officer of OGT. EMS founded a research charity, Kirkhouse Trust, and an educational charity, formerly the EM Southern Trust, now the Edina Trust, using royalty income from licensing the microarray technology. The Kirkhouse Trust supports research and training on crop improvement in semi-arid regions of the developing world. EMS is chairman and a trustee. The Edina Trust supports educational projects which promote an interest in science in primary schools.

Southern blot

A **Southern blot** is a method routinely used in molecular biology to check for the presence of a DNA sequence in a DNA sample. Southern blotting combines agarose gel electrophoresis for size separation of DNA with methods to transfer the size-separated DNA to a filter membrane for probe hybridization. The method is named after its inventor, the British biologist Edwin Southern.[1] Other blotting methods (i.e., western blot, northern blot, southwestern blot) that employ similar principles, but using RNA or protein, have later been named in reference to Southern's name. As the technique was eponymously named, Southern blot should be capitalized, whereas northern and western blots should not.

References

1. ^ Southern, E.M. (1975): "Detection of specific sequences among DNA fragments separated by gel electrophoresis", *J Mol Biol.*, 98:503-517.

Method

1. Restriction endonucleases are used to cut high-molecular-weight DNA strands into smaller fragments.
2. The DNA fragments are then electrophoresed on an agarose gel to separate them by size.
3. If some of the DNA fragments are larger than 15 kb, then prior to blotting, the gel may be treated with an acid, such as dilute HCl, which depurinates the DNA fragments, breaking the DNA into smaller pieces, thus allowing more efficient transfer from the gel to membrane.
4. If alkaline transfer methods are used, the DNA gel is placed into an alkaline solution (typically containing sodium hydroxide) to denature the double-stranded DNA. The denaturation in an alkaline environment provides for improved binding of the negatively charged DNA to a positively charged membrane, separates it into single DNA strands for later hybridization to the probe (see below), and destroys any residual RNA that may still be present in the DNA.
5. A sheet of nitrocellulose (or, alternatively, nylon) membrane is placed on top of (or below, depending on the direction of the transfer) the gel. Pressure is applied evenly to the gel (either using suction, or by placing a stack of paper towels and a weight on top of the membrane and gel), to ensure good and even contact between gel and membrane. Buffer transfer by capillary action from a region of high water potential to a region of low water potential (usually filter paper and paper tissues) is then used to move the DNA from the gel on to the membrane; ion exchange interactions bind the DNA to the membrane due to the negative charge of the DNA and positive charge of the membrane.
6. The membrane is then baked, i.e., exposed to high temperature (60 to 100 °C) (in the case of nitrocellulose) or exposed to ultraviolet radiation (nylon) to permanently and covalently crosslink the DNA to the membrane.
7. The membrane is then exposed to a hybridization probe—a single DNA fragment with a specific sequence whose presence in the target DNA is to be determined. The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye. In some cases, the hybridization probe may be made from RNA, rather than DNA. To ensure the specificity of the binding of the probe to the sample DNA, most common hybridization methods use salmon testes (sperm) DNA for blocking of the membrane surface and target DNA, deionized formamide, and detergents such as SDS to reduce non-specific binding of the probe.
8. After hybridization, excess probe is washed from the membrane, and the pattern of hybridization is visualized on X-ray film by autoradiography in the case of a radioactive or fluorescent probe, or by development of color on the membrane if a chromogenic detection method is used.

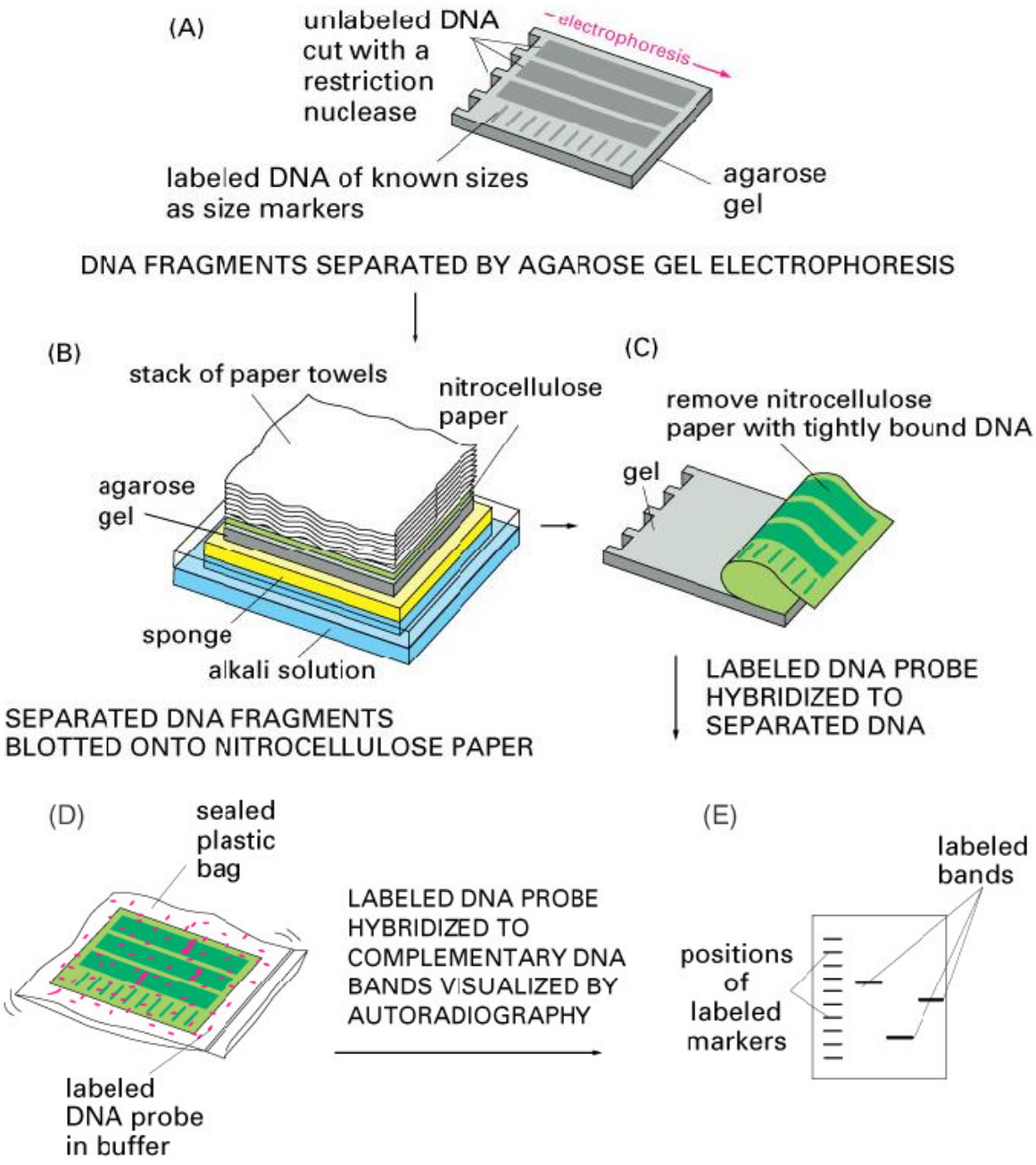
Result

Hybridization of the probe to a specific DNA fragment on the filter membrane indicates that this fragment contains DNA sequence that is complementary to the probe.

The transfer step of the DNA from the electrophoresis gel to a membrane permits easy binding of the labeled hybridization probe to the size-fractionated DNA. It also allows for the fixation of the target-probe hybrids, required for analysis by autoradiography or other detection methods.

Southern blots performed with restriction enzyme-digested genomic DNA may be used to determine the number of sequences (e.g., gene copies) in a genome. A probe that hybridizes only to a single DNA segment that has not been cut by the restriction enzyme will produce a single band on a Southern blot, whereas multiple bands will likely be observed when the probe hybridizes to several highly similar sequences (e.g., those that may be the result of sequence duplication). Modification of the hybridization conditions (for example, increasing the hybridization temperature or decreasing salt concentration) may be used to increase specificity and decrease hybridization of the probe to sequences that are less than 100% similar.

