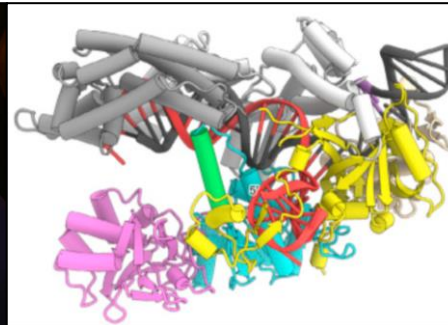
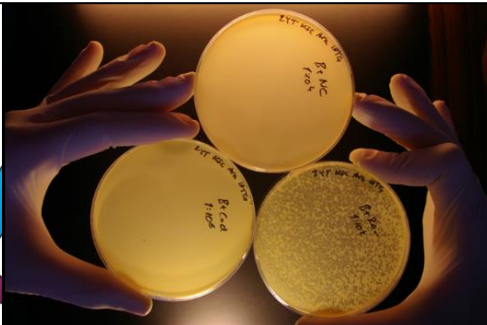
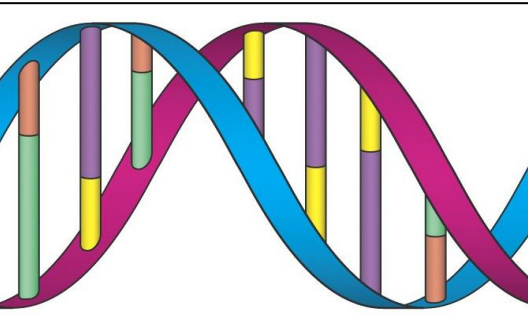


de CRISPR-Cas revolutie

van biologie naar gen therapie



John van der Oost

Laboratory of Microbiology
Wageningen University





- ➔ DNA – wat is het, en waarom is het belangrijk ?
- Genetische ziekte – hemochromatose
 - CRISPR-Cas – van ontdekking tot toepassing
 - Gen therapie – recente voorbeelden
 - Conclusies

DNA – wat is het ?



- DNA is verantwoordelijk voor de opslag van genetische informatie
- DNA chromosomen komen voor in alle organismen
- DNA komt voor in elke levende cel (bacterie, plant, mens)



DNA - wat is het ?

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.F.S. *Discovery II* for their part in making the observations.

¹ Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1925).
² Loquet-Huguin, M. S., *Mon. Not. Roy. Astr. Soc., Geophys. Supp.*, **8**, 226 (1929).
³ Von Arx, W., S., Woods Hole Papers in Phys. Oceanogr. Meteor., **11**, 53 (1951).
⁴ Ekman, V. V., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2**(11) (1935).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey¹. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining 5-D-deoxy-ribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furburg's model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furburg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There

is a residue on each chain every 3.4 Å, in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them. The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally^{2,3} that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{4,5} on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

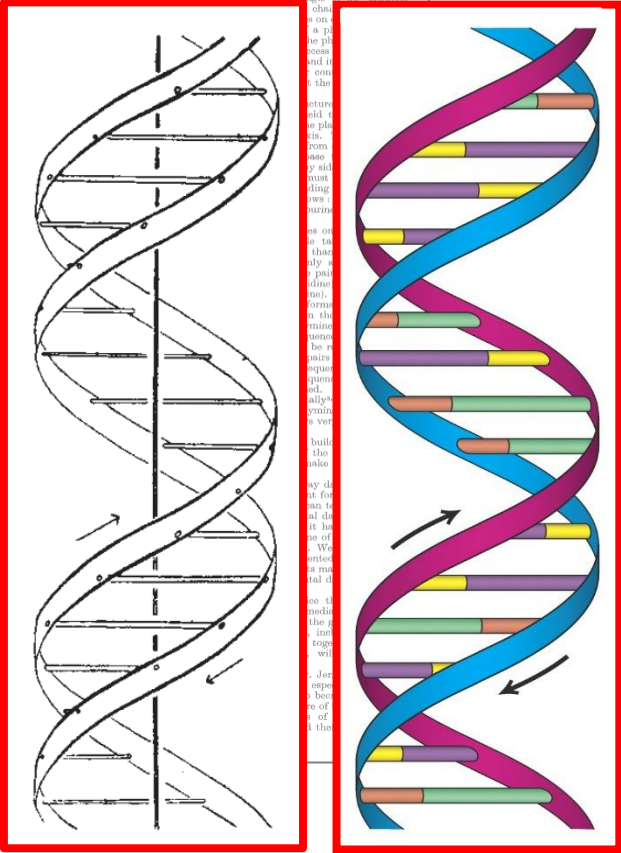
We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at



Diagrammatic. The two ribbons symbolize the two phosphate-sugar chains. The horizontal red lines the pairs of bases holding the chains together. The vertical blue marks the fibre axis.



DNA – wat is het ?



specifieke
base paring
G-C en A-T

“de specifieke base paring van het DNA suggereert een mogelijk kopieer-mechanisme voor genetisch materiaal”



DNA – wat is het ?

No. 4356 April 25, 1953 NATURE 277

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R. F. S. *Discovery II* for their part in making the observations. We have assumed the names of the ships and the names of the crew members as a matter of courtesy.

* Young, P. B., Gerrard, H., and M. S. (1952).
 * Longest, H. (1952).
 * Von Arx, W., S., Woods Hole (1952).
 * Ekman, V. W., *Arktis, Mar. 44*.

MOLECULAR BIOLOGY
NUCLEIC ACIDS
A Structure for Deoxyribose Nucleic Acid

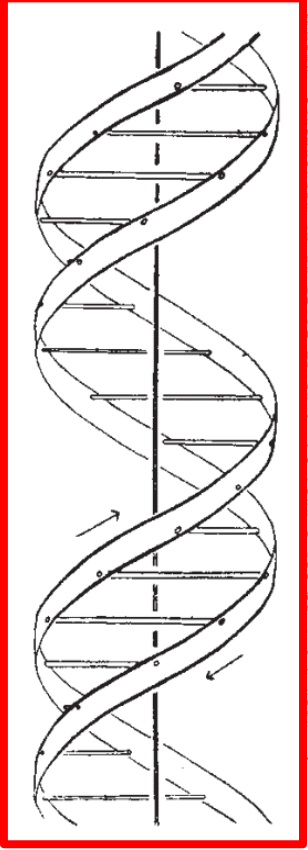
WE wish to suggest a structure for the deoxyribose nucleic acid (DNA) which is biologically important.

