Lecture 5b
Drugs & Genome Editing

- Drugs that inhibit key processes
- How do you edit the genome of an organism

Antibiotics That Inhibit Prokaryotic Translation

Protein Synthesis – tRNA & Ribosomes

Role of different Ribosomal subunits

30S (Small) – RBS & mRNA codon/anticodon
50S (Large) – Peptide bond synthesis
Exit tunnel – new protein emerges

tRNA sites:

A – aminoacyl – next tRNA-AA binds
P – 1st tRNA-Met & growing peptide
E – empty tRNA leave from here

Initiation:

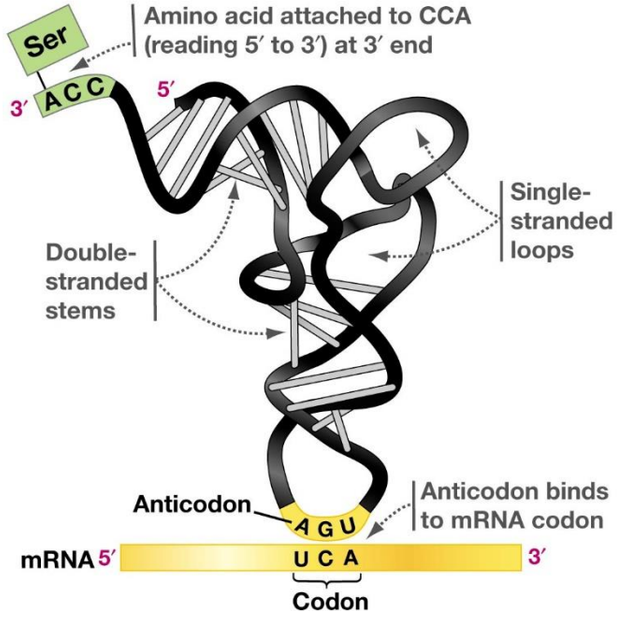
1. mRNA binds to 30S
2. fMet-tRNA binds to P site
3. 50s binds to complete initiation complex

Elongation:

1. New AA-tRNA in A site
2. Peptide bond formation (amino acid in A site added to C-term of peptide in P site)
3. Translocation (tRNA-peptide moves to P site)
4. tRNA exits

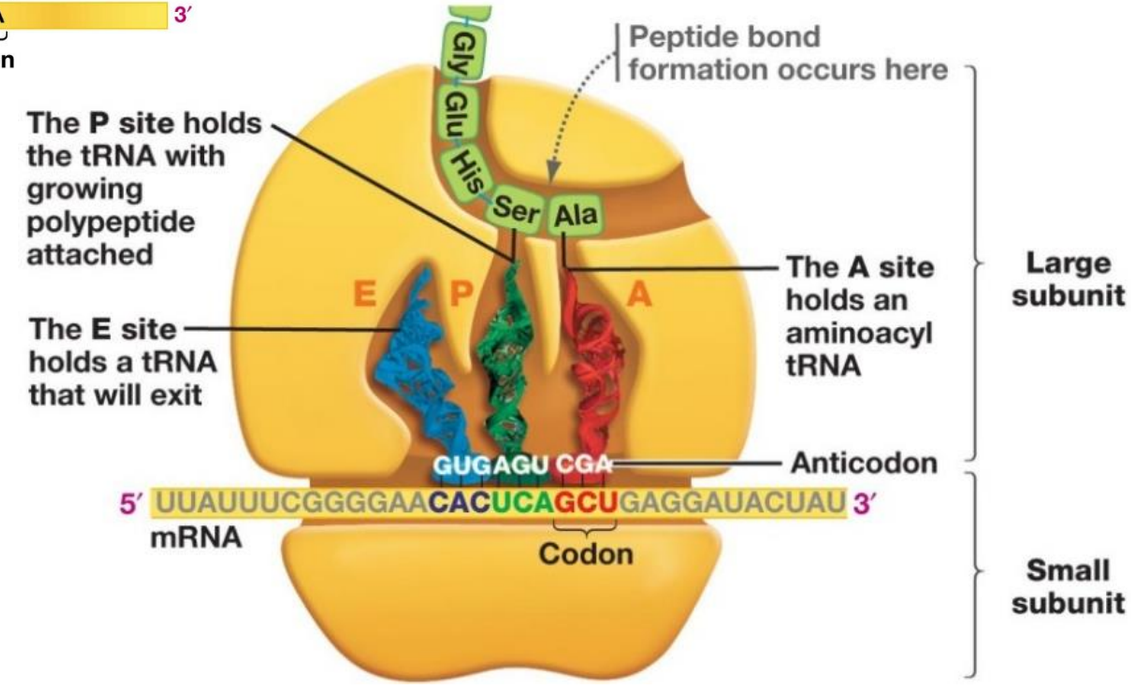
Termination:

1. Stop codon at A site
2. Termination factor (protein) adds water to cleave peptide from last tRNA



tRNAs

- The adapter molecules are called transfer RNAs or tRNAs.
- Contain a CCA sequence at 3’ end where the amino acid is attached
- a triplet anticodon to form base pairs with the appropriate mRNA codon

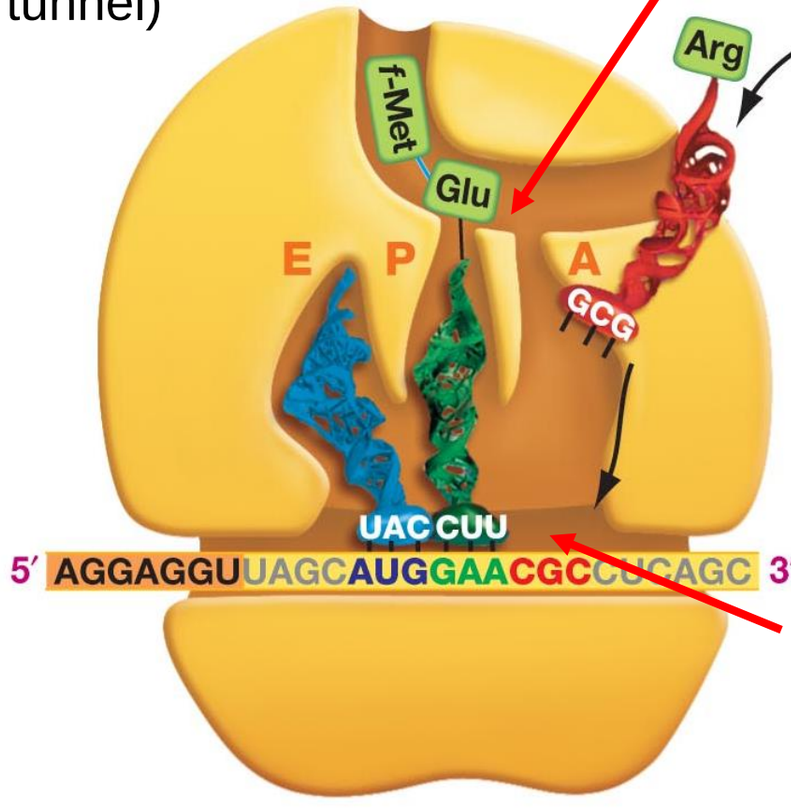


Antibiotics that Inhibit Protein Synthesis

6. Erythromycin (macrolide, blocks exit tunnel)

4. Chloramphenicol (peptide bond formation)

2. Tetracycline (binding of charged tRNA)



5. Viomycin (blocks translocation)

1. Linezolid (blocks 70s formation from two subunits)

3. Kanamycin (Aminoglycoside) (codon/anticodon pairing – wrong amino acid gets added)

Genome Editing – CRISPR Cas9

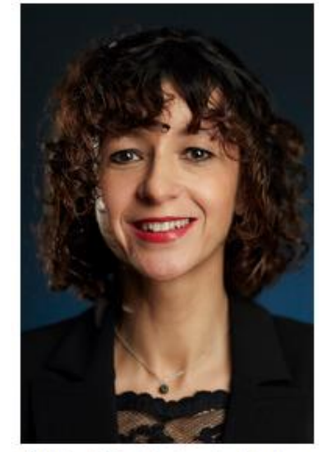
A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]†
Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