Southern Blotting: Gel Transfer



Technika značení DNA sondy nick-translací …

LABELING DEOXYRIBONUCLEIC-ACID TO HIGH SPECIFIC ACTIVITY INVITRO BY NICK TRANSLATION WITH DNA-POLYMERASE I

Dle WoS citováno 11.668-krát

Full Text | Print | E-mail | Add to Marked List | Save to EndNote Web | more options

Nobelova cena 1980

Author(s): RIGBY PWJ, DIECKMANN M, RHODES C, BERG P

Source: JOURNAL OF MOLECULAR BIOLOGY **Volume:** 113 **Issue:** 1 **Pages:** 237-251 **Published:** ~1977

Times Cited: 11,668 **References:** 46

Document Type: Article

Language: English

Addresses: STANFORD UNIV, DEPT BIOCHEM, STANFORD, CA 94305 USA

Publisher: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON, ENGLAND NW1 7DX

Subject Category: Biochemistry & Molecular Biology

(1) DNáza I udělá v DNA „zářezy“
(2) DNA Pol I (má 5‘-3‘ exonukleázovou a 5‘-3‘ polymerázovou aktivitu, včleňuje nové (značené dNTP) nukleotidy

Technika značení DNA sondy multiprime primingem …

A TECHNIQUE FOR RADIOLABELING DNA RESTRICTION ENDONUCLEASE FRAGMENTS TO HIGH SPECIFIC ACTIVITY

Dle WoS citováno 21.171-krát

Full Text | Print | E-mail | Add to Marked List | Save to EndNote Web | more options

Author(s): FEINBERG AP, VOGELSTEIN B

Source: ANALYTICAL BIOCHEMISTRY **Volume:** 132 **Issue:** 1 **Pages:** 6-13 **Published:** 1983

Times Cited: 21,171 **References:** 33

Document Type: Article

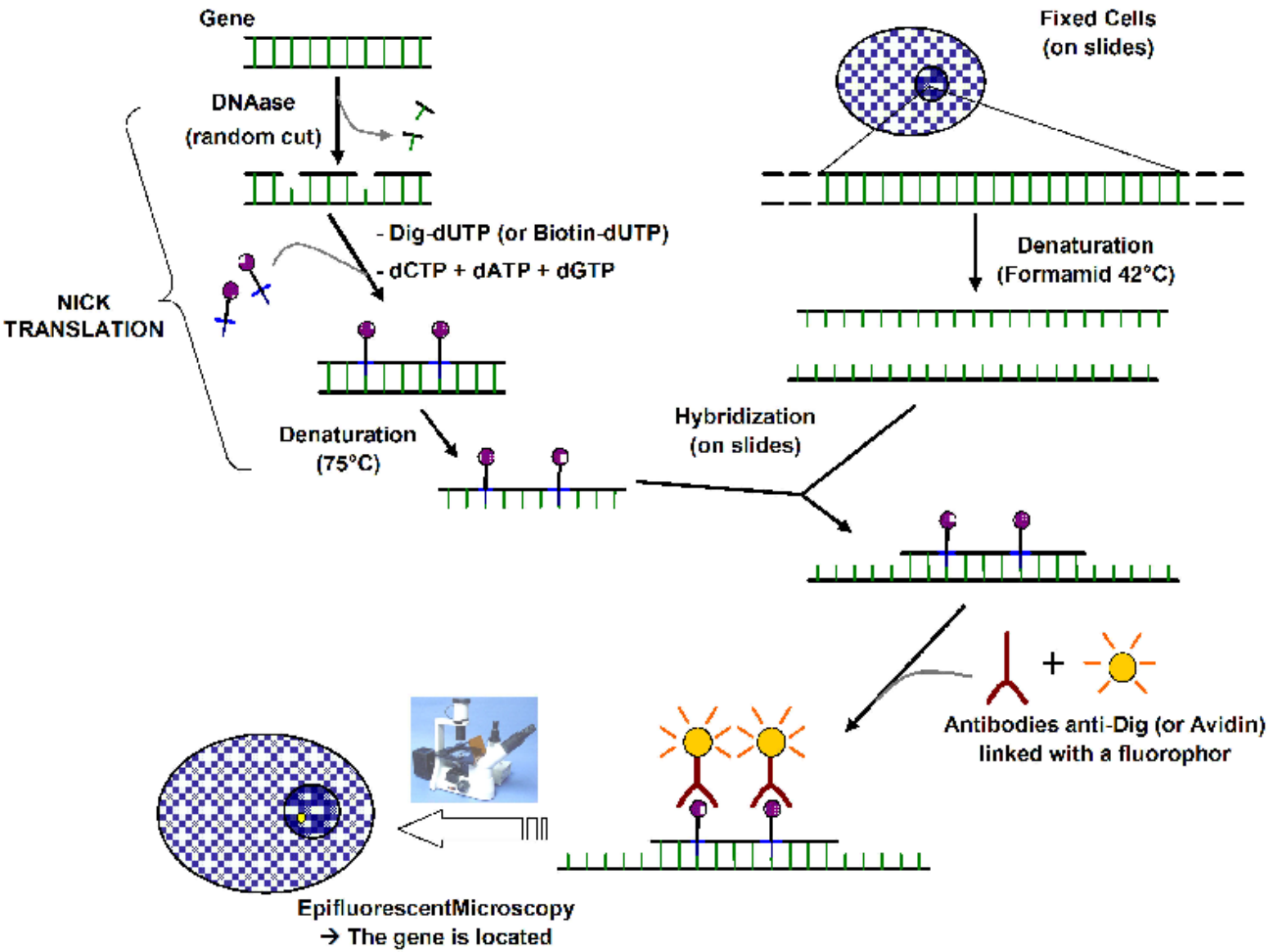
Language: English

Addresses: FEINBERG, AP (reprint author), JOHNS HOPKINS UNIV, SCH MED, CTR ONCOL, CELL STRUCT & FUNCT LAB, BALTIMORE, MD 21205 USA

Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495

(1) denaturace DNA
(2) vazba náhodných oligonukleotidů
(3) Klenow (DNA Pol) dosyntetizovává druhé vlákno se značeným dNTP

Fluorescenční *in situ* hybridizace (FISH)

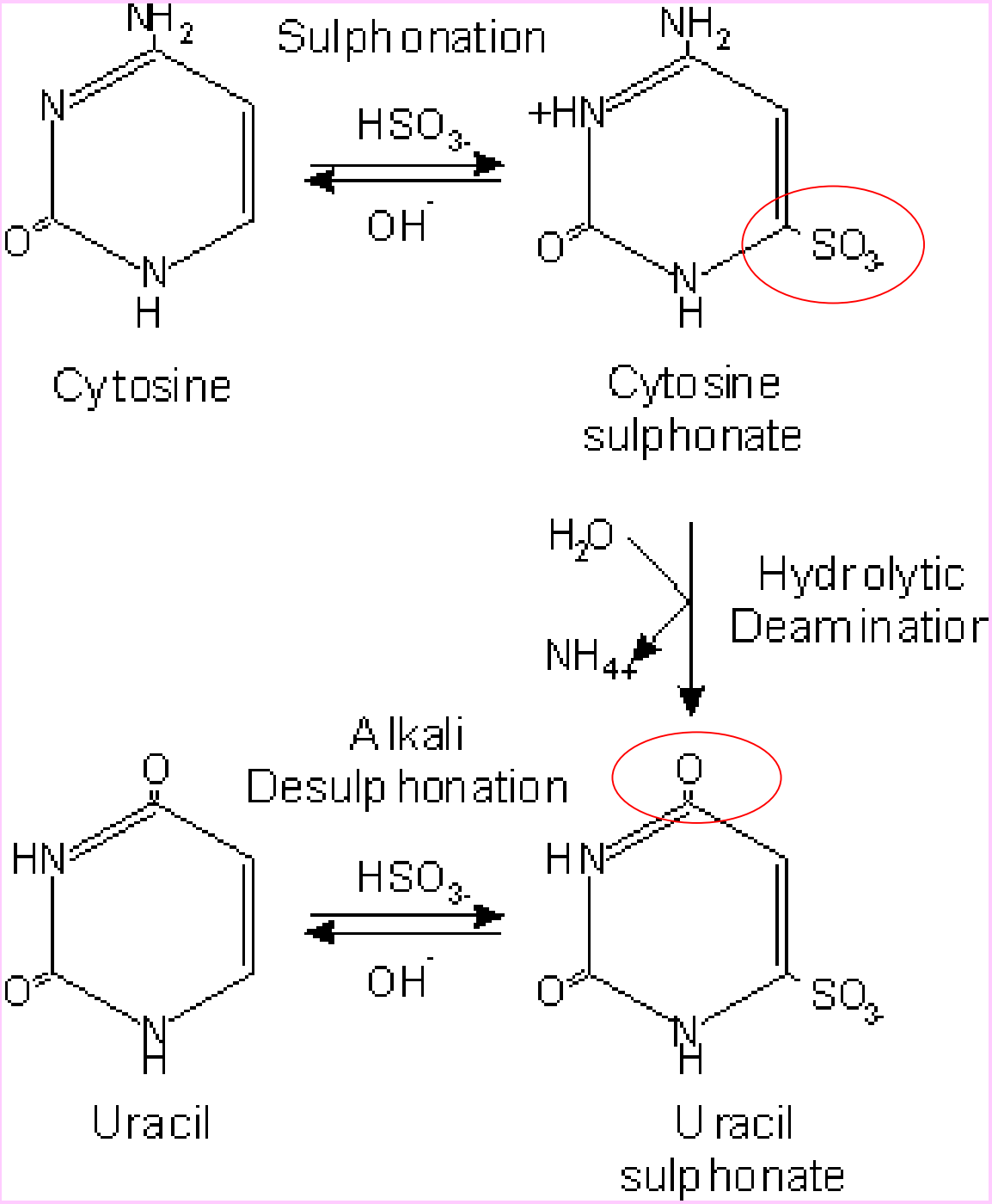


METYLACE DNA lze studovat na základě :

(a) metylačně sensitivních restrikčních endonukleáz,

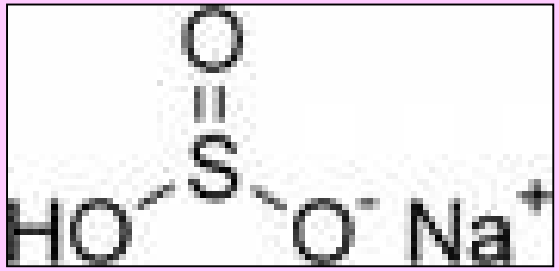
(b) siřičitanového působení na DNA, které deaminuje cytosin na uracil,

