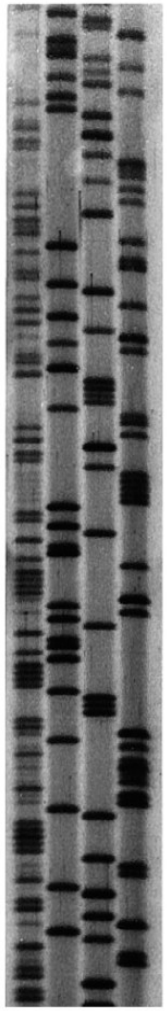
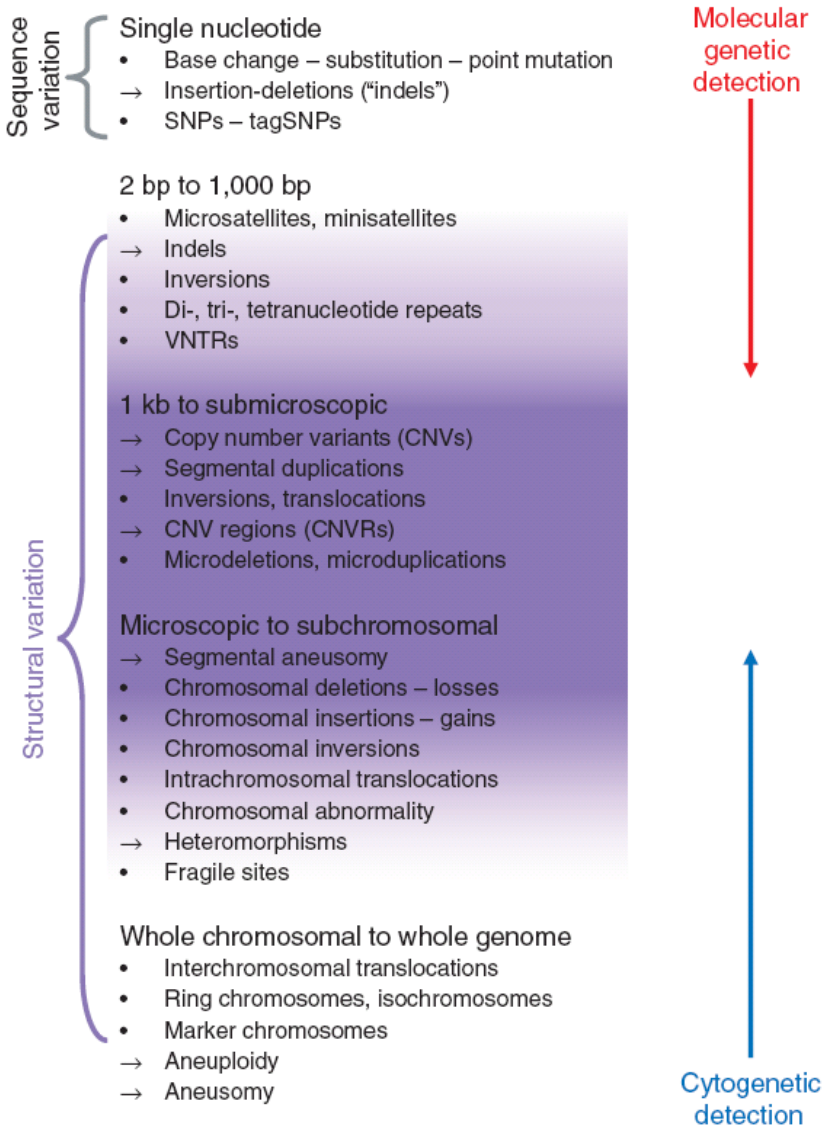


DNA-Analytik III

Genetische
Variabilität

Genetische Variabilität

Lexikon



Scherer et al. Nat Genet Suppl 39:s7 (2007)

Genetische Variabilität

Sequenzvariation

Mutationen (Mikro~)

Basensubstitution

Insertion + Deletion = InDel

Polymorphismen

SNP („single nucleotide" ~)

Polymorphismen resultieren aus **Mutationen**, die sich mit einer Häufigkeit >1% in einer Population etabliert haben.

SNP-Bestimmung

Methoden

Sanger-Sequenzierung

PyroSequenzierung

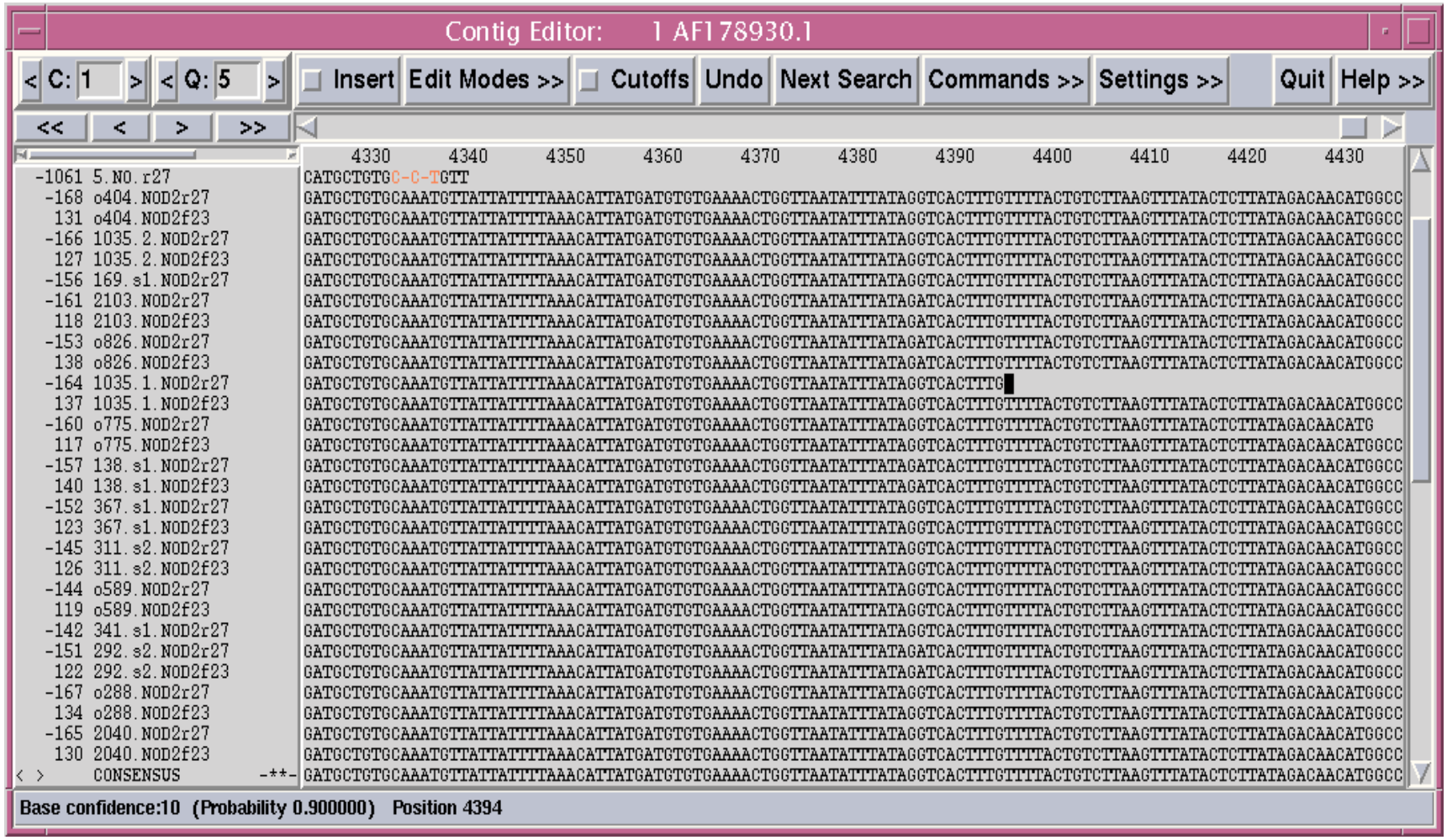
Quantitative PCR („TaqMan“)

Massenspektrometrie („Sequenome“)

DNA-Chip („Affimetrix“)

