Archer VARIANT*Plex* Copy Number Variation Detection and Visualization Enables Exon-Level Resolution Without a Panel of Normals

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Introduction

Copy number variations (CNVs) are genomic alterations in which regions of the genome are present at abnormal copy numbers relative to the expected diploid state. In cancer, CNVs can activate oncogenes, inactivate tumor suppressor genes, drive genomic instability, and influence response to targeted therapies. Therefore, accurate CNV detection is an important component of genomic profiling.

CNVs can be detected using targeted next-generation sequencing (NGS) panels but most methods rely on a matched normal sample or panel of normals (PoN) to establish a baseline for normalization and fold-change calculation. These approaches can increase workflow complexity, cost, and turnaround time while introducing variability depending on normal cohort selection. Additionally, traditional static CNV visualizations can make it difficult to distinguish true signal from noise, particularly for small or borderline events.

To address these limitations, we present a cohortless CNV detection method (CNV 2.0) in Archer Analysis that advances both CNV detection and visualization. This approach derives copy number estimates directly from the sample of interest, eliminating the need for a matched normal sample or PoN. Designed for use with VARIANT*Plex*™ panels, this method simplifies CNV analysis while maintaining accurate detection of copy number gains and losses. Enhanced interactive visualizations improve interpretation and support identification of challenging events, including single-exon deletions.

Here, we provide a comprehensive evaluation of cohortless CNV detection across diverse sample types, including FFPE tumor extracts, cell lines, and reference materials, and across a broad range of clinically relevant CNV events. This evaluation includes copy number alterations characterized using Seracare's Seraseq™ CNV Copy Loss DNA Reference Standard and detection of single-exon *DMD* deletions.

Methods

Raw reads from sequenced Anchored Multiplex PCR (AMP™) libraries were deduplicated to unique fragments using molecular barcodes incorporated during library preparation and aligned to the genome. For general cohortless CNV method validation, the catalog VARIANT*Plex* Pan Solid Tumor (Figure 1) and VARIANT*Plex* Pan Solid Tumor v2 (Figure 2) panels were used. For single-exon deletion studies (Figures 3 and 4), custom primers targeting each deletion event (12 primers for exon 44 and 11 primers for exon 45) were spiked into the catalog VARIANT*Plex* Pan Solid Tumor v2 panel.

Rather than using a matched normal sample or panel of normals (PoN) for normalization, a bootstrap normalization approach combined with a per-primer curve-fit model was used to correct for systematic biases, including GC content, PCR amplification, and sequencing effects. Suspected outlier primers were removed, and the remaining bias-corrected counts were segmented to group adjacent regions with similar copy number states.

The sample baseline copy number was estimated from bias-corrected counts of autosomal primers within the sample. Fold-change values were calculated relative to this estimated baseline rather than a matched normal sample or PoN. Each segment mean was statistically tested against the estimated baseline copy number, and p-values were reported to identify significant copy number gains and losses.

CNV visualizations were redesigned to improve event interpretation and data exploration. Interactive features include pan-and-zoom navigation, gene highlighting to distinguish primers associated with genes of interest or neighboring genes, single-primer resolution views, and tooltip-enabled access to primer- and segment-level metrics, including fold change, primer and gene information, and statistical significance. Called CNV events are clearly annotated, improving identification of both broad and focal alterations.

To characterize the limits of single-exon deletion detection, in silico downsampling studies were performed on representative samples (Figure 4). Reads, primer coverage, and combinations of read depth and primer coverage were systematically reduced to assess the minimum deduplication ratio and exon-level primer representation required for reliable event detection. These analyses establish practical performance thresholds and inform future panel design and sample quality recommendations for single-exon CNV detection.

Results and Performance

Figure 1. Accurate CNV detection in Archer Analysis without panel-of-normal samples

