Recommendations for improving the OncoScan workflow for optimal results

Introduction

The ability to accurately detect copy number (CN) changes such as copy number variations (CNVs) is critical to fully profile solid tumors. Approximately 80% of all cancers are affected by both somatic mutations and CN changes [1]. Genome-wide CN changes can also be used to detect subclones and clonal evolution. The number and complexity of CN aberrations is an indicator of disease progression in many cancers.

Somatic cancer mutational signatures have been extensively studied and categorized. The most recent large-scale analysis, the ICGC/TCGA Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium, used data from more than 23,000 cancer patients to provide a systematic perspective on the repertoire of somatic mutational processes that contribute to the development of human cancer [2].

Understanding the impact of genome-wide alterations in CN on cancer is comparatively in its infancy. In contrast to base-scale mutational signatures, no CN signature is associated with known cancer risk factors [3]. As the field matures, it will become increasingly clear which models are better suited to addressing specific biological questions. To resolve these questions, pan-cancer analyses utilizing all methods will be key, and we present here the first step towards that goal: a mechanism-agnostic pan-cancer compendium of allele-specific CN signatures [3].

CN signatures are very useful for understanding treatment regimen. Ovarian and brain cancers are beginning to reveal actionable signatures, such as homologous recombination deficiency (HRD), indicative of PARP inhibitor treatment response, and chromothripsis for brain cancer prognosis [4,5].

The severity of genomic instability, measured by the number of CN segments, proportion of the genome displaying loss of heterozygosity (LOH), and genome-doubling status vary greatly among cancer types [3]. Understanding complex biomarker signatures that drive genomic instability can require costly genomic tools to supplement next-generation sequencing (NGS). Accurate CN profiling has the potential to lead to more accurate disease diagnosis, prognosis, and novel therapeutic intervention [6].

However, obtaining genome-wide CN and LOH profiles from solid tumor samples is a significant challenge due to the difficulty of working with limited amounts of DNA derived from formalin-fixed, paraffin embedded (FFPE) samples. FFPE treatment modifies DNA into short fragments and can alkylate the bases leading to mispairings and deletions.

Applied Biosystems™ OncoScan™ assays utilize a molecular inversion probe (MIP)–based technology that was originally developed for SNP genotyping, but has subsequently been used to identify other types of genetic variation, including focal insertions and deletions, larger CN alterations, LOH, and most recently, somatic mutations. Compared to other target capture methods, MIP-based assays do not require shotgun library preparation and can be run on relatively low DNA input (<100 ng in some settings). MIP-based assays can achieve high overall specificity due to the targeted probe design and can be performed by both array-based and in-solution methods [7].

Specifically, OncoScan CNV assays provide:

- **Whole-genome CN analysis**—detect structural variants such as deletions, duplications, and unbalanced translocations that are not well characterized by short-read sequencing or targeted sequencing
- **Comprehensive coverage**—whole-genome analysis of genes with established significance and those with emerging evidence, thus helping to reduce future reverification burden
- **An all-in-one assay**—help reduce costs and processing times by detecting chromosomal arm aberrations, focal changes, LOH, and copy-neutral LOH (cnLOH) in a single assay
- **Robust performance**—detect subclones and assess clonal evolution and genetic variations that are known to have important implications in cancer
- **Low sample input requirement and fast results**—go from sample to answer, including data analysis, in just 3 days, using only 80 ng of FFPE-derived DNA

The success of OncoScan assays have led to OncoScan assays being featured in over 300 publications.