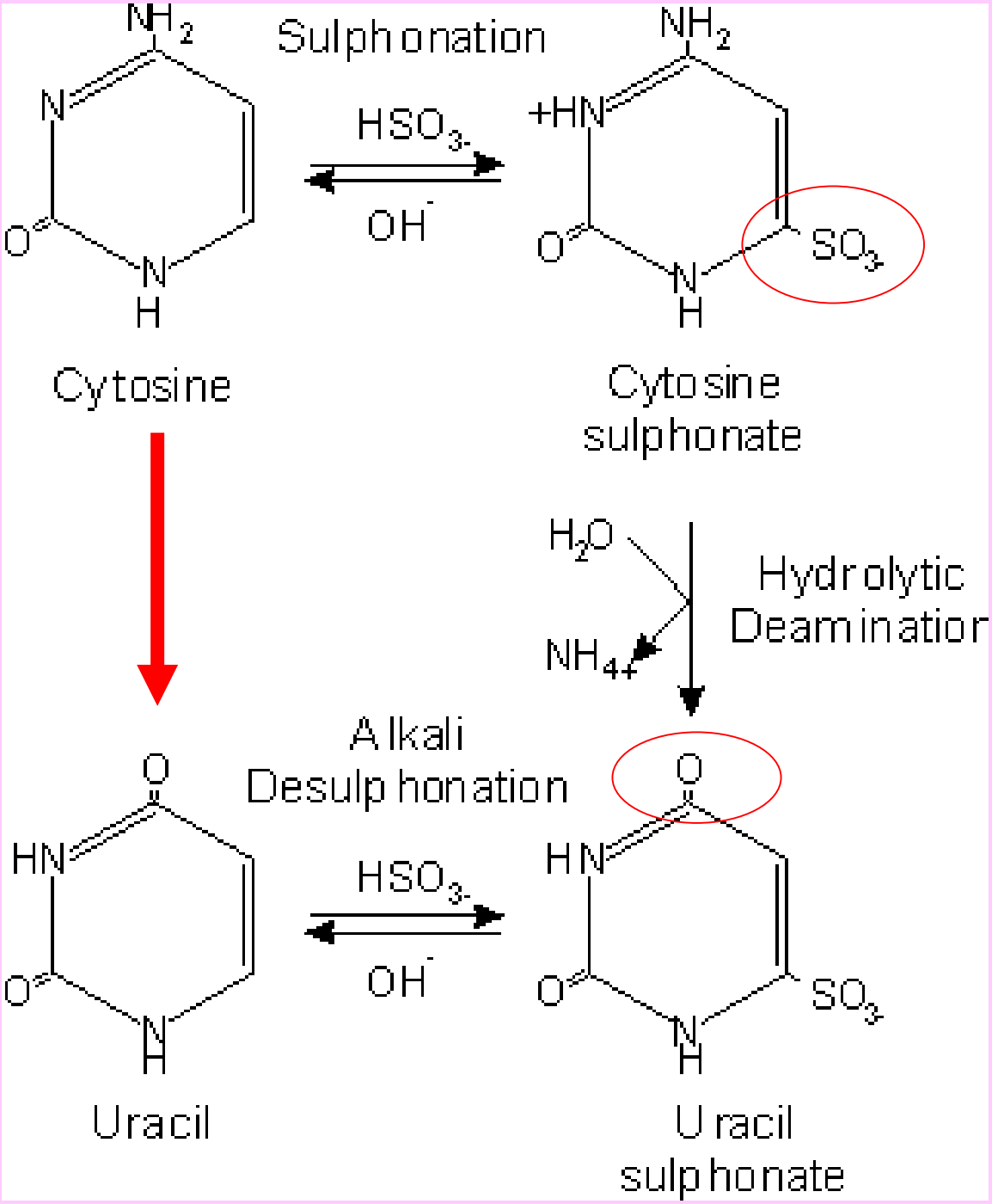
(c) chromatinové imunoprecipitace s využitím protilátky vůči metyl-cytosinu



