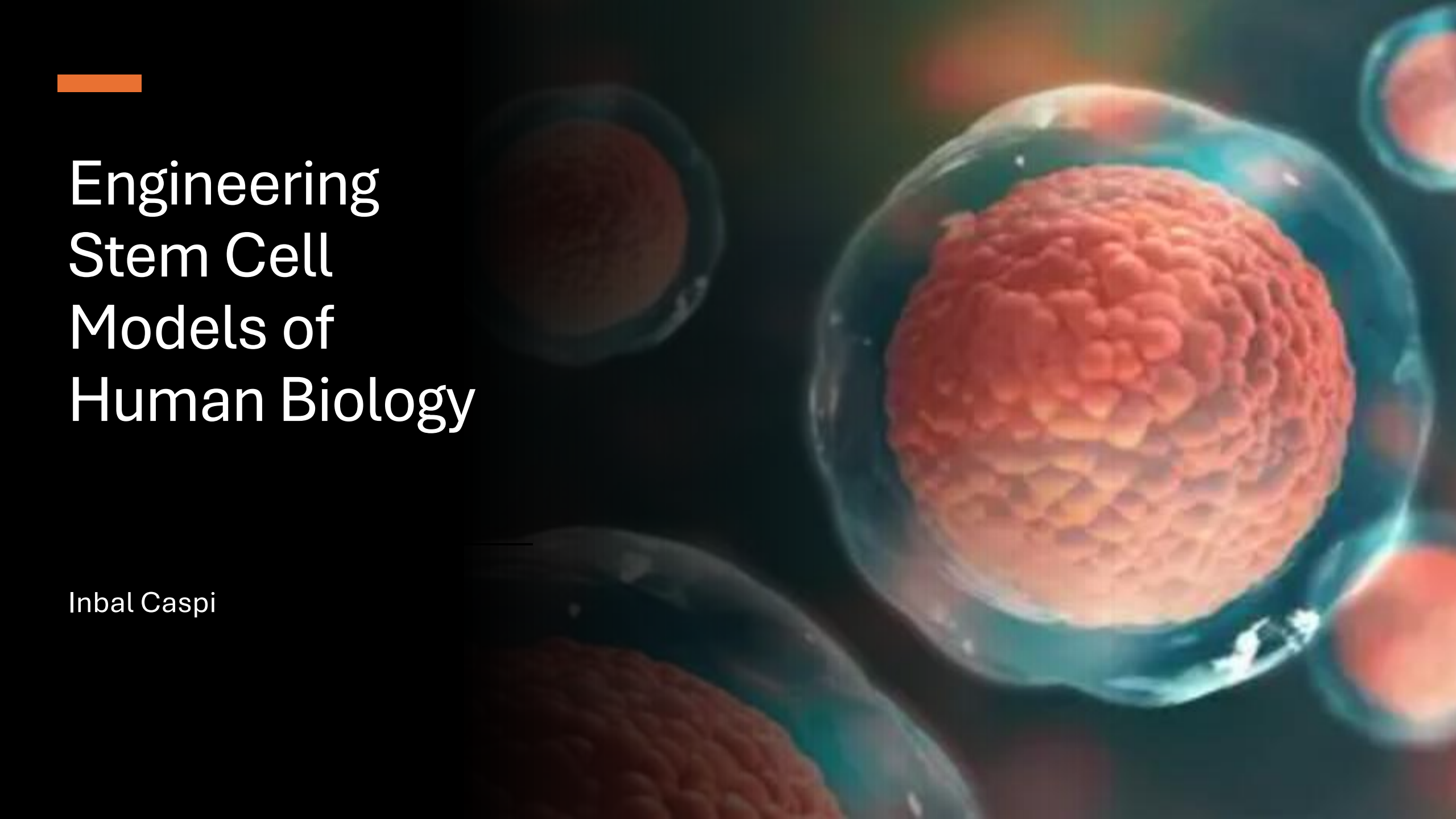
Engineering Stem Cell Models of Human Biology

Inbal Caspi



Applying Genetic Engineering to Cell/Tissue Therapy

Lesson Objectives

- Designing a gene and stem cell–based therapy
- Gain hands-on understanding of how to design a gene correction strategy
- Learn how to choose editing tools
- Practice experimental reasoning — deciding which steps, controls, and assays to use
- Understand how genetically corrected cells can be used for tissue regeneration and transplantation

Focus Case

Retinal degeneration caused by a single mutation

Guiding Question

How would you design, validate, and translate a genetic correction strategy using stem cells as the therapeutic platform?

