WHITE PAPER

# Overcoming hurdles in high-throughput profiling of cancer cell responses

## Introduction

High-throughput methodologies, such as functional genomic screening and cell panel screening, play pivotal roles in deciphering the underlying genetic and molecular pathways involved in cancer etiology and drug resistance. Functional genomic screens are primarily used for interrogating gene function, identifying genetic dependencies, and studying pathways implicated in cellular processes. Numerous techniques are available for modulating genes including systematic gene knockdown, knockout, or over-expression. In contrast, cell panel screening can be used to assess treatment responses across panels of genomically diverse cell lines. Both functional genomic and cell panel screens can be used to gain evidence of drug resistance and sensitivity, aid the selection of efficacious drugs for treating specific cancers, stratify patients for clinical trials, and provide data to inform mechanism of action studies.

Researchers can leverage functional genomic screening and cell panel screening to gain a more comprehensive understanding of biological processes and disease mechanisms and to support drug development efforts. Using examples of research findings, this article explores how scientists are leveraging the strengths of each method to identify potential drug candidates, understand disease mechanisms, and tailor treatments to individual patients in the era of personalized medicine.

## Determining therapeutic targets

Functional genomic screening can be used to identify potential therapeutic targets through the assessment of cellular phenotypes arising from genome-wide perturbations. Screens can be performed in high throughput, with libraries

of small interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA, or CRISPR-Cas9 single guide RNA (sgRNA).

In an investigation conducted by Lawo et al., the team employed a pooled-based CRISPR screening protocol to identify resistance factors against the BRAF kinase inhibitor, vemurafenib.[1] They used lentivirus transduction to deliver both the Cas9 endonuclease and the sgRNA to A375 melanoma cells, which have a BRAF V600E gain-of-function mutation. Following 14 days of treatment with vemurafenib, cells were harvested, genomic DNA extracted, and sgRNA frequency determined using next-generation sequencing (NGS).

Analysis revealed that treatment with vemurafenib caused a general decrease in the representation of sgRNAs in agreement with expectations of a cell population incubated in the presence of an anti-proliferative selection pressure. In a subpopulation of cells, there was an increase in sgRNA counts consistent with a population of cells developing resistance. When the team used a dedicated CRISPR-Cas9

screen analysis platform to perform individual sgRNA analysis and gene level hit ranking, they found that the highest-ranking genes (MED12, NF1, CUL3, NF2, TADA2B, and TADA1) were those whose loss is known to confer resistance to vemurafenib. Additionally, the researchers identified genes related to histone acetyltransferase and mediator complexes as potential resistance factors.

This study underscores the effectiveness of a whole-genome pooled CRISPR-Cas9 screening approach in pinpointing both known and novel drug resistance factors. It also showcases how complete loss of gene function enables researchers to conduct a robust exploration of phenotypes for the discovery of innovative targets in therapeutic research.

## Identifying specific indications and patient cohorts for treatment success

The highly heterogeneous nature of cancer means that individuals with the same type of cancer can respond differently to treatments. Identifying which specific indications and patient populations are most likely to benefit from a particular drug candidate or combination of treatments is therefore essential to maximize the chance of success.

High-throughput cell panel screens are powerful tools for systematically evaluating potential treatments across diverse cell lines. They can be used to identify specific indications and patient cohorts where treatments are most likely to succeed. An example of their utility is the work conducted by Sorrell et al. who employed Revvity's 2D OncoSignature™ panel – a genomically diverse set of 300 clinically relevant cancer cell lines – to identify genomic and lineage-specific predictors of sensitivity to BRAF inhibitors.[2] By treating the panel with the BRAF inhibitors dabrafenib and vemurafenib, the group determined that the two most significant predictors of sensitivity were mutations in BRAF and DAPK1. When they analyzed lineage-specific treatment responses they found that skin and colon cancers – two cancer types where BRAF mutations are prevalent – were the most responsive.

A comparison of treatment response in cell lines harboring the V600E BRAF mutation and those containing wild-type BRAF revealed an increased sensitivity of mutant BRAF-containing cell lines to BRAF inhibition. Lastly, the team confirmed that the current standard of care for patients with BRAF-mutated melanoma (a combination of BRAF and MEK inhibitors) delayed the appearance of resistance.

Together, the study demonstrates the utility of the OncoSignature panel to discern critical predictors of sensitivity to BRAF inhibitors in diverse cancer cell lines. These findings emphasize the significance of tailoring treatments to the right patient populations, ultimately advancing the prospects of successful cancer therapeutics.

## Understanding mechanisms of drug resistance and sensitivity

The development of drug resistance is one of the biggest limiting factors in achieving success in cancer treatment. Understanding which genes and pathways influence resistance to certain treatments is of paramount importance for determining the most appropriate drug or treatment regimen for a specific patient or condition. Le Sage and colleagues showcased the value of combining three distinct CRISPR-Cas9-based genome-wide screening techniques – CRISPR knockout (CRISPRko), CRISPR interference (CRISPRi), and CRISPR activation (CRISPRa) – to explore genetic factors influencing resistance and sensitivity to the BRAF inhibitor, vemurafenib, in two independent studies.[3,4]

Their investigations began with a proof-of-concept screen, leveraging both CRISPRa and CRISPRi, to evaluate which genetic modulations conferred resistance to vemurafenib in A375 melanoma cells. By comparing the outcomes of the gain- and loss-of-function screening datasets, Le Sage and colleagues identified genes that displaying opposing effects when activated or inhibited in the presence of a drug.