17 AUGUST 2012 VOL 337 **SCIENCE** www.sciencemag.org

The Nobel Prize in Chemistry 2020



Emmanuelle Charpentier

Prize share: 1/2

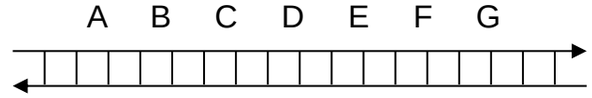
Jennifer A. Doudna

Prize share: 1/2

The Nobel Prize in Chemistry 2020 was awarded jointly to Emmanuelle Charpentier and Jennifer A. Doudna "for the development of a method for genome editing"

Key Concepts in Genome Editing

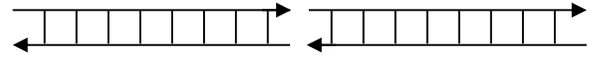
Repair of a ***targeted*** double strand break = modification of the genome at a ***defined location***.



Genomic DNA (A, B, C, D, E, F, G represent short sequences)

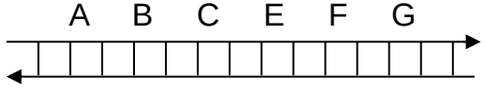
Nuclease (sequence specific)

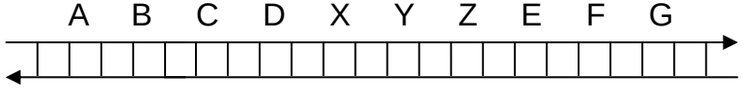
Genomic DNA with double stranded break

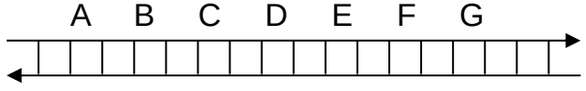


Non-Homologous End Joining (NHEJ)

Repair Method I: Addition or deletion of bases







Deletion

Insertion

together called "***Indels***"

Restoration (undetected)

Original Sequence

```
--ATG......GGGTGGCCGATT...CGATAA—
--Met......GlyTrpProIle...Arg
```

Deletion of one base

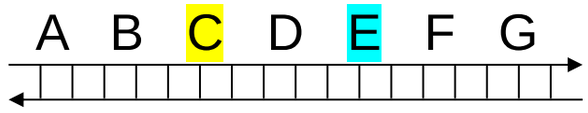
```
--ATG......GGGTGCCGATT...CGATAA—
--Met......GlyCysArgLeu.ArgIle...
```

Indels:

- +/- 3*n* = addition or loss of amino acids
- +/- 1 or 2 bases = Frame shift. changes the amino acid sequence after the indel. The ribosome considers three bases (codon) relative to the start codon.

Key Concepts in Genome Editing

Repair method II – replacement of a segment of DNA



Genomic DNA

Nuclease

Genomic DNA with double stranded break

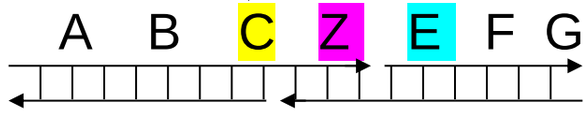
Template C Z E

Homology-Directed Repair (HDR)

C Z E

Template

G-DNA w/DS break



Genomic DNA with staggered nicks (rapidly ligated)

