Success Story: Luxturna Potency Assay Development to Validation

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Analytical and Quality Control
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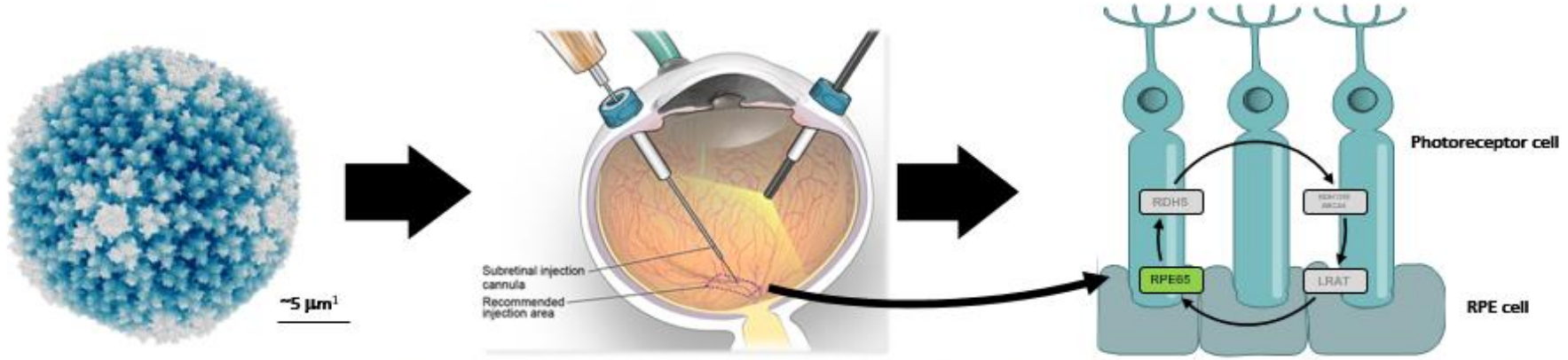
LUXTURNA: First Gene Therapy for a Genetic Disease Approved in the U.S.

- LUXTURNA@ (voretigene neparvovec-rzyl) for subretinal injection[1]
- For the treatment of patients with biallelic *RPE65* mutation-associated retinal dystrophy who have viable retinal cells
- Adeno-associated virus (AAV) serotype 2 vector carrying the *RPE65* transgene
- Formulated to a concentration of $5x10^{12}$vg/mL



Viral vector is generated[2]

- LUXTURNA is a suspension of an adeno-associated virus vector–based gene therapy for subretinal injection
- LUXTURNA is a live, nonreplicating adeno-associated virus serotype 2 that has been genetically modified to express the human *RPE65* gene
- LUXTURNA is derived from naturally occurring adeno-associated virus using recombinant DNA techniques

LUXTURNA for subretinal injection

Transduction of RPE cells[2]

- Injection of LUXTURNA into the subretinal space results in transduction of some retinal pigment epithelial cells with a cDNA encoding normal human RPE65 protein, thus providing the potential to restore the visual cycle[2]

1. LUXTURNA [package insert]. Philadelphia, PA: Spark Therapeutics, Inc., 2017. 2. Trapani et al. *Prog Retinal Eye Res.* 2014;43:108-128.

Biological Products Safe, Pure, and Potent

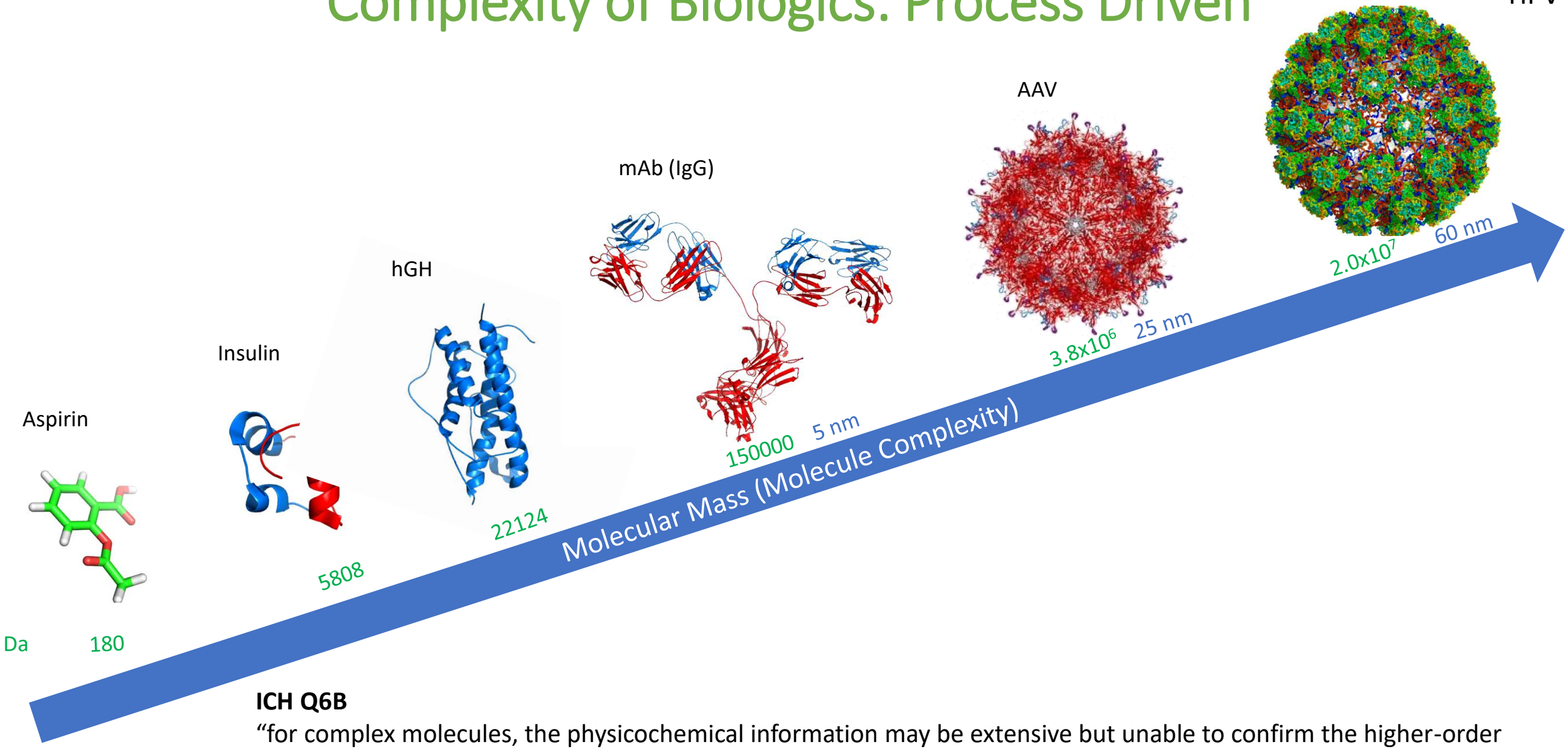
All biological products regulated under section 351 of the PHS Act must meet prescribed requirements of safety, purity and potency for BLA approval; *Federal Food, Drug and Cosmetic Act, (FDC Act), (21 U.S.C. 321 et seq.); (21 CFR 601.2).*

Potency: 21CFR600.3(s) "The word potency is interpreted to mean the specific ability or capacity of the product(…) *to effect a given result*."

Bioassay:

- Evaluate potency/activity of a drug for release/stability purposes
- Assay should reflect/mimic product's known/intended Mechanism of Action (MoA)

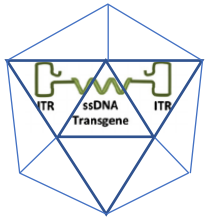
Complexity of Biologics: Process Driven



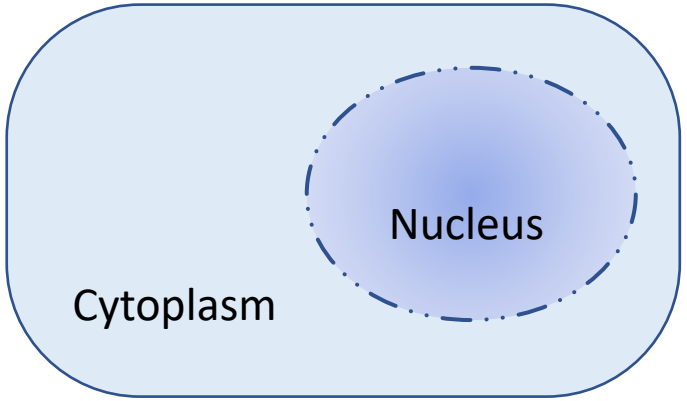
ICH Q6B

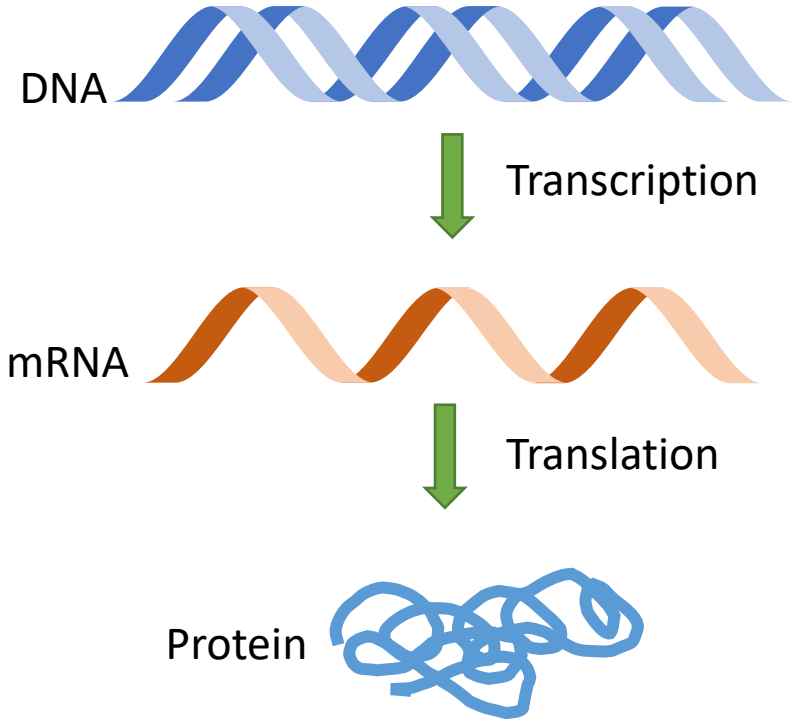
"for complex molecules, the physicochemical information may be extensive but unable to confirm the higher-order structure, which, however, can be inferred from the **biological activity**"

Gene Therapy Mechanism(s) of Action



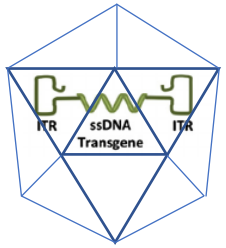
Multi-Step Process Based on “Central Dogma of Molecular Biology”