A structure for nucleic acid was first proposed by Pauling and Corey in their manuscripts available for publication. Their model consisted of two chains, with the phosphate groups on the outside, and the bases on the inside, linked together by hydrogen bonds. This structure is in agreement with the X-ray diagrams of the DNA.

(1) We believe that the structure of the DNA is as follows: the two chains are twisted round each other, and the phosphate groups are on the outside, linked together by hydrogen bonds. The bases are on the inside, linked together by hydrogen bonds. The structure is a right-handed helix, with the phosphate groups on the outside, and the bases on the inside. The distance between two phosphate groups is 3.4 Å. The distance between two bases is 3.4 Å. The distance between two phosphate groups and two bases is 3.4 Å. The distance between two phosphate groups and two bases is 3.4 Å.

Another three-chain structure was suggested by Fraser (in the *Nature*), in which the phosphate groups are on the outside, linked together by hydrogen bonds. This structure is in agreement with the X-ray diagrams of the DNA.

This figure is purely schematic. The two phosphate-sugar chains are shown in red, the base-pairs in blue. The vertical blue marks the three axes.



NATURE VOL. 227 AUGUST 8 1970 561

is with the detailed formation. It states from protein to either

giving four standard the acid. can be stated as the information transfer alphabet to another. ed by the diagrams of at that time, though I (linked) in which all presented by arrows, out the flow of matter d., residues-by-residue, polymer molecule to commonly occurred it a to construct useful us were part of our because it was being ves could not occur. - wise to state these

PROTEIN

ned in 1958. Right arrows possible transfer. The for transfer transfer the three possible arrows

to transfer could be The first group was at or indirect, seemed did arrows in Fig. 2.

in

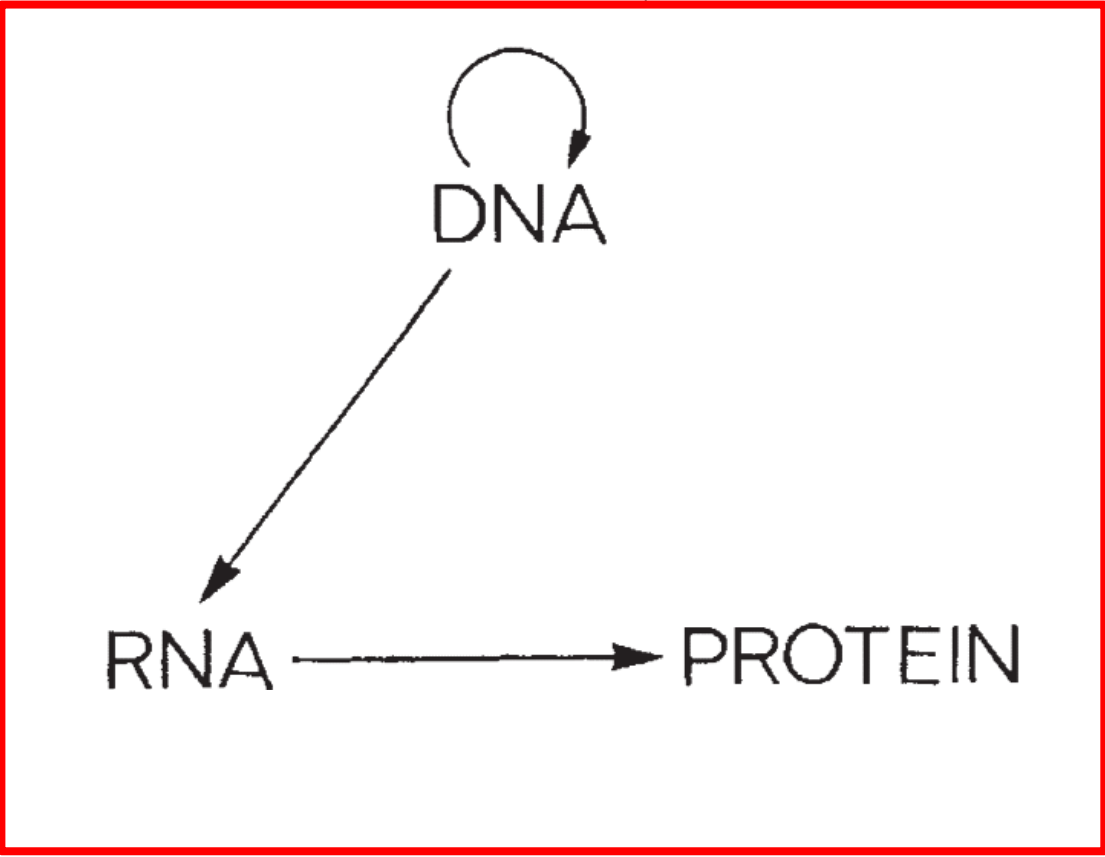
med to occur because in in Fig. 2 as dotted or any experimental requirement. They

ne to Temin's work?

DNA

RNA

PROTEIN



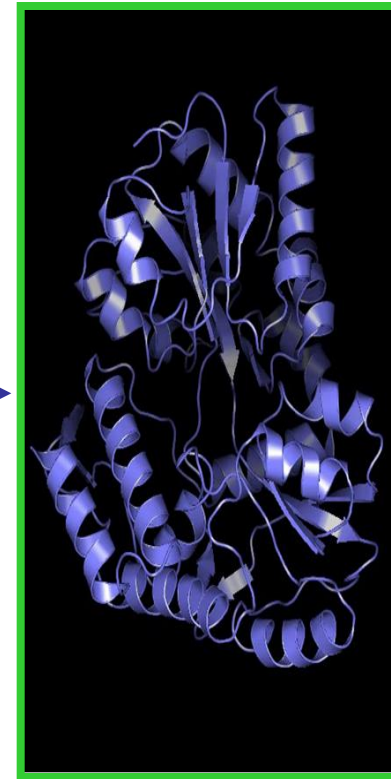
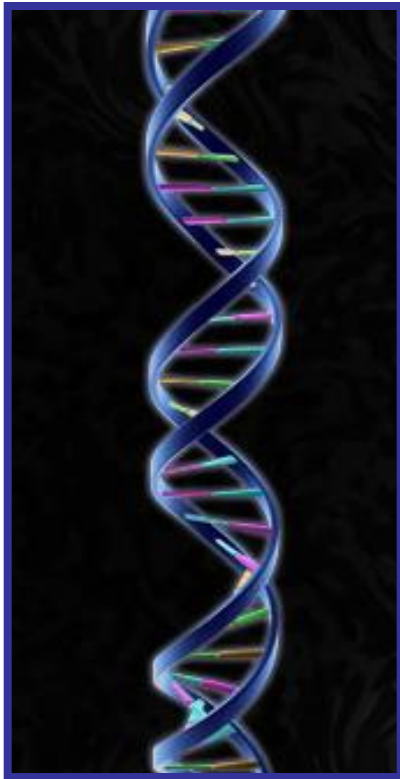
de genetische informatie die is opgeslagen in het DNA kan worden gedupliceerd tijdens cel-deling (DNA > DNA + DNA), maar ook tot expressie komen als eiwitten met specifieke functies (DNA > RNA > eiwit)

DNA > RNA > eiwit



informatie - opslag

biologische functie



DNA

Eiwit

DNA – waarom is het belangrijk ?



