

LAW FIRM OF NAREN THAPPETA

# Gene Editing Basics

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Topics for discussion

# Core Concepts

What does CRISPR do?

Biology Basics

Gene Editing – Basic Concepts

CRISPR – How it works

Future/Issues

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#2

# What does CRISPR do?

## **CRISPR**

**C**lustered **R**egularly **I**nterspaced  
**P**alindromic **R**epeats

Gene Editing Technology

Helps understand basis of many  
diseases (e.g., autism/cancer)

Genetically modified animals and plants  
improve crop yield, size, resistance to  
disease

Future: Cure/treatment (see below  
from Economist of Sep 2022

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## Rare birds

Key gene therapies for rare diseases in development or approved

- Medicines approved in any jurisdiction

Company/therapy name	Disease
BioMarin/Roctavian ●	Haemophilia a
Disease description Blood-clotting disorder	
bluebird bio/eli-cel	Cerebral adrenoleukodystrophy
Rapid loss of neurological function. Often fatal	
bluebird bio/lovo-cel	Sickle-cell disease
Atypical haemoglobin molecules lead to painful condition with wide damage to body and organs	
bluebird bio/Zynteglo* ●	Beta thalassaemia
Blood disorder with reduced levels of working haemoglobin	
CRISPR Therapeutics and Vertex Pharmaceuticals/CTX001	Sickle-cell disease
Atypical haemoglobin molecules lead to painful condition with wide damage to body and organs	
Gensight Biologics/Lumevoq	Leber hereditary optic neuropathy
Mitochondrial genetic disease that causes irreversible and severe vision loss, leading to blindness mostly in teens and young adults	

The Economist, Sept Issue

Company/therapy name	Disease
Novartis/Zolgensma ●	Spinal muscular atrophy
Causes weakening muscles and can be fatal	
Orchard Therapeutics/Libmeldy ●	Metachromatic leukodystrophy
A disorder which degrades the nervous system	
Orchard Therapeutics/Strimvelis ●	ADA-SCID
Inability to fight infections due to lack of white blood cells	
PTC Therapeutics/Upstaza ●	AADC deficiency
Rare genetic disorder of nervous system, interferes with way nerve cells talk to each other	
Roche/Luxturna ●	Inherited retinal disease
Eye disorder that causes vision loss or blindness	
UniQure/EtranaDez†	Haemophilia b
Blood-clotting disorder	
UniQure/Glybera* ●	Lipoprotein lipase deficiency

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## II. BIOLOGY BASICS (Cell)

- Smallest unit that can live on its own
- All living organisms and the tissues of the body
- More than 30 trillion cells total in human body
- More than 200 different types of cells in human body
  - skin cell, hair cell etc.

