Comparative Effects of Transfection Reagents on IL-1 Cytokine Induction and Transfection Efficiency in H1299 Cells

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GSNJS Team Project in Molecular Biology

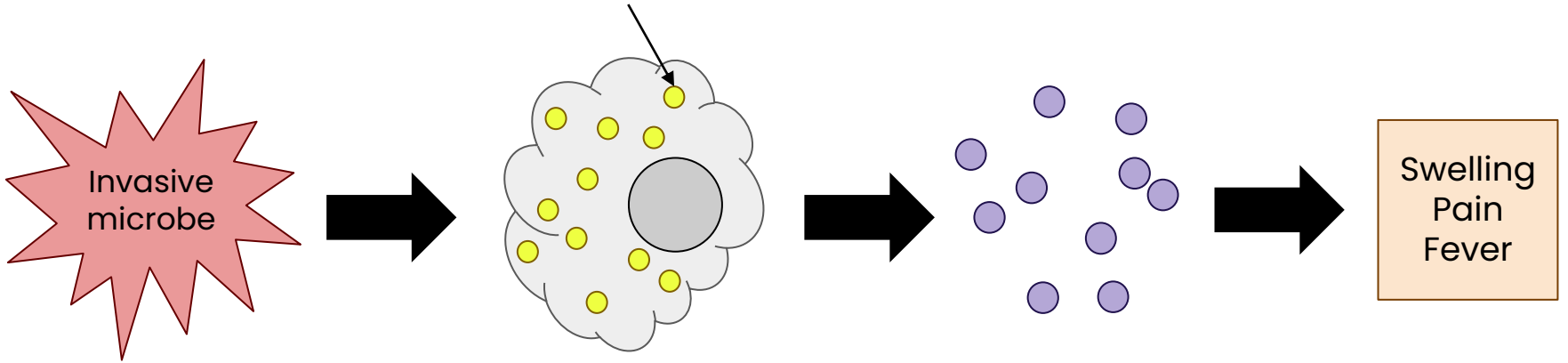
Vaccines

- Take before infection → avoid severe disease, protect community
- COVID mRNA vaccine
- Inflammation from transfection reagent that delivers mRNA

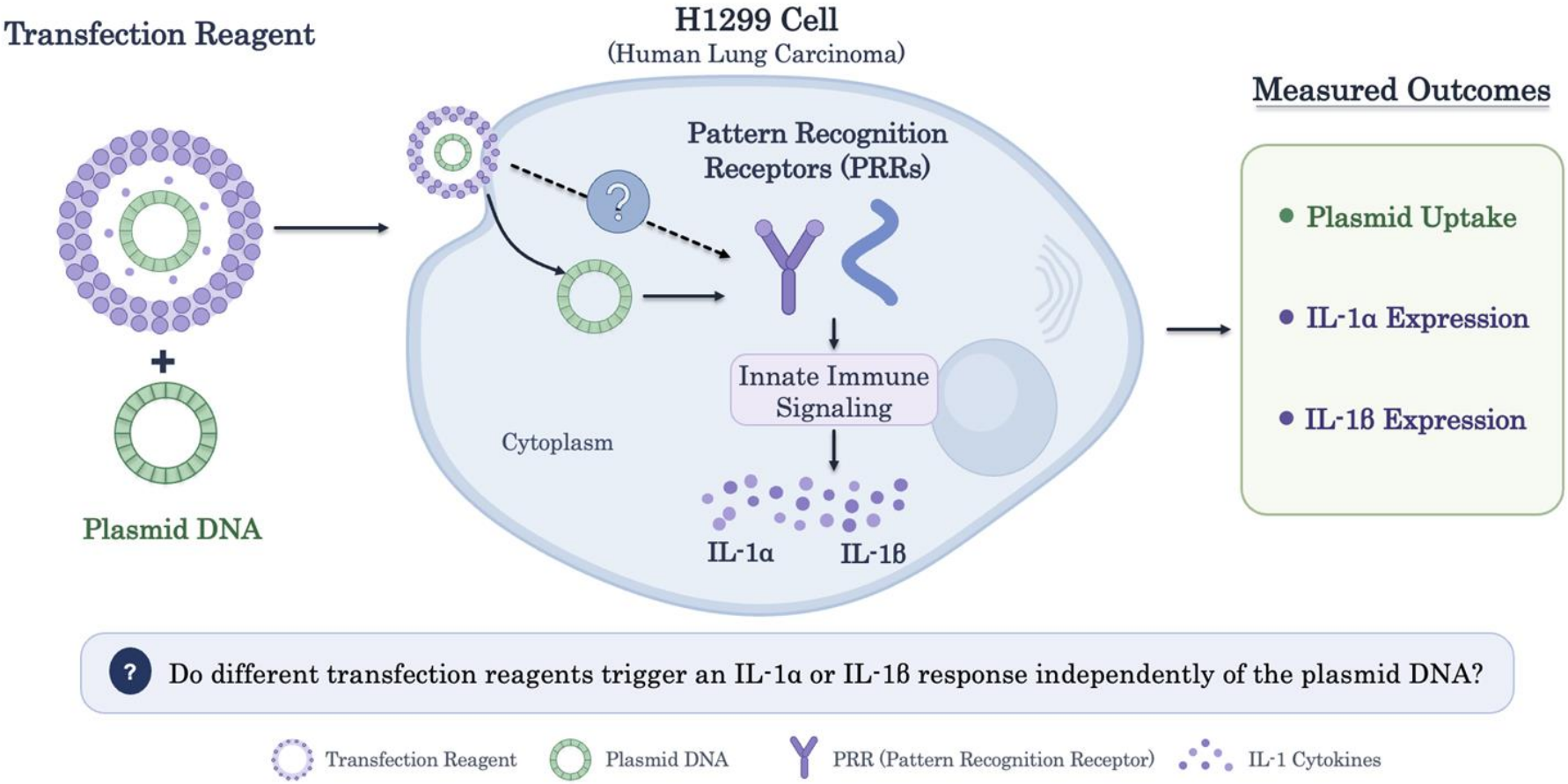
Reagent may activate the cell's innate immune response

Innate Immune Response

Microbe-Associated Molecular Pattern (MAMP) → Pattern Recognition Receptors (PRRs) *Sensory proteins* → Cytokines IL-1α & IL-1β → Inflammation



