NANOMOSAIC

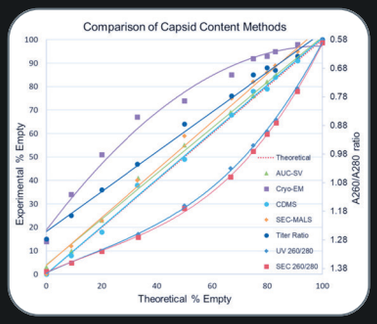
One device for AAV capsid & transgene quantification

STATUS QUO

Gene therapy manufacturing challenges

Getting gene therapies manufactured at scale is a matter of controlling complex biologic & chemical reactions. Having resorted to generic proteomic and genomic analytical methods, the process now often features dozens of different types of process controls executed, each with specific challenges:

Method	Pro	Con
AUC	Highest resolution (F/E/P)	Complex, time-consuming, specialist user
CDMS	F/E/P detection	Complex, time-consuming, specialist user
CIEF	Simultaneous charge profile	Serotype-specific prep
Cryo-EM (TEM)	Visual analysis	No partial determination
DLS/SLS/UV	Low volume — high throughput	Lower specificity
Mass photometry	Low volume — high throughput	Complex data analysis, peak resolving
qPCR/ELISA	Readily available equipment & tech	Compounded error, data reconciliation, dual workflow
SEC-MALS	High-throughput, simultaneous aggregation profile	No partial determination
UV 260/280	Readily available equipment & tech	Indirect measurement, compounded error



Werle et al, Comparison of analytical techniques to quantitate the capsid content of adeno-associated viral vectors, 2021, Mol. Ther. Methods Clin. Dev Borrowed from GTAD 2023 Presentation by Voyager Therapeutics: "Assessment of AAV Empty-Full Methods and their Ability to Examine Partial Species"

The Dilemma: Choose only two.

In order to make analytics and in-process controls useful, AAV manufacturers need to adhere to three core requirements:

Speed.

Results need to be available fast, ideally during the same day. With processes upstream requiring ad hoc changes to biological and physiological inputs, an ideal analytical method allows for <4 hours of processing.

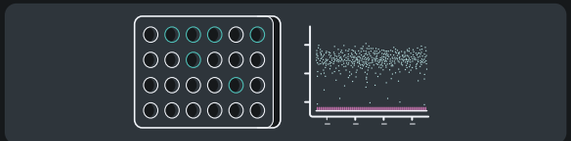
Accuracy.

Accurate insights into what is happening both with capsid and transgene is essential. Understanding sample integrity upstream is a core challenge for scaling up with intact transgenes downstream.

Cost.

Gene therapy products cost multiple 100,000's of dollars per batch. Bringing down the cost per sample analyzed is a core requirement.

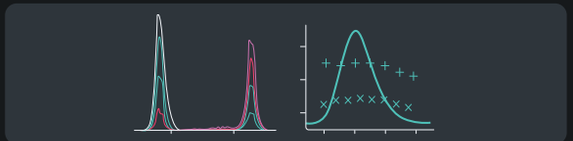
The current gold standard: suboptimal.



Capsid ELISA + dPCR

High-throughput, but inaccurate.

A method that is executable at high-throughput, but introducing compound error due to the use of two different analytical methods. dPCR multiplexing on transgene integrity furthermore offers challenges for analytical teams.



AUC or CD-MS

Accurate, but low throughput.

Highly accurate methods, mass-based, offering great insight into integrity, but require complex training for users. Moreover, price per sample executed is typically only viable for downstream products. Does not work with crude samples.

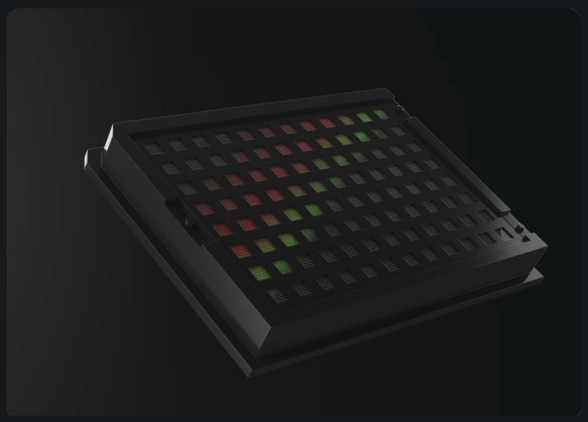
NEXT-GENERATION ANALYTICS

Introducing Tessie & nanoneedle technology

NanoMosaic's team has multiple years of experience developing biomarker analytics across research areas. Now, we have set out to focus on the rapidly evolving gene therapy market and building a fit-for-purpose workflow that combines both capsid and transgene interrogation.