## II. BIOLOGY BASICS (Cell)

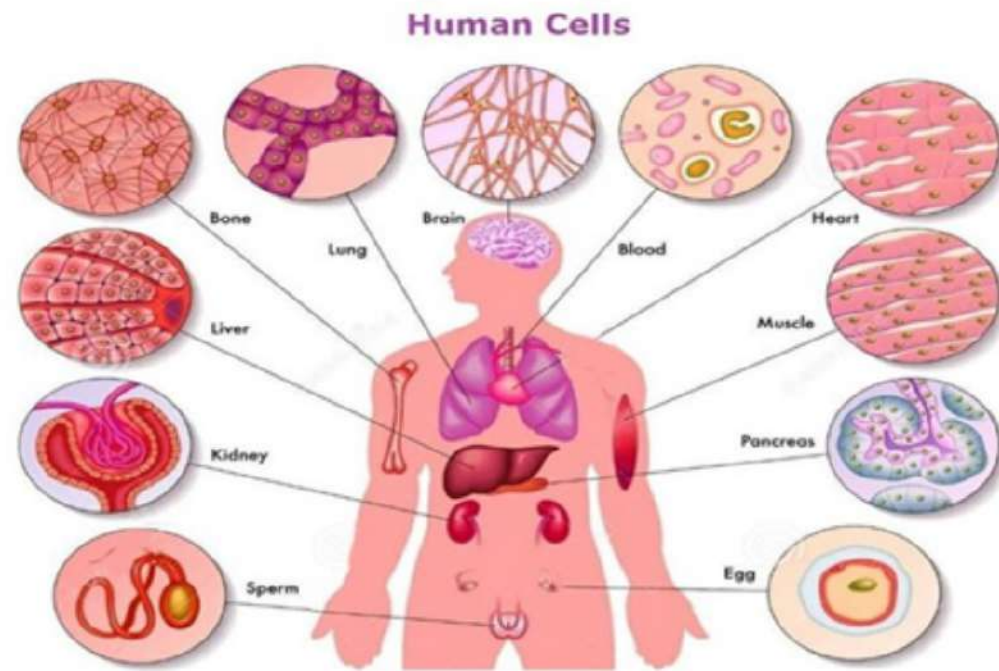
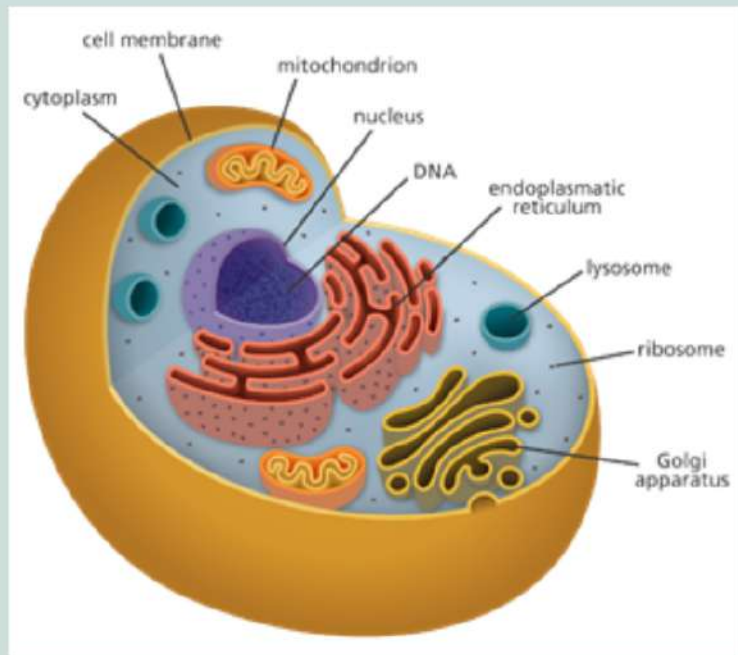


Image of each cell represents the content of the cell type



## II. BIOLOGY BASICS (Cell) Contd...



- **Nucleus**

- function: command center
- contains the genome (in detail below)
  - genome contains the commands

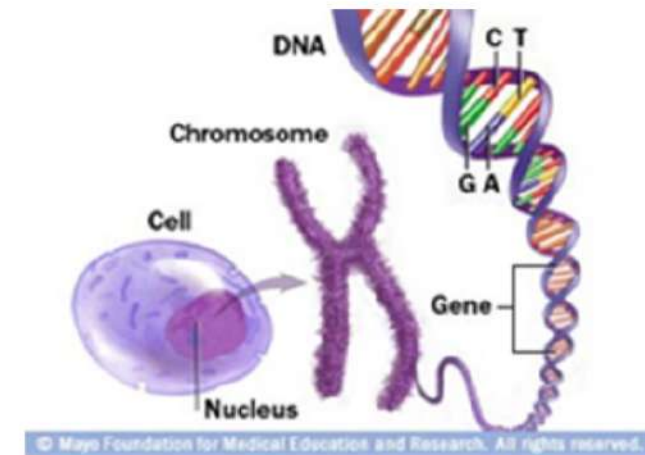
- **Miscellaneous**

- Cytoplasm (surrounds all the cell parts within membrane)
- Mitochondrion (a power source)
- Endoplasmic reticulum (transport materials in cells)
- Ribosomes (creates protein)
- Lysosome (removes cell waste)
- Cell membrane etc.

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## II. BIOLOGY BASICS (Genetic Material)