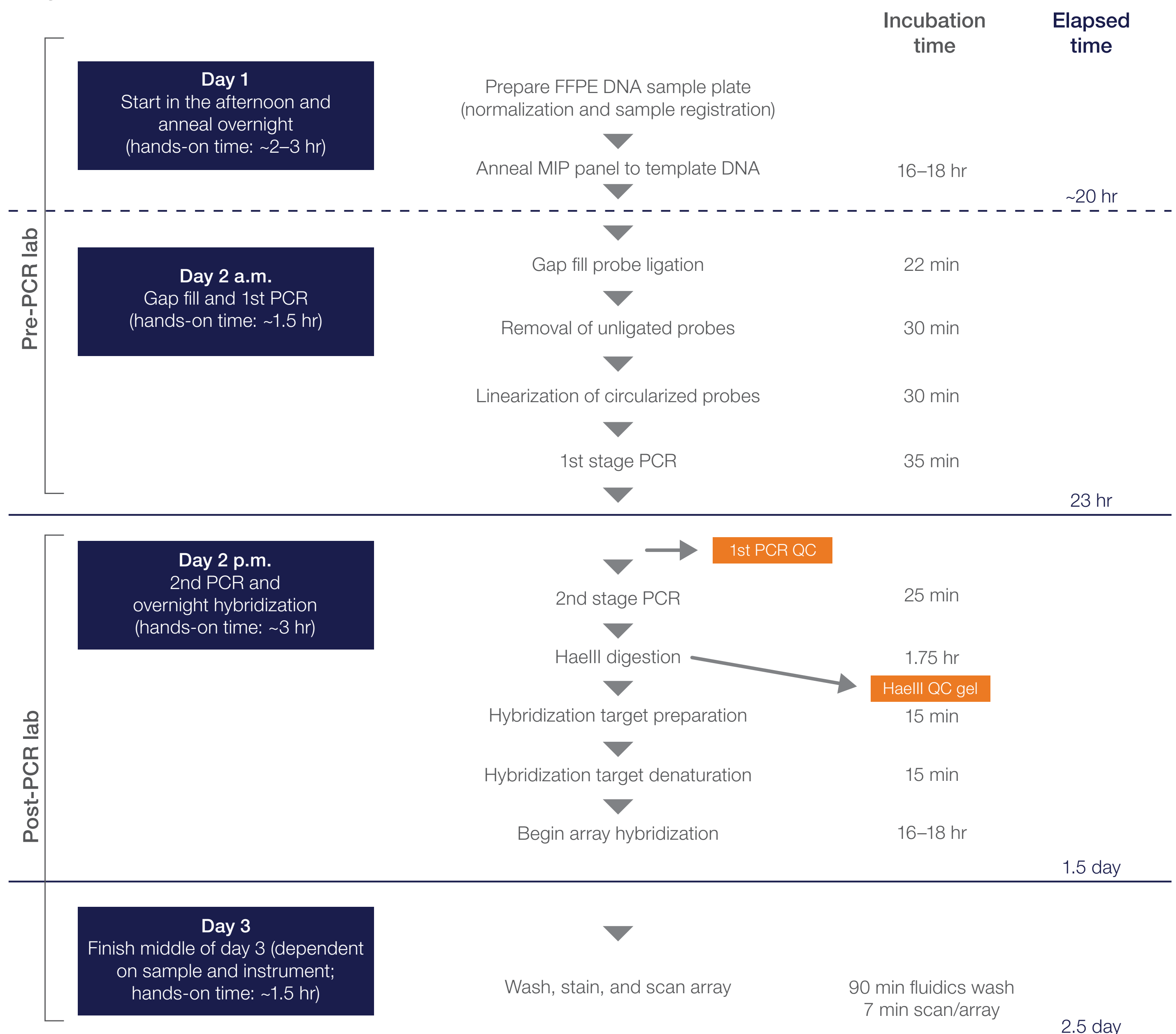

Figure 1. Detailed workflow of the OncoScan assay.

Recommendations to improve the OncoScan workflow

OncoScan assays are powerful tools that provide whole-genome CN analysis, even from samples that are notoriously difficult to work with, including archived FFPE samples. However, the workflow can be complex and lead to inconsistent results (Figure 1). We provide six recommendations that can be easily adapted to your workflow for optimal data collection (Figure 2).

Tip 1: Be alert at package arrival.
Proper and timely handling of reagents and storage is essential for robust performance.

- Check reagent temperature and packaging conditions—make sure your products did not arrive compromised, especially when in cold-chain boxes
- Store all reagents at the recommended temperatures and conditions
- Do not use reagents that have been improperly stored, as storage methods can profoundly impact activity

Tip 2: Instrument maintenance is crucial for success.
Instrumentation plays an important role in the successful execution of OncoScan assays. Therefore, all equipment must be well maintained and routinely calibrated. We strongly recommend bleaching fluidics weekly or after every 4 runs (whichever occurs first), to avoid reduced signal on OncoScan arrays.

Tip 3: Keep your reagents on ice at all times.
Throughout the assay, it is imperative that you keep all reagents on ice. Make sure the reagents are added to chilled PCR plates (placed on the cold block). Whenever a chilling step is called for, chill the plate on the cold block for at least 1 min, and then spin down at 2,400 rpm for 30 sec before adding the reagents.