Cut site

Original Sequence

-AATGCGATGCAAAGGCTGATGGGTACCTAGATGCC-
-TTACGCTACGTTTCCGACTACCCATGGATCTACGG-

C D E

Template sequence

GCGATGCAAATTTTTTTCCTAGAT
CGCTACGTTTAAAAAAAGGATCTA

C Z E

Final Sequence

-AATGCGATGCAAATTTTTTTCCTAGATGCC-
-TTACGCTACGTTTAAAAAAAGGATCTACGG-

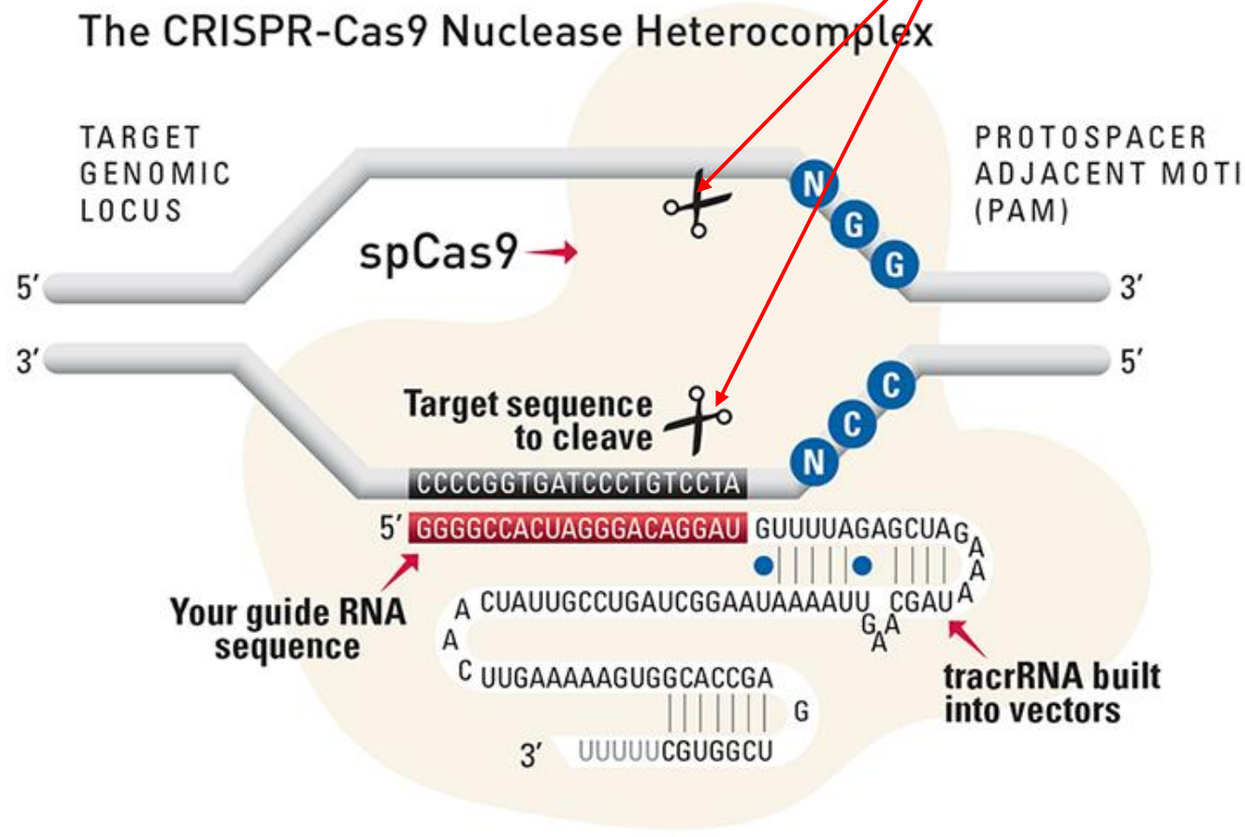
How to Cut at a Defined Location - Cas9 + Guide RNA

Cas9-RNA complex

- Cas9 – nuclease that cuts DNA after activation
- Guide RNA:
  - 5’ end complementary to target sequence
  - 3’ end required for Cas9 activity (tracrRNA)

Double stranded break
(3 bases from PAM)

Step 1. Cas9 Binds to PAM, then checks if RNA is complimentary to DNA sequence 5’ to PAM.



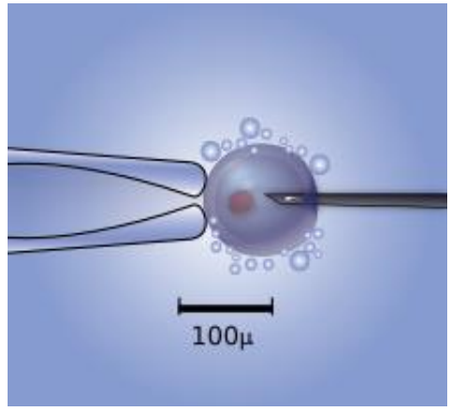
Step 2. After PAM recognition by Cas9, guide RNA unwinds DNA, by pairing with one DNA strand.

