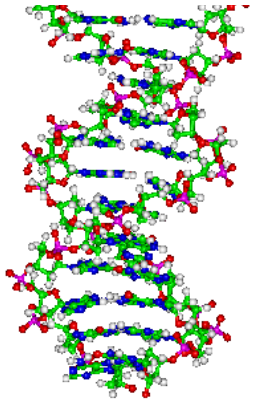

(In)Stabilität von DNA

- grundsätzliche Erwägungen zur Biosicherheit

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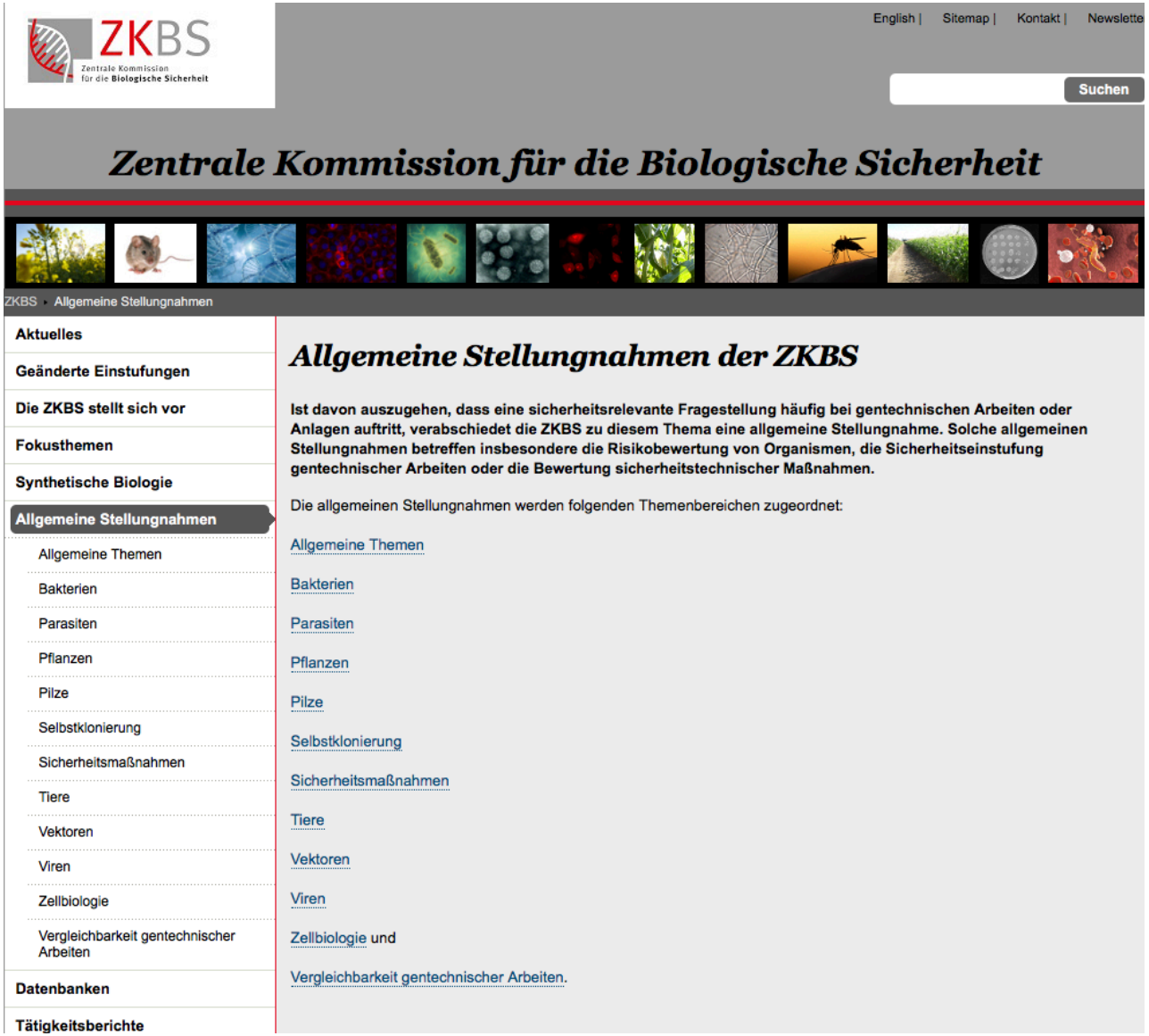
JG|U
JOHANNES GUTENBERG UNIVERSITÄT MAINZ

GenTG und GenTSV

Sicher-heitsstufe	Risikoeinschätzung nach dem Stand der Wissenschaft	Organismus dieser Risikogruppe (Beispiele)
S1	Es ist nicht von einem Risiko für die menschliche Gesundheit und die Umwelt auszugehen	Lactobacillus bulgaris (Joghurt) E. coli K12 (Labor-Sicherheitsstamm)
S2	Es ist von einem geringen Risiko für die menschliche Gesundheit oder die Umwelt auszugehen	Streptococcus mutans (Karies) Herpes Simplex Viren Salmonella Enteritidis
S3	Es ist von einem mäßigen Risiko für die menschliche Gesundheit oder die Umwelt auszugehen	HIV (AIDS) Bacillus anthracis (Milzbrand)
S4	Es ist von einem hohen Risiko oder dem begründeten Verdacht eines solchen Risikos für die menschliche Gesundheit oder die Umwelt auszugehen	Ebola Virus (Hämolyse) Marburg Virus

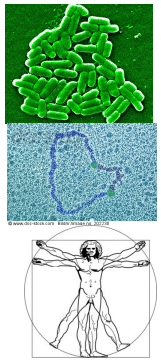
Einschätzung der Sicherheitsstufe



- (In)Stabilität von DNA in Zellen
- Horizontaler Gentransfer
- DNA-Stabilität in der Umwelt

- Nachweis und Identifikation von GVOs

Stabilität von DNA in Zellen



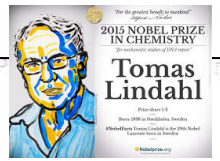
Wirtsgenom
Vektor-DNA
Spender-DNA

→ Stabilität des GVO

Die Stabilität wird v.a. beeinflusst durch

- Mutation
- Rekombination
- Replikation
- Transposition

Mutationsspektrum und -Häufigkeit in menschlichen Zellen



aus: TIG 14(3), 1999

TABLE 1. Endogenous DNA lesions in human cells

Lesion	Mode of formation	Number of residues generated daily per human genome	Genome steady state level in normal, repair-proficient cells
Uracil	Cytosine deamination	400	~1
Thymine (opposite guanine)	5-Methylcytosine deamination	30	10–20
Hypoxanthine	Adenine deamination	10	~1
8-Oxoguanine	Guanine oxidation	~1000	~1
faPy	Guanine oxidation	~200	~5
Thymine glycol and similar oxidized pyrimidines	Pyrimidine oxidation	~500	~5
Etheno C	Lipid peroxidation of cytosine	~200	~5
Etheno A	Lipid peroxidation of adenine	~200	~5
3-Methyladenine	SAM methylation of adenine	600	~5
7-Methylguanine	SAM methylation of guanine	4000	3000
O^5-Methylguanine	Genomic alkylation by endogenous nitrosamines	~200	~1
Abasic site	Hydrolytic depurination	9000	~5

This table was prepared and presented in a talk by Tomas Lindahl (ICRF, UK) one of the organizers of the meeting, who kindly provided it for this report. It is not intended to be an exhaustive list of all the types of damage discussed. The values for hydrolytic and alkylation damage are based on the measured rates of generation and of repair, while the values for oxidative damage are based on measured rates of repair and on approximate rates of generation of lesions estimated from data with microbial mutants.

Häufigkeit von spontanen Punkt-Mutationen

- Rate von Basensubstitutionen auf menschlichem Y-Chromosom in „Echtzeit“ bestimmt
- Individuen 13 Generationen entfernt
- Illumina-Sequenzierung fluoreszenzsortierter Y-DNA von zwei Männern (maximal zeitlich getrennt)

➢**3 x 10^{-8} Mutationen / Nt / Generation** Xue et al. 2009, Curr Biol

Etwa 60 Neumutationen in jedem Neugeborenen

letters to nature

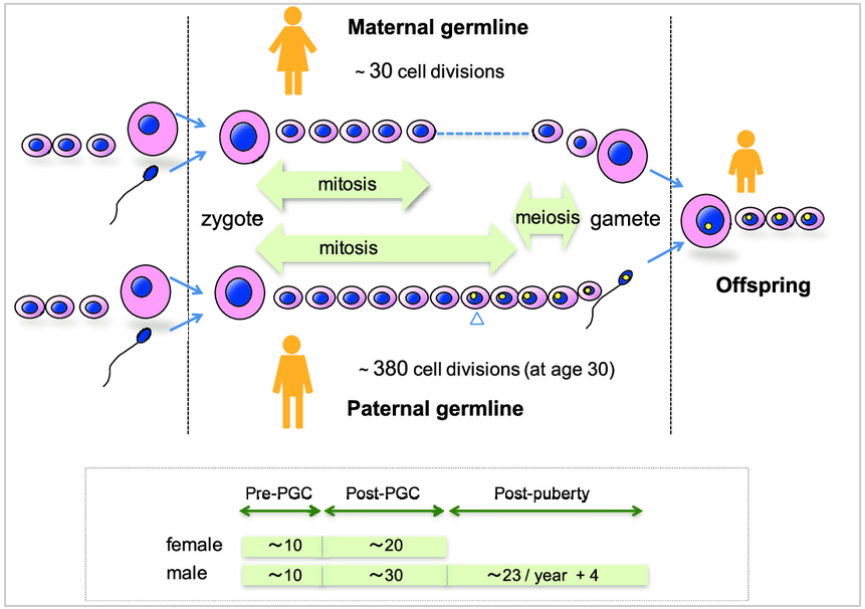
Strong male-driven evolution of DNA sequences in humans and apes

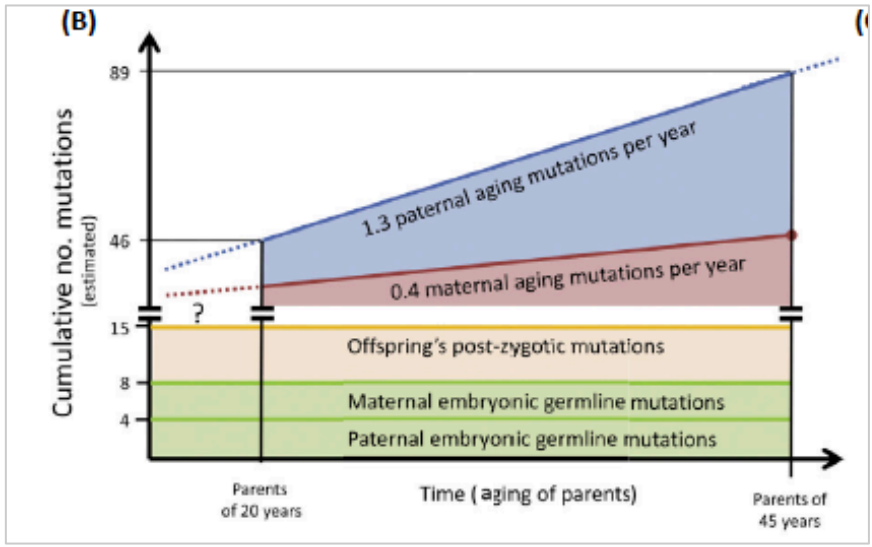
Kateryna D. Makova & Wen-Hsiung Li

Department of Ecology and Evolution, University of Chicago, 1101 East 57th Street, Chicago, Illinois 60637, USA

Male-to-female ratio of mutation rate in primates is 4-6.

Replication errors are dominant.





Goldman et al. (2019) TIG

Female age effect?
Other causes?

https://www.researchgate.net/figure/Human-germ-cell-lineage-De-novo-germline-mutations-occur-when-female-and-male-gametes_fig1_328644153

De novo mutations across 1,465 diverse genomes reveal mutational insights and reductions in the Amish founder population

Michael D. Kessler[a,b,c,d], Douglas P. Loesch[a,b,c], James A. Perry[b,c], Nancy L. Heard-Costa[e,f], Daniel Taliun[g], Brian E. Cade[h,i], Heming Wang[h,i], Michelle Daya[j], John Ziniti[k], Soma Datta[k], Juan C. Celedón[l], Manuel E. Soto-Quiros[m], Lydiana Avila[m], Scott T. Weiss[k,n], Kathleen Barnes[j], Susan S. Redline[h,o,p], Ramachandran S. Vasan[f], Andrew D. Johnson[f,q], Rasika A. Mathias[r,s], Ryan Hernandez[t], James G. Wilson[u], Deborah A. Nickerson[v], Goncalo Abecasis[w], Sharon R. Browning[x], Sebastian Zöllner[y,z], Jeffrey R. O'Connell[b,c], Braxton D. Mitchell[b,c,aa], National Heart, Lung, and Blood Institute Trans-Omics for Precision Medicine (TOPMed) Consortium[1], TOPMed Population Genetics Working Group[2], and Timothy D. O'Connor[a,b,c,d,3]

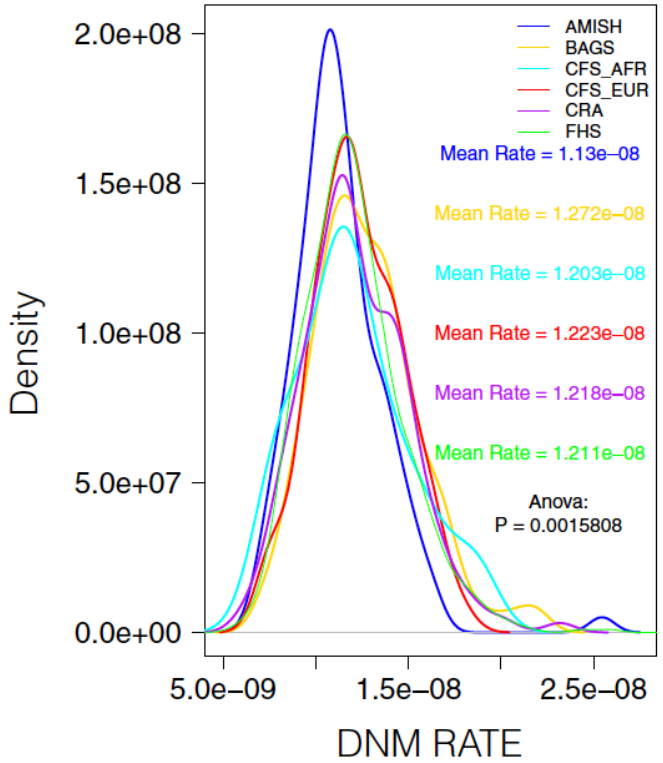


Fig. 3. DNM rates across diverse cohorts. DNM rates per individual show significant differences across cohort, which are driven by a reduction in the Amish.

7% reduced mutation rate in Amish!

> pre-industrial lifestyle?

ARTICLE

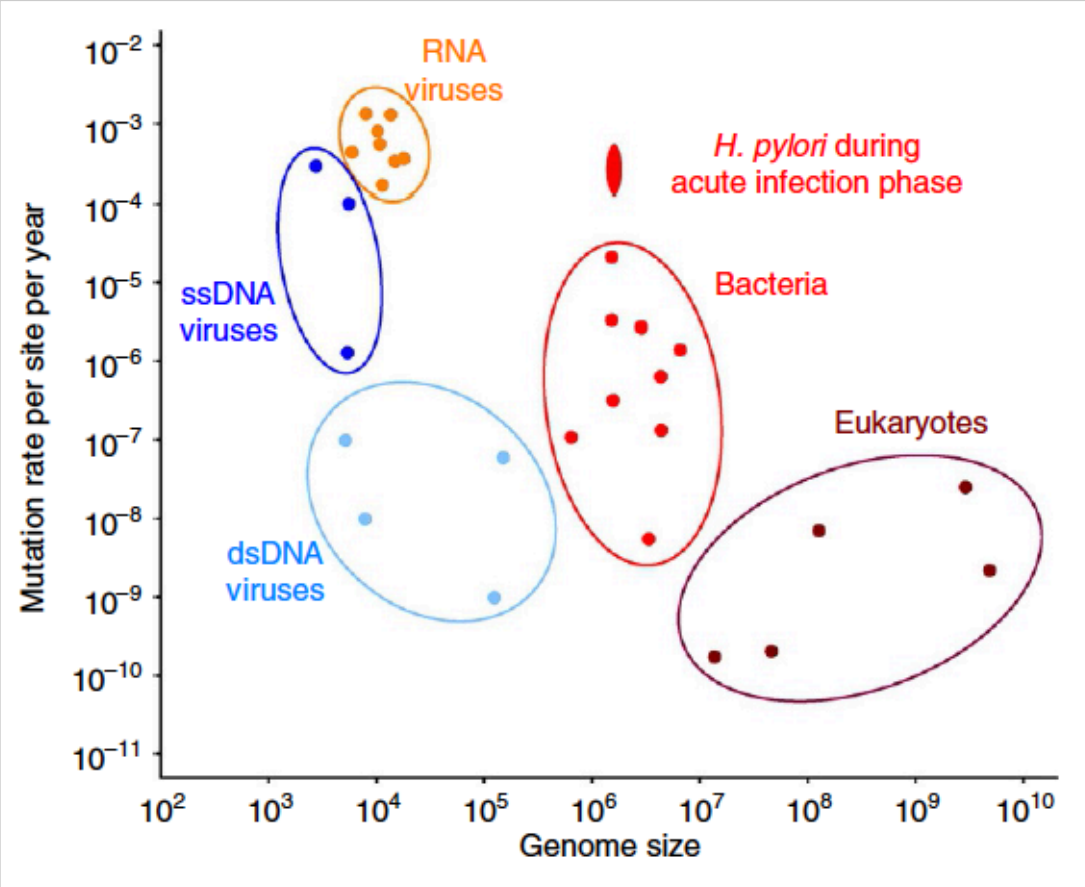
Received 19 Mar 2014 | Accepted 20 May 2014 | Published 13 Jun 2014

DOI: 10.1038/ncomms5165

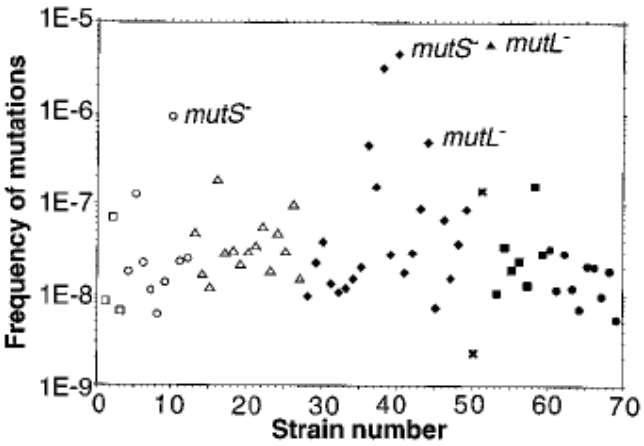
A mutation burst during the acute phase of *Helicobacter pylori* infection in humans and rhesus macaques

Bodo Linz[1,2], Helen M. Windsor[3], John J. McGraw[1,2], Lori M. Hansen[4], John P. Gajewski[1,2], Lynn P. Caylie M. Hake[2], Jay V. Solnick[4,5], Stephan C. Schuster[2,6] & Barry J. Marshall[2,3]

High variability in prokaryotic mutation rates

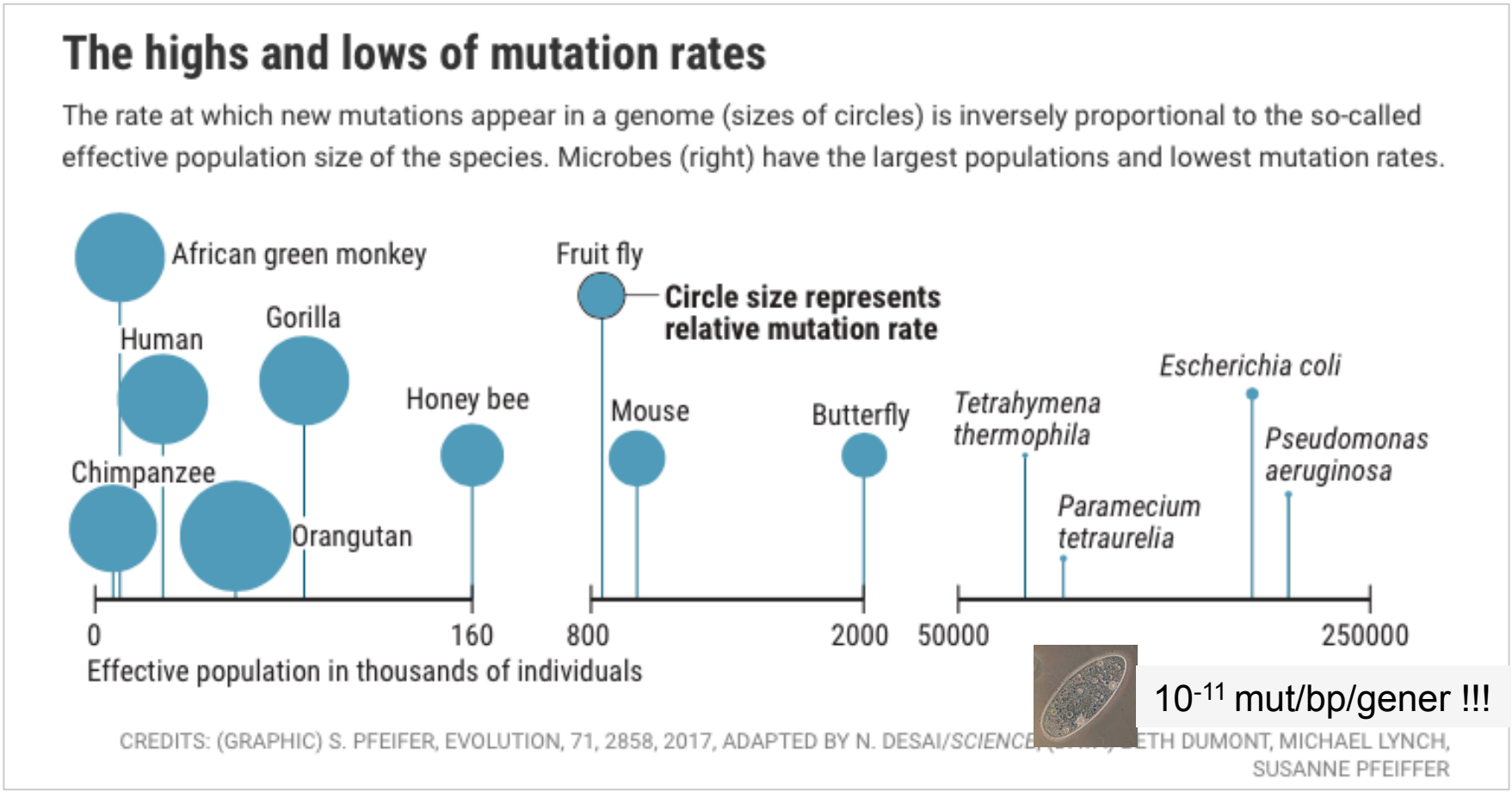


E. coli strains



Matic et al. (1997) Nature

Evolution der Mutationsraten



10^{-11} mut/bp/gener !!!

Small effective pop size > genetic drift > fixation of deleterious allels

BMC Evolutionary Biology 2004

Research article

Open Ac

Moderate mutation rate in the SARS coronavirus genome and its implications

Zhongming Zhao[1,2], Haipeng Li[3], Xiaozhuang Wu[3], Yixi Zhong[3], Keqin Zhang[4], Ya-Ping Zhang[4,5], Eric Boerwinkle[3] and Yun-Xin Fu*[4,3]

cell culture; or 3) using the common variants only. The mutation rate in the SARS-CoV genome was estimated to be 0.80 – 2.38 × 10^{-3} nucleotide substitution per site per year which is in the same order of magnitude as other RNA viruses. The non-synonymous and synonymous substitution

Clin Infect Dis. 2020 Mar 4. pii: ciaa203. doi: 10.1093/cid/ciaa203. [Epub ahead of print]

Genomic diversity of SARS-CoV-2 in Coronavirus Disease 2019 patients.

Shen Z[1,2], Xiao Y[3], Kang L[1,2], Ma W[1,2], Shi L[1,2], Zhang L[1], Zhou Z[4], Yang J[1,2], Zhong J[1,2], Yang D[5], Guo L[3], Zhang G[6], Li H[7], Xu Y[5], Chen M[8], Gao Z[5], Wang J[3], Ren L[3], Li M[1,9].

⊕ Author information

Abstract

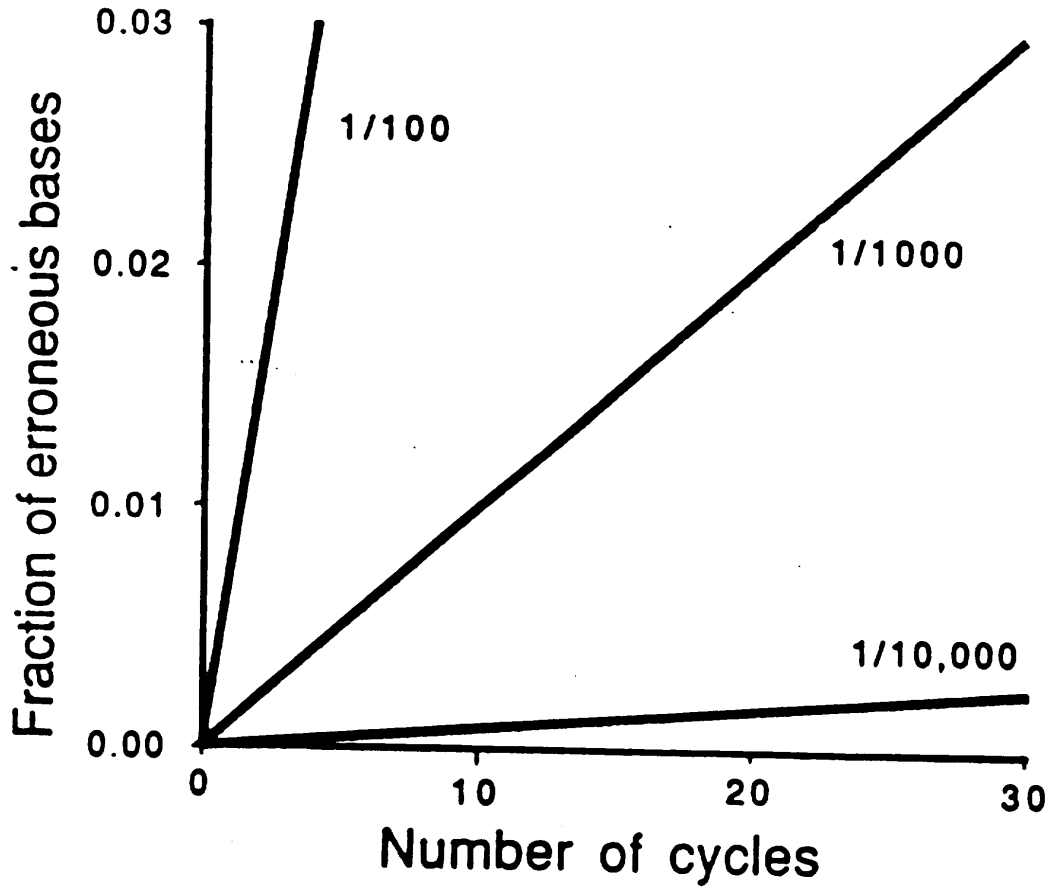
BACKGROUND: A novel coronavirus (SARS-CoV-2) has infected more than 75,000 individuals and spread to over 20 countries. It is still unclear how fast the virus evolved and how the virus interacts with other microorganisms in the lung.

METHODS: We have conducted metatranscriptome sequencing for the bronchoalveolar lavage fluid of eight SARS-CoV-2 patients, 25 community-acquired pneumonia (CAP) patients, and 20 healthy controls.

RESULTS: The median number of intra-host variants was 1-4 in SARS-CoV-2 infected patients, which ranged between 0 and 51 in different samples. The distribution of variants on genes was similar to those observed in the population data (110 sequences). However, very few intra-host variants were observed in the population as polymorphism, implying either a bottleneck or purifying selection involved in the transmission of the virus, or a consequence of the limited diversity represented in the current polymorphism data. Although current evidence did not support the transmission of intra-host variants in a person-to-person spread, the risk should not be overlooked. The microbiota in SARS-CoV-2 infected patients was similar to those in CAP, either dominated by the pathogens or with elevated levels of oral and upper respiratory commensal bacteria.

CONCLUSION: SARS-CoV-2 evolves in vivo after infection, which may affect its virulence, infectivity, and transmissibility. Although how the intra-host variant spreads in the population is still elusive, it is necessary to strengthen the surveillance of the viral evolution in the population and associated clinical changes.

Artifizielle Mutationen durch PCR



Kary Mullis

1944-2019

FIG. 1. Fraction of bases expected to be in error in a cloned PCR product as a function of the number of cycles. Three potential misincorporation rates are shown (solid lines).