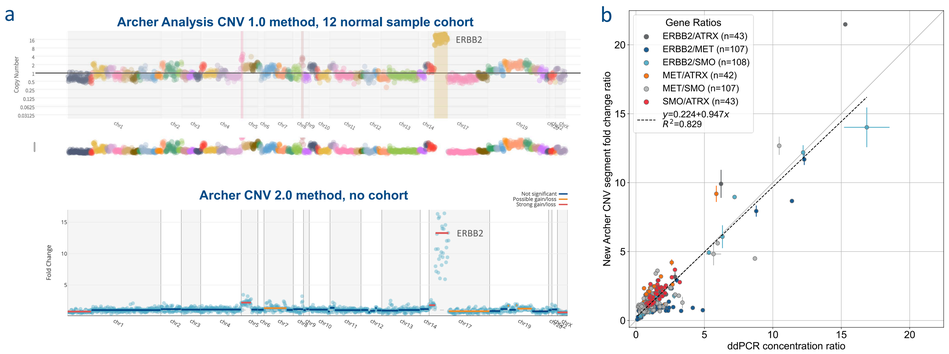
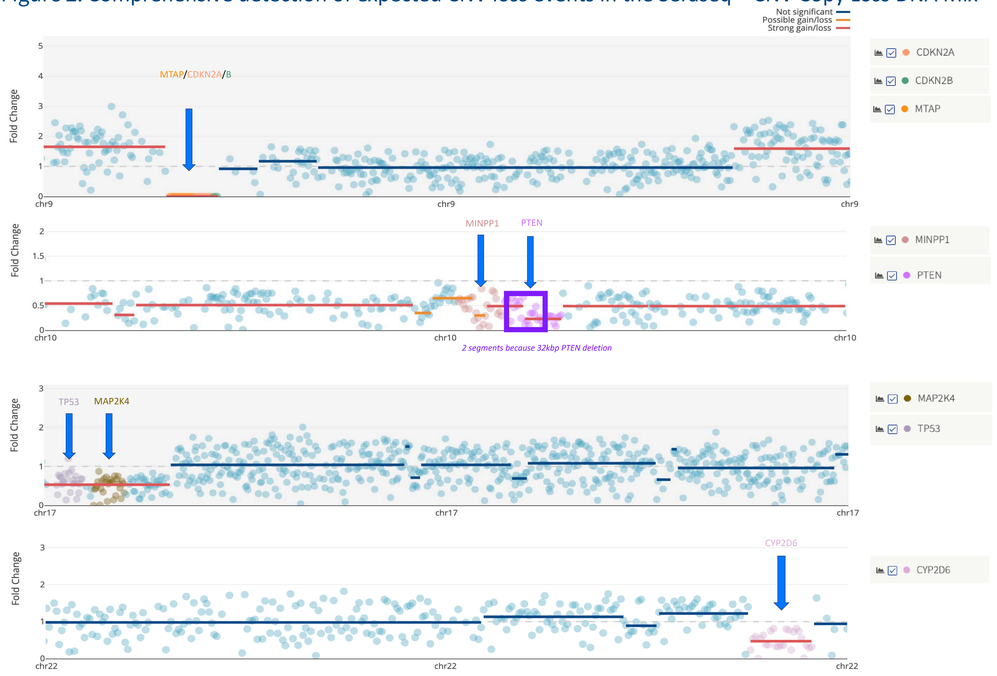


Figure 1. (a) Comparison of cohortless and panel-of-normals CNV analysis for the same library. 50 ng of DNA from FFPE tissue was prepared with our catalog VARIANT*Plex* Pan Solid Tumor panel. Each image is a cropped screenshot of Archer Analysis CNV results for the same library generated using either CNV 1.0 method with a panel of normals (top) or CNV 2.0 cohortless method (bottom). The panel of normals used for the CNV 1.0 method consisted of 12 unrelated normal-adjacent tissue samples, whereas CNV 2.0 uses only intra-sample data for CNV detection. Points represent primers. In the CNV 2.0 visualization (bottom), red bars indicate CNV segments. For the CNV 1.0 method (top), shaded vertical regions indicate events that pass the default filtering thresholds. A large *ERBB2* gain and a *GNAS* gain (not visible in the cropped images) are detected by both approaches. The CNV 2.0 results corroborate all events identified by the CNV 1.0 method while also detecting additional events that were not identified or did not meet the significance thresholds of the panel-of-normals workflow. **(b)** Concordance of CNV 2.0 segment fold-change ratios with ddPCR measurements for 108 unique inputs, including tumor FFPE extracts, cell lines, and reference materials with a variety of amplifications and deletions. Points are colored according to the pair of genes compared. The x and y values are the ratios (or mean ratios when available) calculated from ddPCR concentrations or CNV segment fold changes for segments that overlap with the coordinates of the ddPCR probes used. Standard deviation error bars are drawn, and mean ratio plotted, when there were replicate libraries, replicate ddPCR results, or both. Inputs were assayed with 4 ddPCR probes located in *MET, ERBB2, SMO*, and *ATRX* and with VARIANT*Plex* libraries prepared using panels ranging in size from approximately 900 to 10,000 primers. The number of points for each gene ratio pair is unequal because not all panels used to prepare libraries covered the regions that were targeted by ddPCR, e.g., all panels covered *ERBB2* and *SMO*, but some panels did not also cover ATRX. The agreement between our segment fold change ratios and the ddPCR concentration ratios indicates that our modelling and segmentation methods perform well across this diverse set of inputs.