Innate Immune Recognition During Transfection



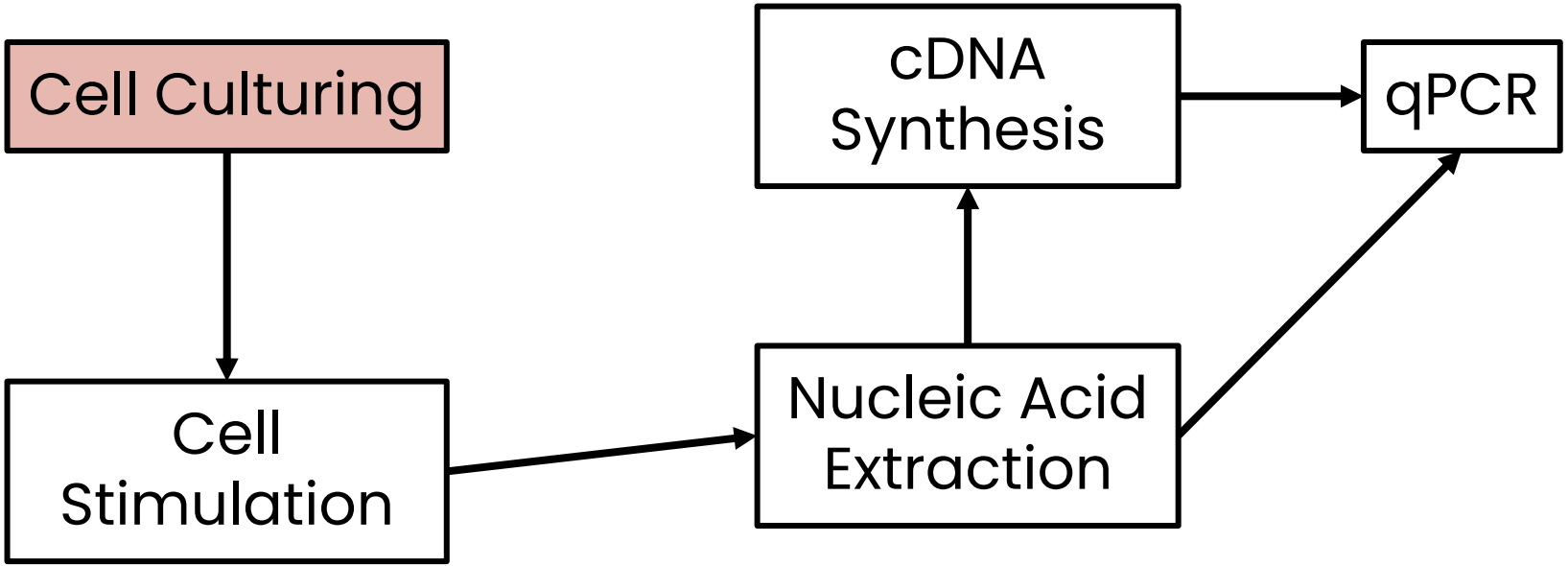
Central Question

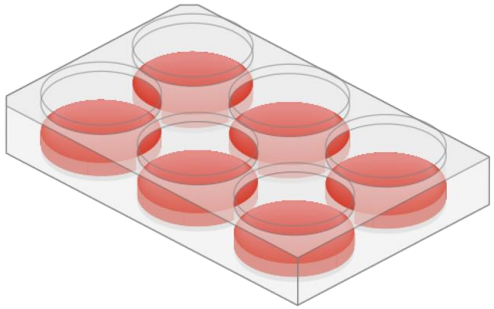
Does the transfection reagent itself induce an innate immune response?

Hypothesis

Cytokine release will vary by reagent type due to reagent-induced innate immune responses