SNP-Bestimmung

DyeTerminator-Sequenzierung

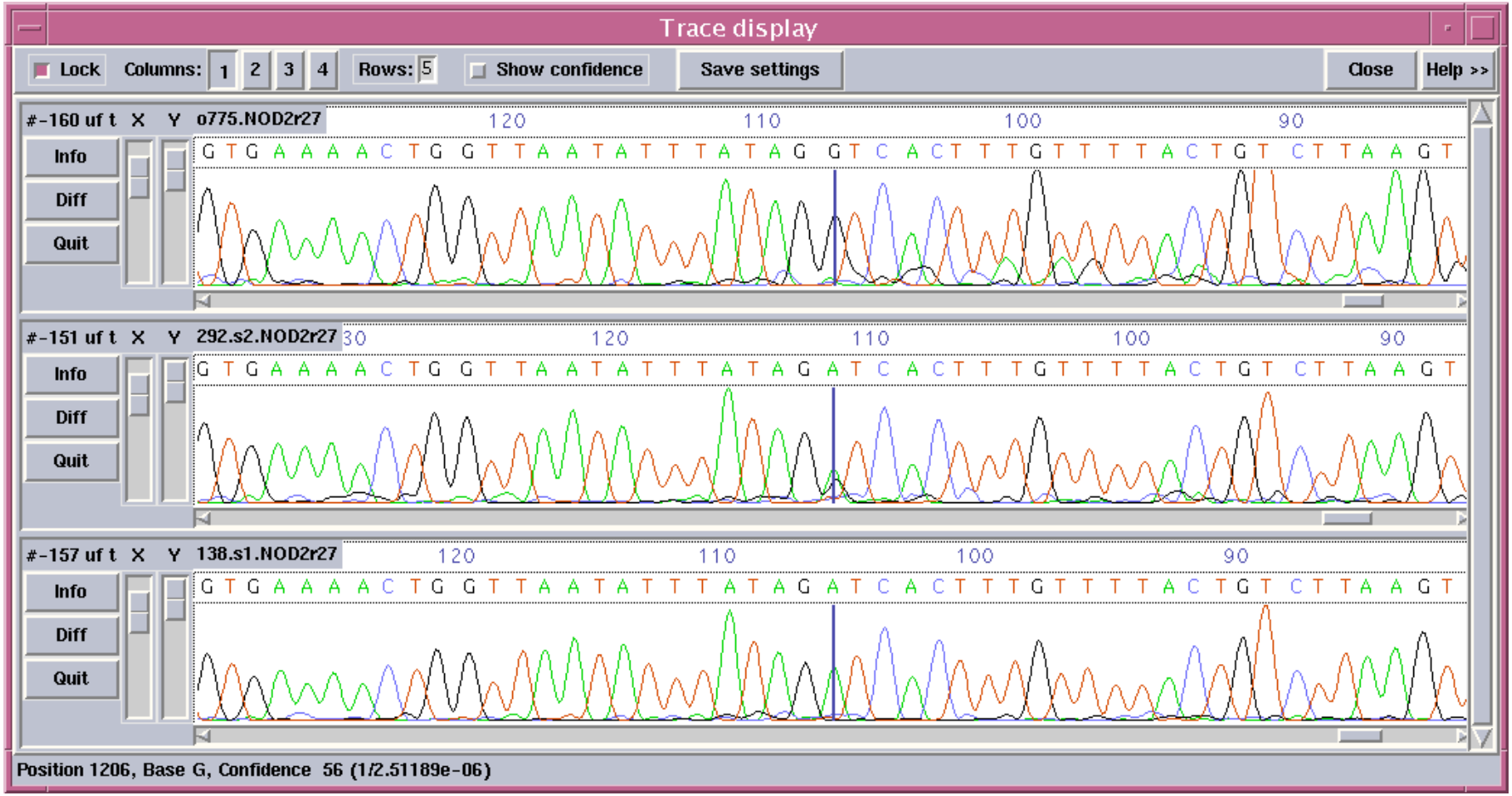


SNP-Bestimmung

DyeTerminator-Sequenzierung

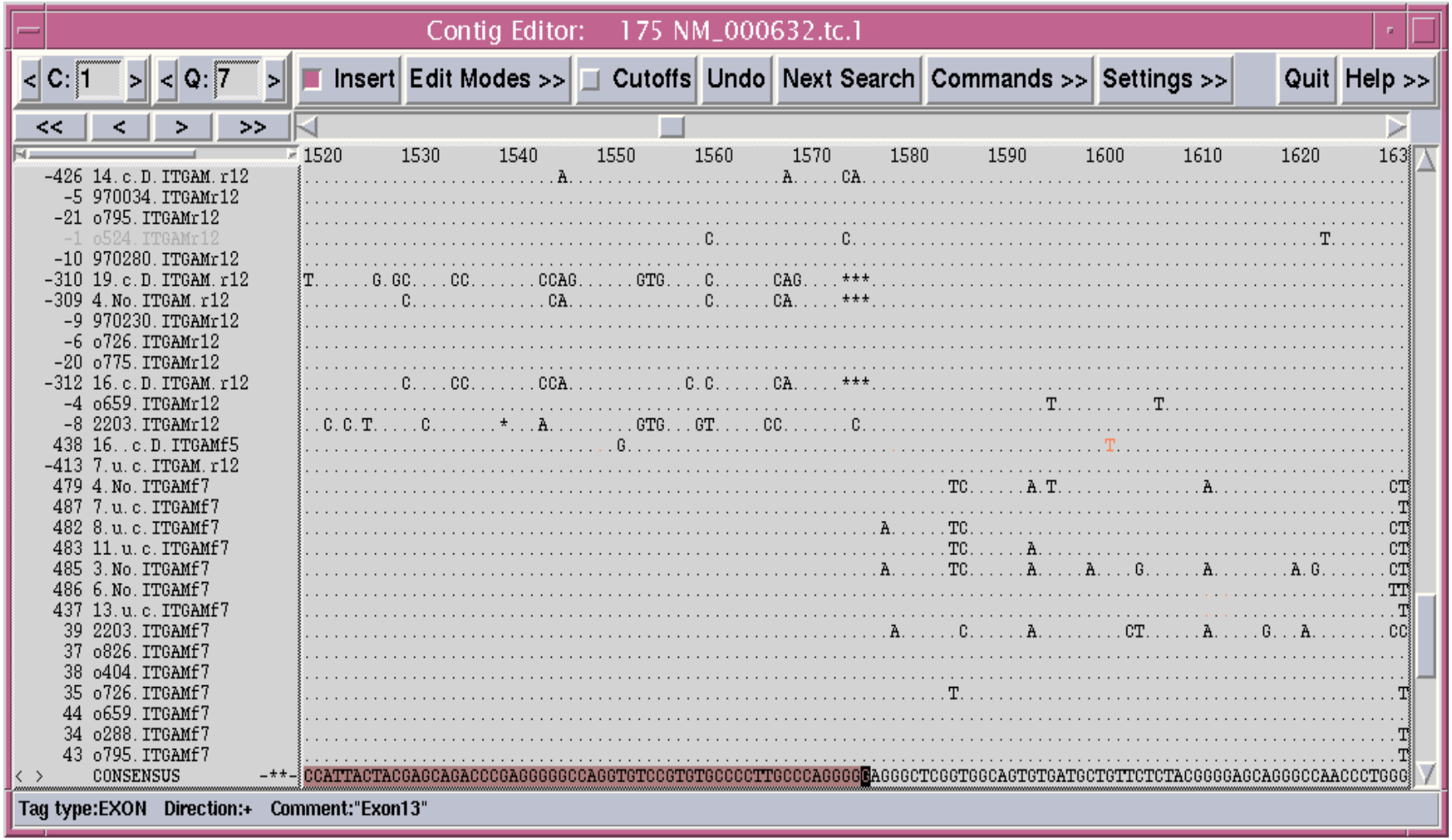
SNP-Bestimmung

DyeTerminator-Sequenzierung



InDel-Bestimmung

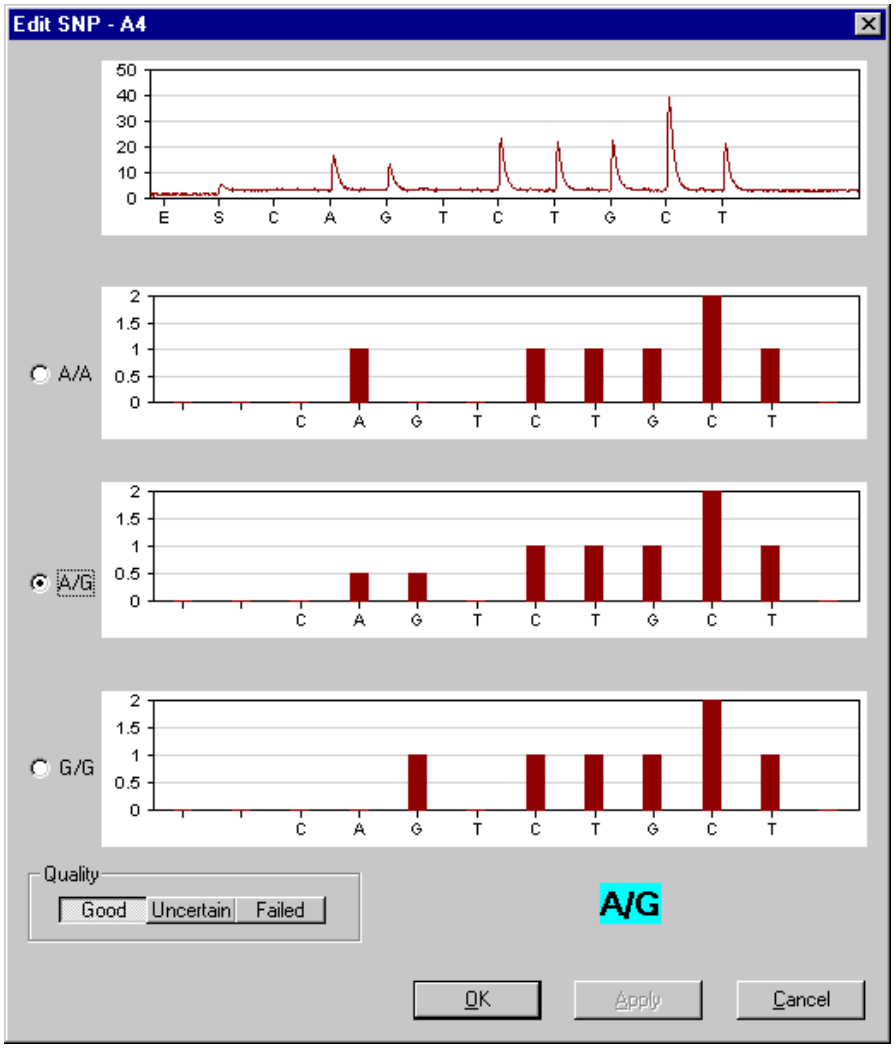
DyeTerminator-Sequenzierung



InDel-Bestimmung

DyeTerminator-Sequenzierung

Pyrosequencing



sequence to analyze:

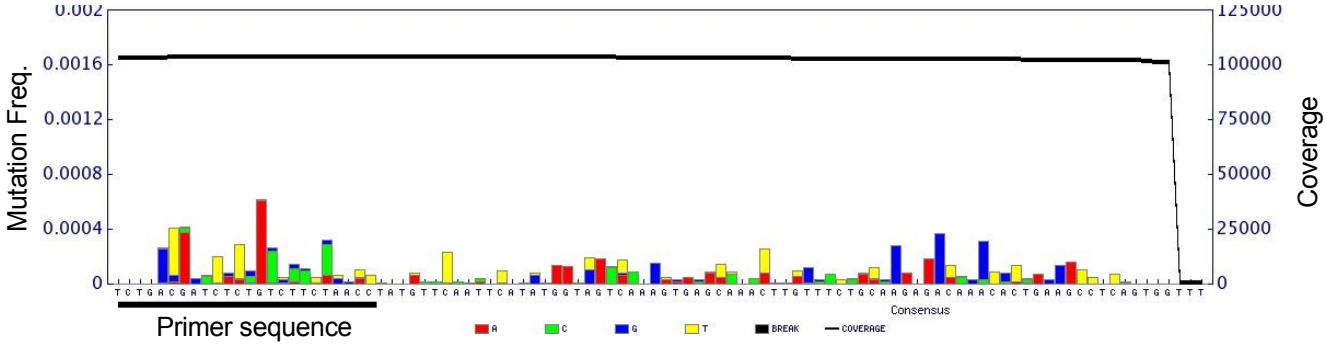
A/GCTGCCT

PYROSEQUENCING

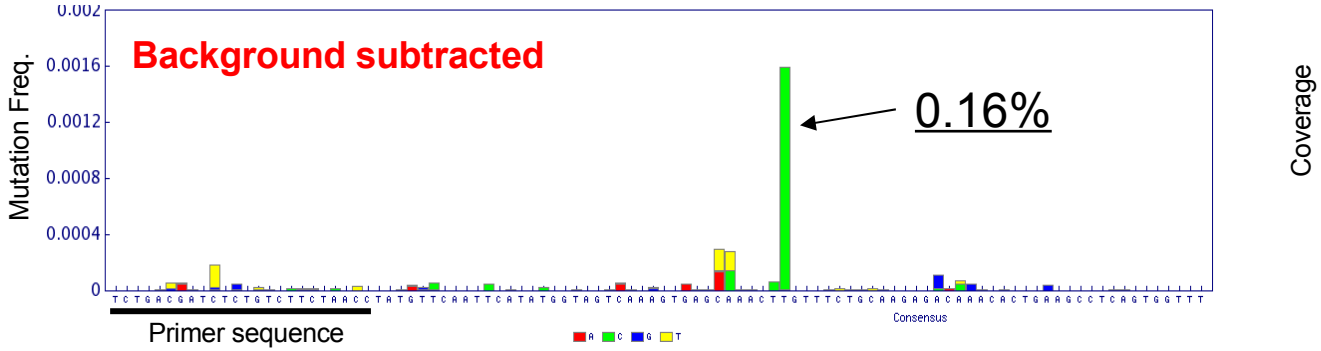
Bestimmung seltener Mutationen/Variationen

Ultra deep 454 sequencing

T only

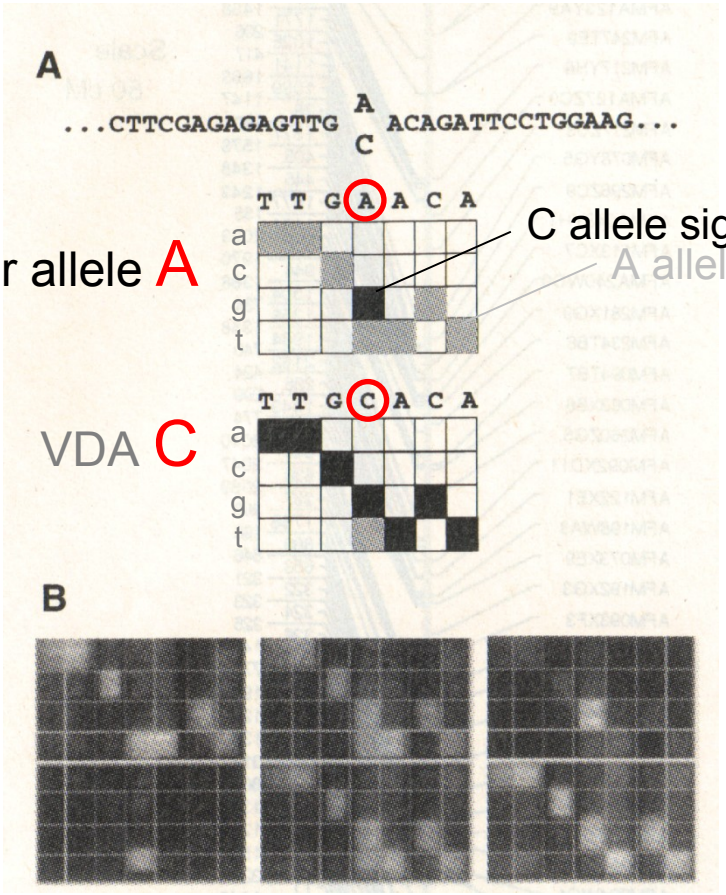


C to T ratio: 1/500



SNP-Bestimmung

Genotypisierung per Chip



Variant Detection Array for allele A

VDA C

VDA A

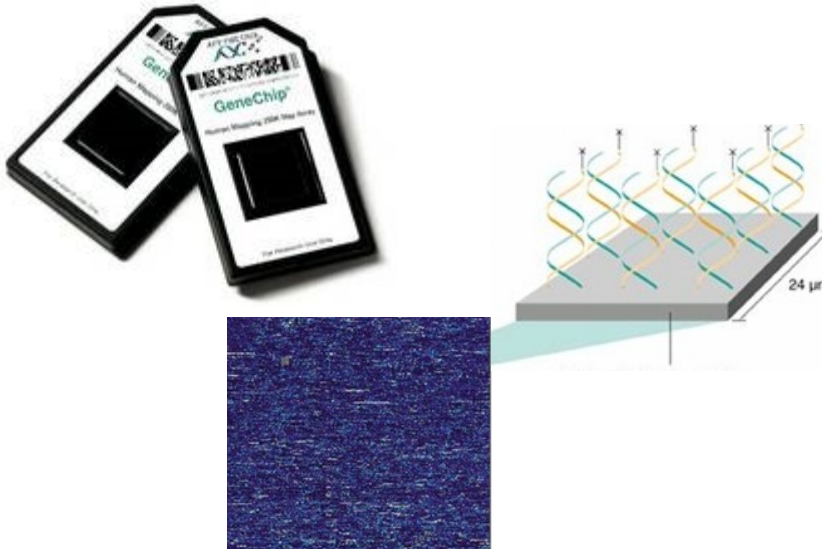
VDA C

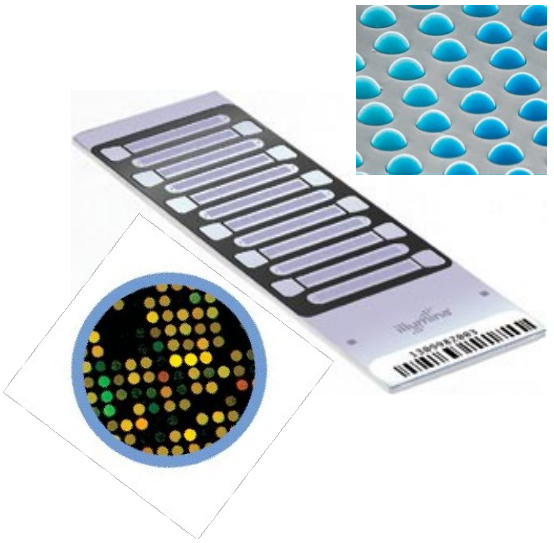
A/A **A/C** **C/C**

Wang et al. Science 280:1077 (1998)