Step 3. Cas9 cleaves both strands near site, generating a double strand break.

Step 4. Double stranded break triggers DNA repair, using injected replacement DNA for homologous repair

Altering the Genome Sequence with Cas9-CRISPR

Components to microinject:
1. Cas9 enzyme (nuclease)
2. Guide RNA, specific for site of cleavage, bound to the Cas9 protein
3. Copy of replacement DNA sequence (dsDNA)



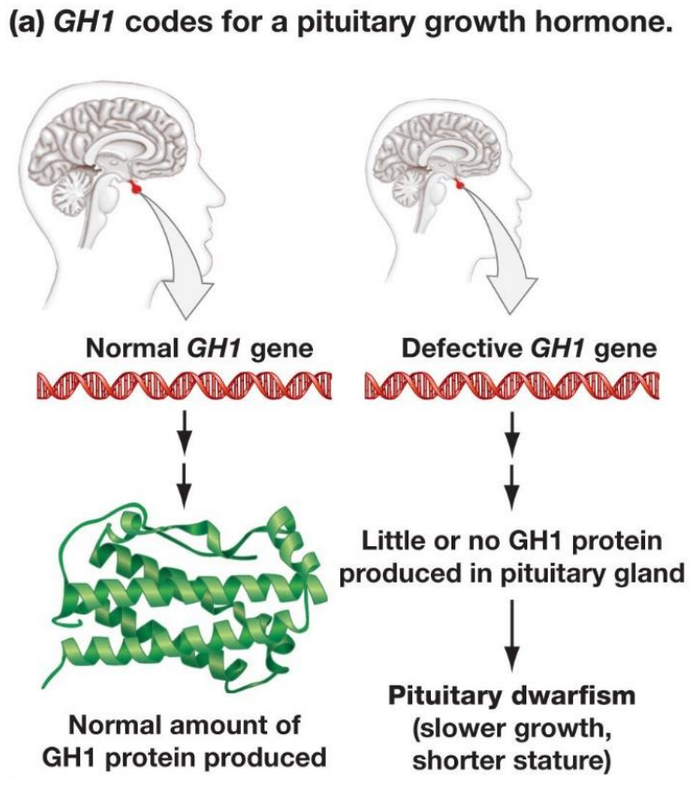
1. Guide RNA directs Cas9 to desired site, by pairing with one DNA strand.
2. CRISPR cleaves both strands near site, generating a double strand break.
3. Double stranded break triggers DNA repair, using injected replacement DNA for homologous repair

(Video originally from Nature)

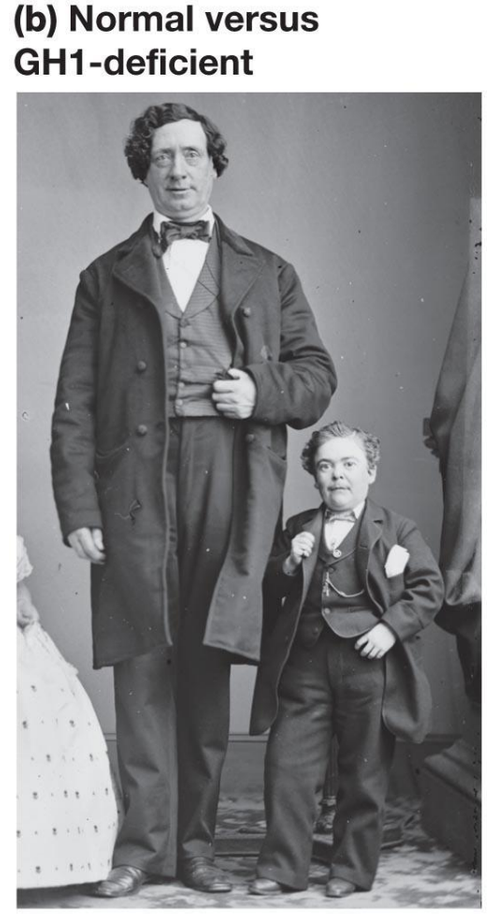
Also view:
https://wyss.harvard.edu/media-post/gene-editing-mechanism-of-crispr-cas9/

