Syndrom fragilního X chromozómu (FXS)

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- Syndrom fragilního X chromozómu je nejčastější příčinou dědičných mentálních retardací s frekvencí výskytu 1:4000 u mužů a 1:8000 u žen.
- Je způsoben expanzí CGG repetic v 5′UTR fragile X mental retardation genu (*FMR1*, Xq27).
- První dokázaný příklad choroby způsobené expanzí trinukleotidových repetic.

Část 1: Klinické projevy

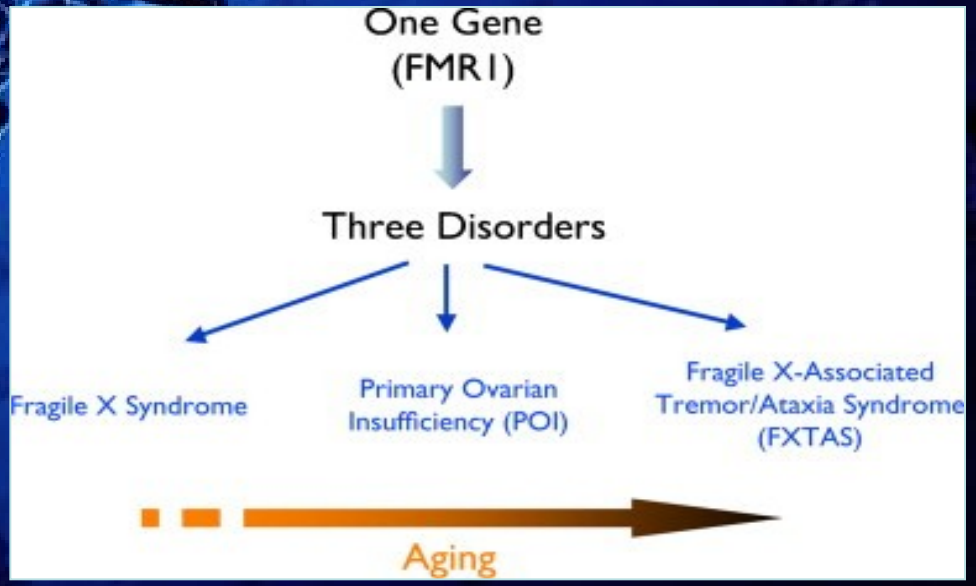
Mutace ve *FMR1* genu vedou ke třem rozdílným projevům.

• Normální lidský *FMR1* gen má počet CGG repetic v rozmezí 5 - 54.

• Rozsáhlé expanze na 200x CGG vedou k metylaci CpG ostrovů a transkripčnímu umlčení *FMR1* genu → **syndrom fragilního X chromozómu** (FXS).

• Expanze CGG repetic mezi 55 až 200 opakováními (premutace) je spojena s progresivním neurodegenerativním onemocněním zvaným **fragile X-associated tremor/ataxia syndrome (FXTAS)** (onemocnění manifestující se v nebo po paté dekádě života). U žen nosiček se přítomnost premutace může projevit **předčasnou ovariální insuficiencí (FXPOI)**.

• Nemetylované expanze 55-200 jednotek CGG jsou nestabilní během meiózy a mohou expandovat do plné mutace. K expanzím dochází pouze při maternálním přenosu.



IMPORTANCE OF REPEAT SIZE IN PREMUTATED INDIVIDUALS: RISK FOR THE OFFSPRING

Number of Maternal Premutation CGG Repeats	Total Maternal Transmissions	Expansions to Full Mutations (%) [1]
55-59	27	1 (3.7%)
60-69	113	6 (5.3%)
70-79	90	28 (31.1%)
80-89	140	81 (57.8%)
90-99	111	89 (80.1%)
100-109	70	70 (100%)
110-119	54	53 (98.1%)
120-129	36	35 (97.2%)
130-139	18	17 (94.4%)
140-200	19	19 (100%)

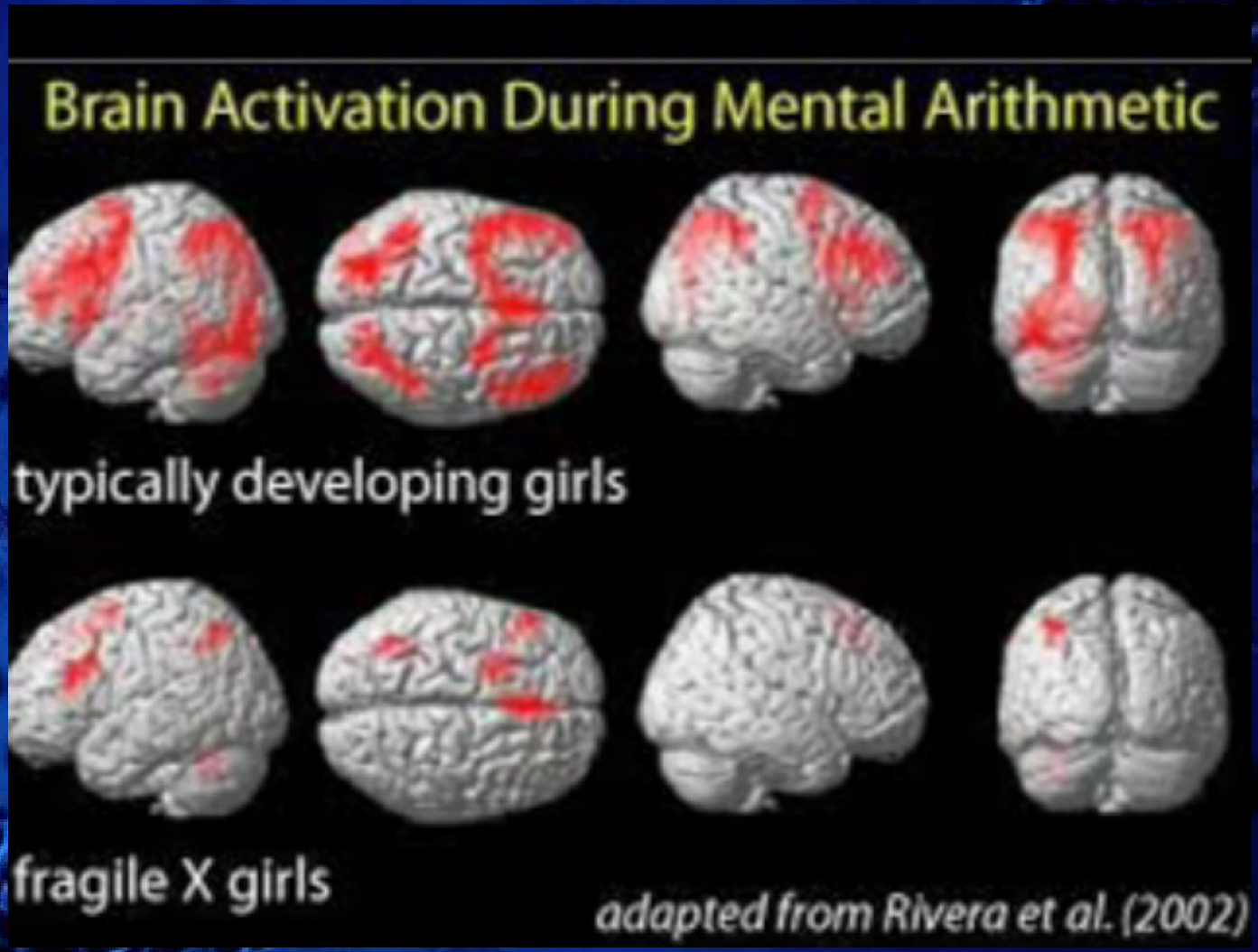
Saul, RA and Tarleton JC. FMR1-related disorders GeneReviews 2008

Morfologické projevy FXS

- normal structure
- broad forehead
- elongated face
- large prominent ears
- strabismus (crossed eyes)
- highly arched palate
- hyperextensible joints
- hand calluses (from self-abuse)
- pectus excavatum (indentation of chest)
- mitral valve prolapse (benign heart condition)
- enlarged testicles
- hypotonia (low muscle tone)
- soft, fleshy skin
- flat feet
- seizures (in about 10 percent)

Psychosociální projevy FXS

- Snížené IQ.
- Hyperaktivita, hypersenzitivita spojená se senzorickými podněty, úzkost a zpoždění vývoje.
- 30% pacientů jsou autisté (2–5% autistických dětí má FXS).
- 25% pacientů trpí epilepsií.