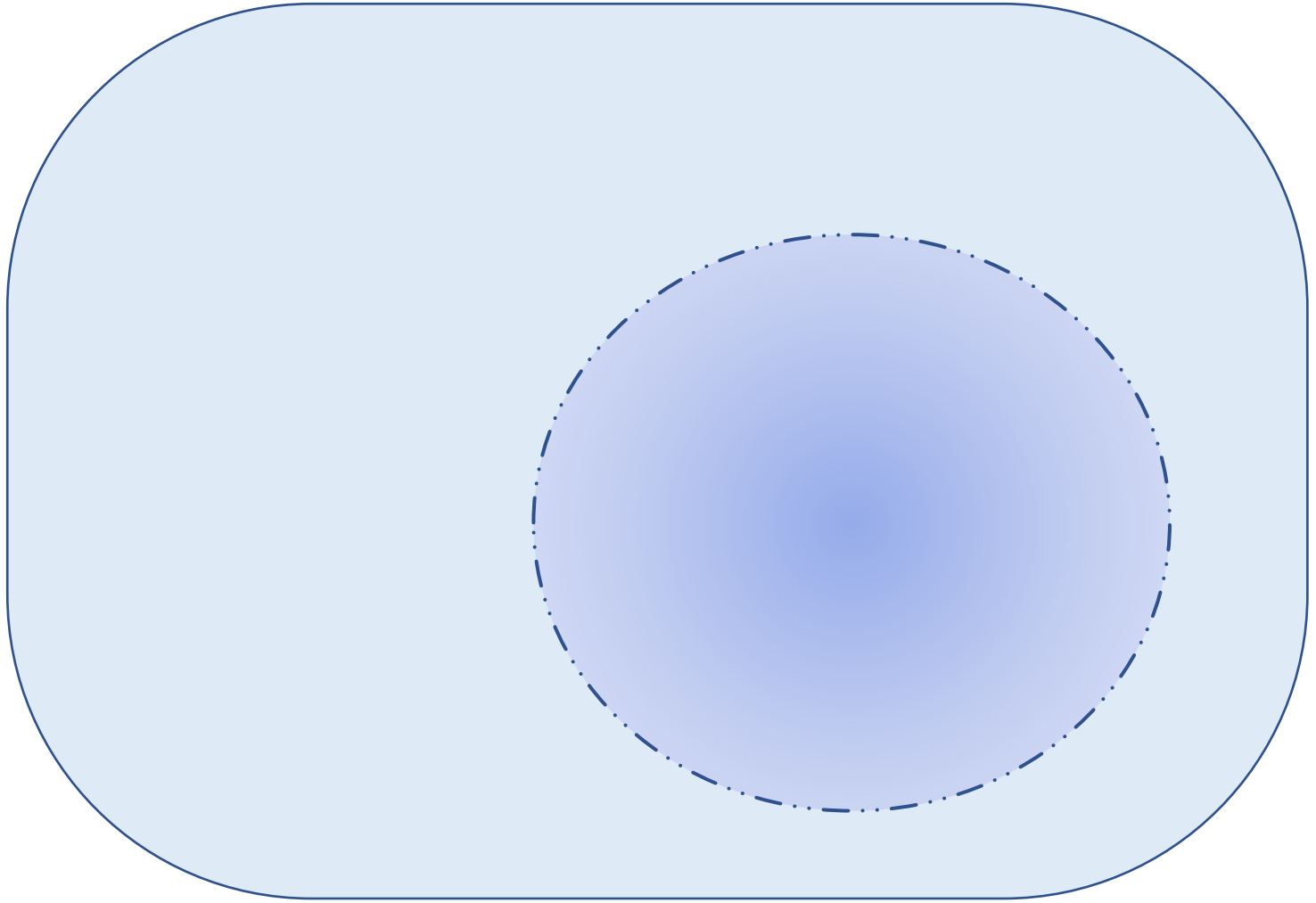




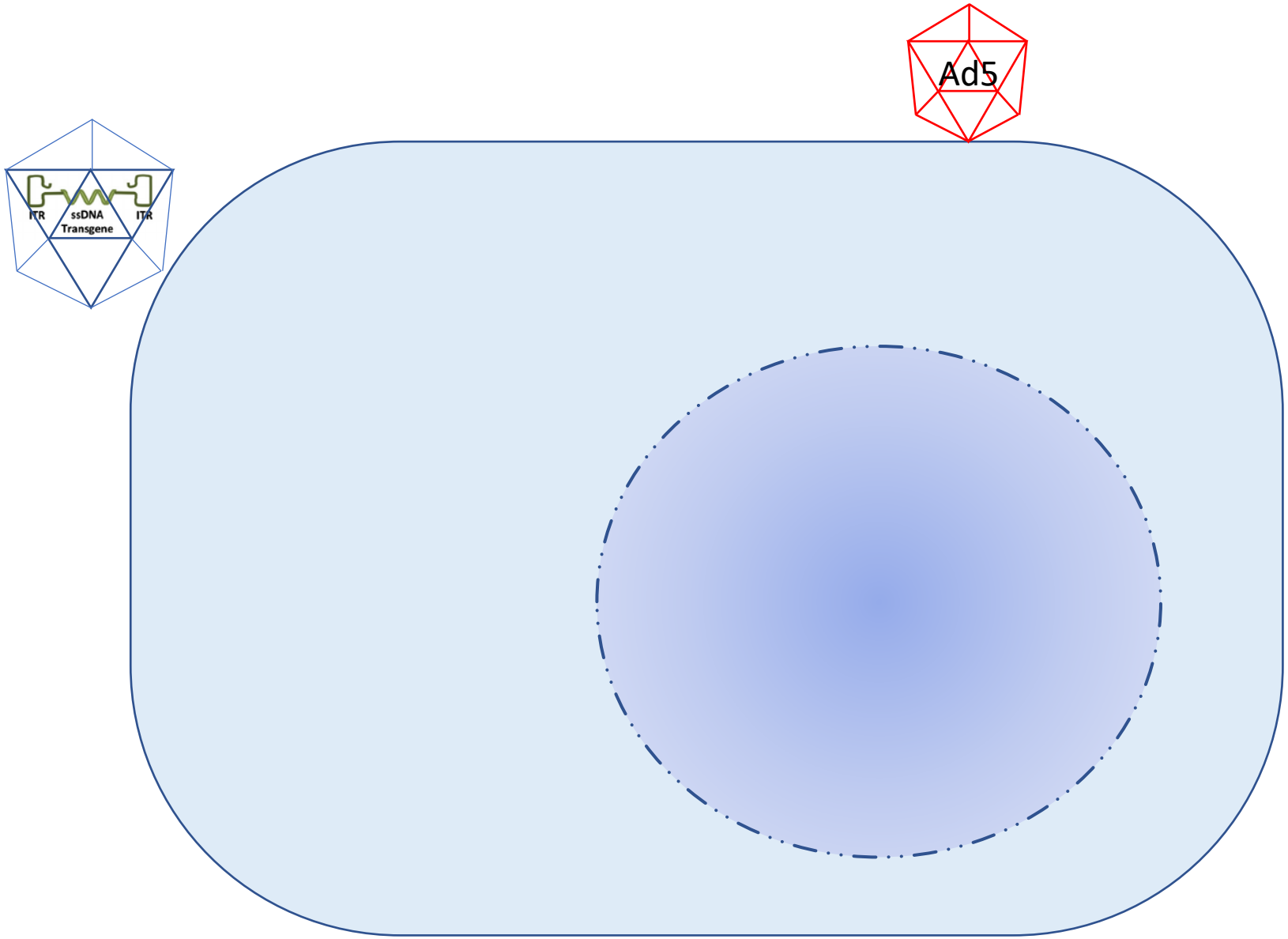
1. Gene Delivery
2. Gene Transcription to mRNA
3. mRNA Translation to Protein
4. Functional Protein