Artifizielle Mutationen durch PCR

TABLE II

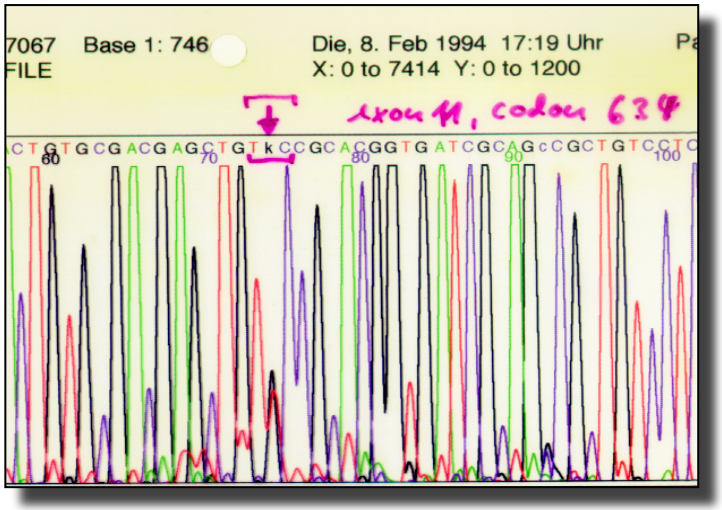
FRACTION OF MUTANT MOLECULES IN FINAL AMPLIFICATION PRODUCT

Number of starting molecules[a]	Cycle of PCR in which mutation first appears			
	1	2	5	10
1	0.5	0.25	0.003	0.001
10	0.05	0.025	0.0003	0.0001
100	0.005	0.0025	0.00003	0.00001
1000	0.0005	0.00025	0.000003	0.000001

[a] Number of starting molecules refers to the number of intact single-stranded template molecules.

Artifizielle Mutationen durch PCR

- Kein Problem bei direkter Sequenzierung von PCR-Matrizen (z. B. bei der Gendiagnose)



- Aber: Problem bei „Vereinzelung“ von PCR-Produkten durch Klonierung (z. B. zum Zwecke der Genexpression in Bakterien)

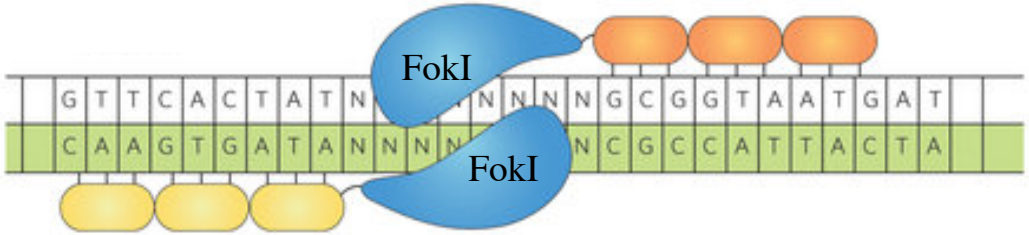
Mutationsraten verschiedener hitzestabiler DNA-Polymerasen

TABLE 6.1. Thermostable DNA polymerases differ in their enzymatic activities

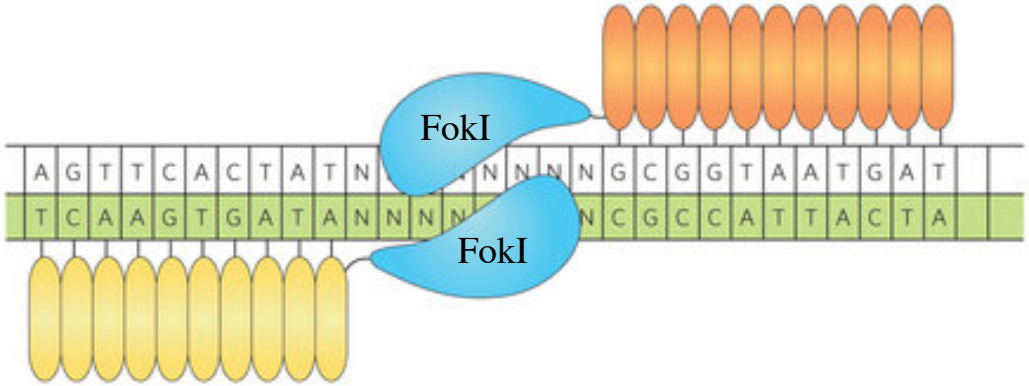
Enzyme	Relative efficiency[a]	Error rate[b]	Processivity[c]	Extension rate[d]	3′ to 5′ exo	5′ to 3′ exo
Taq Pol	88	2×10^{-4}	55	75	no	yes
Tli Pol (Vent)	70	4×10^{-5}	7	67	yes	no
Pfu Pol	60	7×10^{-7}	n.d.	n.d.	yes	no
r*Tth*	n.d.	n.d.	30	60	no	yes

[a] Percent conversion of template to product per cycle.
[b] Frequency of errors per base pairs incorporated.
[c] Average number of nucleotides added before dissociation.
[d] Average number of nucleotides added per second.
n.d = not determined.

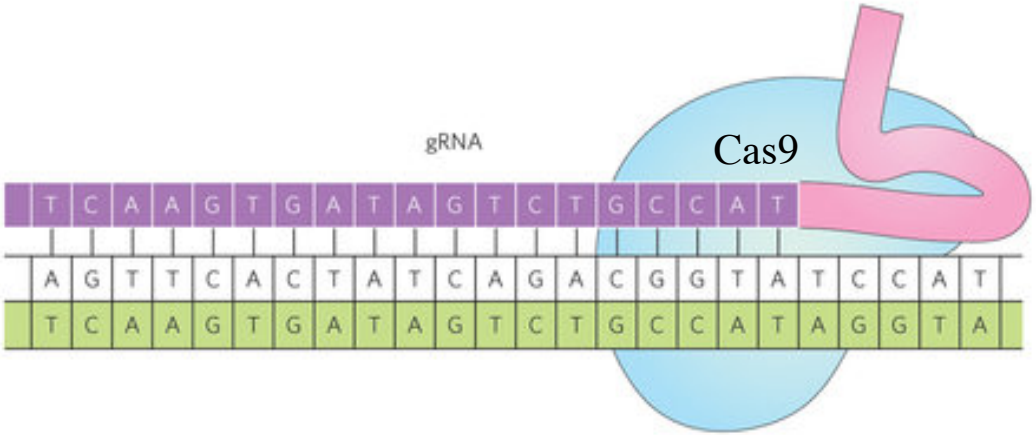
Mutagenese durch Genome Editing



Zink-Finger-
Nukleasen (ZFN)



TALE-
Nukleasen (TALEN)



CRISPR-
Cas9

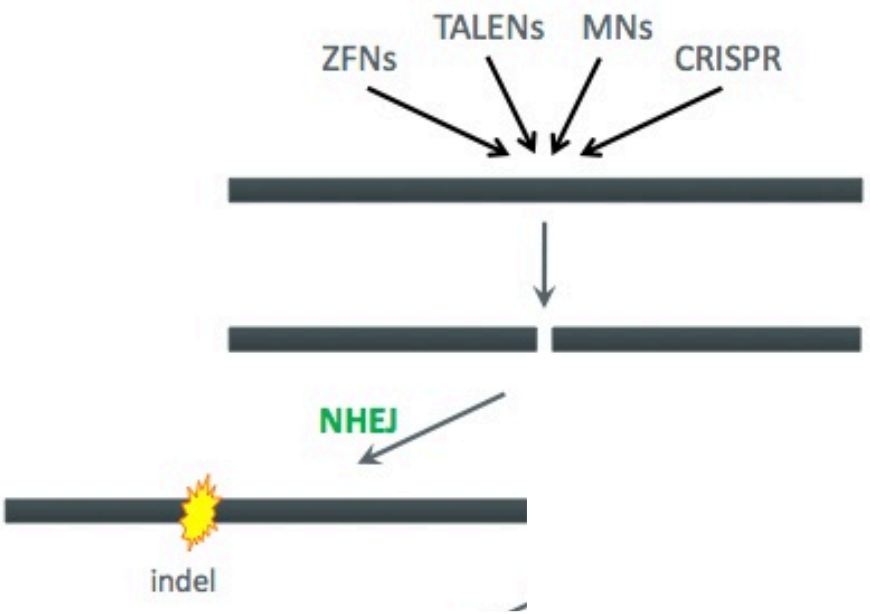
There's CRISPR in Your Yogurt

We've all been eating food enhanced by the genome-editing tool for years.

By Kerry Grens | January 1, 2015

ANDRZEJ KRAUZE

Genome Editing



Gezielte Erzeugung eines Doppelstrang-Bruchs

Fehlerhafte Reparatur

Mutationen (InDels) führen meist zu Gen-Knockout!

= chemische/physikalische Mutagenese

Gesetz zur Regelung der Gentechnik (Gentechnikgesetz - GenTG)
§ 3 Begriffsbestimmungen

Im Sinne dieses Gesetzes sind

1. Organismus
jede biologische Einheit, die fähig ist, sich zu vermehren oder genetisches Material zu übertragen, einschließlich Mikroorganismen,

1a. Mikroorganismen
Viren, Viroide, Bakterien, Pilze, mikroskopisch-kleine ein- oder mehrzellige Algen, Flechten, andere eukaryotische Einzeller oder mikroskopisch-kleine tierische Mehrzeller sowie tierische und pflanzliche Zellkulturen,

2. gentechnische Arbeiten

   a) die Erzeugung gentechnisch veränderter Organismen,

   b) die Vermehrung, Lagerung, Zerstörung oder Entsorgung sowie der innerbetriebliche Transport gentechnisch veränderter Organismen sowie deren Verwendung in anderer Weise, soweit noch keine Genehmigung für die Freisetzung oder das Inverkehrbringen zum Zweck des späteren Ausbringens in die Umwelt erteilt wurde,

3. gentechnisch veränderter Organismus
ein Organismus, mit Ausnahme des Menschen, dessen genetisches Material in einer Weise verändert worden ist, wie sie unter natürlichen Bedingungen durch Kreuzen oder natürliche Rekombination nicht vorkommt; ein gentechnisch veränderter Organismus ist auch ein Organismus, der durch Kreuzung oder natürliche Rekombination zwischen gentechnisch veränderten Organismen oder mit einem oder mehreren gentechnisch veränderten Organismen oder durch andere Arten der Vermehrung eines gentechnisch veränderten Organismus entstanden ist, sofern das genetische Material des Organismus Eigenschaften aufweist, die auf gentechnische Arbeiten zurückzuführen sind,

3a. Verfahren der Veränderung genetischen Materials in diesem Sinne sind insbesondere

   a) Nukleinsäure-Rekombinationstechniken, bei denen durch die Einbringung von Nukleinsäuremolekülen, die außerhalb eines Organismus erzeugt wurden, in Viren, Viroide, bakterielle Plasmide oder andere Vektorsysteme neue Kombinationen von genetischem Material gebildet werden und diese in einen Wirtsorganismus eingebracht werden, in dem sie unter natürlichen Bedingungen nicht vorkommen,

   b) Verfahren, bei denen in einen Organismus direkt Erbgut eingebracht wird, welches außerhalb des Organismus hergestellt wurde und natürlicherweise nicht darin vorkommt, einschließlich Mikroinjektion, Makroinjektion und Mikroverkapselung,

   c) Zellfusionen oder Hybridisierungsverfahren, bei denen lebende Zellen mit neuen Kombinationen von genetischem Material, das unter natürlichen Bedingungen nicht darin vorkommt, durch die Verschmelzung zweier oder mehrerer Zellen mit Hilfe von Methoden gebildet werden, die unter natürlichen Bedingungen nicht vorkommen,

3b. nicht als Verfahren der Veränderung genetischen Materials gelten

   a) In-vitro-Befruchtung,

   b) natürliche Prozesse wie Konjugation, Transduktion, Transformation,

   c) Polyploidie-Induktion,

   es sei denn, es werden gentechnisch veränderte Organismen verwendet oder rekombinante Nukleinsäuremoleküle, die im Sinne von den Nummern 3 und 3a hergestellt wurden, eingesetzt.
   Weiterhin gelten nicht als Verfahren der Veränderung genetischen Materials

   a) Mutagenese und

   b) Zellfusion (einschließlich Protoplastenfusion) von Pflanzenzellen von Organismen, die mittels herkömmlicher Züchtungstechniken genetisches Material austauschen können,

   es sei denn, es werden gentechnisch veränderte Organismen als Spender oder Empfänger verwendet,

3c. sofern es sich nicht um ein Vorhaben der Freisetzung oder des Inverkehrbringens handelt und sofern keine gentechnisch veränderten Organismen als Spender oder Empfänger verwendet werden, gelten darüber hinaus nicht als Verfahren der Veränderung genetischen Materials

   a) Zellfusion (einschließlich Protoplastenfusion) prokaryotischer Arten, die genetisches Material über bekannte physiologische Prozesse austauschen,

   b) Zellfusion (einschließlich Protoplastenfusion) von Zellen eukaryotischer Arten, einschließlich der Erzeugung von Hybridomen und der Fusion von Pflanzenzellen,

   c) Selbstklonierung nicht pathogener, natürlich vorkommender Organismen, bestehend aus

      aa) der Entnahme von Nukleinsäuresequenzen aus Zellen eines Organismus,

      bb) der Wiedereinführung der gesamten oder eines Teils der Nukleinsäuresequenz (oder eines synthetischen Äquivalents) in Zellen derselben Art oder in Zellen phylogenetisch eng verwandter Arten, die genetisches Material durch natürliche physiologische Prozesse austauschen können, und

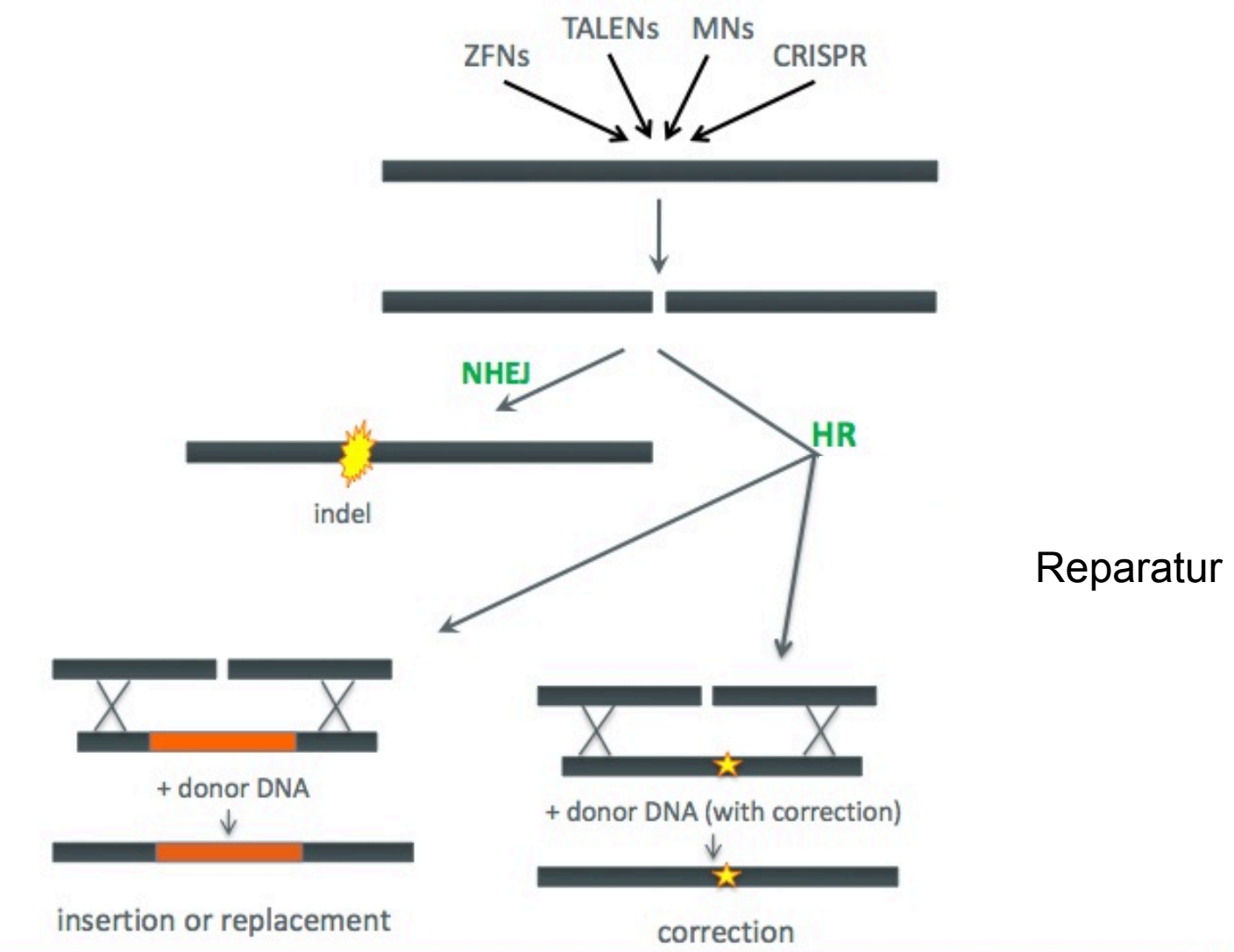
      cc) einer eventuell vorausgehenden enzymatischen oder mechanischen Behandlung.

      Zur Selbstklonierung kann auch die Anwendung von rekombinanten Vektoren zählen, wenn sie über lange Zeit sicher in diesem Organismus angewandt wurden,

4. gentechnische Anlage

Mutagenese ist keine Gentechnik!

Genome Editing



Reparatur „mit Vorlage“

„Genome Editing ist Gentechnik“!

trans parenz
GENTECHNIK
PFLANZEN · FORSCHUNG · LA

AKTUELL | FORSCHUNG | ANBAU | LEBENSMITTEL | SICHERHEIT | RECHT

Alles Gentechnik. Das Urteil der Richter und die Versäumnisse der Politik

(26.07.2018) Der Europäische Gerichtshof hat ein überraschendes Urteil gefällt: Anders als der Generalanwalt in seinem Plädoyer haben die Richter die neuen Züchtungstechniken wie Genome Editing und CRISPR als Gentechnik eingestuft – mit allem, was an gesetzlichen Auflagen und gesellschaftlicher Ablehnung dazu gehört. Damit sind in Europa erst einmal konkrete Anwendungen verbaut. Doch das Urteil konnte nur deswegen so ausfallen, weil die Politik sich seit Jahren um längst überfällige Entscheidungen gedrückt hat.

Robert Habeck, Bundesvorsitzender B90/Grüne) stellt das pauschale Nein seiner Partei zur Gentechnik in Frage. Die Gen-Schre CRISPR/Cas „bringt kein artfremdes Gen ein, sondern simuliert einen natürlichen Prozess im Schnellverfahren“. (FAZ, 18.07.2018).

Foto: Raimund Spekking, CC BY-SA 4.0.

Bundesumweltministerin Svenja Schulze (SPD, links im Bild) sieht sich selbst auf Seiten der Gentechnik-Gegner: „Ich will keine Gentechnik durch die Hintertür!“ Hier bei der Übergabe der Unterschriftenliste der Aktion FOODprint mit Josef Wilhelm (Rapunzel) und Elke Röder (BNN).

Foto: BNN; großes Foto oben: welcomia / 123RF


ZKBS
Zentrale Kommission für die Biologische Sicherheit
English | Sitemap | Kontakt | Newsletter
Suchen
Zentrale Kommission für die Biologische Sicherheit
ZKBS ▸ Fokusthemen ▸ Genome Editing & EuGH-Urteil
Aktuelles
Geänderte Einstufungen
Die ZKBS stellt sich vor
Fokusthemen
DIYbio - Do it yourself-Biologie
Gene-Drive-Systeme
Genome Editing & EuGH-Urteil
Influenzaviren
Genome Editing - Auswirkungen des EuGH-Urteils auf die Pflanzenzüchtung
Warum verursacht das EuGH-Urteil so viel Wirbel?
Wissenschaftlicher Hintergrund
Das EuGH-Urteil
Problem des Nachweises
Fazit der ZKBS
1. Der EuGH stellt fest, „... dass durch Mutagenese gewonnene Organismen GVO im Sinne der GVO-Richtlinie sind, da durch die Verfahren und Methoden der Mutagenese eine auf natürliche Weise nicht mögliche Veränderung am genetischen Material eines Organismus vorgenommen wird.“ (Pressemitteilung des EuGH, Hervorhebungen durch die ZKBS; vgl. Urteil Randnummer 29)
Diese Feststellung ist naturwissenschaftlich nicht begründbar. Mutationen, ausgelöst durch die Anwendung klassischer Mutagene wie Strahlung oder Chemikalien, erzeugen Erbgutveränderungen, wie sie unter natürlichen Bedingungen durch natürliche Strahlung und andere Umwelteinflüsse auch auftreten. Es liegen dieselben Mechanismen zugrunde. Konkret können natürliche Mutationen durch folgende Prozesse entstehen:
biologische Sicherheit
(siehe auch Pressemitteilung des EuGH). Sie fallen somit unter die Regularien der EU-Richtlinie 2001/18/EG über die absichtliche Freisetzung von GVO in die Umwelt (GVO-Richtlinie). Allerdings sind die mittels der konventionellen Mutagenese erzeugten GVO vom Anwendungsbereich der Richtlinie ausgenommen. Das Urteil stellt eine juristische Auslegung der GVO-

Modrzejewski et al. Environ Evid (2019) 8:27
https://doi.org/10.1186/s13750-019-0171-5

Environmental Evidence

SYSTEMATIC MAP Open Access

What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map

Dominik Modrzejewski*, Frank Hartung, Thorben Sprink, Dörthe Krause, Christian Kohl and Ralf Wilhelm

Abstract

Background: Within the last decades, genome-editing techniques such as CRISPR/Cas, TALENs, Zinc-Finger Nucleases, Meganucleases, Oligonucleotide-Directed Mutagenesis and base editing have been developed enabling a precise modification of DNA sequences. Such techniques provide options for simple, time-saving and cost-effective applications compared to other breeding techniques and hence genome editing has already been promoted for a wide range of plant species. Although the application of genome-editing induces less unintended modifications (off-targets) in the genome compared to classical mutagenesis techniques, off-target effects are a prominent point of criticism as they are supposed to cause unintended effects, e.g. genomic instability or cell death. To address these aspects, this map aims to answer the following question: What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects? This primary question will be considered by two secondary questions: One aims to overview the market-oriented traits being modified by genome-editing in plants and the other explores the occurrence of off-target effects.

Methods: A literature search in nine bibliographic databases, Google Scholar, and 47 web pages of companies and governmental agencies was conducted using predefined and tested search strings in English language. Articles were screened on title/abstract and full text level for relevance based on pre-defined inclusion criteria. The relevant information of included studies were mapped using a pre-defined data extraction strategy. Besides a descriptive summary of the relevant literature, a spreadsheet containing all extracted data is provided.

Results: Altogether, 555 relevant articles from journals, company web pages and web pages of governmental agencies were identified containing 1328 studies/applications of genome-editing in model plants and agricultural crops in the period January 1996 to May 2018. Most of the studies were conducted in China followed by the USA. Genome-editing was already applied in 68 different plants. Although most of the studies were basic research, 99 different market-oriented applications were identified in 28 different crops leading to plants with improved food and feed quality, agronomic value like growth characteristics or increased yield, tolerance to biotic and abiotic stress, herbicide tolerance or industrial benefits. 252 studies explored off-target effects. Most of the studies were conducted using CRISPR/Cas. Several studies firstly investigated whether sites in the genome show similarity to the target sequence and secondly analyzed these potential off-target sites by sequencing. In around 3% of the analyzed potential off-target

....wenig Anhaltspunkte für off-target Effekte.

Anzalone et al. Nature, Oct 2019

Prime Editing: eine neue Dimension!

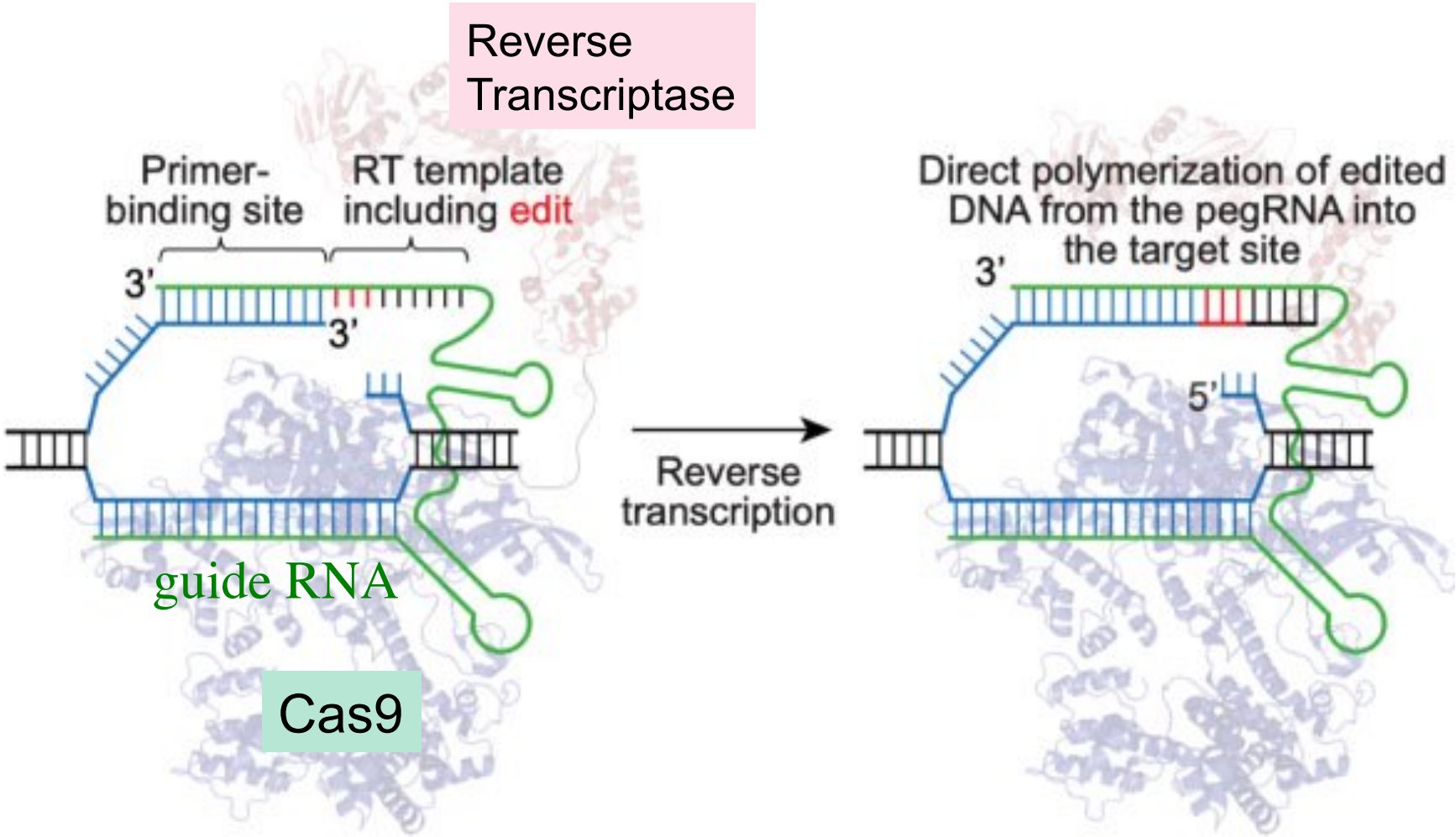
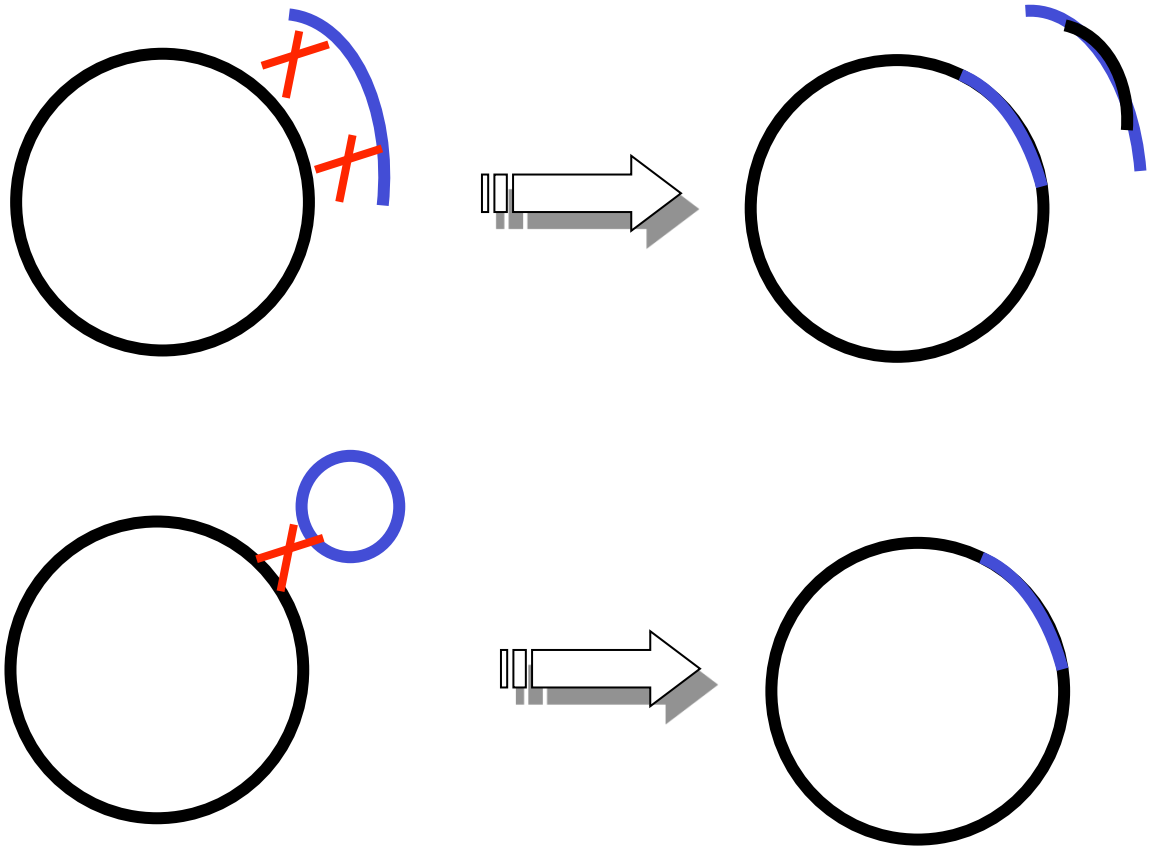


Figure 2: The prime editor with the pegRNA. The Cas9 portion of the editor cuts the genomic DNA and the reverse transcriptase portion polymerizes DNA onto the nicked strand based on the pegRNA sequence. Image from David Liu with permission.

