

A straightforward solution for transfection complexes size kinetics follow-up: Videodrop allows to better characterize, optimize and control the transfection step

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Transfection efficiency remains a crucial hurdle in viral vector production (LV or AAV) using transfection reagent-based methods. Several factors influence this efficiency, including the ratio of transfection reagent to DNA, DNA concentration, and incubation time. Studies¹ have shown a clear correlation between the size of the formed transfection reagent /DNA complexes and transfection efficiency. Consequently, being able to monitor this size kinetics is essential to have access to a key parameter for the transfection step process development, scale up and in process control.

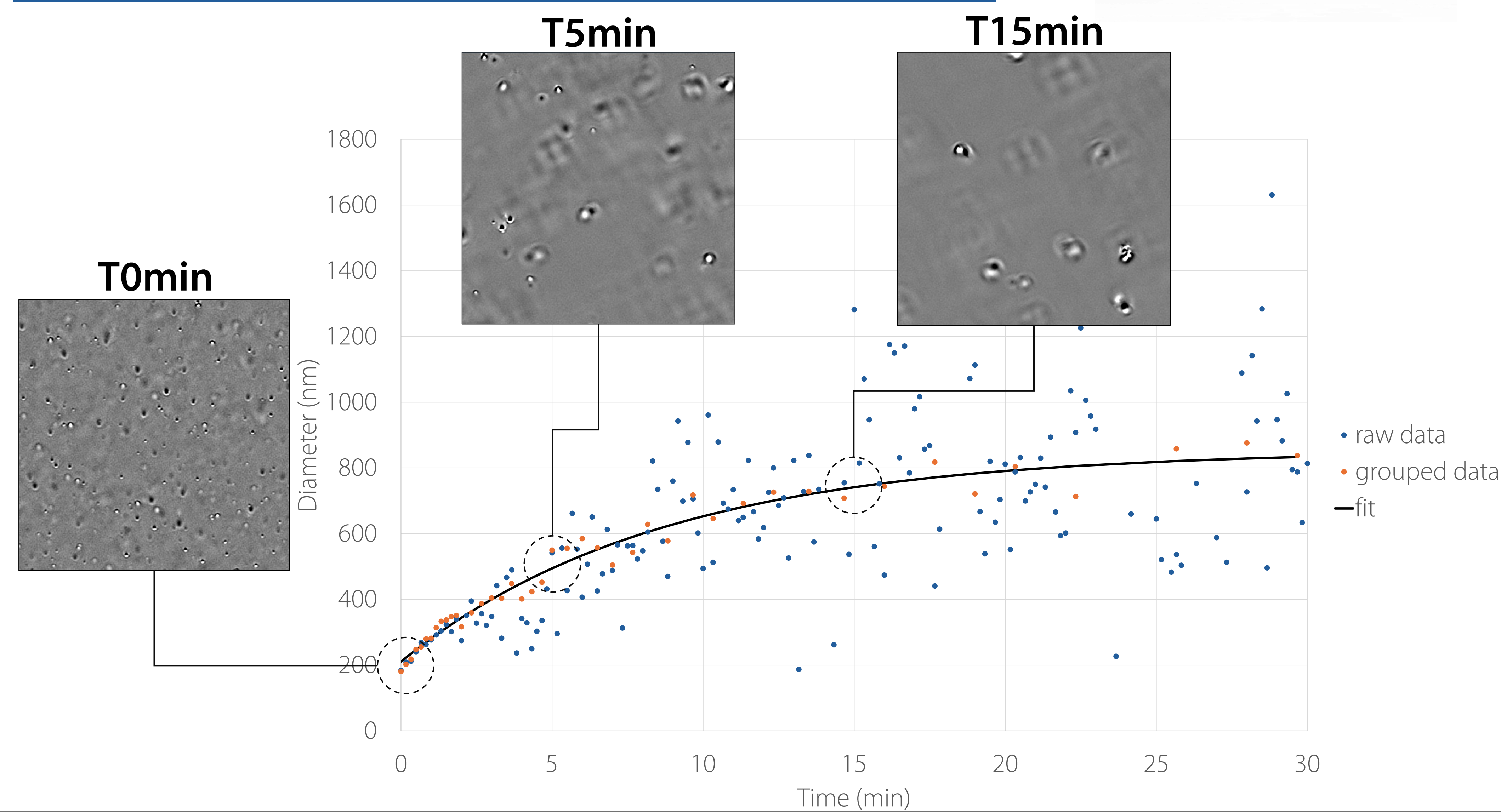
Videodrop is a novel approach that empowers scientists with real-time monitoring capabilities to address these challenges. Videodrop offers a new metric focusing on a critical quality attribute (CQA) – Temporal evolution of particle size – to ultimately improve transfection efficiency, reproducibility and reduce plasmid/polymer costs.

1. Hu, Y. *et al.* Size-Controlled and Shelf-Stable DNA Particles for Production of Lentiviral Vectors. *Nano Lett.* **21**, 5697–5705 (2021).

Viral vector production process



Videodrop : Direct visualization of transfection mix complexation



Videodrop, a very fast microscope transformed as an interferometer allows to visualize in real-time the complexation of plasmid DNA/transfection reagent over time. A **measure** of nanoparticles size **every 15s** enables a **precise size kinetic evaluation**.