Methodology

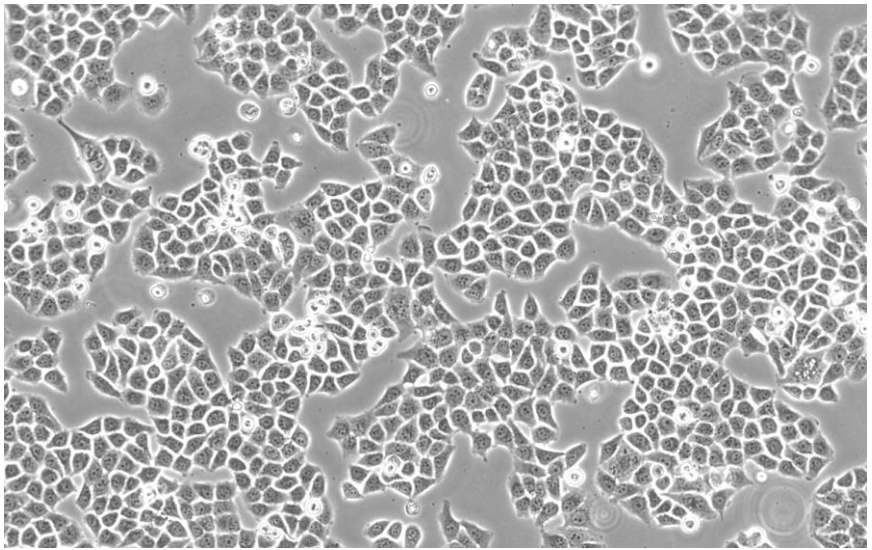




Cell Culturing

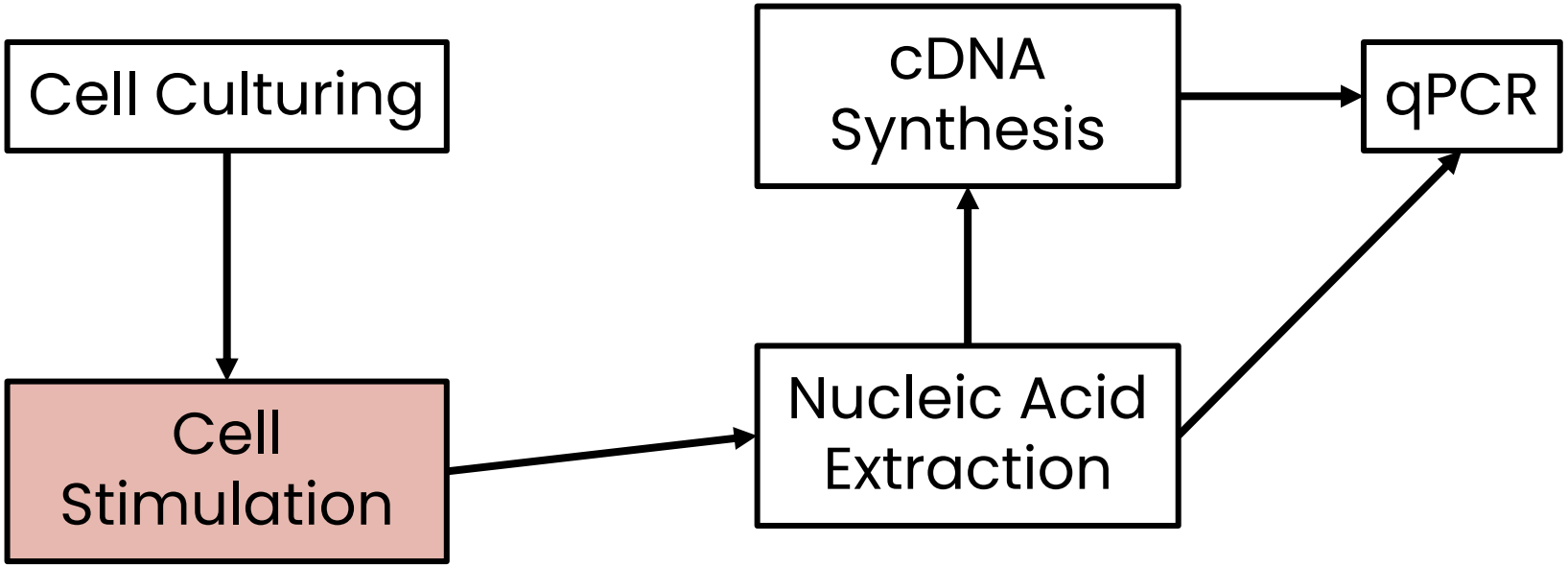
H1299

- Significant immune response

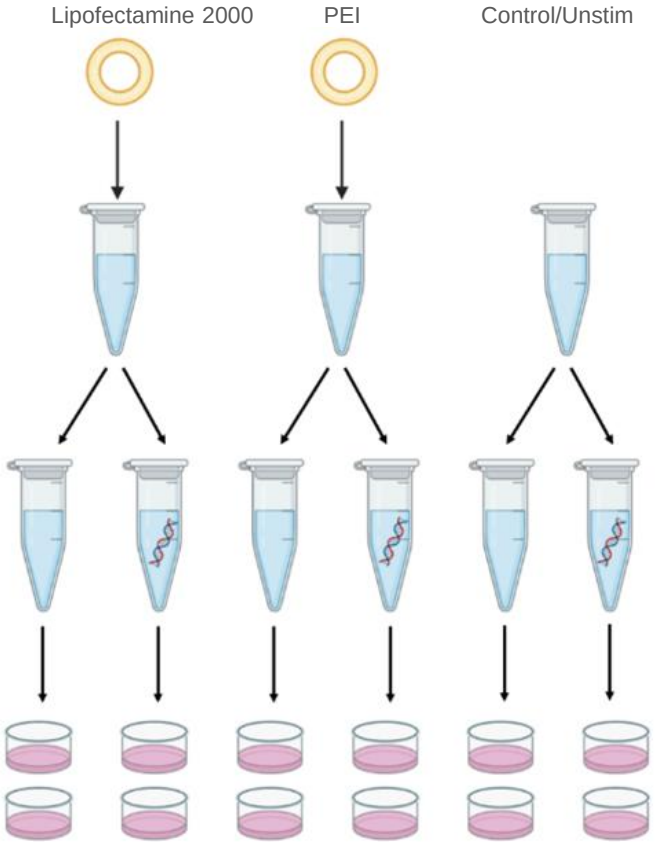


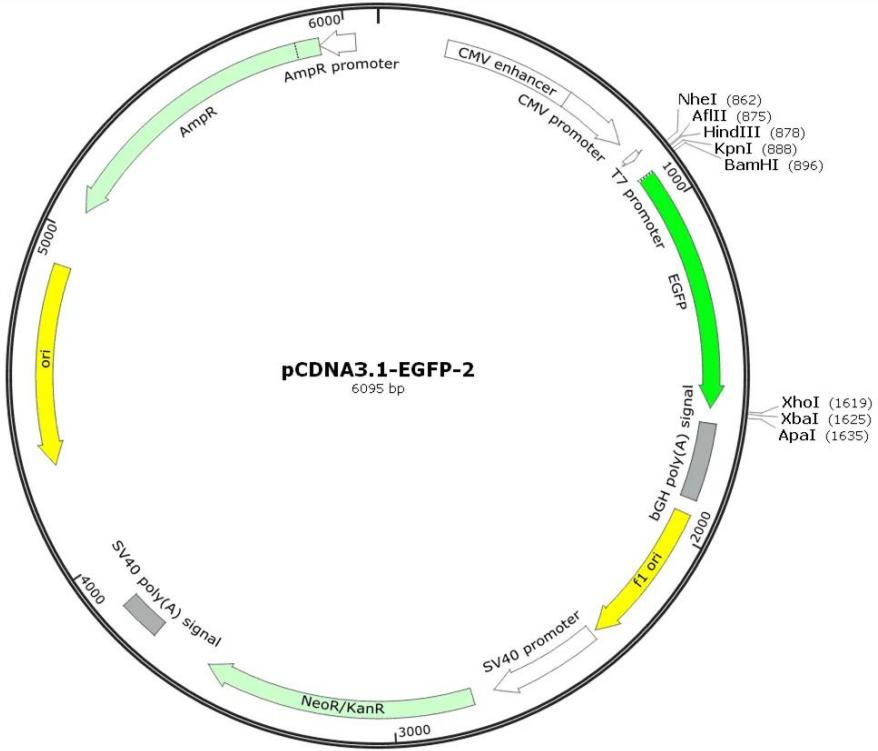
https://www.abmgood.com/Cas9-Stably-Expressing-H1299-Cell-Line-T9610.html