Rekombination

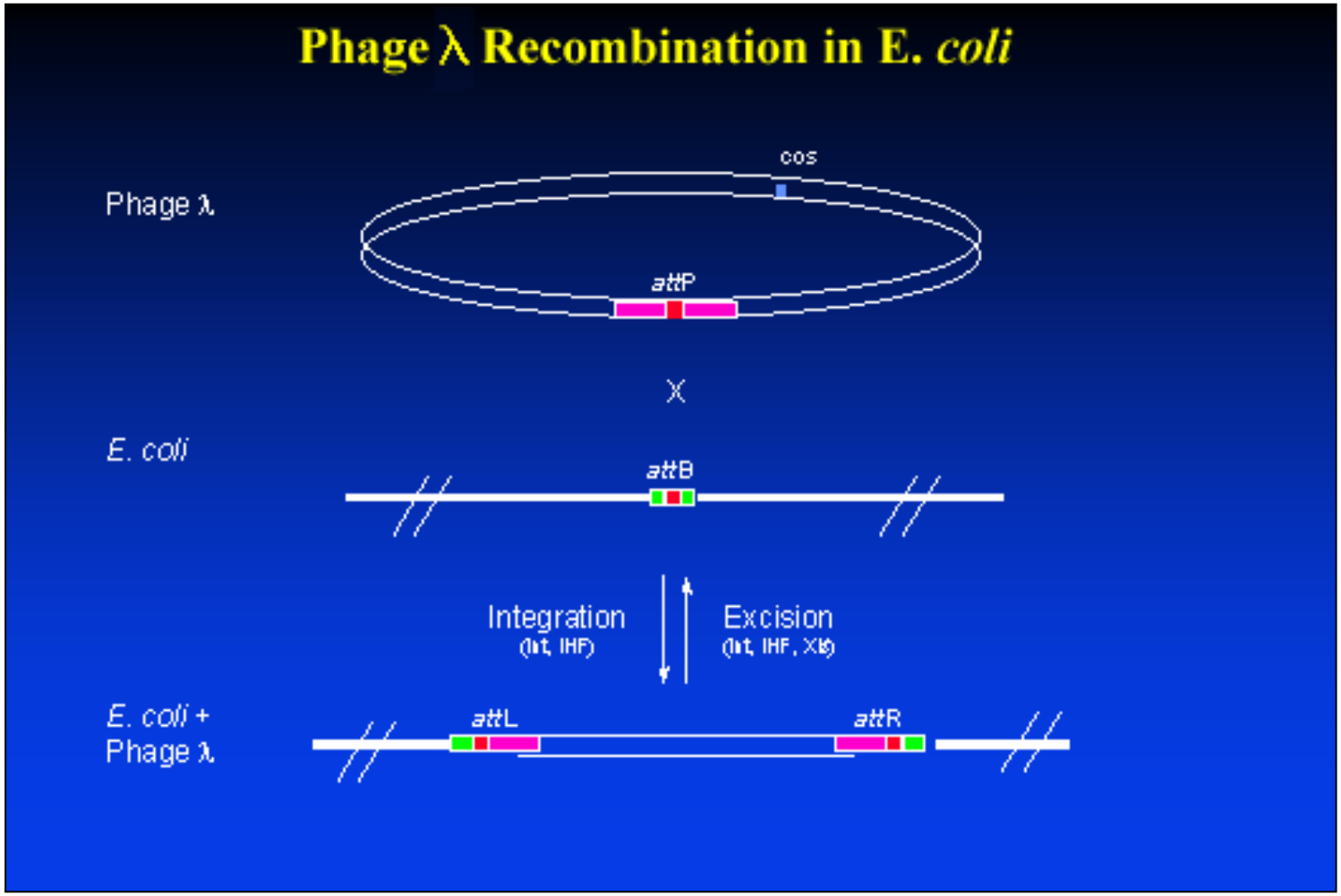
- zwischen Chromosomen
- zwischen Chromosomen und extrachromosomaler DNA
- zwischen zwei extrachromosomalen Elementen

Homologe Rekombination

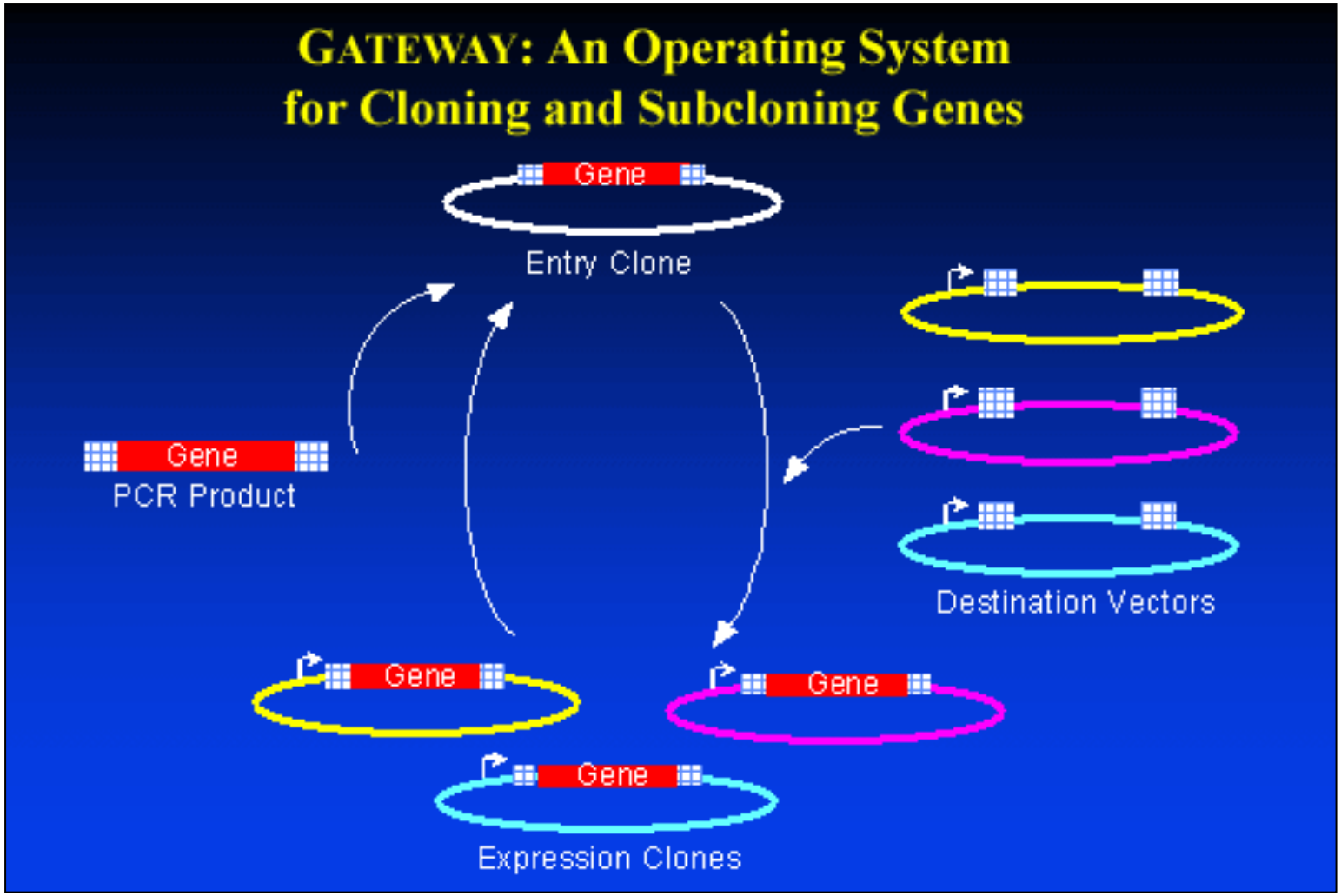


...besonders effizient in Mikroorganismen

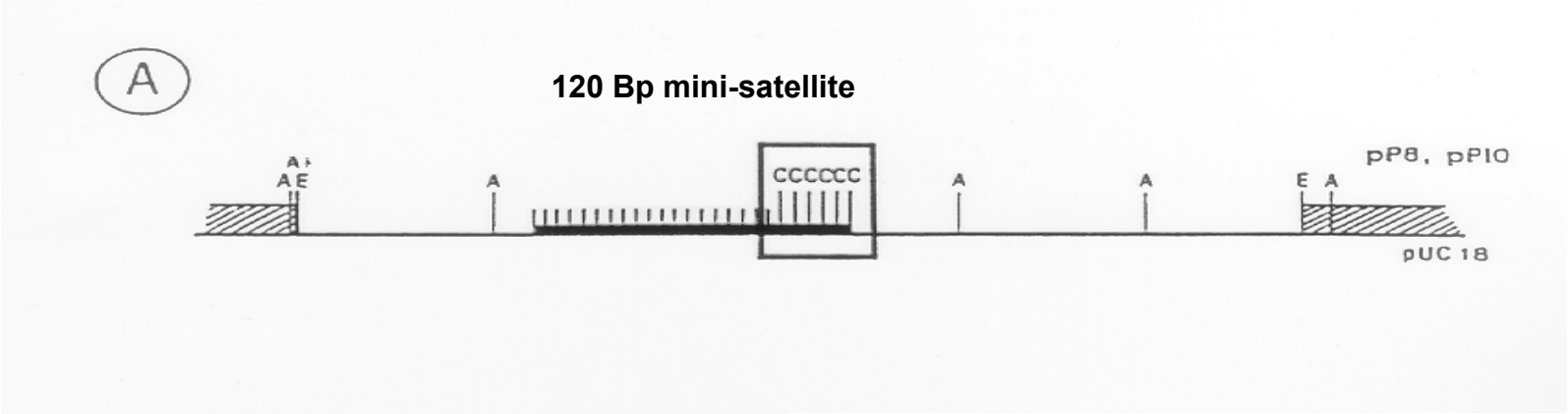
Klonieren mit Hilfe der ortsspezifischen Rekombination

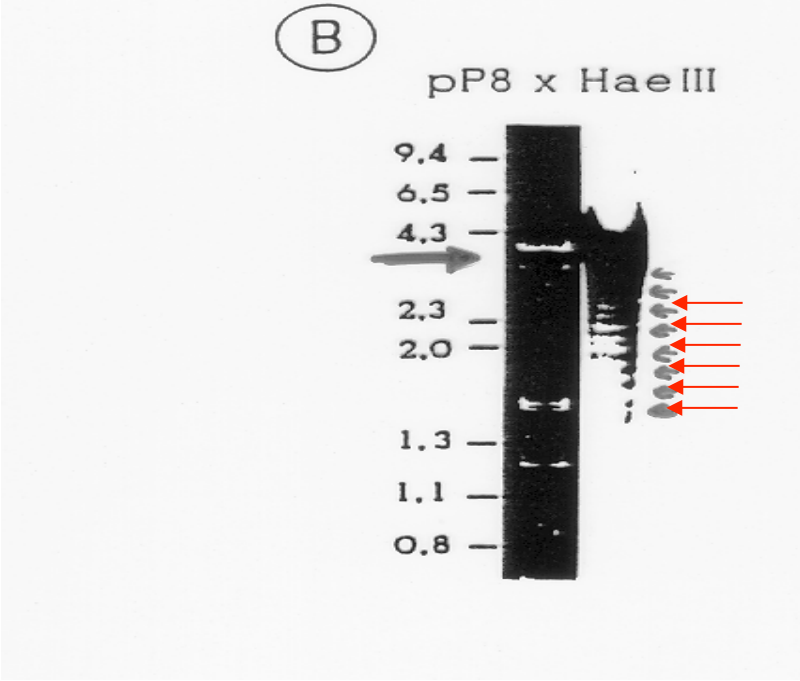


Klonieren mit Hilfe der ortsspezifischen Rekombination

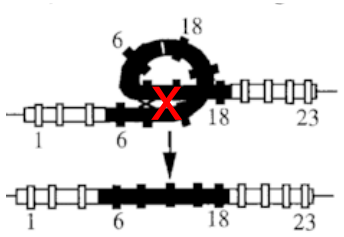


Instabilität klonierter DNA

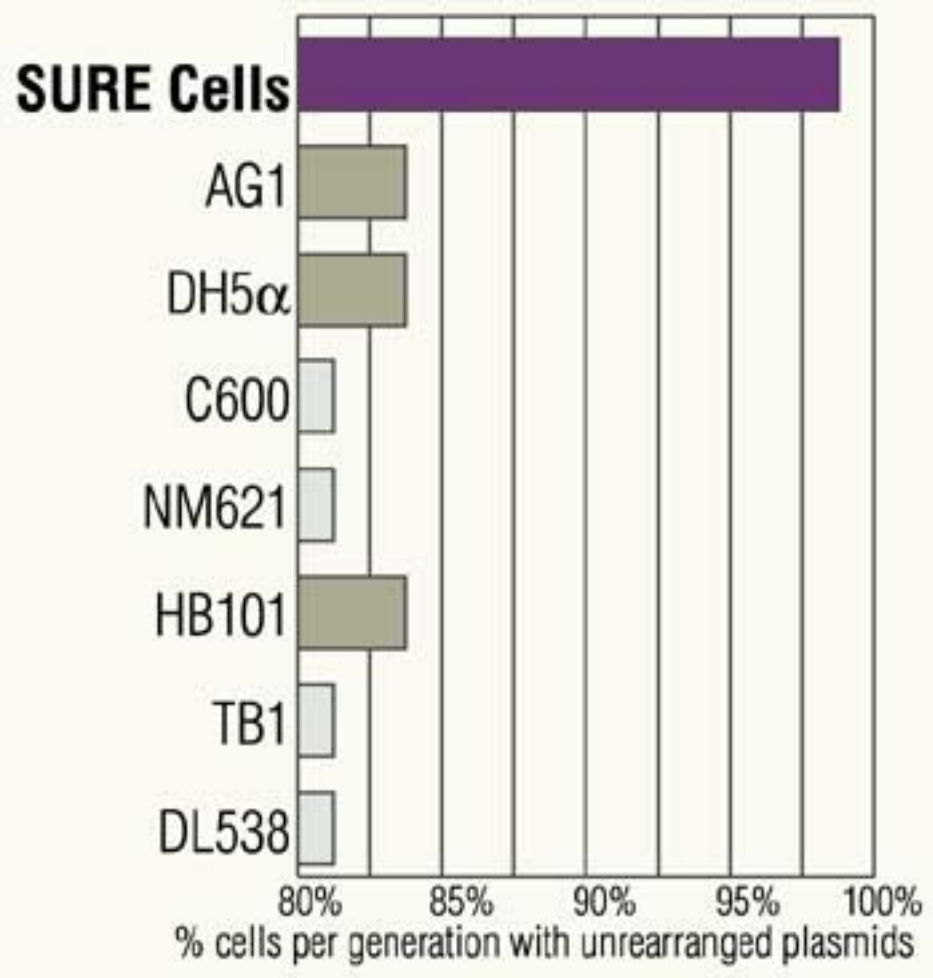




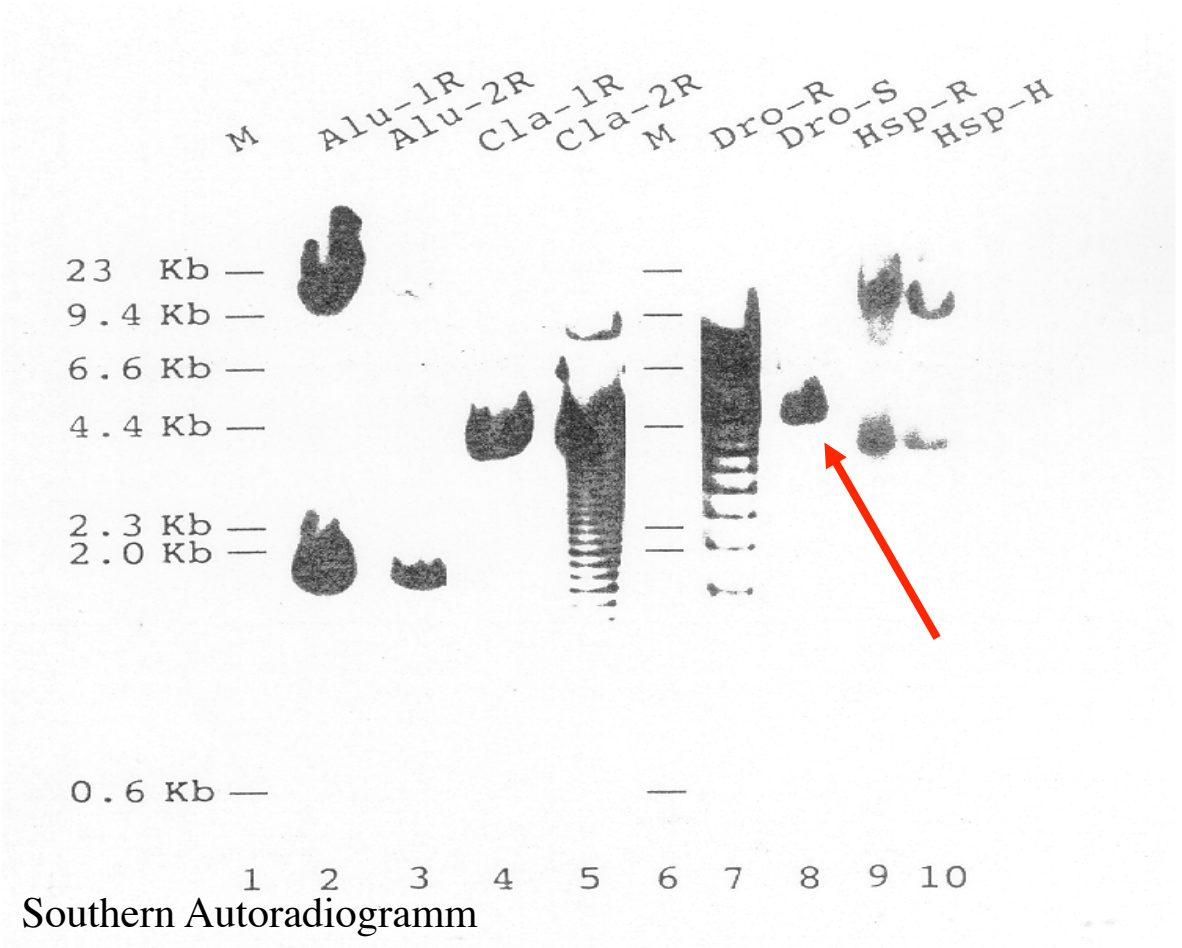
Vermutlich intra-chromosomale Rekombination zwischen tandem repeats



Stabilisierung von Repetitionen in E. coli

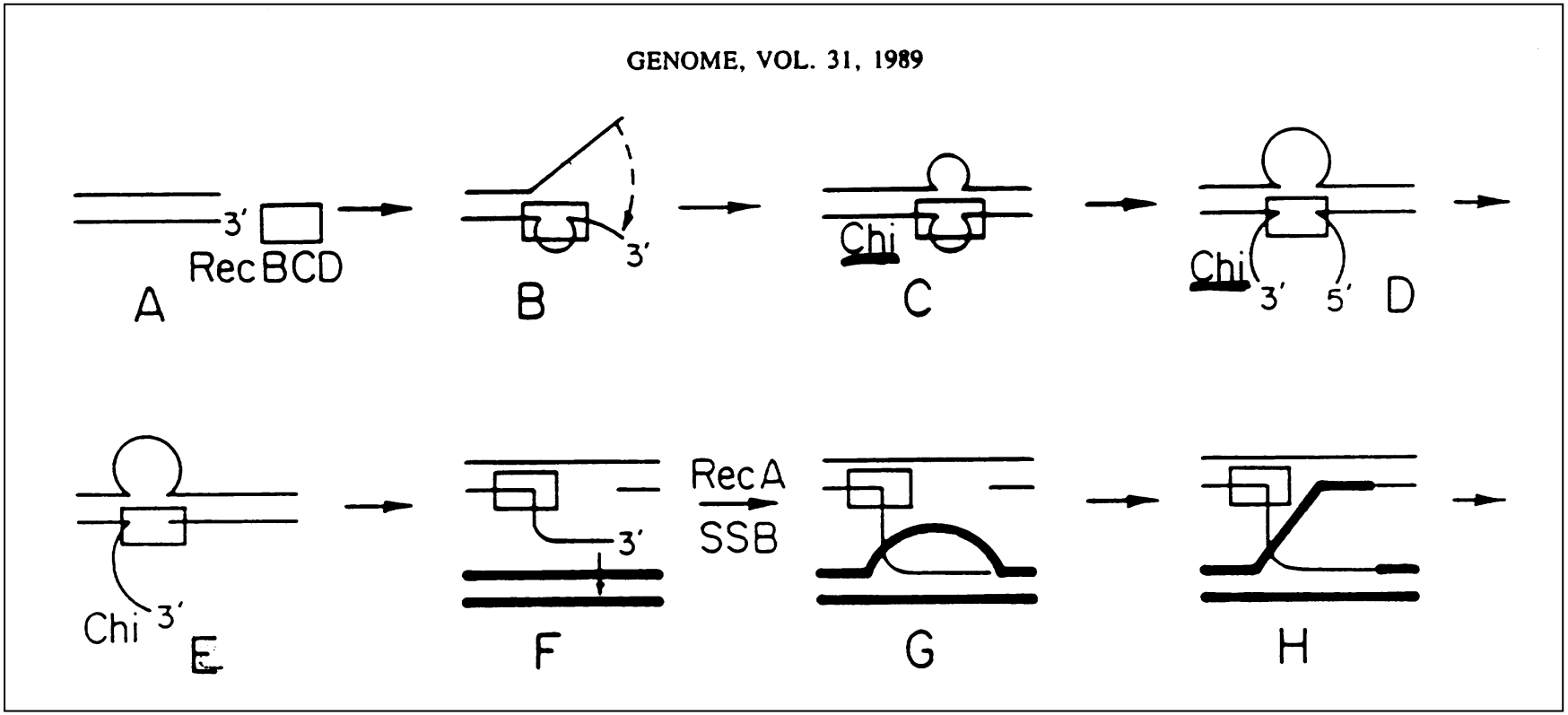


Stabilisierung von Tandem-Repetitionen durch E. coli SURE



Southern Autoradiogramm

HOTSPOTS der Rekombination



Crossover
Hotspot
Instigator

GCTGGTGG

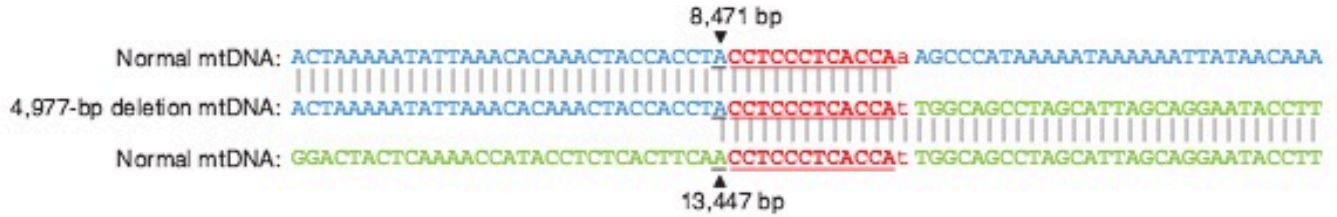
HOTSPOTS der Rekombination

A common sequence motif associated with recombination hot spots and genome instability in humans

Simon Myers[1,2], Colin Freeman[2], Adam Auton[2,3], Peter Donnelly[2,4] & Gil McVean[2]

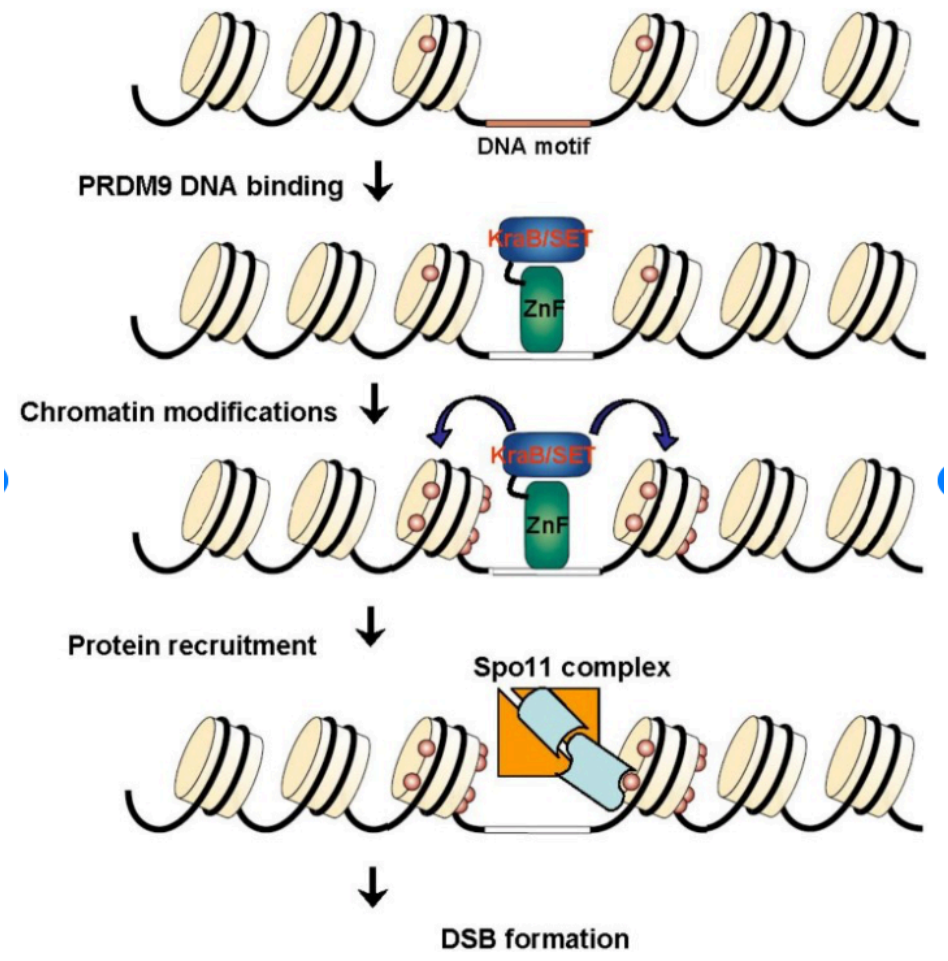
In humans, most meiotic crossover events are clustered into short regions of the genome known as recombination hot spots. We have previously identified DNA motifs that are enriched in hot spots, particularly the 7-mer CCTCCCT. Here we use the increased hot-spot resolution afforded by the Phase 2 HapMap and novel search methods to identify an extended family of motifs based around the degenerate 13-mer CCNCCNTNNCCNC, which is critical in recruiting crossover events to at least 40% of all human hot spots and which operates on diverse genetic backgrounds in both sexes. Furthermore, these motifs are found in hypervariable minisatellites and are clustered in the breakpoint regions of both disease-causing nonallelic homologous recombination hot spots and common mitochondrial deletion hot spots, implicating the motif as a driver of genome instability.

„...a driver of genome instability."



Nat Genet 2008

HOTSPOTS der Rekombination

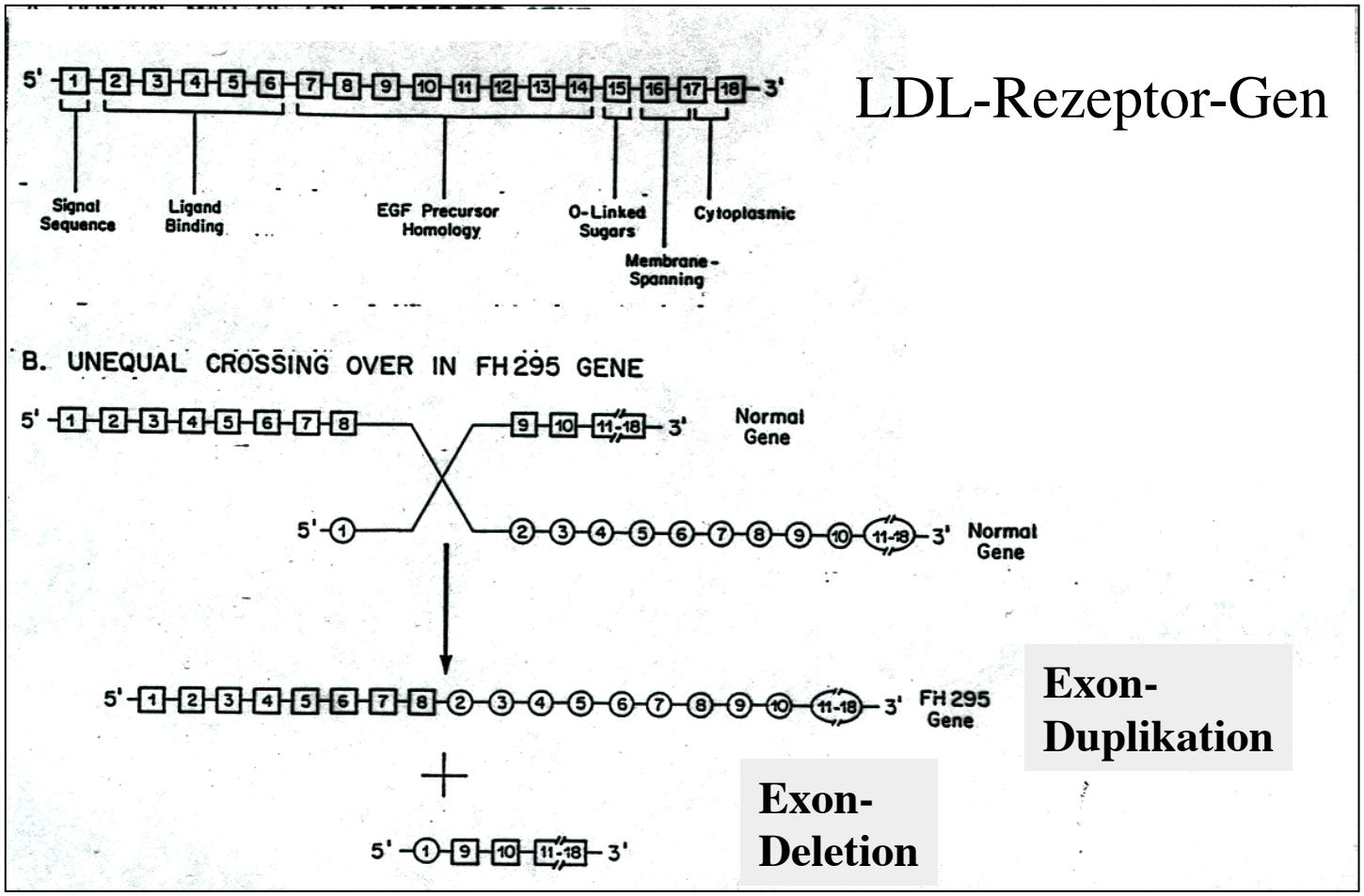


Hotspot specification by PRDM9: PRDM9 binds to a target sequence through its zinc finger domain and brings about H3K4Me3 at adjacent nucleosomes. Additional modifications and proteins recruitment may follow either by interaction with H3K4Me3 and/or with PRDM9, allowing the recruitment of the SPO11 complex and DSB formation.