Brain Activation During Mental Arithmetic
typically developing girls
fragile X girls
adapted from Rivera et al. (2002)

Fragile X-assosiated tremor/ataxia syndrom (FXTAS)

- The physical symptoms of FXTAS include an intention tremor, ataxia, Parkinsonism (this includes small, shuffling steps, muscle rigidity and slowed speech), as well as neuropathic symptoms. As the disease progresses to the more advanced stages, an individual with FXTAS is also at risk of autonomic dysfunction. This includes hypertension, bowel and bladder dysfunction, as well as impotence.
- An individual with FXTAS may also exhibit the following symptoms: a decrease in cognition, which includes diminishing short-term memory and executive function skills, declining math and spelling abilities and decision-making abilities. FXTAS may also result in changes in personality, due to alterations of the limbic area in the brain. This includes increased irritability, angry outbursts, and impulsive behaviour.

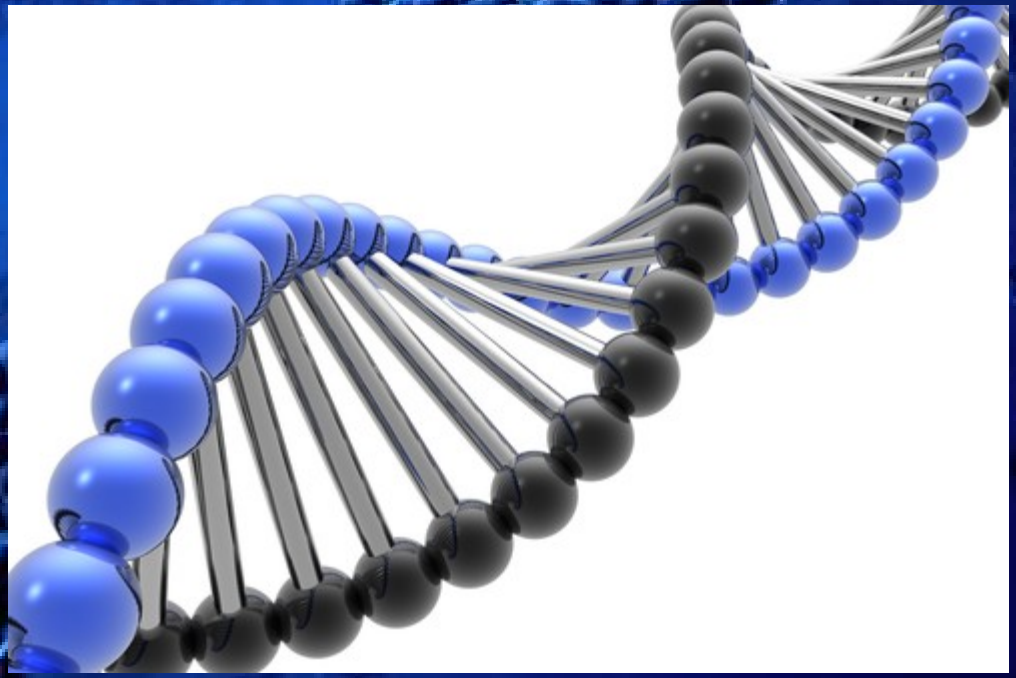
(Hagerman, PJ; Hagerman RJ (2004). "Fragile X-associated tremor/ataxia syndrome (FXTAS)". Mental Retardation and Developmental Disabilities Research Reviews 10 (1): 25–30.)

Fragile X associted primary ovarian insufficiency (FXPOI)

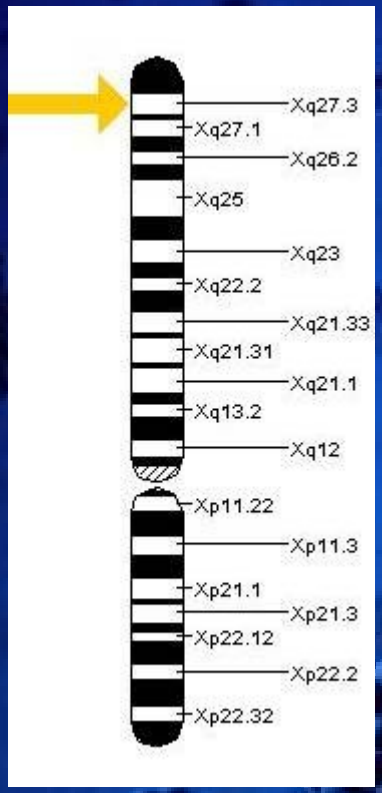
- Approximately 20-28% of women with an FMR1 premutation (55-200 CGG repeats) experience fragile x primary ovarian insufficiency and another 23% experience early menopause (i.e., menopause before the age of forty five).

(http://www.fragilex.org/html/menopause.htm)

Část 2: Molekulární podstata



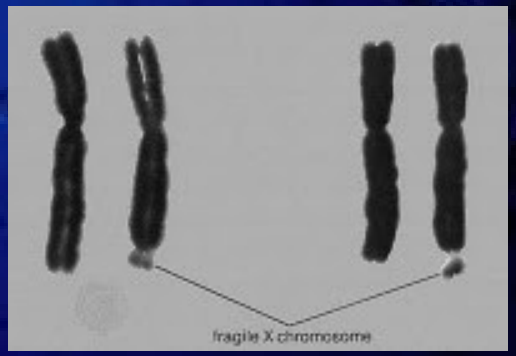
FMR1 gene



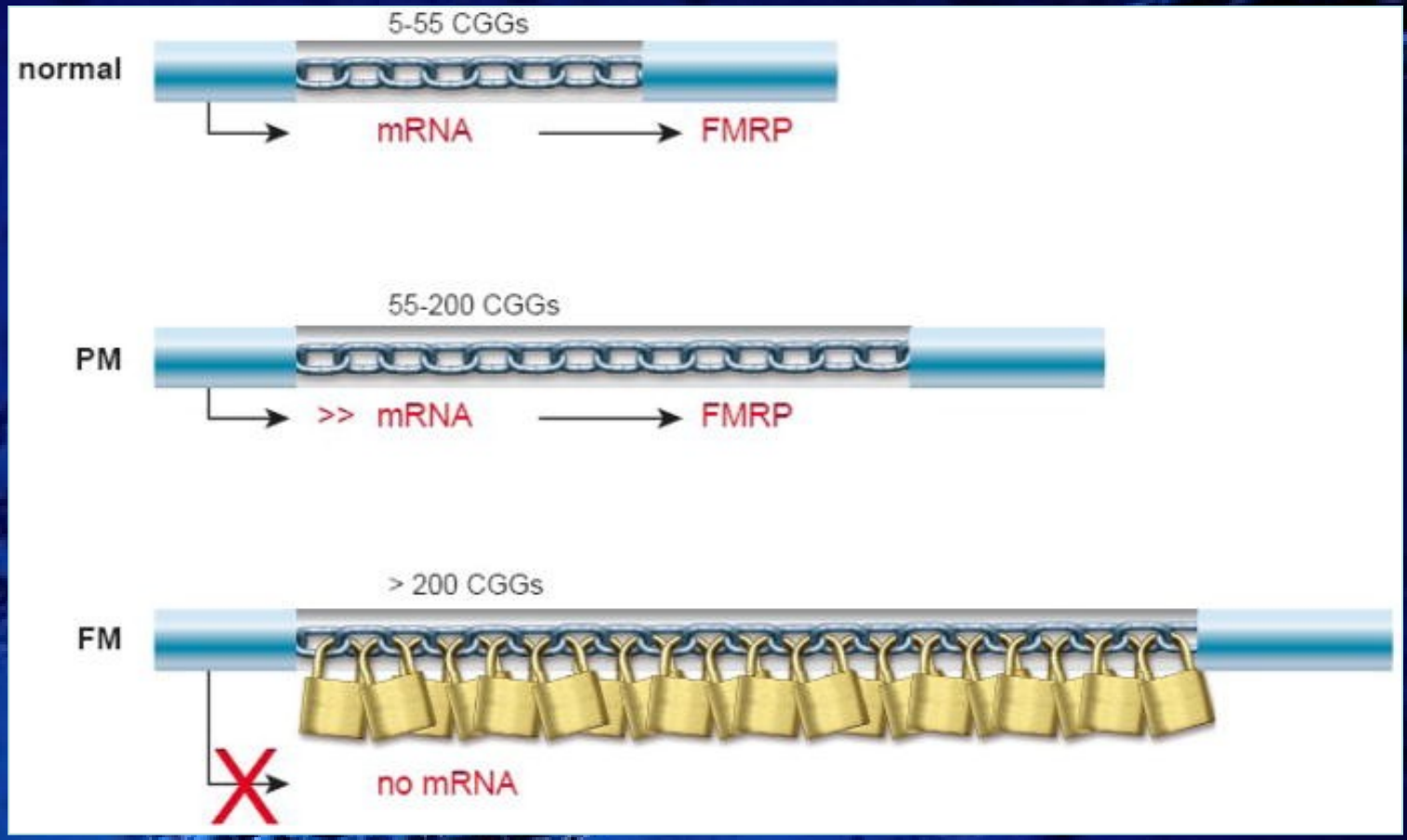
http://en.wikipedia.org/wiki/File:Fmr1.jpeg

http://www.erasmusmc.nl/47421/51019/2004677/hoogeveen-figuur.JPG

- Xq 27.3
- 39,179 bp
- Několik sestřihových variant
- CGG repetitivní oblast v 5'-UTR



http://4.bp.blogspot.com/_FoiEZNQLqOI/S-6APdSyIII/AAAAAAAABfM/pZLSLHtrpC0/s1600/fragile43.jpg