Methodology

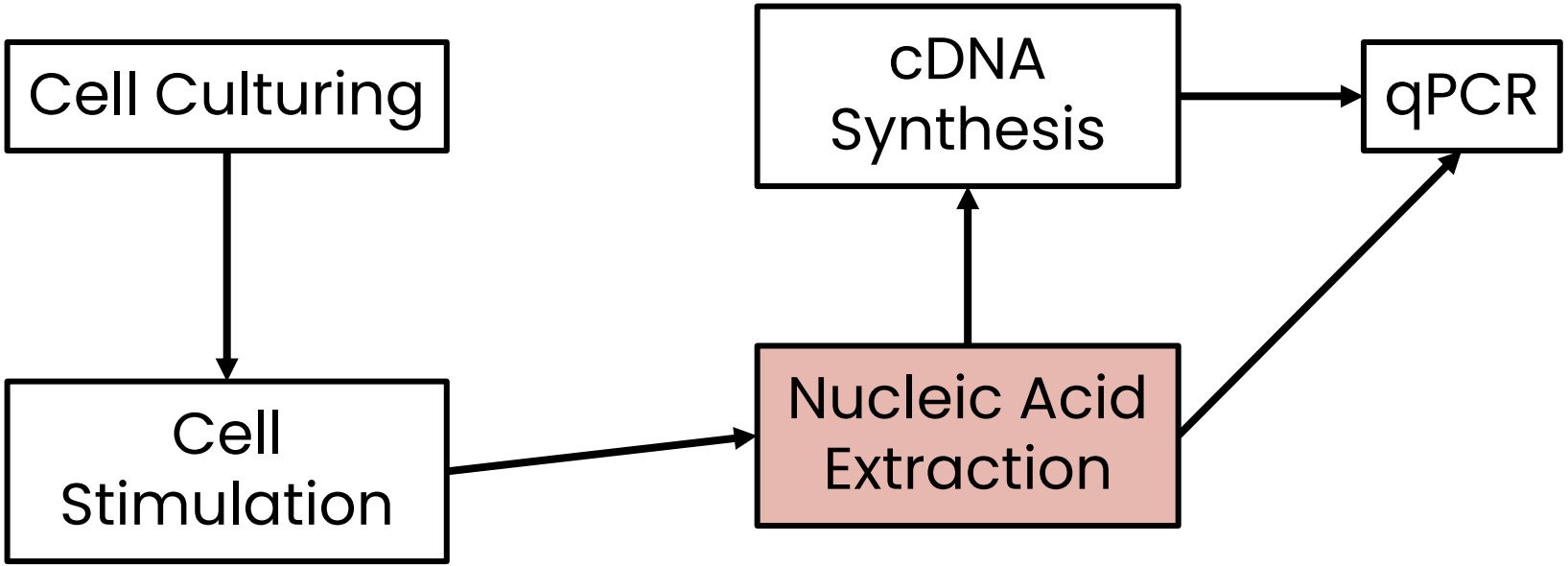


Cell Stimulation

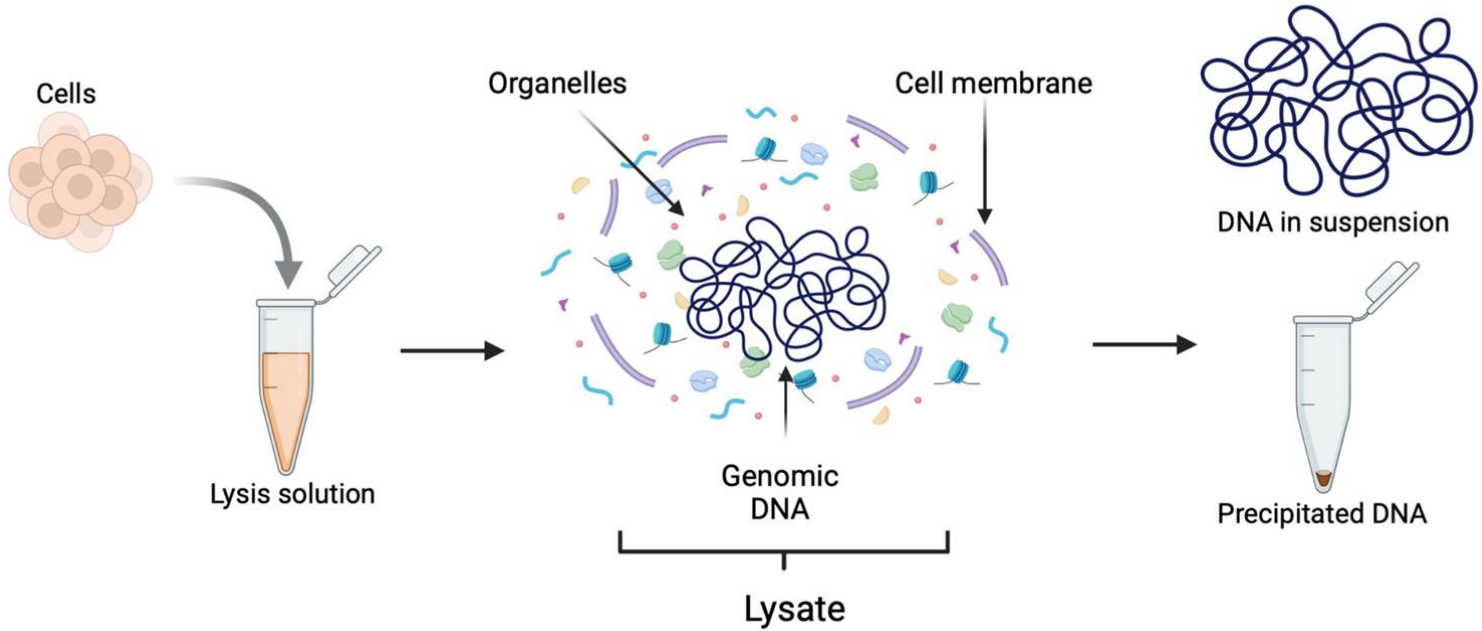




Methodology

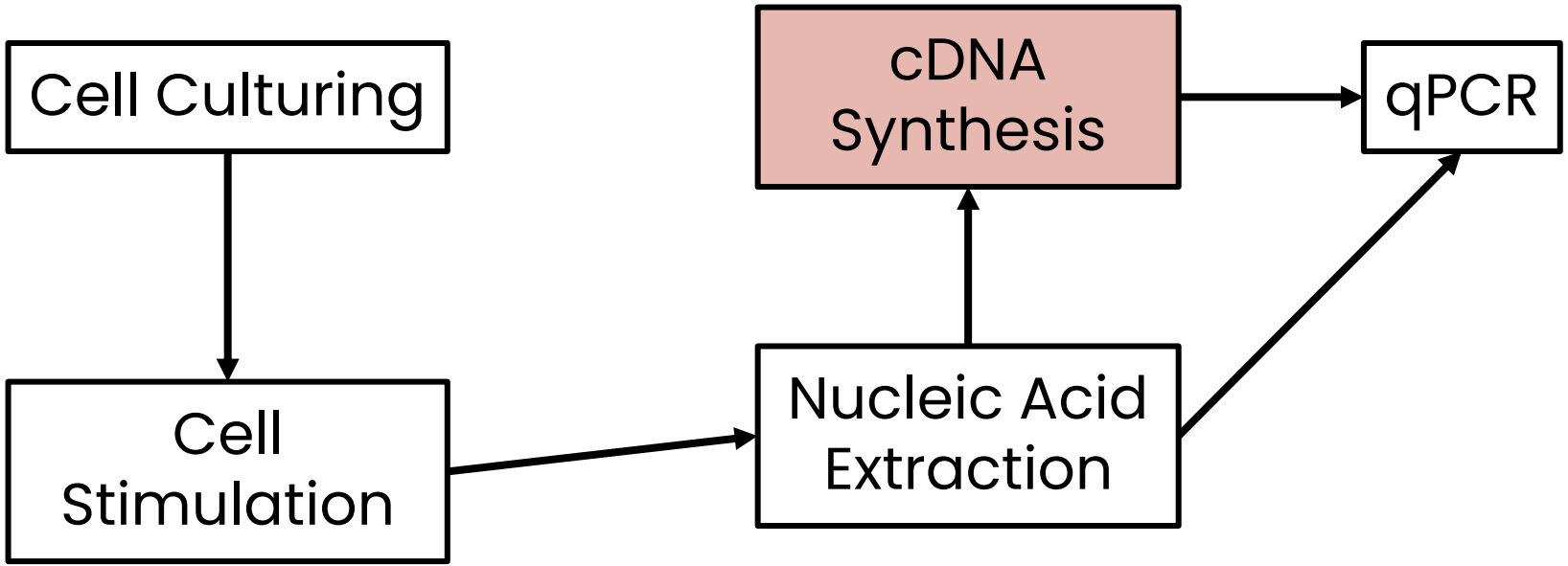


Nucleic Acid Extraction

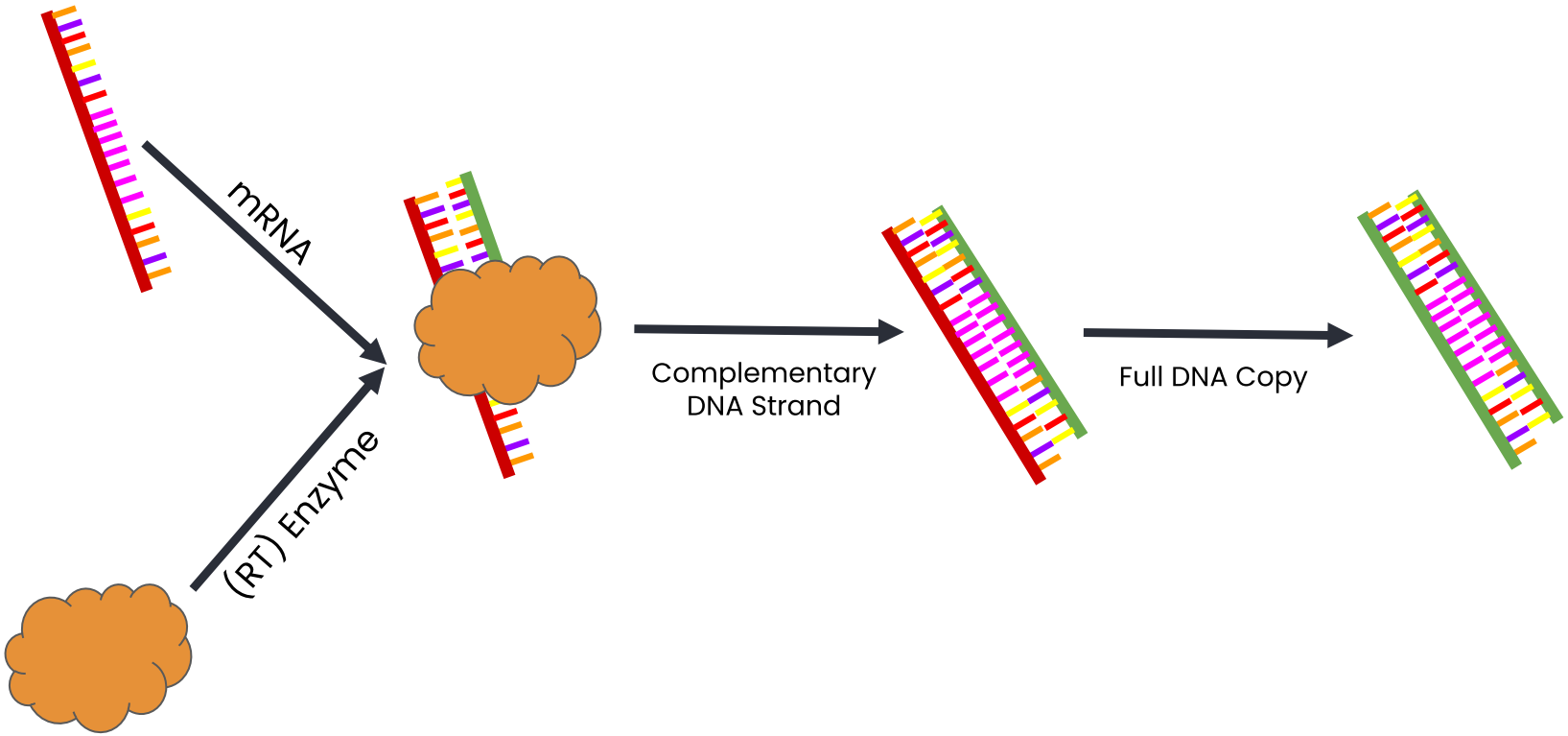


https://eaglepubs.erau.edu/cellandmoleclab/chapter/chapter-1/

