Case Study

UCSF optimizes viral sequencing with dynamic PCR control

The Challenge: When fixed PCR cycles waste good data

High-throughput viral sequencing depends on precise library amplification. Too few cycles, and libraries fail. Too many, and artifacts overwhelm valuable reads.

At the UCSF Center for Advanced Technology (CAT), Professor Eric Chow and his team routinely process dozens to hundreds of research and surveillance samples, many with highly variable input quality and concentration. In workflows like viral sequencing, the standard approach relies on long, fixed-cycle PCR steps designed to average out these differences.

The result: unnecessary over-amplification, hidden primer dimers, wasted sequencing capacity, and slow turnaround times.

A unique feature of the CAT facility is their focus on R&D and evaluating new technologies that will better serve the UCSF community. Dr. Chow identified the potential for an alternative mode of amplification control—one that could adapt to each sample automatically—to make sequencing workflows cheaper, better, and faster. So, they decided to put it to the test.

Meet the Team

Dr. Eric Chow
Adjunct Associate Professor, UCSF | Director, Center for Advanced Technology | Focus: Genomics technology development, NGS workflows

UCSF Center for Advanced Technology (CAT)
Founded in 2003 | Supports Illumina and PacBio sequencing | ~50% staff time dedicated to R&D and workflow optimization

PCR: The hidden bottleneck in viral sequencing workflows

The ARTIC protocol, widely used for viral genome sequencing, relies on multiplex PCR followed by indexing PCR. To account for variable sample inputs, the multiplex step traditionally runs for ~35 cycles with long extensions—often lasting up to four hours.

The typical workflow looks like this:

Traditional ARTIC prep:

1. Multiplex PCR (35 cycles, endpoint)
2. Indexing PCR (fixed cycles)
3. QC and quantification
4. Sequencing

This approach has several drawbacks:

- Over-amplification produces "bubble products" and primer dimers
- Primer dimers hybridize to library molecules and escape size-based cleanup
- Dimers, which show up as a spike in G-bases, reduce usable reads
- Extensive cleanup and QC adds time and cost
- Most errors appear only after sequencing

"Once you over amplify samples, it can lead to artifacts... that can make size selection really troublesome."

— Eric Chow

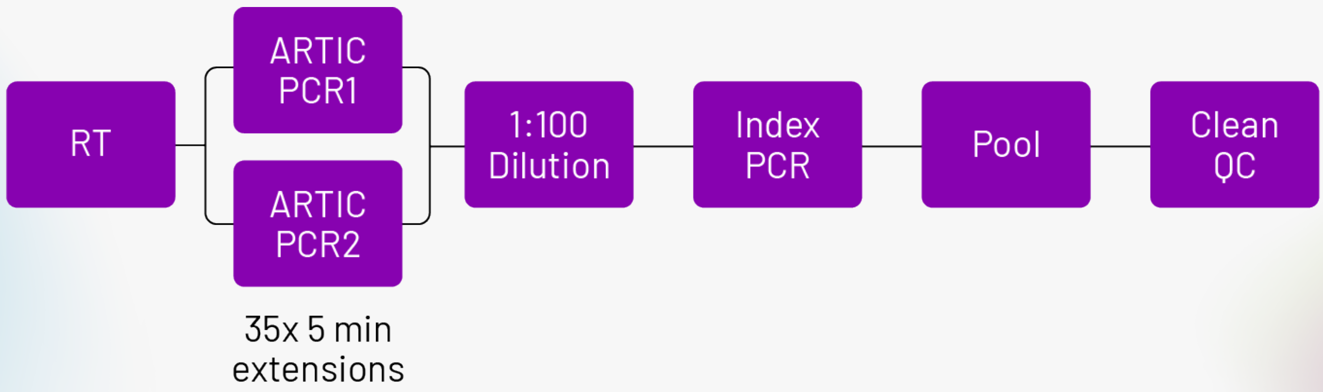

Figure 1. Tailed ARTIC workflow developed by Daryl Gohl at the University of Minnesota

Introducing dynamic amplification with icon96™ + AutoNorm™

The CAT team evaluated the ability of the icon96 instrument to regain control over amplification. Instead of running a preset number of cycles, the icon96 system leverages AutoNorm technology to monitor every well in real time and stop each reaction automatically when it reaches a user-defined endpoint. In practice, this means: Low-input, high-input, and degraded samples all reach optimal amplification—without manual intervention.