- DNA is verantwoordelijk voor de opslag van genetische informatie
- DNA chromosomen komen voor in (bijna) alle organismen
- DNA komt voor in elke levende cel (bacterie, plant, mens)
- DNA wordt verdubbeld voor celdeling (als organisme groeit)
- DNA kopieer-fouten komen voor (soms in mensen, vaak in virussen)
- DNA veranderingen kunnen voordelig zijn (evolutie)
- **helaas**, DNA veranderingen kunnen ook nadelig zijn (genetische ziektes)

de CRISPR-Cas revolutie

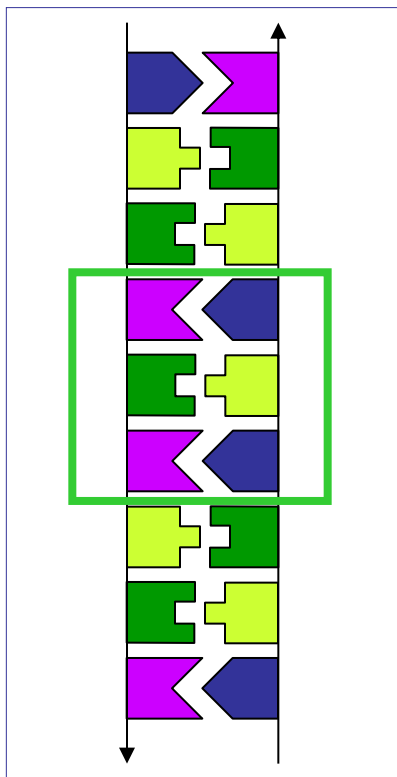


- DNA – wat is het, en waarom is het belangrijk ?

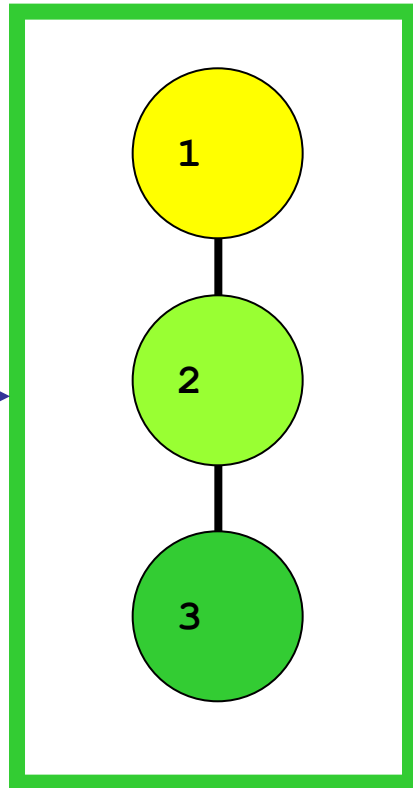
Genetische ziekte – hemochromatose

- CRISPR-Cas – van ontdekking tot toepassing
- Gen therapie – recente voorbeelden
- Conclusies