SNP-Bestimmmung

High-throughput array-based genotyping





Affymetrix

Human SNP Array 6.0

>1.8 million markers

906,600 SNPs

946,000 for CNVs

Illumina

Human 660W-Quad BeadChip

2.6 million markers / four samples

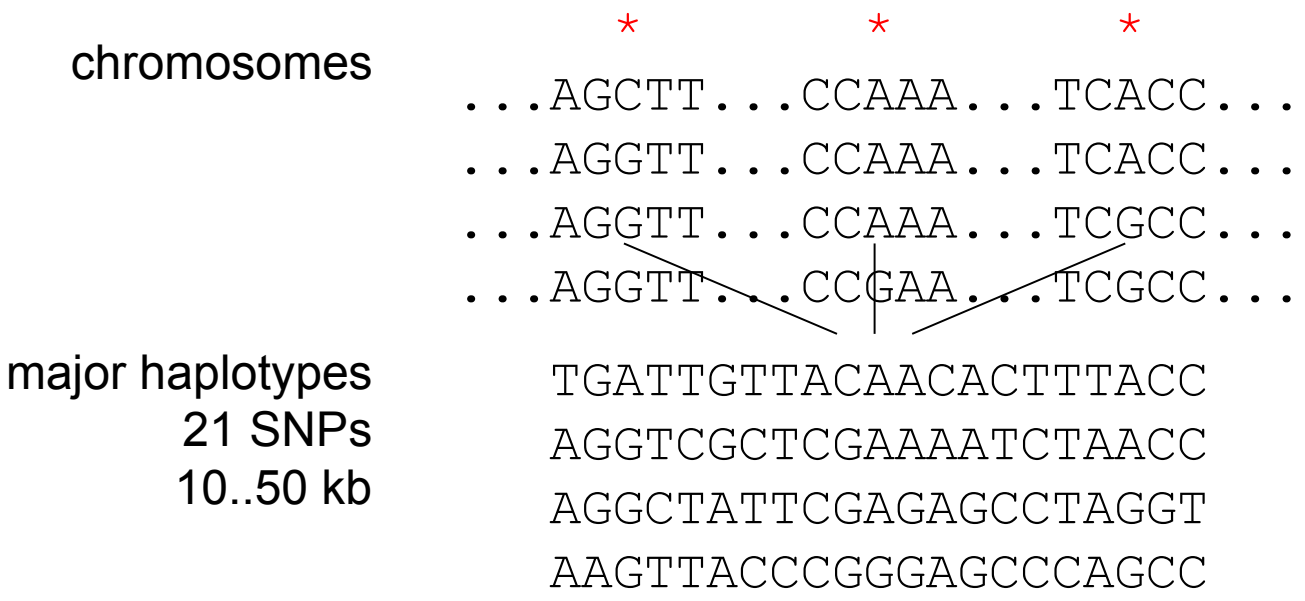
550,000 tag SNPs

100,000 for CNVs

5,000 common CNVs

Haplotype

= set/region physically linked polymorphism



Genetische Variabilität

Haplotypen

Vol 449|18 October 2007|doi:10.1038/nature06258 nature

ARTICLES

A second generation human haplotype map of over 3.1 million SNPs

The International HapMap Consortium*

We describe the Phase II HapMap, which characterizes over 3.1 million human single nucleotide polymorphisms (SNPs) genotyped in 270 individuals from four geographically diverse populations and includes 25–35% of common SNP variation in the populations surveyed. The map is estimated to capture untyped common variation with an average maximum r^2 of between 0.9 and 0.96 depending on population. We demonstrate that the current generation of commercial genome-wide genotyping products captures common Phase II SNPs with an average maximum r^2 of up to 0.8 in African and up to 0.95 in non-African populations, and that potential gains in power in association studies can be obtained through imputation. These data also reveal novel aspects of the structure of linkage disequilibrium. We show that 10–30% of pairs of individuals within a population share at least one region of extended genetic identity arising from recent ancestry and that up to 1% of all common variants are untaggable, primarily because they lie within recombination hotspots. We show that recombination rates vary systematically around genes and between genes of different function. Finally, we demonstrate increased differentiation at non-synonymous, compared to synonymous, SNPs, resulting from systematic differences in the strength or efficacy of natural selection between populations.

TIHC. Nature 449:851 2007

HapMap Projekt

Gegenstand

270 individuals from 4 geographically diverse populations:

YRI Africans: 30 trios (Yoruba in Ibadan, Nigeria)
CEU European: 30 trios of northern/western ancestry (Utah, US; CEPH collection)
CHB Chinese: 45 unrelated Han individuals (Beijing, China)
JPT Japanese: 45 unrelated individuals (Tokyo, Japan)

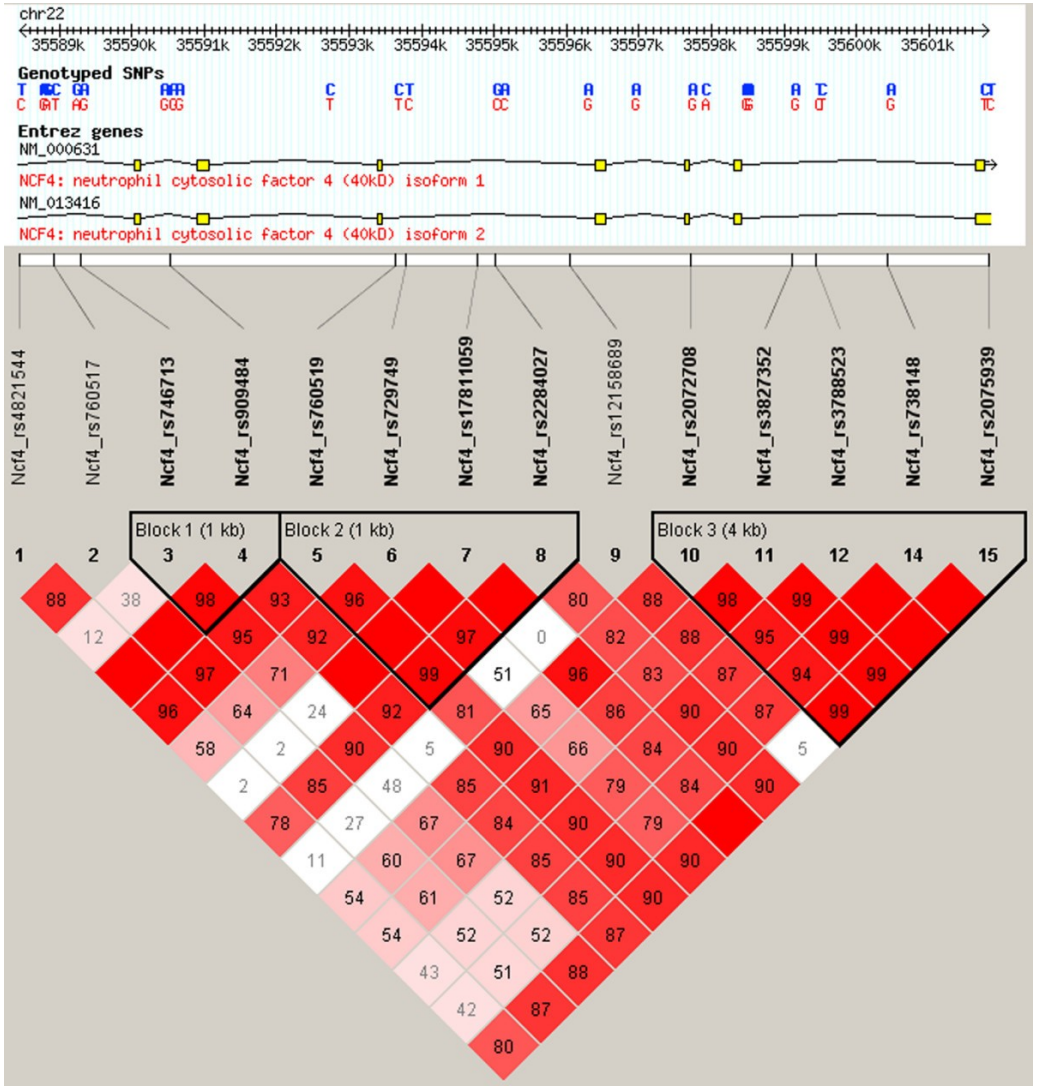
3.1 million human SNPs genotyped

~25–35% of common SNP variation in the populations surveyed

TIHC. Nature 449:851 2007

HapMap Projekt

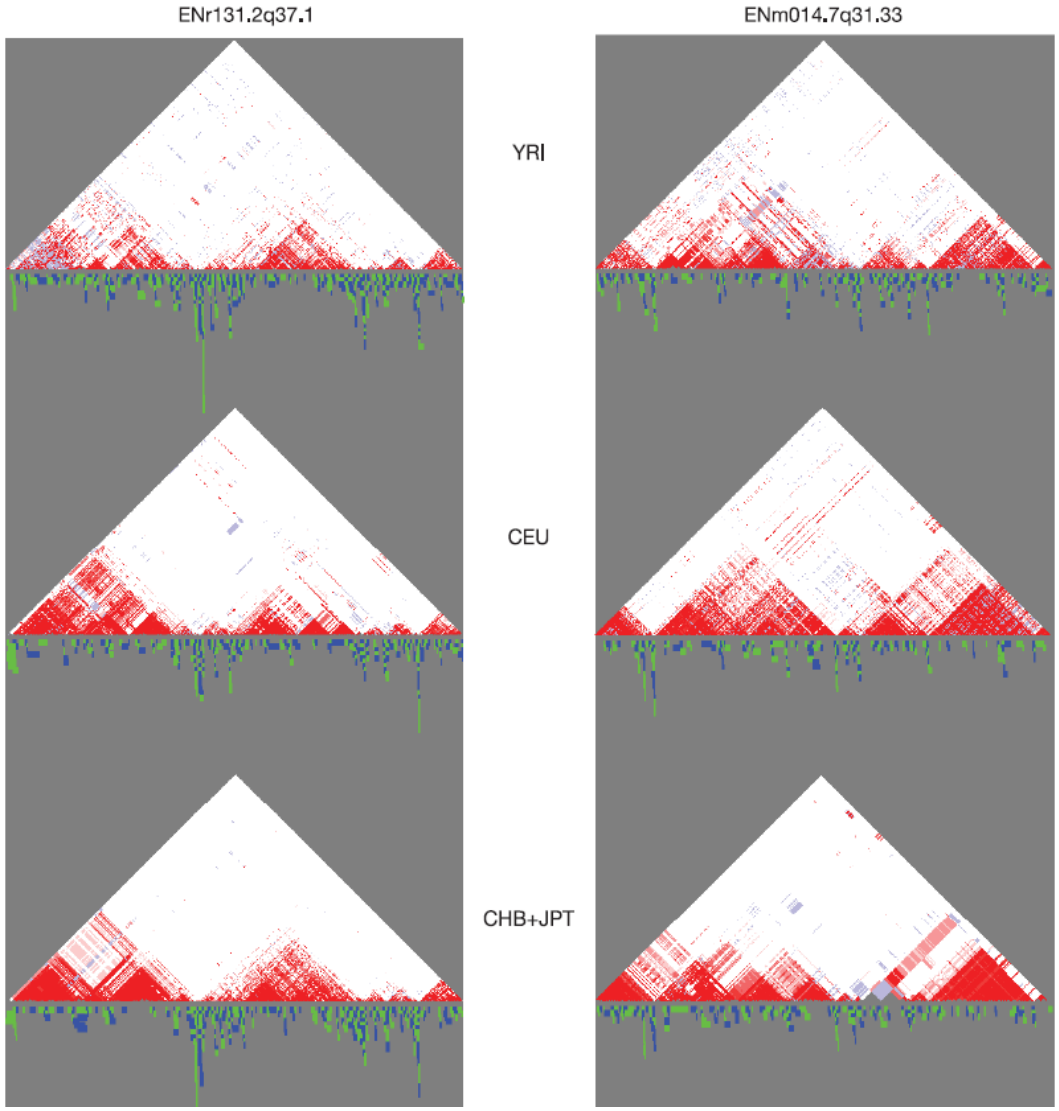
Haplotyp-Blöcke



Olsson et al. Arthritis Res & Ther 9:r98 2007

HapMap Projekt

Haplotyp-Blöcke



Africans

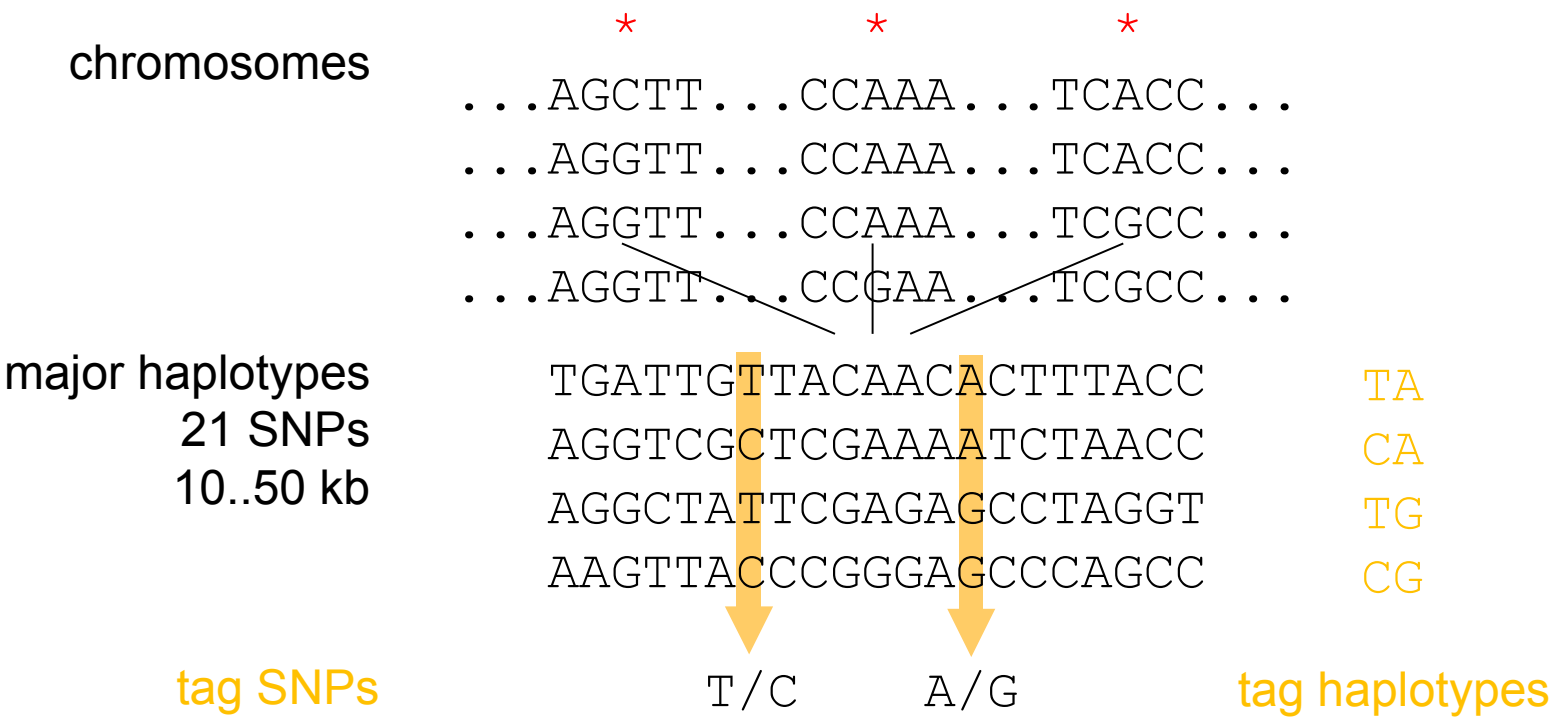
Europeans

Asians

TIHC. Nature 437:1299 2005

Haplotype

= set/region physically linked polymorphism



HapMap Projekt

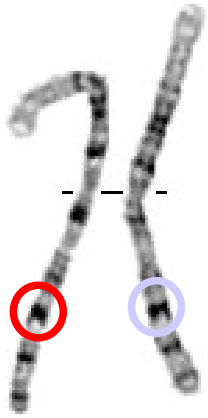
Schlussfolgerungen

- HapMap is estimated to capture the 65-75% untyped common SNPs with a likelihood of 90-96% depending on population.
- Current generation of commercial genome-wide genotyping products captures 3.1 million HapMap SNPs with 80% in African and up to 95% in non-African populations.