NanoMosaic's Nanoplate

A simple consumable that plays nicely with existing lab equipment. Our Nanoplates offer 96-, 384-, or 1536-well capability. Each well is built on a single silicon surface, offering 10,000s of binding opportunities for proteins or nucleic acids.

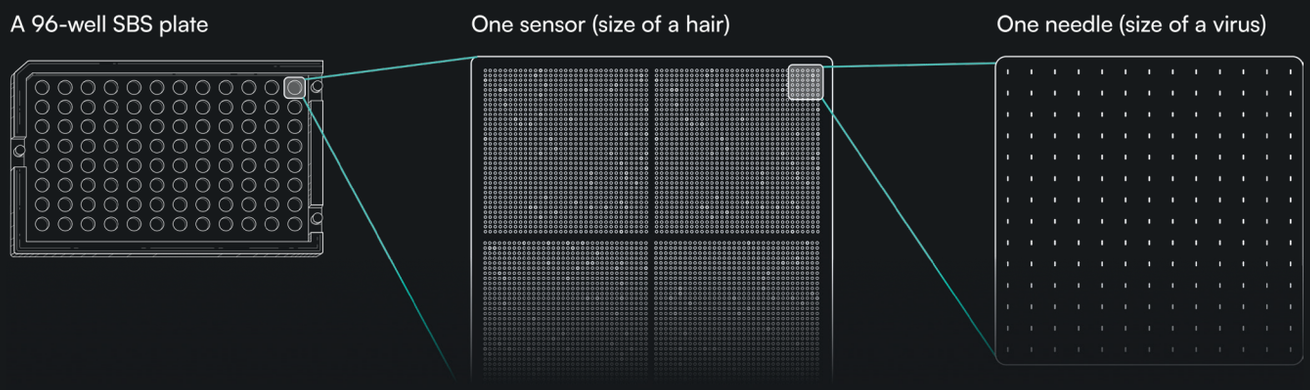


NanoMosaic's Tessie

Our benchtop reader, with a ten-minute read time, supports even the largest gene therapy throughputs, making it capable of handling them with a single instrument.

Nanoscale analytics. Manufacturing scale throughput.

Each well supports an array with 10,000's of binding points. The surface conjugates much like standard microplates in ELISA workflows. The binding of the analyte to a nanoneedle changes the property of the needle, and is used to quantify the amount of analyte in samples. With high amounts of binding opportunity, our consumables support direct-from-crude analysis, reducing the time to result without sacrificing throughput.



GENE THERAPY ANALYTICS, AT SCALE

Fit-for-purpose gene therapy assays

Based on our platform technology, we have developed Tessie and specific assays to fit gene therapy workflows.

Capsid, transgene. One workflow.

Two assays that can be executed in similar times, from a single sample, enabling accurate F/E/P ratio generation at scale.

Results in 3 hours.

As easy, and as fast, as a simple ELISA assay. For the transgene assay, a simple lysis step is added and results for both capsid and transgene are generated in 3 hours.

No purification, direct from crude.

No sample preparation, no purification. Work directly from very low sample volume.

High-resolution, transgene integrity.

Our transgene assay helps teams understand the transgene integrity at scale. Quantification only occurs in the case of both ends of a full-length transgene being present.

Wide dynamic range.

A broader dynamic range than both dPCR and ELISA makes Tessie capable of supplanting both analytical methods.

Robust, scalable.

Coefficient of variation (CVs) lower than 10% for both capsid and transgene assays make our method scalable to your laboratory team.

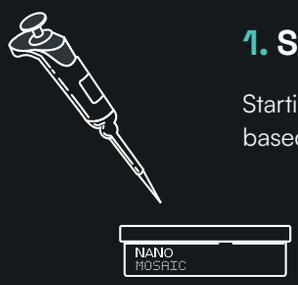
Simple data analysis.