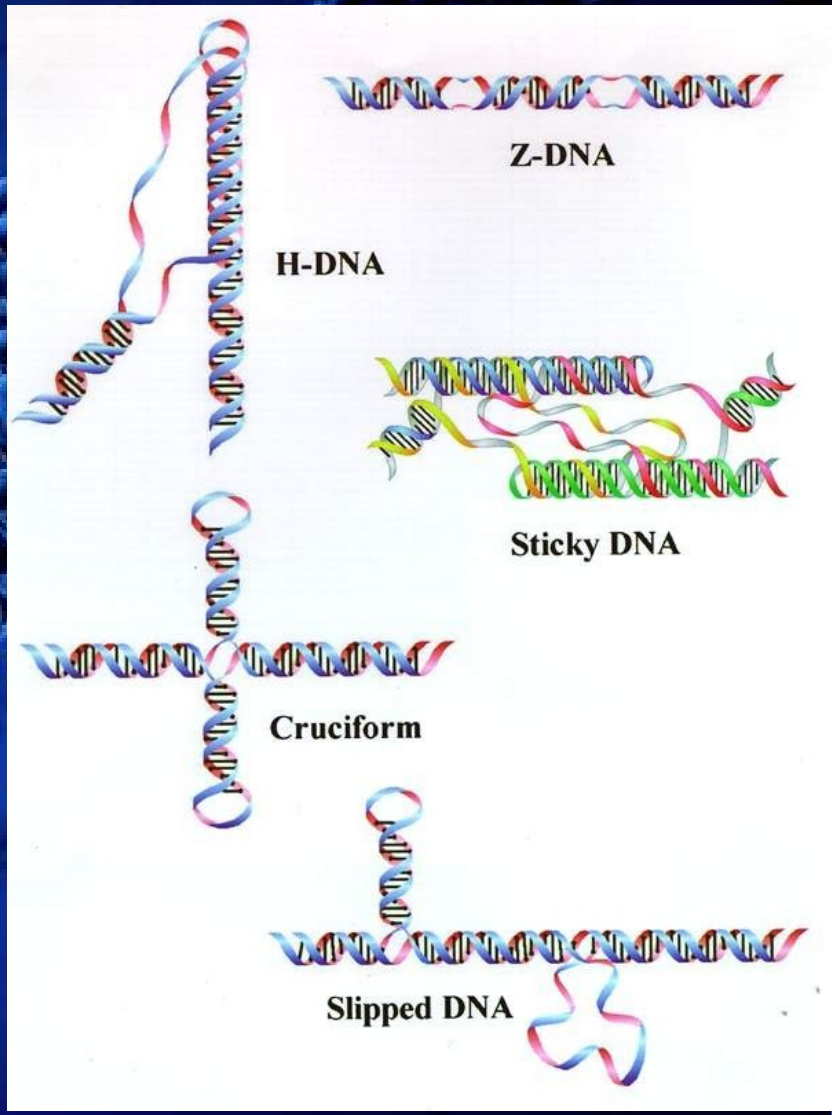
The CGG repeat in the *FMR1* gene Schematic representation of normal, PM (premutation) and FM (full mutation) alleles of the *FMR1* gene and the effect of the expansion on transcription and translation. Methylation due to extensive elongation of the CGG repeat in the 5′-ÚTR of the *FMR1* gene is depicted as a lock.

Biochim Biophys Acta. 2009 June; 1790(6): 467–477.

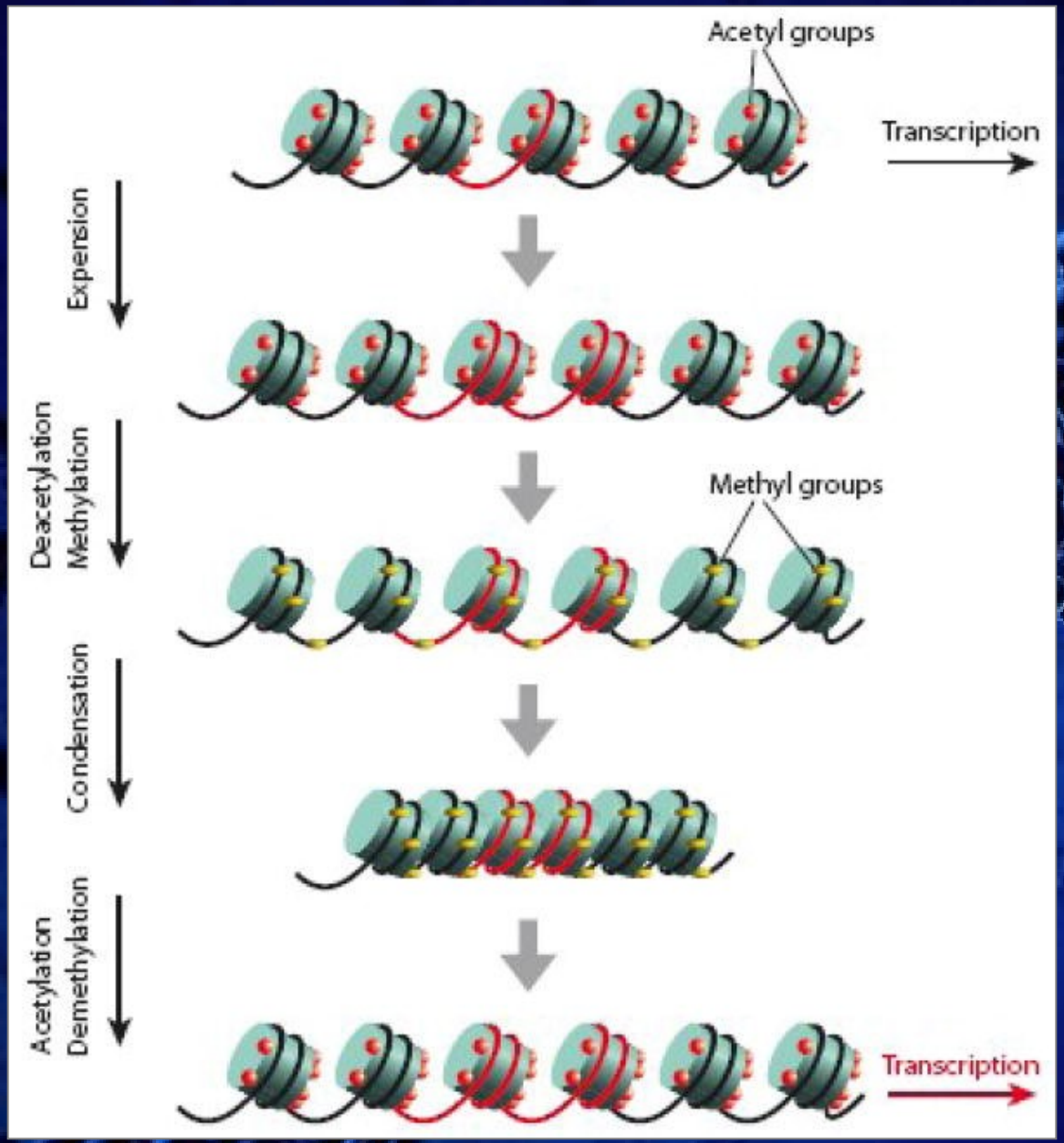
• Trinucleotide repeat sequences (TRS) - dynamic mutations - the number of repeats tends to increase in size over generations.

• Several factors contribute to mutational dynamics - number of repeats, composition and length of the repeating motif, presence of interruptions within the sequence and the rate of intracellular processes such as replication, transcription, repair, or recombination.