PRDM9 bindet an DNA-Motive und initiiert Rekombination über Modifizierung von Histon H3

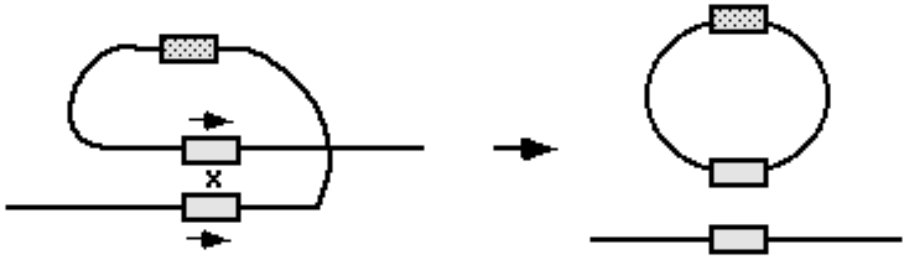
Kumar & de Massy 2010

Instabilität durch inäquales Crossingover zwischen Repeats

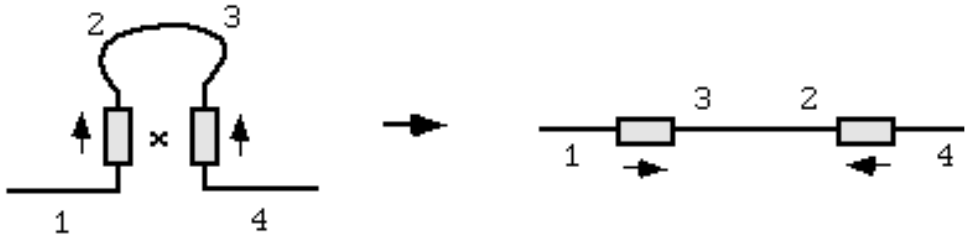


Lehman et al. (1987) Cell 48, pp.827

Weitere Mechanismen der Genom-Instabilität via Rekombination



Deletion durch Intra-Chromatid-Rekombination zwischen ‚direct repeats'



Inversion durch Intra-Chromatid-Rekombination zwischen ‚inverted repeats'

Genom-Rearrangements als Ursache genetischer Erkrankungen

TABLE 1. Physical features of regions associated with genomic disorders

Trait	Rearrangement type	Distance between repeats (kb)	Repeat length (bp)
Color blindness	DEL	0	39 000
α-Thalassemia	DEL	3.7 or 4.2	4000
Growth hormone deficiency	DEL	6.7	2200
Debrisoquine sensitivity	DEL	9.3	2800
Hunter mucopolysaccharidosis	INV	20	3000
Glucocorticoid-remediable aldosteronism	DUP	45	10 000
Hemophilia A	INV	500	9500
CMT1A/HNPP	DUP/DEL	1500	24 011
X-linked ichthyosis	DEL	1900	20 000
Williams syndrome	DEL	~2000	>30 000
Smith–Magenis syndrome/dup(17)(p11.2)	DEL/DUP	~5000	>200 000

Abbreviations: DEL, deletion; DUP, duplication; INV, inversion.

TIG 14(10), 417ff

Spezialproblem:

Rekombination durch *template switch* bei reverser Transkription

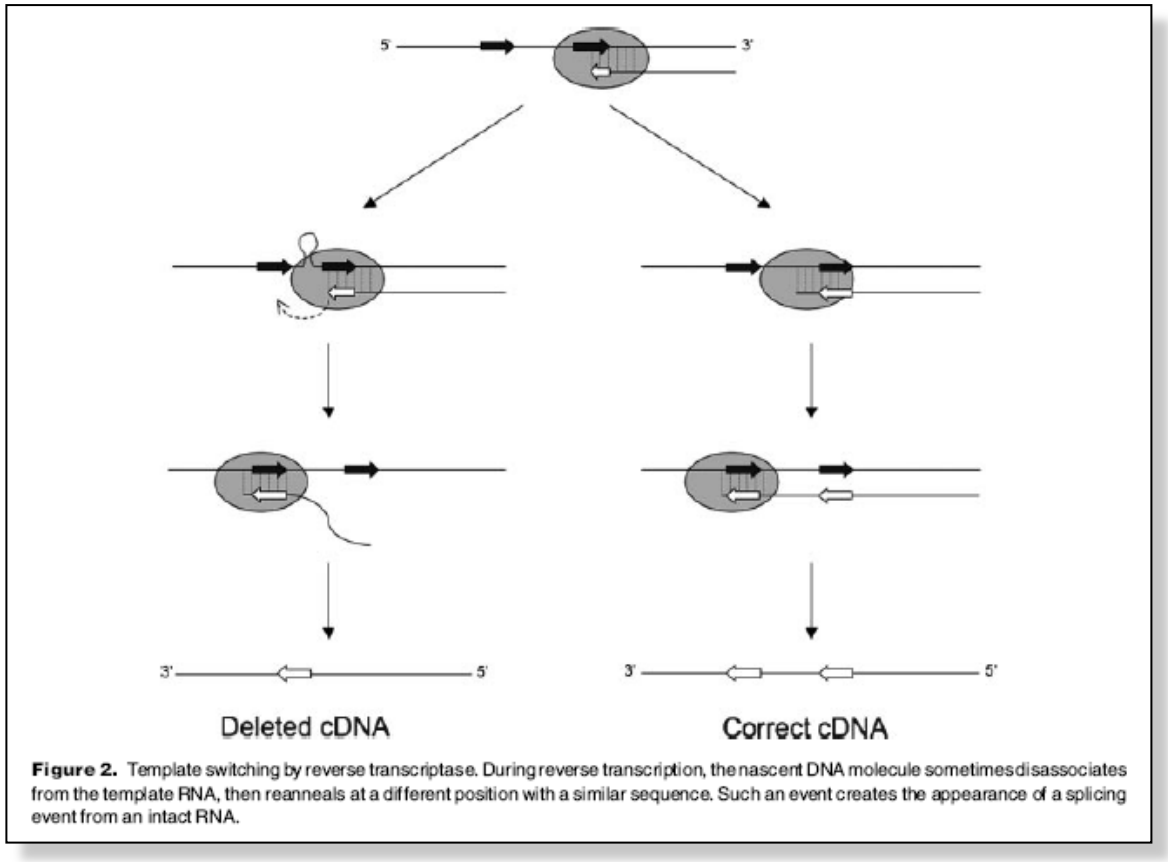
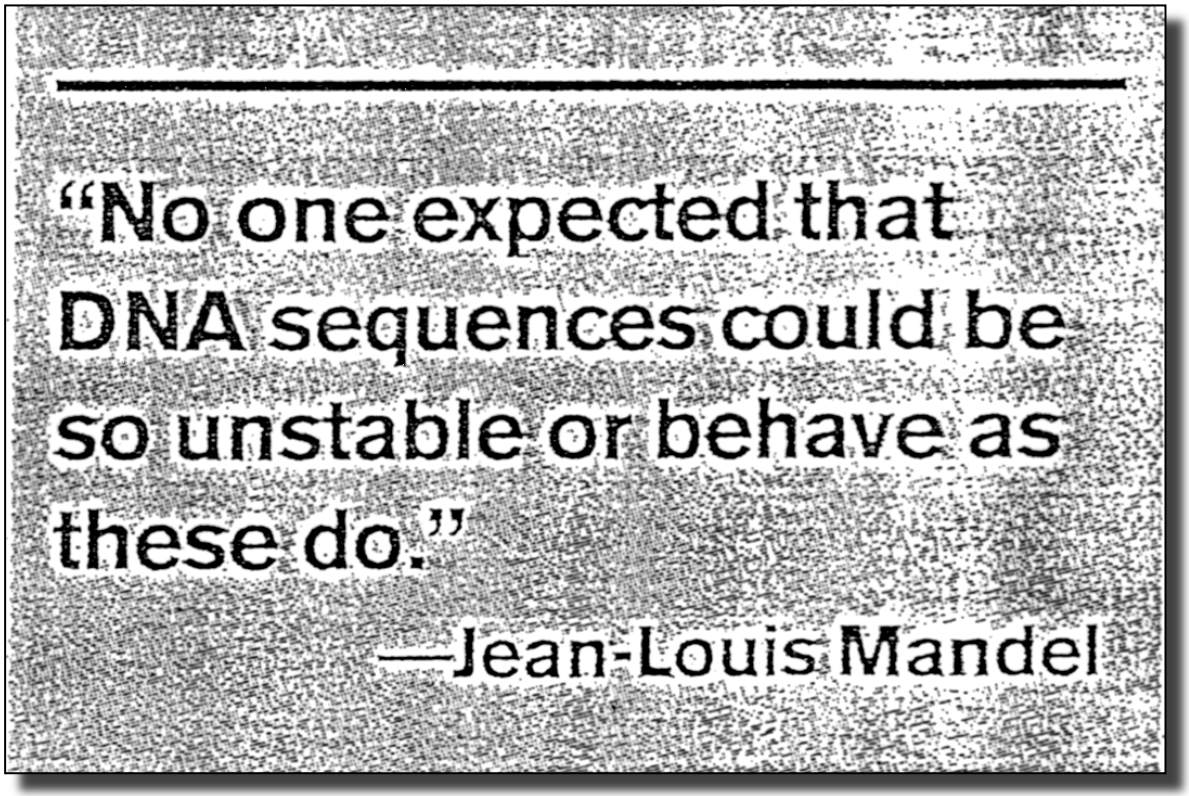


Figure 2. Template switching by reverse transcriptase. During reverse transcription, the nascent DNA molecule sometimes disassociates from the template RNA, then reanneals at a different position with a similar sequence. Such an event creates the appearance of a splicing event from an intact RNA.

…1-2% von cDNAs sind artifizielle Chimären

BioEssays 30: 601 (2008)

Instabilität bei Replikation und Transkription



Science 1993

Cell, Vol. 72, 971–983, March 26, 1993, Copyright © 1993 by Cell Press

A Novel Gene Containing a Trinucleotide Repeat That Is Expanded and Unstable on Huntington's Disease Chromosomes

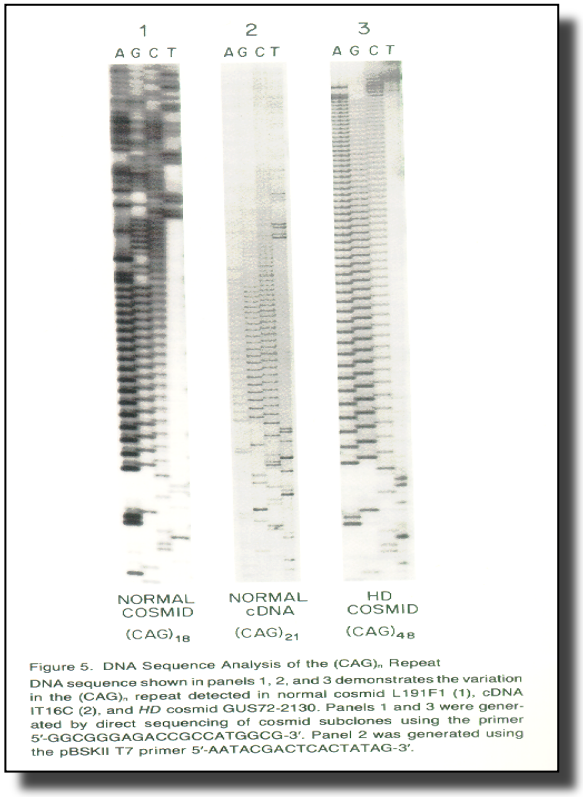


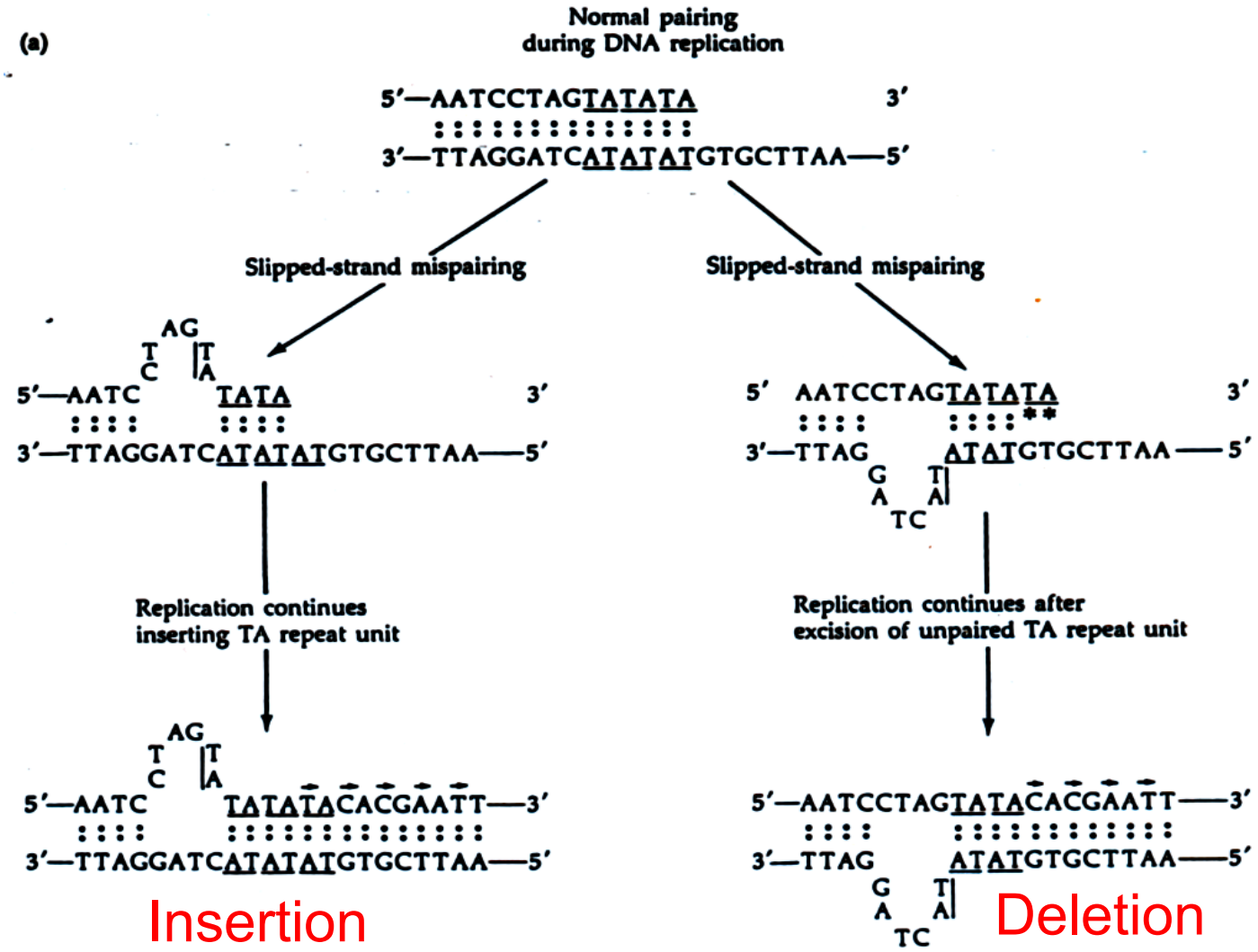
Figure 5. DNA Sequence Analysis of the $(CAG)_n$ Repeat

DNA sequence shown in panels 1, 2, and 3 demonstrates the variation in the $(CAG)_n$ repeat detected in normal cosmid L191F1 (1), cDNA IT16C (2), and *HD* cosmid GUS72-2130. Panels 1 and 3 were generated by direct sequencing of cosmid subclones using the primer 5′-GGCGGGAGACCGCCATGGCG-3′. Panel 2 was generated using the pBSKII T7 primer 5′-AATACGACTCACTATAG-3′.

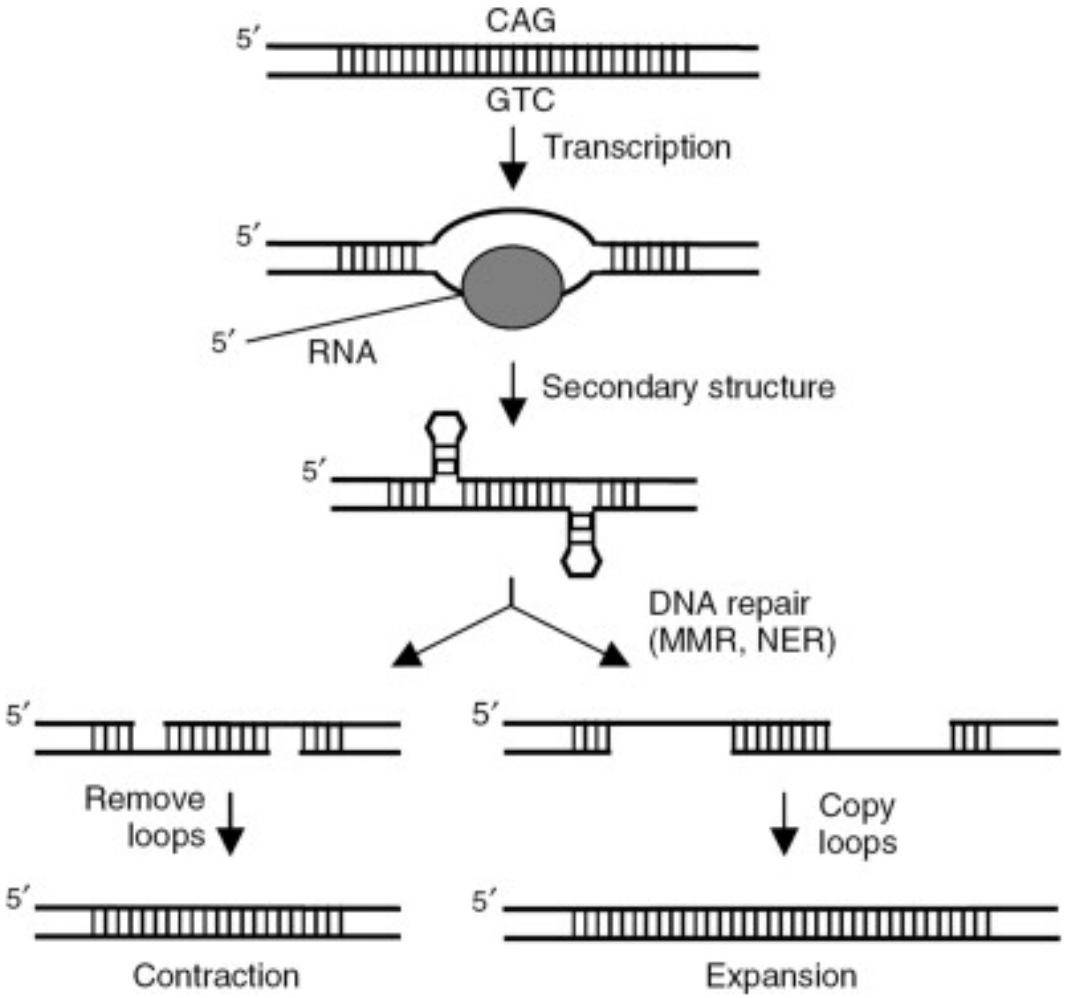
(CAG)n

- HD Allel > 36 x CAG
- Wildtyp 6-34 x CAG

„Slippage“ bei der Replikation



Transkriptionsgekoppelte Instabilität von Simple Repeats



Trinukleotid-Erkrankungen

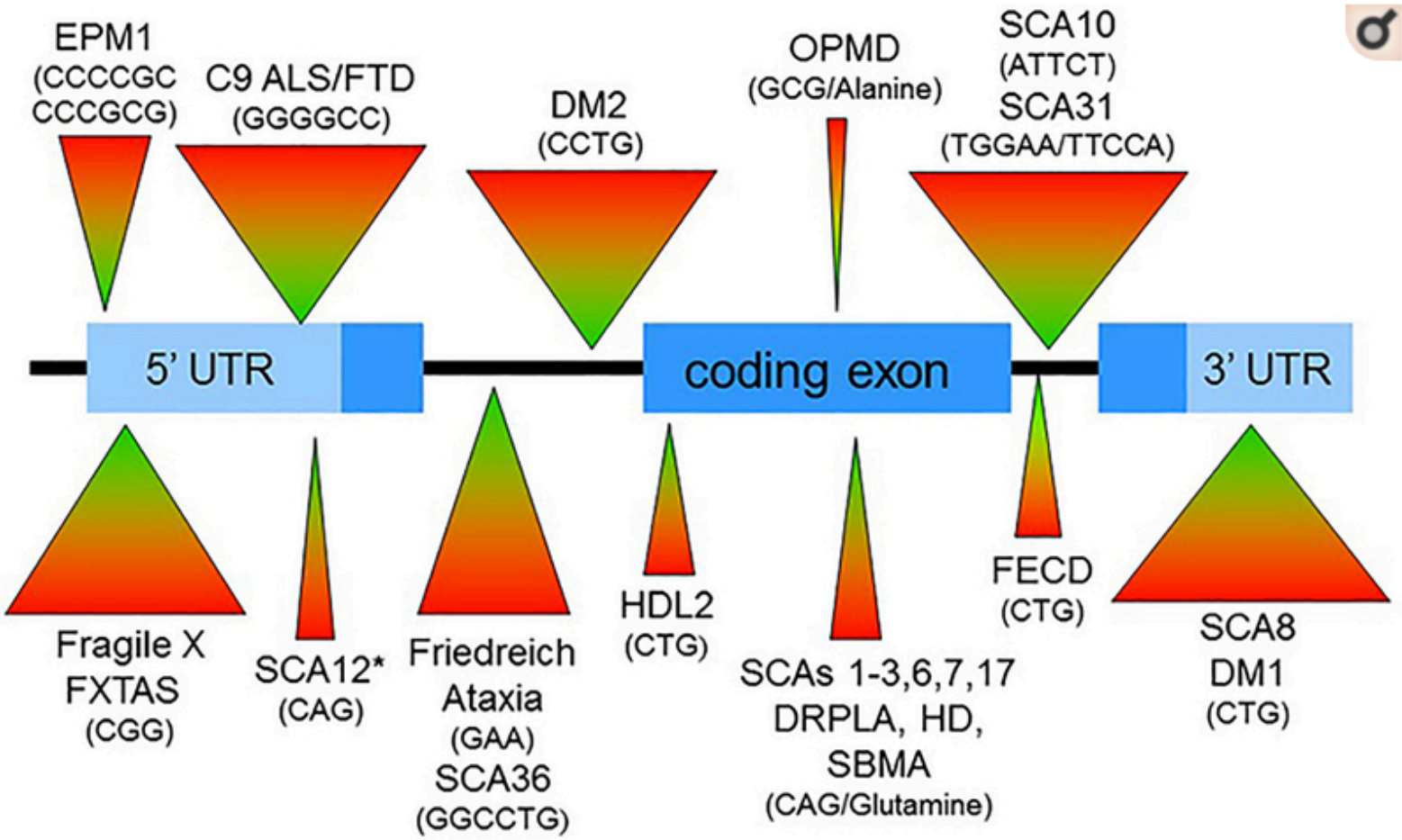
Table 1. Summary of triplet repeat diseases in humans

Repeat sequence	Condition	Gene	Repeat localisation	Repeat number	
				Normal	Disease
CGG	FRAXA	FMR-1	5′-Untranslated	6–52	200–1000
CGG	FRAXE	?		6–25	200–1000
CGG	FRA11B (Jacobsen syndrome)	CBL2	?	~11	400–800
CAG	SBMA (Kennedy disease)	Androgen receptor	ORF	12-33	<100
CAG	Huntington	Huntington	ORF	9–30	<150
CAG	SCA 1	Ataxin	ORF	9–39	<100
CAG	DRPLA/HRS	Atropin	ORF	9–23	<100
CAG	Machado-Joseph	MJD 1	ORF	16–36	<100
CTG	Myotonic dystrophy	DM kinase (DM-1)	3′-Untranslated	5–40	200–4000

ORF, open reading frame.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6485936/pdf/nihms-1017348.pdf

Repeat Expansion - Erkrankungen



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6485936/pdf/nihms-1017348.pdf

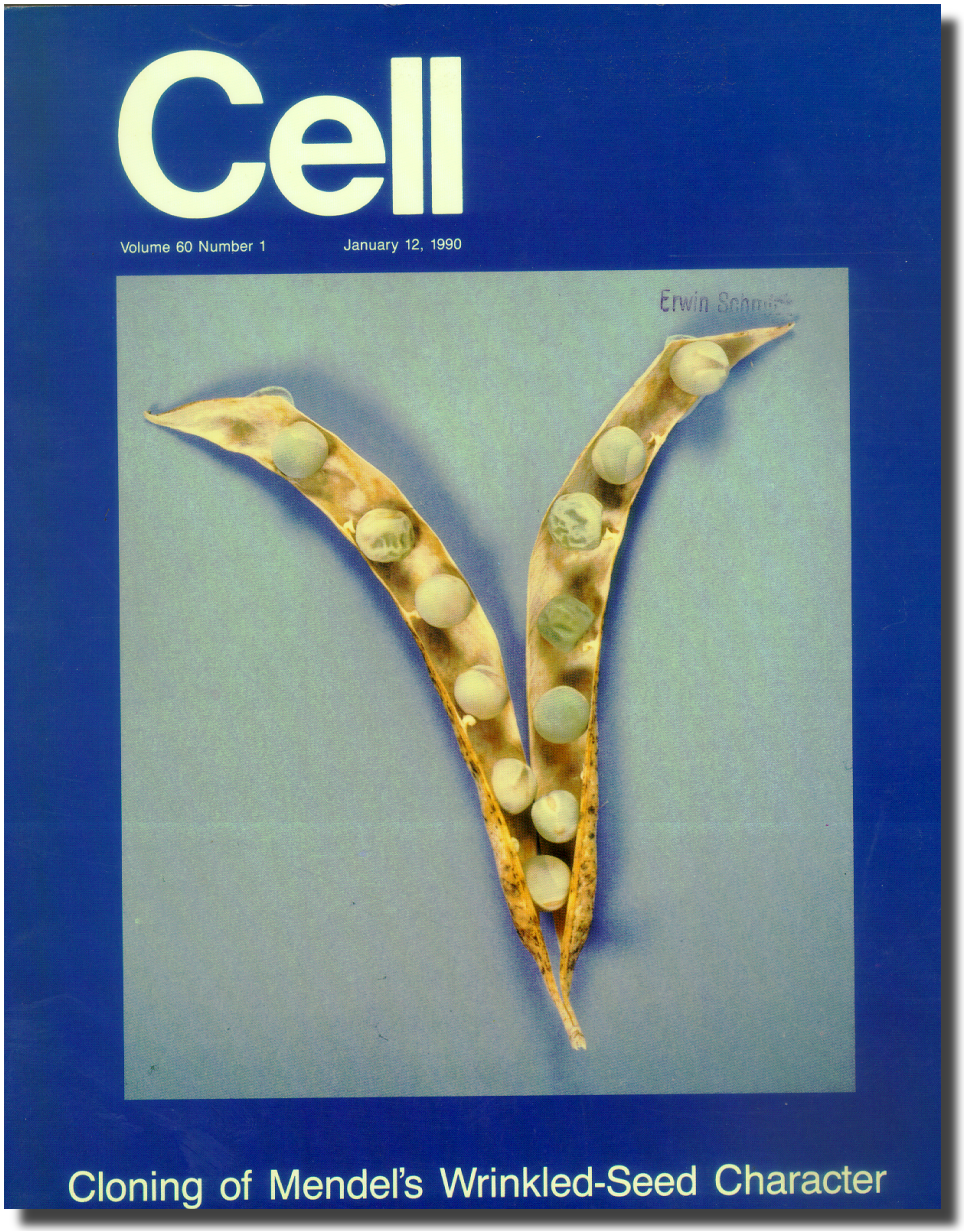
Instabilität ist speziesabhängig

Stability of an expanded trinucleotide repeat in the androgen receptor gene in transgenic mice

Peter M. Bingham[1], Marion O. Scott[2], Suping Wang[2], Michael J. McPhaul[3], Elizabeth M. Wilson[4], James Y. Garbern[2], Diane E. Merry[2] & Kenneth H. Fischbeck[2]

The expansion of trinucleotide repeat sequences underlies a number of hereditary neurological disorders. To study the stability of a trinucleotide repeat and to develop an animal model of one of these disorders, spinal and bulbar muscular atrophy (SBMA), we have generated transgenic mice carrying either the normal or expanded repeat human androgen receptor (AR) gene. Unlike the disease allele in humans, the AR cDNA containing the expanded repeat in transgenic mice showed no change in repeat length with transmission. Expression of the SBMA AR was found in transgenic mice, but at a lower level than normal endogenous expression. The lack of a physiological pattern of expression may explain why no phenotypic effects of the transgene were observed.