Aplikace hydrogensiřičitanu sodného na vzorek DNA při vysokém pH

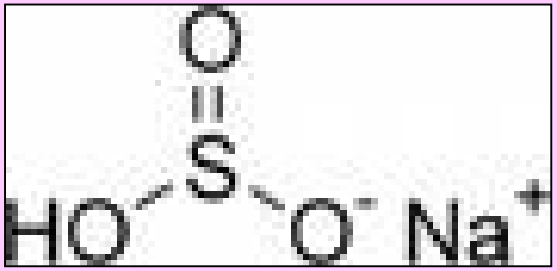
$NaHSO_3$ = hydrogensiřičitan sodný

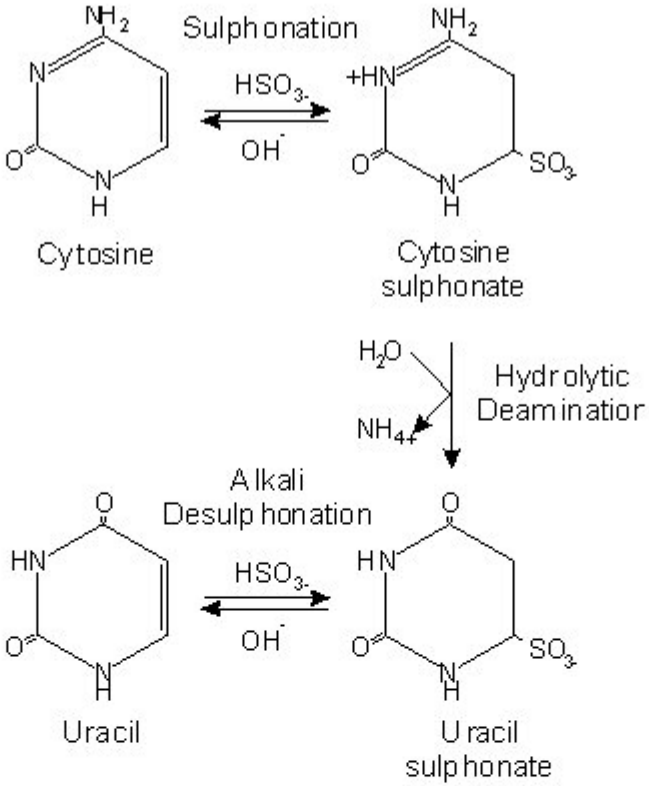




$NaHSO_3$ =

hydrogensiřičitan sodný

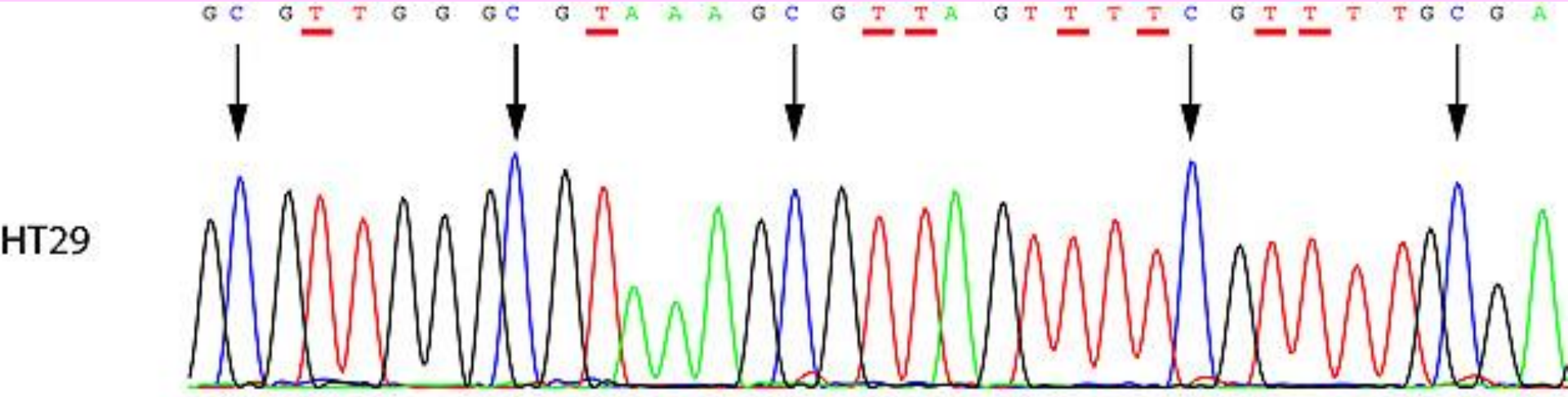




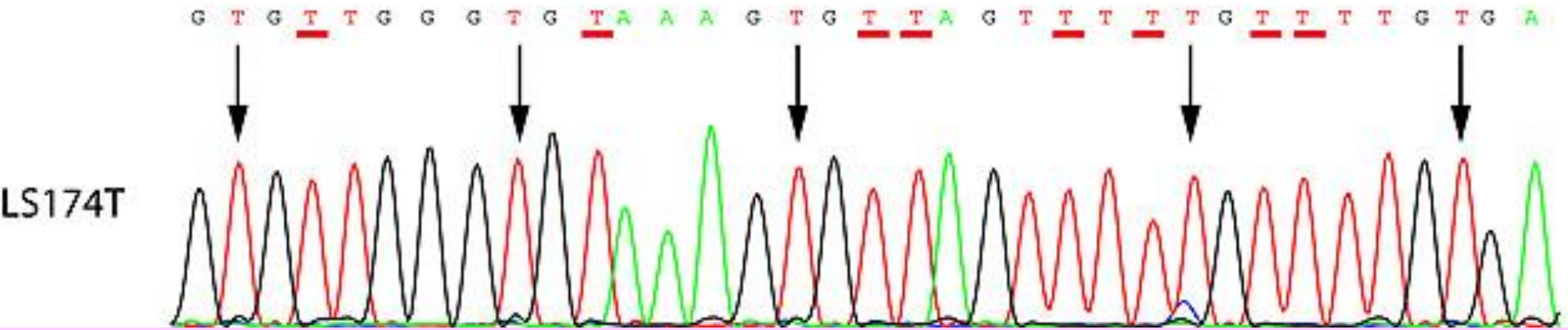
Protocol

1. Isolate genomic DNA with the quality sufficient for restriction enzyme digestion.
2. Digest DNA (50 to 200 ng) with any enzyme which does not cut the region of interest, but resulting in as short fragments as possible in smallest possible volume. *Note: increasing the amount of DNA will make denaturing not efficient enough and therefore make bisulphite reaction incomplete!*
3. Stop the reaction by boiling DNA for 5 min.
4. Add 10N NaOH to 0.3N final concentration and denature DNA 37° C for 15 min.
5. Prepare 4-5 eppendorf tubes with cold mineral oil.
6. Add 2x volume of 2% low melting agarose to the DNA solution, mix by pipetting up and down.
7. Form agarose beads by pipetting 10 µl aliquots of DNA/agarose mixture into cold mineral oil. *Note: Don't pipett the second aliquot in the tube where you already have one bead!*
8. Transfer beads in the tube containing 1 ml of modifying solution (5M sodium bisulphite (2.5M sodium metabisulphite), 100mM Hydroquinon).
9. Incubate the tubes 4 h at 50° C in the dark.
10. Wash the beads 6 times for 15 min in TE pH 8.0.
11. Complete the modification by incubating the beads 2 times for 15 min in 0.2 N NaOH.
12. Wash the beads 3 times for 15 min in double distilled H_2O.
13. Use one ul of the obtained DNA for PCR with selected primers.

Kontrolní vzorek DNA indikuje pozice nukleotidů – sekvence bez siřičitanového působení

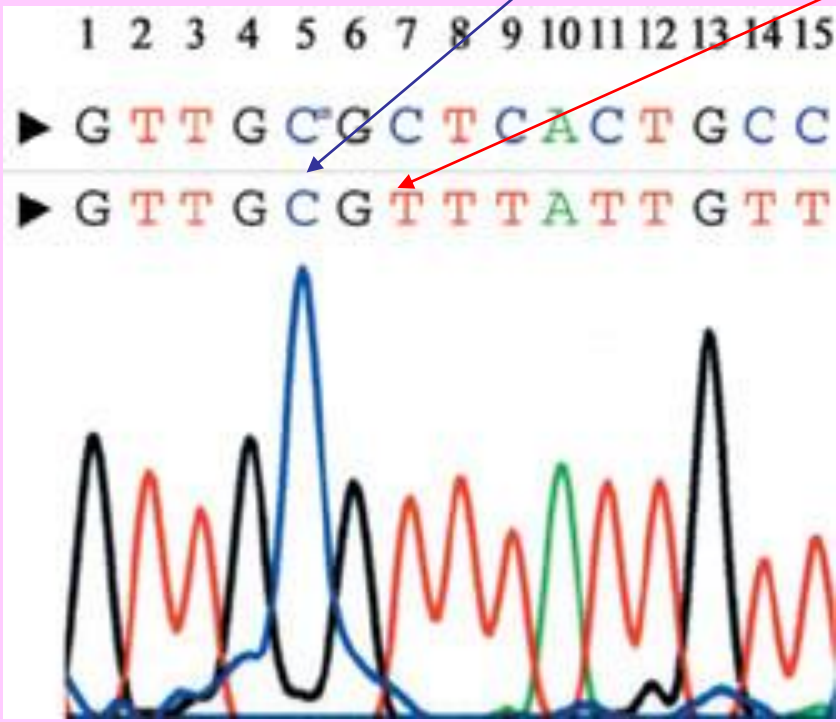


Nezbytná kontrola tranzice všech cytosinů po působení siřičitanu



Stejná DNA po působení siřičitanu ukazuje tranzice všech cytosinů v uracil (tymin)

Genomové sekvenování (po aplikaci siřičitanu) ukazuje tranzice cytosinu v tymin, zatímco metylovaný cytosin je rezistentní a projeví se jako cytosin



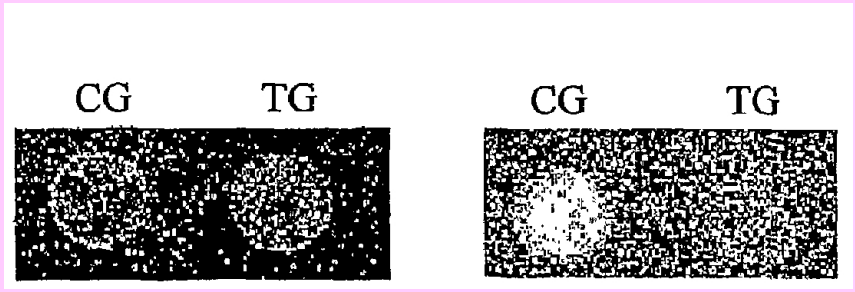
… původní sekvence DNA

... po genomovém sekvenování

Methylation-Specific Oligonucleotide Microarray (MSO)

A new high-throughput methylation analysis method … využívá techniky siřičitanové konverze C-T v analyzovaném vzorku DNA a jeho následnou (chipovou) hybridizaci na komerčně připravené oligonukleotidy markerových genových sekvencí