• **A significant feature of tandem repeats is ability to form unusual DNA structures (left-handed Z-DNA, cruciforms, slipped-stranded DNA, triplexes, and tetraplexes). Such non-B-DNA structures potentially may be hazardous for genome stability if not removed by repair mechanisms.**



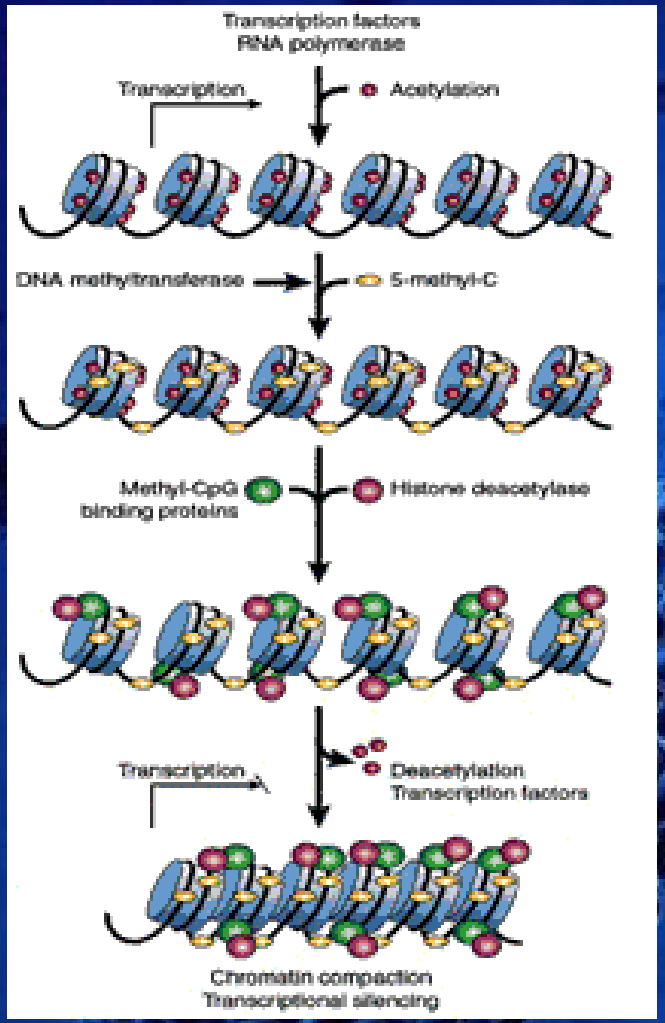
The simplest explanation of an repeat expansion – an slippage of DNA polymerase during DNA replication.



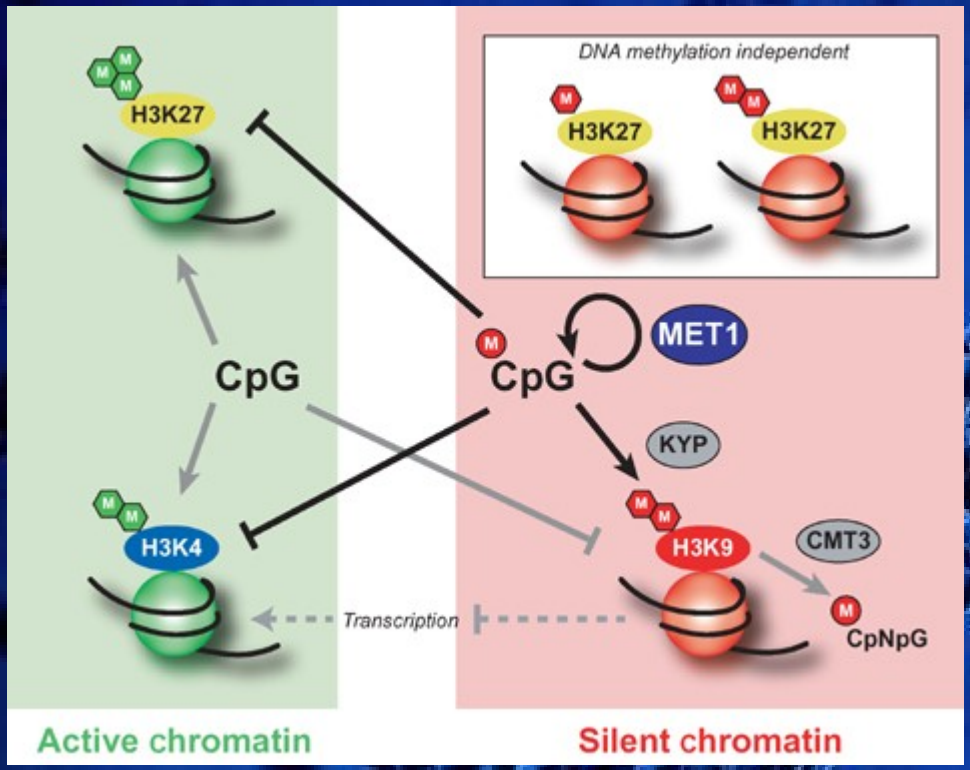
Schematic representation of the chromatin structure of the *FMR1* gene

In the normal situation the active gene has an open chromatin structure. **When the CGG repeat (red line) is expanded, deacetylation and methylation of the promoter and CGG region takes place leading to a packaged and less accessible chromatin structure causing inactivation of the *FMR1* gene.** Treatment with 5-azadC results in demethylation and acetylation leading to an open chromatin structure and transcription will be (partly) restored.

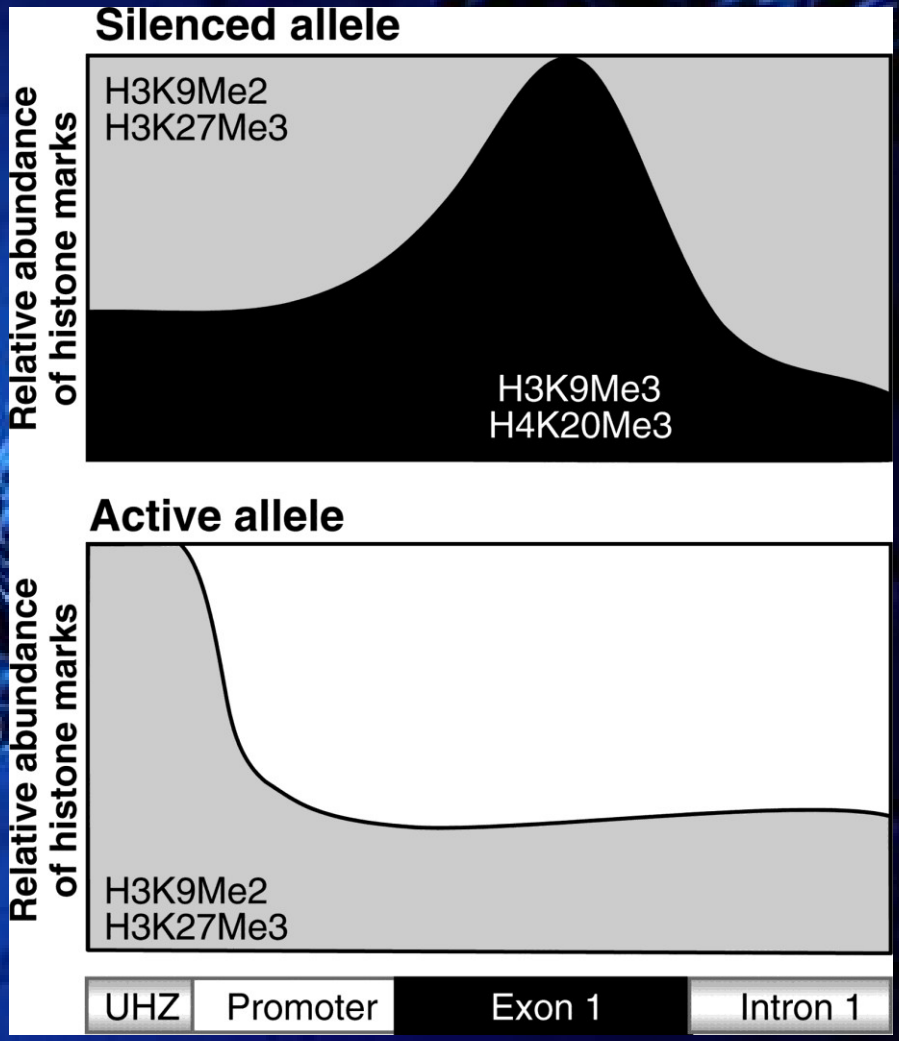
Biochim Biophys Acta. 2009; 1790(6): 467–477.