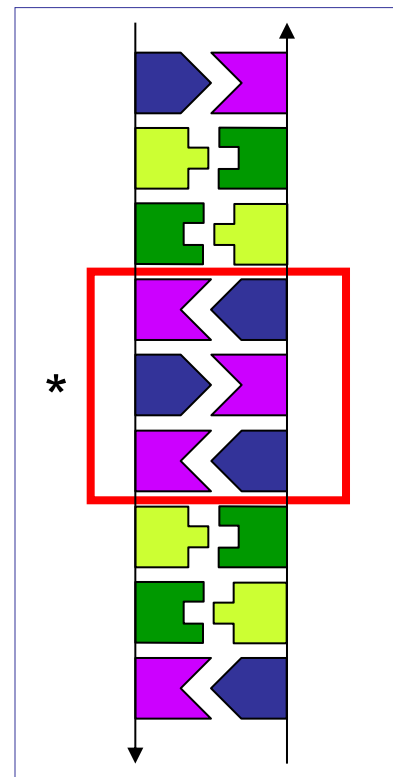
Genetische ziekte



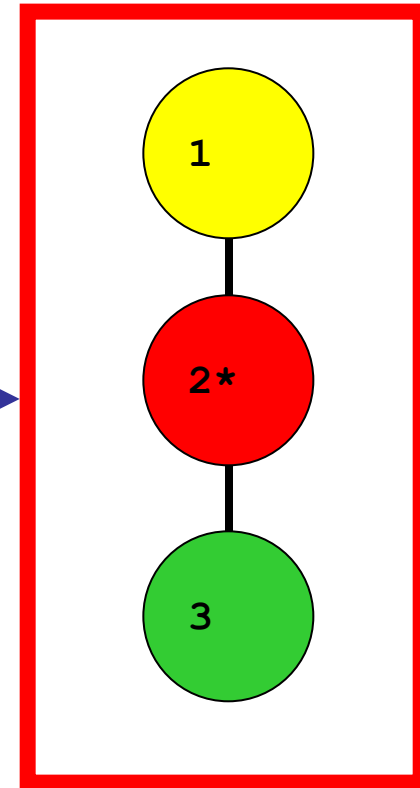
gen



eiwit



mutant gen*

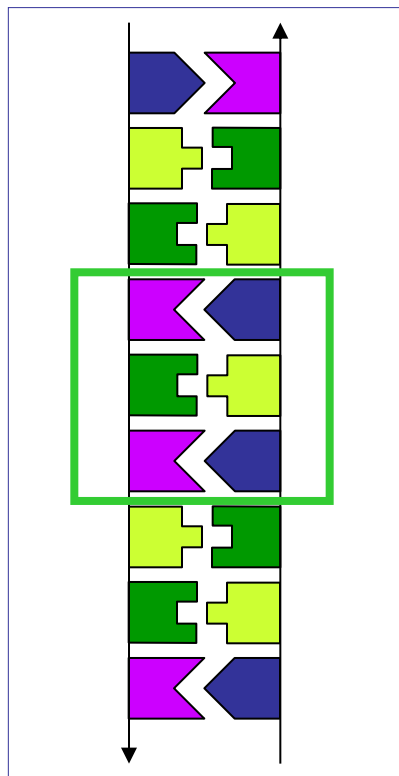


mutant eiwit*

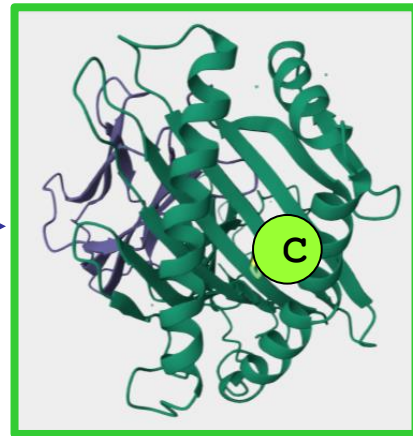
Hematochromatose - HFE (High Fe) eiwit



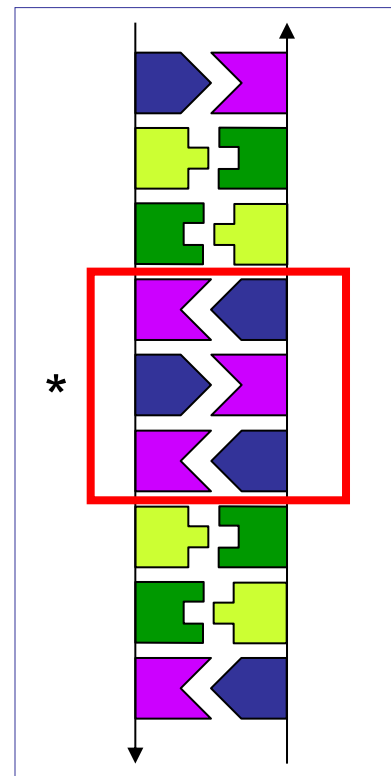
hfe gen codeert voor HFE proteïn (regulatie ijzer opname)



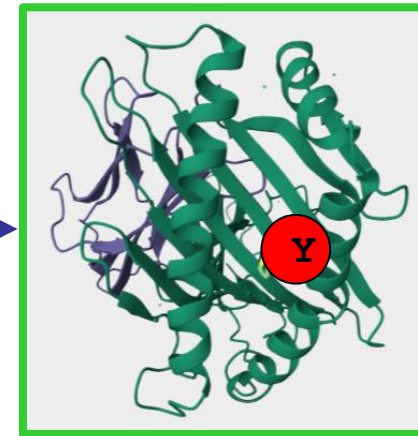
hfe gen



HFE eiwit



hfe gen*
(G845A)

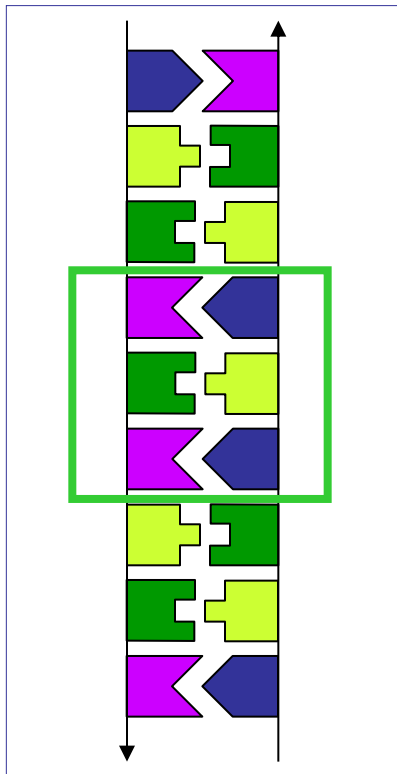


HFE eiwit*
(C282Y)

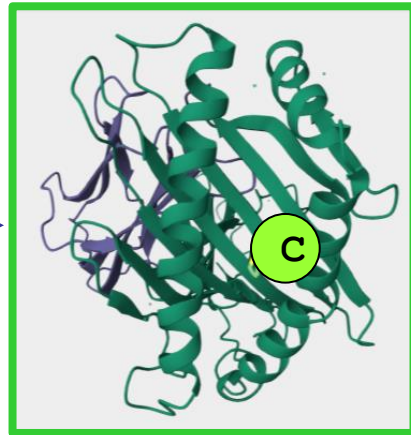
Hematochromatose - HFE (High Fe) eiwit



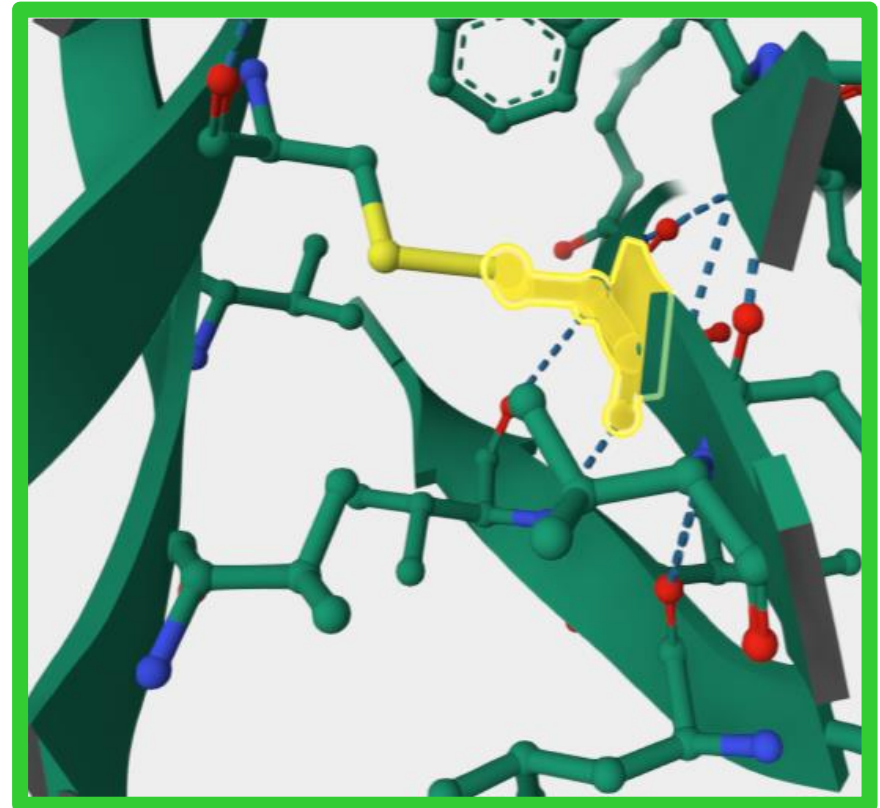
hfe gen codeert voor HFE proteïn (regulatie ijzer opname)