TIHC. Nature 449:851 2007

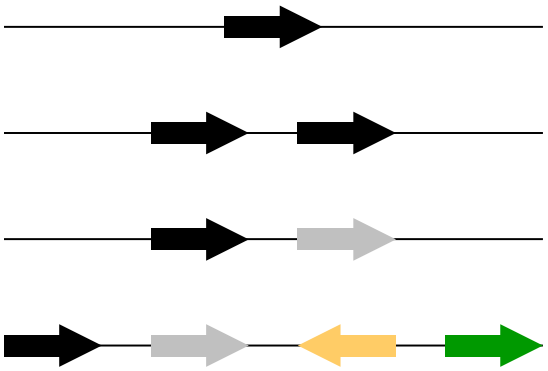
Genetische Variabilität

Strukturelle Variationen



Chromosom A

Chromosom B

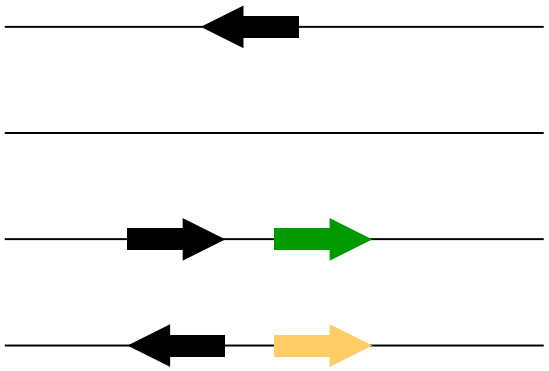


Inversion

InDel

Allel-Variation

Kombination



Segmentale Duplikation

Definition

genomic regions >**1kb**

with nt identity >**90%**

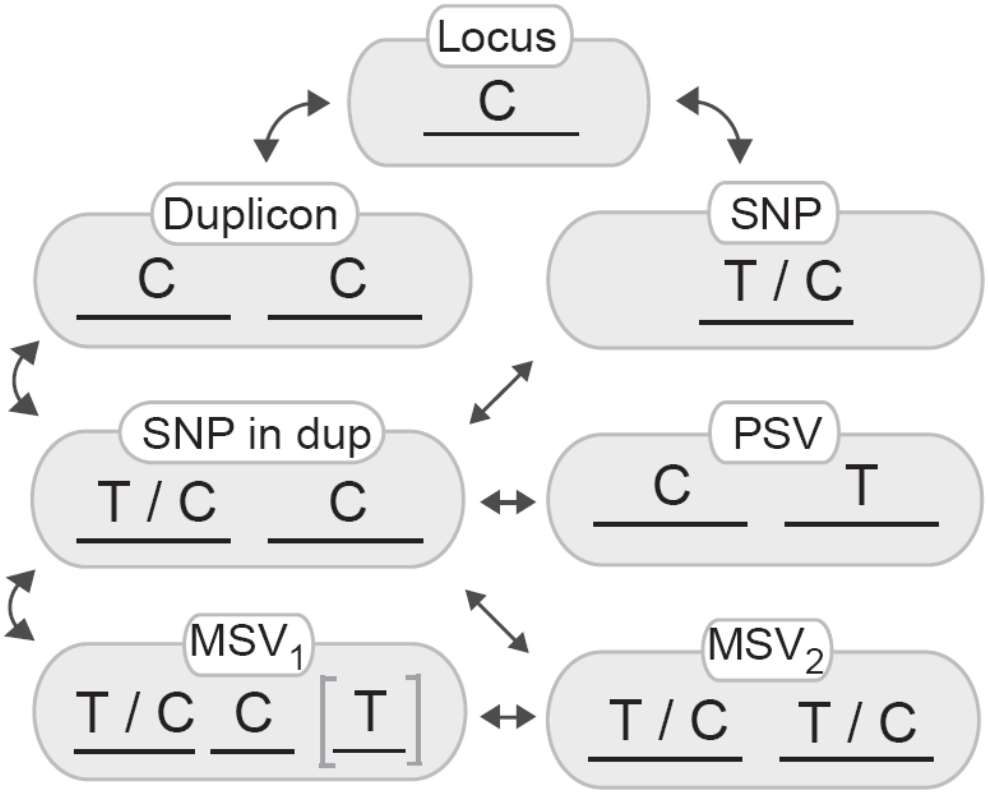
Human genome

5.3% segmentally duplicated

87% of all segmental duplications >**50 kb**

Segmentale Duplikation

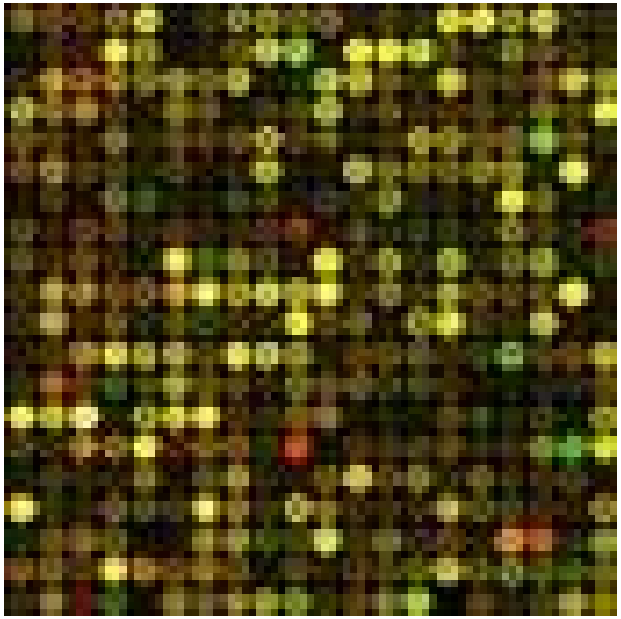
SNVs

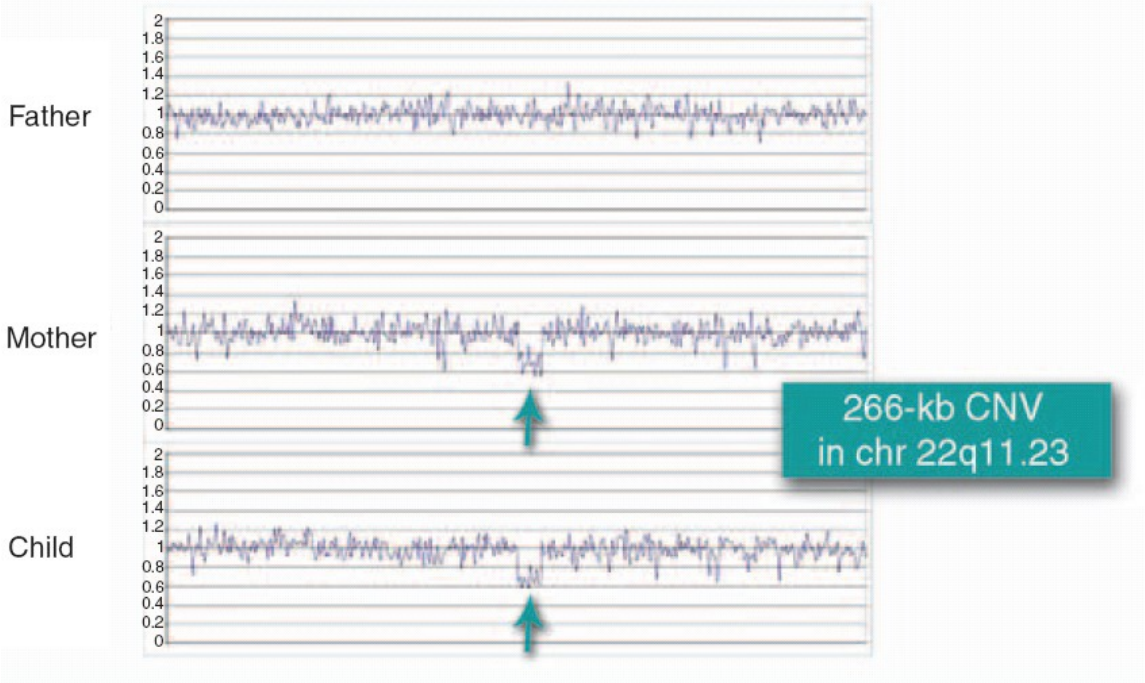


Fredman et al., Nat Genet 36, 861 (2004)

Copy number variation (CNV)

Detection by DNA microarrays



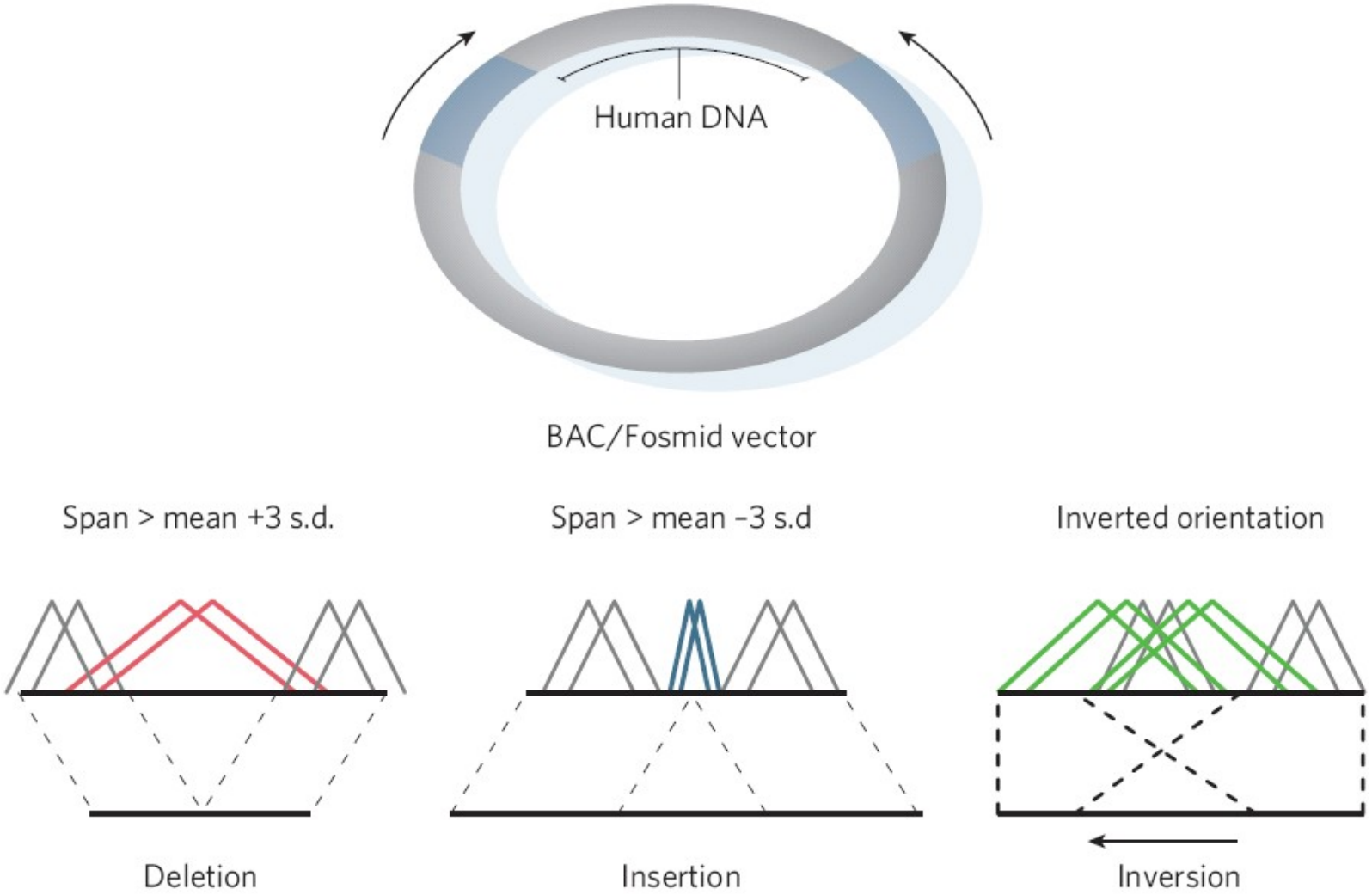


- **0.5-2 Mio data points**
- **comparative hybridization vs. a reference**

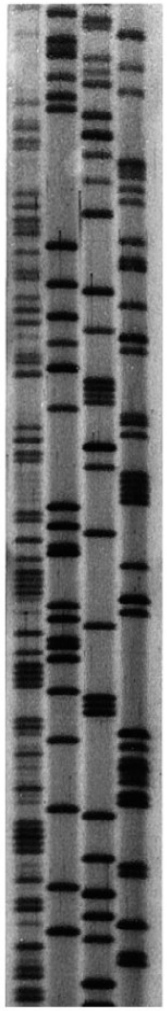
Lee et al., Nat Genet Suppl 39:s48 (2007)

Strukturelle Variationen

Kartierung



Eichler et al., Nature 447:161 (2007)



DNA-Analytik

epigenetische Modifikationen

METHYL MAGIC

Maximum Health Through Methylation

METHYLATION

Prevents Heart Disease and Stroke
Boosts Brain Power
Cures Depression
Treats Arthritis and Many Other Diseases
Prevents Cancer
Slows Down Aging

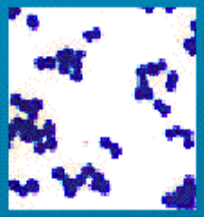
Craig Cooney, Ph.D.
with Bill Lawren
Foreword by Kilmer S. McCully, M.D.