Using CRISPR-Cas9 to Correct Genetic Diseases
Human growth hormone (hGH)

Pituitary Dwarfism

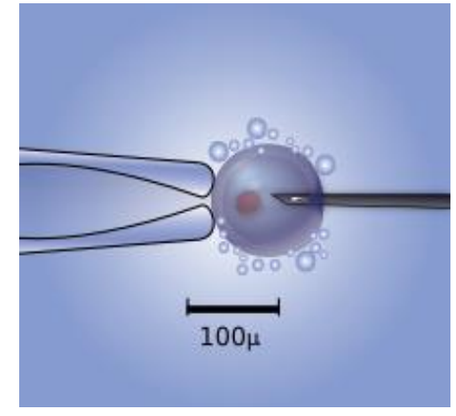


Between one in 14,000 and one in 27,000 babies born each year have some form of dwarfism.



1860 William Harrison and Charles Stratton - comedians and performers.

Components to microinject:
1. Cas9 enzyme (nuclease)
2. Guide RNA, specific for site of cleavage, bound to the Cas9 protein
3. Copy of replacement DNA sequence (dsDNA)



CRISPR Repair of Grown Hormone Gene

Human growth hormone gene

```
>M13438.1:497-2129 Human growth hormone gene (HGH-N), complete cds
AGGATCCCAAGGCCCAACTCCCCGAACCACTCAGGGTCCTGTGGACAGCTCACCTAGCTGCAATGGCTAC  70
AGGTAAGCGCCCCTAAAATCCCTTTGGCACAATGTGTCCTGAGGGGAGAGGCAGCGACCTGTAGATGGGA 140
CGGGGGCACTAACCCTCAGGGTTTGGGGTTCTGAATGTGAGTATCGCCATCTAAGCCCAGTATTTGGCCA 210
ATCTCAGAAAGCTCCTGGCTCCCTGGAGGATGGAGAGAGAAAAACAAACAGCTCCTGGAGCAGGGAGAGT 280
GTTGGCCTCTTGCTCTCCGGCTCCCTCTGTTGCCCTCTGGTTTCTCCCCAGGCTCCCGGACGTCCCTGCT 350
CCTGGCTTTTGGCCTGCTCTGCCTGCCCTGGCTTCAAGAGGGCAGTGCCTTCCCAACCATTCCCTTATCC 420
AGGCTTTTTGACAACGCTATGCTCCGCGCCCATCGTCTGCACCAGCTGGCCTTTGACACCTACCAGGAGT 490
TTGTAAGCTCTTGGGGAATGGGTGCGCATCAGGGGTGGCAGGAAGGGGTGACTTTCCCCCGCTGGAAATA 560
AGAGGAGGAGACTAAGGAGCTCAGGGTTTTTCCCGACCGCGAAAATGCAGGCAGATGAGCACACGCTGAG 630
CTAGGTTCCCAGAAAAGTAAAATGGGAGCAGGTCTCAGCTCAGACCTTGGTGGGCGGTCCTTCTCCTAGG 700
AAGAAGCCTATATCCCAAAGGAACAGAAGTATTCATTCCTGCAGAACCCCCAGACCTCCCTCTGTTTCTC 770
AGAGTCTATTCCGACACCCTCCAACAGGGAGGAAACACAACAGAAATCCGTGAGTGGATGCCTTCTCCCC 840
AGGCGGGGATGGGGGAGACCTGTAGTCAGAGCCCCCGGGCAGCACAGCCAATGCCCGTCCTTGCCCCTGC 910
AGAACCTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCGTGGCTGGAGCCCGTGCAGTTCCTCAG 980
GAGTGTCTTCGCCAACAGCCTGGTGTACGGCGCCTCTGACAGCAACGTCTATGACCTCCTAAAGGACCTA 1050
GAGGAAGGCATCCAAACGCTGATGGGGGTGAGGGTGGCGCCAGGGGTCCCCAATCCTGGAGCCCCACTGA 1120
CTTTGAGAGACTGTGTTAGAGAAACACTGGCTGCCCTCTTTTTAGCAGTCAGGCCCTGACCCAAGAGAAC 1190
TCACCTTATTCTTCATTTCCCCTCGTGAATCCTCCAGGCCTTTCTCTACACTGAAGGGGAGGGAGGAAAA 1260
TGAATGAATGAGAAAGGGAGGGAACAGTACCCAAGCGCTTGGCCTCTCCTTCTCTTCCTTCACTTTGCAG 1340
AGGCTGGAAGATGGCAGCCCCCGGACTGGGCAGATCTTCAAGCAGACCTACAGCAAGTTCGACACAAACT 1410
CACACAACGATGACGCACTACTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGGACATGGACAAGGT 1480
CGAGACATTCCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTCTAGCTGCCCGGGTGG 1550
CATCCCTGTGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCACCAGCCTTG 1620
TCCTAATAAAATTAAGTTGCATCATT
```