Alexander Olek, EPIGENOMICS AG



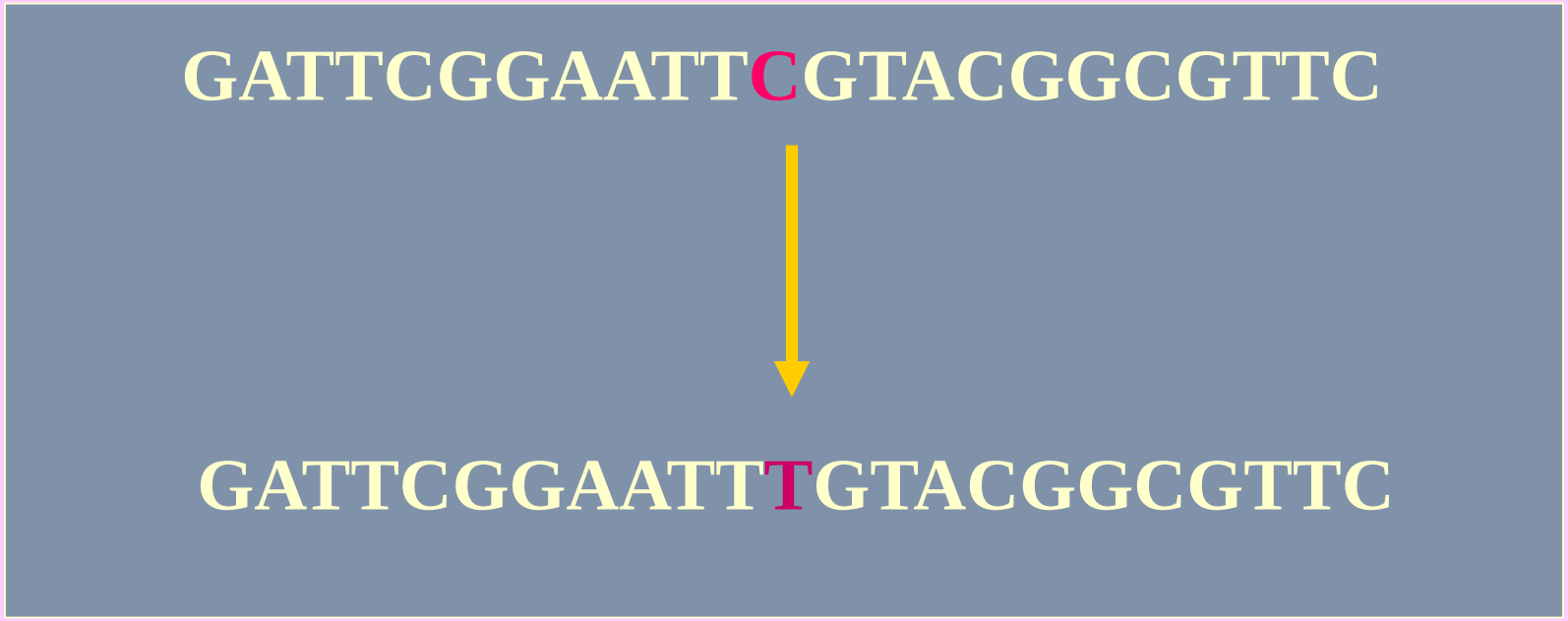
epigenomics

C → T

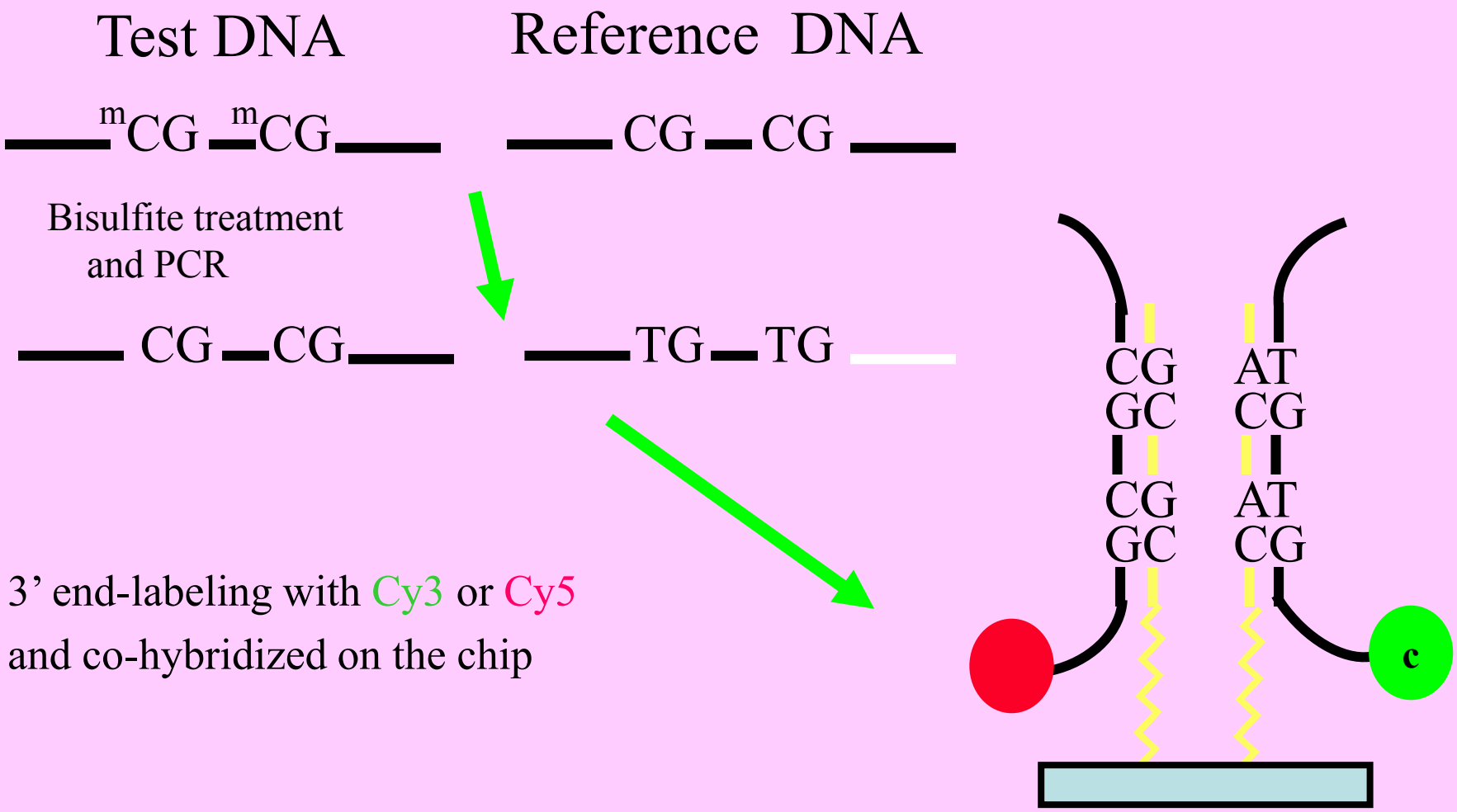
mC → C

Oligonucleotide-Based Hybridization Microarray

Oligonucleotide microarray for detecting single nucleotide polymorphisms and gene mutations



Methylation-Specific Oligonucleotide Microarray (MSO)



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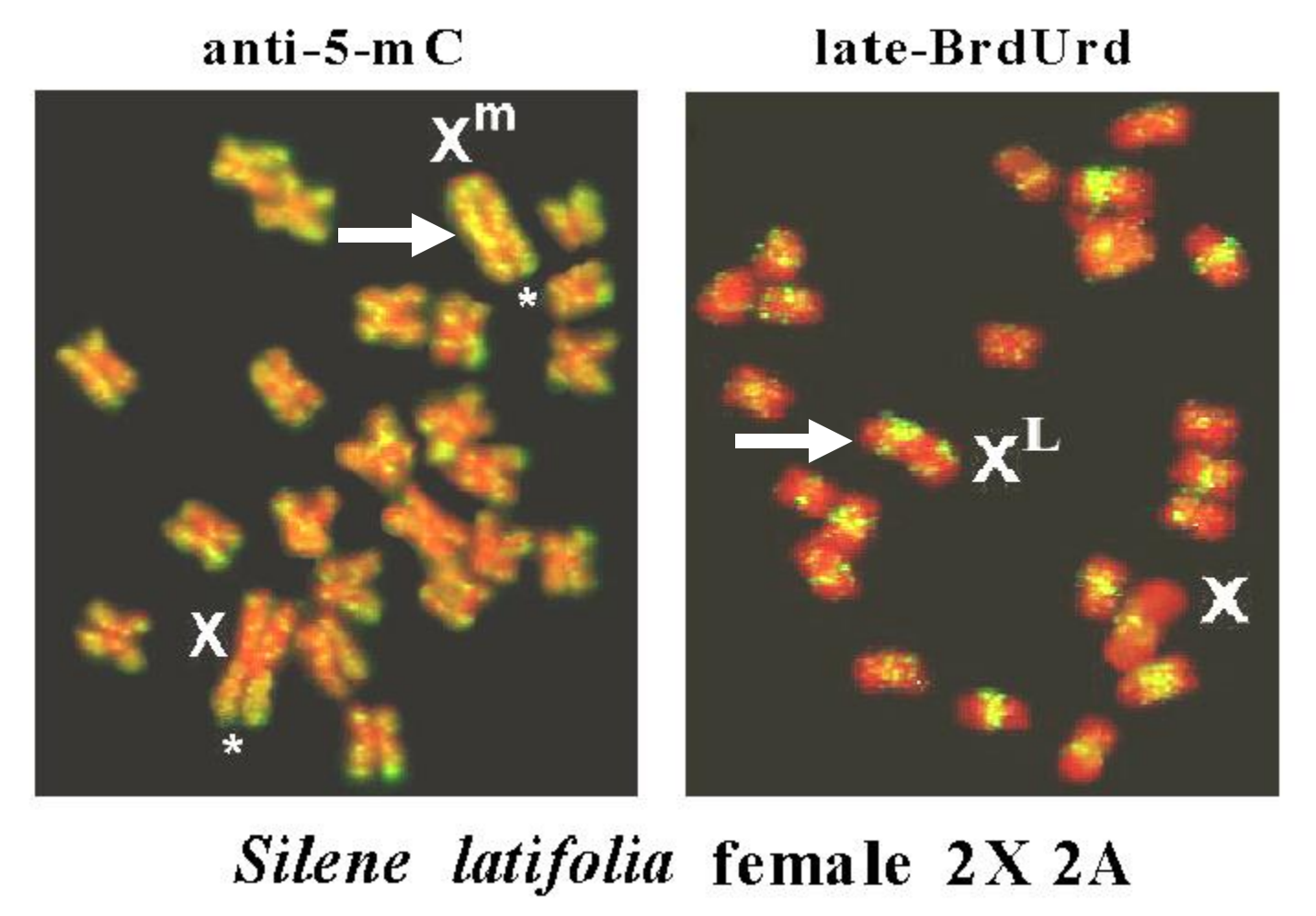
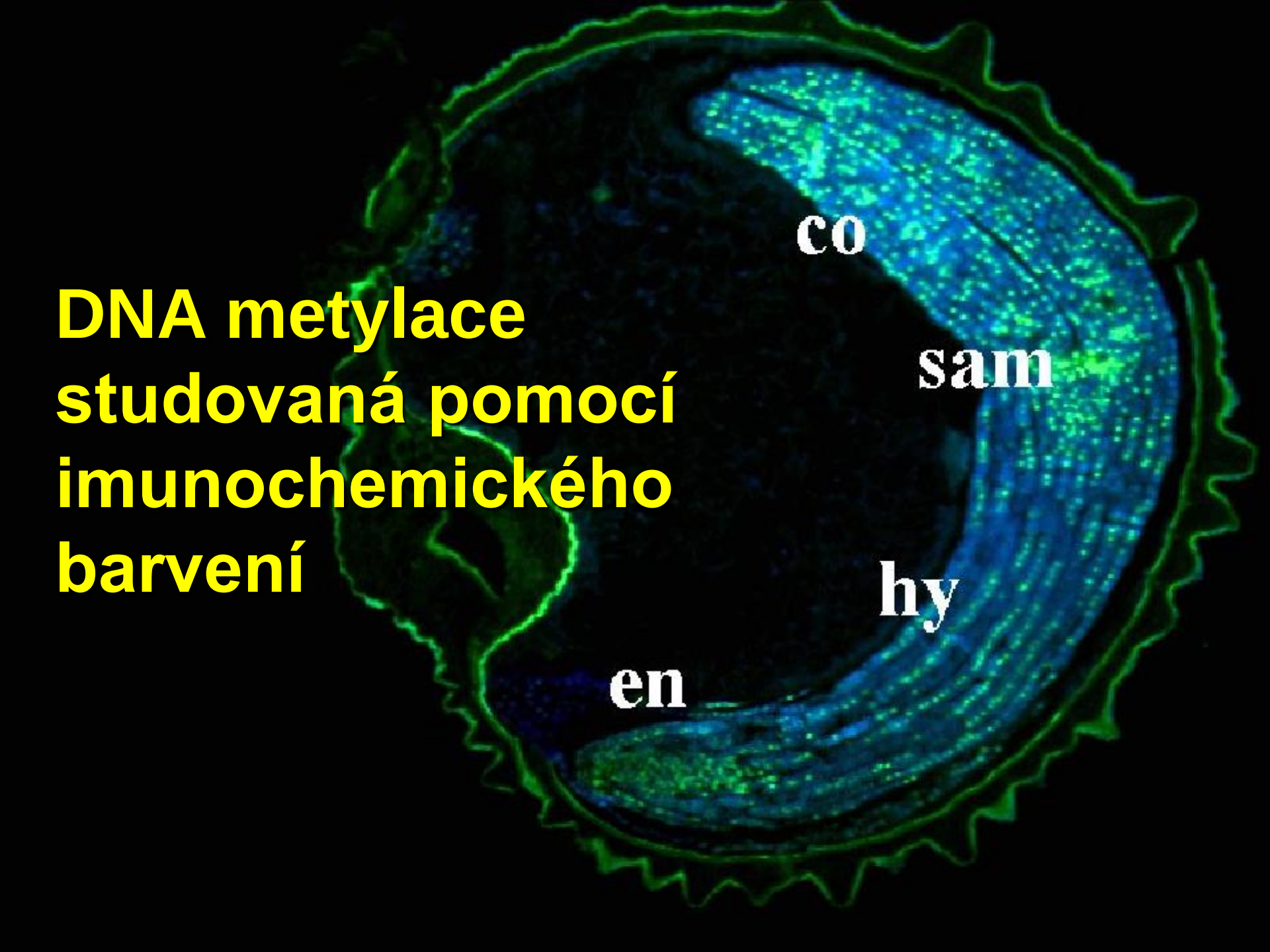
(b) siřičitanového působení na DNA, které deaminuje cytosin na uracil,