- Pre-chill reagents that have been thawed on ice
- Add all additional reagents while tubes are on ice
- For pre-PCR steps, chill plates on the cold block for >10 min
- For post-PCR steps, chill plates on the cold block for >30 min

Tip 4: Avoid cross-contamination by identifying the sources and locations where it may happen.
Following the recommendations in the user manual can help reduce the risk of sample and dNTP contamination:

- Separate the lab into pre-PCR and post-PCR areas
- Have a separate lab space for the dNTP-mix preparation step
- Change gloves between the AT and GC mixing steps
- Make sure to count the number of freeze/thaw cycles for key enzymatic steps
- Do not downsize sample size (kits are designated for 3 sets of 8 samples, not 4 sets of 6 samples)

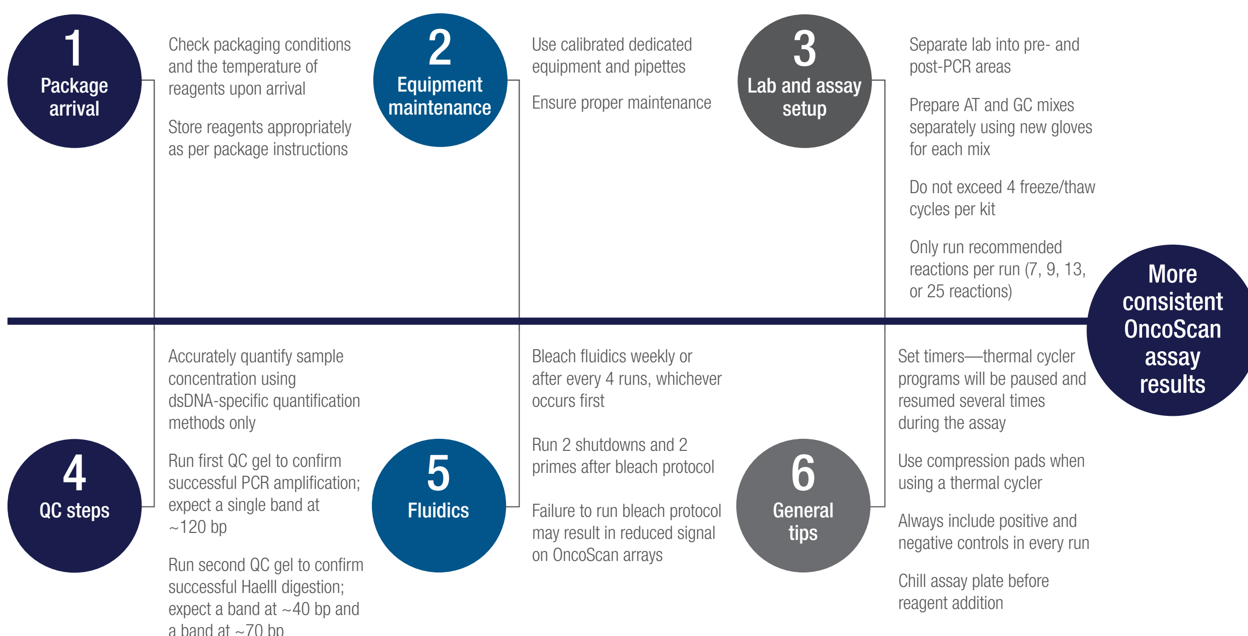

Figure 2. Recommendations to improve the OncoScan workflow for more consistent results. Refer to the OncoScan user manual for detailed guidelines and contact your local field application specialist for in-depth training.

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Tip 5: Perform all in-process QC steps.

We strongly recommend performing all 3 in-process QC steps for a successful assay. All genomic DNA samples should be normalized to 12 ng/µL using low-EDTA TE (1X) buffer, along with running 2 QC gels during the assay:

- Quantify dsDNA concentration
- First QC gel—run 1st stage PCR product on gel to identify samples that did not amplify (distinct band at 120 bp in all samples except negative control)
- Second QC gel—run gel after HaeIII digestion to confirm the expected pattern of a band at ~40 bp and at ~70 bp

Tip 6: General tips.

- Set timers—during the pre-PCR portion of the assay, the thermal cycler program will be paused and resumed multiple times; set timers to pause the thermal cycler before the program proceeds to the next step
- Use compression pads with the Applied Biosystems™ Veriti™ Thermal Cycler and GeneAmp™ PCR System 9700 throughout the assay in both labs
- Include positive controls in every run, especially when working with FFPE samples; poor sample quality can cause samples to fail

Conclusions

OncoScan assays are powerful tools that provide whole-genome CN analysis. OncoScan assays utilize molecular inversion probe (MIP)–technology, proven for identifying CN alternations, LOH, cnLOH, and somatic mutations. The data obtained using OncoScan assays have had a strong impact in oncology; however, the complex workflow requires careful preparation. Several recommendations are provided to mitigate the challenging workflow, to provide consistent and accurate results from OncoScan assays.

Ordering information

Description	Quantity	Cat. No.
Product		
OncoScan CNV Assay	24 reactions	902695
OncoScan CNV Plus Assay Training Kit	18 reactions	902305
OncoScan product training	3 days	000.878
Services		
OncoScan FFPE Services		000.879
OncoScan CNV FFPE Services		000.901

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