Nat. Genet. (1995) 9:191



Transposition

- Mendels r-Lokus („rugosus“)
- starch-branching enzyme (SBEI)

Cell (1990) 60: 115-122

Alu-Retroposons erzeugen Mutationen in menschlichen Genen

A *de novo Alu* insertion results in neurofibromatosis type 1

Margaret R. Wallace*, Lone B. Andersen, Ann M. Saulino, Paula E. Gregory†, Thomas W. Glover† & Francis S. Collins‡

Howard Hughes Medical Institute, and the Departments of Internal Medicine and Human Genetics, and †Pediatrics and Communicable Diseases, University of Michigan, 4570 MSRB II, 1150 W. Medical Center Drive, Ann Arbor, Michigan 48109-0650, USA

NEUROFIBROMATOSIS type 1 (NF1) is a common autosomal dominant disorder with a high mutation rate and variable expression, characterized by neurofibromas, *café-au-lait* spots, Lisch nodules of the iris, and less frequent features including bone deformities and learning disabilities[1]. The recently cloned *NF1* gene encodes a transcript of 13 kilobases from a ubiquitously expressed locus on chromosome 17 (refs. 2–4). Most NF1 patients are expected to have unique mutations, but only a few have so far been characterized, restricting genetic and functional information and the design of DNA diagnostics. We report an unusual *NF1* mutation, that of a *de novo Alu* repetitive element insertion into an intron, which results in deletion of the downstream exon during splicing and consequently shifts the reading frame. This previously undescribed mechanism of mutation indicates that *Alu* retrotransposition is an ongoing process in the human germ line.

The 31-year-old male patient (D.D.) exhibits several features of NF1, including one cutaneous neurofibroma, axillary freckling, Lisch nodules, cervical nerve root tumours, and macrocephaly. *Café-au-lait* spots are not present. His parents show no signs of NF1, and DNA fingerprinting analysis found no evidence of nonpaternity. Part of the *NF1* complementary DNA detected an abnormal Southern blot pattern in the patient's DNA after digestion with several restriction enzymes[2]. This was consistent with a small insertion (300–500 basepairs (bp)) in a 3.8-kilobase (kb) *Eco*RI fragment which contains six *NF1* exons

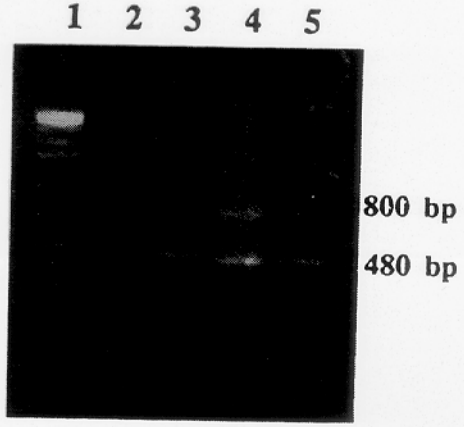


FIG. 1 Ethidium bromide staining of a 1.0% agarose gel demonstrates the insertion in the exon 6 PCR product. Lane 1 contains the BRL 1-kb ladder, lane 2 contains a water (negative) control, lanes 3 and 5 are products from the patient's father and mother, respectively, and the patient's PCR products are shown in lane 4. All show the normal fragment of 480 bp, but the patient also has an abnormal fragment of ~800 bp. DNA from both the patient's leukocytes and from an established lymphoblastoid line gave the same result (data not shown).

METHODS. Genomic DNA from the patient and his parents was extracted as described[2]. Genomic DNA (100–500 ng) was amplified using the exon 6 primers already described[3], with 35 cycles (each cycle entailed 1 min each at 94°C for denaturation, 65°C for annealing, and 72°C for extension) using standard buffers and reagents recommended by Cetus. One-tenth of each PCR reaction was loaded per lane.

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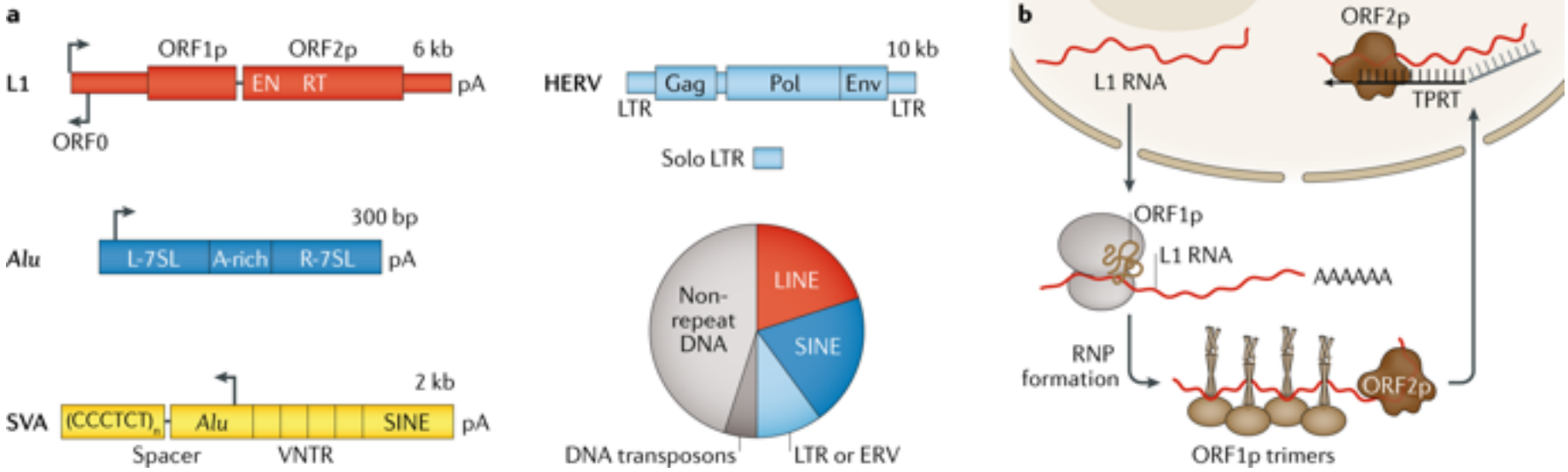
‡ To whom correspondence should be addressed.

Nat Rev Genet. Dec 2019

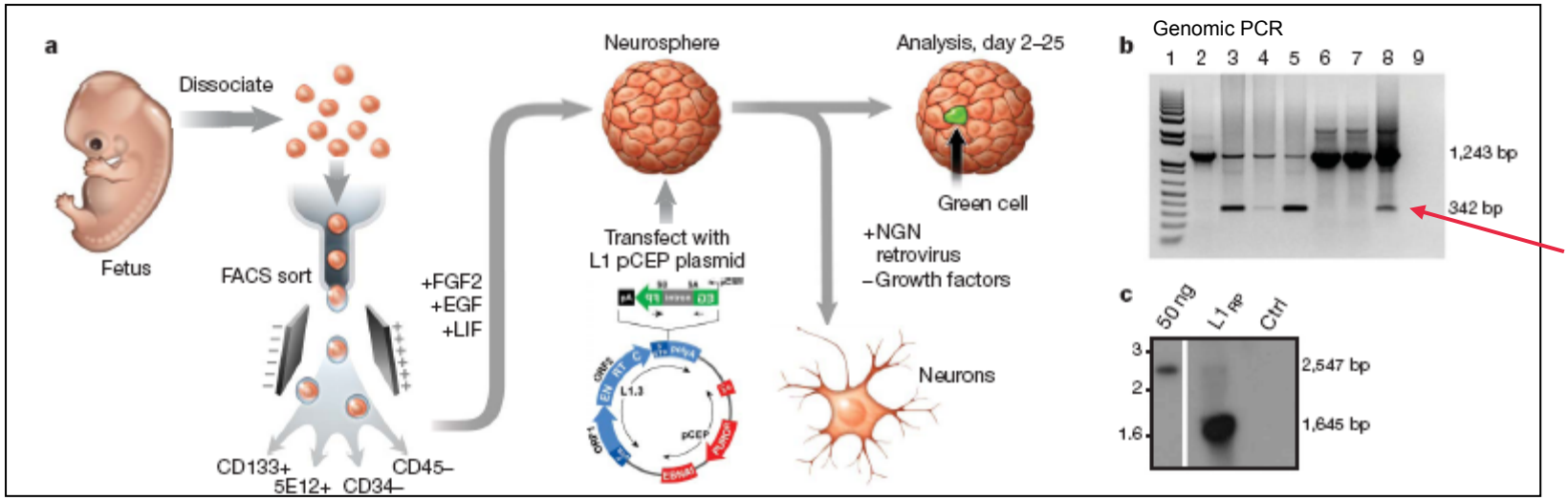
REGULATORY ELEMENTS

Transposable elements in human genetic disease

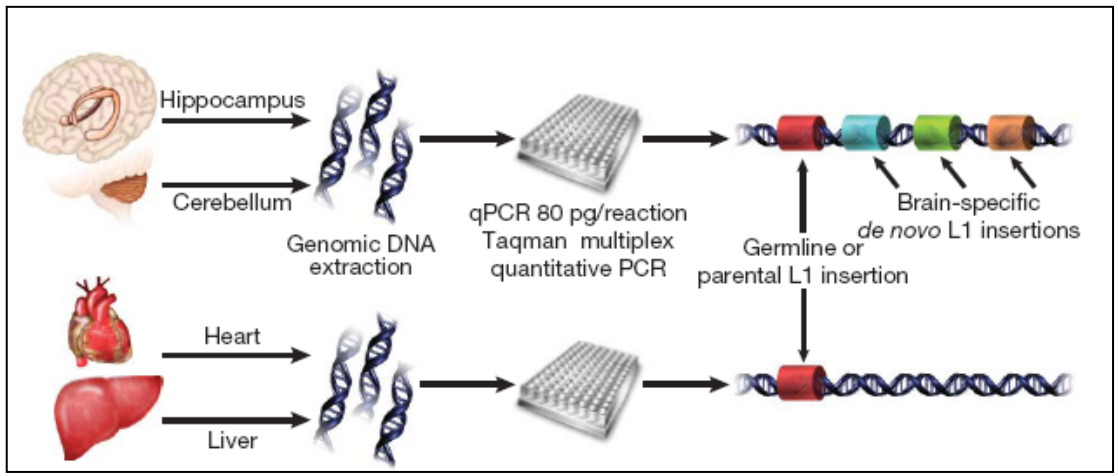
*Lindsay M. Payer[1] and Kathleen H. Burns[1,2]**



L1 Transposons may shape our brain



Somatic (!!) transposition events



ca. 100 additional L1 transposons per neuron?

Does somatic transposition affect neuronal function and diversity??

Coufal et al. 2009; Kurnosov et al. 2015

Transposition als Artefakt beim Klonieren

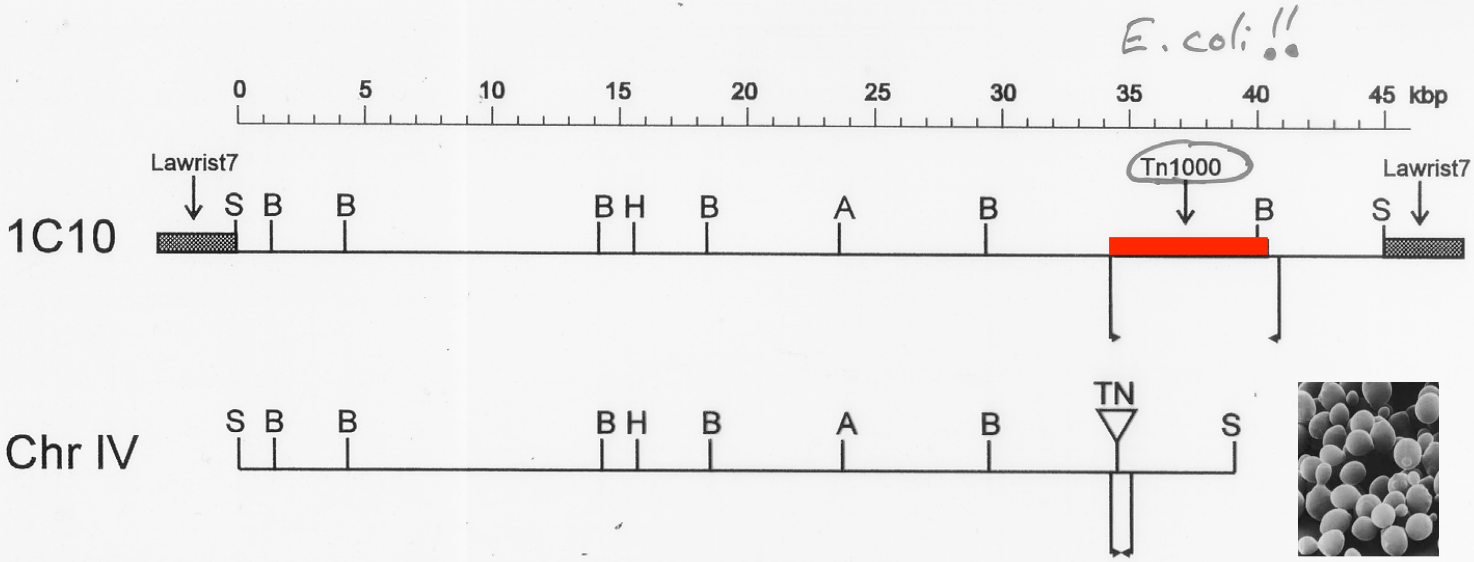


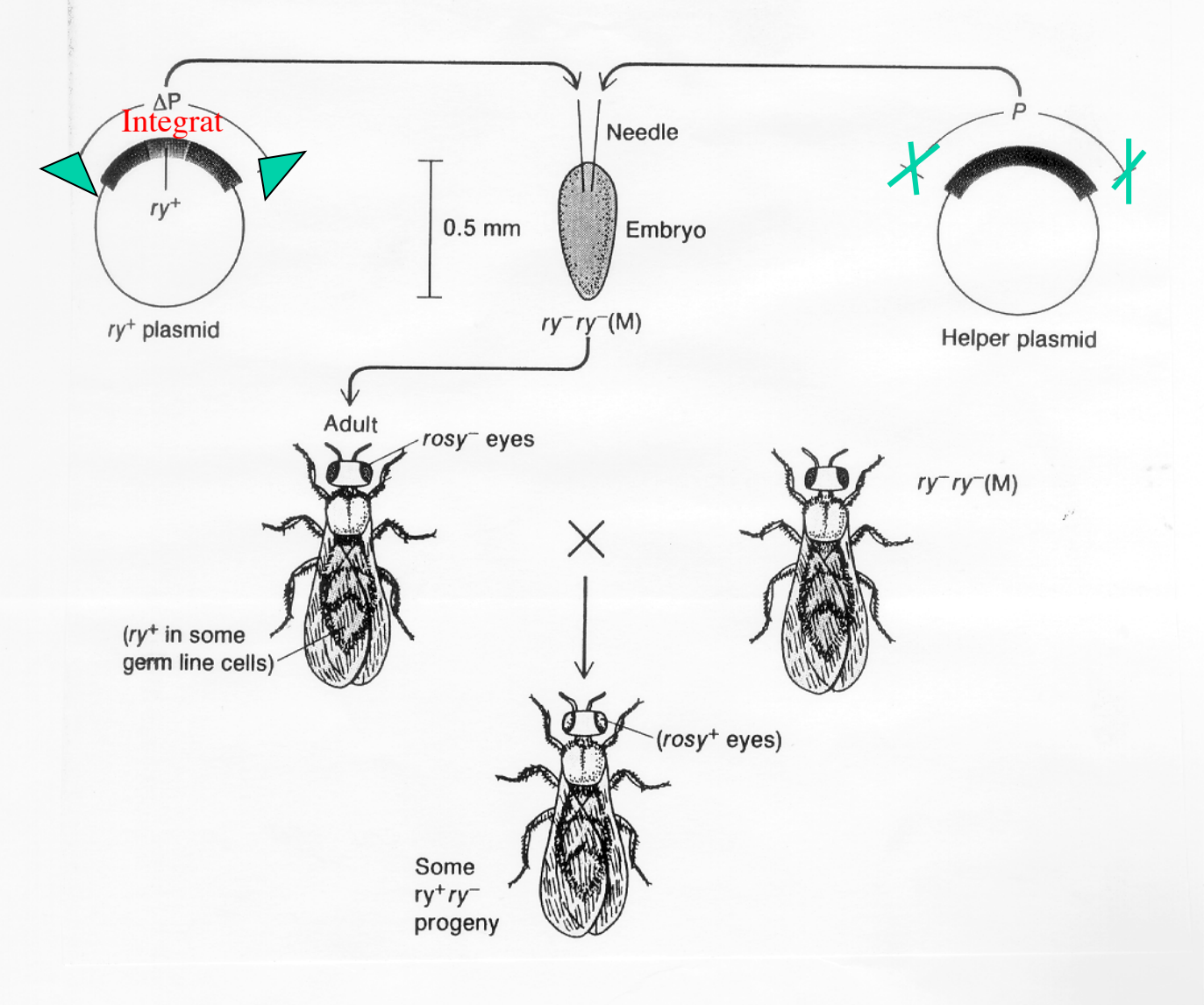
Abb. 3.11: Bestimmung des Integrationsorts des Transposons 1000 mittels PCR

Mit Hilfe von Oligonukleotidprimern, die links und rechts des Transposons im Cosmid liegen, konnte der Integrationsort des Transposons aus genomischer Hefe-DNA mittels PCR amplifziert werden. Mit diesen Oligonukleotidprimern, die im Cosmid 6,4 kbp auseinanderliegen, amplifiziert man aus genomischer DNA ein 395 bp langes Fragment. Das Transposon ist also in genomischer Hefe-DNA nicht vorhanden. Aus dem Vergleich der Nukleotidsequenz des Amplifizierungsprodukts mit der des Cosmids 1C10 ließ sich der Integrationsort des Transposons exakt bestimmen. Das Transposon wird von einer 5 bp umfassenden "target site"Duplikation flankiert.

A = Acc I, B = Bam HI, H = Hind III, S = Sau 3AI, ▬ = Transposon 1000, ▬ = Vektoranteil des Cosmids (Lawrist7)

TN ▽ = Integrationsort des Transposons 1000 im Cosmid 1C10, ↓ ↓ = Position der Oligonukleotidprimer

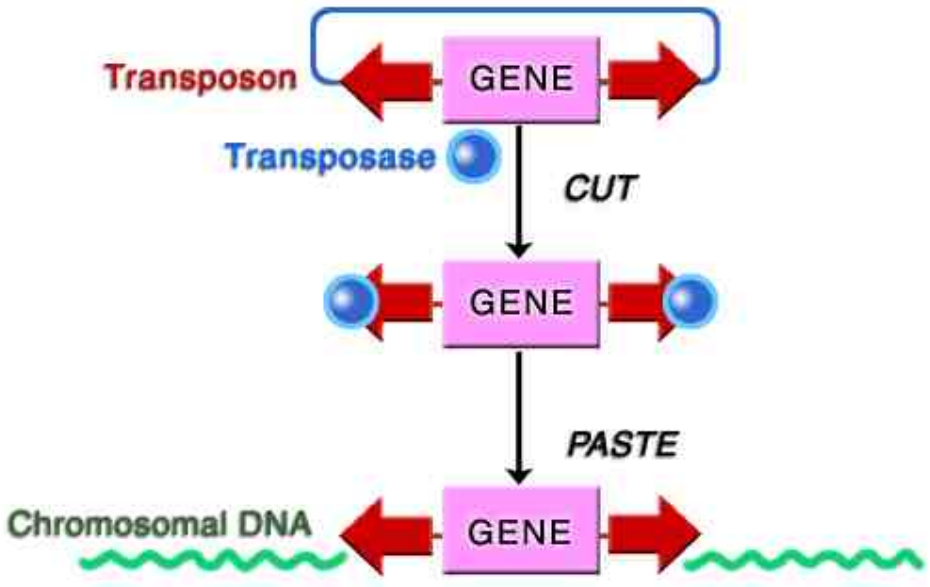
Transposons als Vektoren



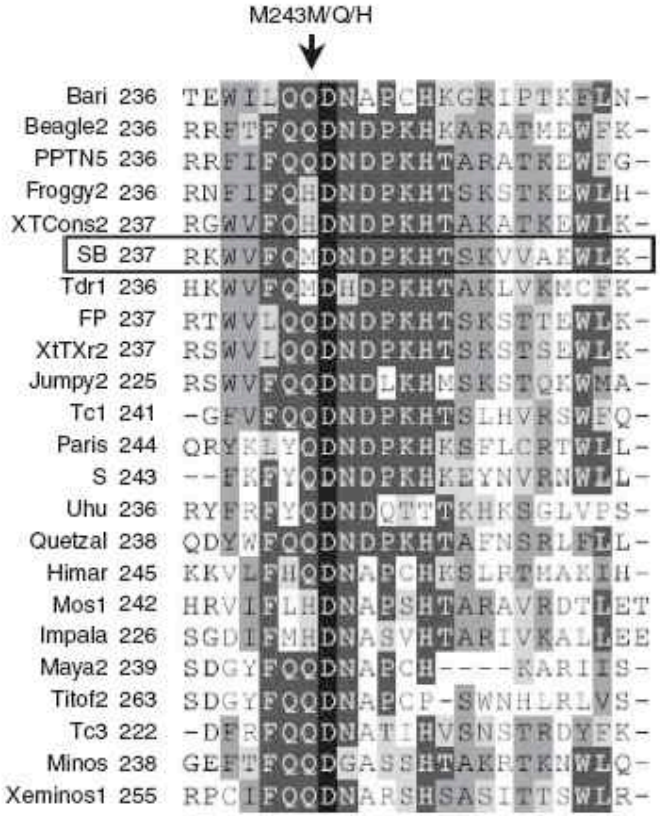
...auch für Gentherapie!

Sleeping Beauty - ein synthetisches Transposon-System

Ivics et al. 1997



Transfektion durch hydrodynamische Injektion (z.B. in Leber in vivo)



Optimierung der Transposase durch Molekulare Evolution

Instabilität durch Horizontalen Gentransfer

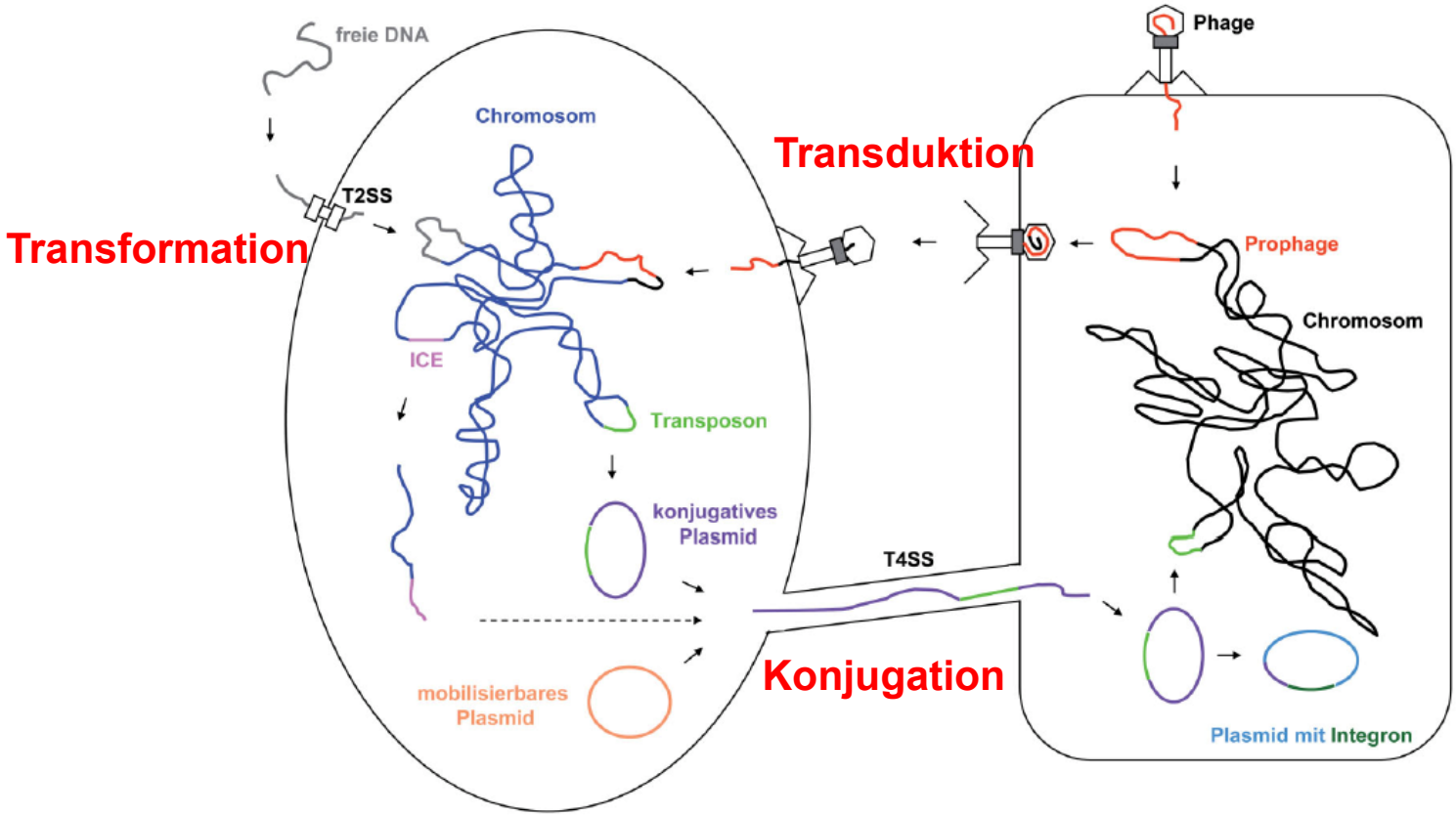


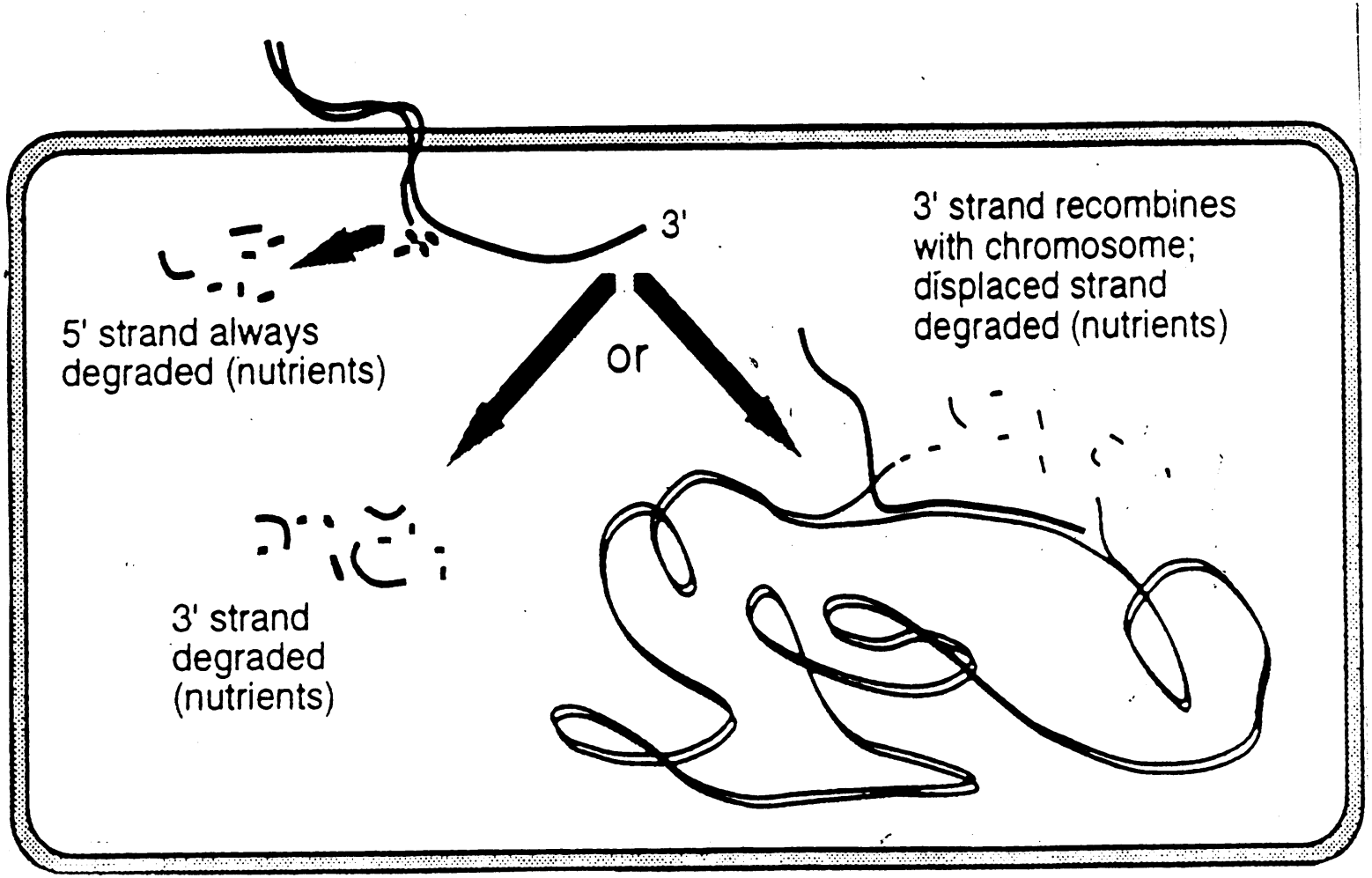
ABB. 1 Mechanismen des horizontalen Gentransfers in Bakterien. Die natürliche ▸ Kompetenz zur Aufnahme von freier DNA aus der Umwelt wird häufig über Typ II-Transportsysteme (T2SS) vermittelt, der konjugative Transfer von DNA erfolgt über Typ IV-Transportsysteme (T4SS). ICEs (integrative und konjugative Elemente) und konjugative Plasmide sind Beispiele für konjugativ übertragbare genetische Elemente. Nichtkonjugative Plasmide können durch konjugative Elemente mobilisiert werden. Ein lysogener ▸ Phage kann nach Integration ins Wirtsgenom (Prophage) und anschließender Replikation Chromosomenabschnitte mitverpacken und an spätere Wirtszellen weitergeben (Transduktion). Transponierbare genetische Elemente wie Transposons und ▸ IS-Elemente können ihre Lokalisation zwischen verschiedenen genetischen Elementen wechseln. ▸ Integrons sind selbst nur in Kombination mit ▸ MGEs (mobilen genetischen Elementen) mobil, können aber Genkassetten aufnehmen.