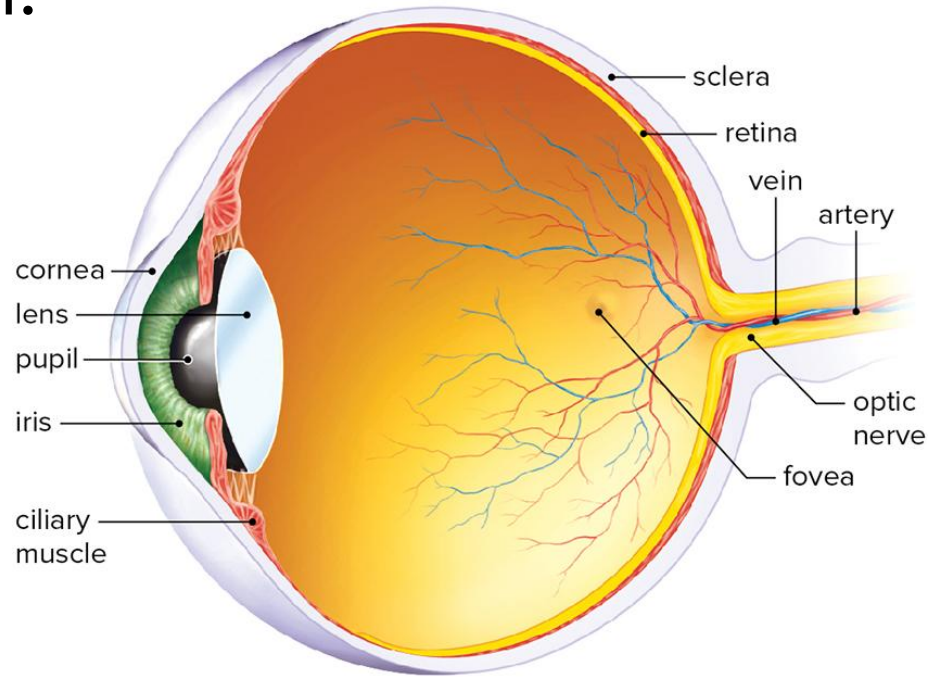
Eye Physiology

- **Cornea and Lens:** Focus incoming light onto the retina.
- **Iris and Pupil:** Control how much light enters the eye.
- **Retina:** Light-sensitive layer containing **photoreceptors:**

❖**Rods** - low-light and black-and-white vision.

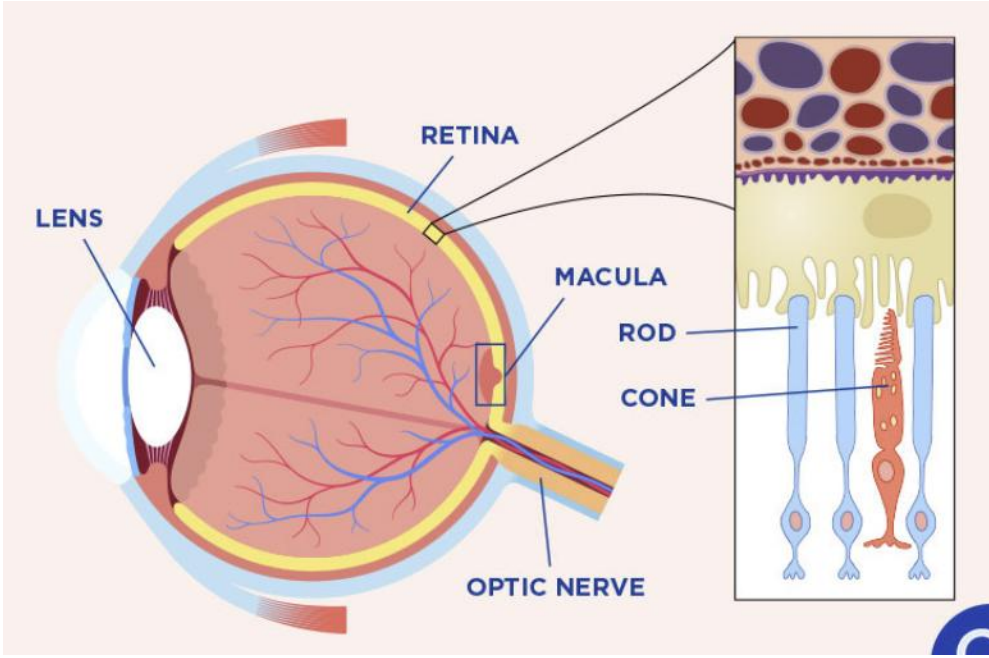
❖**Cones** - color and sharp vision.