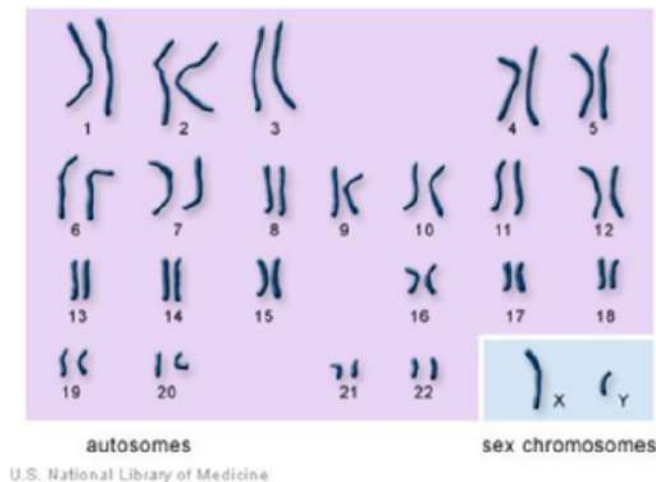
- **Genome (the sum total of information in a cell)**
  - instruction manual of the body
  - divided into 46 DNA strands called chromosomes
    - DNA is a biomolecule that holds genetic information
    - DNA is akin to computer hard-drive storing lots of data
  - RNA: single-stranded intermediate of DNA- makes proteins
- **Chromosome**
  - supercoiled DNA
  - Can read from DNA, but not chromosome
  - Chromosome=closed book; DNA=open book ready to read
- **Genes**
  - segment of DNA that has A known function
  - estimated more than 20,000 genes in humans
  - sequence of Nucleotides
    - 4 types: A, T, C, G (alphabet for genes)
    - A always paired with T
    - C always paired with G
    - Duplicate information in each gene
  - Hurdle in each gene editing:
    - Genes are being discovered
    - full function(s) of each gene is still not clear





## II. BIOLOGY BASICS (Chromosomes)

- 46 total chromosomes in each human cell typically
  - difference may manifest as a disease
- each chromosome is a compressed molecule of DNA
- every cell has all 46 chromosomes
- 23rd pair sex chromosomes
- XX-> inherit female characteristics
- XY -> inherit male characteristics



## II. BIOLOGY BASICS (Understanding the Challenge)

- Every cell of ONE human being
  - 3.2 billion nucleotides (in the form of 46 chromosomes)
  - substantially same sequence (99%+)
  - 50- 250 million nucleotides in each chromosome
- Cell type is determined by which part (genes) of the sequence provides commands
- Across humans the sequences are 99.9% similar
- Difference between 46 chromosomes within a cell?
  - substantial difference between chromosome types within the cell
  - Length also different
  - Each chromosome contains a specific portion of the genome.
  - Ex. The gene for the proteins that carries oxygen in the blood is on chromosome 11 and 16
- Difference between chromosomes across cell types within a human
  - The same set of chromosomes (genome) are in each cell type
  - Cell type: Only a portion of the genome is expressed in each cell
  - estimated mutation rate 175 mutations per genome per generation
  - most of the time this mutation leads to no change
  - accumulation of mutations over many (millions) years leads to evolution

## II. BIOLOGY BASICS (Understanding the Challenge) Contd...

- Difference between chromosomes across humans?
  - Apart from the males having an xy chromosomes and females xx
  - 0.001% average difference in human genomes
  - Unique identifiers of each human still!
- Difference between chromosomes between monkey (bonobo) and human?
  - 1.2% genetic difference between humans and bonobos
  - that difference believed to be the basis for our superior learning
- Summary of challenge:
  - knowing gene and corresponding function(s)
  - desired modification of that gene
    - setting the boundary
    - cut/paste

### III. GENE EDITING BASICS

0. Conceptual Inputs: (I1) DNA sought to be edited; (I2) effector complex specifying the gene target

1. Locate the gene target in the DNA

- Acquire DNA (I1) from organism
- unwind and unzip DNA strand with enzyme
  - enzyme: a biomolecule that can cut chemical bonds between the complementary pairs of nucleotides
  - Ladder split into two vertical parts
  - find target DNA specified by (I2) at target site

2. Mutation

- cut the target site
  - enzyme makes double stranded break in DNA
- Introduce donor nucleotide sequence in the cut area

4. Ends of DNA are rejoined

5. Common for CRISPR, TALEN and Zinc Finger Nucleases (ZFN) gene editing technologies

6. CRISPR: Gene editing complex carries out 1 and 2 above

- RNA manufactured for annealing with (finding and latching onto) the target
- CAS9 for effecting a double stranded break (at two points per latched boundaries)



## IV. CRISPR Patent 8697359 – Claim

**Assignee:** MASSACHUSETTS INSTITUTE OF TECHNOLOGY and Broad Institute  
Issued - 15-Apr-2014; Earliest Priority: 12-Dec-2012 (pre-AIA Applicable)  
Interference Parties: Junior Party: Applicant; Senior Party: Sigma-Aldrich Co.  
(USA/Missouri based)

15. An engineered, programmable, non-naturally occurring Type II CRISPR-Cas system comprising a **Cas9 protein and at least one guide RNA that targets and Cas9 protein and atleast one guide RNA that targets and hybridizes to a target sequence of a DNA molecule in a eukaryotic cell,** wherein the DNA molecule encodes and the eukaryotic cell expresses at least one gene product and the Cas9 protein cleaves the DNA molecules, whereby expression of the at least one gene product is altered; and, wherein the Cas9 protein and the guide RNA do not naturally occur together.