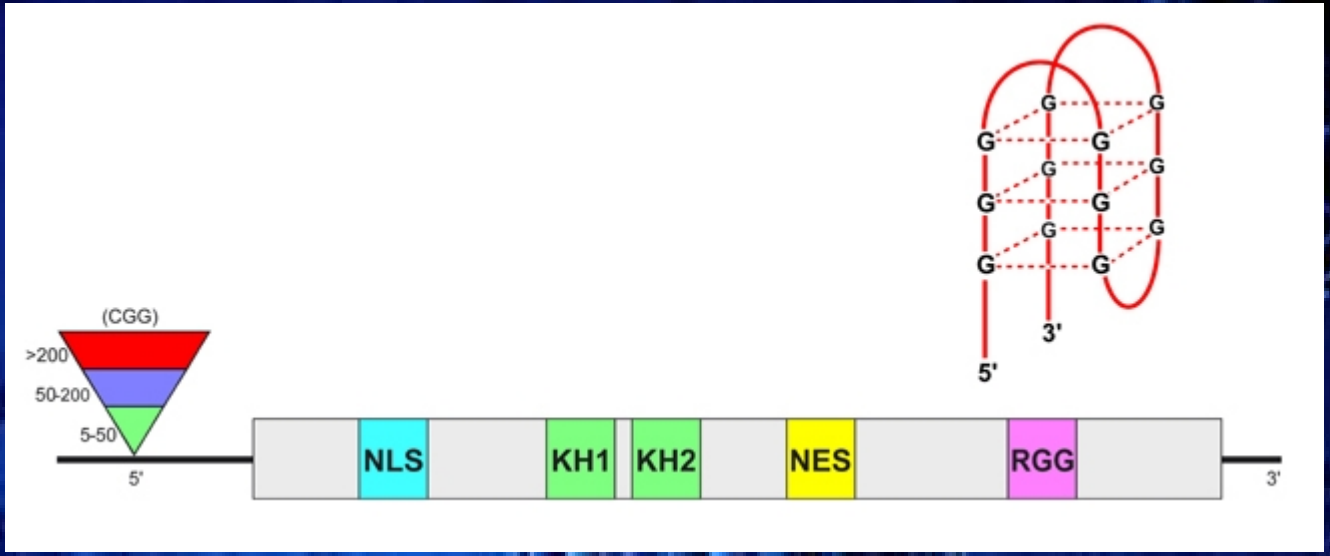
Transcriptionally active chromatin regions tend to be hyperacetylated and hypomethylated. If a region of DNA or a gene is destinated for silencing, chromatin remodeling enzymes such as histone deacetylases and ATP-dependent chromatin remodelers likely begin the gene silencing process. One or more of these activities may recruit DNA methyltransferase resulting in DNA methylation, followed finally by recruitment of the methyl-CpG binding proteins. The region of DNA will then be heritably maintained in an inactive state.



http://www.nature.com/emboj/journal/v24/n15/images/7600743f6.jpg



Kumari D , Usdin K Hum. Mol. Genet. 2010;hmg.ddq394



http://www.erasmusmc.nl/47421/51019/2004677/hoogeveen-figuur.JPG

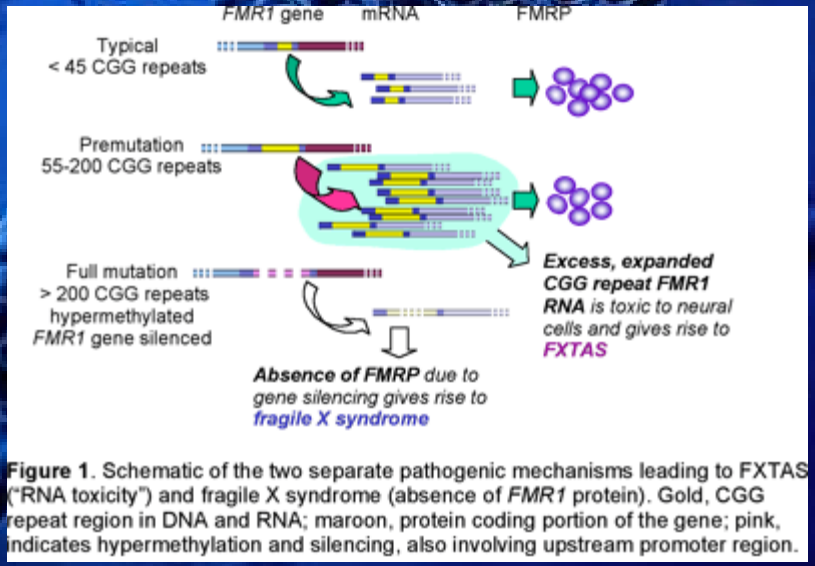
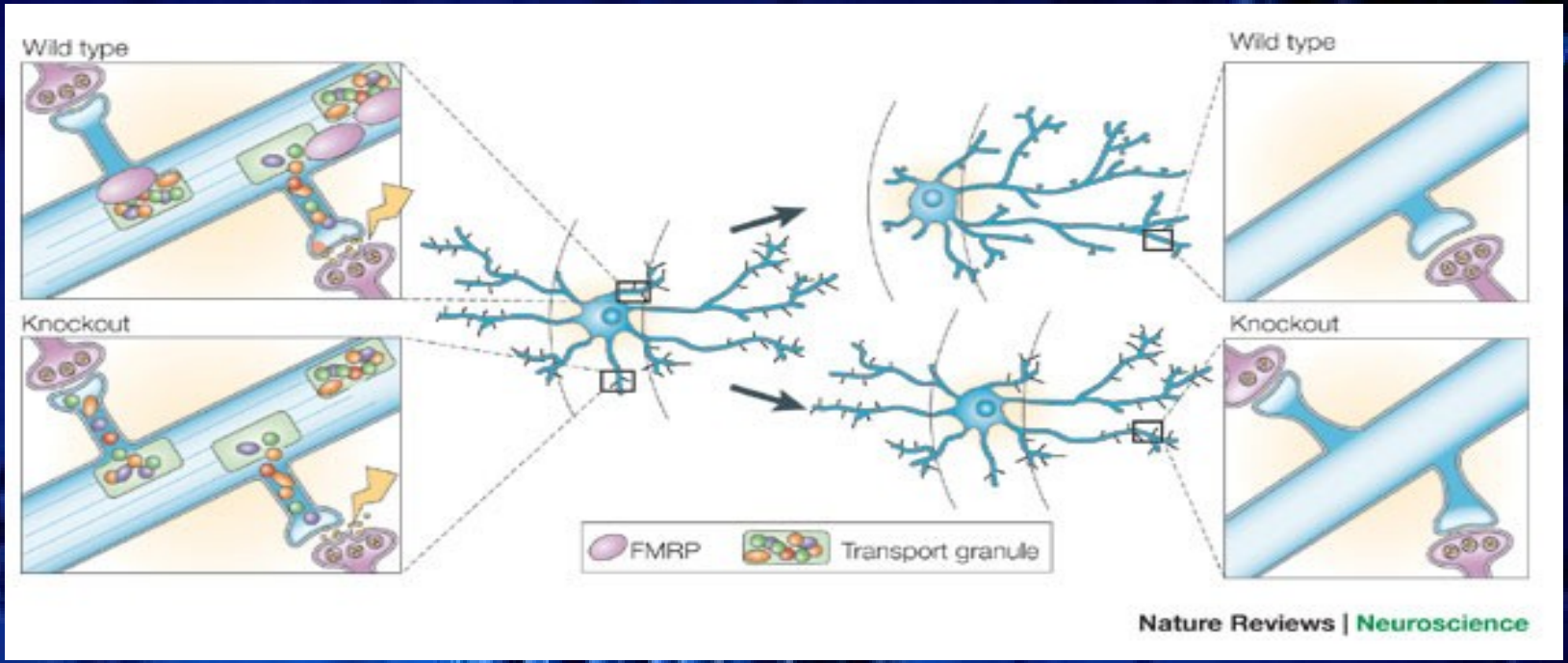