HGT bei Mikroorganismen nachgewiesen...

- in Abwasser
- in Flußwasser
- in der Rhizosphäre
- im Boden
- in Nahrungsmitteln (Käse)
- im menschlichen Darm

Duncan et al. (1992) Genetic exchange in natural microbial communities. Adv Microbial Ecol

Genes for Breakfast...



Nature (2000) 405, 299

review article

Lateral gene transfer and the nature of bacterial innovation

Howard Ochman*, Jeffrey G. Lawrence† & Eduardo A. Groisman‡

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Unlike eukaryotes, which evolve principally through the modification of existing genetic information, bacteria have obtained a significant proportion of their genetic diversity through the acquisition of sequences from distantly related organisms. Horizontal gene transfer produces extremely dynamic genomes in which substantial amounts of DNA are introduced into and deleted from the chromosome. These lateral transfers have effectively changed the ecological and pathogenic character of bacterial species.

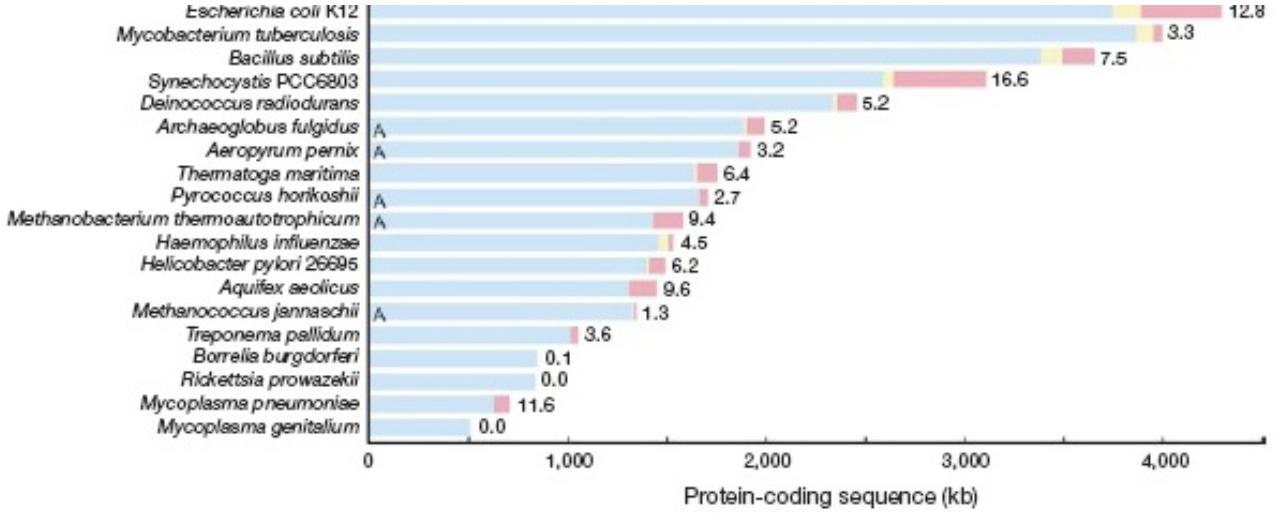


Figure 2 Distribution of horizontally acquired (foreign) DNA in sequenced bacterial genomes. Lengths of bars denote the amount of protein-coding DNA. For each bar, the native DNA is blue; foreign DNA identifiable as mobile elements, including transposons and bacteriophages, is yellow, and other foreign DNA is red. The percentage of foreign DNA is noted to the right of each bar. 'A' denotes an Archaeal genome.

HGT bei pathogenen Bakterien

BGI Sequencing news: German EHEC strain is a chimera created by horizontal gene transfer

by David Tribe on 2 June 2011

Molecular genetics in China is providing answers in the frantic effort to solve the urgent food safety crisis in EU.

The chimera of Arezzo (courtesy of Wikipedia/Lucarelli)

Rapid work in China has applied third generation DNA decoding technologies to decode the German outbreak disease bacterium genome. It has revealed the germ to be a hybrid (which can be described alternatively as a chimera, a true natural GMO). But before readers get excited about what this implies, they need to consider that all *E. coli* strains are chimeras.

The novel germ has some virulence abilities of a class of pathogenic *E. coli bacteria* called entero-aggregative *E. coli* (#EAEC). It has similarities to a bacterial strain called EAEC 55989 , which was isolated in the Central African Republic and is known to cause serious diarrhea. EAEC typically carry extra mini-chromosomes called plasmids. The German outbreak strain has the typical plasmid genes of EAEC bacteria as well as shigatoxin genes seen in EHEC (sometimes called STEC, or

VTEC) germs

...und bei Eukaryoten?

Fliegengene beim Menschen

Überwindung der Artenschranke / Auslöser für Nervenleiden

Das Erbgut ist nicht starr, sondern befindet sich in ständigem Fluß. Es gibt Gene, deren Position innerhalb der Erbmasse nicht festgelegt ist. Sie springen gleichsam umher. Solche „springenden Gene" wurden bei vielen Lebewesen entdeckt, auch beim Menschen. Es kann mitunter sogar vorkommen, daß diese mobilen genetischen Elemente von einer Spezies zur anderen übertragen werden. Amerikanische Forscher haben jetzt ein Gen aus der Fliege im menschlichen Erbgut entdeckt, wo es neurologische Erkrankungen hervorrufen kann.

Das springende Gen mit Namen „mariner" enthält den genetischen Code für ein Enzym, das die gesamte Einheit aus dem Erbgut ausschneidet und an anderer Stelle wieder einsetzt. Auf den Menschen ist dieses Fliegengen wahrscheinlich durch Viren

Tooth-Syndrom liegt eine der beiden Kopien dieses Gens verdoppelt vor, bei der Neuropathie fehlt sie dagegen.

Duplikationen und Deletionen dieser Art können im Laufe der Reifeteilung von Eizellen und Spermien auftreten. Bei diesem Vorgang lagern sich die beiden Partner jedes Chromosomenpaares aneinander und tauschen Abschnitte vom Erbgut aus. Das erhöht die genetische Variabilität und ist somit durchaus von Nutzen für das langfristige Überleben einer Spezies. Vereinzelt kann es jedoch vorkommen, daß sich die gleichartigen Chromosomen nicht exakt aneinanderlagern. Nach der Zellteilung trägt dann eine Tochterzelle zwei Kopien des gleichen Abschnitts, während der anderen dieser Bereich verlorengeht.

Die beiden erblichen Nervenleiden sind

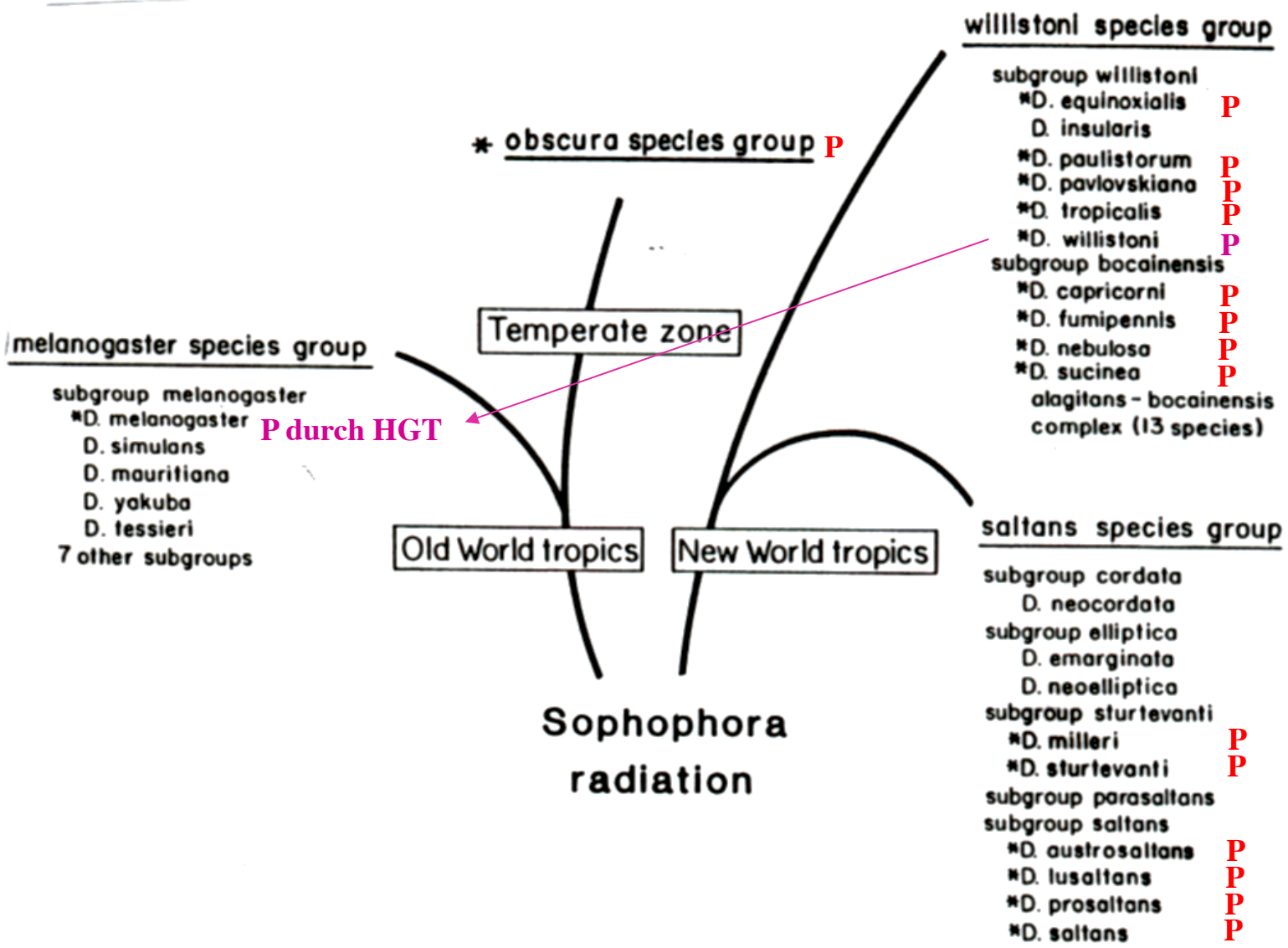
Auch falsch: „300 Bakteriengene im Humangenom“

Table 24 Probable vertebrate-specific acquisitions of bacterial genes

Human protein (accession)	Predicted function	Known orthologues in other vertebrates	Bacterial homologues		Human origin confirmed by PCR
			Range	Best hit	
AAG01853.1	Formiminotransferase cyclodeaminase	Pig, rat, chicken	*Thermotoga*, *Thermoplasma*, *Methylobacter*	*Thermotoga maritima*	Yes
CAB81772.1 AAB59448.1	Na/glucose cotransporter	Rodents, ungulates	Most bacteria	*Vibrio parahaemolyticus*	Yes (CAB81772, AAC41747.1) NT* (AAB59448.1, AAA36608.1)
AAA36608.1 AAC41747.1					
BAA1143.21	Epoxide hydrolase (α/β-hydrolase)	Mouse, *Danio*, fugu fish	Most bacteria	*Pseudomonas aeruginosa*	Yes
CAB59628.1	Protein-methionine-*S*-oxide reductase	Cow	Most bacteria	*Synechocystis sp.*	Yes
BAA91273.1	Hypertension-associated protein SA/ acetate-CoA ligase	Mouse, rat, cow	Most bacteria	*Bacillus halodurans*	NT*
CAA75608.1	Glucose-6-phosphate transporter/ glycogen storage disease type 1b protein	Mouse, rat	Most bacteria	*Chlamydophila pneumoniae*	Yes
AAA59548.1 AAB27229.1	Monoamine oxidase	Cow, rat, salmon	Most bacteria	*Mycobacterium tuberculosis*	Yes
AAF12736.1	Acyl-CoA dehydrogenase, mitochondrial protein	Mouse, rat, pig	Most bacteria	*P. aeruginosa*	Yes
AAA51565.1 IGI_M1_ctg19153_147	Aldose-1-epimerase	Pig (also found in plants)	*Streptomyces*, *Bacillus*	*Streptomyces coelicolor*	Yes

Nature 2001

P-Transposons: HGT vor ca. 70 Jahren



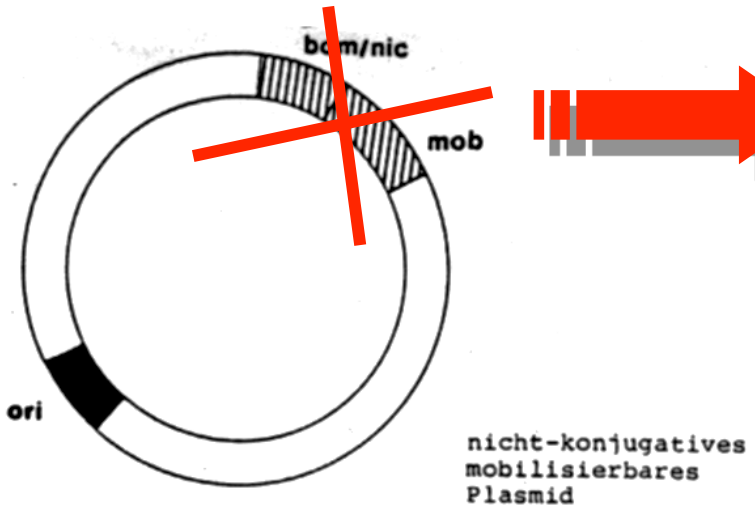
BIUZ 5/2008

HGT: weitere Fälle

BEISPIELE FÜR HGT ZWISCHEN ENTWICKLUNGSGESCHICHTLICH WEIT ENTFERNTEN ORGANISMEN

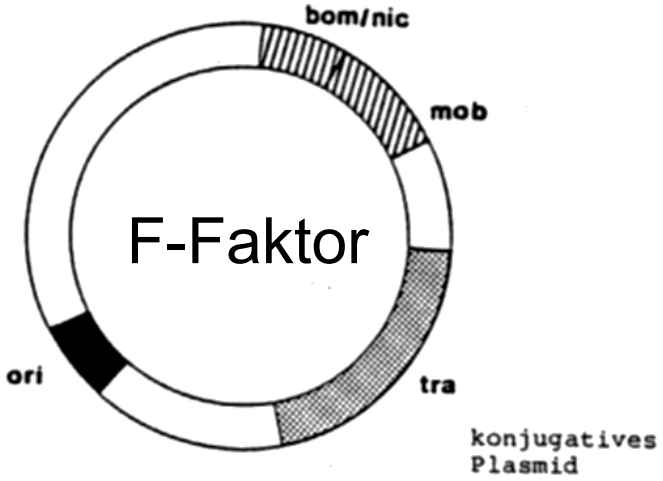
Donor	Rezipient	Besonderheiten
Agrobacterium tumefaciens	mindestens 90 dikotyledone Pflanzenfamilien	Übertragung einer tumorinduzierenden DNA über ein Typ IV-Transportsystem, Weitervererbung nur bei Keimbahntransfer.
Wolbachia	*Drosophila ananassae*	Integration eines fast vollständigen *Wolbachia*-Genoms in das Genom einer Taufliege; Expression einzelner Gene gezeigt.
Fibrobacter succinogenes (Bakterium im Pansen von Wiederkäuern)	*Orpinomyces joyonii* (Pilz im Pansen von Wiederkäuern)	Verschiedene Glycosylhydrolase-Gene zum Abbau von Cellulose und anderen pflanzlichen Polysacchariden, Pansen als gemeinsames Habitat von Bakterium und Pilz mit extrem hohen Populationsdichten.
Magnaporthe grisea (Filamentöser Pilz)	*Phytophthora* (Oomyctes)	HGT zwischen entfernt verwandten Eukaryoten.
E. coli (gramnegativ)	*Clostridium* (grampositiv)	Konjugativer Transfer von Plasmiden zwischen weit entfernten Bakterienarten; für genetische Manipulationen nutzbar.
Verschiedene Bakterien und Eukaryoten	Phycodnaviren (infizieren z.B. Algen), Mimiviren (infizieren z.B. Amöben)	Große Viren mit Genomgrößen zwischen 0,3 und 1,2 Megabasen, die sowohl bakterielle als auch eukaryotische Gene erworben haben.
Verschiedene Bakterien	*Methanosarcina mazei* (Archaee)	30% des Genoms der Archaee *Methanosarcina mazei* stammt vermutlich aus dem Reich der Bakterien.
Verschiedene Archaeen	Verschiedene Bakterien	Die systematische Analyse der Genome von 73 pathogenen Bakterien erbrachte mindestens 43 Gentransfers aus dem Reich der Archaeen, vor allem von Stoffwechselgenen.
Eukaryot	*Microcystis aeruginosa* (Cyanobakterium)	Transfer eines eukaryotischen Aktin- und Profilin-Gens in ein Bakterium; aktive Expression und Funktionswandel; nur in individuellem Isolat.

Sicherheit moderner Plasmide



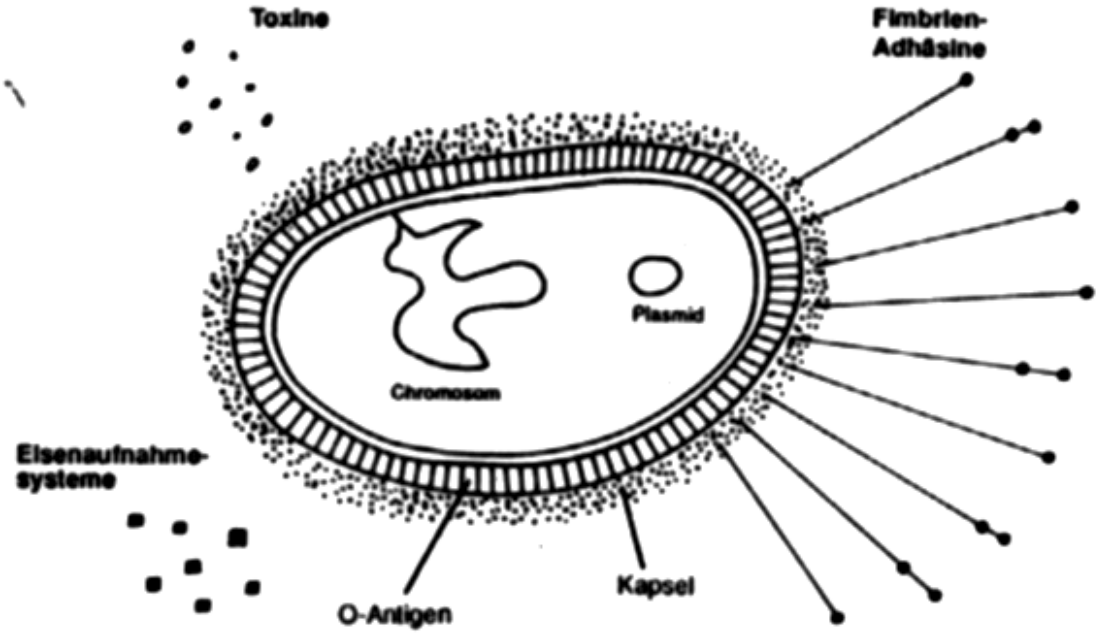
pUC18

- nicht-konjugativ
- nicht mobilisierbar



- konjugativ

Sicherheit von E. coli K12



- keine Toxine
- keine Fimbrien-Adhäsine
- keine Eisenaufnahmesysteme
- keine Kapsel
- reduziertes O-Antigen
- keine Plasmide
- kleineres Genom

Sicherheit von E. coli K12

letters to nature

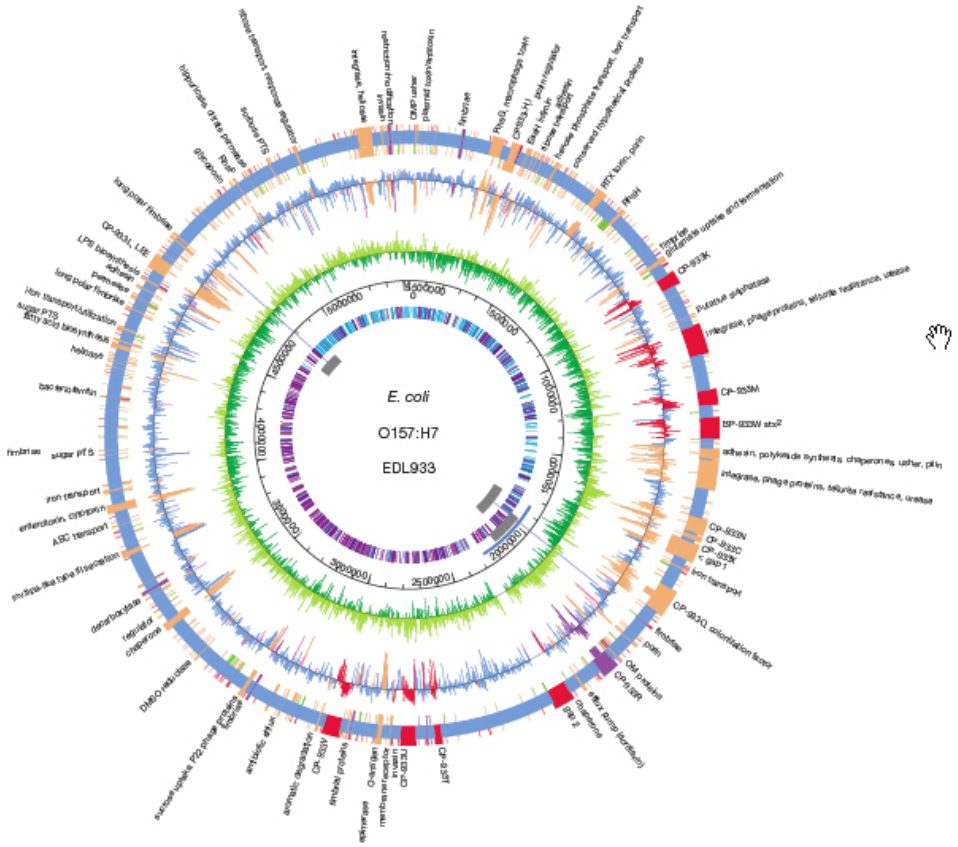
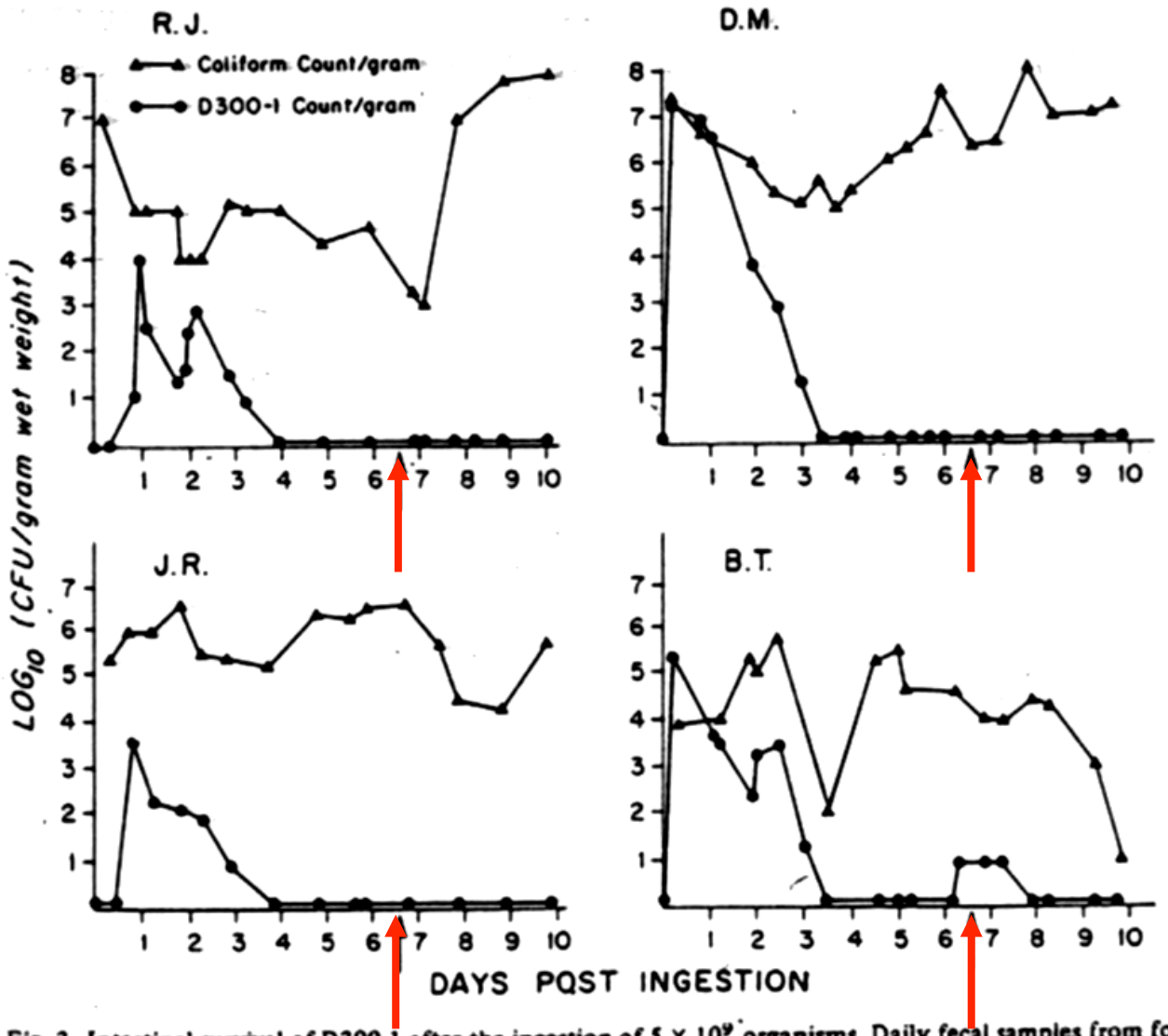


Figure 1 Circular genome map of EDL933 compared with MG1655. Outer circle shows the distribution of islands: shared co-linear backbone (blue); position of EDL933-specific sequences (O-islands) (red); MG1655-specific sequences (K-islands) (green); O-islands and K-islands at the same locations in the backbone (tan); hypervariable (purple). Second circle shows the G+C content calculated for each gene longer than 100 amino acids, plotted around the mean value for the whole genome, colour-coded like outer circle. Third circle shows the GC skew for third-codon position, calculated for each gene longer than 100 amino acids: positive values, lime; negative values, dark green. Fourth circle gives the scale in base pairs. Fifth circle shows the distribution of the highly skewed octamer Chi (GCTGGTGG), where bright blue and purple indicate the two DNA strands. The origin and terminus of replication, the chromosomal inversion and the locations of the sequence gaps are indicated. Figure created by Genvision from DNASTAR.

Pathogener Stamm O157:H7 (EDL933) hat

1387 Gene mehr

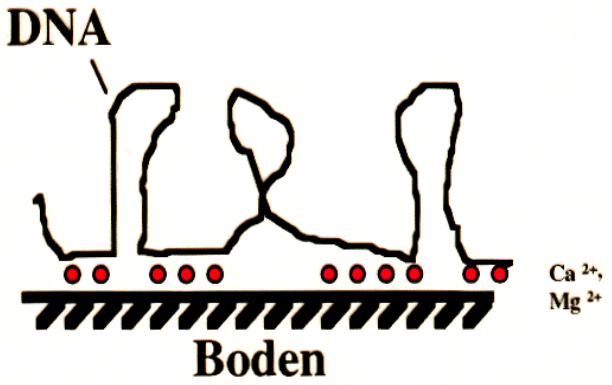
als E. coli K12!!