> "Each individual well is its own thermocycler... you can monitor and control each well independently."
>
> — Eric Chow

How icon96 works:

- Real-time fluorescence tracking in every well
- Independent thermal control per sample
- Automated stop cycle based on:
  - Target RFU
  - Amplification slope
  - Fold-change over baseline
- Built-in pooling calculations

Rebuilding the ARTIC workflow

The CAT team first replaced fixed-cycle indexing PCR with AutoNorm-controlled amplification. They then expanded the approach to both multiplex and indexing steps. The result: No manual cycle optimization. No individual quantification. No guesswork.

ARTIC workflow with icon96:

1. Multiplex PCR (AutoNorm-controlled)
2. Indexing PCR (AutoNorm-controlled)
3. Single cleanup
4. Automated pooling
5. Sequencing

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Case Study

The Results: Cleaner libraries, faster turnaround

1. Improved sequencing quality

Under the fixed cycle ARTIC workflow, artifacts that survived cleanup appeared during sequencing as dark bases and reduced usable reads. Using AutoNorm for both PCR steps produced the cleanest profiles, with minimal shoulders and artifacts, revealing a direct link between dynamic endpoint control and cleaner sequencing output.

Libraries prepared with AutoNorm showed:

- Dramatic reduction in primer dimers
- Fewer off-target products
- Higher proportion of desired amplicons
- Elimination of late-cycle G-base spikes

> "We're not seeing those primer dimers show up and we're getting a lot more usable reads."
>
> — Eric Chow

2. Faster library preparation

Beyond cleaner libraries, AutoNorm significantly accelerated the overall workflow. By delivering more consistent amplification across samples, the equal-volume pooling step the ARTIC protocol already relies on finally lived up to its promise — producing more even sequencing coverage without added quantification.

Workflow gains included:

- Reduction from 35 cycles to ~17–22 cycles
- ~1 hour saved in PCR run time
- ~1.5 hours saved per library prep
- Same-day library prep and sequencing
- Reduced risk of handling errors

> "We could cut about 13 cycles... saving about an hour and a half in our entire workflow."
>
> — Eric Chow