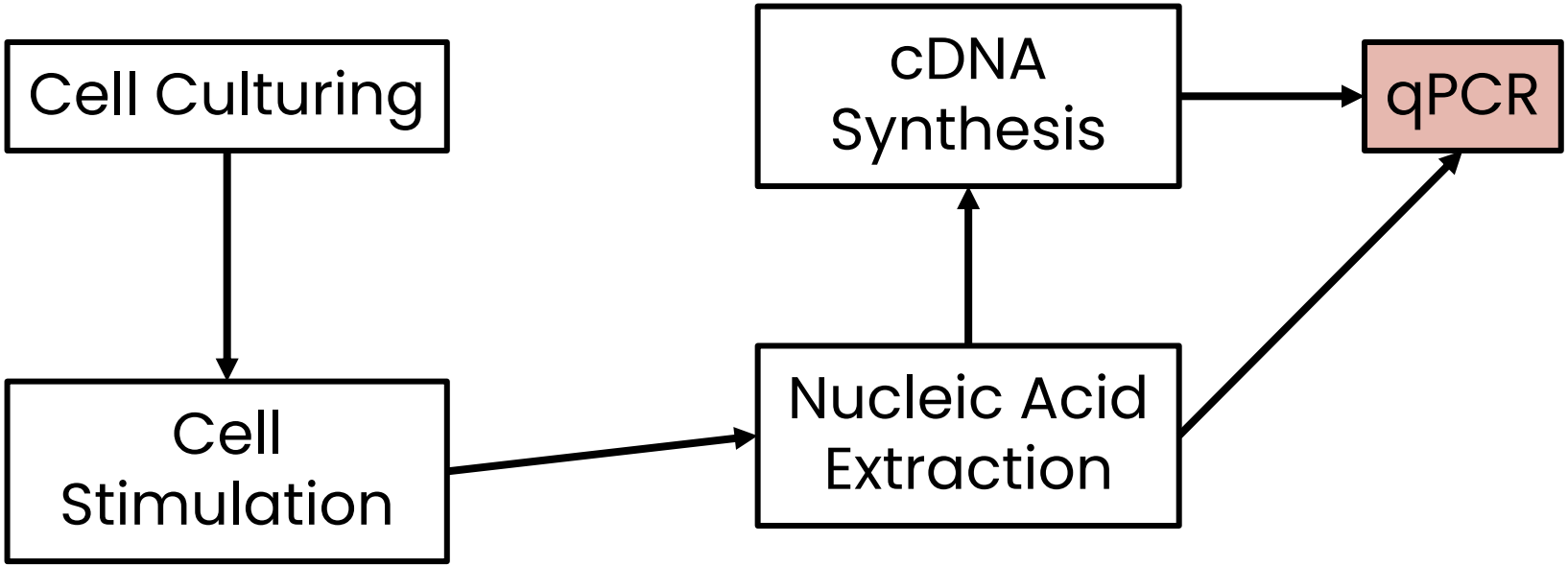
Methodology



cDNA Synthesis

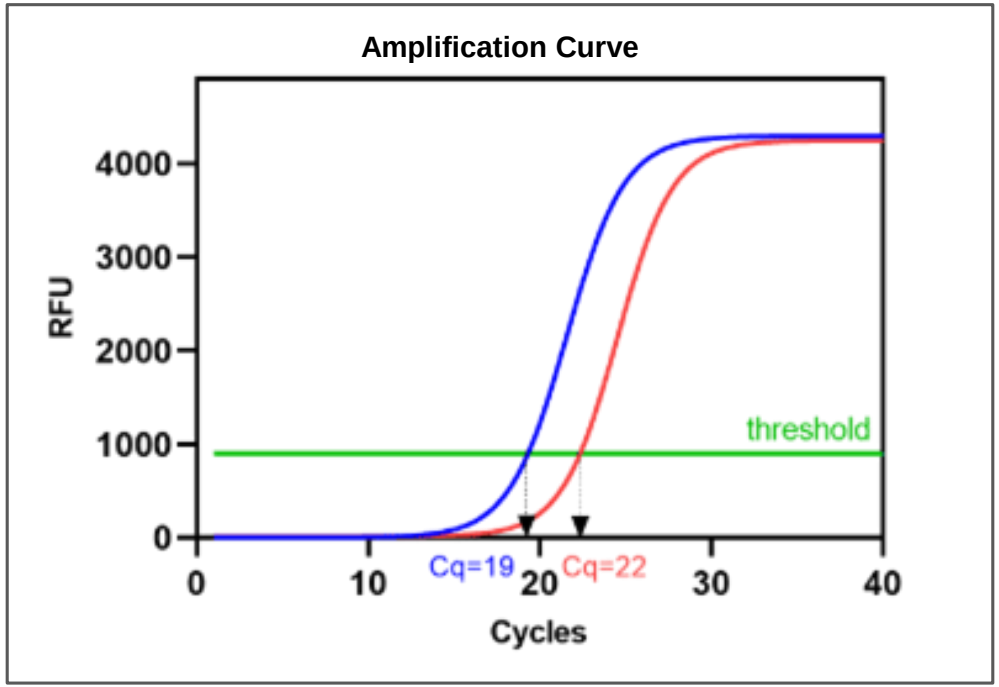


Methodology



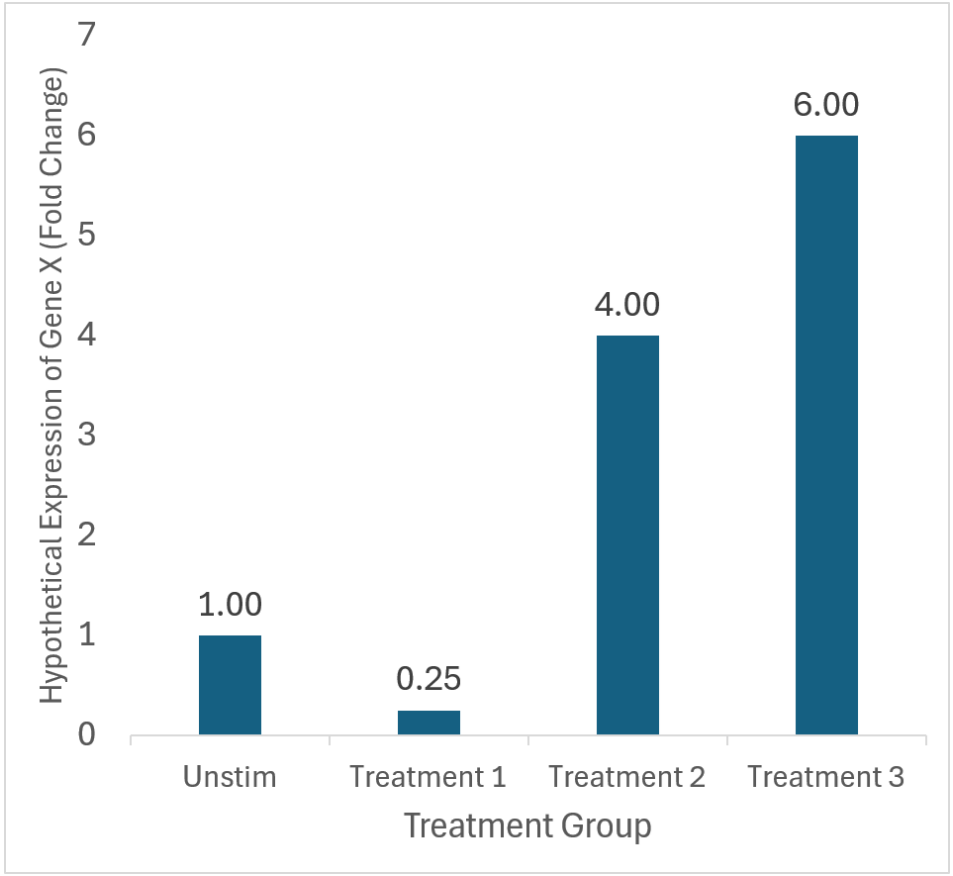
qPCR

Quantitative **P**olymerase **C**hain **R**eaction



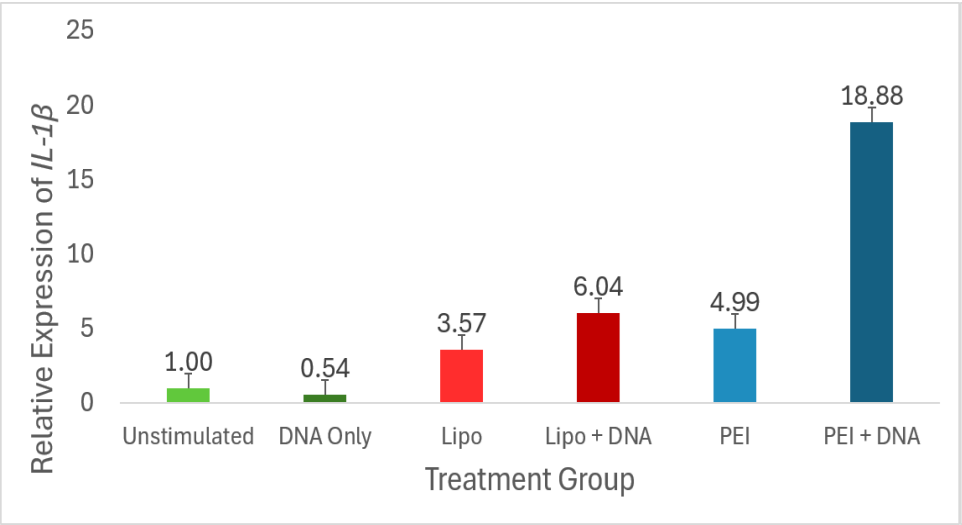
https://www.oncolines.com/mechanistic-cell-biology/quantitative-polymerase-chain-reaction-qpcr/#