***(What was argued as new)***

## IV. CRISPR Patent 8697359 – Advancement

### **History**

- Genome engineering there since 1970
- CAS9 (cutting) present since 2010s
- Improvements primarily to locating the gene target in the DNA

### **Specification**

The CRISPR/Cas or the CRISPR-Cas system (both terms are used interchangeably throughout this application) does not require the generation of customized proteins to target specific sequences but rather a single Cas enzyme can be programmed by a short RNA molecule to recognize a specific DNA target, in other words the Cas enzyme can be recruited to a specific DNA target using said short RNA molecule.



## V. CRISPR Patent 8697359 – Enabling Disclosure

### CRISPR parts

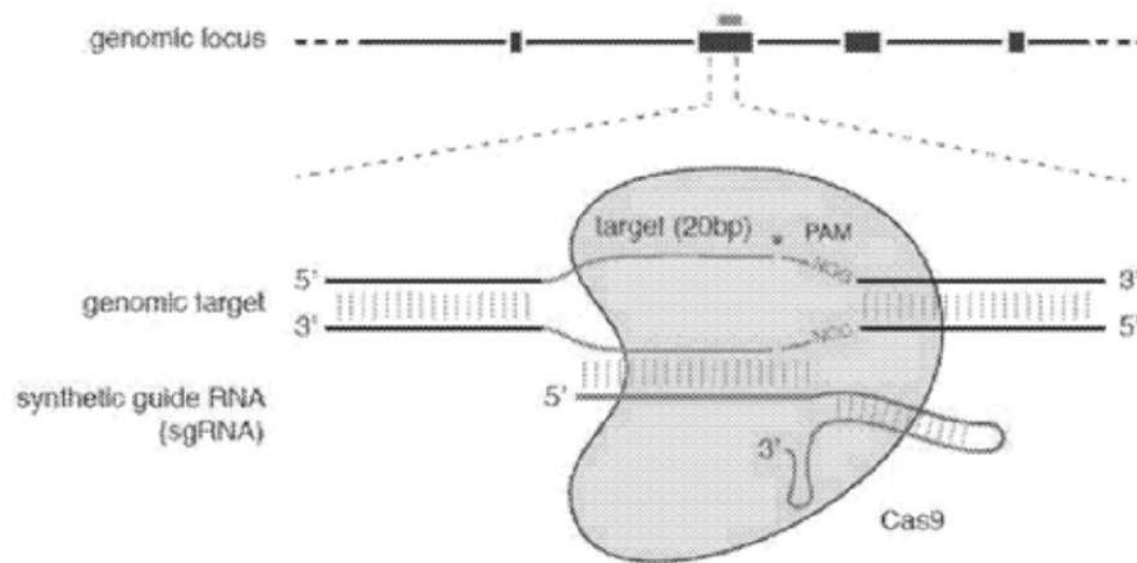
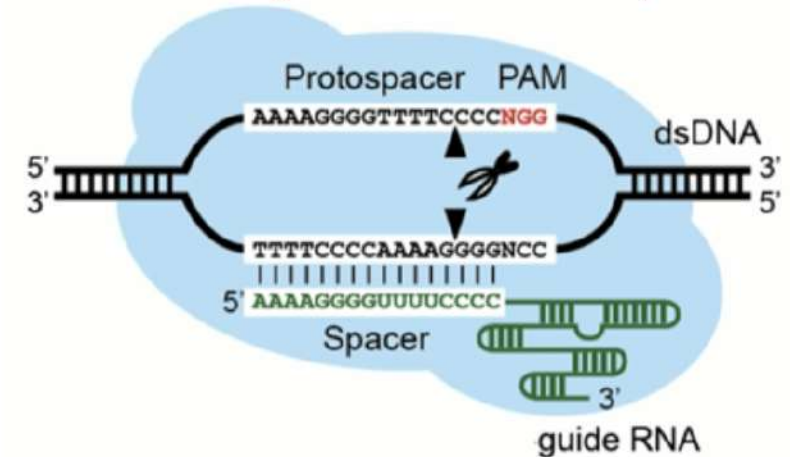