No complex collation of separate analytical methods in convoluted spreadsheets. The transgene and capsid titers are harmonized and presented as ratio determinations.

EXECUTABLE BY YOUR TEAM

A workflow everyone knows, with more analytical depth.

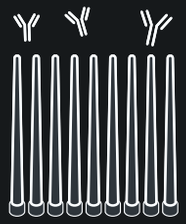
Based on our platform technology, we have developed Tessie and specific assays to fit gene therapy workflows.



1. Spin and dilute samples

Starting directly from crude samples, you can spin and dilute based on where you are in the manufacturing process.

2. Conjugate capture antibody



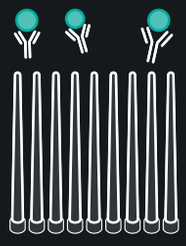
Much like a simple ELISA, conjugate the antibody to our Nanoplate.

Skip step if you use pre-coated Nanoplates.

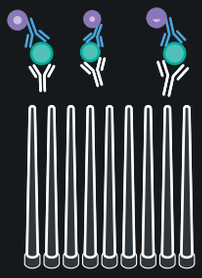
3. Add sample

Add your sample (2 µL to 10 µL) to the Nanoplate, cover with the plate cap & let incubate at room temperature for 30 minutes. For transgene, a simple lysis step is added here.

4. Add detection antibody

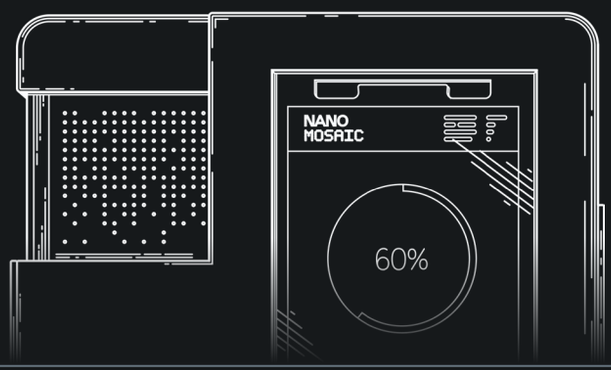


Add detection antibody, let plate dry, remove plate gasket, dry again.



5. Add Nano Amplifier

Add our proprietary Nano Amplifier reagent, incubate for five minutes, wash and dry.



6. Ten minute read

Automated plate read on NanoMosaic platform & review data. The analysis software automatically interpolates control and test values to determine the ratios of interest.

CAPSID ASSAY

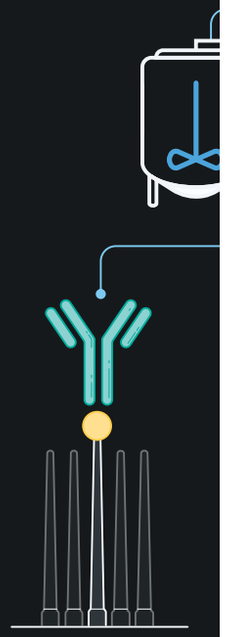
Understanding capsid quality.

One

1

Capsid binds.

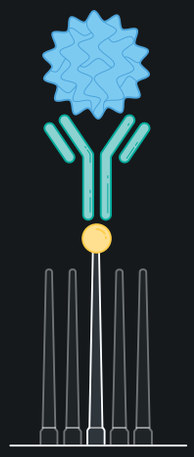
Capsid binds to conjugated antibodies, with the capability of using AAV serotype-specific antibodies or custom antibodies matched to your workflow.



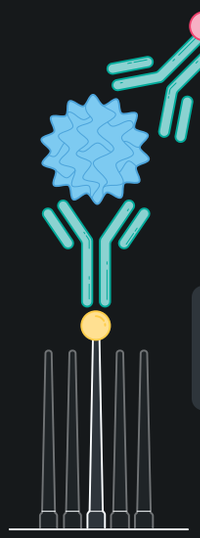
2

Detection antibody binds.

Detection antibody attaches to the surface of the bound capsid. An affinity tag is linked to the detection antibody.