(c) chromatinové imunoprecipitace s využitím protilátky vůči metyl-cytosinu … kvantifikace metylace na DNA přímo izolované z genomu … MeDIP (methylated DNA immunoprecipitation) copuled with comparative genomic hybridization (CGH) microarray

KOMPENZACE DÁVKY GENŮ VÁZANÝCH NA CHROMOZOM X U ROSTLIN ?

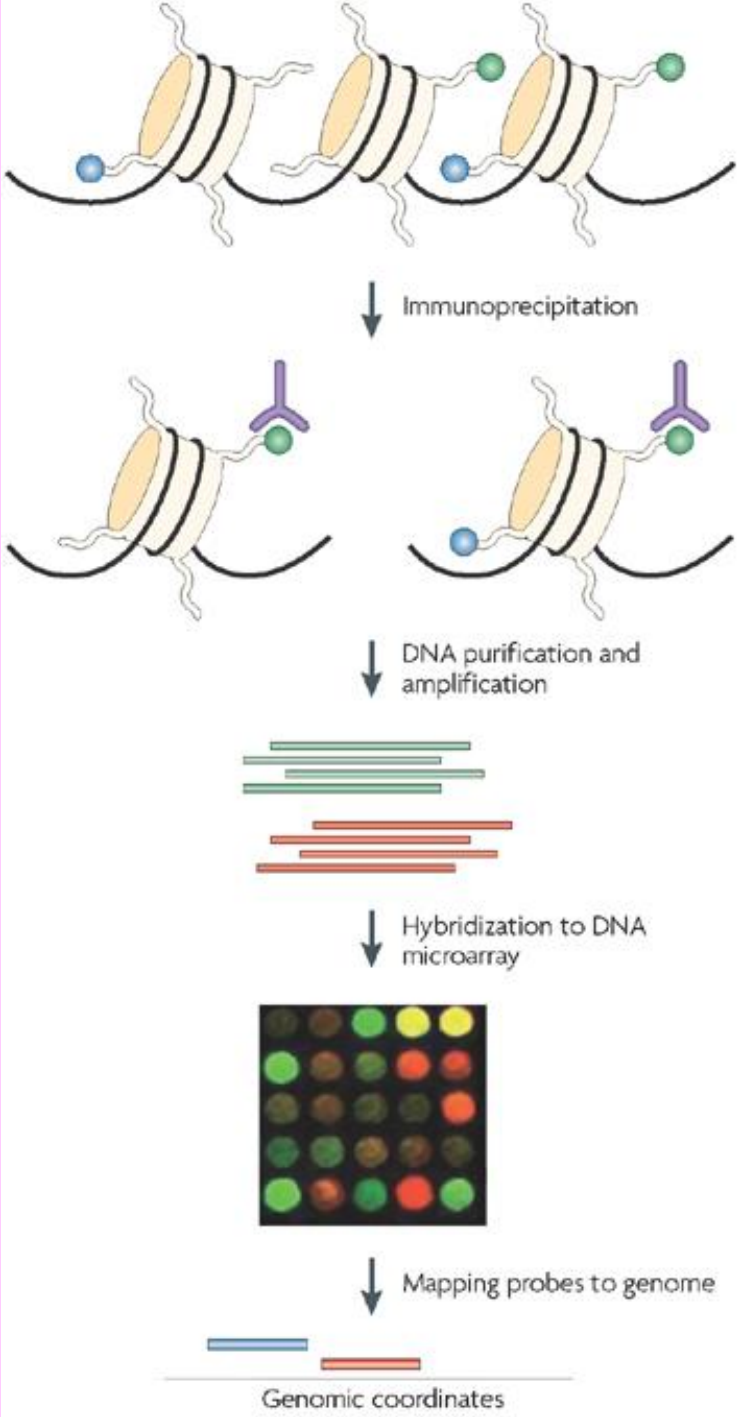




DNA metylace studovaná pomocí imunochemického barvení

Metody studia epigenetických jevů

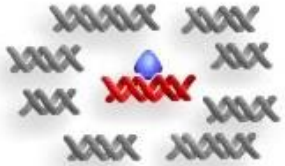
- analýza genové exprese:
  Northern, *in situ* hybridizace,
  RT-PCR, *microarrays*
- analýza metylace DNA:
  restrikční enzymy, nukleázy, Southern,
  genomové sekvenování
- analýza modifikací histonů:
  Western, imunobarvení,
  chromatinová imunoprecipitace
- aplikace inhibitorů modifikujících enzymů



Chromatin Immunoprecipitation

Chromatin immunoprecipitation, or ChIP, refers to a procedure used to determine whether a given protein binds to or is localized to a specific DNA sequence *in vivo*. The diagram below illustrates the basic steps of this procedure.

DNA-binding proteins are crosslinked to DNA with formaldehyde in vivo.

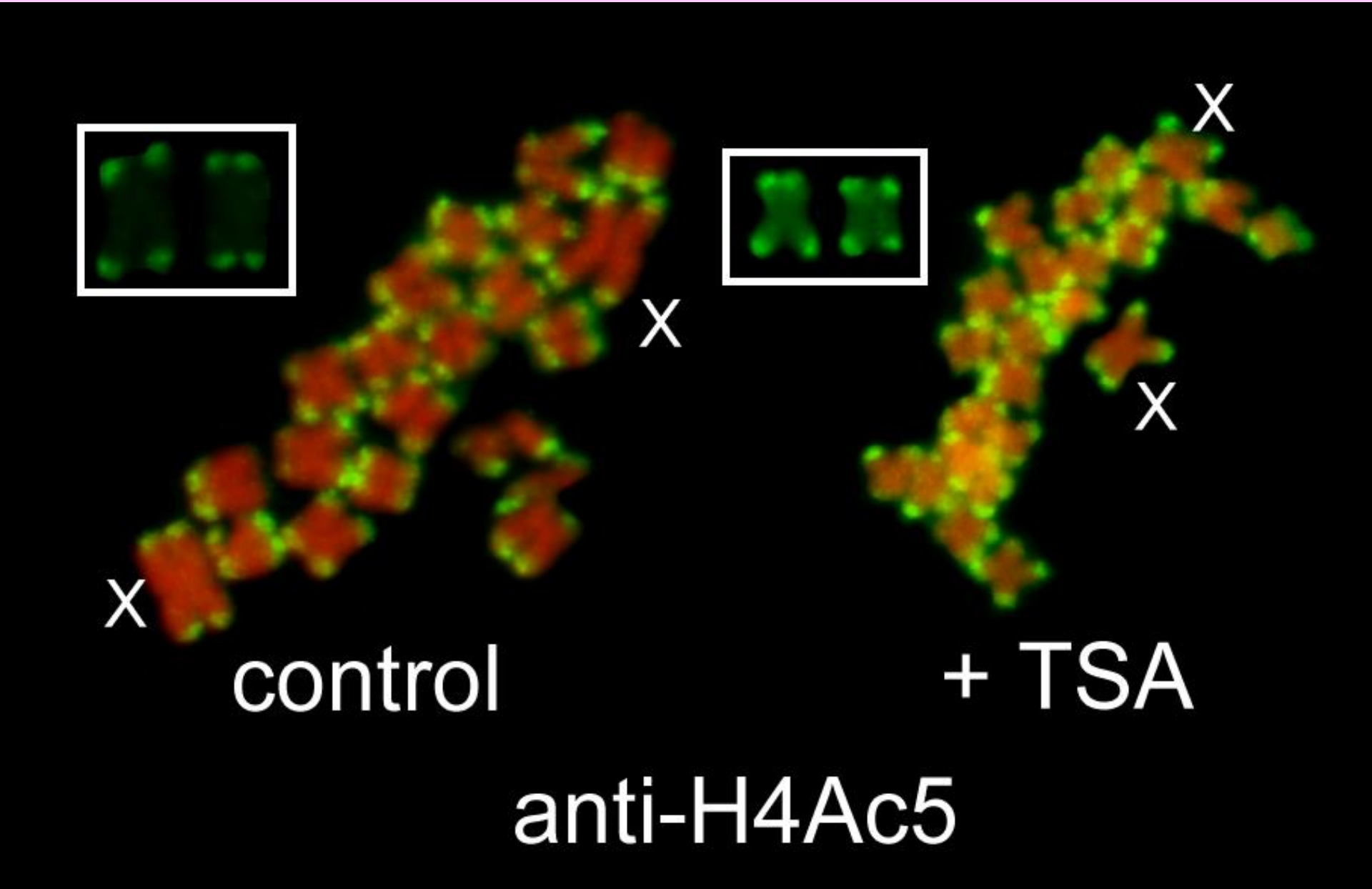


Isolate the chromatin. Shear DNA along with bound proteins into small fragments.