Kenntnisstand

- in den meisten Organismen sind ~5% aller Cytosine in Position 5 des Pyrimidinringes methyliert
- Cytosinmethylierung ist kodierungsneutral und dient als Markierung der DNA
- diese Markierung der DNA erfüllt in unterschiedlichen Organismengruppen unterschiedliche Aufgaben
- in Säugern führt die *in-vitro* Methylierung von Promotorbereichen zur transkriptionellen Inaktivierung des betreffenden Gens *in-vivo*
- die mit aktiven Genen assoziierten *CpG islands* sind unmethyliert
- der 5-Methylcytosingehalt von DNA ist gewebespezifisch

Kenntnisstand

Methylierung in unterschiedlichen Organismen



Bakterien

- Restriktions-/Modifikationssystem
- Reparatursystem
- Markierung des Replikationsorigins

Pilze

- Erkennung von Duplikationen
- *host-defence-mechanism (?)*

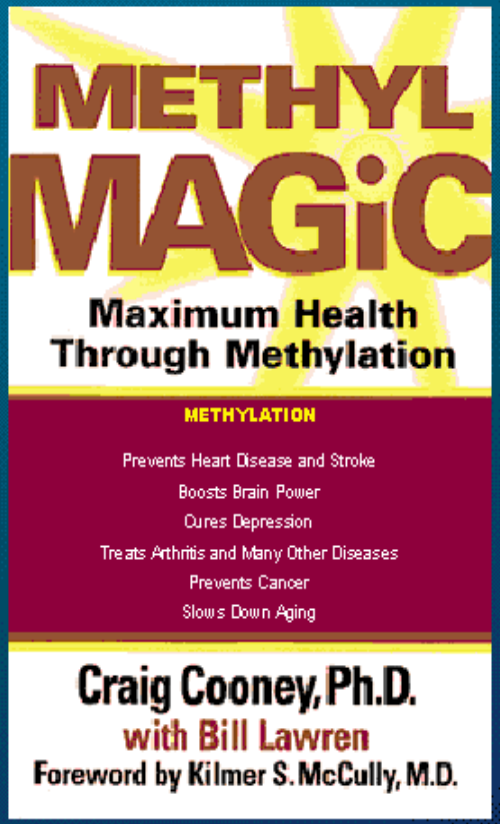
Pflanzen

- *host-defence-mechanism (?)*
- Geninaktivierung
- Hemmung des Elongationsschrittes der Transkription

Tiere

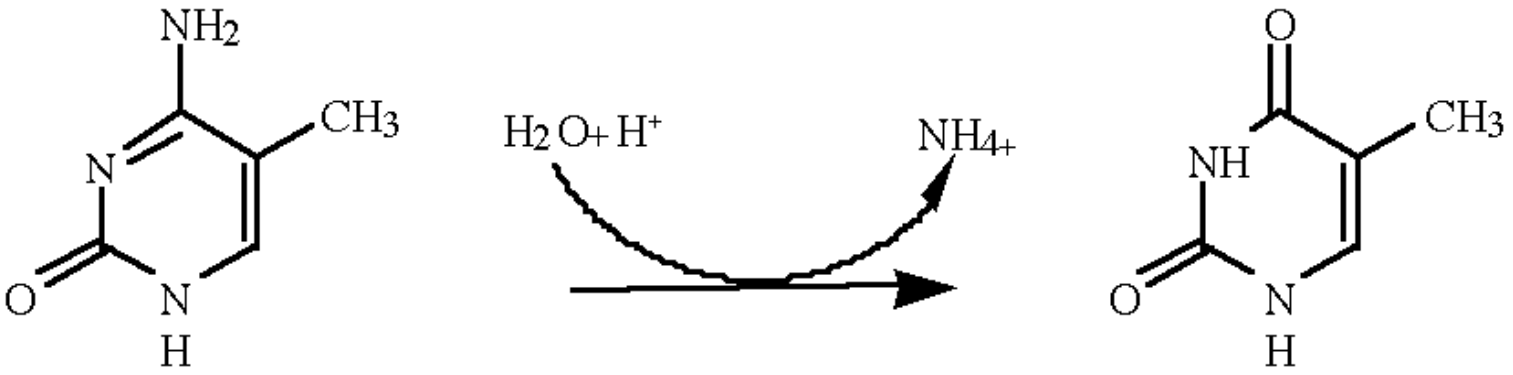
- *host-defence-mechanism (?)*
- Geninaktivierung, Heterochromatisierung
- Hemmung des Initiationsionsschrittes der Transkription

Mensch:



Methylierung

spontane Desaminierung



5-Methyl-cytosin

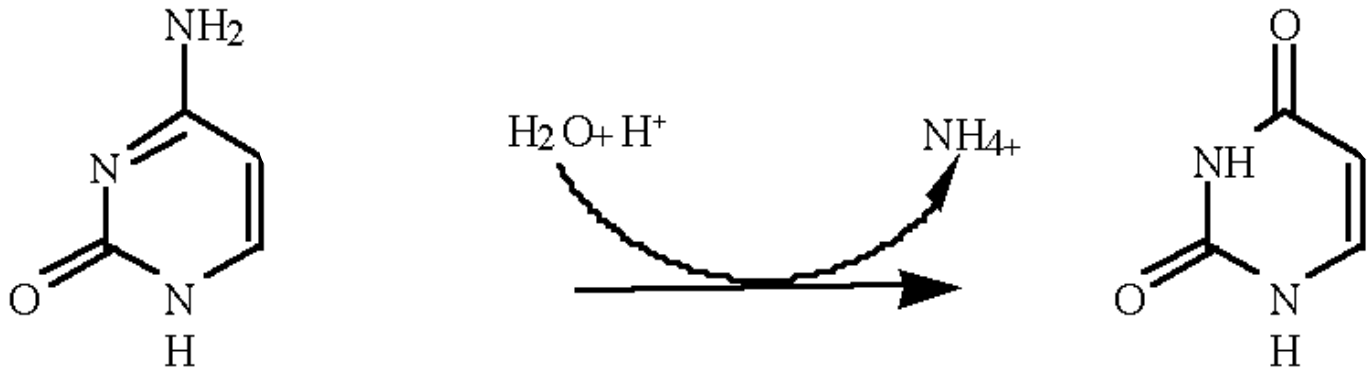
Thymin

Was sind *CpG islands*?

- CpG = Cytosin, in 5‘-Richtung gefolgt von einem Guanin
- CpG-Paare sind im Genom der Eukaryoten etwa 5fach unterrepräsentiert.
- Bereiche in denen CpG-Paare mit der statistisch zu erwartenden Häufigkeit auftreten, werden *CpG islands* genannt.
- C+G-Dichte und CpG/GpC Verhältnis zur *in-silico* Identifizierung genutzt (observed/expected > 0,8; G+C > 60%)
- 5‘-Bereiche von 60% aller Gene sind mit *CpG islands* assoziiert.
- In Vertebraten sind nur Cytosine in CpG-Paaren methyliert.
- *CpG islands* sind i.d.R. unmethyliert.

Bisulfit Sequenzierung

chemische Desaminierung

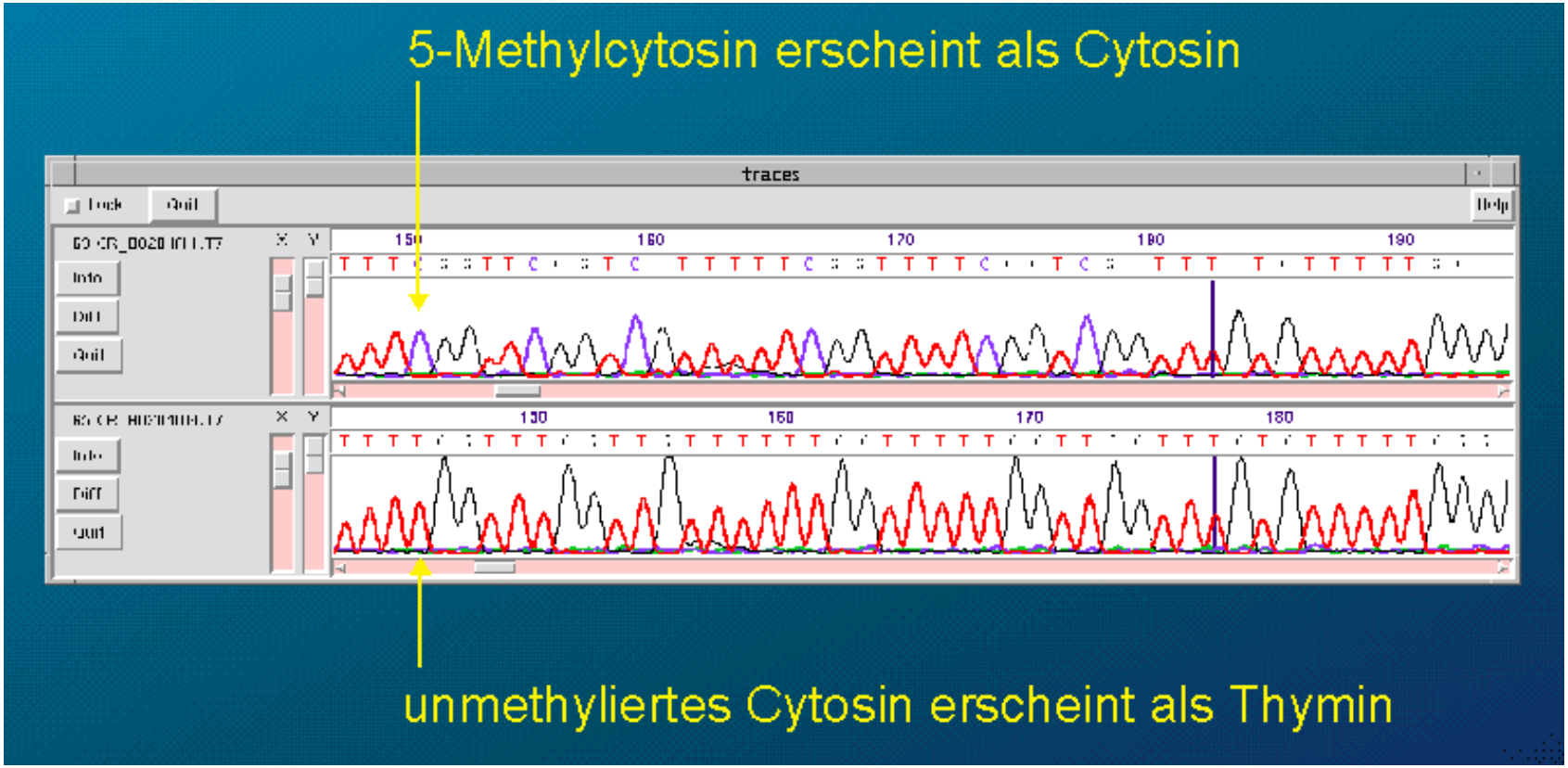


Cytosin

Uracil

Bisulfit Sequenzierung

Nachweis mit DyeTerminatoren



Bisulfit Sequenzierung

Genomweite Analyse

Vol 462|19 November 2009|doi:10.1038/nature08514 nature

ARTICLES

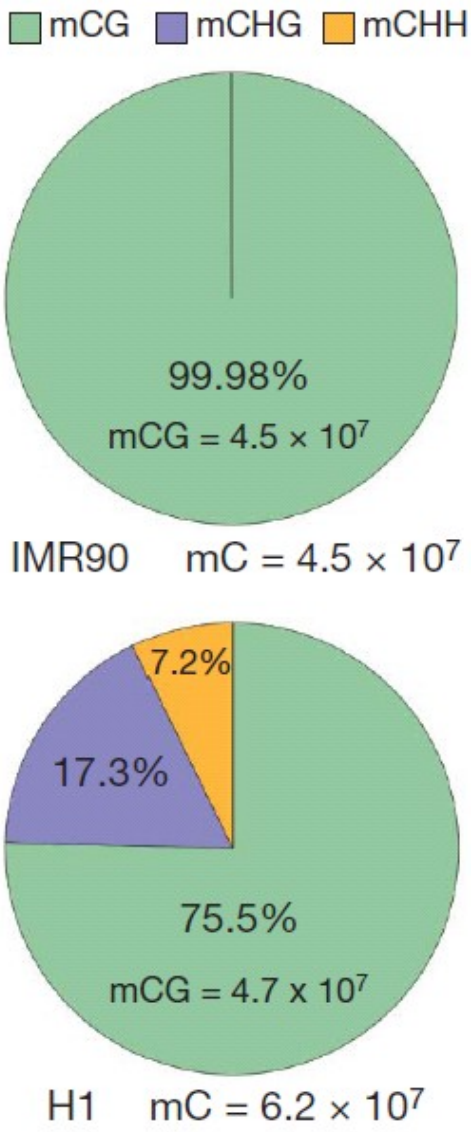
Human DNA methylomes at base resolution show widespread epigenomic differences