hfe gen



HFE eiwit



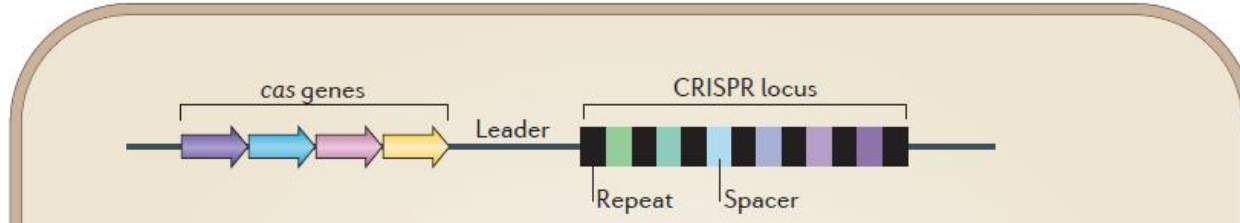
Cys-282 vormt een zwavel-brug met Cys-225
(cruciaal voor een stabiele eiwit structuur)

de CRISPR-Cas revolutie



- DNA – wat is het, en waarom is het belangrijk ?
- Genetische ziekte – hemochromatose
- ➡ **CRISPR-Cas – van ontdekking tot toepassing**
- Gen therapie – recente voorbeelden
- Conclusies

