Case Study

HudsonAlpha Institute reduces RNA-seq costs by 30% in fruit maturation study

The challenge: hidden quality in every apple

In the quest for RNA biomarkers for fruit maturity, a robust and cost-effective workflow is key. Accurate harvest timing is critical for apple quality and shelf life—but growers currently rely on their eye and a simple starch test. The result: ~30% of harvested fruit is discarded in storage due to quality issues.

Molecular biomarkers could change the game. But identifying them means sequencing hundreds of apple samples. RNA extraction is historically messy in plants, and since we're talking about farming, not pharma, costs have got to be low.

Meet the Team

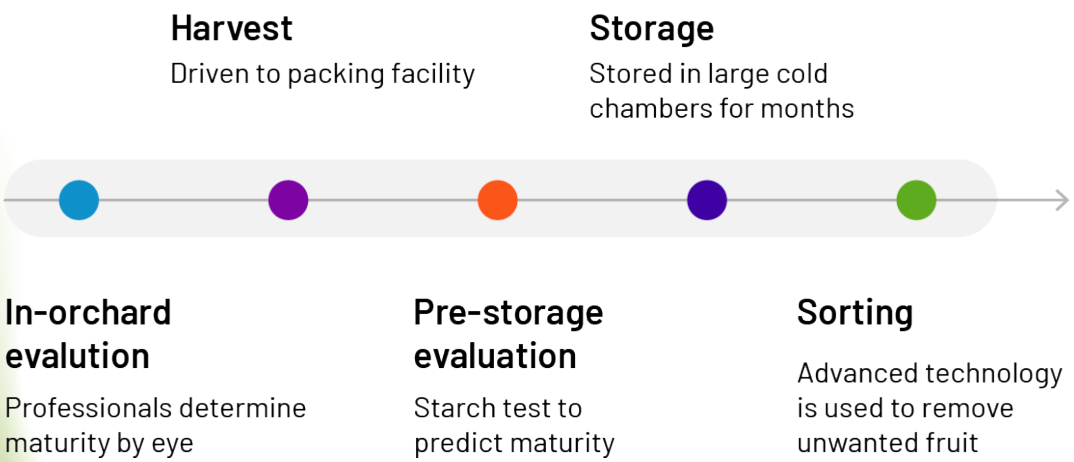
Dr. Alex Harkess
Faculty Investigator, HudsonAlpha
Research focus: Plant reproduction, evolutionary genomics

Dr. Nicole Szeluga
Postdoc, Harkess Lab, HudsonAlpha
Project lead, industry partnerships

Dr. Loren Honaas
Research Molecular Biologist, USDA ARS
Field expertise, sample collection

> "I'm at a genomics institute with genome sequencers in my own lab, and still a major bottleneck for every apple biomarker project is data generation."
>
> **— Dr. Alex Harkess, HudsonAlpha Institute for Biotechnology**

Commercial Apple Supply Chain



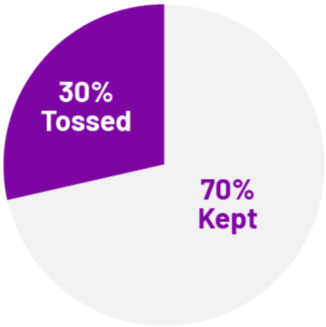
In-orchard evaluation
Professionals determine maturity by eye

Harvest
Driven to packing facility

Pre-storage evaluation
Starch test to predict maturity

Storage
Stored in large cold chambers for months

Sorting
Advanced technology is used to remove unwanted fruit



RNA normalization: the hidden labor drain

Before the HudsonAlpha team can sequence the fruit-extracted RNA samples to search for biomarkers, they have to go through several processing steps to ensure they are getting the most out of each sequencing run. Each step costs them time, money (in reagents and people hours), and increases the risk of errors that could impact data quality.