Infectivity Assay

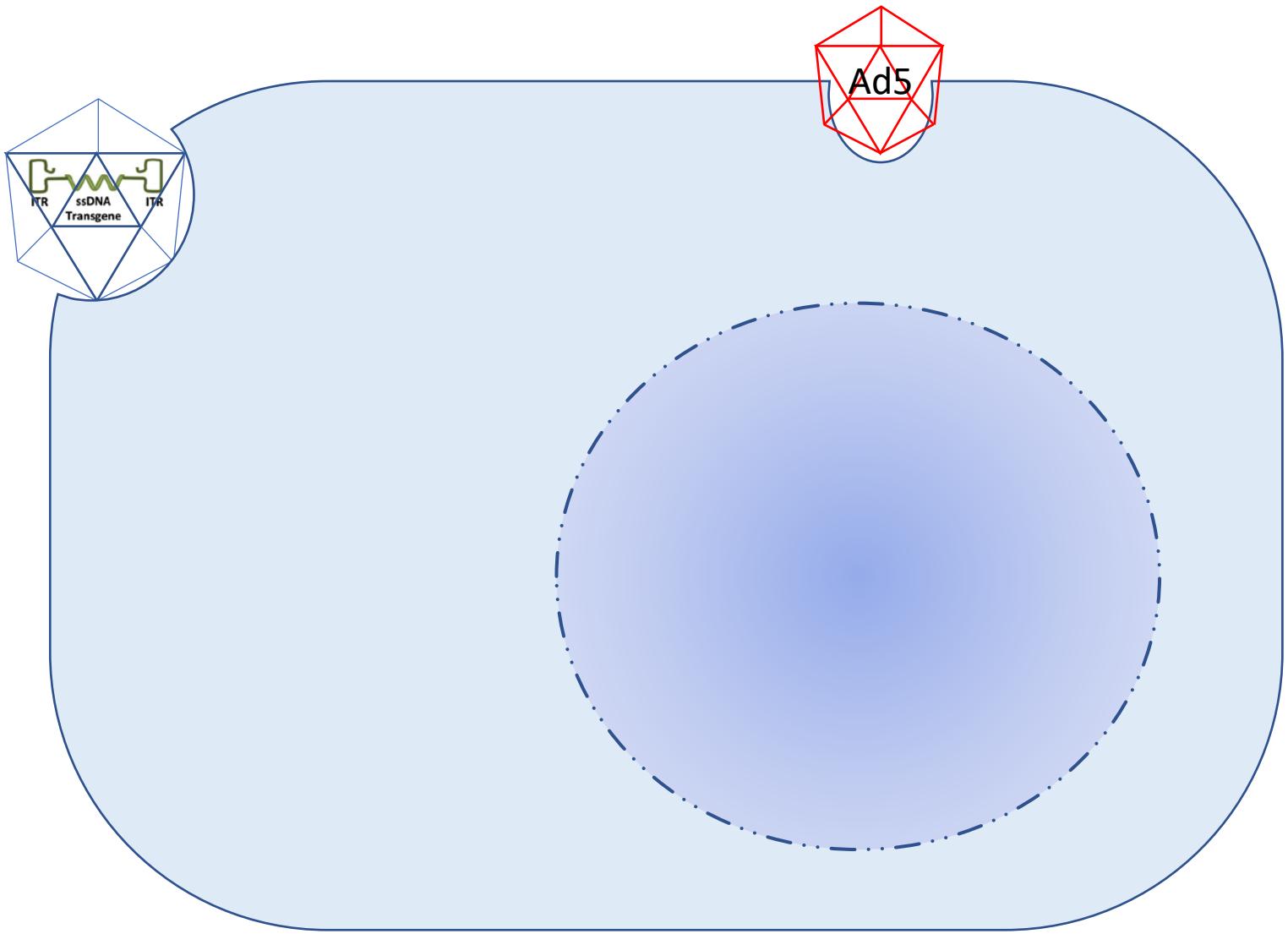




Infectivity Assay



Infectivity Assay



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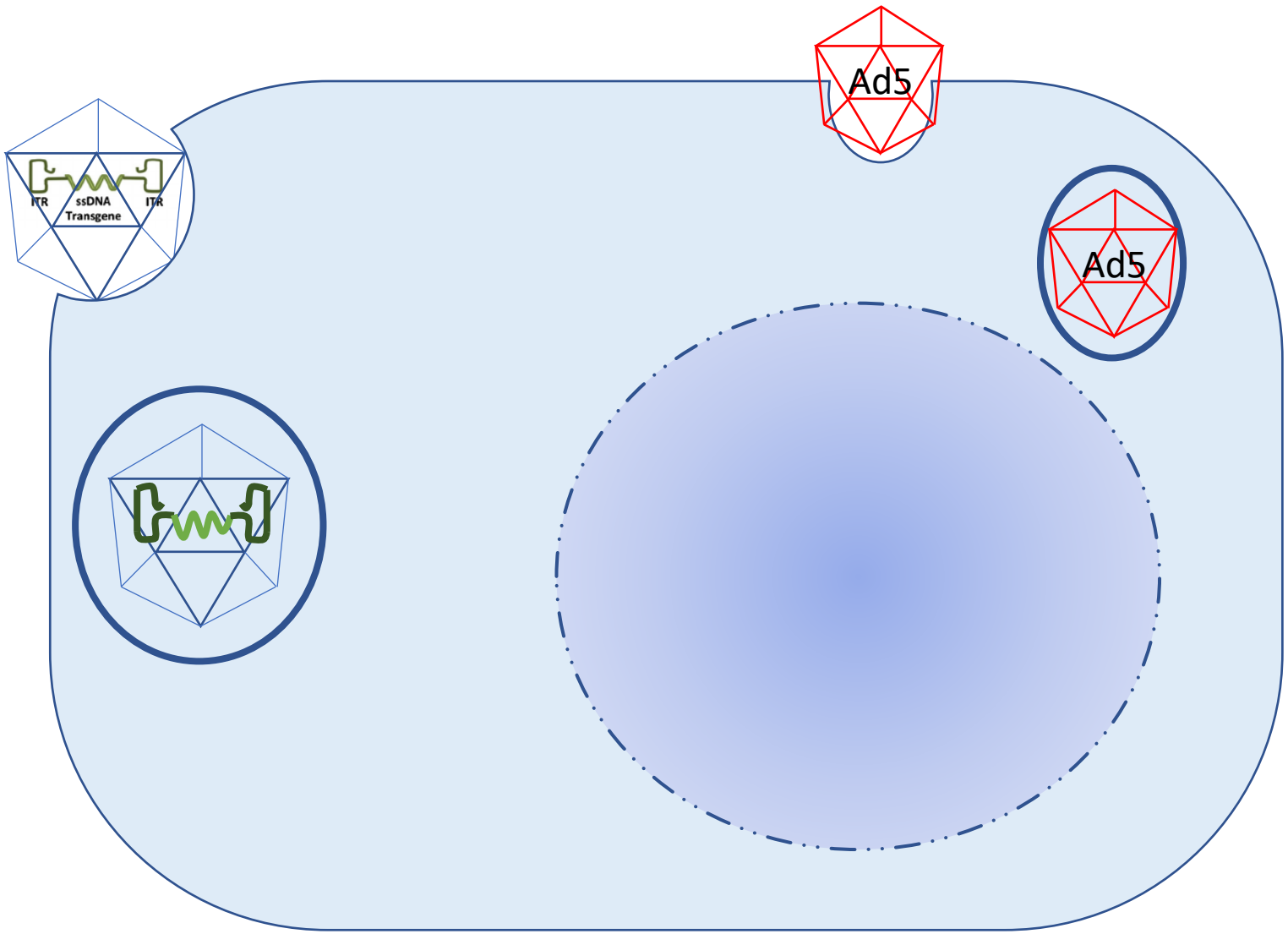

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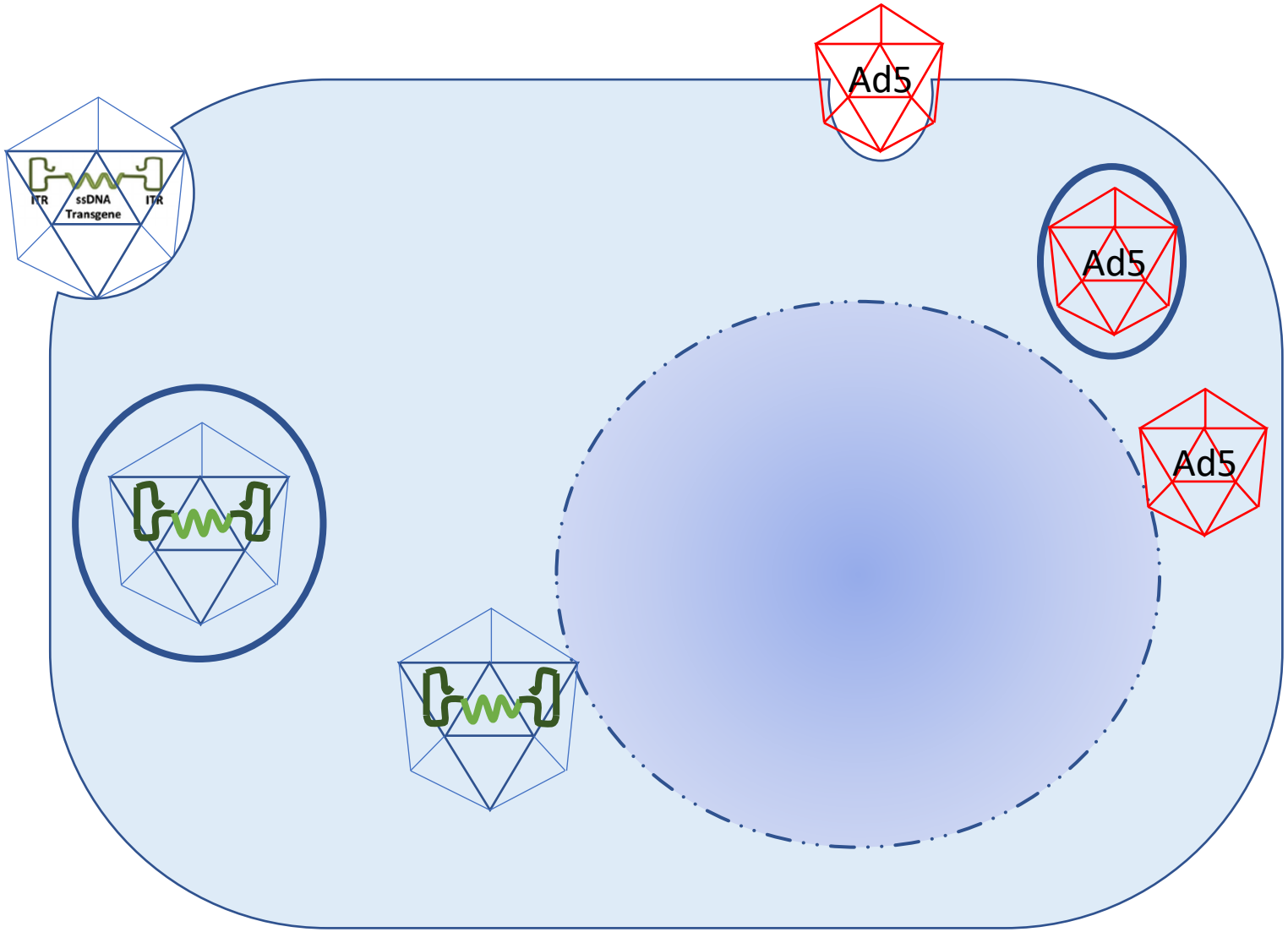

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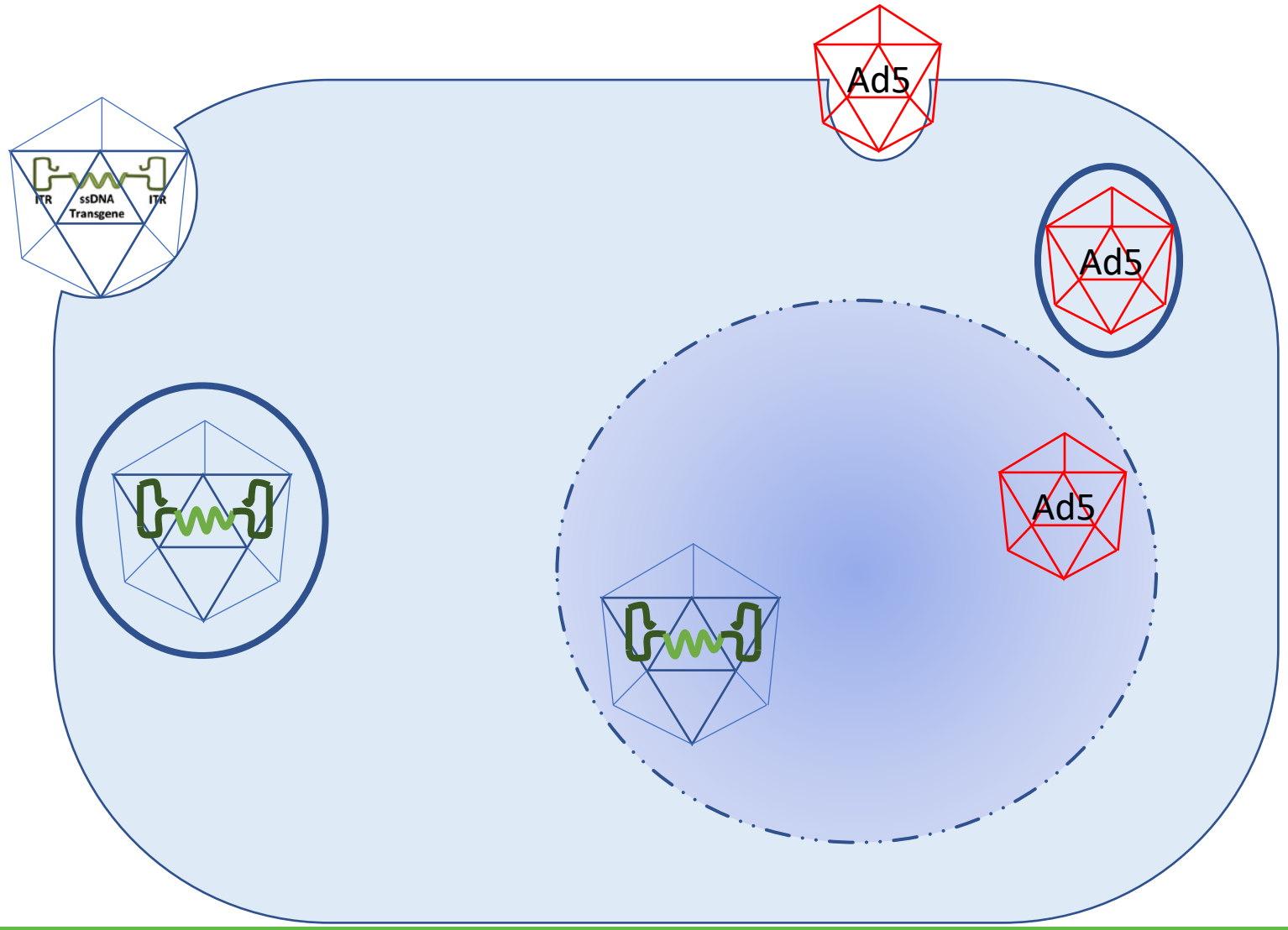

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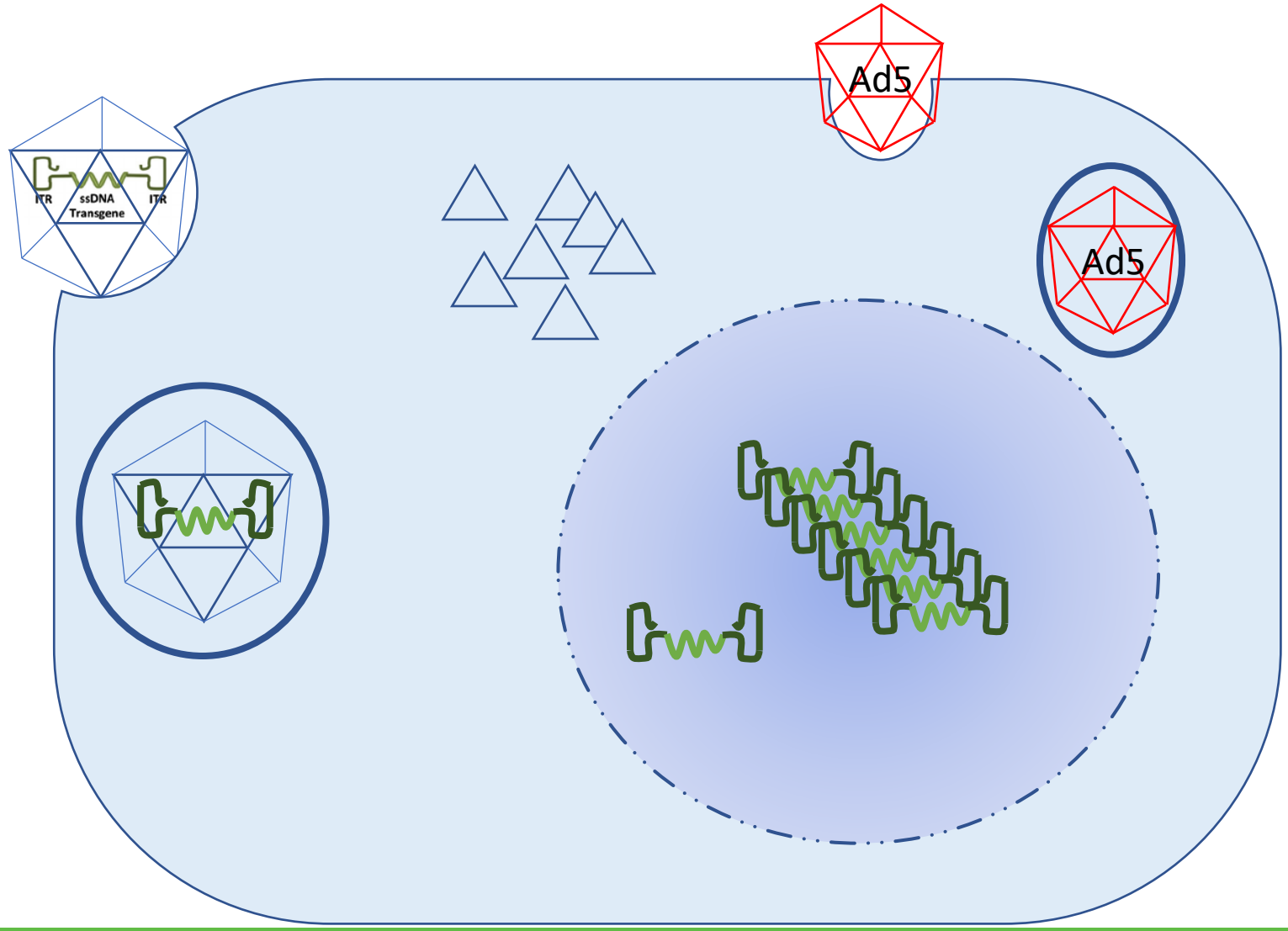