Possible PAM Sites

Location of mutation
Isoleucine (I) to Asparagine (N)

Wild type (normal)

R L E D G S P R T G Q I F K Q T Y S

-CTTTGCAGAGGCTGGAAGATGGCAGCCCCCGGACTGGGCAGATCTTCAAGCAGACCTACAGCAA-
-GAAACGTCTCCGACCTTCTACCGTCGGGGGCCTGACCCGTCTAGAAGTTCGTCTGGATGTCGTT-

Mutant (growth hormone non-functional)

R L E D G S P R T G Q N F K Q T Y S

-CTTTGCAGAGGCTGGAAGATGGCAGCCCCCGGACTGGGCAGAACTTCAAGCAGACCTACAGCAA-
-GAAACGTCTCCGACCTTCTACCGTCGGGGGCCTGACCCGTCTTGAAGTTCGTCTGGATGTCGTT-

- The cut site needs to be close to site of mutation so that the injected dsDNA repair template can be as short as possible.
- A NGG (PAM site) is needed for Cas9 to bind.
- There are four possible PAM sites in the DNA sequence on the bottom left. The PAM site closest to the mutation was selected so that the cut site is close to mutation site.

CRISPR Repair of Grown Hormone Gene

- The PAM site closest to the mutation was selected so that the cut site is close to mutation site.
- The targeting section of the guide RNA should have the same sequence as 5’ to the XGG, 18 bases are required:

  5’AGAUGGCAGCCCCCGGAC---------- plus additional RNA needed for Cas9 function

- This RNA would cause cleavage of both the wild-type or mutant sequence since they are identical in this region. This is OK since the repair DNA will contain the wild-type sequence.
- The site of Cas9 cleavage is between the PAM and the guide RNA sequence.
- The injected DNA contains sequences on both sides of the ds break, causing the replacement of the sequences at the double stranded break due to repair.

Injected dsDNA for Homologous Repair

```
CTTTGCAGAGGCTGGAAGATGGCAGCCCCCGGACTGGGCAGATCTTCAAGCAGACCTACAG
GAAACGTCTCCGACCTTCTACCGTCGGGGGCCTGACCCGTCTAGAAGTTCGTCTGGATGTC
```