Fold Change

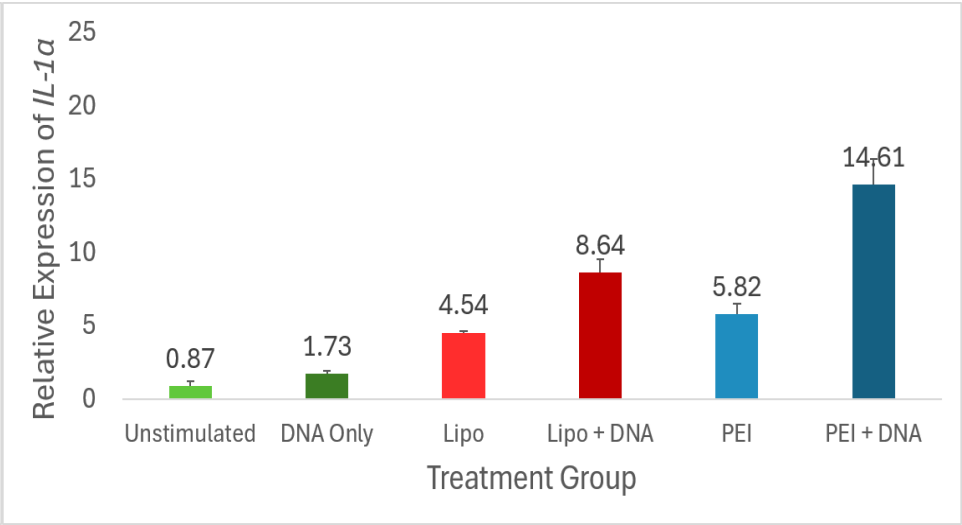

Hypothetical Expression of Gene X (Fold Change)
0
1
2
3
4
5
6
7
1.00
0.25
4.00
6.00
Unstim
Treatment 1
Treatment 2
Treatment 3
Treatment Group

Does the transfection reagent alone induce an innate immune response?