1. Isolate RNA from hundreds of apple samples
2. Quantify each sample (concentration varies 5-fold or more)
3. Manually normalize to equal concentrations
4. Prepare sequencing libraries with consistent amplification
5. Normalize library pools before sequencing

Conventional

Isolate RNA
↓
Quantify
↓
Normalize
↓
Amplify
↓
Normalize Again
↓
Sequence

iconPCR

Isolate RNA
↓
Amplify
↓
Sequence

> "We have RNA inputs ranging from 0.3 to 1.5 micrograms—a fivefold difference. Normally, we'd want these identical before sequencing. This is labor-intensive and often insufficient."
>
> **— Dr. Alex Harkess, HudsonAlpha Institute for Biotechnology**

Introducing intelligent amplification with icon96™ and AutoNorm™

icon96 with AutoNorm technology changes everything. Rather than setting a fixed PCR cycle count in advance, the system monitors each well in real-time. The result? All libraries reach identical amplification targets, regardless of starting RNA quantity.

How icon96 works:

- Real-time monitoring tracks amplification curves for each sample
- User-defined fluorescence threshold triggers stop-cycle automatically
- Input can be chaotic—output is perfect

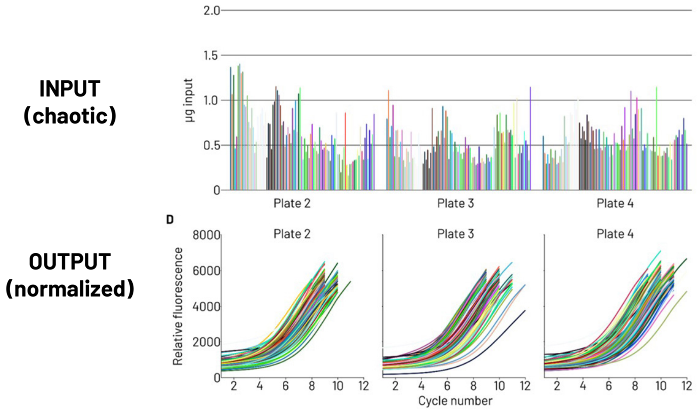


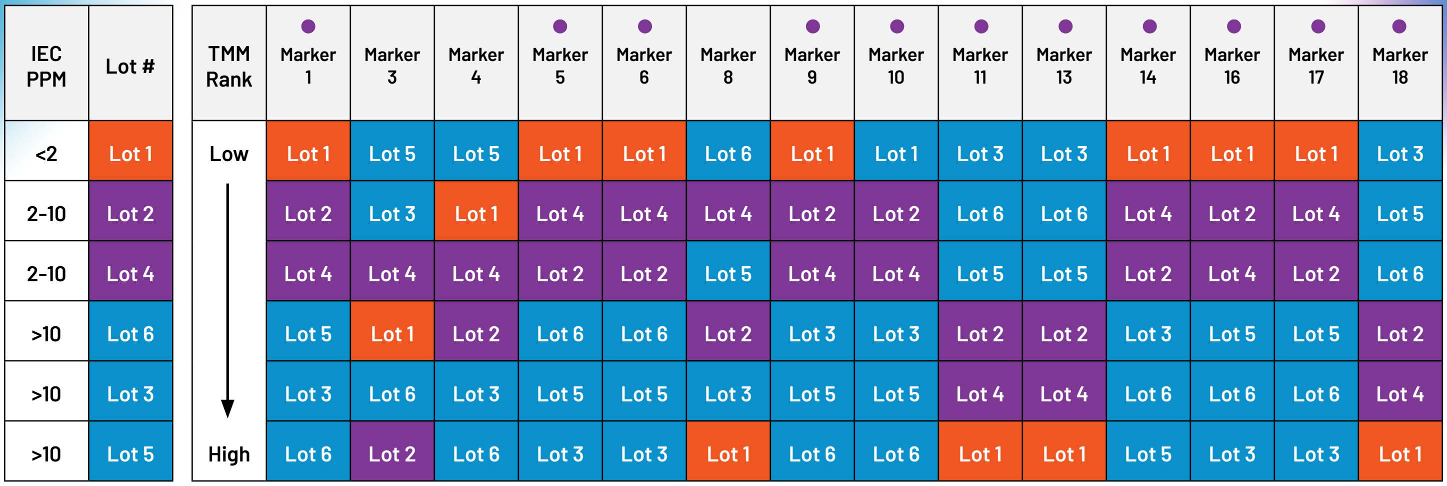
Figure 1. Plant-derived RNA samples ranging from 0.5-1.5 micrograms (INPUT) were amplified by icon96. The stop cycle for each sample was dynamically selected using AutoNorm based on the slope of the amplification curves (OUTPUT).