Add
Sample to nanoplate

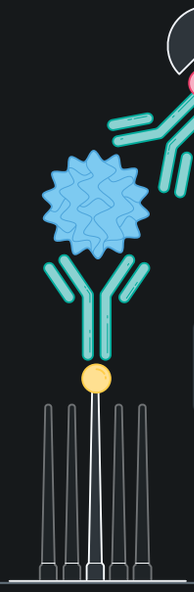


Add
Detection antibody

3

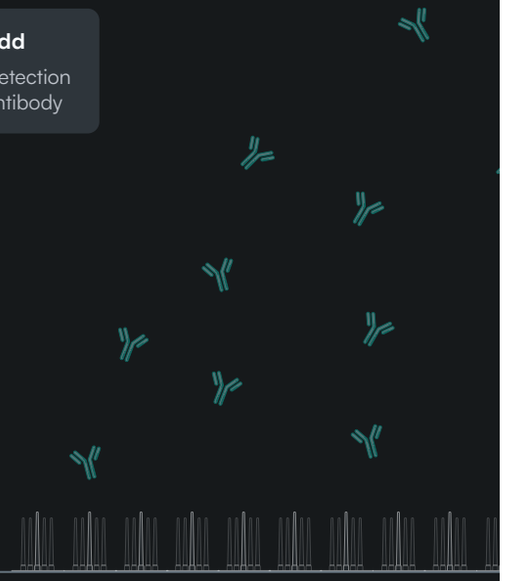
Nano Amplifier step.

The Nano Amplifier changes the property of nanoneedles that have capsid bound to them, enabling the reader to quantify the analyte.



Add
Nano Amplifier

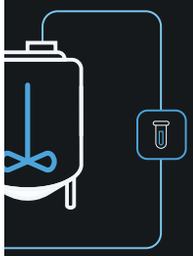
Read plate
After drying



TRANSGENE ASSAY

Detailed insight into transgenes.

workflow



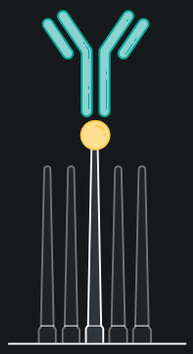
Sample
2 µl to 10 µl crude extract

4

Probe transgene.

Transgene is probed with 2 affinity tags, one of which binds to the end of our conjugated capture antibody.

Bind
Capture antibody with Nanoplate

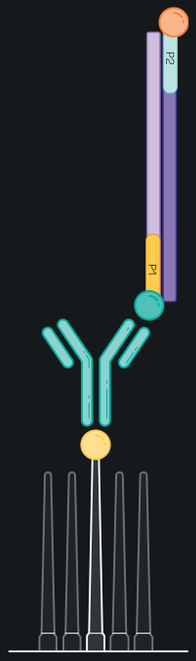


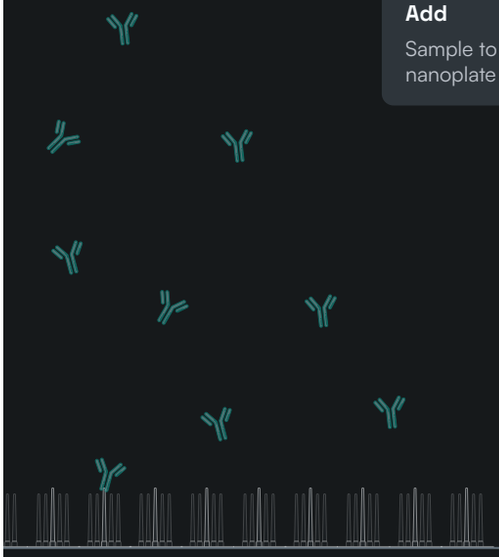
2

Probed transgene captured.

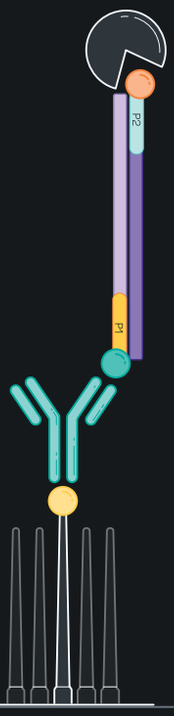
The probed transgene binds to the capture antibody.