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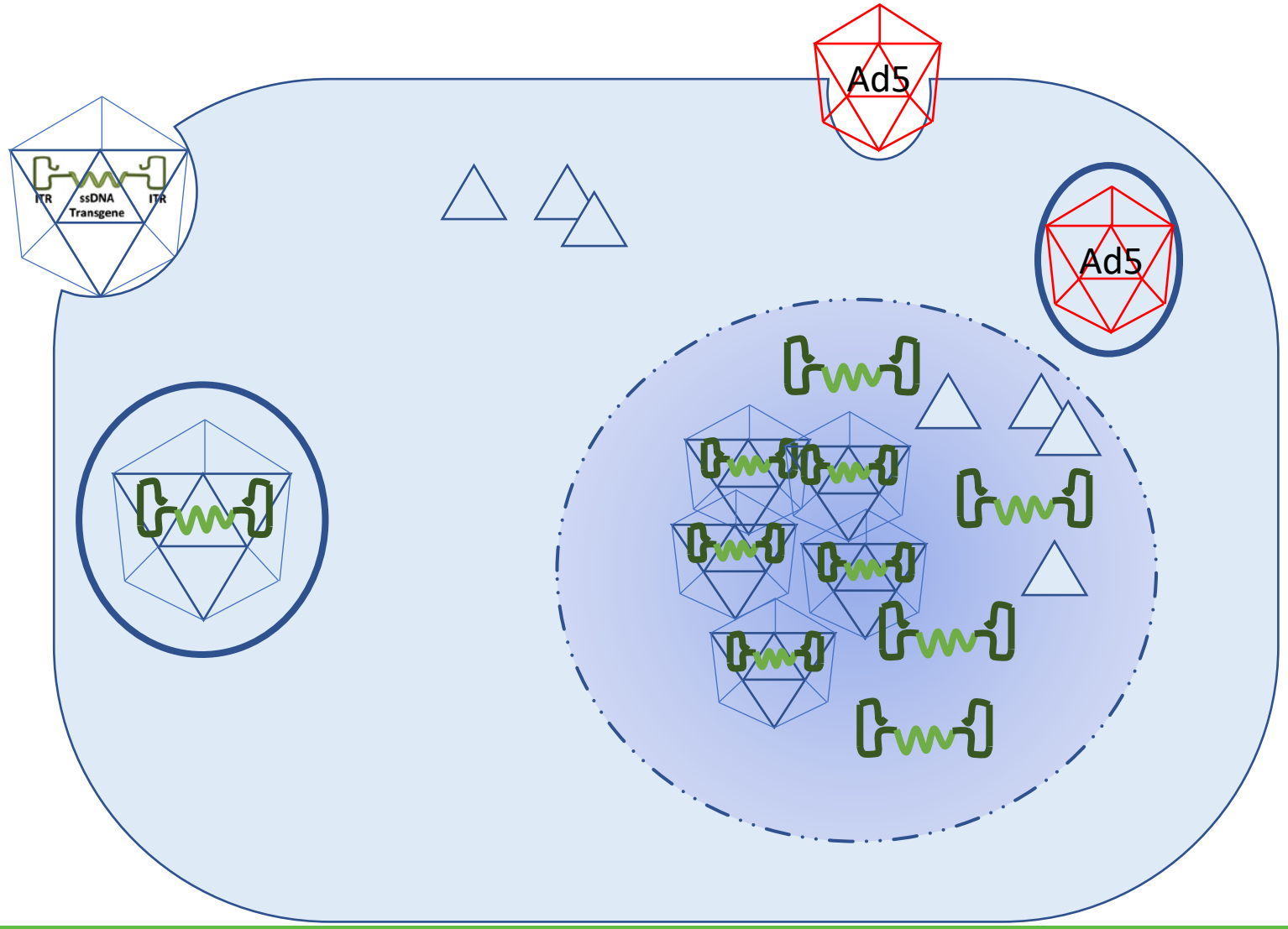

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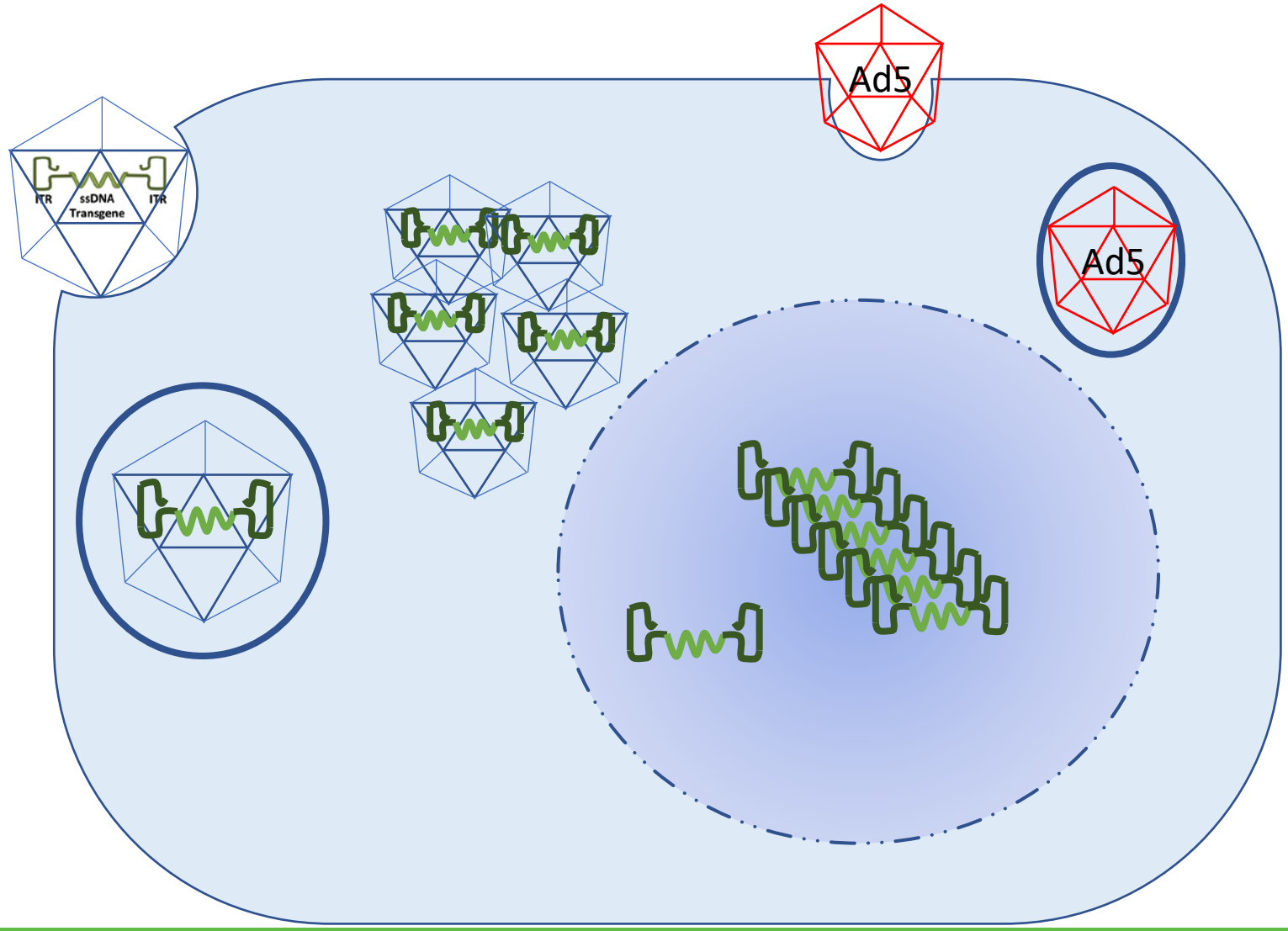

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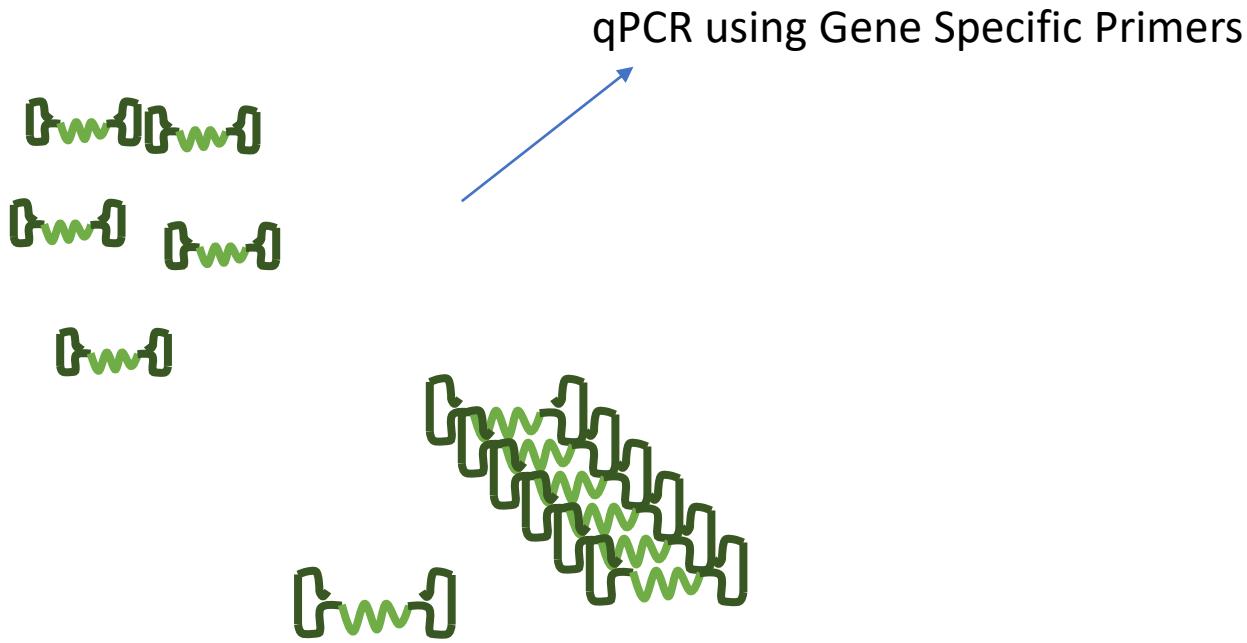