Today, despite the **critical role** and the **cost** of the transfection preparation step, quality controls are often missing. Scientists currently rely only on supplier recommendations without any visualization.

By enabling scientists to observe and **understand transfection mix complexation**, Videodrop contributes to improved control of the upstream bioproduction process.

Material & Method

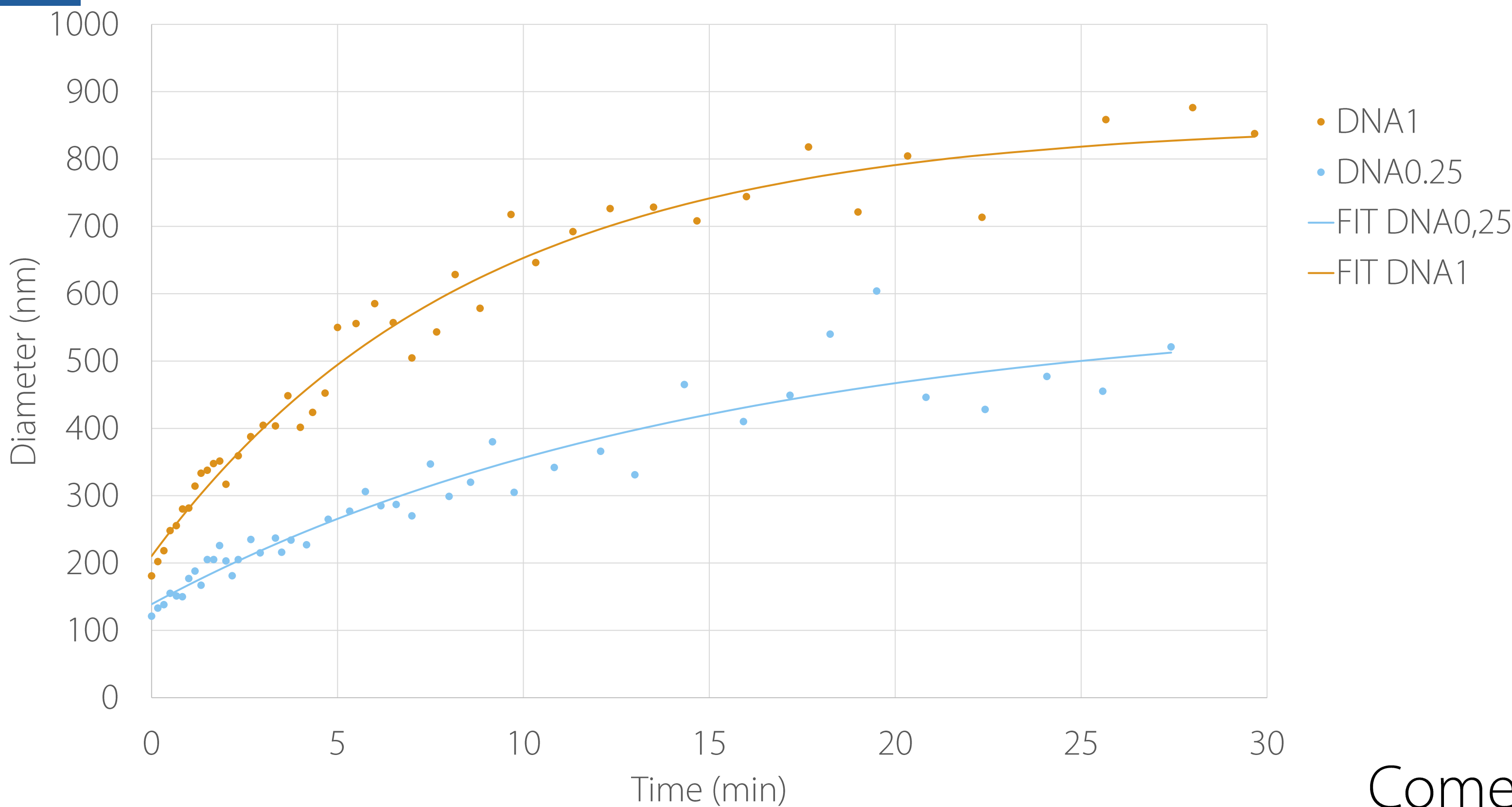
	Ratio DNA/PEIPro	Ratio plasmids (GFP,RC9,Helper)	Complexation buffer	Repetition	Experiment
DNA1	1/1	2/2/1	PBS	5 times, different days	Mix [plasmid/PBS] + [PEIPro/PBS] ; vortex then size kinetics on Videodrop for 30min
DNA0.25	0.25/1	2/2/1	PBS	1 time	Mix [plasmid/PBS] + [PEIPro/PBS] ; vortex then size kinetics on Videodrop for 30min

Videodrop : Screening of different transfection mix conditions

Videodrop's user-friendly interface and automated kinetic monitoring enable the analysis of various transfection mix conditions. To illustrate different size kinetics, this experiment focused on two DNA/PEIPro ratios. Results indicated that a higher DNA/PEIPro ratio correlates with larger complexes and faster growth.

These findings suggest numerous possibilities for further exploration, including:

- Different media (composition, serum, pH),
- DNA ratios and quantities,
- transfection reagent products.



Videodrop is a new suitable instrument, adapted to USP team constraints and reality, to characterize the size kinetics of transfection complexes. It gives a new insight into the optimization and control of the transfection step.

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