Add
Sample to nanoplate





Add
Nano Amplifier



3

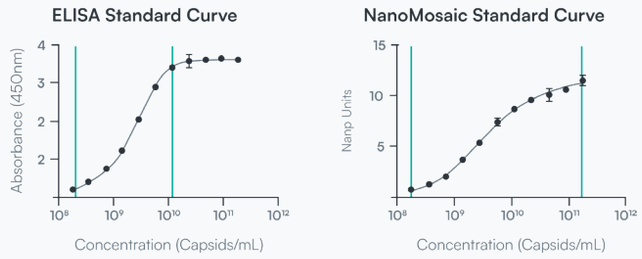
Nano Amplifier step.

The Nano Amplifier changes the property of nanoneedles that have transgenes bound to them, enabling the reader to quantify the analyte.

Read plate
After drying

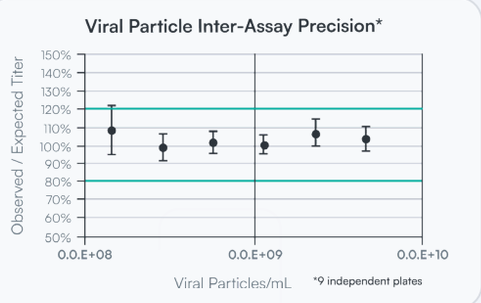
CAPSID ASSAY

Beyond ELISA results.



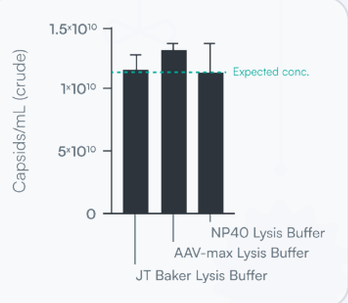
Wider dynamic range than ELISA

Capsid binds to conjugated antibodies, with the capability of using commercially available or custom-developed antibodies matched to your workflow.



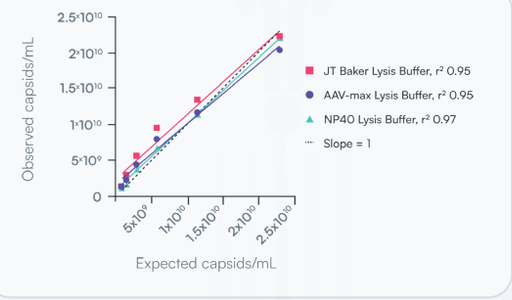
Inter-assay precision

NanoMosaic's assay is within 10% of the expected titers, starting from low concentrations going all the way to higher concentrations. Across 9 different plates, 3 users, and 3 different instruments our experiments resulted in a low coefficient of variation of 7%.



Accurate in crude conditions

After lysing HEK293T cells using multiple different lysis buffers and within this media and lysate background, we spiked-in a known number of capsids. When comparing the number of capsids and the different lysis media, we concluded that it is very close to the expected value when measured in crude conditions.

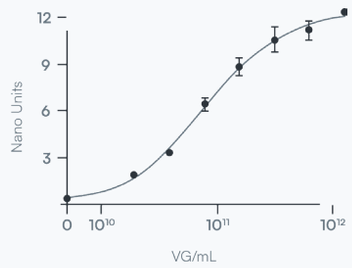


Across popular lysis methods

By testing three different lysis conditions, the data show excellent linearity of samples estimated in crude cell background down to 1.0e+09 capsids/mL.

TRANSGENE ASSAY

Transgene integrity information.