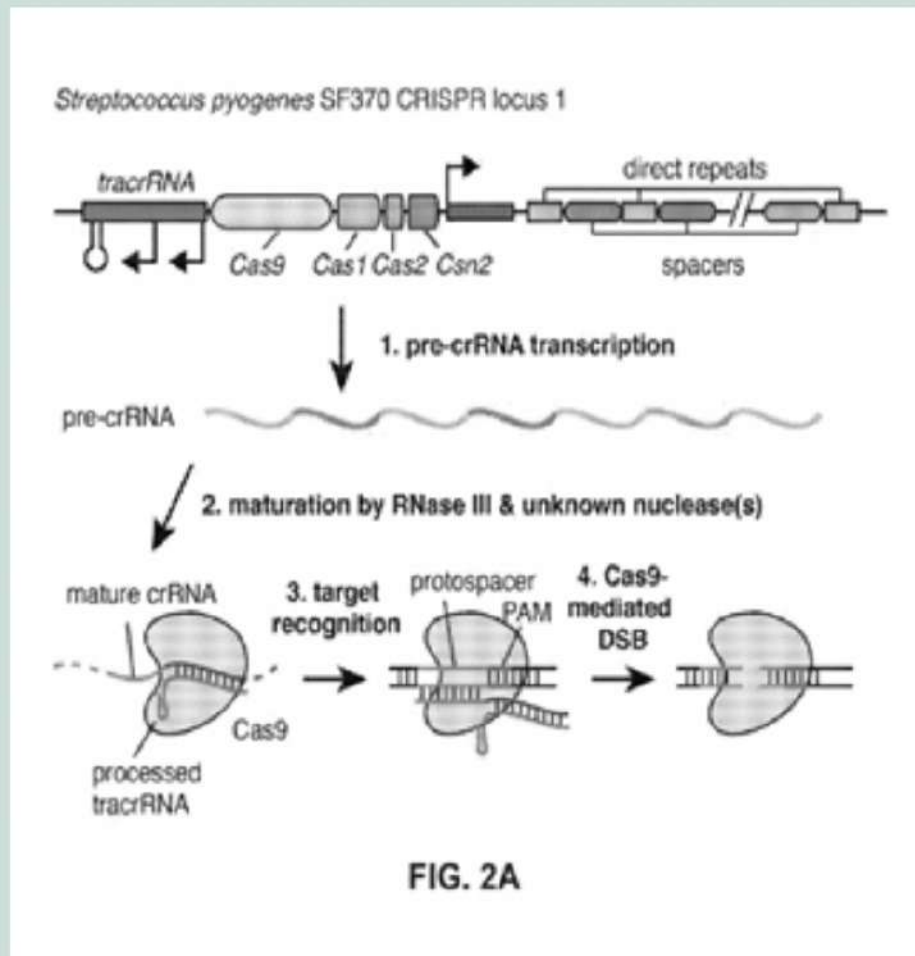
FIG. 1

### Enlargement of effector parts



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## V. CRISPR Patent 8697359 – Enabling Disclosure (Contd...)



- Biogenesis of CRISPR RNA
  - CRISPR RNA transcribed into long RNA transcript (pre- crRNA)
  - pre-CRNA processed into mature crRNA consisting of spacer and flanking sequence
  - Individual crRNAs bind to Cas protein to form a complex
- RNAase III cuts pre-crRNA into mature crRNA
  - Mature crRNA = 1 repeat + spacer
- Target phase
  - crRNA is a guide to recognize the DNA target because it is complementary to the target
  - crRNA + tracrRNA + cas9 bind to DNA target
- 4. DNA target is cut
  - a. Cas9 enzyme creates a double stranded break (DSB) in the DNA

## CRISPR Patent 8697359 – Enabling Disclosure (Contd...)

- PAM (protospacer adjacent motif) helps ensure the cas9 system doesn't try to cut its own gRNA strand.
- tracrRNA is complementary to the palindromic (direct) repeats.
- tracrRNA + crRNA = sgRNA
- Figures 3A onwards primarily establish the observed results for specific component operation

## V. Advancements/Issues

- Major Advancements
  - Cutting multiple targets in one round
  - More comprehensive and affordable gene sequencing
  - xeno-transplantation from pig to human
- Unforeseen consequences of gene editing
  - Functions of gene not completely understood
- Ethical Issues
  - Eugenics
  - Environmental impact
  - Expensive - Access to all



# Thank You!

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