Infectivity Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	100 µL Media	50µL Ad5 + 50µL media							100 µL Media			100 µL Media
B	100 µL Media	50 µL Ad5 +50 µL Dil 5	50 µL Ad5 +50 µL Dil 6	50 µL Ad5 +50 µL Dil 7	50 uL Ad5 +50 uL Dil 8	50 µL Ad5 +50 µL Dil 9	50 µL Ad5 +50 µL Dil 10	50 µL Ad5 +50 µL Dil 11	50 µL Ad5 +50 µL Dil 12	50 µL Ad5 +50 µL Dil 13	50 µL Ad5 +50 µL Dil 14	100 µL Media
C	100 µL Media											100 µL Media
D	100 µL Media											100 µL Media
E	100 µL Media											100 µL Media
F	100 µL Media											100 µL Media
G	100 µL Media											100 µL Media
H	100 µL Media											100 µL Media

Viral Particles Dilutions

Cells transduced with Serial 10-dilutions in 7 replicates of Test Article and Ad5

Viral Particles Dilutions

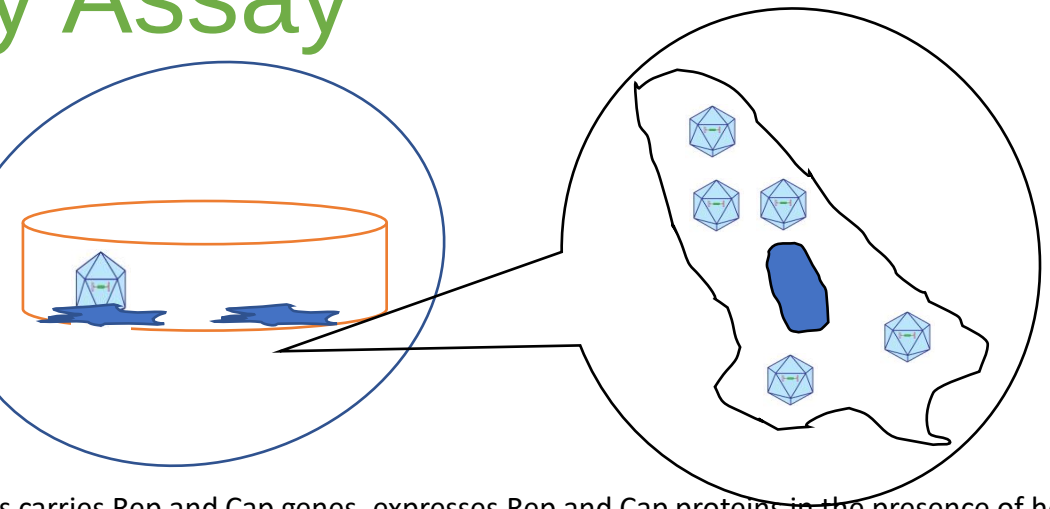
B	218,1133	96,5178	25,952	18,608	32	2	0	0	0	0
C	287,1291	95,363	33,397	19,940	3,252	0	0	0	0	0
D	208,020	85,999	21,297	8,736	861	4,478.	0	0	0	0
E	280,9809	57,847	30,098	6,777	589	5,086	0	0	0	0
F	312,269	236,798	51,491	4,592	857	5	233	0	0	0
G	331,257	93,915	31,164	5,505	1,874	8,745	4,669	0	0	0
H	121,827	52,358	23,273	4,189	4,236	3	0	0	0	0

Kärber method for TCID50 Calculations

1. Infectious Titer (IU/mL): $10^{[1+D(S-0.5)]}$/ V [mL]*

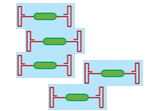
D: Logarithm of the dilution factor
S: Logarithm of the initial dilution plus the sum of ratios of infectious-positive wells per total wells at each subsequent dilution
V: Volume of the diluted Test Article used for inoculation

2. Particle-to-Infectious Particle Ratio

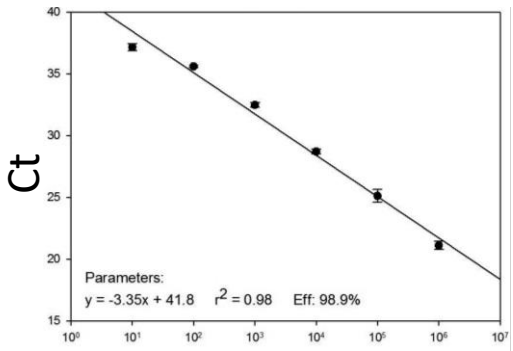


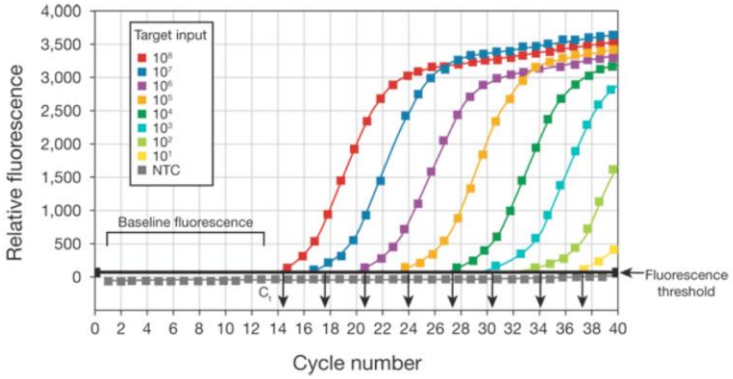
Cells carries Rep and Cap genes, expresses Rep and Cap proteins in the presence of helper Ad5
Helper dependent amplification of infected AAV viral Particles

Cell lysed and Transgene specific Primers used for qPCR

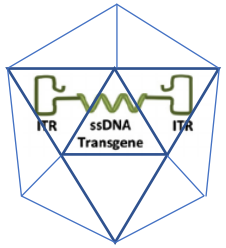


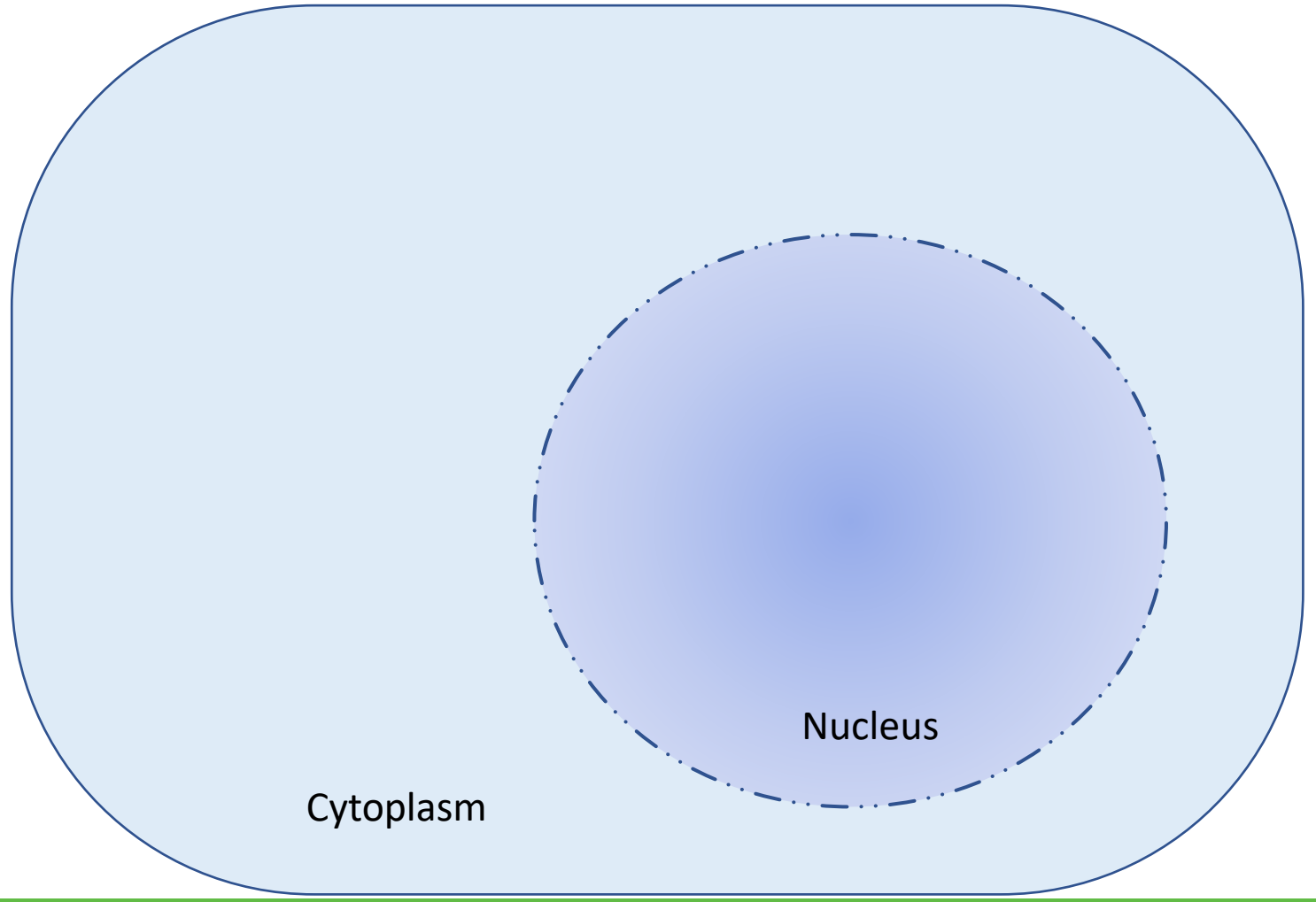
Cut off = 64 Copies





Activity and Gene Expression Assay





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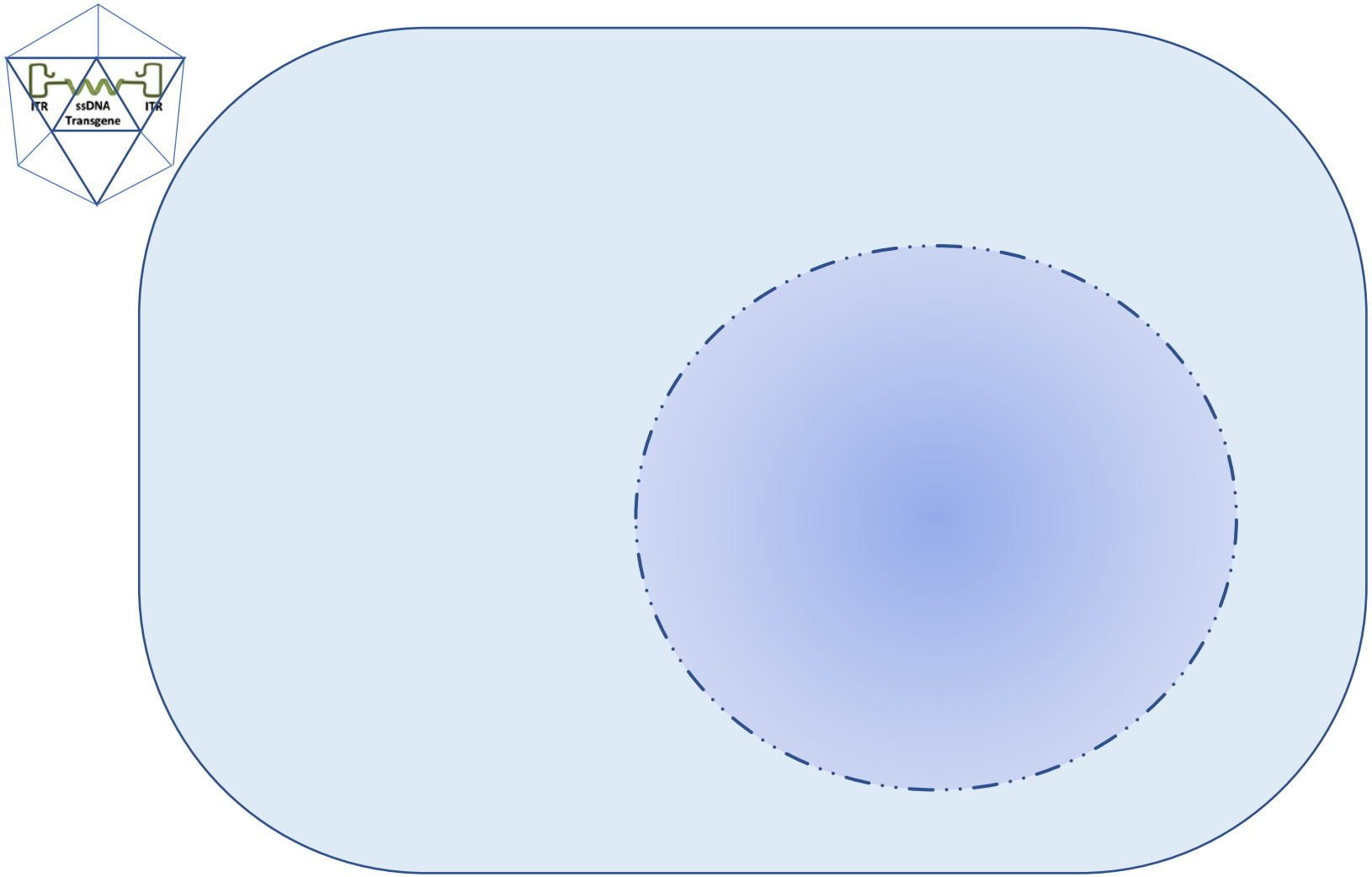