- **Optic Nerve:** Sends visual signals from retina to the brain.



Leber Congenital Amaurosis (LCA)

- A group of rare, inherited retinal diseases that cause severe vision loss from birth or early infancy.
- Autosomal recessive inheritance
- ~1 in 30,000–50,000 newborns
- Caused by mutations in 25+ different genes.
- Over time, photoreceptors degenerate, leading to worsening vision or blindness.



Leber Congenital Amaurosis 10 (LCA10)

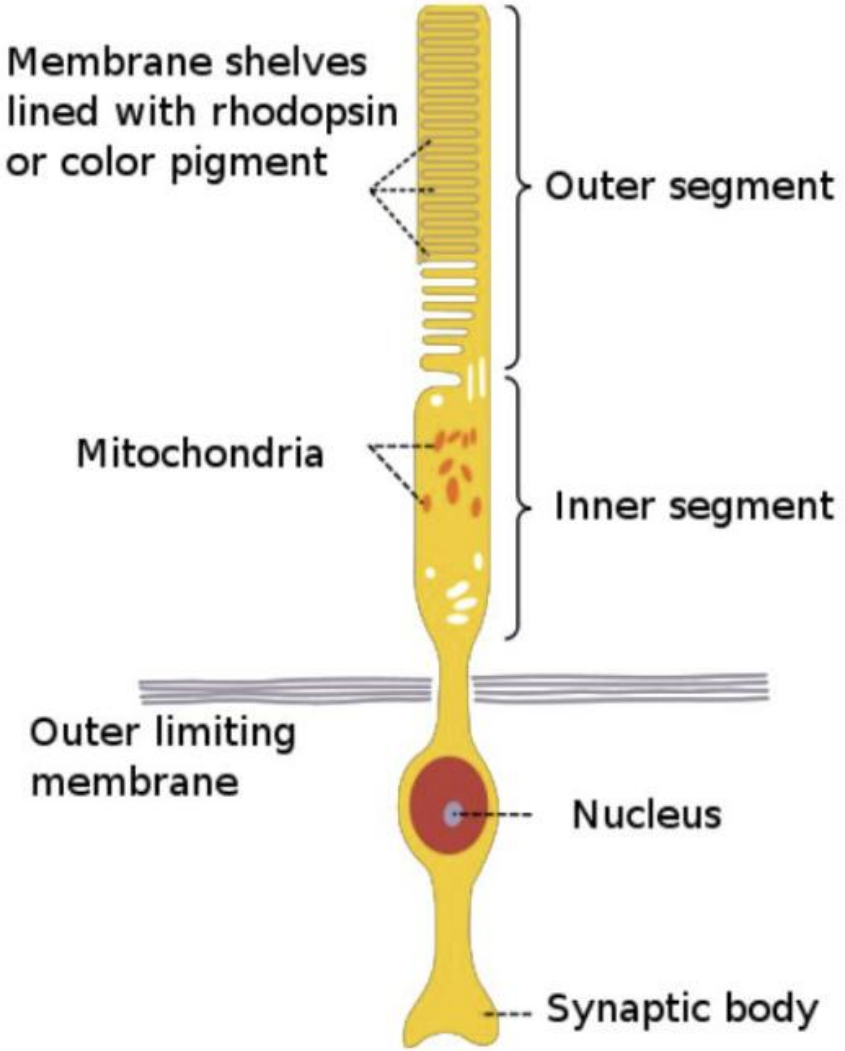
- LCA10 is the most frequent genetic subtype of LCA (30% of all LCA cases).
- Early-onset severe visual impairment (infancy)
- Symptoms: severe photophobia, high hyperopia, and visual acuity reduced to hand movements or perception of light.
- Cone-rod dystrophy pattern
- Retina often appears normal early, then degenerates
- Progression:

❖Childhood: rod degeneration → white retinal flecks.

❖Adolescence/early adulthood: pigmentary retinopathy.

The Photoreceptor Structure

- **Outer Segment:** Detects light (rhodopsin/photopsins).
- **Inner Segment:** Produces energy and proteins.
- **Connecting Cilium:** Transfers proteins to outer segment.
- Signal sent to brain via synapse.