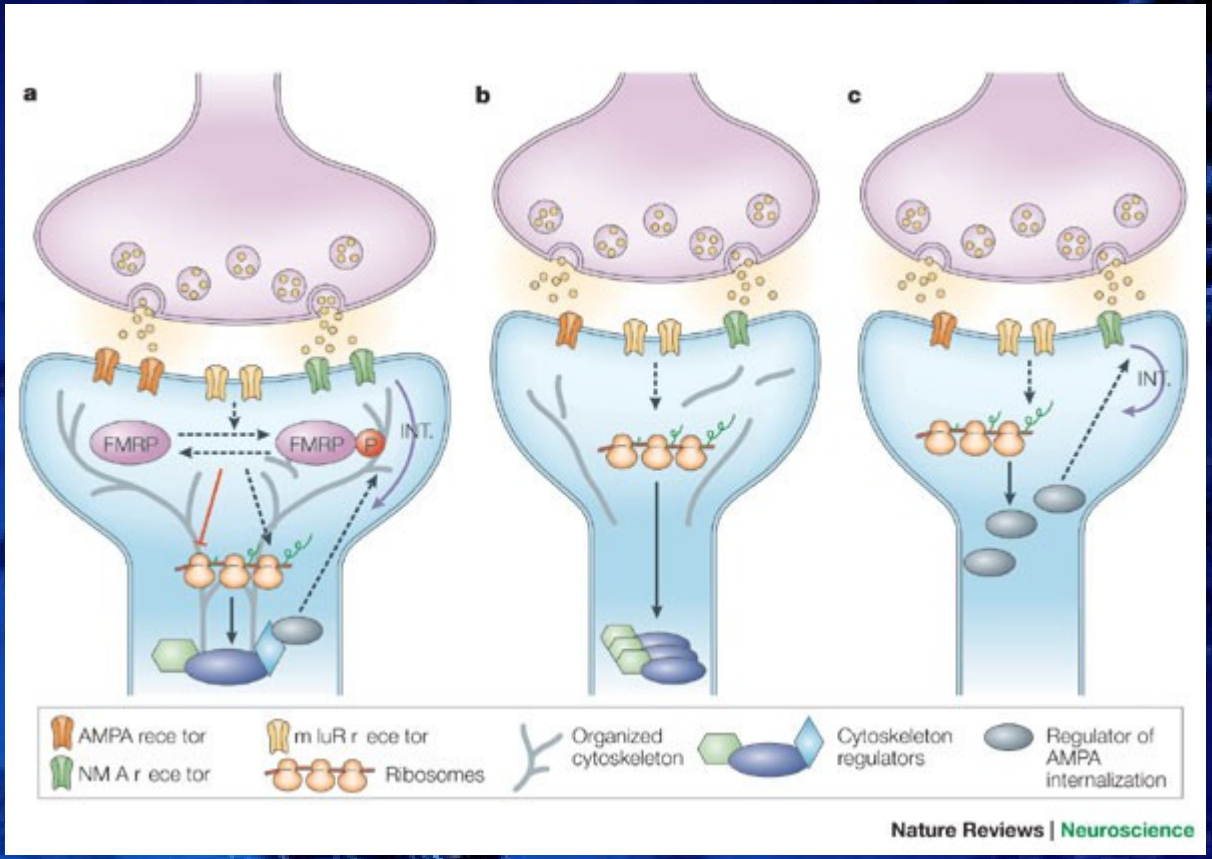
Figure 1. Schematic of the two separate pathogenic mechanisms leading to FXTAS ("RNA toxicity") and fragile X syndrome (absence of *FMR1* protein). Gold, CGG repeat region in DNA and RNA; maroon, protein coding portion of the gene; pink, indicates hypermethylation and silencing, also involving upstream promoter region.

http://www.ucdmc.ucdavis.edu/ntri/images/body/figure1.gif



The figure shows a hypothetical mechanism through which the absence of fragile X mental retardation protein (FMRP) could lead to failure of synapse pruning and, as a consequence, dendrite pruning, in a typical spiny stellate neuron in a whisker barrel (centre). The model assumes that FMRP regulates the synthesis of structural proteins (for example, postsynaptic density protein 95 (PSD-95)) or signalling proteins that form part of a complex that is important for stabilizing and maturing developing synapses (see next fig. for one possible conceptualization of this process). When FMRP is present, this stabilization complex (carried by the transport granule) is selectively targeted to active synapses (upper left), which results in selective maturation and stabilization of spines (upper right) and pruning of non-stabilized synapses. In the absence of FMRP (lower left), the stabilization complex is equally targeted to active and inactive synapses, which results in a weaker form of maturation and stabilization, and gives rise to greater numbers of synapses and an immature morphology (lower right).

From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Claudia Bagni & William T. Greenough
Nature Reviews Neuroscience **6, 376-387 (May 2005)**



At synapses, protein synthesis is initiated by different cellular stimuli, and this leads to an independent response of a single synapse that can influence synaptic plasticity. **a** | In a wild-type spine, stimulation of metabotropic glutamate receptors enhances the synthesis of fragile X mental retardation protein (FMRP), which could act to negatively regulate the translation of proteins that are involved in ionotropic receptor internalization during long-term depression and of proteins that regulate the cytoskeleton (such as microtubule-associated protein 1B (MAP1B), activity-regulated cytoskeletal-associated protein (ARC), arginine-binding protein 2 (ARGBP2), postsynaptic density protein 95 (PSD-95) and Rac1). This receptor-coupled signalling pathway might also be responsible for FMRP phosphorylation and the consequent release of mRNAs from translational inhibition and/or the activation of translation of other specific dendritic mRNAs. The correct balance between synthesis and degradation of these proteins would promote and maintain the mature shape of the synapse. **b** | In a spine of a patient with fragile X syndrome, or in the mouse model of the syndrome, the absence of FMRP would lead to an increase and/or decrease in the translation of protein regulators of the cytoskeleton, both of which might have an effect on the lengthening of dendritic spines. **c** | The absence of FMRP could also lead to an increase in the translation of proteins that are involved in ionotropic (AMPA (-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (***N***-methyl-**D**-aspartate)) receptor internalization (INT.) during hippocampal long-term depression, which could lead to fewer receptors being present on the postsynaptic membrane and to thinner spines. mGluR, metabotropic glutamate receptor.

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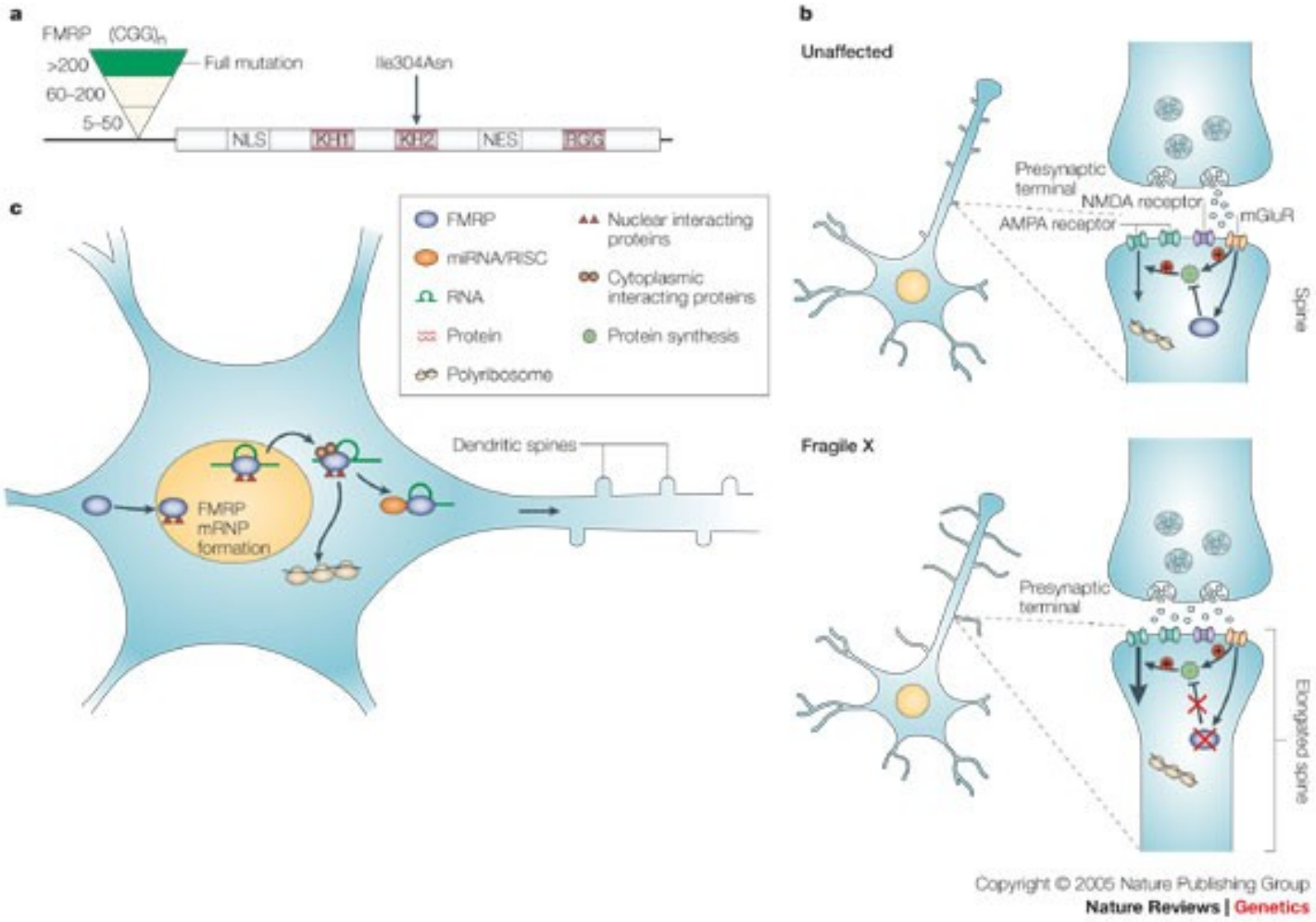
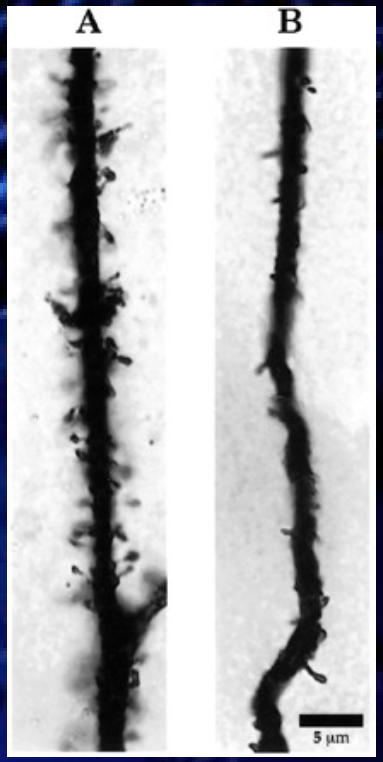