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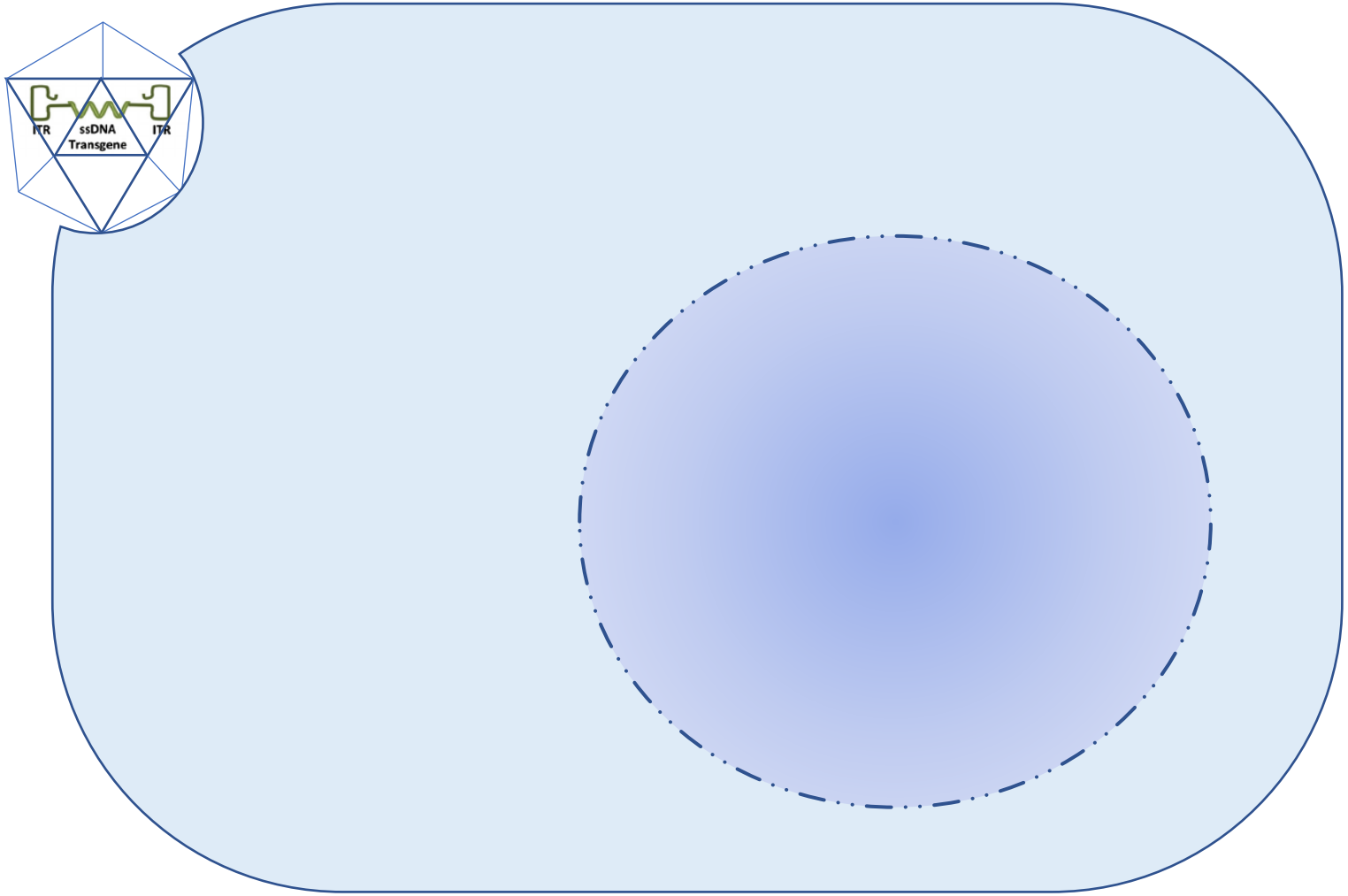

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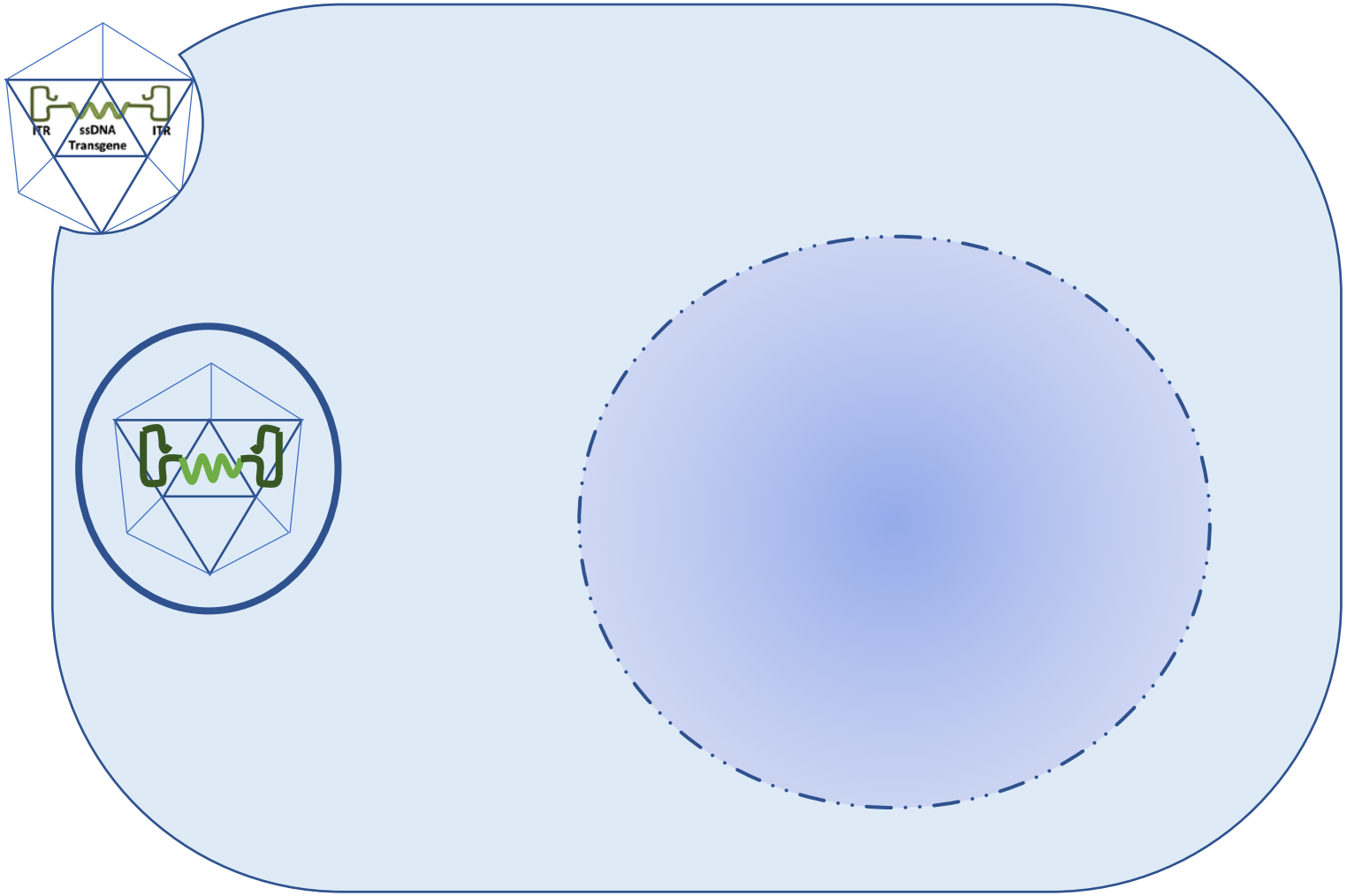

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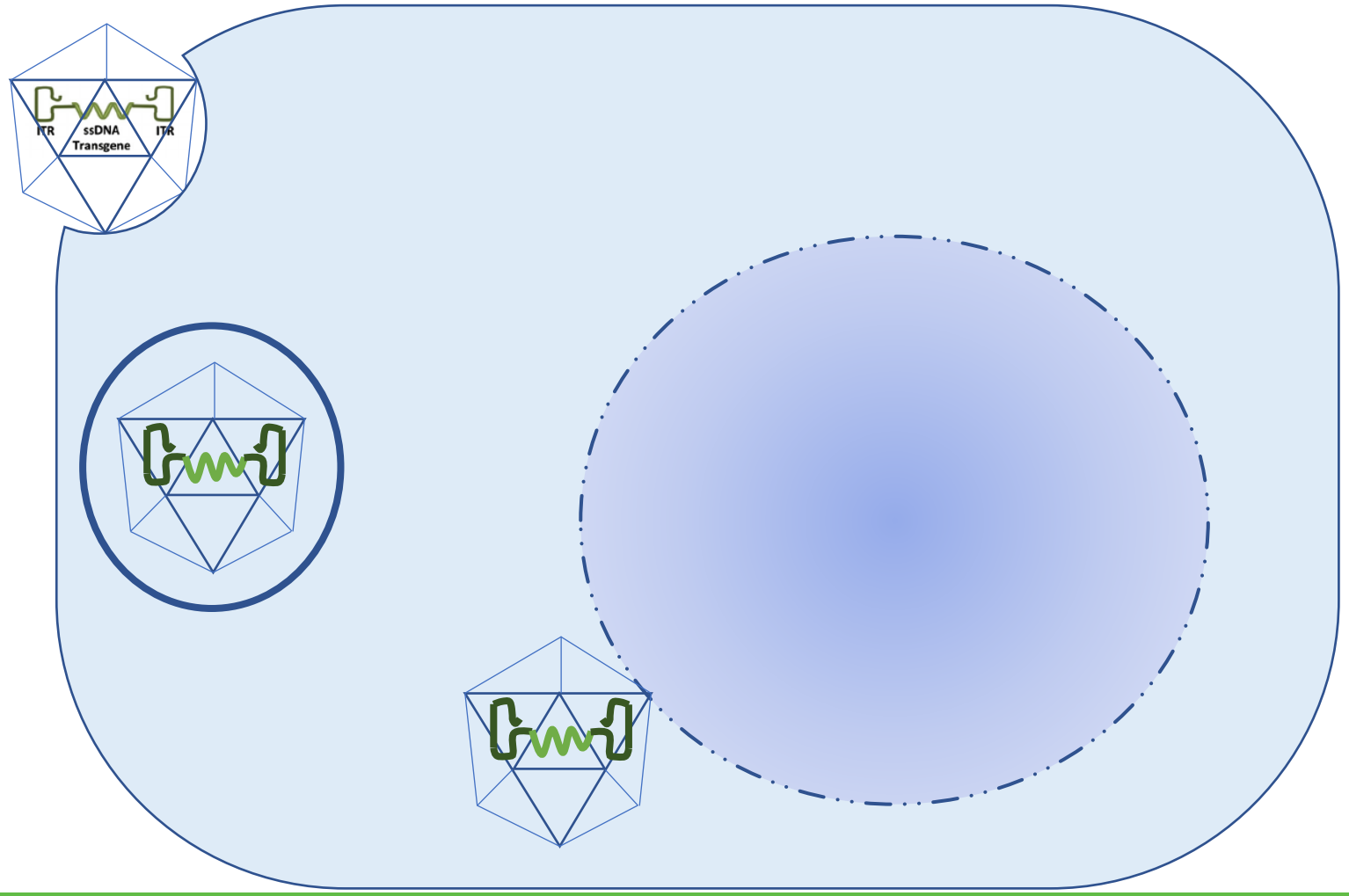