The CRISPRi loss-of-function screen yielded a series of validated and novel vemurafenib resistance hits, while the CRISPRa screen revealed that vemurafenib resistance was associated with the activation of genes involved in receptor tyrosine kinase, G-protein coupled receptor, and integrin signaling pathways. When the team combined and compared the CRISPRi and CRISPRa datasets, they were able to validate drug resistance targets and identify pathway modulators that influenced both drug sensitivity and resistance. For instance, MYC was shown to sensitize cells to vemurafenib in the CRISPRa screen, but conferred resistance in the CRISPRi screen. The dual screening also led to the direct identification of the molecular target of vemurafenib, BRAF. Interestingly, the BRAF gene displayed resistance to drug treatment when overexpressed in the CRISPRa screen, but also when knocked down in the CRISPRi screen. These findings showcase how the parallel analysis of CRISPRi and CRISPRa datasets enables the identification of pathway modulators that affect both drug sensitivity and resistance, even when sgRNAs are not reciprocally lost or increased in each screen.

Overall, Le Sage and colleagues demonstrated the value of a dual CRISPRi and CRISPRa screening approach for identifying genetic factors that influence resistance to vemurafenib. By combining loss- and gain-of-function datasets, they were able to enhance their understanding of the complex gene networks controlling drug response, while also gaining confidence in their target identification and validation analyses.

## Investigating drug combinations for improved outcomes

While pooled CRISPRko screening is a valuable tool for target identification, it can be more challenging to use this approach to distinguish between gene deletions that cause cell death or those that cause cell cycle arrest in the presence of a given drug. This is because both lead to a loss of cells from the expanding cell population. Determining whether cell death has occurred is of great importance when investigating potential drug combinations and reducing the development of drug resistance.

Walter and colleagues used an adapted pooled CRISPR screening protocol to identify potential therapeutic targets for combination therapy in the treatment of triple-negative breast cancer (TNBC) with PI3K inhibitors.[5] These inhibitors are known to induce proliferative arrest in cancer cells rather than cell death, leading to the development of drug resistance. Therefore, the researchers aimed to find genes that, when targeted, can induce cell death in the presence of PI3K inhibitors, thereby improving treatment outcomes and reducing drug resistance.

To achieve this, the researchers conducted a positive selection CRISPR cell death screen in TNBC MDA-MD-231 cells. Cells were infected with Revvity's optimized CRISPR lentiviral library, selected, expanded for 10 days, and then treated with the pan PI3K inhibitor GDC-0941 for 24 hours. Following treatment, the cells were separated into Annexin V positive and Annexin V negative populations, an approach that is used to determine between healthy and apoptotic cells, respectively. These two populations were then analyzed for sgRNA abundance using NGS.

The cell death screen enabled the identification of genes whose loss synergizes with PI3K inhibition in the TNBC cell line and those required for cell death induction in the presence of these inhibitors. For example, the epidermal growth factor receptor (EGFR) was identified as one of the top enriched genes in the Annexin V positive population.

EGFR inhibition is known to work synergistically with PI3K inhibitors and is currently being investigated in clinical trials. These findings therefore have implications for the development of new drug combinations and a better understanding of the mechanisms behind drug resistance to single-agent PI3K inhibitors.

High-throughput cell panel screens are also useful tools for screening single agents or drug combinations. While these screens have traditionally been conducted in 2D monolayer formats, researchers are increasingly adopting 3D approaches to complement and validate 2D findings. Scales et al. demonstrated the utility of Revvity's 3D OncoSignature arrayed cell panel screening platform, which is an adaption of the existing 2D OncoSignature platform, for compound screening across a panel of 200 cell lines in a spheroid format.[6] They compared the results of the 3D screen to 2D assay readouts to identify any differences in outcomes.

Analysis revealed enhanced sensitivities to single agent MEK and EGFR inhibition or combined suppression of the MEK and PI3K pathways in the 3D environment compared with the 2D assay. The authors also demonstrated that their platform was amenable to screening patient-derived organoids, which display more cellular heterogeneity than spheroids. This demonstrates the power of 3D cell panel screening in organoids for validating 2D and spheroid-based 3D screens and showcases the evolving landscape of cell panel screening.

## Conclusion

In the fast-paced drug discovery industry, gathering rapid information to identify promising molecular targets and advance assets is critical for securing funding and advancing toward the clinic. This article has explored how high-throughput methodologies like functional genomic screening and cell panel screening offer valuable insights into gene function, genetic dependencies, and cancer-related cellular pathways. These techniques empower researchers to identify novel therapeutic targets, tailor treatments to specific patient populations, unravel drug resistance mechanisms, and explore promising drug combinations.

Revvity's innovative screening tools and services are designed to help identify genetic drivers behind diseases, develop and validate diagnostic workflows, and deliver new precision medicine therapies. Our comprehensive portfolio includes integrated solutions for CRISPR knockout, activation, inhibition, and RNAi technologies, as well as 2D and 3D cell panel screens. We specialize in providing screening services that leverage the know-how of our experienced scientists and cutting-edge platforms to generate high-quality, reproducible data required to help you make the right decisions.

**Discover how our services can support your therapeutic development process today.**

## References


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