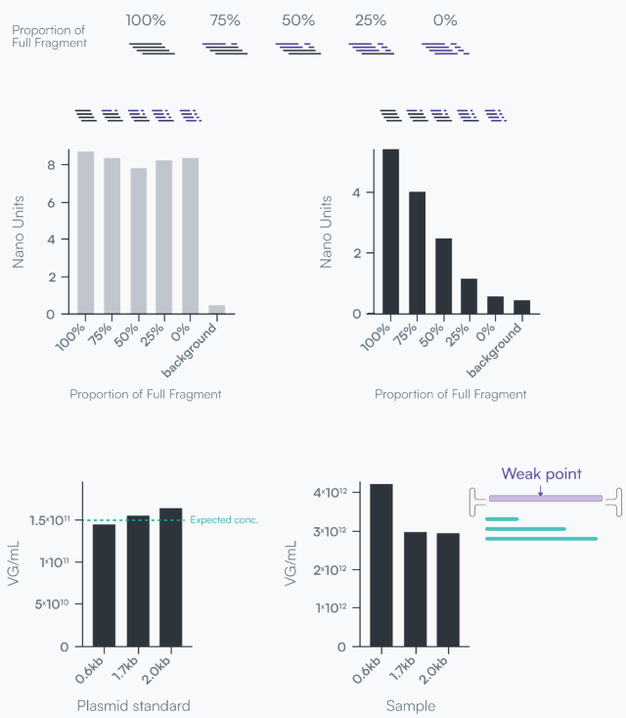


Repeatable & reproducible

Plasmid DNA was used as the standard to test the repeatability and reproducibility of the NanoMosaic assay. The assay provides good quantification and a low coefficient of variation at 6.4%, performed over 4 different plates and 3 different users.

Reference transgene concentration

- NanoMosaic: 1.2e12 vg/mL
- ddPCR: 1.2e12 vg/mL
- Expected: 1.2e12 vg/mL



Accurate transgene information

Plasmid DNA was digested to isolate the transgene segment, analyzed via dPCR and NanoMosaic assays, yielding identical results.

From plasmid DNA, we prepared samples with intact and truncated genomes mixed in ratios of 25% intact to 75% partial. Probes specific for full and partial regions were used during testing.

NanoMosaic two-probe assay accurately quantifies intact transgenes. Grey boxes show detection of partial genomes, while black boxes represent full-length genome detection, showing signal decrease with more partial genomes.

Using dPCR (targeting the ITR region) and NanoMosaic (targeting the full length), the probed regions determined what was measured in the sample. Probes were designed for the full-length (2 kb) and two partial fragments (1.7 kb and 0.6 kb). When examining plasmid DNA (containing only the full length), the expected outcome aligned with reality, with all three probes yielding identical results. However, when testing the sample, we observed a higher signal from the 0.6 kb fragment and lower signals from the 1.7 kb and 2.0 kb fragments, suggesting a higher concentration towards the left of the 1.7 kb fragment.

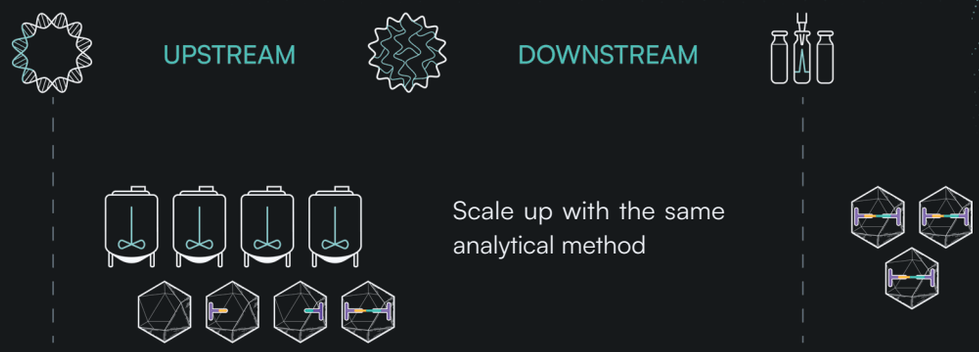
IMPACT FOR AAV MANUFACTURERS

A new analytical paradigm.

Our aim is to usher in a new golden standard for AAV gene therapy manufacturers. Enabling teams upstream to increase the resolution of their information, and syncing up analytical methods with teams downstream.

Such information will ultimately:

- Simplify challenging DOE experiments.
- Make products homogeneous faster.
- Bring gene therapies to patients at affordable prices.



"NanoMosaic's goal is to consolidate all the different platforms into a single platform where a continuum is created between R&D to upstream and to downstream"

Dr. Qimin Quan
(Co-founder and Chief Scientific Officer)

Contact us for a demo

Our team of application specialists helps your team get set up, with real samples, in a matter of weeks. Collaborate on concordance studies, and understand the simplicity of the workflow, right in your own laboratories.