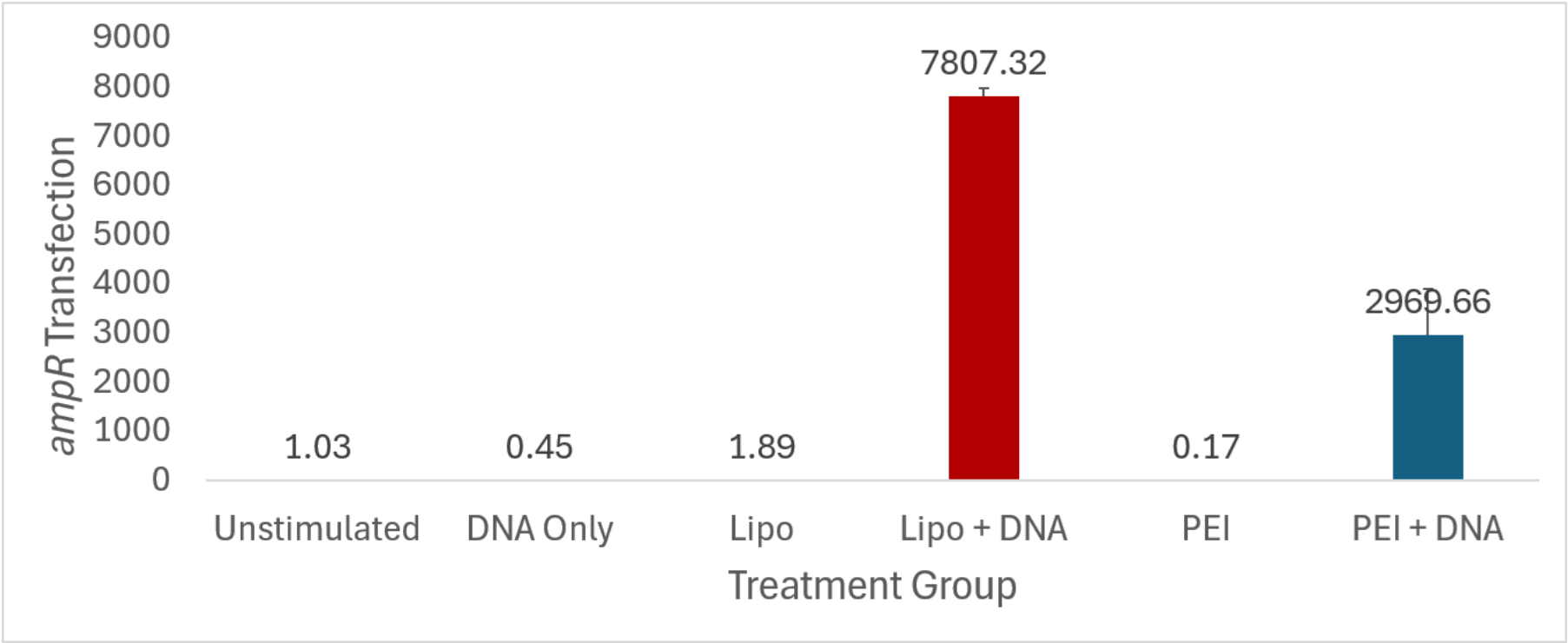
Cytokine IL-1β Fold Change



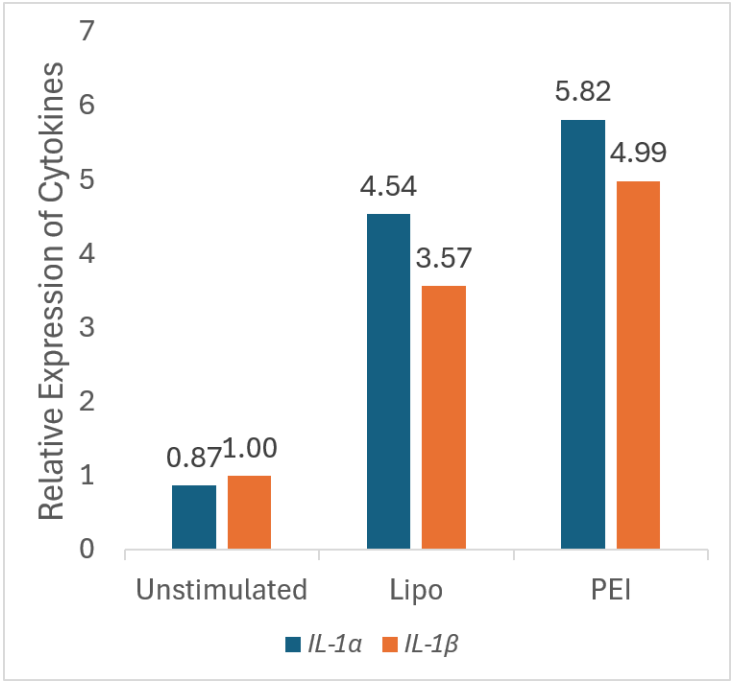
Cytokine IL-1α Fold Change

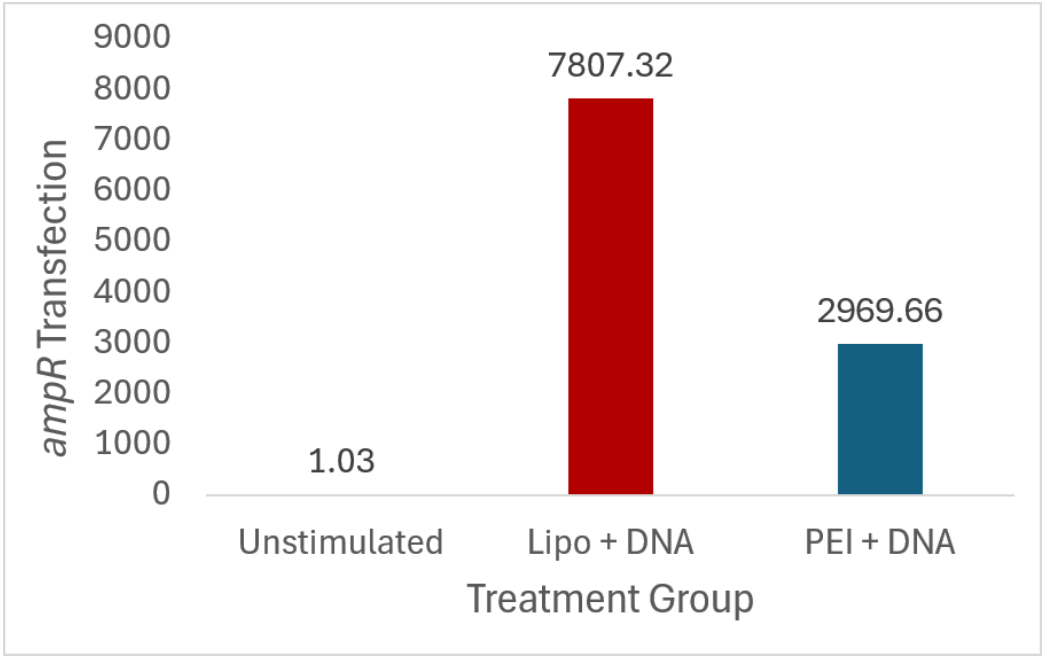


Does the transfection reagent used impact the amount of *ampR* transfection?



Preliminary data suggests that bigger inflammatory responses cause less transfection. Cytokine induction may be inhibiting DNA uptake.





Hypothesis

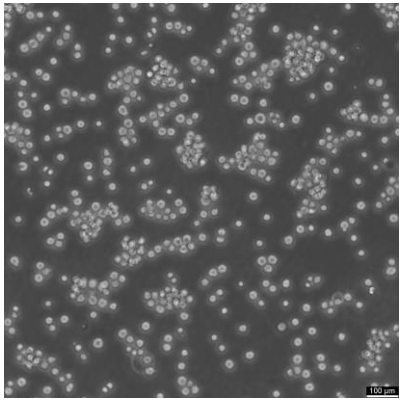
Cytokine release will vary by reagent type due to reagent-induced innate immune responses.

Takeaway

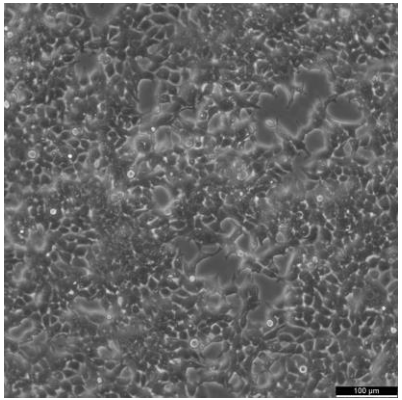
- Transfection reagents trigger an independent immune response.
- The reagent with the higher cytokine release has lower transfection rates

Future Directions

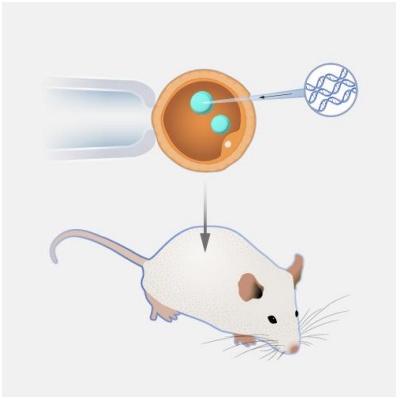
- Nucleic acid vaccine development
- Other conventional transfection reagents (e.g., FuGENE, LyoVec)
- Multiple cell lines, model organisms
- Broader cytokine & chemokine measurements



