

We've rebranded some of our products. *PhenoCycler®* is now *PhenoCycler™*.

FAQs for Nolan Colorectal Cancer Study

Aug 11, 2020

FOR INTERNAL USE ONLY. NOT FOR DISTRIBUTION

1. What is the elevator pitch for this study?

- The study is a conceptual framework for how the commercial PhenoCycler system could be applied to the study of tumor FFPE samples and TMA cores with a highly multiplexed panel.
- The study also shows how high levels of multiplexing can uncover new insights in the tumor microenvironment and can help explain how spatial relationships of tumor and immune cells can correlate to prognosis and treatment response - an ideal case study for oncology prospects.
- **CELLULAR NEIGHBORHOODS:** In simple terms, the authors draw parallels between the neighborhoods in a city and cellular neighborhoods in the tumor microenvironment.
 - A city is organized into neighborhoods with discrete functions (e.g., industrial, financial, residential, and so on) and each individual contributes to that neighborhood's output (e.g., construction worker, banker, teacher). Similarly, the tumor microenvironment is also organized into cellular neighborhoods that are composed of individually contributing cell types
 - **This study places an emphasis on analyzing tissue biology at two different levels - the individual interacting cell types and their spatial context as well as the tissue regions within which they are organized.** A detailed study of both levels of tissue architecture and behavior is now possible with a high dimensional imaging platform such as the PhenoCycler System.

2. How many TMA cores can a customer run on the PhenoCycler platform?

Contact the PhenoCycler support team for this information.

3. The study cites a 56-marker panel with 2 nuclear stains. Can a PhenoCycler customer currently run a 56-marker panel on our commercial platform?

20 of the 56 markers overlap with Akoya's inventoried antibodies and 11 are an exact clone match. At this point a PhenoCycler customer can run a panel of 40 markers using a mix of inventoried PhenoCycler antibodies and third-party antibodies conjugated to PhenoCycler barcodes. We will be releasing more barcodes in coming months however the additions planned over the next 6 months will not increase the panel size beyond 44 markers.

4. What if a customer wants to use the published barcode sequences in the Supplemental table and order them directly from IDT for custom conjugation?

Customers can theoretically use the barcode sequences and have them manufactured on their own. However, Akoya does not recommend that users manufacture PhenoCycler barcodes on their own for the following reasons:

- **Barcode optimization isn't trivial:** Our commercial PhenoCycler barcodes have gone through proprietary chemical modifications and a manufacturing screening process to ensure reproducibility across different tissue types and formats (Human FF tonsil, Human FFPE, Mouse FF). If customers choose to make their own barcodes or combine barcodes with ours, we cannot guarantee that they will not interfere with each other, causing multiple antibodies to appear as one.
- **Are you really saving \$\$\$?:** Customer-derived oligos are typically expensive to synthesize to the length and purity level needed for PhenoCycler applications, so it's unlikely customers could be saving money to manufacture and screen barcodes at the throughput needed by most labs.
- **Akoya Support:** The Akoya Barcodes are also fully supported by our Field Service and Support team. If a user decides to use barcodes directly from IDT then we wouldn't be able to troubleshoot their experiments.
- **Focus on the biology:** Researchers can focus on the biology and publish new findings with PhenoCycler technology instead of spending additional time on optimizing barcodes from an academic lab. It's not a trivial process.

5. Are those barcode sequences the same as our commercially available PhenoCycler barcodes?

Our barcodes have evolved since their initial versions created in the Nolan lab. We've optimized them during the commercial development process for different tissue types and formats. As mentioned, users are free to experiment with the Nolan lab barcodes but we wouldn't be able to troubleshoot their experiments. We also would not be able to guarantee that commercially available PhenoCycler Barcodes and inventoried PhenoCycler antibodies would be compatible with antibodies conjugated with Nolan lab barcodes. Additionally, Akoya's conjugation process differs from the Nolan lab, where we have made improvements for stability and yield.