Contact us

Contact us via
www.nanomosaicllc.com

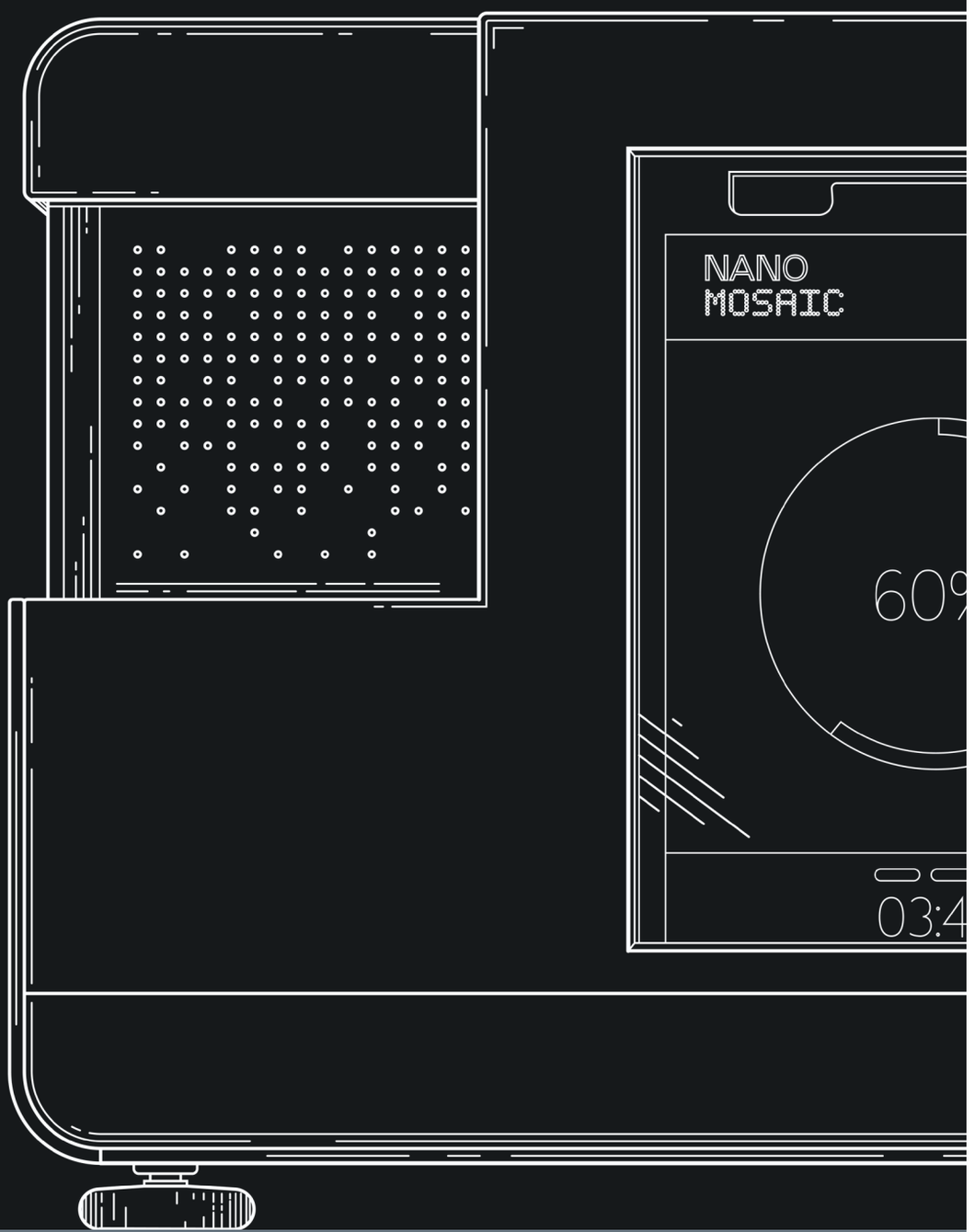
Consult and scope

Our team is available to help scope your demo to quickly adopt your new assay, seamlessly.

Concordance results

Our team generates data with yours to prove concordance with improved workflows.

NANOMOSAIC



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