Jurkat cells



HEK293T cells



Mouse model

https://www.cytion.com/us/ca/Jurkat-Cells/302147, https://www.cytion.com/HEK293T-Cells/300189, https://www.genome.gov/genetics-glossary/Mouse-Model

Acknowledgements

- State of NJ: Office of the Secretary of Higher Education
- BASF Environmental Catalyst and Metal Solutions
- Bristol Myers Squibb
- The Garden State Initiative
- Overdeck Family Foundation
- Novartis Pharmaceuticals Corp.
- Dr. Brianne Barker
- Brenna Hezel
- Emily Shirk

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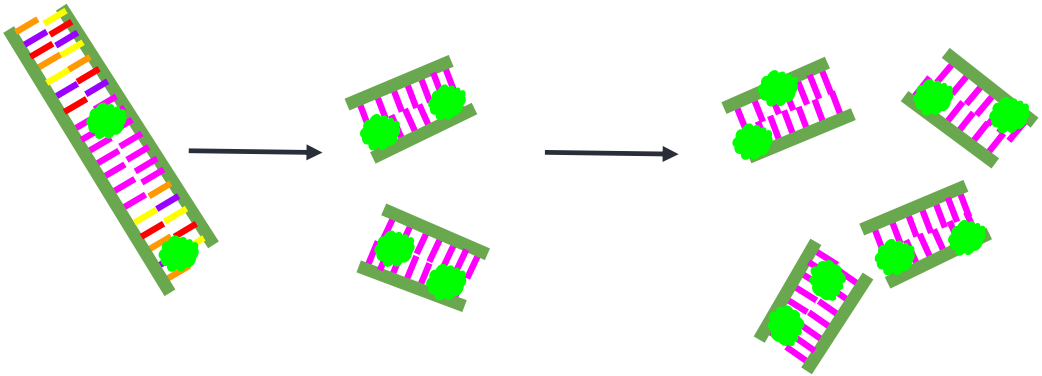
Limitations

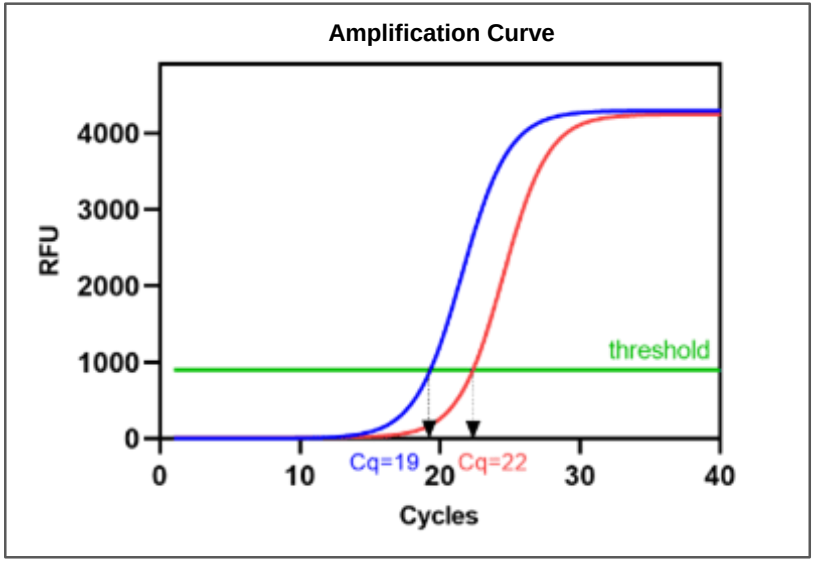
- Laboratory procedures
- LNPs are proprietary data
- Limitations of H1299 cell line
- Accuracy of *in vitro* models

Statistical Analysis

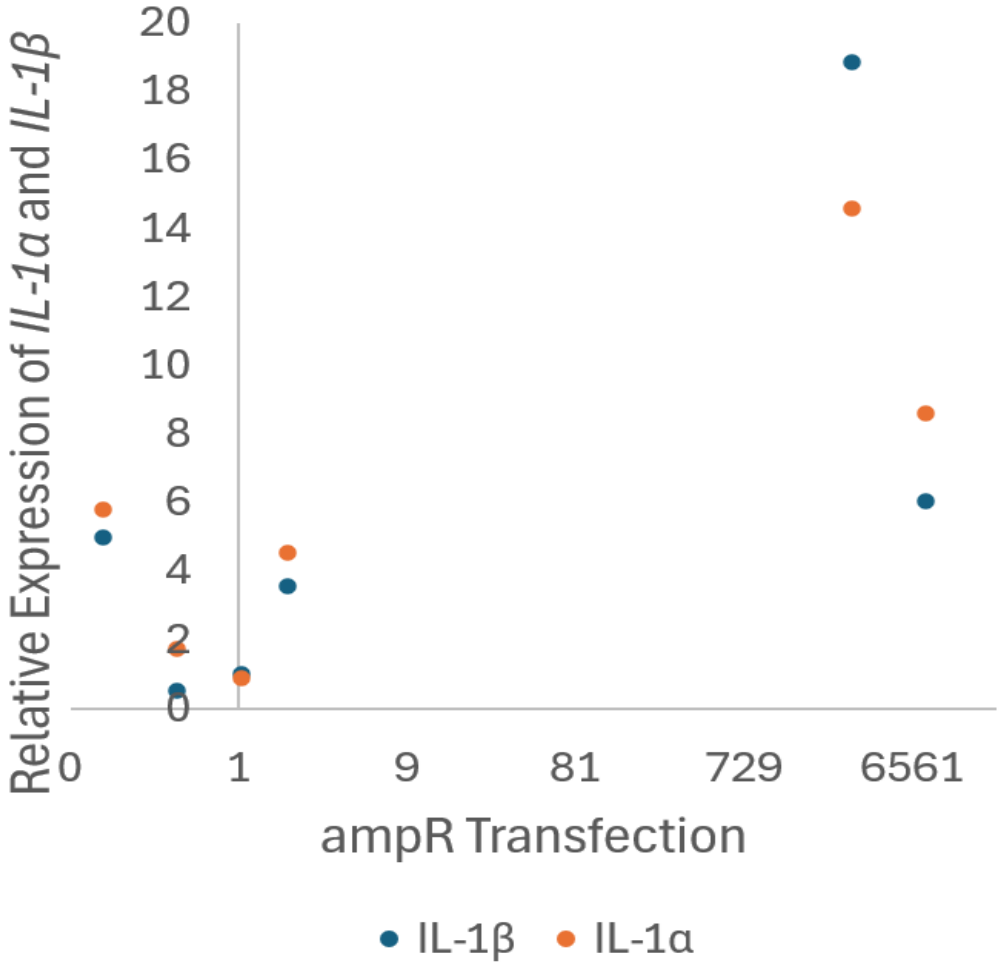
- Welch's t-test: *ampR* transfection vs. unstimulated controls (Lipo, PEI: $p < 0.05$)
- Two-way ANOVA (Reagent × DNA): *IL-1α, IL-1β* Fold Change
    - Reagent significant for both *IL-1α, IL-1β* Fold Change
    - Reagent × DNA interaction significant for *IL-1β* ($p < 0.05$)

qPCR





https://www.oncolines.com/mechanistic-cell-biology/quantitative-polymerase-chain-reaction-qpcr/#



Cytokine induction has no statistically significant correlation with the amount of DNA transfected into the cell, which stands to reason that it may be the lipid instead.

IL-1α vs. *ampR*: r = 0.38

IL-1β vs. *ampR*: r = 0.57