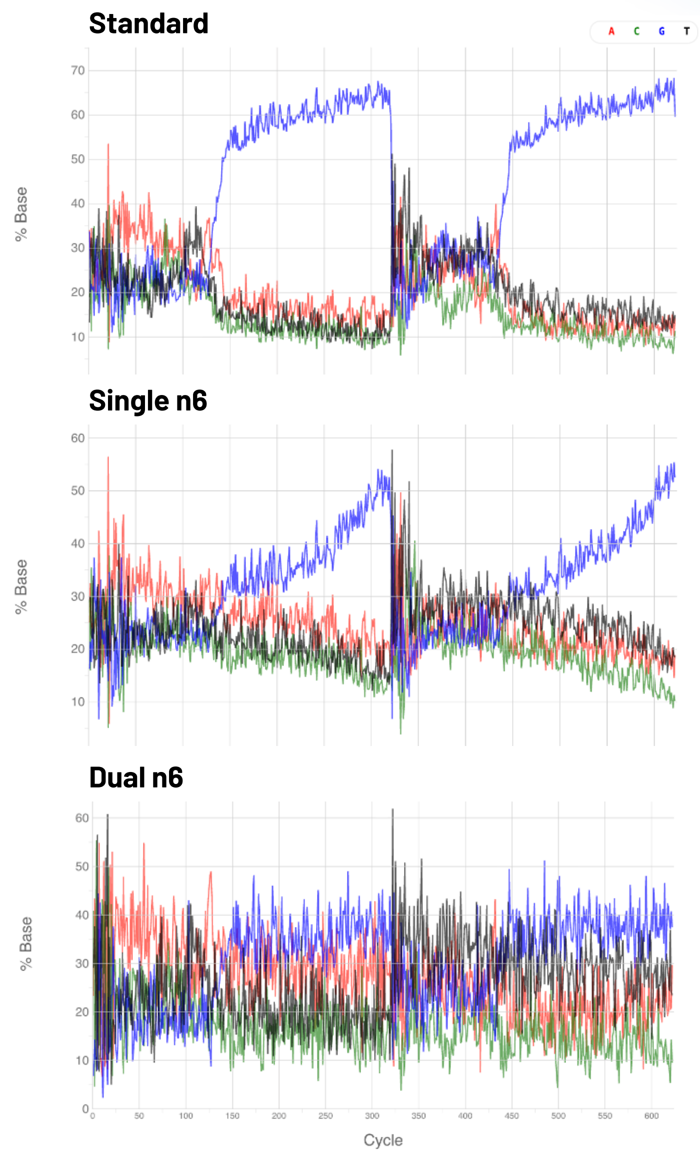

Figure 2. End of read G basecalls confirm virtual elimination of "dark base" (G base) signals. Top figure = standard ARTIC workflow with fixed cycle PCR. Middle = AutoNorm for 1 reaction. Bottom = AutoNorm for both

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3. Reliable data across variable inputs

To test whether variable-cycle amplification affected comparability, the team ran a controlled RNA-seq study using K562 cells and liver tissue. Each sample was split into low-, medium-, and high-input aliquots and amplified using AutoNorm. The results confirm that dynamic amplification preserves quantitative relationships, even across 16-fold input differences.

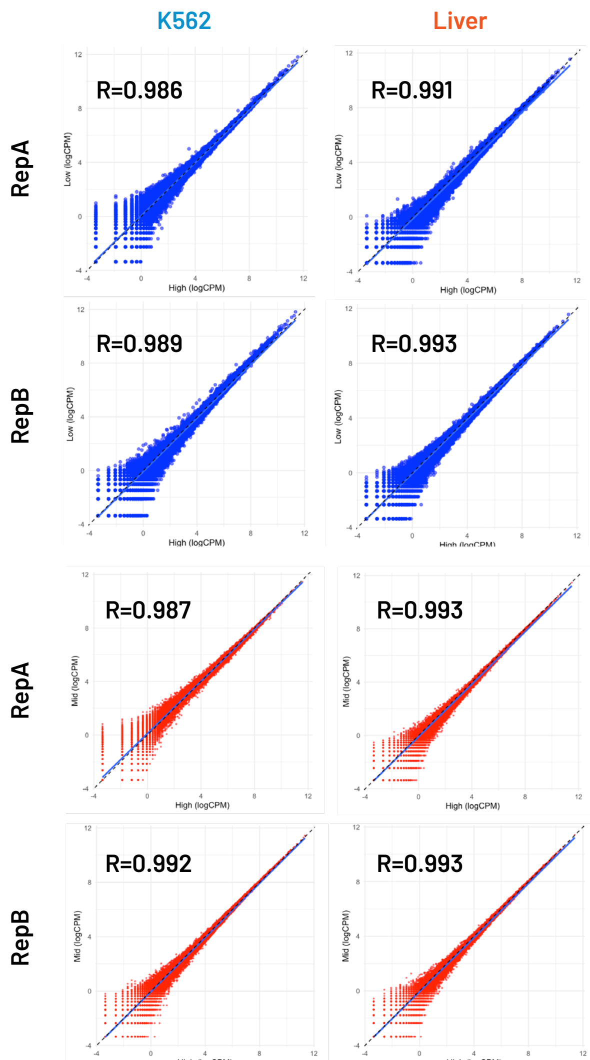

Figure 3. Correlation between dilutions confirms that variable cycle number does not impact comparability.

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> "The replicates are incredibly tight...with an R^2 generally around 0.99 or higher."
>
> — Eric Chow

Data consistency outcomes included:

- 13+ cycles saved using AutoNorm
- Tight PCA clustering by biological origin
- R^2 values >/= 0.99 across dilutions
- Consistent expression profiles
- No detectable bias from variable cycle amplification

Beyond ARTIC: A platform for variable biology

Given the success of the ARTIC trial and comparability study, the UCSF team next plans to expand icon96 use into additional applications, particularly those with low or variable input. Dr. Chow noted that the icon system also supports process and assay development work, including temperature optimization and future melt curve-based QC.

Future applications:

- RNA-seq
- 10x Visium spatial transcriptomics
- FFPE libraries
- ChIP-seq
- 16S sequencing
- Metagenomics

Learn more about **icon96** for viral and RNA sequencing

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