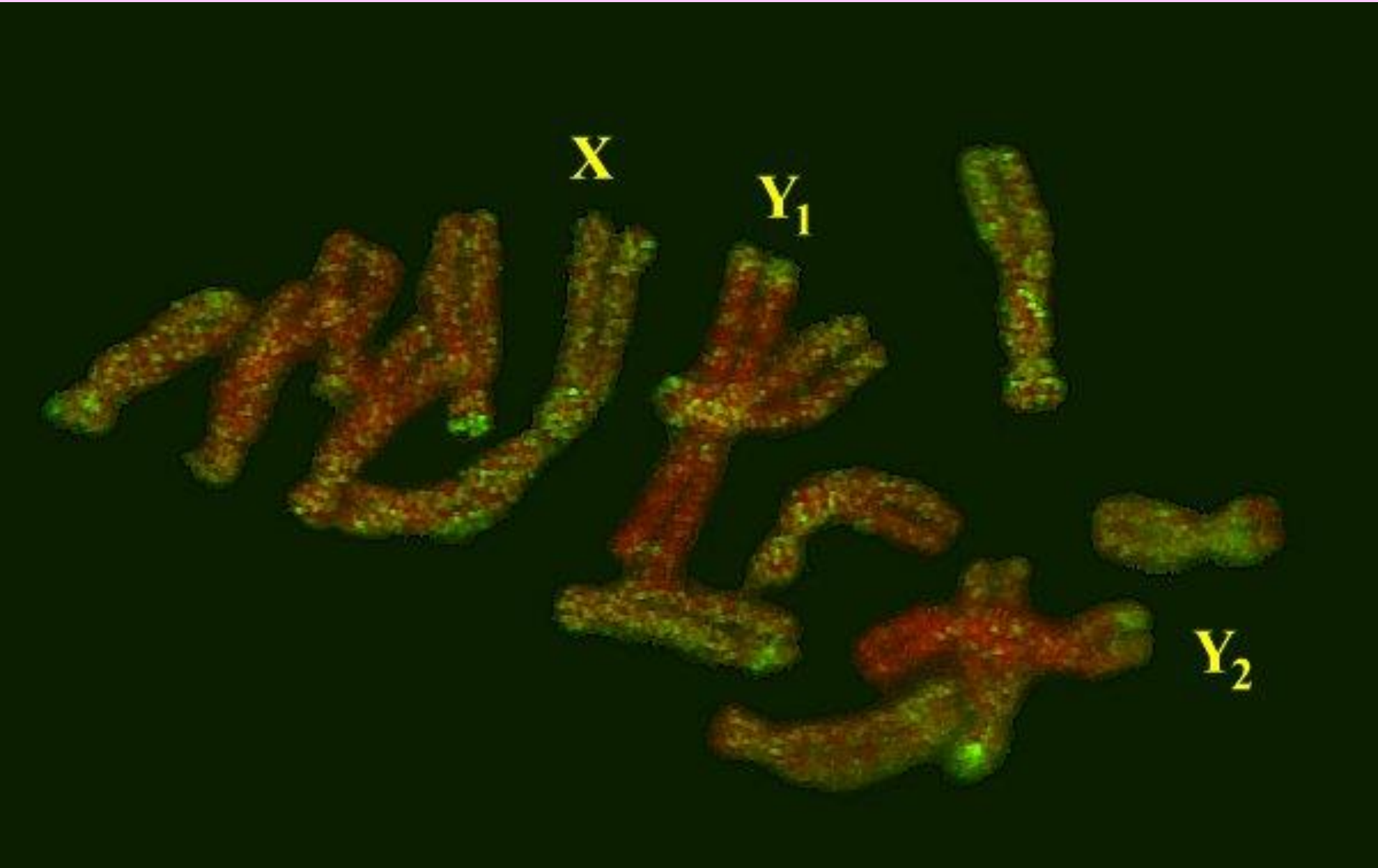
Bind antibodies specific to the DNA-binding protein to isolate the complex by precipitation. Reverse the cross-linking to release the DNA and digest the proteins.

Use PCR to amplify specific DNA sequences to see if they were precipitated with the antibody.

HISTON H4 ACETYLACE CHROMOZÓMŮ



Heterochromatické chromozomy Y *Rumex acetosa* jsou H4 hypoacetylované



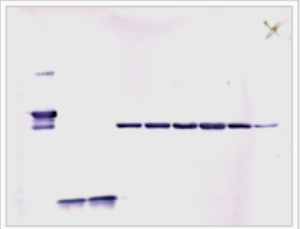
Western blot

...pedia

The **western blot** (alternately, **immunoblot**) is a method of detecting specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) (Figure 1) or by the 3-D structure of the protein (native/ non-denaturing conditions). The proteins are then transferred to a membrane (typically nitrocellulose or PVDF), where they are probed (detected) using antibodies specific to the target protein. There are now many reagent companies that specialize in providing antibodies (both monoclonal and polyclonal antibodies) against many thousands of different proteins. Commercial antibodies can be expensive, though the unbound antibody can be reused between experiments. This method is used in the fields of molecular biology, biochemistry, immunogenetics and other molecular biology disciplines.

Other related techniques include using antibodies to detect proteins in tissues and cells by immunostaining and enzyme-linked immunosorbent assay (ELISA).

The method originated from the laboratory of George Stark at Stanford. The name **western blot** was given to the technique by W. Neal Burnette[1] and is a play on the name Southern blot, a technique for DNA detection developed earlier by Edwin Southern. Detection of RNA is termed northern blotting.



A Western blot.

Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: A method for studying antibody specificity and antigen structure

(simian virus 40/T antigens/virion proteins/reversible gel crosslinks/*Staphylococcus aureus* protein A)

JAIME RENART*, JAKOB REISER, AND GEORGE R. STARK

Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Communicated by Paul Berg, April 4, 1979

ABSTRACT We describe a rapid and very sensitive method for detecting proteins as antigens after their separation in polyacrylamide/agarose composite gels, with or without sodium

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 7, pp. 3116–3120, July 1979
Biochemistry

Podle WOS práce citována 500-krát

Analytical Biochemistry
Volume 112, Issue 2, April 1981, Pages 195-203

Abstract | References | PDF (1198 K)

doi:10.1016/0003-2697(81)90281-5 Cite or Link Using DOI

"Western Blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A

W. Neal Burnette

Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104, USA

Podle WOS práce citována 7.186-krát

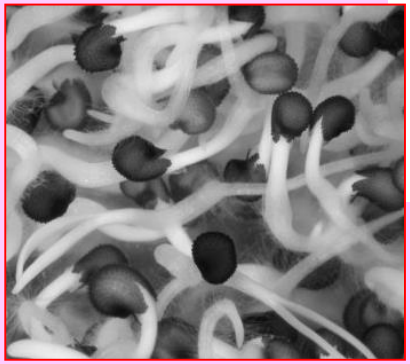
Received 20 May 1980. Available online 07 December 2004.