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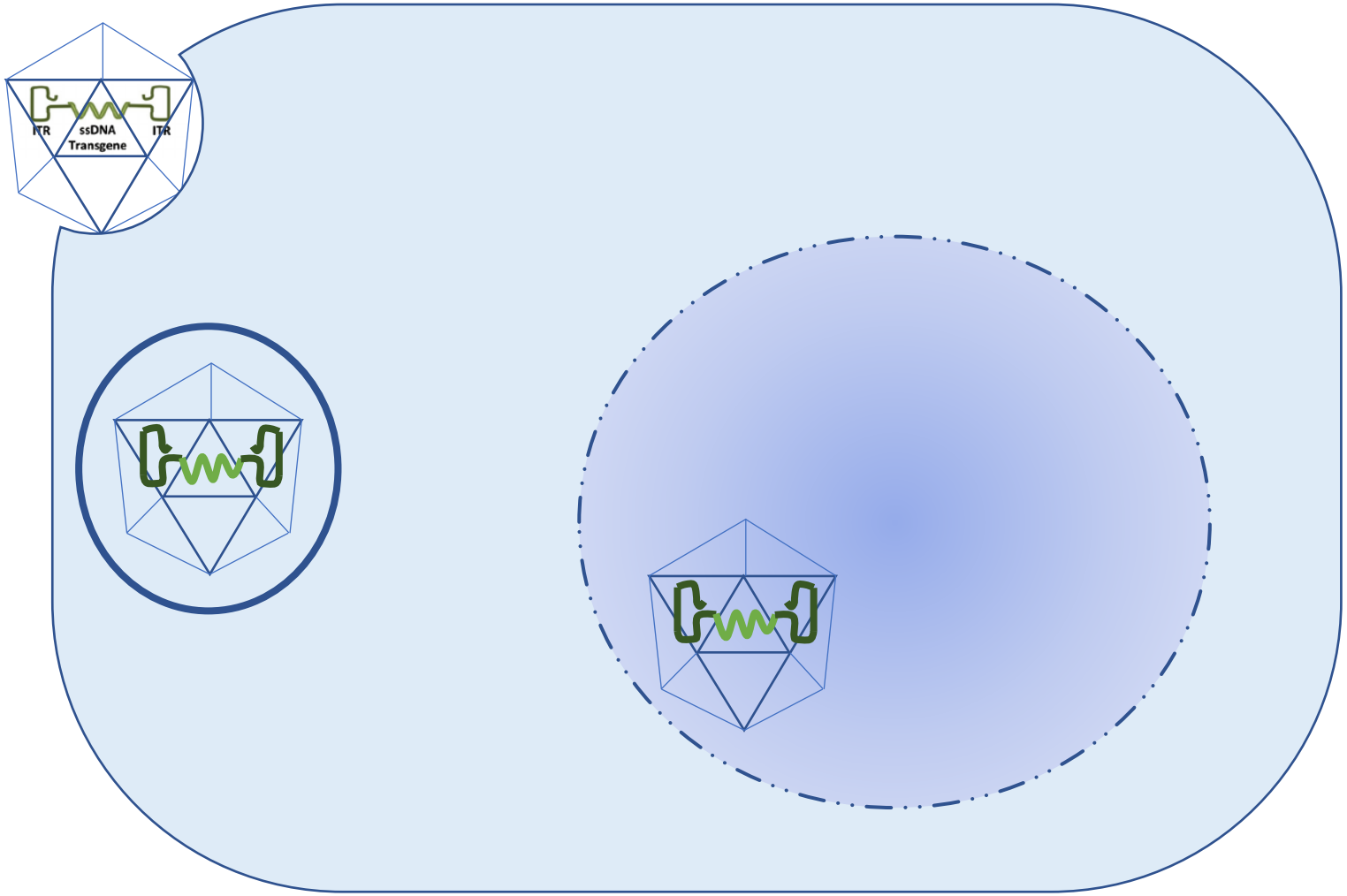

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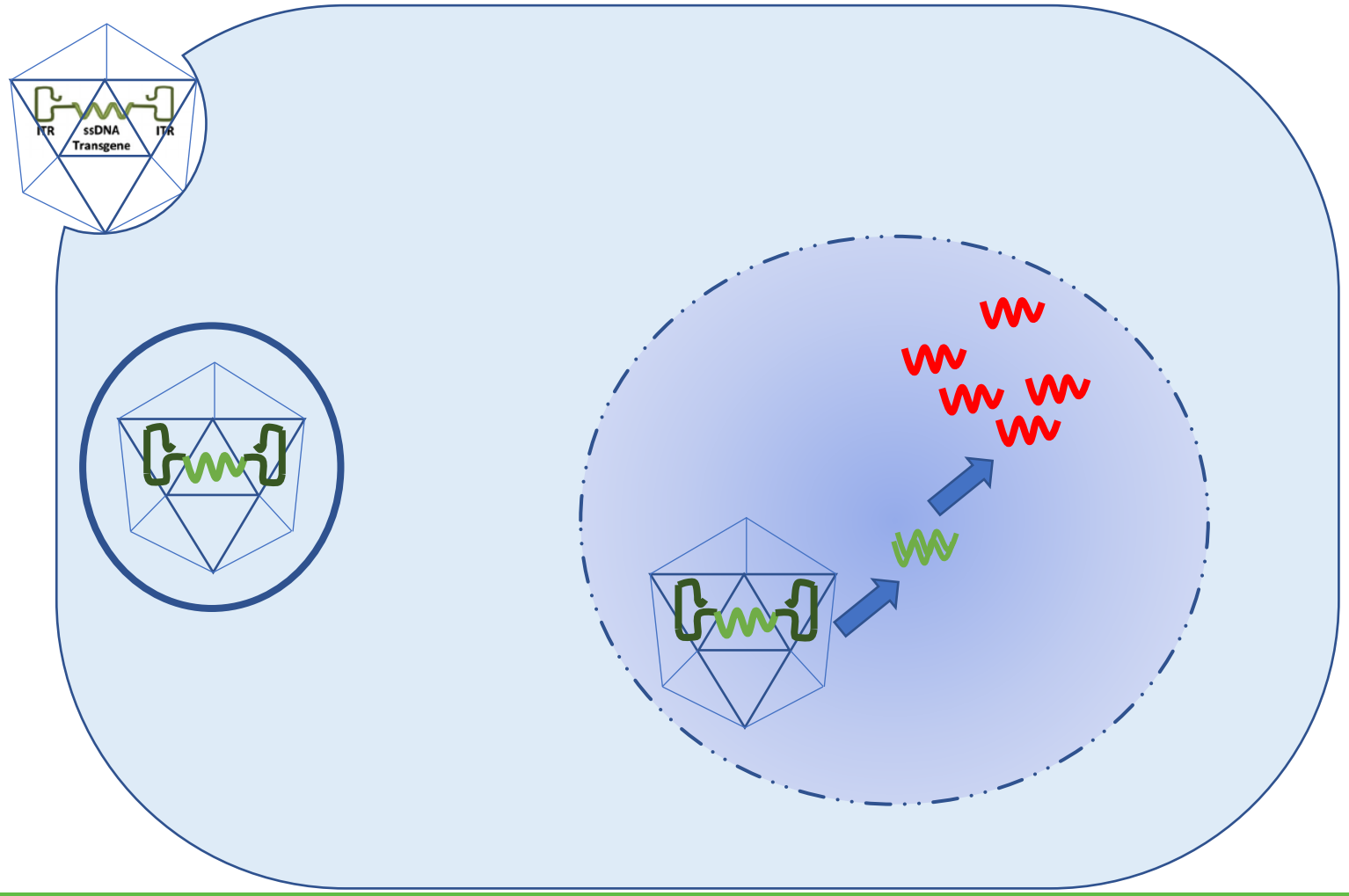

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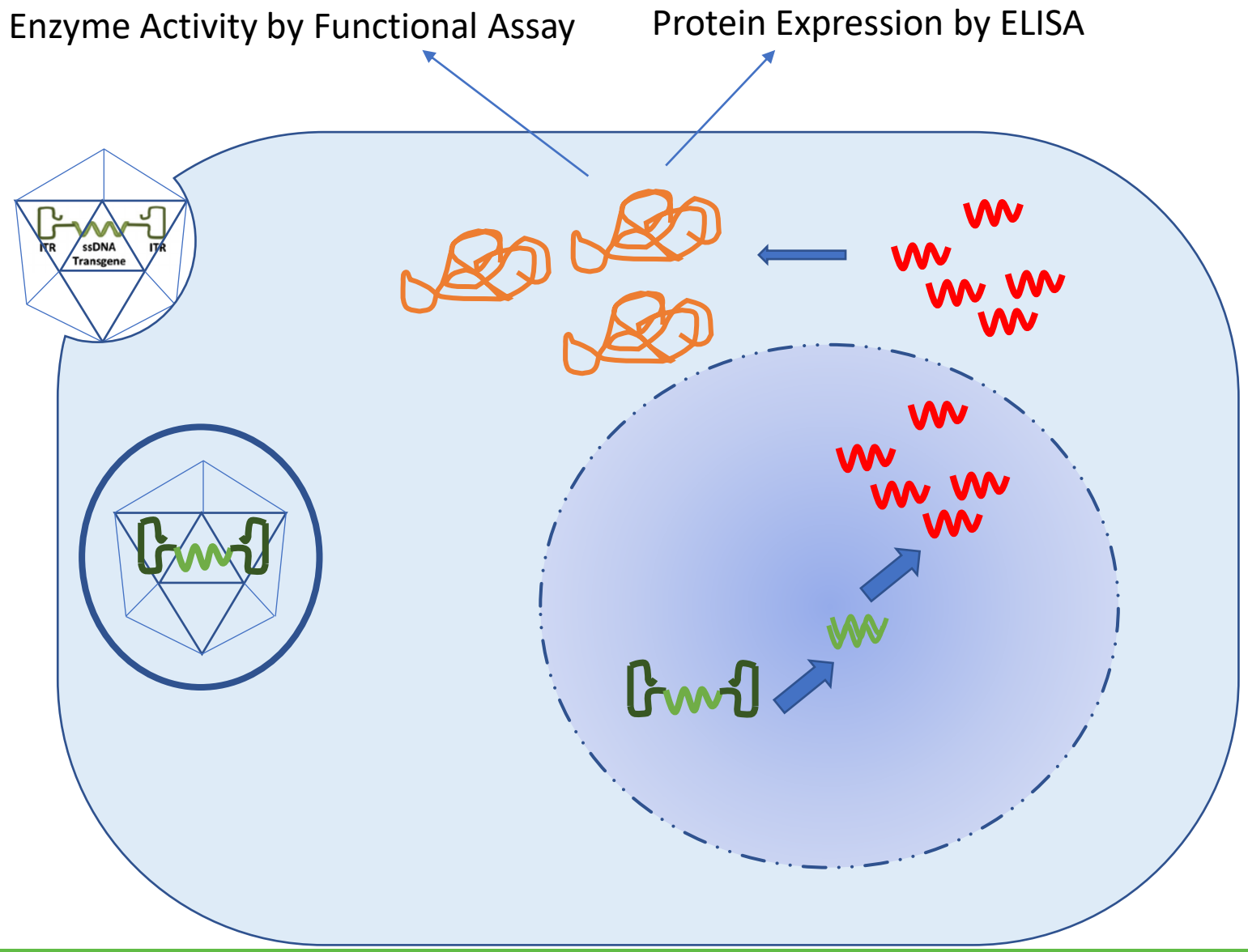
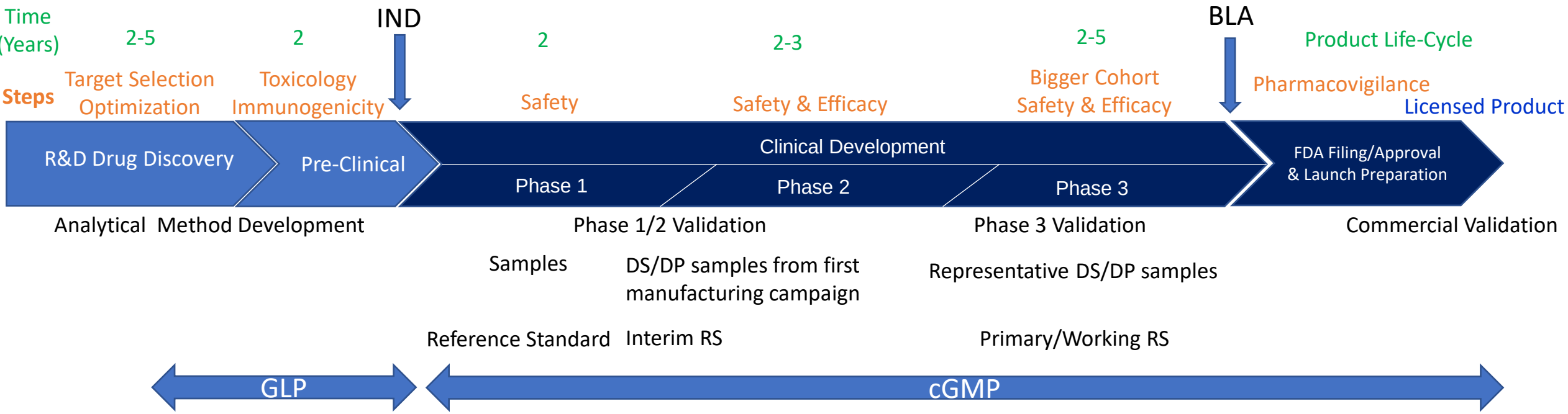