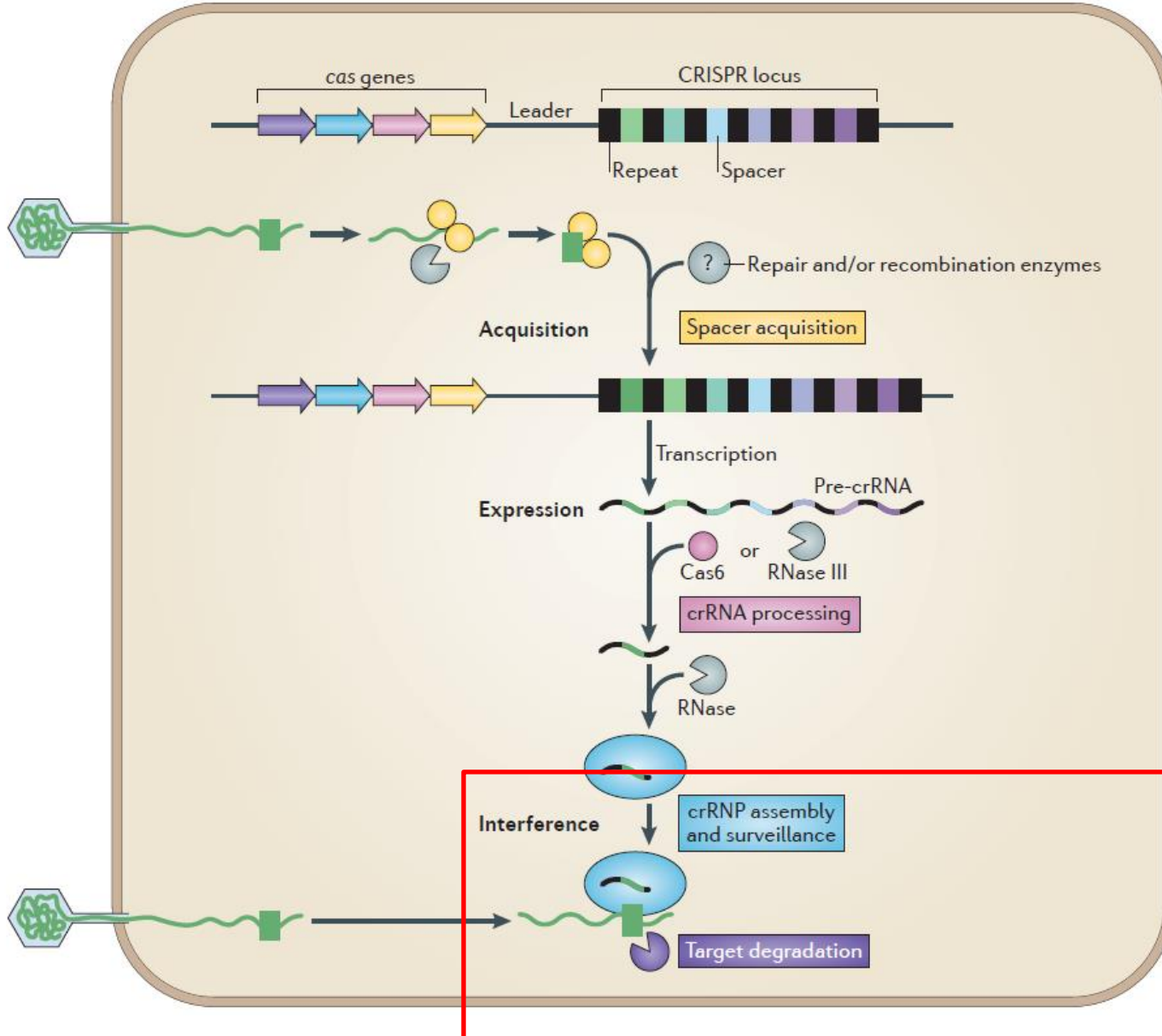
CRISPR-Cas mechanisme



CRISPR = clustered regularly interspaced short palindromic repeats

Cas = CRISPR-associated proteins

CRISPR-Cas mechanism

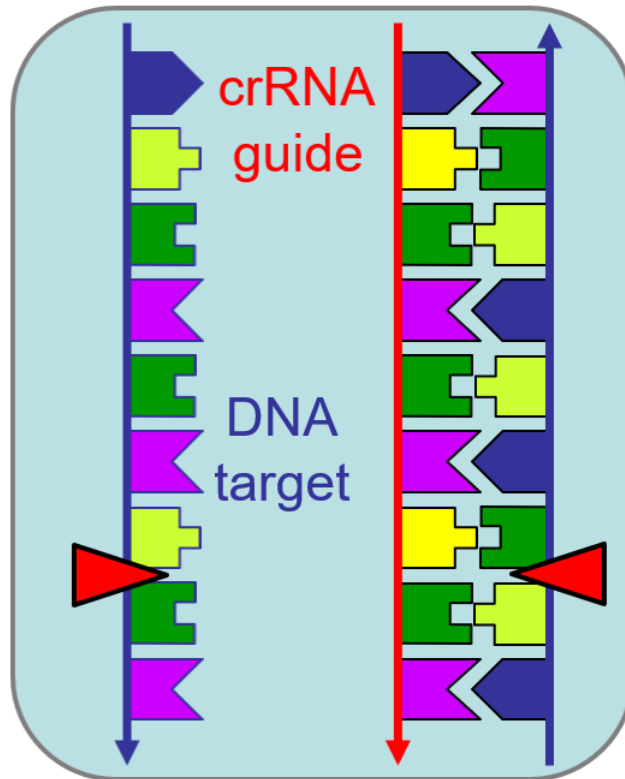


1. Spacer inbouw

2. Guide expressie

3. Target aanval

crRNA guides Cas nuclease to DNA target



Cas nuclease

in dit voorbeeld is
een crRNA guide gebruikt
van 9 letters
(GATCTCATC),
dit “woord” komt gemiddeld 10.000x
voor in het humane genome ...

in een echt CRISPR systeem,
zijn guides vaak 20 letters
(GATCTCATCATGATCTCATC),
zo'n lang “woord” komt gemiddeld
slechts 1x
voor in het humane genome ...

dus, we kunnen een guide
ontwerpen die heel specifiek
één enkele plek (in één enkel gen)
kan vinden en knippen
op het humane DNA
(net als Ctrl-F / Ctrl-X)

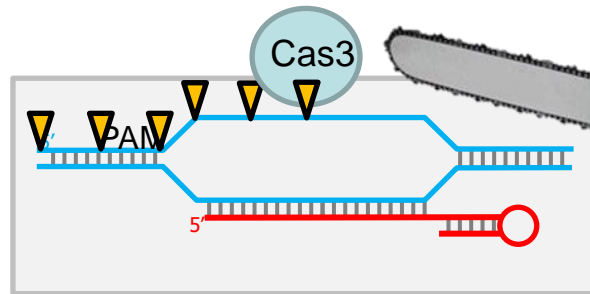
dit maakt specifieke
genome editing mogelijk

CRISPR-Cas – DNA editing



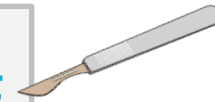
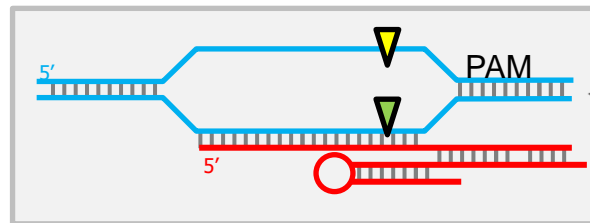
Class-1

Type I
Cascade

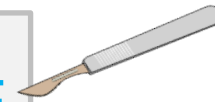
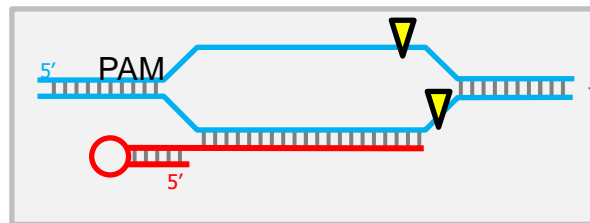


Class-2

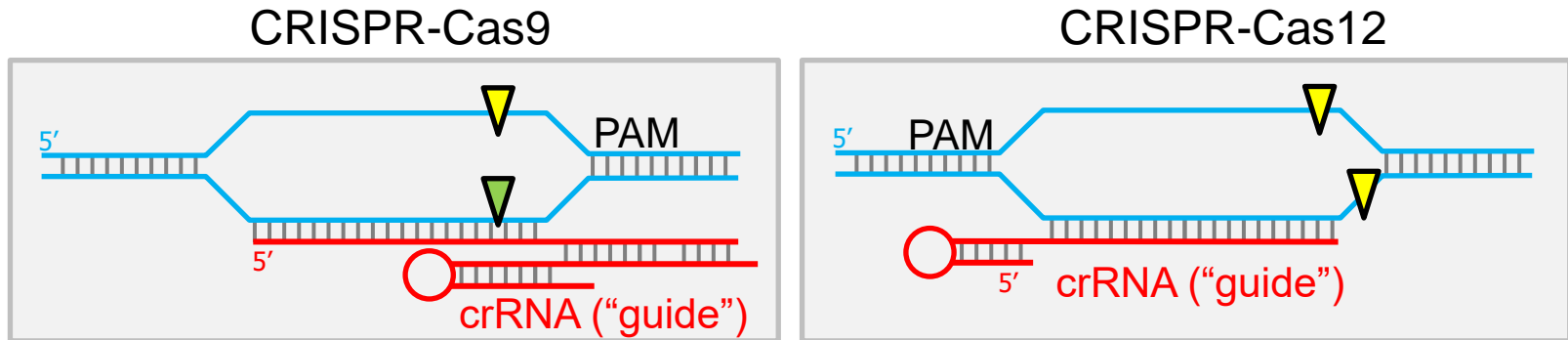
Type II
Cas9



Type V
Cas12a

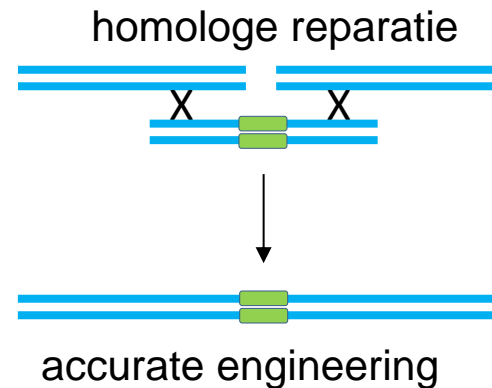
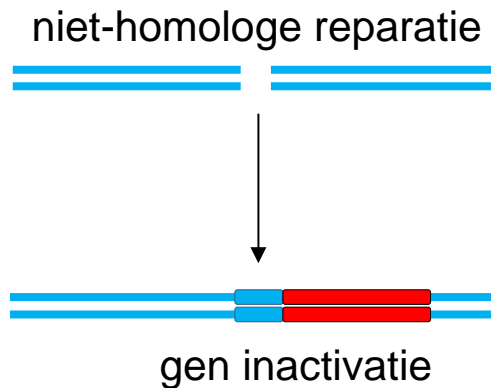


CRISPR-Cas – DNA editing



met Cas9 / Cas12 en een design gids-RNA (“guide”)
kan op één enkele plek een breuk gemaakt worden in het DNA van een cel

daarna zullen reparatie-systemen
van de cel de breuk weer herstellen



de CRISPR-Cas revolutie



- DNA – wat is het, en waarom is het belangrijk ?
- Genetische ziekte – hemochromatose
- CRISPR-Cas – van ontdekking tot toepassing
- ➔ **Gen therapie – recente voorbeelden**
- Conclusies



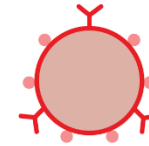
Base editing: Revolutionary therapy clears girl's incurable cancer

11 December 2022



How does the treatment work?