The outcome: A practical solution for RNA-based biomarker testing

In a pilot test, the HudsonAlpha team sequenced 384 apple peel samples following library preparation on icon96. They identified 14 biomarkers for fruit maturation that correlated strongly with internal ethylene concentration (IEC) - the industry standard for maturity assessment. 11 of the 14 biomarkers consistently predicted harvest timing, proving that they work in real world conditions. And, speaking of the real world, they calculated that their sequencing costs were reduced by 30%, meaning that icon96 is both an impactful and a practical solution.

Key Metrics

- ✓ 384 apple peel samples processed
- ✓ 5-fold variation in input RNA controlled
- ✓ ~30% reduction in RNA-seq costs
- ✓ Strong correlation between RNA-seq biomarkers and fruit maturity metrics
- ✓ 11 of 14 biomarkers consistently predict harvest timing



IEC PPM	Lot #	TMM Rank	● Marker 1	Marker 3	Marker 4	● Marker 5	● Marker 6	Marker 8	● Marker 9	● Marker 10	● Marker 11	● Marker 13	● Marker 14	● Marker 16	● Marker 17	● Marker 18
<2	Lot 1	Low	Lot 1	Lot 5	Lot 5	Lot 1	Lot 1	Lot 6	Lot 1	Lot 1	Lot 3	Lot 3	Lot 1	Lot 1	Lot 1	Lot 3
2-10	Lot 2	↓	Lot 2	Lot 3	Lot 1	Lot 4	Lot 4	Lot 4	Lot 2	Lot 2	Lot 6	Lot 6	Lot 4	Lot 2	Lot 4	Lot 5
2-10	Lot 4		Lot 4	Lot 4	Lot 4	Lot 2	Lot 2	Lot 5	Lot 4	Lot 4	Lot 5	Lot 5	Lot 2	Lot 4	Lot 2	Lot 6
>10	Lot 6		Lot 5	Lot 1	Lot 2	Lot 6	Lot 6	Lot 2	Lot 3	Lot 3	Lot 2	Lot 2	Lot 3	Lot 5	Lot 5	Lot 2
>10	Lot 3		Lot 3	Lot 6	Lot 3	Lot 5	Lot 5	Lot 3	Lot 5	Lot 5	Lot 4	Lot 4	Lot 6	Lot 6	Lot 6	Lot 4
>10	Lot 5	High	Lot 6	Lot 2	Lot 6	Lot 3	Lot 3	Lot 1	Lot 6	Lot 6	Lot 1	Lot 1	Lot 5	Lot 3	Lot 3	Lot 1

Figure 2. Ranking six lots by relative expression of maturity biomarkers measured by RNA-seq. 11 of 14 biomarkers (●) consistently correlate with ethylene concentration.

From lab bench to grower's toolkit

This study establishes a blueprint for scalable biomarker discovery in agriculture. The USDA and HudsonAlpha team demonstrated that by combining icon96 with automated normalization, it's feasible to use transcriptional biomarkers in the field to help growers make data-driven harvest decisions, reducing waste and improving fruit quality across the supply chain.

Key Takeaways

- ✓ Reproducible NGS data generation is achievable at scale
- ✓ Molecular biomarkers can reliably predict fruit maturity
- ✓ Workflow efficiency directly impacts research timelines AND costs
- ✓ The approach extends beyond apples—any variable RNA input benefits from this solution

Case Study

icon96 + AutoNorm: transforming RNA workflows

The apple biomarker project showcases icon96's power in RNA-seq workflows beyond agriculture. Whether preparing libraries from FFPE tissues, single-cell suspensions, cell-free DNA (cfDNA), or challenging plant samples, AutoNorm eliminates the manual guesswork of cycle optimization. The result: cleaner data, faster decisions, and scalable workflows.

> "The ultimate result to you is that we've achieved about a 30% reduction in the cost of generating this RNA-seq data for apple and pear biomarker projects."
>
> **— Dr. Alex Harkess, HudsonAlpha Institute for Biotechnology**

Learn more about icon96 applications for RNA-Seq