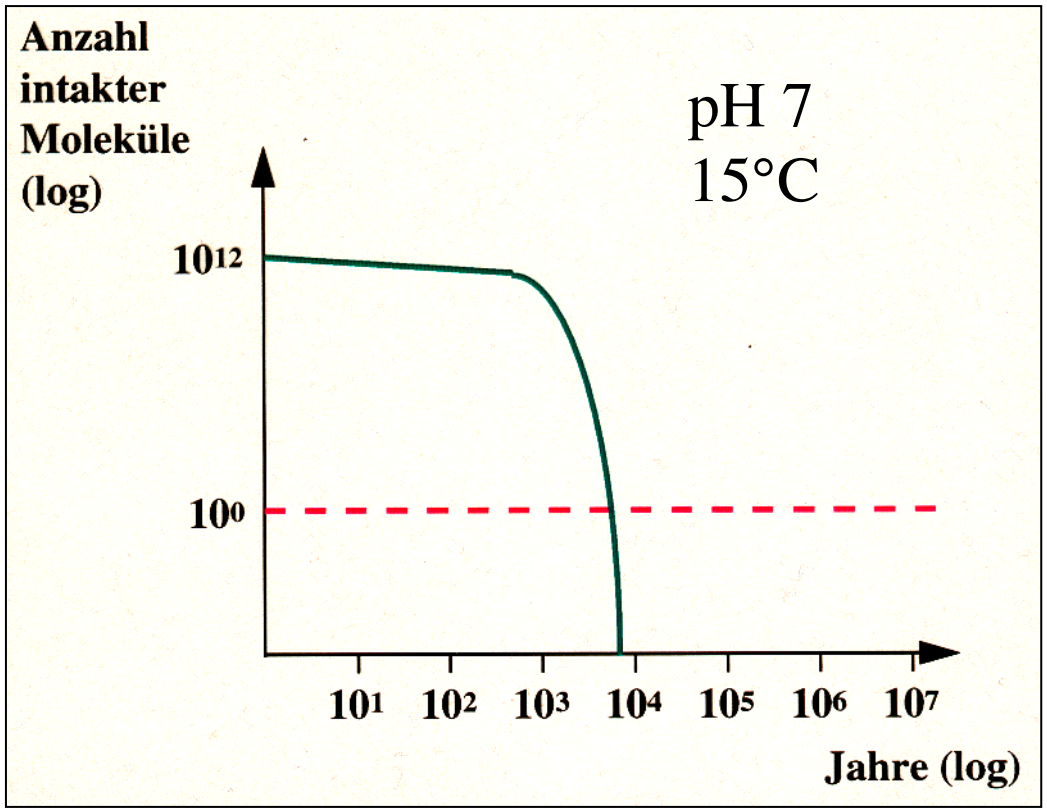


GENE 14, 145-154

Fig. 3. Intestinal survival of D300-1 after the ingestion of 5×10^9 organisms. Daily fecal samples from four human volunteers (see Table II) were analyzed quantitatively and qualitatively according to the protocol outlined in Fig. 1. The minimal detectable level by quantitative analyses was 10 cfu (colony-forming units)/g (wet weight) feces. Arrow (↑) denotes the initiation of tetracycline therapy (1.5 g orally over 1.5 days).

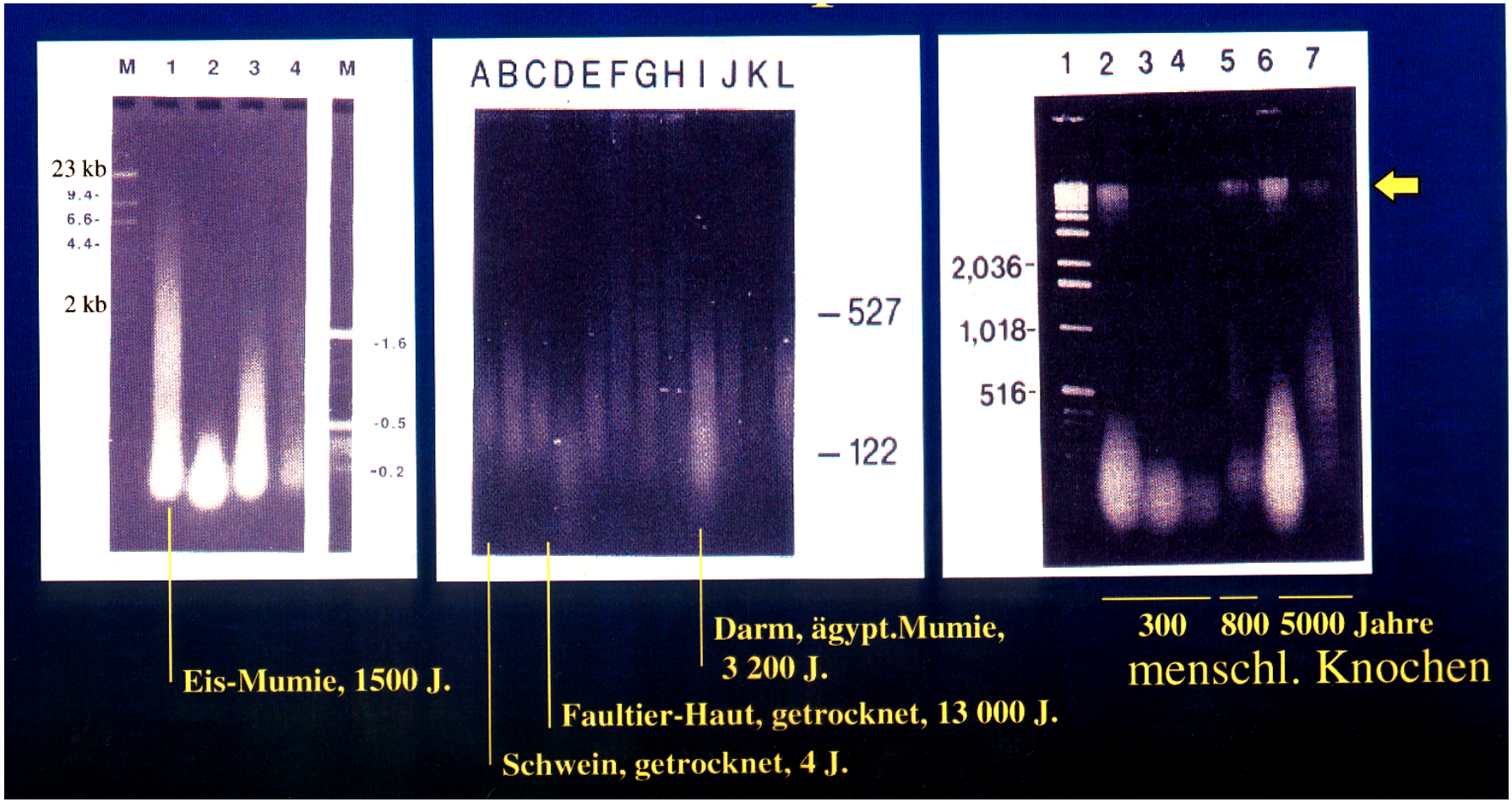
DNA-Stabilität in der Umwelt



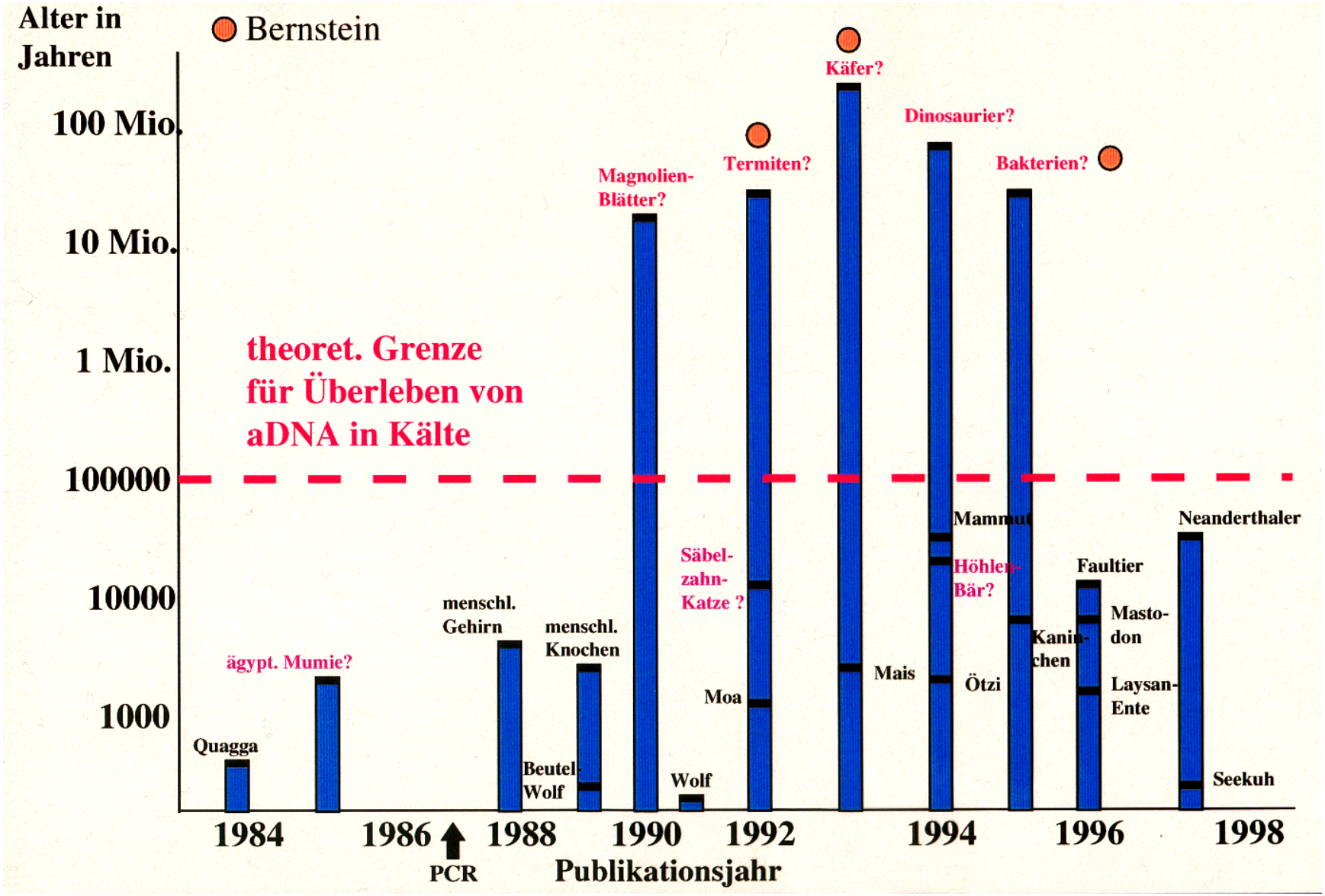


Nach 6000 Jahren Moleküle weitgehend depuriniert

Stabilität von ancient DNA



aDNA: Science und Fiction



LETTER

doi:10.1038/nature12323

Recalibrating *Equus* evolution using the genome sequence of an early Middle Pleistocene horse

Ludovic Orlando[1]*, Aurélien Ginolhac[1]*, Guojie Zhang[2]*, Duane Froese[3], Anders Albrechtsen[4], Mathias Stiller[5], Mikkel Schubert[1], Enrico Cappellini[1], Bent Petersen[6], Ida Moltke[4,7], Philip L. F. Johnson[8], Matteo Fumagalli[9], Julia T. Vilstrup[1], Maanasa Raghavan[1], Thorfinn Korneliussen[1], Anna-Sapfo Malaspinas[1], Josef Vogt[6], Damian Szklarczyk[10]†, Christian D. Kelstrup[10], Jakob Vinther[11]†, Andrei Dolocan[12], Jesper Stenderup[1], Amhed M. V. Velazquez[1], James Cahill[5], Morten Rasmussen[1], Xiaoli Wang[2], Jiumeng Min[2], Grant D. Zazula[13], Andaine Seguin-Orlando[1,14], Cecilie Mortensen[1,14], Kim Magnussen[1,14], John F. Thompson[15], Jacobo Weinstock[16], Kristian Gregersen[1,17], Knut H. Røed[18], Véra Eisenmann[19], Carl J. Rubin[20], Donald C. Miller[21], Douglas F. Antczak[21], Mads F. Bertelsen[22], Søren Brunak[6,23], Khaled A. S. Al-Rasheid[24], Oliver Ryder[25], Leif Andersson[20], John Mundy[26], Anders Krogh[1,4], M. Thomas P. Gilbert[1], Kurt Kjær[1], Thomas Sicheritz-Ponten[6,23], Lars Juhl Jensen[10], Jesper V. Olsen[10], Michael Hofreiter[27], Rasmus Nielsen[28], Beth Shapiro[5], Jun Wang[2,26,29,30] & Eske Willerslev[1]

The rich fossil record of equids has made them a model for evolutionary processes[1]. Here we present a 1.12-times coverage draft genome from a horse bone recovered from permafrost dated to approximately 560–780 thousand years before present (kyr BP)[2,3]. Our data represent the oldest full genome sequence determined so far by almost an order of magnitude. For comparison, we sequenced the genome of a Late Pleistocene horse (43 kyr BP), and modern genomes of five domestic horse breeds (*Equus ferus caballus*), a Przewalski's horse (*E. f. przewalskii*) and a donkey (*E. asinus*). Our analyses suggest that the *Equus* lineage giving rise to all contemporary horses, zebras and donkeys originated 4.0–4.5 million years before present (Myr BP), twice the

from an interglacial organic unit associated with the Gold Run volcanic ash, dated to 735 ± 88 kyr BP[2,3] (Fig. 1b). Relict ice wedges below the unit indicate persistent permafrost since deposition (Supplementary Information, section 1.1), whereas the organic unit, hosting the fossil, indicates a period of permafrost degradation, or a thaw unconformity[7], during a past interglacial as warm or warmer than present[3], and rapid deposition during either marine isotope stage 19, 17 or 15. This indicates that the fossil dates to approximately 560–780 kyr BP. The metapodial shows typical caballine morphology, consistent with Middle rather than the smaller Late Pleistocene horse fossils from the area (Fig. 1c and Supplementary Information, section 1.2). This age is con-

RESEARCH ARTICLE

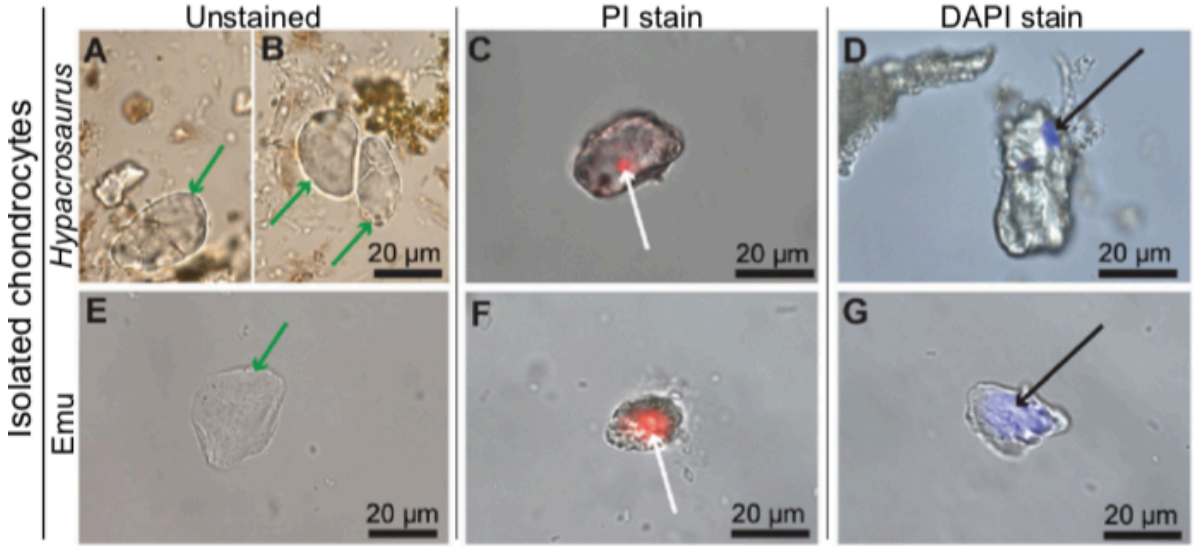
National Science Review
0: 1–8, 2020
doi: 10.1093/nsr/nwz206
Advance access publication 12 January 2020

MOLECULAR BIOLOGY & GENETICS

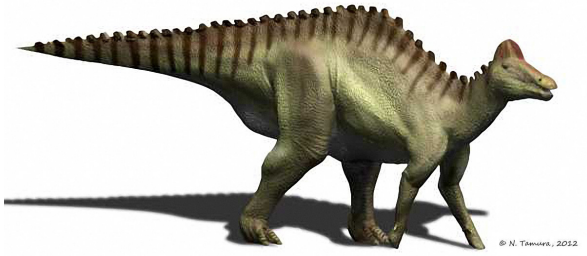
Evidence of proteins, chromosomes and chemical markers of DNA in exceptionally preserved dinosaur cartilage

Key Laboratory of
ertebrate Evolution
nd Human Origins,
nstitute of Vertebrate
aleontology and
aleoanthropology,

Alida M. Bailleul [1,2,*], Wenxia Zheng[3], John R. Horner[4], Brian K. Hall[5], Casey M. Holliday[6] and Mary H. Schweitzer[3,7,8]



Wird der Traum wahr?



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0099-2240/93/030712-06$02.00/0

Vol. 59, No. 3

Abundance of Virus-Sized Non-DNase-Digestible DNA (Coated DNA) in Eutrophic Seawater

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Received 4 September 1992/Accepted 8 December 1992

Total DNA concentration in 0.2-μm-pore-size Nuclepore filter filtrates (<0.2-μm fraction) of Tokyo Bay water was estimated to be 9 to 19 ng/ml by an immunochemical quantification method. Almost 90% of the DNA in the <0.2-μm fraction was found in the size fractions larger than 3.0×10^5 Da and 0.03 μm, and most was not susceptible to DNase digestion, that is, consisted of non-DNase-digestible DNA (coated DNA). A significant amount of DNA was obtained from the <0.2-μm fraction of the seawater by three different methods: polyethylene glycol precipitation, direct ethanol precipitation, and ultrafilter concentration. Gel electrophoresis analysis of the isolated DNAs showed that they consisted mainly of coated DNAs with a similar molecular sizes (20 to 30 kb [1.3×10^7 to 2.0×10^7 Da). The abundance of the ultramicron virus-sized coated DNA in natural seawater suggests that these DNA-rich particles can be attributed to marine DNA virus assemblages and that they may be a significant phosphorus reservoir in the environment.

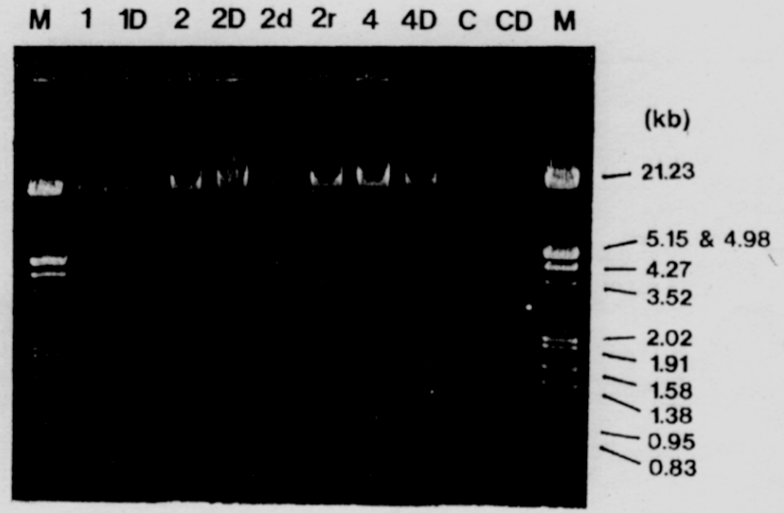
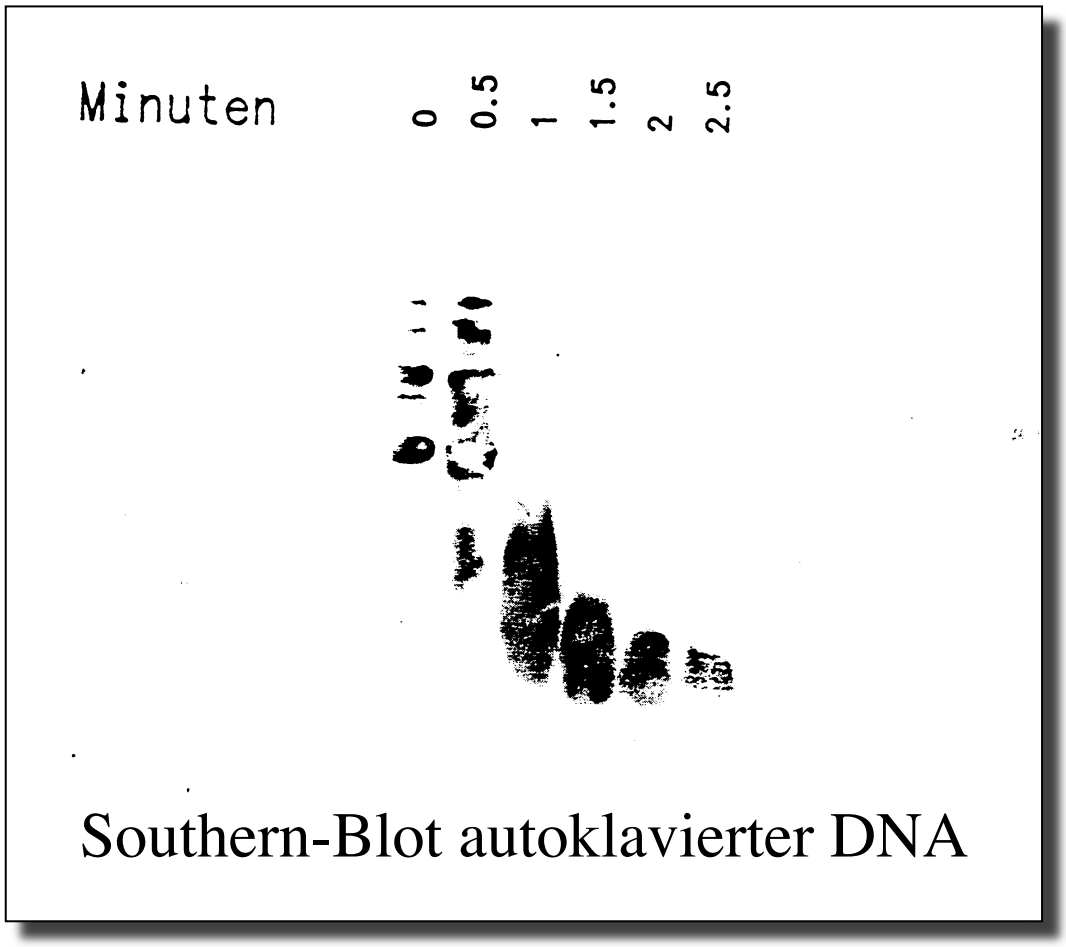


FIG. 2. Gel electrophoresis of DNA materials isolated by 10% PEG precipitation from the <0.1-μm (lanes 1 and 1D), <0.2-μm (lanes 2, 2D, 2d, and 2r), and <0.4-μm (lanes 4 and 4D) fractions of Tokyo Bay water (sample taken on 10 June 1991). Samples were treated with DNase before addition of EDTA and SDS (D) or with DNase (d) or RNase (r) immediately before electrophoresis. Lanes C and CD, reagent control; lanes M, λ phage DNA *Eco*RI and *Hin*dIII digests.

DNA in der Umwelt

Verhinderung „genetischer“ Umweltverschmutzung



Southern-Blot autoklavierter DNA

- nicht vom GenTG gefordert
- aber gute Laborpraxis

Typisierung von GVOs mit molekularbiologischen Techniken

- PCR — Amplifikation variabler DNA-Bereiche
- Hybridisierung — (DNA-Fingerprinting, Spezies-DotBlot)
- Sequenzanalyse — NGS !!

PCR-Nachweis von E. coli K12

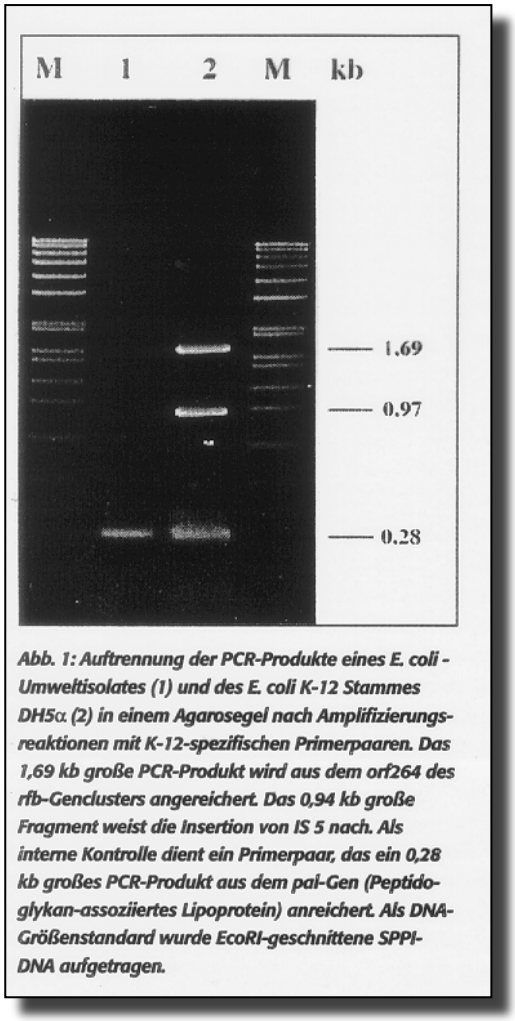
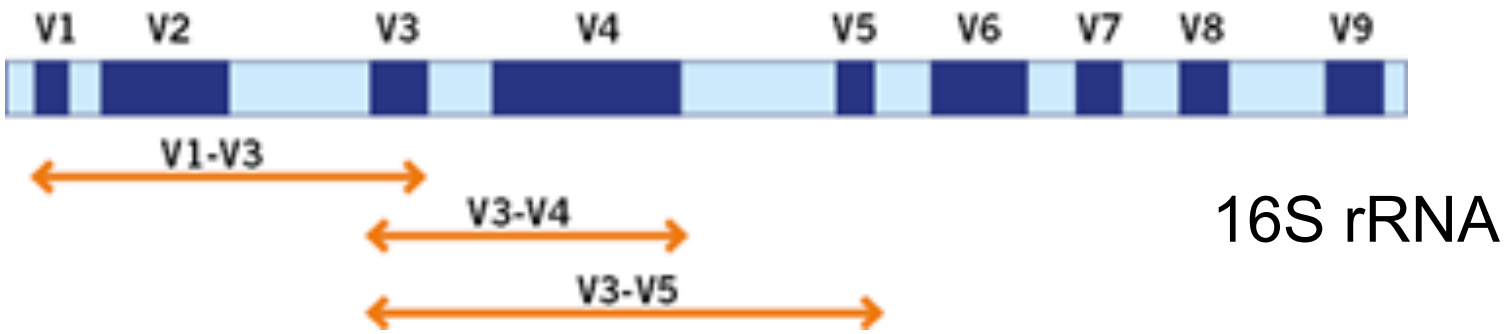


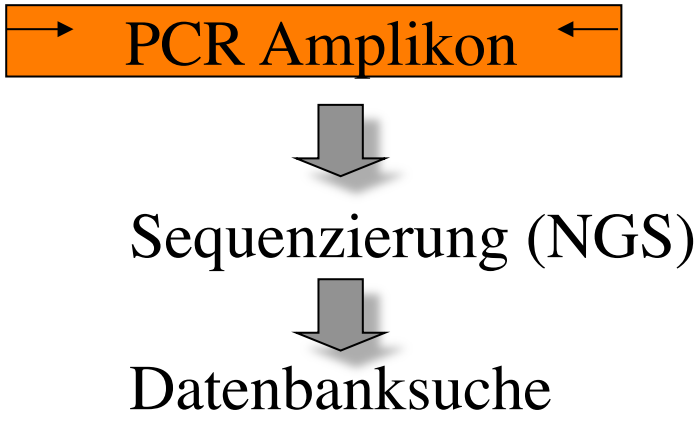
Abb. 1: Auftrennung der PCR-Produkte eines E. coli - Umweltisolates (1) und des E. coli K-12 Stammes DH5α (2) in einem Agarosegel nach Amplifizierungs-reaktionen mit K-12-spezifischen Primerpaaren. Das 1,69 kb große PCR-Produkt wird aus dem orf264 des rfb-Genclusters angereichert. Das 0,94 kb große Fragment weist die Insertion von IS 5 nach. Als interne Kontrolle dient ein Primerpaar, das ein 0,28 kb großes PCR-Produkt aus dem pal-Gen (Peptido-glykan-assoziiertes Lipoprotein) anreichert. Als DNA-Größenstandard wurde EcoRI-geschnittene SPPI-DNA aufgetragen.

Rfb-Gen / IS 5

Kuhnert et al. 1995

Speziestypisierung bei Bakterien





Polymerase chain reaction for the rapid identification of *Clostridium botulinum* type A strains and detection in food samples

P. Fach, D. Hauser[1], J.P. Guillou[2] and M.R. Popoff[1]

Centre National d'Etudes Vétèrinaires et Alimentaires/Laboratoire Central d'Hygiène Alimentaire, Paris, [1] Institut Pasteur, Toxines microbiennes, Paris and [2] Centre National d'Etudes Vétèrinaires et Alimentaires/Laboratoire Central de Recherches Vétèrinaires, Maisons-Alfort, France

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1993, p. 2161–2165
0099-2240/93/072161-05$02.00/0

Vol. 59, No.

Direct Polymerase Chain Reaction Detection of *Campylobacter jejuni* and *Campylobacter coli* in Raw Milk and Dairy Products

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APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1993, p. 631–635
0099-2240/93/020631-05$02.00/0

Vol. 59, No.

Detection of Enteric Viruses in Oysters by Using the Polymerase Chain Reaction

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APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1993, p. 556–560
0099-2240/93/020556-05$02.00/0

Vol. 59, No.

Rapid Polymerase Chain Reaction Method for Detection of *Vibrio cholerae* in Foods

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The polymerase chain reaction was used to selectively amplify sequences within the cholera toxin operon from *Vibrio cholerae* O1. Oysters, crabmeat, shrimp, and lettuce were seeded with *V. cholerae* and then homogenized or washed with alkaline peptone water, followed by short-term (6- to 8-h) enrichment. A detection limit of as few as 1 *V. cholerae* CFU per 10 g of food was obtained with amplification reactions from crude bacterial lysates. The method is extremely rapid and obviates the need for DNA isolation from a variety of complex food matrices.