1 Alyssa had T-cell leukaemia



T-cells, a type of white blood cell, destroy threats in the body

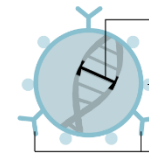
Alyssa's were out of control

2 Doctors used 'base editing' to engineer her therapy



Base editing changes one letter in the genetic code

3 Donor T-cells were edited in three ways



DNA altered to resist chemotherapy

Markings removed to protect donor T-cells

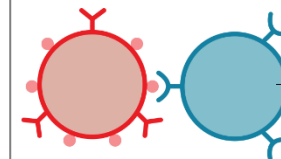
Receptors removed to prevent donor T-cells attacking the body

4 T-cells further modified to attack cancer



T-cell rearmed with new receptors

5 Battle of the T-cells



Modified T-cells find and destroy cancerous T-cells

Source: BBC research



2023 – ex vivo CRISPR therapie (SCD)



BIOMICEDINE

Tweaking genes with CRISPR or viruses fixes blood disorders

People with sickle cell disease or beta-thalassemia could be freed of debilitating symptoms for a lifetime

UK first to approve CRISPR treatment for diseases: what you need to know

The landmark decision could transform the treatment of sickle-cell disease and β -thalassaemia – but the technology is expensive.



GOEDGEKEURD VOOR THERAPIE

- **UK** - Medicines and Healthcare products Regulatory Agency (**MHRA**)
- **US** - Food and Drug Administration (**FDA**)
- **EU** - European Medicines Agency (**EMA**)



Eerste Nederlandse patiënt behandeld met CRISPR-Cas9-infuus

Innovatie

Patiëntenzorg

Zeldzame ziekten

Genen

afleveren van CRISPR-Cas9 (mRNA) in lever met “nano-particles”: specifieke *in vivo* gen therapie

“hereditair angio-oedeem” is een zeldzame erfelijke aandoening, veroorzaakt door een tekort aan het eiwit *C1-esteraseremmer*. Als deze remmer ontbreekt, kunnen er op allerlei plekken in het lichaam plotselinge zwellingen ontstaan. **CRISPR-Cas9** onderdrukt de aanmaak van het pre-kallikreïne eiwit, zodat het signaal dat leidt tot zwellingen niet meer kan worden doorgegeven.”

Hematochromatose - HFE (High Fe) eiwit



HFE gen = 1047 bp (letters)

Human genom = 3.000.000.000 letters

uitdaging van gen therapie:

hoe kan je de mutatie vinding,

en hoe die dan te repareren (A845 > G845)

kan dat met CRISPR-Cas ??

TYPE 1 HEMOCHROMATOSE

HFE* gene (G845A)

```
ATGGGCCCGCGAGCCAGGCCGGCGCTTCTCCTCCTGATGCTTTTGCAGACCGGGTCC
TGCAGGGGCGCTTGCTGCGTTCACACTCTCTGCACTACCTCTTCATGGGTGCCTCAGA
GCAGGACCTTGGTCTTTCCCTTGTGTTGAAGCTTGGGCTACGTGGATGACCAGCTGTTC
GTGTTCTATGATCATGAGAGTCGCCGTGTGGAGCCCCGAAGTCCATGGGTTTCCAGTA
GAATTTCAAGCCAGATGTGGCTGCAGCTGAGTCAGAGTCTGAAAGGGTGGGATCAGAT
GTTCACTGTTGACTTCTGGACTATTATGAAAATCACAACCACAGCAAGGAGTCCAC
ACCCTGCAGGTATCCTGGGCTGTGAAATGCAAGAAGACAACAGTACCGAGGGCTACT
GGAAGTACGGGTATGATGGGCAGGACCACCTTGAATTCTGCCCTGCACACTGGATTG
GAGAGCAGCAGAACCAGGGCTGGCCACCAAGCTGGAGTGGGAAAGGCACAAGATT
CGGGCCAGGCAGAACAGGGCCTACCTGGAGAGGGACTGCCCTGCACAGCTGCAGCAGT
TGCTGGAGCTGGGGAGAGGTGTTTGGACCAACAAGTGCCCTCCTTTGGTGAAGGTGAC
ACATCATGTGACCTCTTCACTGACCACTCTACGGTGTCCGGCCCTTGAACACTACCCC
CAGAACATCACCATGAAGTGGCTGAAGGATAAGCAGCCAATGGATGCCAAGGAGTTGC
AACCTAAAGACGTATTGCCCAATGGGGATGGGACCTACCAGGGCTGGATAACCTTGGC
TGTACCCCTGGGGAAGAGCAGAGATATACGACAGGTGGAGCACCCAGGCCTGGAT
CAGCCCCCATTGTGATCTGGGAGCCCTCACCGTCTGGCACCCCTAGTCATTGGAGTCA
TCAGTGAATTGCTGTTTTGTGTCGTCATCTTGTTCATGGAATTTTGTTCATAATATT
AAGGAAGAGGCAGGGTTCAAGAGGAGCCATGGGGCACTACGTCTTAGCTGAACGTGAG
TGA
```

HFE protein

```
MGPRARPALLLLMLLQTAVLQGRLLRSHSLHYLFMGASEQDLGLSLFEALGYVDDQLF
VFYDHESRRVEPRTPWVSSRISSQMWLQLSLSLKGWDHMFVTDFWTIMENHNHSHKESH
TLQVILGCEMQEDNSTEGYWKYGYDGQDHLEFCPDTLDWRAAEPRAWPTKLEWERHKI
RARQNRAYLERDCPAQLQLLELGRGVLDQVPPPLVKVTHHVTSVVTTLRCRALNYYP
QNI TMKWLKDKQPMDAKEFEPKDVLPNGDGT YQGWI TLAVPPGEEQRYTQVEHPGLD
QPLIWIWEPSPSGTLVIGVISGIAVFVILF IIGILF I I LRKRQGRSGAMGHYVLAERE
```