homology regions required for repair

Possible PAM Sites

DNA cuts by cas9

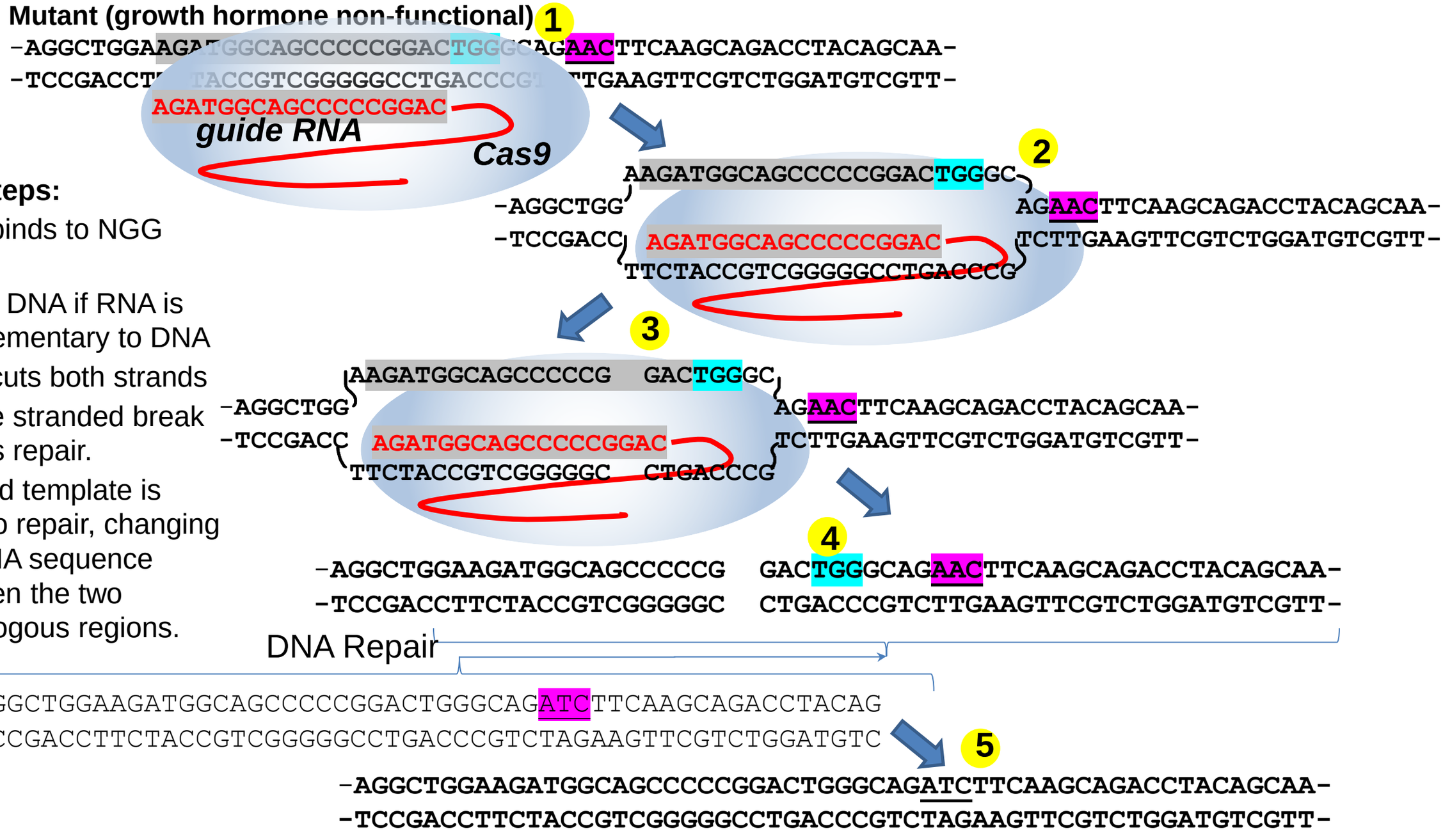
Wild type (normal)

```
          R  L  E  D  G  S  P  R  T  G  Q  I  F  K  Q  T  Y  S
-CTTTGCAGAGGCTGGAAGATGGCAGCCCCCGGACTGGGCAGATCTTCAAGCAGACCTACAGCAA-
-GAAACGTCTCCGACCTTCTACCGTCGGGGGCCTGACCCGTCTAGAAGTTCGTCTGGATGTCGTT-
```

Mutant (growth hormone non-functional)

```
          R  L  E  D  G  S  P  R  T  G  Q  N  F  K  Q  T  Y  S
-CTTTGCAGAGGCTGGAAGATGGCAGCCCCCGGACTGGGCAGAACTTCAAGCAGACCTACAGCAA-
-GAAACGTCTCCGACCTTCTACCGTCGGGGGCCTGACCCGTCTTGAAGTTCGTCTGGATGTCGTT-
```

Note that Cas9 will cut both the wild-type and the mutant, but repair will insert the wild-type sequence.



Editing Steps:

1. Cas9 binds to NGG (PAM)
2. Opens DNA if RNA is complementary to DNA
3. Cas9 cuts both strands
4. Double stranded break causes repair.
5. Injected template is used to repair, changing the DNA sequence between the two homologous regions.