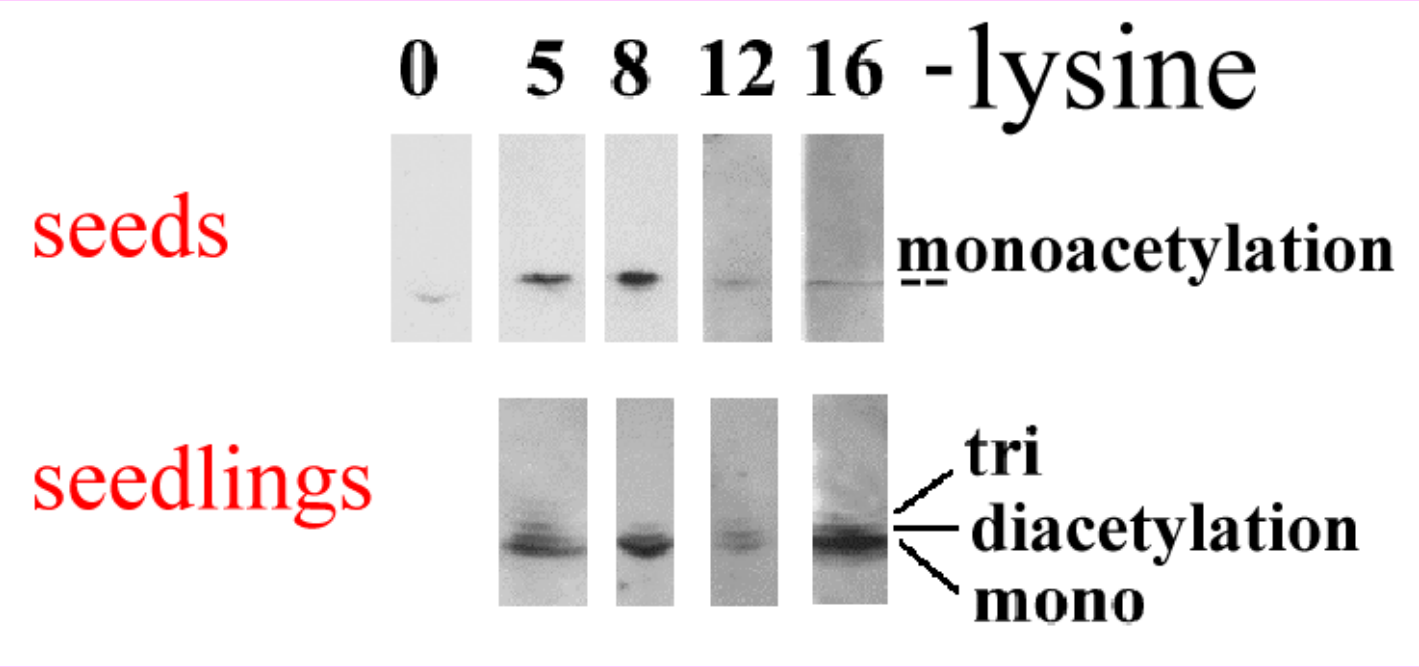
Abstract

A simple and efficient procedure was employed for the electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to sheets of pure, unmodified nitrocellulose. Immobilized proteins could then be radiographically visualized *in situ* by reaction with specific antibody and the subsequent binding of radioiodinated *Staphylococcus* protein A to the immune complexes. The detection of murine leukemia virus antigens in complex cellular lysates was used to demonstrate the efficacy of this technique.

Dynamika H4 acetylace
v průběhu klíčení semen *S. latifolia*

Western-analýza na AUT gelu





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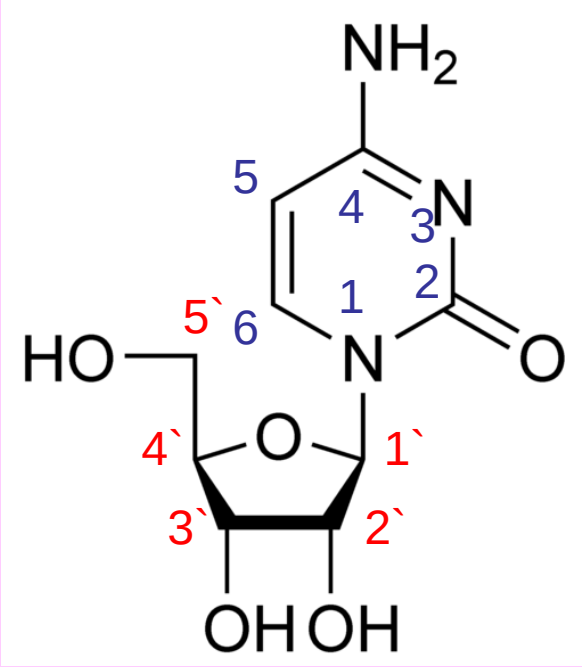
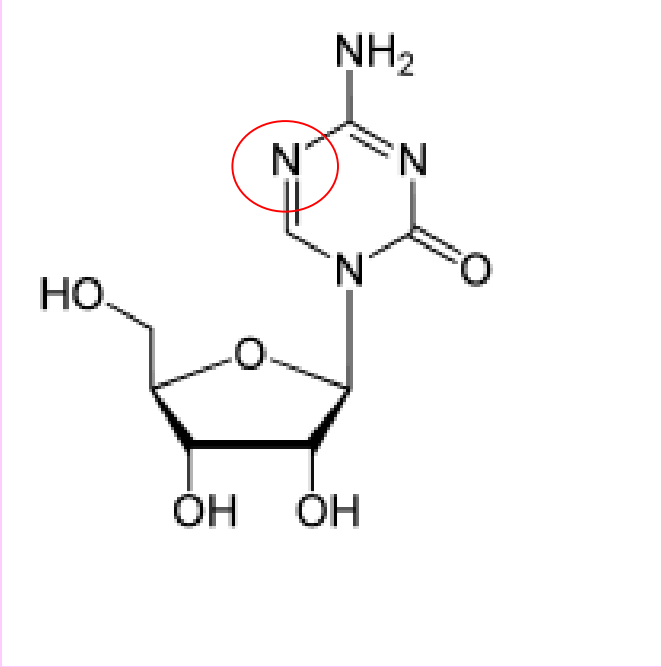
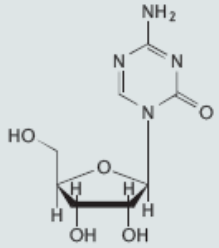
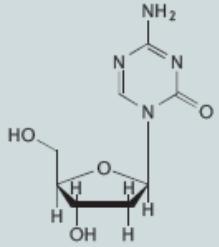
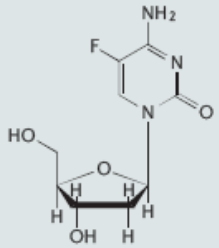
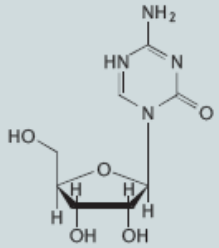
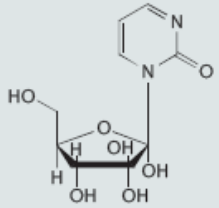


cytidin



5-azacytidin
(inhibitor DNA metyltransferáz)

DNA-methylation inhibitors: nucleoside analogues

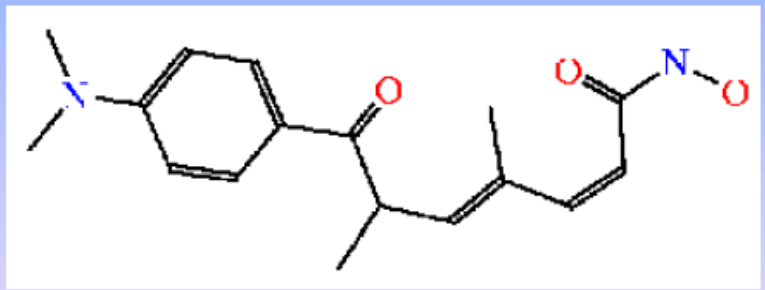
Inhibitor	Structure	Dose range	Clinical trials
5-Azacytidine		μM	Phase I, II, III: haematological malignancies
5-Aza-2′-deoxycytidine		μM	Phase I, II, III: haematological malignancies; cervical, non-small-cell lung cancer
5-Fluoro-2′-deoxycytidine		μM	Phase I
5,6-Dihydro-5-azacytidine		μM	Phase I, II: ovarian cancer and lymphomas
Zebularine		μM–mM	Preclinical

Epigenetika 21. století

EPIGENETICKÉ TERAPIE ?

ovlivňování exprese genů modulací acetylace histonů

Trichostatin A (TSA) - antifungální antibiotikum ze *Streptomyces platensis*, inhibuje histon deacetylásy, indukuje apoptotickou smrt nádorových buněk, zastavuje Huntingtonovu choreu u drosofily

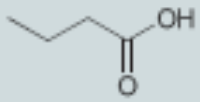
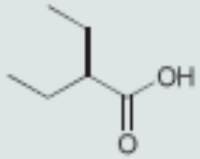
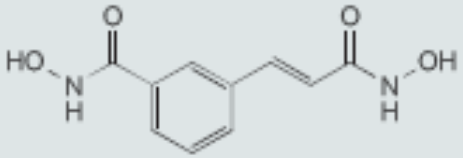
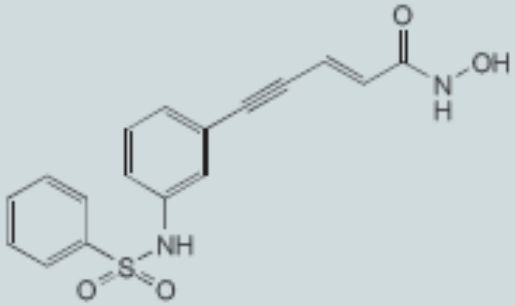
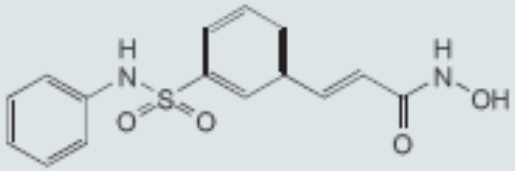
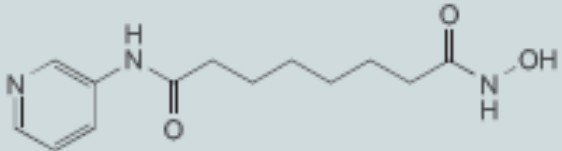


Butyrát sodný (NaBT) – potenciální inhibitor nádorového bujení, léčení muskulární dystrofie

$H_3C - (CH_2)_2 - COONa$

Tropoxin

Histone-deacetylase inhibitors: short-chain fatty acids and hydroxamic acids

Inhibitor	Structure	Dose range	Clinical trials
Short-chain fatty acids			
Butyrate		mM	Phase I, II: colorectal
Valproic acid		mM	Phase I: AML, leukaemias
Hydroxamic acids			
m-Carboxy cinnamic acid bishydroxamic acid (CBHA)		μM	Preclinical
Oxamflatin		μM	Preclinical
PDX 101		μM	Phase I
Pyroxamide		nM	Preclinical