Method Development and Phase Appropriate Validation



Phase 1/2
- Limited Quantitative information
- Wider Acceptance Range
- Assay Development/Qualification

Phase 3
- Assay Validated
- Narrower limits to ensure lot-to-lot consistency
- Stability testing of validation lots to establish expiry dating prior to licensure

Luxturna Bioassay Timeline

Transfer of the technology from Children's Hospital of Philadelphia (CHOP)

IND (2007)

Pivotal Phase 3 Clinical Trial Initiated (Nov. 2012)

In-vivo Potency Assay: Pupillary Light Response

Qualitative

1 DS and DP for PPQ Data for Additional Lots Provided After Approval

Development
In-vitro Potency

Quantitative

Validation
In-vitro Potency

Life Cycle Management of Bioassays

Phase Appropriate Method Validation Performed

https://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM592083.pdf
https://sparktx.com/wp-content/uploads/product-timeline.pdf

Transition from Qualitative to Quantitative Potency Assay

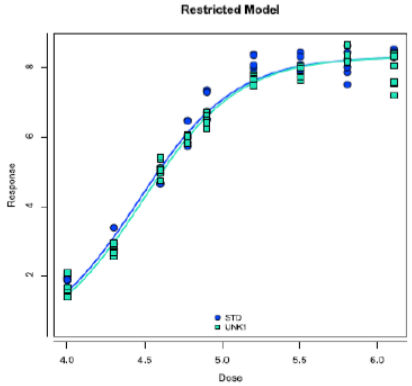
Phase I/II/III

Potency
In vivo Pupillary Light Response

- Animal-based
- Physiological Relevant
- Complex, Highly Variable
- Long Assay Time (4-6 Weeks)
- Qualitative

Commercialization

Potency
In vitro Potency Assay



4PL Fit

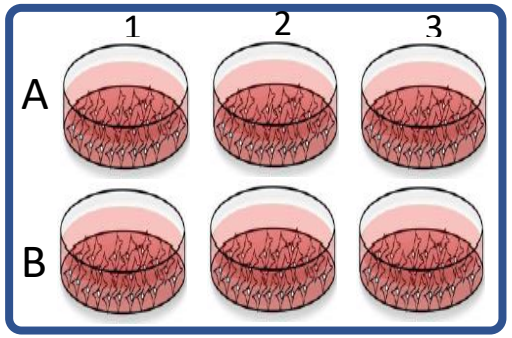
$$Y = \frac{(A - D)}{\left(1 + \left(\frac{X}{C}\right)^{B}\right)} + D$$

A= Upper Asymptote
B= Slope
C= ED50
D= Lower Asymptote

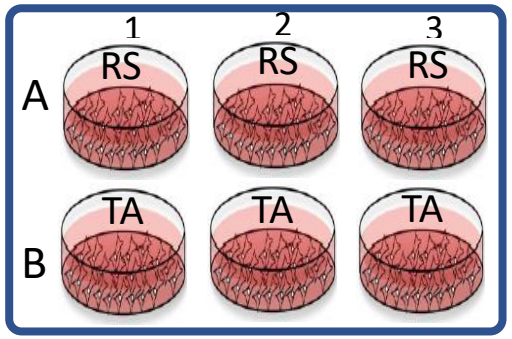
$$\text{Potency} = \left\{ \frac{\text{Sample ED50}}{\text{Reference ED50}} \right\}$$

- ❑ Cell-based
- ❑ Less Variable Compared to animal-based assay
- ❑ Short Assay Time (1 Week)
- ❑ Quantitative
- ❑ Broad Dynamic Range
- ❑ Support Principles of 3Rs

The AAV2-hRPE65v2 Isomerohydrolase Activity Potency Assay



Cell Plated



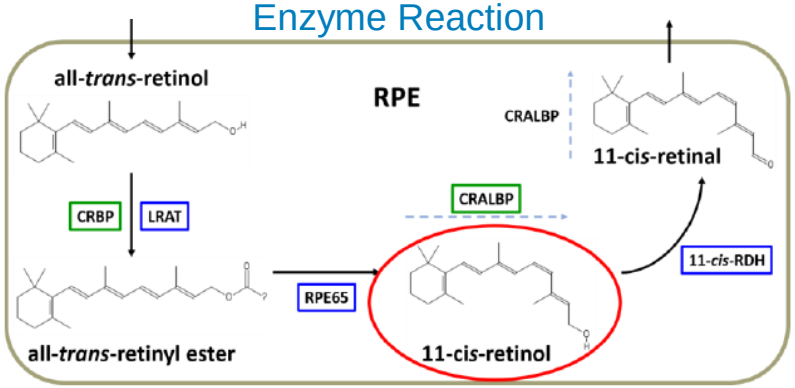
Transduction

Harvest

Resuspend Pellet

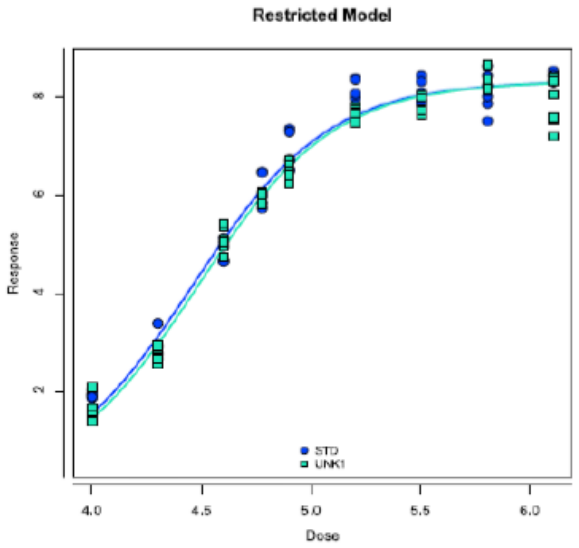
Cell Lysis

Enzyme Reaction



LRAT: Lecithin Retinol AcylTransferase
CRBP: Cellular retinol binding protein
CRALBP: Cellular RetinALdehyde-Binding Protein

LC-MS/MS
11-*cis*-retinol



PLA-Analysis

Cascella et al. Archives of Biochem and Biophys. 2014;539:187-95. Data on File. Spark Therapeutics, Inc. Philadelphia, PA.

Development to Validation

Cell selection: Support MOA of the Drug

Cell Line Selection and Cell Bank Qualification

- Cell Line's History from Origin to Banking
- Sterile
- Mycoplasma Free
- Growth Characteristics
- Morphology

Development

Cell number

Dose-response

Incubation time

Critical reagents

Readout

Representative Sample/RS

Identify variables

⟨1032⟩ DESIGN AND DEVELOPMENT OF BIOLOGICAL ASSAYS

Validation

ICH Q2 (R1)

⟨1033⟩ BIOLOGICAL ASSAY VALIDATION

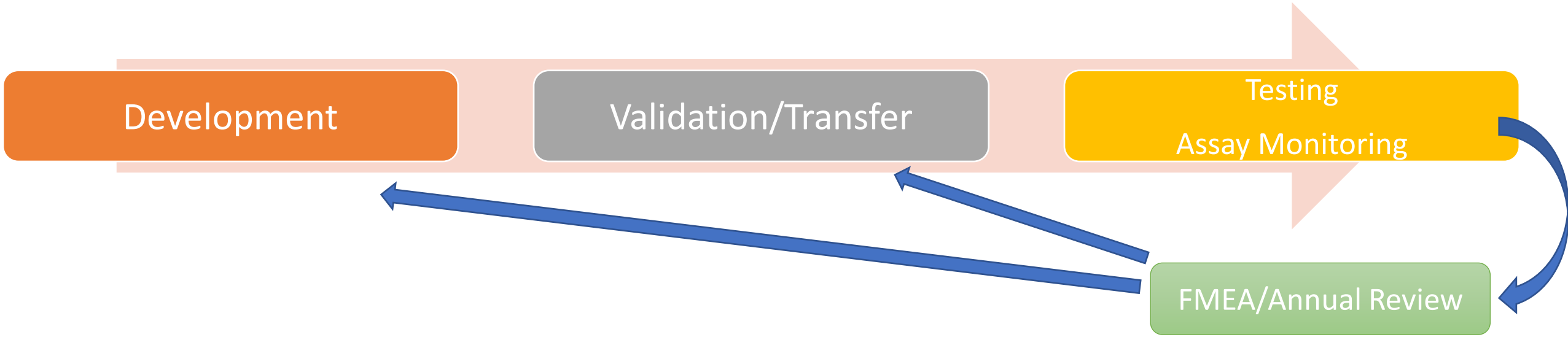
⟨1034⟩ ANALYSIS OF BIOLOGICAL ASSAYS

- Specificity
- Linearity
- Precision
- Accuracy
- Range
- Robustness

ICH Q5D: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products. *US. Fed. Reg.* 63(182) 1998: 50244–50249.

USP<1032> DESIGN AND DEVELOPMENT OF BIOLOGICAL ASSAYS

Analytical Method Life-Cycle



Analytical method performance may drift over the time due to

- Critical Reagents Change
- Equipment Change
- Vendor Change
- Analysts

Proactive Assay Tracking/Trending/Monitoring is Important to Keep the Assay Performance in Controlled State

Summary

- Designed a statistically sound phase-appropriate validation
  - Use historical data or development data for acceptance criteria
- Started early for development of qualitative potency assay
- Identified critical reagents and variables effecting the assay
- Established and characterized the cell bank
- Method life cycle management
  - Methods evolve during product development and product life cycle
  - Continuously monitor the assay performance, control chart
  - Annual Method Review

Thank you!