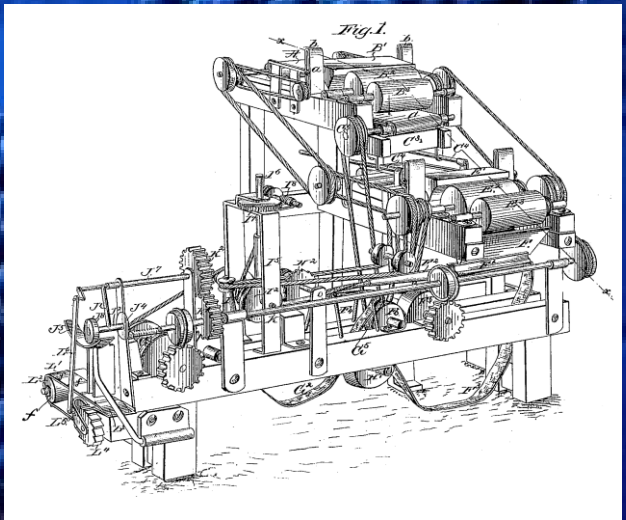


Typical spine morphologies from (A) a human afflicted by fragile-X syndrome and (B) an unaffected control.

Abnormal dendritic spine morphology in patients with FRAXA (an increased density of long, immature dendritic spines). During transport FMRP-mRNA, FMRP suppresses translation. Stimulation of postsynaptic glutamate receptors (mGluRs) results in increased protein synthesis. FMRP, which is also upregulated by mGluRs, serves to dampen this process. The absence of FMRP in FRAXA results in over-amplification of this response.

Dendritic Spine Structural Anomalies in Fragile-X Mental Retardation Syndrome: Scott A. Irwin, Roberto Galvez and William T. Greenough. Cereb. Cortex (2000) 10 (10): 1038-1044.

Část 3: Metodické přístupy - diagnostika



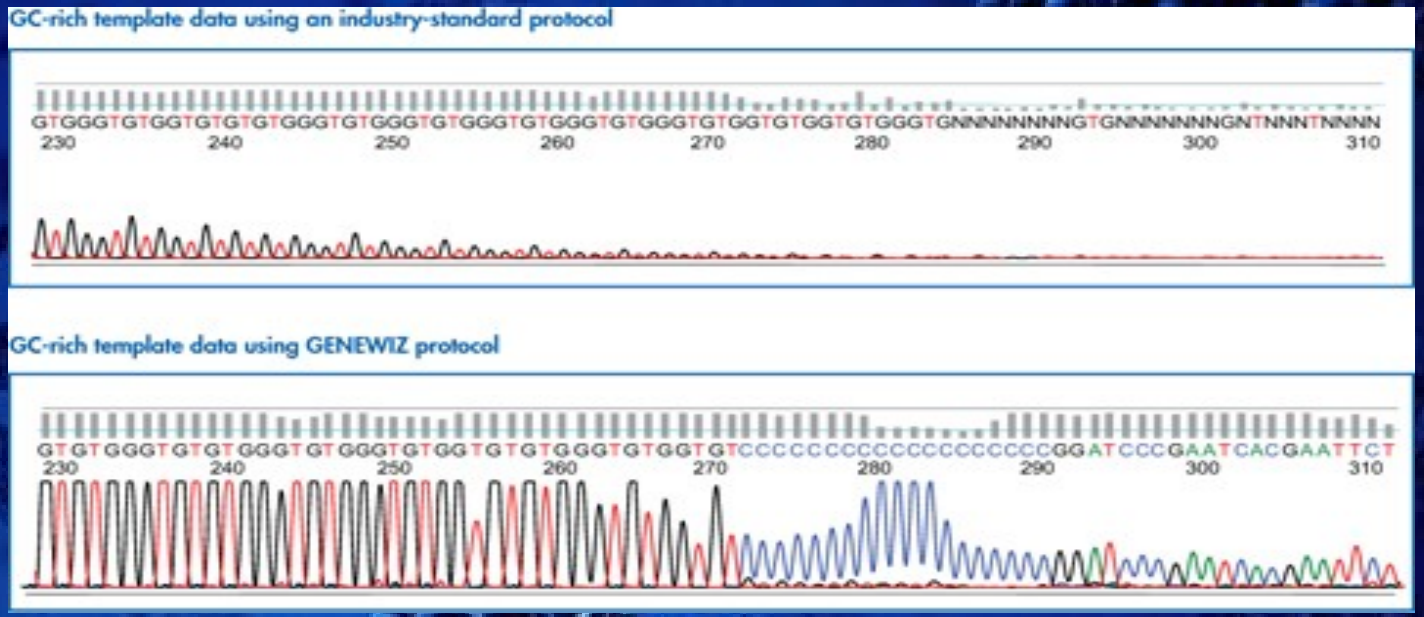
Spektrum metod

- Home-made metody
- GC-rich PCR systémy
- Metylačně specifická PCR
- Triplet-Primed PCR (Abbott Molecular)
- Sizing PCR (Abbott Molecular)
- Southern blotting

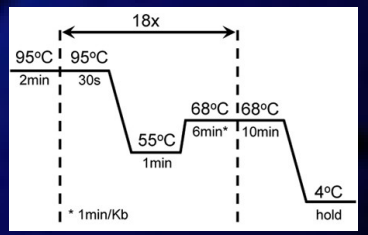
Home-made metody

- Většinou založeny na prosté amplifikaci daného úseku
- Většinou odhalí pouze alely o normální velikosti
- Nutno použít polymerázy s *proof reading* aktivitou