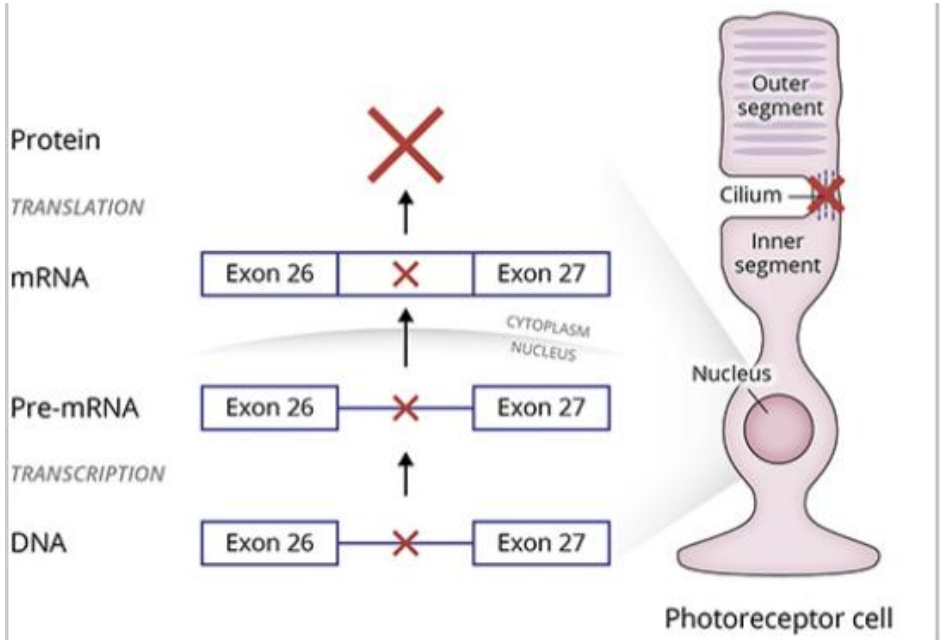


CEP290 Mutation

- CEP290 plays an important role in centrosome and cilia development.
- c.2991+1655A>G intronic mutation results with the insertion of a “pseudo-exon”.
- A premature stop codon produces a truncated CEP290 protein.
- The retina is uniquely sensitive to CEP290 dysfunction:

❖Photoreceptors depend heavily on ciliary transport.

❖The retina receives a bigger reduction in functional CEP290.



Designing a Therapy for LCA10

Your PhD project aims to develop a therapy for **LCA10**, a severe childhood blindness caused by a mutation in the **CEP290** gene.

- Design the main steps of your project.

- Can you test a therapy if you haven't shown the disease phenotype in your system?
- Do we fully understand the defect?
- Can we test a therapy without a way to measure success?
- What would we need in the lab to start working on LCA10?

Project Roadmap

☑ **Understand the disease -** Gene, mutation, affected cells, and mechanism

2. **Establish a disease model -** System to study the mutation and test treatments

How Will We Model LCA10?

Considerations when choosing a model:

- **Relevance:** Does it accurately mimic LCA10 in humans?
- **Cells or Organism?** eye cells, retinal organoids, animals, etc.
- **Feasibility:** Time, cost, and technical difficulty.
- **Ethical & Practical Limits:** What is allowed and realistic to do?
- **Measurable Readouts:** Can we detect improvement or rescue of function?

Possible Models & Their Trade-Offs

Model Option	Advantages	Limitations
Cell line (e.g., HEK293)	Easy, fast, cheap; simple genetic manipulation	Not retinal cells → poor disease relevance
Patient-derived fibroblasts	Carry the real mutation; accessible	Not retinal cells; limited vision-related readouts
iPSCs from LCA10 patients	Human, patient-specific; can differentiate into retinal cells	More time, cost, and expertise required
Retinal organoids (iPSC-derived)	3D retina-like tissue; contains photoreceptors; high disease relevance	Takes months to grow; technically complex
Animal model (mouse/zebrafish)	Whole-eye and behavioral vision studies; allows in vivo testing	Species differences; mutation may not fully replicate human LCA10
Retinal cell line (RPE or photoreceptor precursor)	Eye-relevant cell type; easier than organoids	May not model the exact mutation or full retinal structure

What Do Researchers Usually Choose?

- Patient-derived iPSCs differentiated into retinal cells/organoids
- Why this model is preferred:

❖Contains the patient's real CEP290 mutation.

❖Can be differentiated into photoreceptors, the affected cell type.