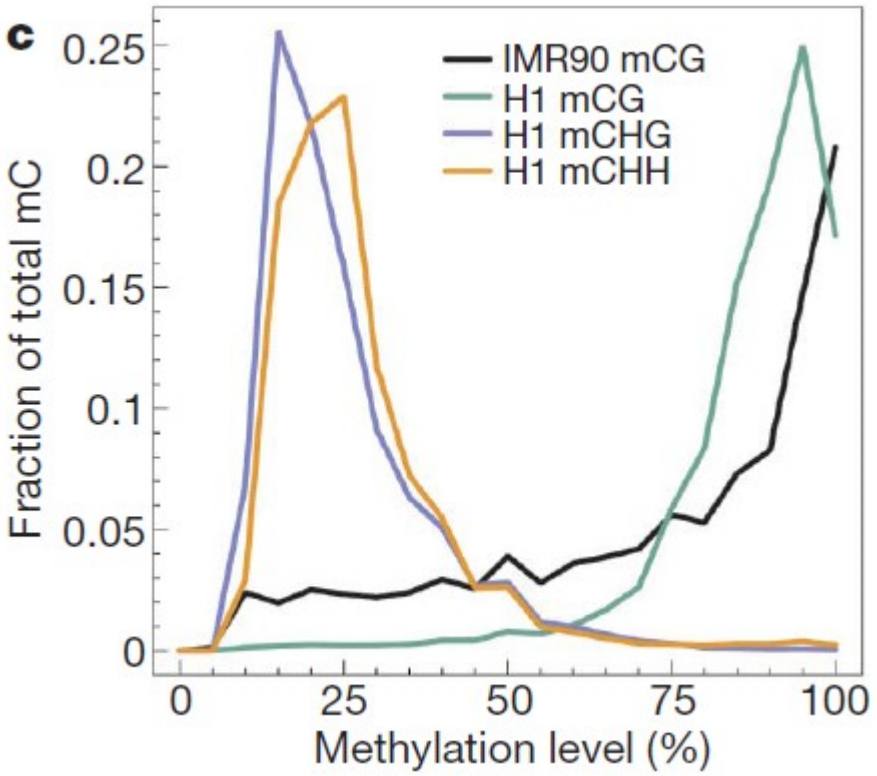
Ryan Lister[1]*, Mattia Pelizzola[1]*, Robert H. Dowen[1], R. David Hawkins[2], Gary Hon[2], Julian Tonti-Filippini[4], Joseph R. Nery[1], Leonard Lee[2], Zhen Ye[2], Que-Minh Ngo[2], Lee Edsall[2], Jessica Antosiewicz-Bourget[5,6], Ron Stewart[5,6], Victor Ruotti[5,6], A. Harvey Millar[4], James A. Thomson[5,6,7,8], Bing Ren[2,3] & Joseph R. Ecker[1]

DNA cytosine methylation is a central epigenetic modification that has essential roles in cellular processes including genome regulation, development and disease. Here we present the first genome-wide, single-base-resolution maps of methylated cytosines in a mammalian genome, from both human embryonic stem cells and fetal fibroblasts, along with comparative analysis of messenger RNA and small RNA components of the transcriptome, several histone modifications, and sites of DNA–protein interaction for several key regulatory factors. Widespread differences were identified in the composition and patterning of cytosine methylation between the two genomes. Nearly one-quarter of all methylation identified in embryonic stem cells was in a non-CG context, suggesting that embryonic stem cells may use different methylation mechanisms to affect gene regulation. Methylation in non-CG contexts showed enrichment in gene bodies and depletion in protein binding sites and enhancers. Non-CG methylation disappeared upon induced differentiation of the embryonic stem cells, and was restored in induced pluripotent stem cells. We identified hundreds of differentially methylated regions proximal to genes involved in pluripotency and differentiation, and widespread reduced methylation levels in fibroblasts associated with lower transcriptional activity. These reference epigenomes provide a foundation for future studies exploring this key epigenetic modification in human disease and development.

DNA Methylierung

Fibroblasten *vs.* ES

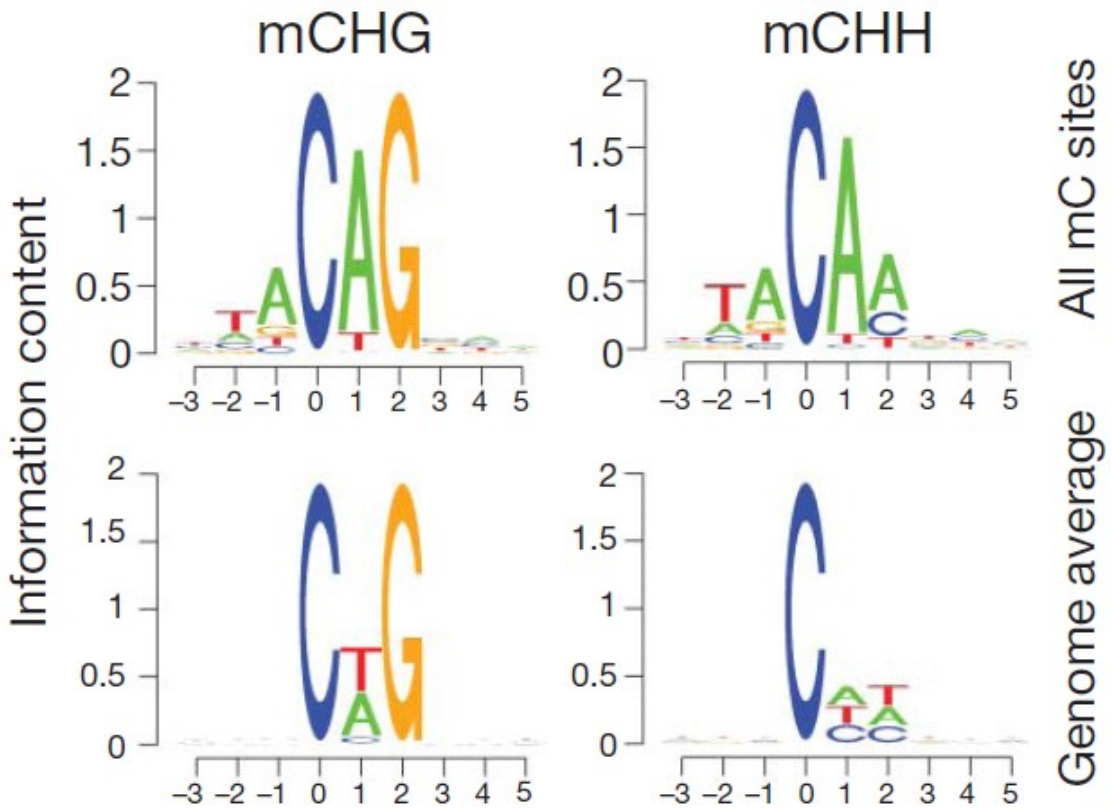




Lister et al., Nature 462:315 (2009)

DNA Methylierung

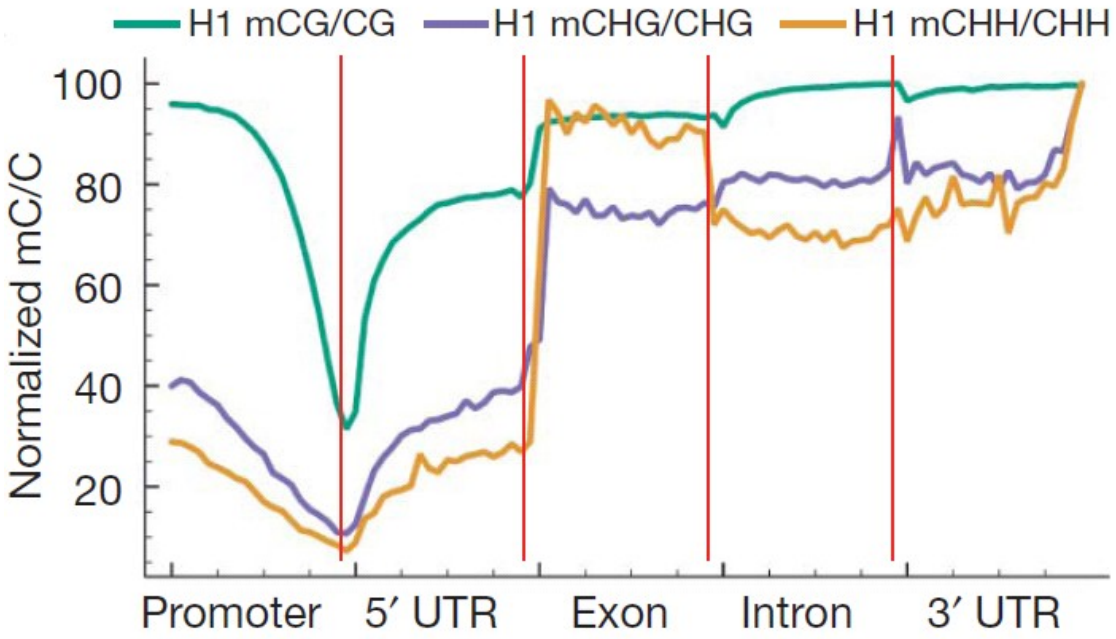
ES



Lister et al., Nature 462:315 (2009)

DNA Methylierung

ES

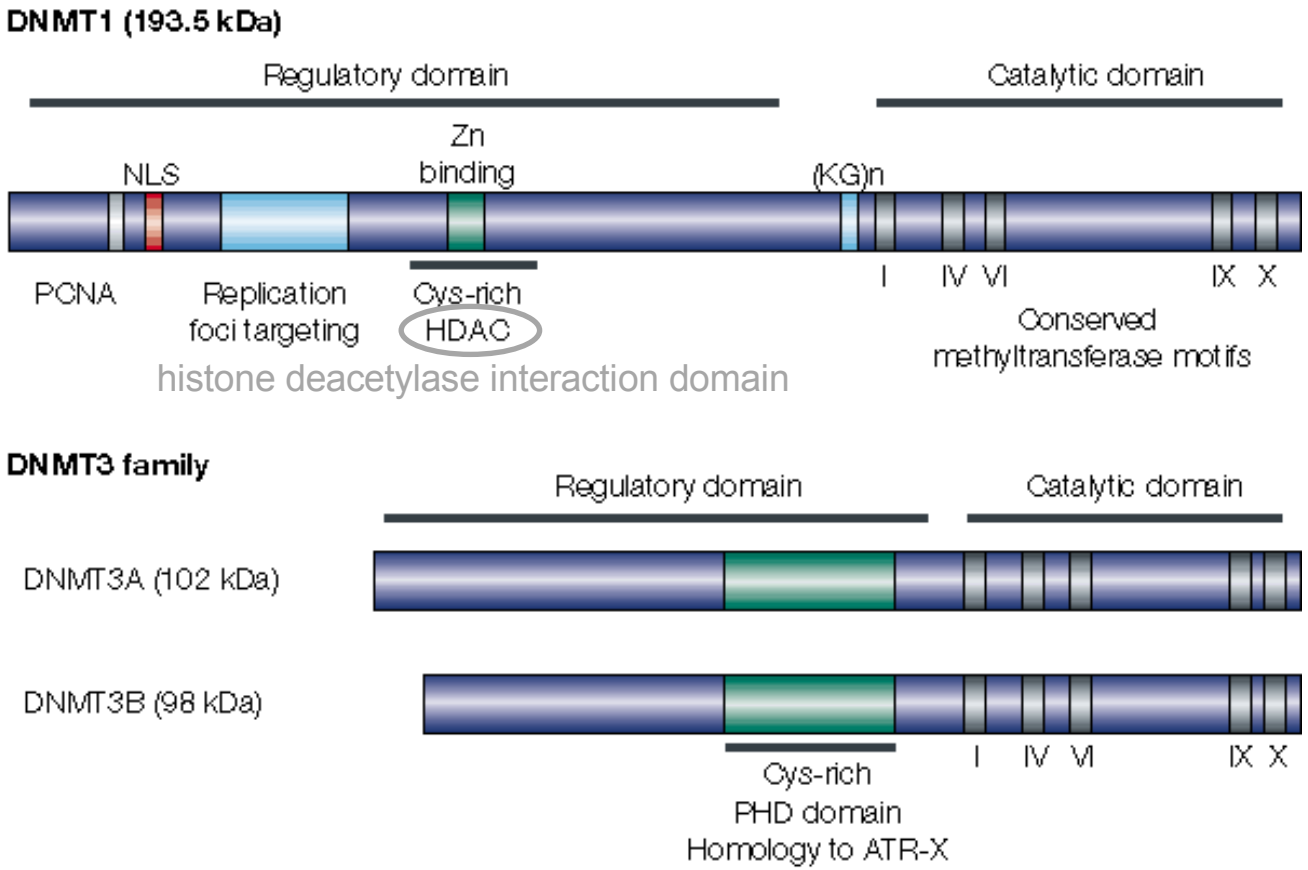


Lister et al., Nature 462:315 (2009)