GC-rich PCR systémy



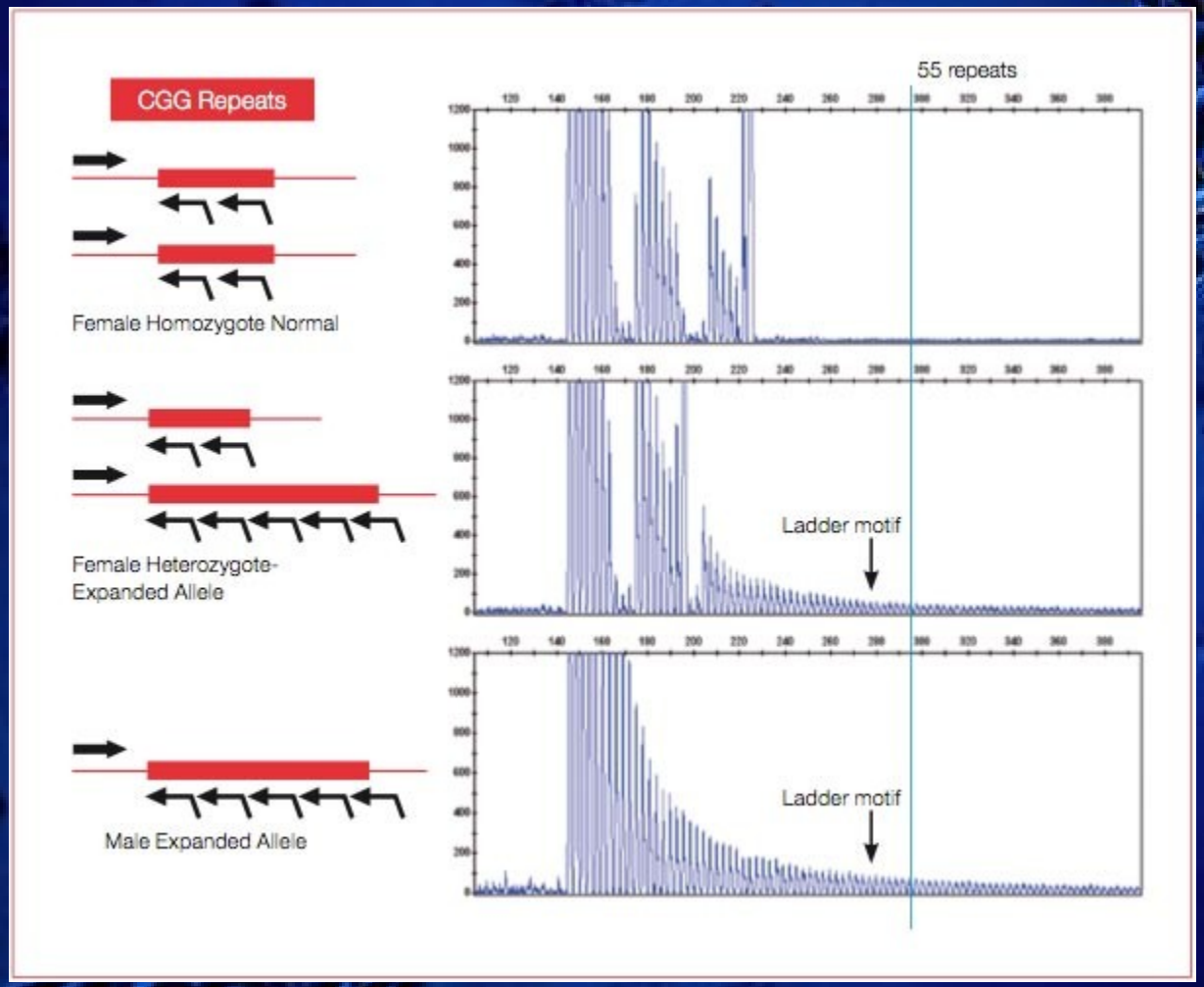
- Amplifikace GC-rich templátů složitá – speciální pufry, speciální polymerázy, speciální PCR programy
- Většinou pouze alely o normální velikosti, dolní hranice premutací



Metylačně specifická PCR

http://www.youtube.com/watch?v=zgNsmY6Led4

Tripled-primed PCR (Abbott)

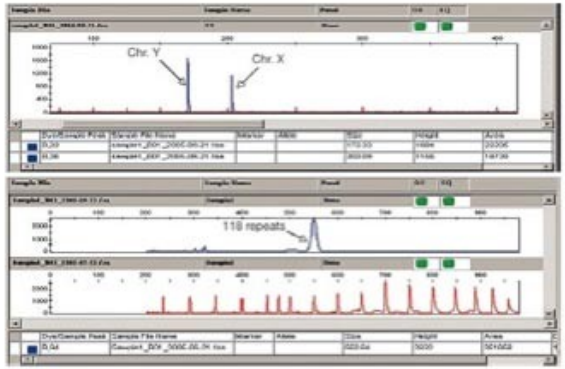


- Malé vstupní množství DNA
- PCR + fragmentační analýza (2 + 1 h)
- Robustní, rychlá, jednoduchá
- Drahá

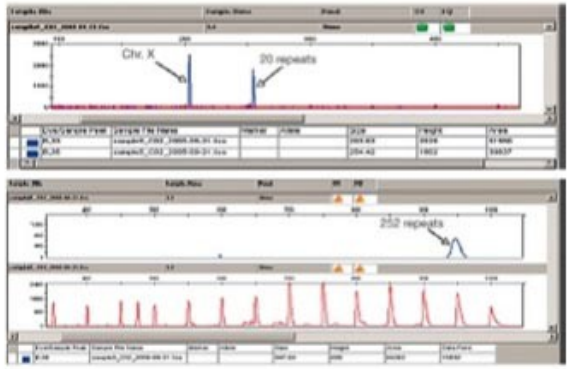
Sizing PCR (Abbott)

CGG Repeats

Male premutation (118 repeats)



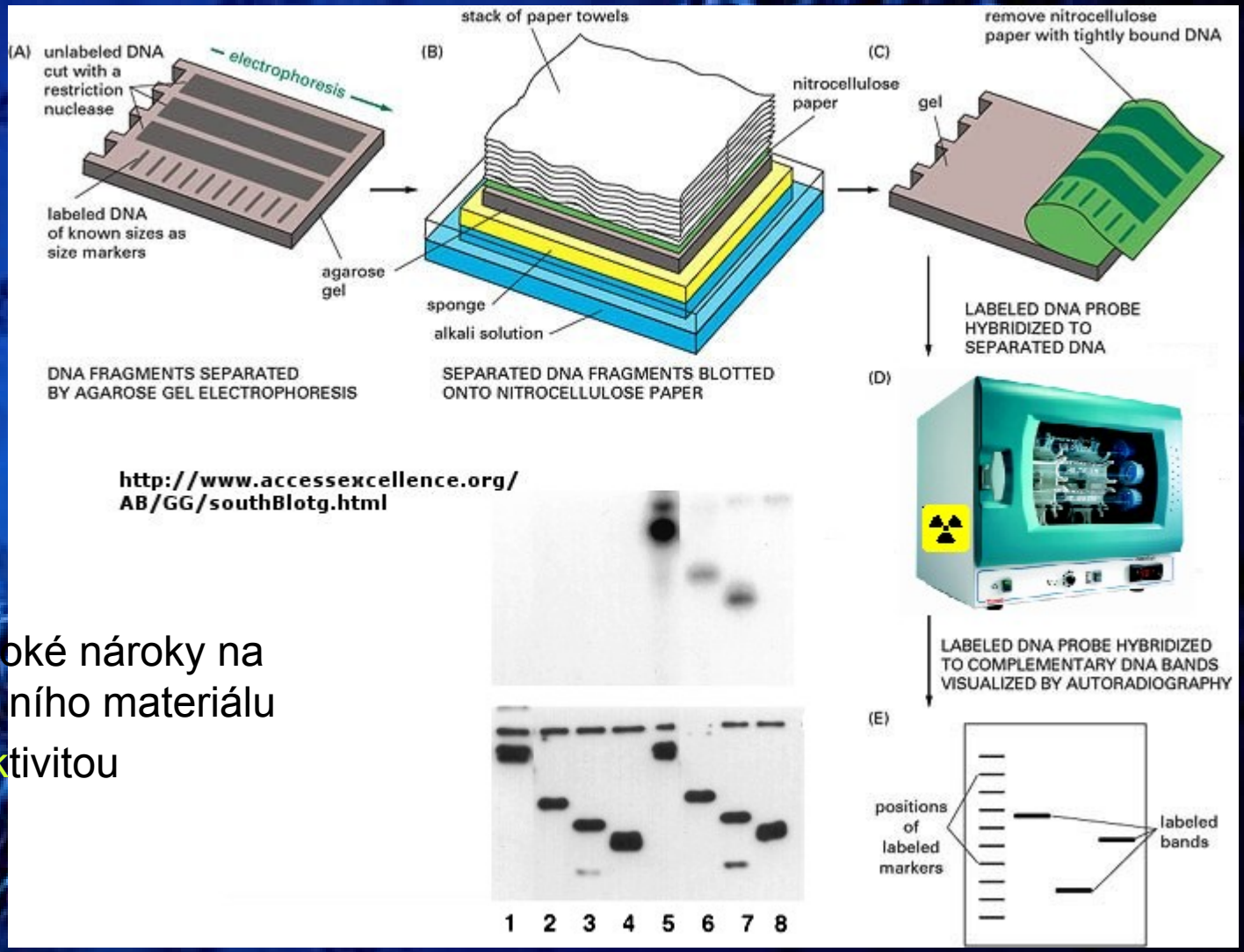
Female Heterozygote-Expanded Allele (20/252 repeats)



The FMR1 Sizing PCR simultaneously amplifies gender specific targets in the X and Y chromosomes as well as the CGG triplet repeat region within the X chromosome's FMR1 gene. A test developed using these reagents may assist the laboratory in the detection and measurement of FMR1 alleles without reflexing to Southern Blot.

- Malé vstupní množství DNA
- PCR + fragmentační analýza (4 + 2 h)
- Robustní, rychlá, jednoduchá
- Velmi drahá

Southern blotting



- Zdlouhavé, vysoké nároky na množství vstupního materiálu
- Práce s radioaktivitou
- Poměrně levné


Děkuji za pozornost