❖Allows direct testing of whether a therapy rescues the defect

❖Enables measurable readouts:

✓ Corrected splicing of CEP290
✓ Restoration of cilia/outer segment structure
✓ Protein localization
✓ Light-response assays (in advanced organoids)

Validate the Disease Model

- What should be the next step?
- What do we need to do *before* we start testing a treatment?
- What information must we gather from our model?

Validate the Disease Model

- Before testing any therapy, we must:
  Confirm that our model actually shows the LCA10 disease features.
- Why this step is essential:

❖Ensure the model replicates the CEP290 defect

❖Establish measurable readouts to detect improvement

❖Create a baseline to compare treatment effects

- Key Goals at this Stage:

❖Demonstrate the mutation causes a defect in the model

❖Identify how to measure rescue (molecular, structural, functional)

How Do We Know Our Model Shows LCA10?

- What measurements or “readouts” would show that the model truly reflects the LCA10 disease?
- **Think about:**
- What is **wrong** in LCA10 that we should detect in the model?
- What could we measure at the **molecular level**?
- What could we measure at the **cellular/structural level**?
- What could we measure at the **functional level**?

Common Readouts to Confirm an LCA10 Model

Molecular Readouts

- Aberrant CEP290 mRNA splicing (pseudo-exon insertion).
- CEP290 protein levels are low.

Cellular / Structural Readouts

- Defective connecting cilium structure.
- Poor outer segment formation or organization.
- Mislocalization of photoreceptor proteins (e.g., opsins).

Functional Readouts

- Reduced or absent photoreceptor activity.
- Impaired phototransduction markers.

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3. **Select a therapeutic strategy -** CRISPR editing, gene therapy, stem cells…

- **How should we approach treating LCA10?**
- Should we try to **fix the mutation**, **replace the gene**, or **support the cells** in another way?
- What type of therapy do you think could work for LCA10, and why?

Comparing Therapeutic Strategies

- **Gene Replacement Therapy**
- **CRISPR Gene Editing**
- **Antisense Oligonucleotides (ASOs)**
- **Stem Cell–Based Therapy**

Which Strategy Makes the Most Sense for LCA10?

Before choosing a therapy, consider these questions:

- At what stage of disease will the therapy be used?
- Are the photoreceptors still present, or have many already died?
- Does the therapy fix the gene, or does it restore lost cells?
- Will the treatment work if few photoreceptors remain?
- If photoreceptors have already degenerate would correcting the gene alone be enough?

Which Strategy Makes the Most Sense for LCA10?

Why stem cell therapy is needed:

- Gene-based treatments require **living photoreceptors** to work
- In LCA10, **photoreceptors gradually die**, especially cones
- Stem cell–derived photoreceptors can **replace lost cells**
- Offers a path to **restore vision even in late-stage disease**

Which Stem Cells Can Be Used to Treat LCA10?

Stem Cell Type	Description	Pros	Cons
Embryonic Stem Cells (ESCs)	Derived from early embryos; pluripotent		
Induced Pluripotent Stem Cells (iPSCs)	Adult cells reprogrammed to pluripotency		
Retinal Progenitor Cells (RPCs)	Precursor cells committed to retinal fate		

Which Stem Cells Can Be Used to Treat LCA10?

Stem Cell Type	Description	Pros	Cons
Embryonic Stem Cells (ESCs)	Derived from early embryos; pluripotent	Can become any cell type; reliable differentiation	Ethical issues; immune rejection risk
Induced Pluripotent Stem Cells (iPSCs)	Adult cells reprogrammed to pluripotency	Patient-specific (no rejection); can make retinal cells	Time-consuming to generate and differentiate
Retinal Progenitor Cells (RPCs)	Precursor cells committed to retinal fate	Naturally form photoreceptors; good integration potential	Limited expansion; less flexible than iPSCs

How Should We Correct the CEP290 Mutation in Patient iPSCs?

- What type of gene-editing approach should we use?
- Do we want to *repair* the mutation or *remove its effect*?
- How important is precision vs. efficiency?
- How do we minimize damage to the genome?
- Temporary editing tools vs. permanent DNA changes?

Gene Editing Options

Method	Key Idea	Notes
CRISPR-Cas9	Creates a DNA break → cell repairs with provided template	Higher efficiency, but risk of unwanted edits
Base Editing	Directly changes one base without cutting DNA	Very precise for single-letter changes, limited to certain base conversions
Prime Editing	Programmable edit with no double-strand break	Very precise, flexible edits, currently lower efficiency

Which Editing Method Fits Best for LCA10?