DNA Methylierung

DNA-Methyl-Transferase

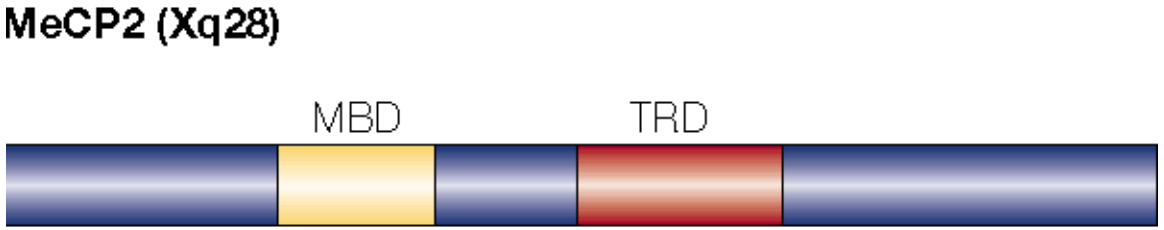
Aufrecht-
erhaltung



de novo

DNA Methylierung

Methyl-C bindende Proteine



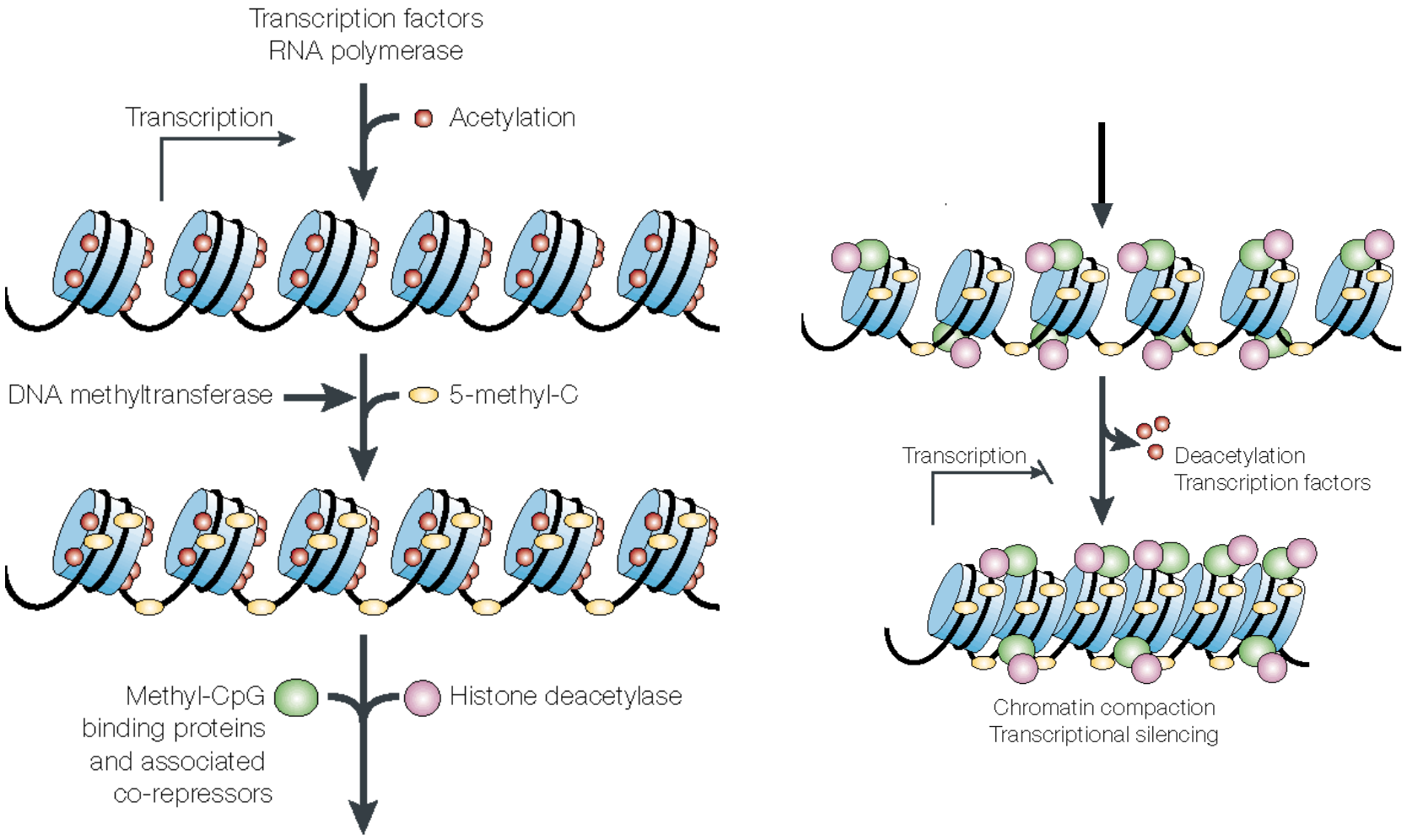
Methyl-CpG Binding Domain

Transcriptional Repression Domain

Nature Reviews | Genetics

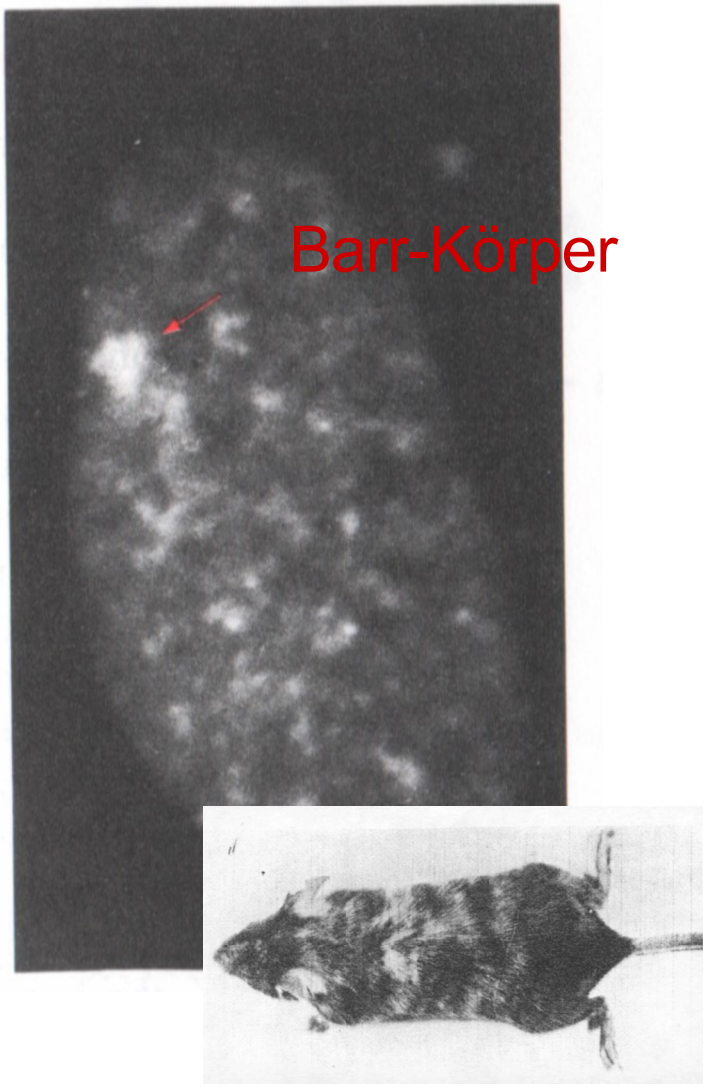
DNA Methylierung

Geninaktivierung & Heterochromatisierung



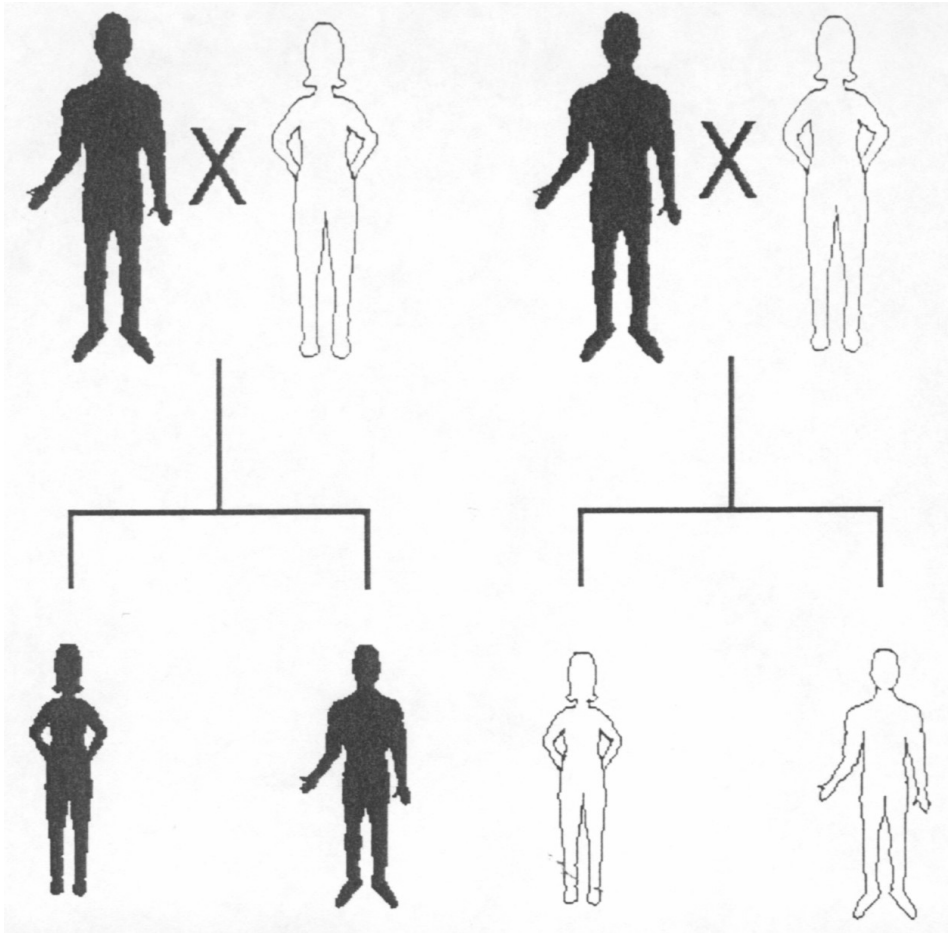
DNA Methylierung

X-Inaktivierung



DNA Methylierung

Imprinting / Prägung



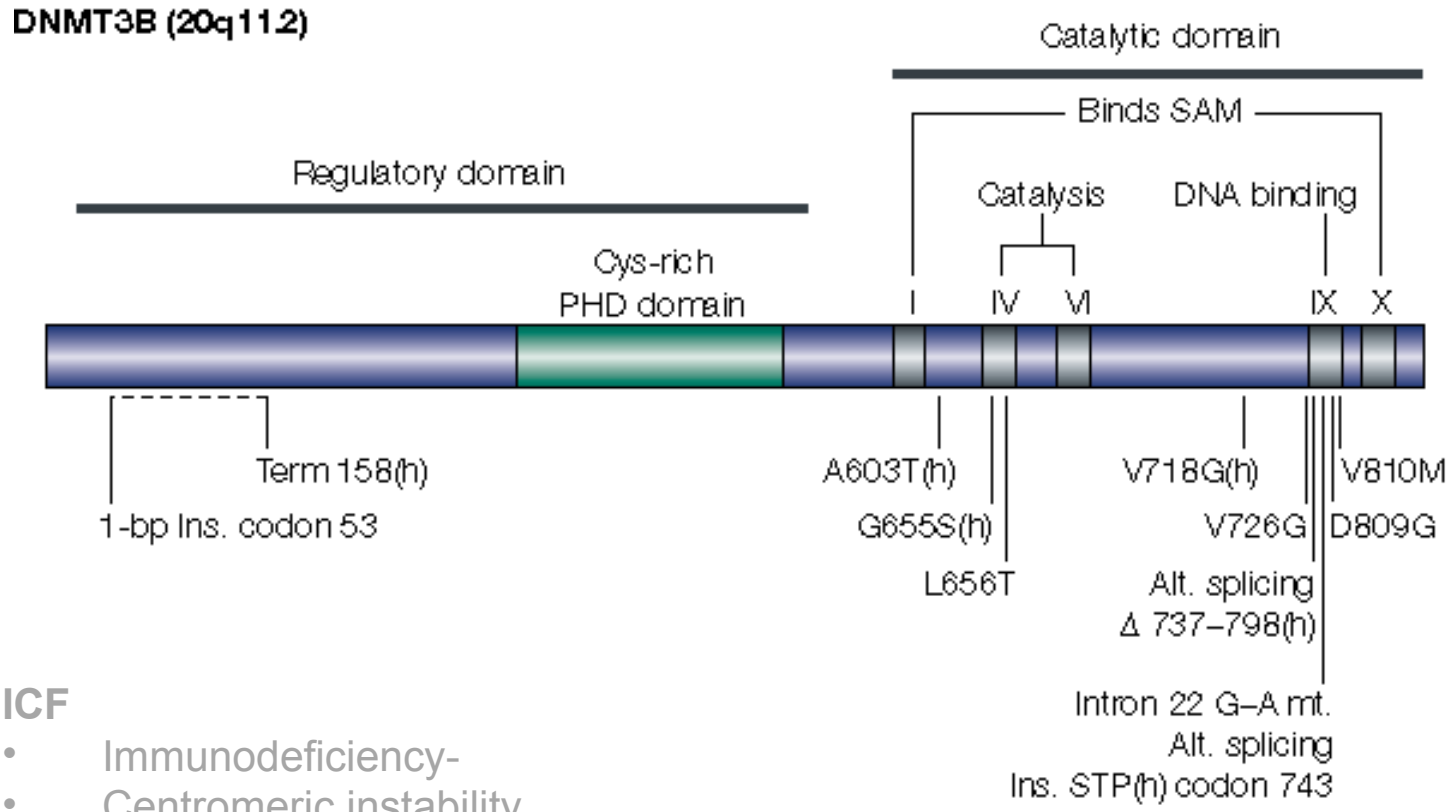
mütterliche
maternales

väterliche
paternales

Gen unterliegt der Prägung / Inaktivierung
Imprinting

DNA Methylierung & Krankheit

ICF-Syndrom

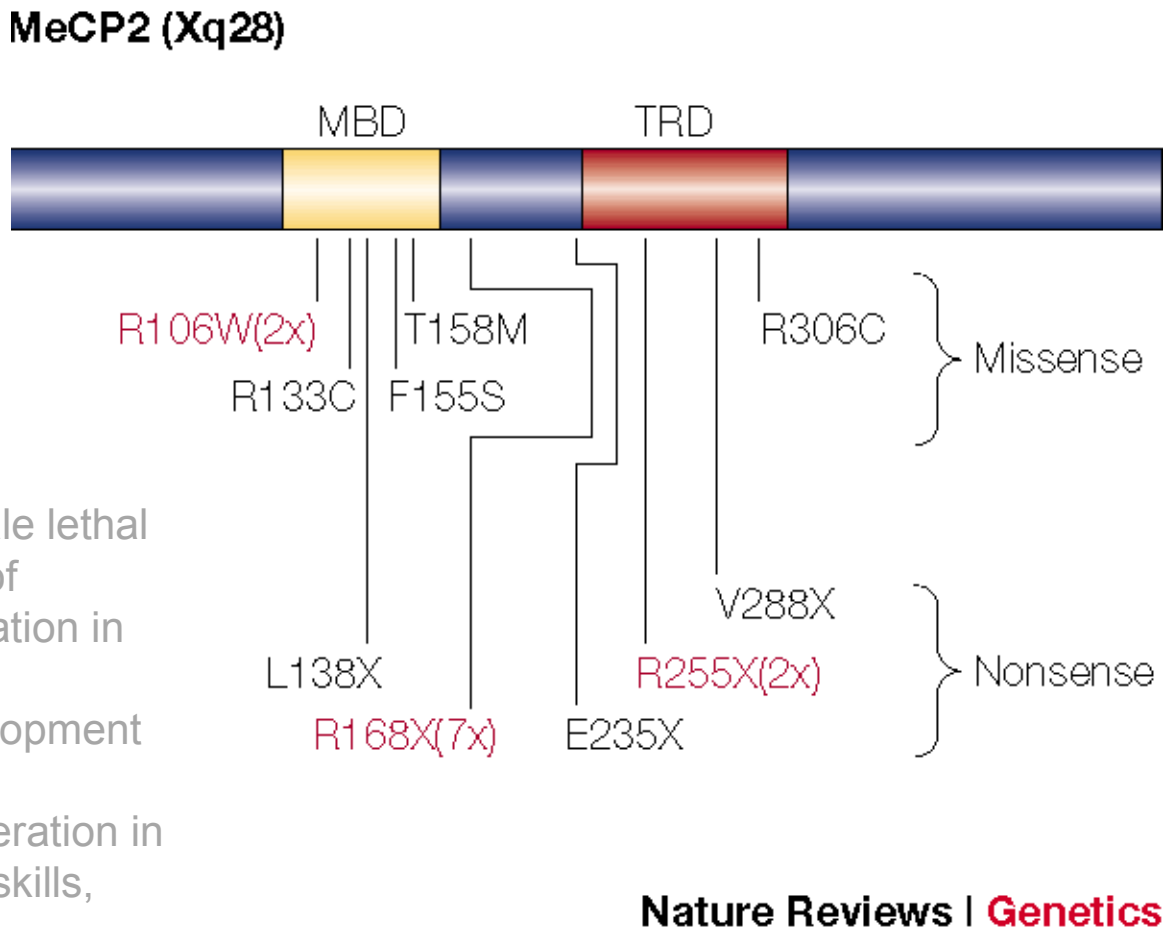


ICF

- Immunodeficiency-