Spezielles Problem:

Zellkulturen

NEWSFOCUS

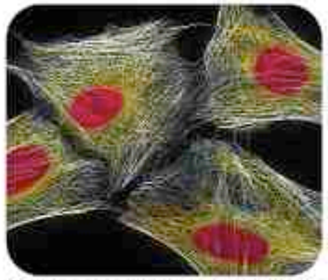
Cases of Mistaken Identity

For decades, biologists working with contaminated or misidentified cell lines have wasted time and money and produced spurious results; journals and funding agencies say it's not their job to solve this problem

IN THE 1980S, WHEN HE WAS A postdoctoral fellow at the Scripps Research Institute in San Diego, California, Reinhard Kofler received what was supposed to be a human cancer cell line from a collaborator. "We cultured it, we cloned genes into it," he recalls, then "[we] genotyped it and realized it was 100% mouse."

After scores of similar experiences with misidentified cells, Kofler and his colleagues at the Tyrolean Cancer Research Institute in Innsbruck, Austria, now authenticate every line as soon as it arrives at the institute. And periodically afterward, they use a simple, cheap, quick, and reliable DNA fingerprinting technique to verify that each cell line continues to be what it should be. "It's an absolute must now," says Kofler. His lab "repeatedly" encounters problems with cell line contamination, and without this constant vigilance, Kofler says, "I wouldn't be confident about our work."

Not every biologist is so wary. A 2004 survey of nearly 500 biologists by Gertrude Buehring of the University of California, Berkeley, and her colleagues, showed that less than 50% of researchers regularly verify the identities of their cell lines using any of the standard techniques such as DNA fingerprinting. "Everybody is in denial" about the widespread problem of cell line cross contamination, says Charles Patrick Reynolds of the University of Southern California and the Children's Hospital Los Angeles' Institute for Pediatric Clinical Research, who establishes new pediatric cancer cell lines and tests potential cancer drugs on existing lines.



Early warning. HeLa cells have contaminated scores of cell lines for more than 4 decades.

Indeed, many studies have shown that a surprisingly large number of cell lines have become contaminated, often by older, more well-established cancerous cells. For example, according to a 1999 paper by Roderick MacLeod and his colleagues at the German Cell Bank (DSMZ) in Braunschweig, 18% of 252 lines donated to the bank were misidentified or contaminated. The extent of the problem "always seems to come as a surprise for people," says John Masters of University College London, president of the European Tissue Culture Society.

And even though biologists read and hear about cross contamination, "people just think that this is not a problem in *my* lab," says Reynolds. If contaminated cell lines are used merely as "test tubes" to express proteins, a lab's work may not be affected. But, say Masters and others, research with contaminated lines continues to obscure potential drug leads and

CREDITS (TOP TO BOTTOM): DSMZ; TORSTEN WITTMANN/PHOTO RESEARCHERS INC.

Science 16.2.2007

…ein lange erkanntes Problem

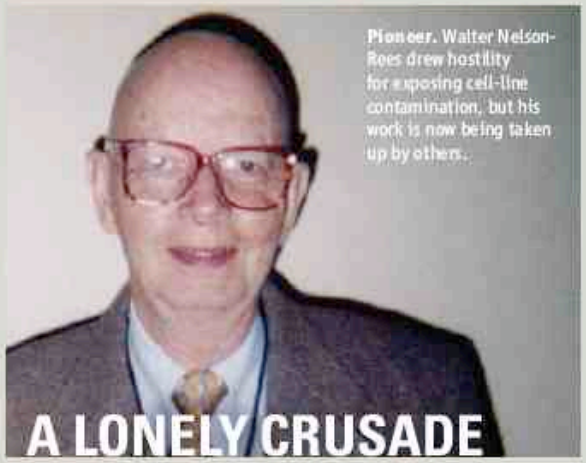
Nelson-Rees et al.

Table 3. Interspecies cell line contamination.

Designation	Reference	Source		Method of determination
		Purported	Actual	
GPS-PDδ and GSP-M	*106*	Guinea pig spleen, adult	Mouse, L-M strain of L cells (*109*)	Serologic and karyologic (conventional staining) (*106*)
Suitor's clone of *Aedes aegypti*	*116*	Mosquito	Moth, Grace's *Antheraea eucalypti* (*117*)	Immunologic, karyologic and isoenzyme electrophoresis (*1*
Culisita inornata		Mosquito	Same as above	
Aedes vexans		Mosquito	Same as above	
LT-1	*118*	Grass frog renal adenocarcinoma	Contaminated with two different cells: TH, box turtle heart (*119*) and FHM, fat head minnow (*120*)	Chromosome analysis, isoenzyme electrophoresis (*121*
CHB	*122*	Human, astrocytoma	Rat, has some characteristics of glial cells; not C-6 strain (*123*)	Chromosome analysis, isoenzyme electrophoresis (*124*
HBC	*125*	Human, invasive duct cell carcinoma, breast	Rat, altered	Chromosome banding, isoenymze electrophoresis, immunofluorescence (*78*)
HEL-R66	*126*	Human	Monkey, *Cercopithecus aethiops* (*127*)	Chromosome banding, isoenzyme electrophoresis, immunofluorescence (*128*)
FQ	*129*	Human, Hodgkin's spleen cells	Owl monkey, *Aotus trirgatus*, kidney cell line, OMK-210 (*130*)	Chromosome banding, isoenzyme electrophoresis (*131*
SpR				
RB				
CaMa (clone 15)	*62, 67*	Human, carcinoma, breast	Syrian hamster, *Mesocricetus auratus* (*95*)*	Chromosome banding isoenzyme electrophoresis, immunofluorescence (*95*)
McCoy (1968)	See note added in proof: S. M. McConnell	Human	Mouse, strain L	Conventional karyology (*95*)
McCoy (1981)	R. W. Eimmons from J. Schacter	Human	Mouse, strain L†	Chromosone analysis, immunofluorescence (*95*)
McCoy's RA (1981)	P. Price, from Dr. Wong, from D. Alexander	Human	Mouse, strain L	Chromosome analysis, immunofluorescence (*95*)

*CaMa, not to be mistaken with Cama 1 (*102*) has been suspected by us of being HeLa. Cells of the original culture are not available. In the present situation a "par tal" culture was thought to be that of Syrian hamster whereas two substrains were clearly of murine origin as shown by chromosome and isoenzyme results [S. Po in communication to M. Green (*67*)]. †N. J. Schmidt indicated that while these cells are said to be human they are positive for murine cells by fluorescent antib

Kontamination in Zellkulturen



Pioneer. Walter Nelson-Rees drew hostility for exposing cell-line contamination, but his work is now being taken up by others.

A LONELY CRUSADE

In 1951, a 31-year-old African-American woman was admitted to Johns Hopkins Hospital in Baltimore, Maryland, for treatment for cervical cancer. The hospital sent a sample of her cancerous tissue to Hopkins tissue culture expert George Gey, who successfully cultured it in his lab. Henrietta Lacks's ferocious cancer cells spread throughout her body and eventually killed her. And her immortalized cells, named HeLa cells after her, quickly spread through labs across the world—and not always because researchers had requested a sample for study.

In 1966, Stanley Gartler of the American Type Culture Collection found that 18 of the first 20 human cell lines established were chromosomally and biochemically identical to HeLa cells. All 18 lines were known to have come from Caucasian individuals. Yet Gartler found that each had a genetic variant of an enzyme found only in the small percentage of African-American population that Lacks had belonged to. Gartler published his findings in *Nature* in 1968, marking the first reported case of HeLa contamination. It was only the beginning.

A few years later, Walter Nelson-Rees began discovering contaminations in lines from laboratories across the world. At the time, he was at the Cell Culture Laboratory of the University of California, Berkeley, at Oakland, characterizing, storing, and distributing cell lines for the U.S. National Cancer Institute (NCI). Over more than 10 years, he counted 279 contaminated lines from 45 different laboratories. Many were contaminated with cells from other species, but the bulk—more than 40 individual lines—had been overcome by HeLa cells. "This sort of scenario happened many, many times; people who thought they were working with one type of cells [were later found to be] working with HeLa cells," he says.

Nelson-Rees published his results in a series of papers in *Science* in the 1970s, urging scientists to stop using contaminated cell lines, re-evaluate their previous research, and employ simple quality-control practices such as regularly verifying their lines' authenticity.

Nelson-Rees's revelations threw the community into a frenzy. Many studies were called into question, and Nelson-Rees was naming names. Some biologists reacted with hostility, and *Nature* in an editorial called Nelson-Rees a "self-appointed vigilante." In a 2001 commentary on cell line authentication, Stephen O'Brien of NCI in Bethesda, Maryland, who had worked with Nelson-Rees, recalled the tension: "Human emotions were on edge, red faces were appearing in the most prestigious laboratories, and discussions of the problem lost any semblance of civility." Nelson-Rees even remembers an anonymous telegram offering to send him a one-way ticket to South Africa. "My aim was to clear up a morass of contamination, and it wasn't easy," he says.

The attacks ultimately took their toll. In 1981, Nelson-Rees quit science and opened an art gallery in San Francisco.

HeLa continues to spread today. In 2004, Gertrude Buehring of the University of California, Berkeley, and her colleagues surveyed 485 researchers from 48 countries who were working with specific cell lines and found that 49 were using seven lines that others had shown to be contaminated by HeLa. When Buehring conducted a PubMed search to identify the number of publications from researchers wrongly using HeLa-contaminated lines as though they still had cells of the original line, she found a total of 220 papers between 1969 and April 2004. And the number of publications on research using cell lines shown to have become contaminated by HeLa had increased by a factor of 10 between 1969 and 2004, whereas the total number of publications had increased by only a factor of 2.7.

Eponymous. HeLa cells came from Henrietta Lacks's cervical cancer.

But perhaps Nelson-Rees will finally get his due. Other scientists are now taking up his fight against cell line contamination (see main text). And in 2004, the Society for In Vitro Biology publicly recognized his contribution to science with a lifetime achievement award.

–R.C.

The FASEB Journal article fj.14-266718. Published online June 26, 2015.

The FASEB Journal • Research Communication

Genetic profiling reveals an alarming rate of cross-contamination among human cell lines used in China

Fang Ye,*,† Chuguang Chen,‡ Jian Qin,‡ Jie Liu,‡ and Congyi Zheng*,†,1

*China Center for Type Culture Collection and †State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China; and ‡Beijing Microread Genetics Company, Limited, Beijing, China

ABSTRACT **Cell lines are widely used as *in vitro* model systems in biologic and medical research. However, much of the research has been invalidated by the unwitting use of false cell lines. A significant proportion of the research involving human cell lines was initiated in China. Paradoxically, the cell lines used in China have never been authenticated. Here, we present a comprehensive survey of cross-contamination in 380 samples from 113 independent sources in China using short tandem repeat profiling methods. High levels of cross-contamination were uncovered (95 of 380, 25%). Notable false cell lines (*e.g.*, KB and WISH) are still actively used under their false identity and tissue attributions. Most strikingly, 85.51% of lines established in China were misidentified (59 of 69) and accounted for over half of the misidentifications (59 of 95, 62.11%). Further, 93.22% of the contaminants in cell lines established in laboratories of China were HeLa cells or a possible hybrid of HeLa with an unknown cell line. Results from these misidentified lines have been published in thousands of potentially erroneous articles and may have distorted the findings visible to the scientific community. False lines have been used in drug screening, potentially leading to unusable or even harmful therapeutic strategies. We also noted the causes of contamination and provided suggestions for remediation.—Ye, F., Chen, C, Qin, J., Liu, J., Zheng, C. Genetic profiling reveals an alarming rate of cross-contamination among human cell lines used in China. *FASEB J.* 29, 000–000 (2015). www.fasebj.org**

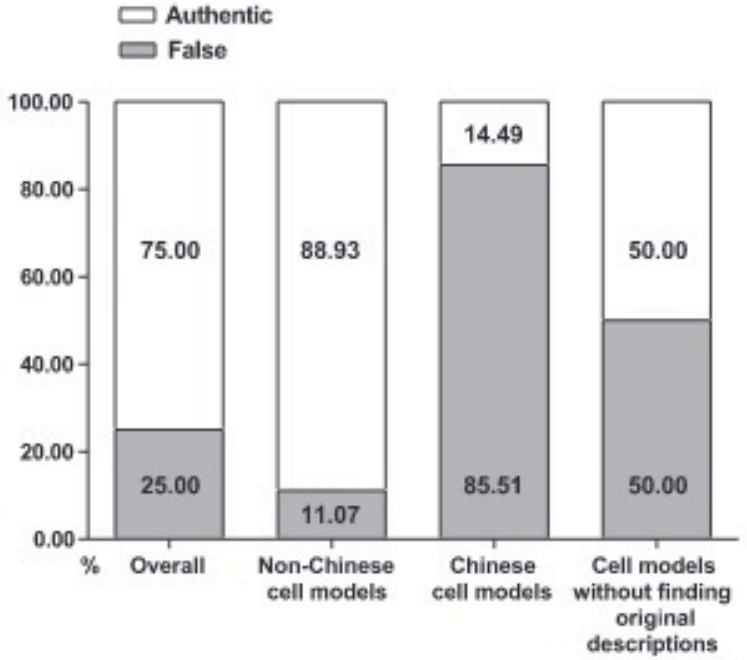


Figure 1. Incidence of misidentification among cell lines used in laboratories of China. From 113 independent sources in China, 380 samples were divided into 3 groups according to their original sources: non-Chinese cell models (n = 307), Chinese cell models (n = 69), and those for which original descriptions could not be found (n = 4).

IJC
International Journal of Cancer

Short Report

False and mycoplasma-contaminated leukemia–lymphoma cell lines: time for a reappraisal

Hans G. Drexler, Wilhelm G. Dirks, Roderick A.F. MacLeod and Cord C. Uphoff

Department of Human and Animal Cell Lines, Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

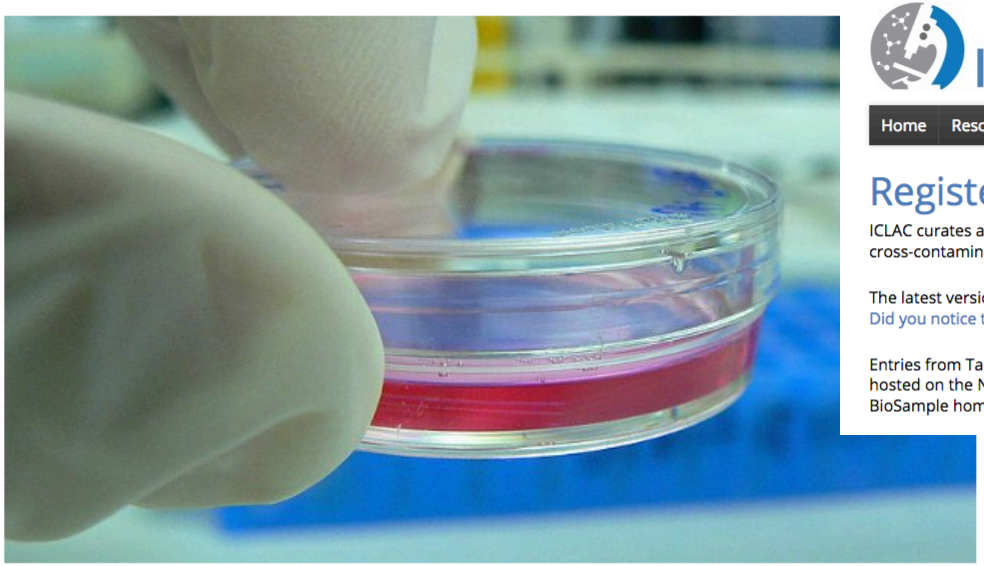
Leukemia–lymphoma cell lines are important research tools in a variety of fields. To represent adequate model systems it is of utmost importance that cell lines faithfully model the primary tumor material and are not cross-contaminated with unrelated cell material (or contaminated with mycoplasma). As it has been previously reported that cross-contaminated cell lines represent a significant problem, it is of interest to know whether any improvement in the prevalence of such "false cell lines" had occurred since we called the alert in 1999. A retrospective review of our data archives covered 848 cell lines received from 1990 to 2014 from 290 laboratories in 23 countries spanning the spectrum of leukemia–lymphoma entities. Two variables were considered: authenticity and freedom from mycoplasma infection. Regarding provenance, we separately considered primary sources (original investigators having established the cell lines or reference repositories) and secondary sources. The percentages of mycoplasma-contaminated cell lines decreased significantly over the 25-year timespan. Among primary sourced material: mycoplasma-contamination fell from 23% to 0%; among secondary sourced: from 48% to 21%. The corresponding figures for cross-contamination declined from 15% to 6%, while among material obtained from secondary sources prevalence remained remarkably high, throughout the time periods at 14–18%. Taken together, our data indicate that using non-authenticated cell lines from secondary sources carries a risk of about 1:6 for obtaining a false cell line. The use of authentic leukemia–lymphoma cell lines holds important translational value for their model character and the reproducibility of the laboratory data in the clinical arena.

The Scientist
EXPLORING LIFE, INSPIRING INNOVATION

Papers Based on Misidentified Cell Lines Top 32,000

An analysis of contaminated literature finds that tens of thousands of papers used cell lines of questionable origins—and these were in turn cited by hundreds of thousands of other papers.

By Kerry Grens | October 16, 2017



WIKIMEDIA, KAIBARA87

Cell line misidentification is rampant throughout biomedical research, and a new analysis quantifies its impact on the scientific literature, finding more than 32,000 papers used lines with no known original stock. "In this case, it must be assumed that all primary literature could be based on false grounds and should at least be treated with caution," the authors write in their report, published in *PLOS ONE* October 12.

The authors based their study on a list 451 cell lines flagged by the International Cell Line Authentication Committee as not having authenticated stock, meaning they are likely mislabeled. They then went through the Web of Science literature database to grab papers based on these lines. "As we only searched for cell lines known to be misidentified, this constitutes a conservative estimate of the scale of contamination in the primary literature," they wrote.

ICLAC

INTERNATIONAL CELL LINE AUTHENTICATION COMMITTEE

Register of Misidentified Cell Lines

ICLAC curates a Register of cell lines that are known to be misidentified through cross-contamination or other mechanisms (e.g., mislabelling).

The latest version is Version 8.0, released 1 December 2016 | Release notes v8.0
Did you notice the name change? From Database to Register

Entries from Table 1 (misidentified cell lines with no known authentic stock) are hosted on the NCBI BioSample database. A link to the data can be found on the BioSample home page here.

Useful Resources

- ICLAC Register of Misidentified Cell Lines
- Advice to Scientists: Incorporating Authentication into Everyday Culture Practice
- Cancer Moonshot Letter
- Cell Line Checklist for Manuscripts and Grant Applications
- Definitions
- Guide to Human Cell Line

Genotypisierung von Individuen durch short tandem repeats (STR)

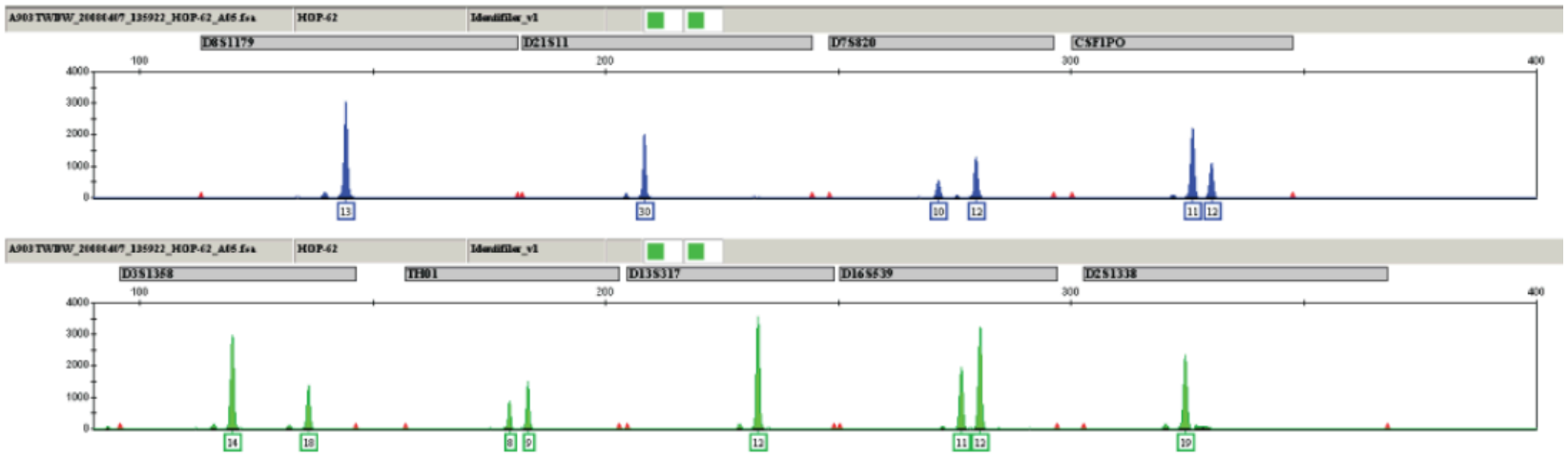
polymorphe Loci

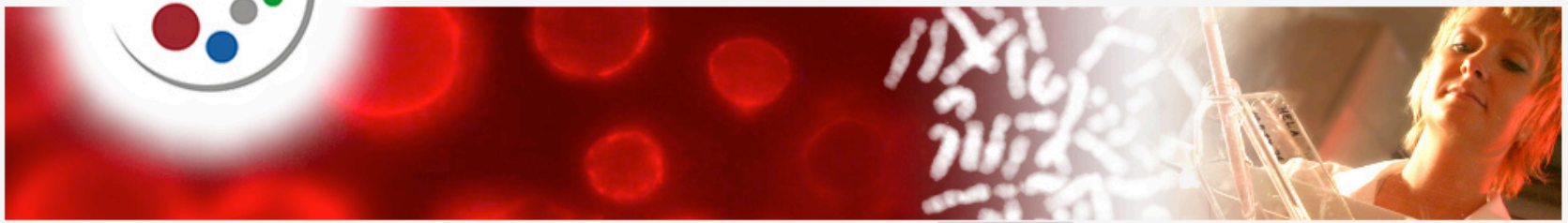
⇩

Multiplex-PCR

⇩

Größenbestimmung durch Elektrophorese





Authentication of Human Cell Lines

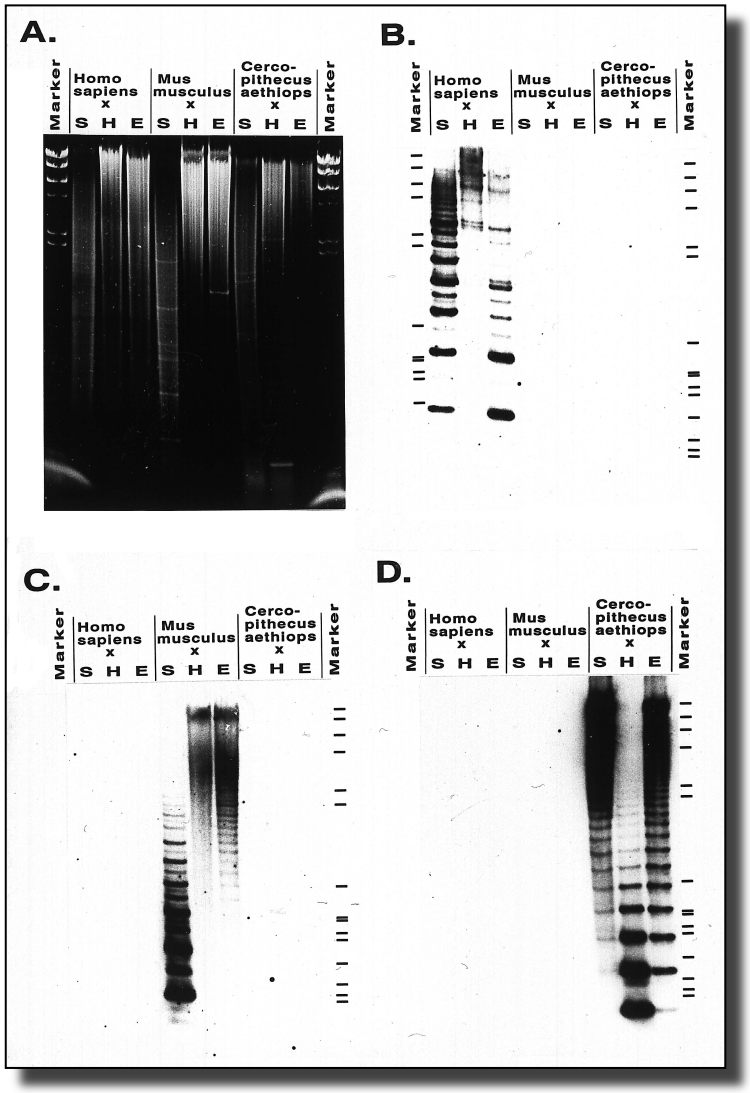
Submission form

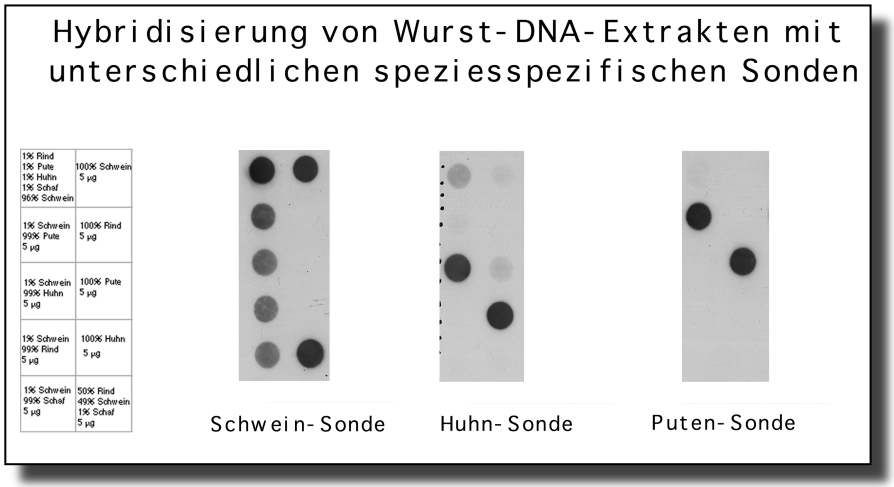
Download our service submission form

The number of false cell lines in circulation is unacceptably high. Use of cancer cell line models is impaired by reliance on misidentified examples representing entities with biological characteristics different from those supposed. To let researcher check the true identity of their cell lines - a requirement increasingly imposed by scientific journals and funding agencies - the DSMZ offers a cell line authentication service.

For detailed information how to submit samples for testing, please fill in the submission form.

Spezies-Typisierung mit artspezifischen DNA-Sonden (*oldschool*)

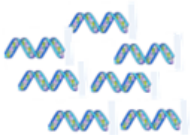




oder andere biologische Gemische....

DNA-Extraction

DNA-shearing



Library preparation

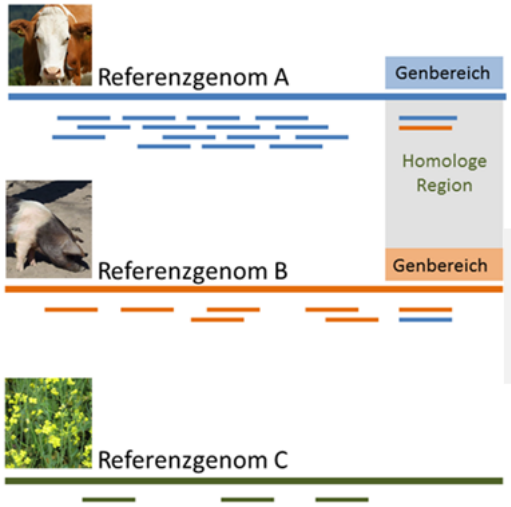
All Food Seq Pipeline

Whole genome shotgun metagenomics

Next Generation Sequencing (NGS)

Millions of short (100Bp) DNA reads

Mapping of reads against known database Sequences



Ripp et al. 2014; Liu et al. 2017; Hellmann et al. 2019

Typisierung durch NGS...

All-Food-Seq

Species	Target value [%]	Proportion [%]	
		AFS-quant	AFS-spec
Cattle	35	36.05 ± 0.04	41.16 ± 0.02
Horse	1	1.27 ± 0.01	1.45 ± 0.01
Pig	9	7.22 ± 0.05	7.59 ± 0.09
Sheep	55	54.76 ± 0.09	49.71 ± 0.08
Waterbuffalo	0	0.64 ± 0.03	0.07 ± 0
Total	100		

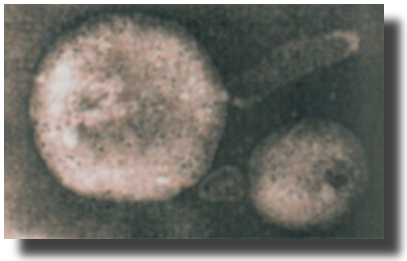
fp

Quantitative species analysis obtained by Illumina sequencing of DNA from the "KalD" reference s
compared. Each dataset tested contained 1 mio of paired-end sequence reads, randomly selected
and mean values plus standard deviations are displayed. "Difference abs." shows the difference b
expected amounts existing in the sample ("target value"). "Difference rel." is calculated by dividin

Ripp *et al.*

Ripp *et al. BMC Genomics* 2014 **15**:639 doi:10.1186/1471-2164-15-639

Mycoplasmenbefall in Zellkulturen



zellwandlose Kleinstbakterien

- weltweit 5-35 % aller Zellkulturen kontaminiert
- verändern zelluläre Prozesse
- begünstigen Tumorentstehung
- sind pathogen für den Menschen

→ PCR-Analytik oder NGS

http://www.dsmz.de/mutz/mutzmyco.htm