- Most suitable choice is **base or prime editing**: to precisely fix the CEP290 splicing mutation with minimal DNA damage.

❖Avoids double-strand DNA breaks.

❖Reduces risk of unwanted insertions/deletions.

❖More precise correction for a single-nucleotide defect.

❖Safer for cells intended for transplantation.

- CRISPR-Cas9? Still possible, but less preferred due to DNA cutting and higher risk of off-target effects

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4. **Design the treatment –** Create the cell line and correct the mutation

Establishing and Characterizing Patient-Derived iPSCs

1. **Sample collection and mutation verification**

- Obtain fibroblasts from LCA10 patient and wild-type (healthy donor) control.
- Sequence the CEP290 gene to identify and confirm biallelic mutations.

2. **CEP290 characterization in fibroblasts**

- Western blot: detect altered protein size due to truncation
- Immunofluorescence: assess CEP290 localization at the ciliary base
- Flow cytometry: quantify CEP290 or related ciliary markers
- RT-PCR: evaluate aberrant CEP290 splicing or reduced transcript levels (Use antibody targeting the region before the truncation site).

3. **Reprogramming and validation**

- Reprogram patient and control fibroblasts into iPSCs.
- Validate iPSCs: Morphology, pluripotent markers, karyotype.

Correcting Patient-Derived iPSCs

1. **Identify and characterize the mutation**

- Confirm the CEP290 variant (c.2991+1655A>G) by sequencing the patient iPSC line
- Retrieve reference sequence NCBI

2. **Design the editing system (Prime Editing or Base Editing)**

- Use tools such as PrimeDesign, pegFinder, or CHOPCHOP to design pegRNA or sgRNA
- Include off-target prediction and PAM site validation

3. **Prepare and deliver editor into iPSCs**

- Deliver the machinery into iPSCs as: plasmid (easy but risk of genomic integration), mRNA (transient, high safety), or RNP complex (immediate action, minimal off-target risk).
- Delivery method: Electroporation (high efficiency, transient), Lipid nanoparticle (less stress, lower efficiency), Viral like AAV/lentivirus (high expression, integration risk).
- Unedited patient iPSC control - baseline with abnormal splicing.

Correcting Patient-Derived iPSCs

4. Screen and isolate corrected clones

- PCR amplify and sequence around the mutation site
- Identify correctly edited clones and exclude indels or off-target events
- Re-plate single cells to expand independent corrected lines
- Mock-edited cells control - Cas9-only electroporation control or unsuccessfully edited clones.

5. Validate the genetic correction

- RT-PCR across intron 26 to confirm restoration of normal *CEP290* splicing
- qPCR and Western blot to verify CEP290 mRNA and protein expression
- Gene expression profile to check for bystander edits or off-target effects

6. Assess cell quality and function

- Test pluripotency markers (OCT4, NANOG, TRA-1-60)
- Confirm normal karyotype
- Evaluate cilia morphology and trafficking (ARL13B, acetylated tubulin staining)

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5. **Create target cells and evaluate safety and effectiveness -** Check off-target effects, cell health, and long-term function

Differentiation into Retinal Lineage and Functional Validation

- **Directed Differentiation**
- Use stepwise cues mimicking eye development
- **Validation of RPE cells**
- Immunostaining, WB, Flow Cytometry for RPE and pluripotent markers
- qPCR for RPE and pluripotent markers
- **Functional Assays**
- Cilia morphology
- Ciliary trafficking: rhodopsin or peripherin localization
- Electrophysiology: patch-clamp / calcium imaging for light responses
- Transcriptomics: single-cell RNA-seq for photoreceptor identity

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6. **Preclinical and clinical progression -** Animal studies, regulatory approval, clinical trials

Precilical Safety and Efficacy Studies in Animal Model

- Sub-retinal transplantation of RPE cells in animal model (rat) helps to evaluate the efficacy and safety of these cells *in vivo*.
- **Functional assays of the photoreceptors:**

1. Histology / Immunohistochemistry - Hematoxylin and eosin (H&E) and immunohistochemical staining for detecting histopathological changes.
2. Anatomy and survival - confirm graft survival, localization, and structure over time.
3. Functional Readouts – is the transplant restores visual signaling.
4. Behavioral tests: Show whether restored retinal output improves visual behavior.
5. Integration and mechanism - determine cell replacement vs trophic support.
6. Human mRNA detection: Confirms donor gene expression within host retina.
7. Safety and immune response – to ensure translation feasibility and rule out harm.

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