- Centromeric instability
- Facial anomalies

DNA Methylierung & Krankheit

Rett-Syndrom

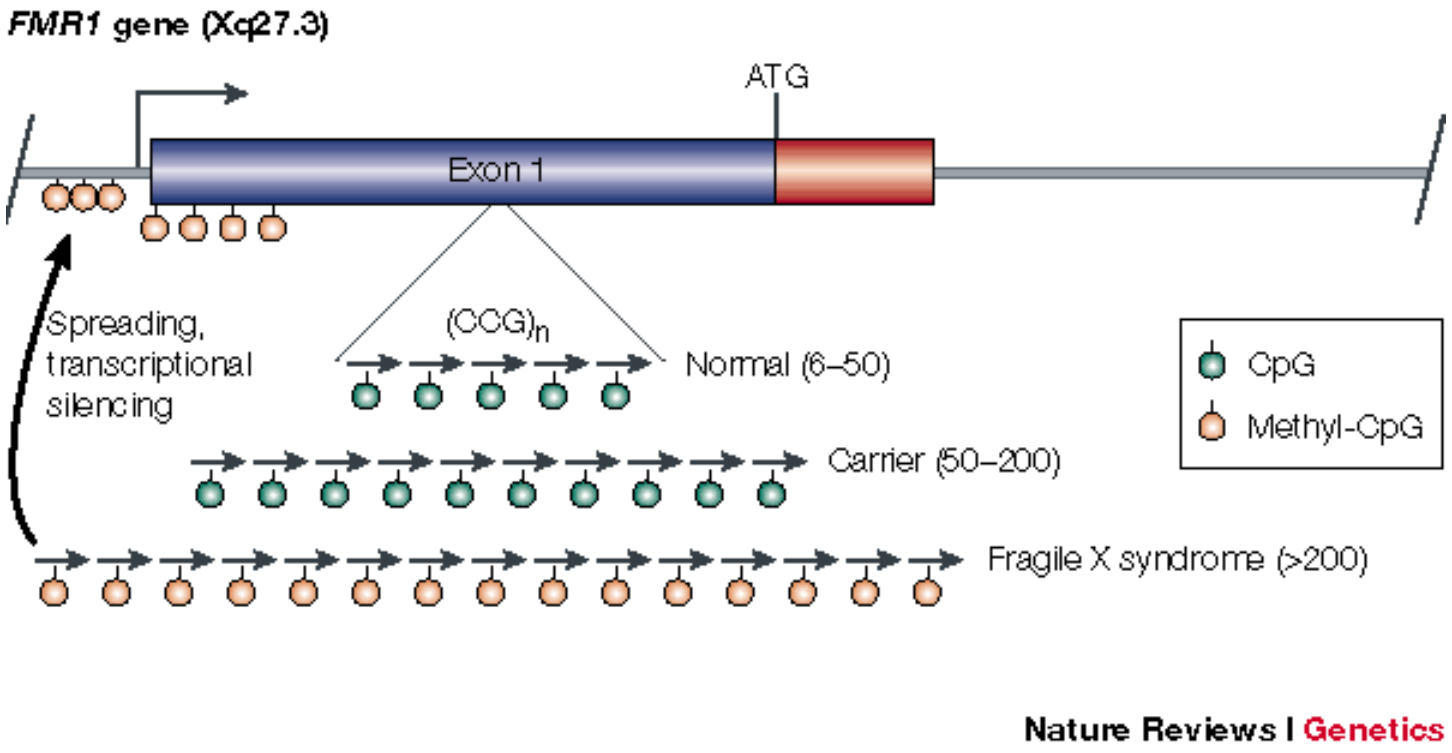


RETT

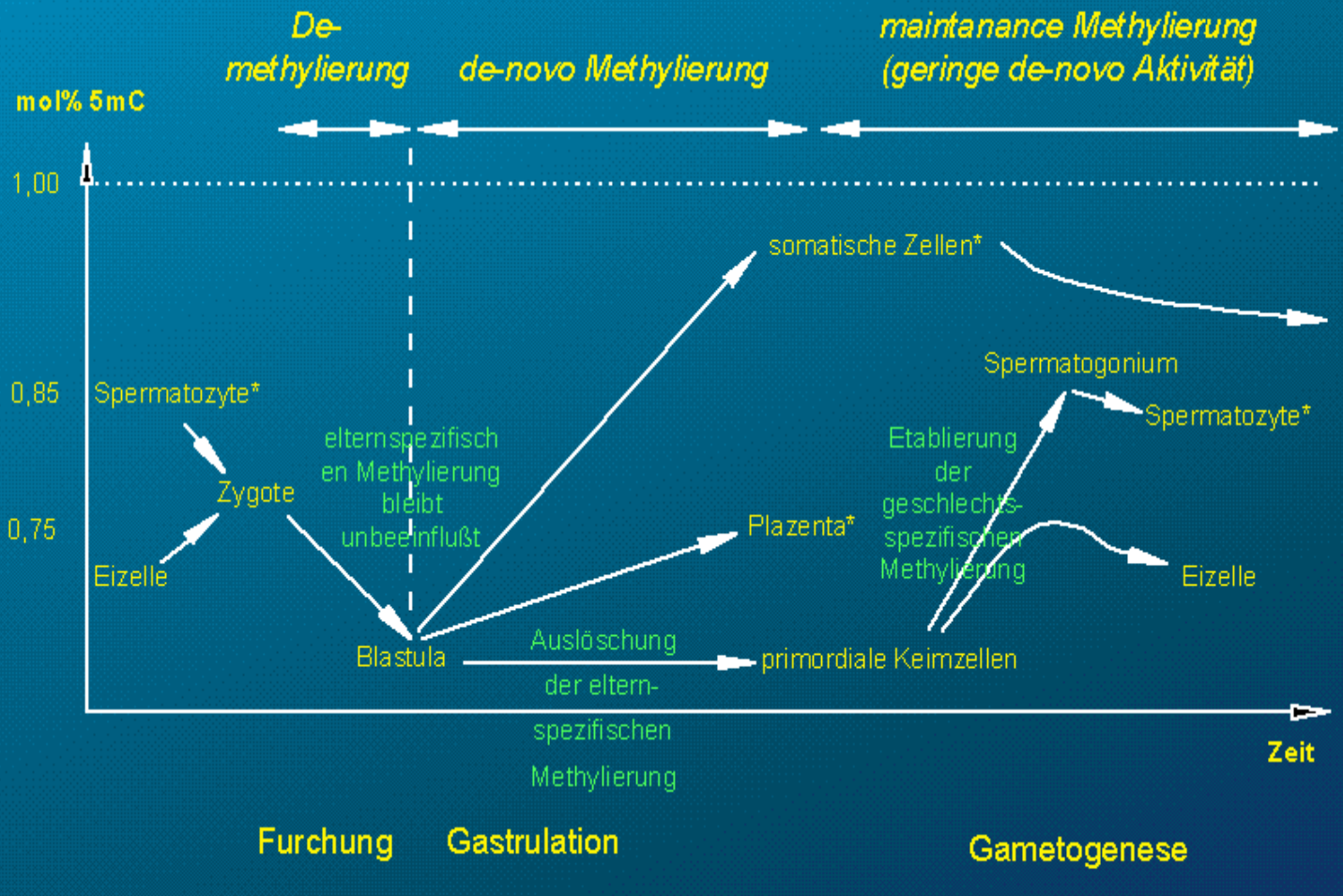
- X-linked dominant, male lethal
- most common cause of sporadic mental retardation in females
- period of normal development followed by
  - progressive degeneration in speech and motor skills,
  - seizures, autism,
  - stereotypical, repetitive hand movements

DNA Methylierung & Krankheit

Fragiles X-Syndrom

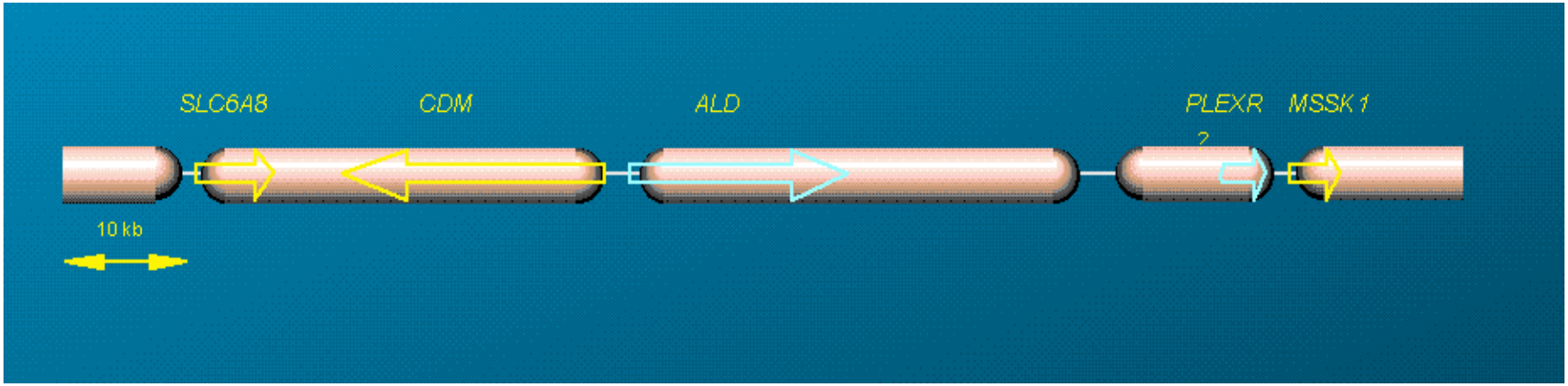


5mC-Gehalt während der frühen Embryogenese

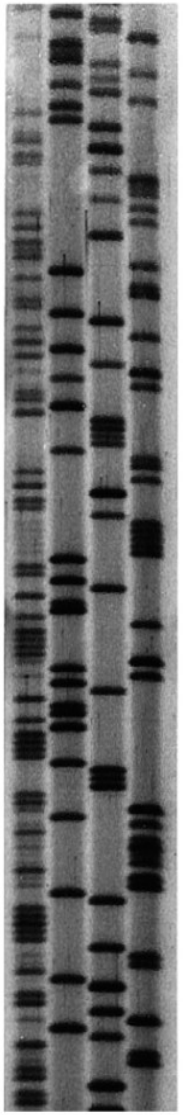


DNA Methylierung

Initiation der Transkription



Mittel zur Reduzierung genomischer Komplexität ?



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