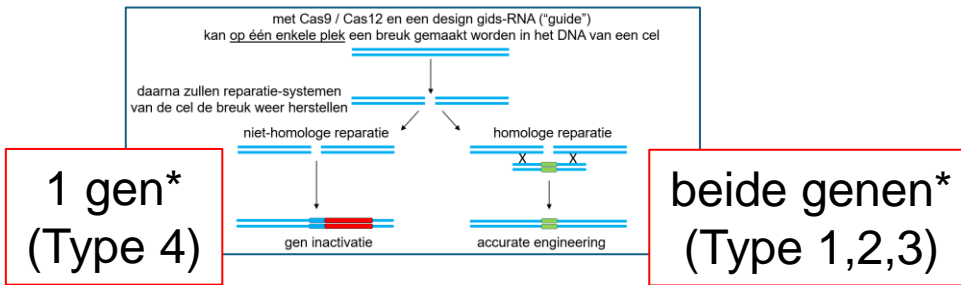
*

Hematochromatose - HFE (High Fe) eiwit



het zou moeten kunnen met Cas9 om een knip aan te brengen op de juiste plek in het genoom, **maar de efficiëntie van editing is (nog) te laag**

(i) DSB + Cas9/sgRNA + repair template + HDR



TYPE 1 HEMOCHROMATOSE

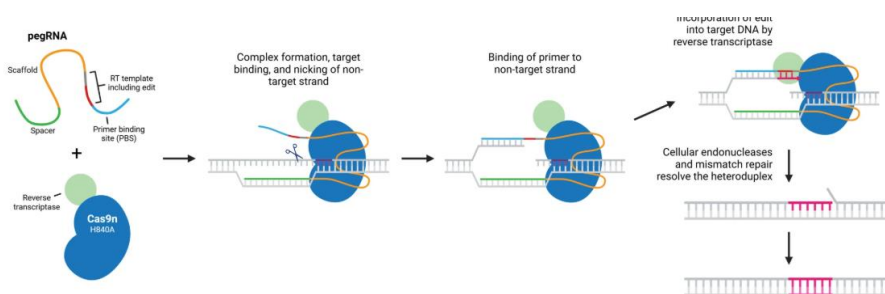
HFE* gene (G845A)

ATGGGCCCGCGAGCCAGGCCGGCGCTTCTCCTCTGATGCTTTTGCAGACC GGGTCC
 TGCAGGGGCGCTTGCTGCGTTCACACTCTCTGCACTACCTCTTCATGGGTGCTCAGA
 GCAGGACCTTGGTCTTTCCTTGTGTTGAAGCTTGGGTACGTGGATGACCAGCTGTTC
 GTGTTCTATGATCATGAGAGTCGCCGTGTGGAGCCCCGAACCTCCATGGGTTTCCAGTA
 GAATTTCAAGCCAGATGTGGCTGCAGCTGAGTCAGAGTCTGAAAGGGTGGGATCAGAT
 GTTCACTGTTGACTTCTGGACTATTATGGAATAACCAACCACAGCAAGGAGTCCAC
 ACCCTGCAGGTATCCTGGGCTGTGAAATGCAAGAAGACAACAGTACCGAGGGCTACT
 GGAAGTACGGGTATGATGGCGAGGACACCTGAAATCTGCCCTGCACACTGGATTG
 GAGAGCAGCAGAACCAGGGCTGGCCACCAAGCTGGAGTGGGAAAGGCACAAGATT
 CGGGCCAGGCAGAACAGGGCTACCTGGAGAGGGACTGCCCTGCACAGCTGCAGCAGT
 TGCTGGAGCTGGGGAGAGGTGTTTGGACCAACAAGTGCCCTCCTTGGTGAAGGTGAC
 ACATCATGTGACCTCTCAGTGACCACTCTACGGTGTCCGGCCCTGAACTACTACCCC
 CAGAACATCACCATGAAGTGGCTGAAGGATAAGCAGCCAATGGATGCCAAGGAGTTCCG
 AACCTAAAGACGTATTGCCCAATGGGGATGGGACCTACCAGGGCTGGATAACCTTGGC
 TGTACCCCTGGGGAAGAGCAGAGATACGTTACCAAGTGGAGCACCCAGGCCTGGAT
 CAGCCCCCATTGTGATCTGGGAGCCCTCACCGTCTGGCACCCCTAGTCATTGGAGTCA
 TCAGTGAATTGCTGTTTTGTGCTCATCTTGTTCATGGAAATTTGTTTCATAATATT
 AAGGAAGAGGCAGGGTTCAAGAGGACCCATGGGGCACTACGTCTTAGCTGAACGTGAG
 TGA

HFE protein

MGFRARPALLLLMLLQTAVLQGRLLRSHSLHYLFMGASEQDLGLSLFEALGYVDDQLF
 VFYDHERRVEPRTPVWSSRISSQMWLQLSLSKGDHMFVTDFWTIMENHNHSHKESH
 TLQVILGCEMQEDNSTEGYWKYGYDGDHLEFCPDTLDWRAEPRAWPTKLEWERHKI
 RARQNRAYLERDCPAQLQLLELGRGVLDDQVPLVKVTHHVTSSVTTTLRCRALNYYP
 QNI TMKWLKDKQPMDAKEFEPKDVLPNGDGT YQGWITLAVPPGEEQRYTQVEHPGLD
 QPLIWIWEPSPGTLVIGVISGIAVFVILFIFILFIILRKRQGRSGAMGHYVLAERE
 *

(ii) SSB + Cas9/RT/pegRNA



de CRISPR-Cas revolutie



- DNA – wat is het, en waarom is het belangrijk ?
- Genetische ziekte – hemochromatose
- CRISPR-Cas – van ontdekking tot toepassing
- Gen therapie – recente voorbeelden

 Conclusies

conclusies



CRISPR-Cas is een uniek afweersysteem van bacteriën

Genoom editing met CRISPR-Cas is revolutie in biologie & biotechnologie

Gen-therapie met CRISPR-Cas mogelijk voor enkele genetische ziektes

Pogingen om CRISPR te gebruiken voor hemochromatosis zijn mij niet bekend

Disclaimer: CRISPR is voor de meeste genetische ziektes nog geen oplossing

samenwerking



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