Figure 2. Comprehensive detection of expected CNV loss events in the Seraseq™ CNV Copy Loss DNA Mix



	CDKN2A	CDKN2B	CYP2D6	MAP2K4	MINPP1	MTAP	PTEN	TP53
10ng SC CNV Copy Loss DNA Mix	Loss	Loss	Loss	Loss	Loss	Loss	Loss*	Loss
# gene copies per Archer Analysis CNV 2.0	0	0	1	1	1	0	1	1

*large deletion in between the two partial loss segments; all of PTEN is deleted

Figure 2. VARIANT*Plex*-HT Pan Solid Tumor v2 libraries prepared using 10ng Seracare's Seraseq CNV Copy Loss DNA Mix (0710-4109) and sequenced on Illumina NextSeq2000 platform to recommended read depth (33M reads, deduplication ratio of 6.2). Each image is a cropped screenshot of Archer Analysis CNV 2.0. All expected variants were detected: 8 CNV Loss events and deletions in the *PTEN* and *TP53* genes. Deletion in *TP53* called using the SNV/Indel pipeline, while the *PTEN* deletion is evident in the CNV 2.0 pipeline (gene loss is broken into 2 segments corresponding to a 32kbp deletion). These findings demonstrate comprehensive CNV detection with CNV 2.0, including focal deletions and larger copy-loss events in a commercially available standard, using a single targeted sequencing workflow to achieve a complete genomic profile.

Figure 3. Cohortless CNV detection enables identification of single-exon *DMD* deletions

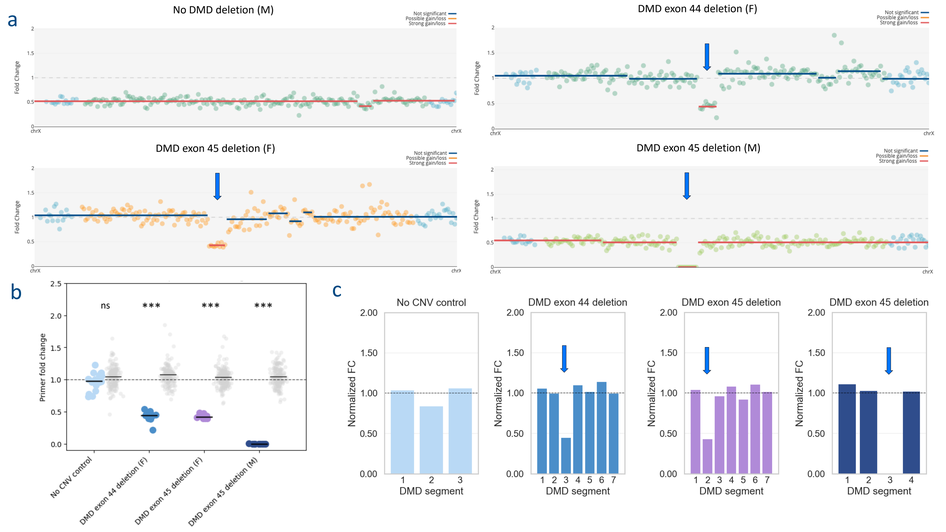


Figure 3. (a) Detection of single-exon deletions in *DMD*. Each image is a cropped screenshot from the Archer Analysis CNV 2.0 visualization, zoomed to the *DMD* locus. Each point represents a primer; *DMD* primers are colored green or orange. Primers targeting other genes are shown in blue. Because *DMD* is located on chromosome X, male samples have a baseline copy number of approximately 0.5 and female samples approximately 1.0. Results are shown for four 10 ng gDNA inputs: (1) mGIAB no-CNV control; (2) NA04315, female exon 44 deletion; (3) NA05117, female exon 45 deletion; and (4) NA05115, male exon 45 deletion. Libraries were prepared using custom primers targeting each deletion event spiked into the catalog VARIANT*Plex* Pan Solid Tumor v2 panel. Arrows indicate deleted exons. **(b)** *DMD* primer fold change for the samples shown in (a). Each point represents a primer, and the dashed line indicates a baseline of 1.0; reduced values within the deleted exon indicate copy-number loss relative to the no-CNV control. Male samples were normalized to a baseline of 1.0 to account for the expected single-copy chromosome X state. Statistical significance from a Welch's t-test between CNV event primers and a downsampled DMD background set matched for sample size is shown by significance stars (*** = $p < 0.001$; ns = not significant). **(c)** Per-segment normalized fold change across *DMD* for the samples shown in (a). Each bar represents a *DMD* segment, and the dashed line indicates a baseline of 1.0. Male samples were normalized to a baseline of 1.0 to account for the expected single-copy chromosome X state. Deletion-positive samples show reduced segment fold change relative to the no-CNV control. These results demonstrate that cohortless CNV analysis, combined with targeted custom primer spike-ins, enables reliable detection of single-exon deletions using targeted NGS.

Figure 4. Read depth and primer coverage requirements for single-exon CNV detection

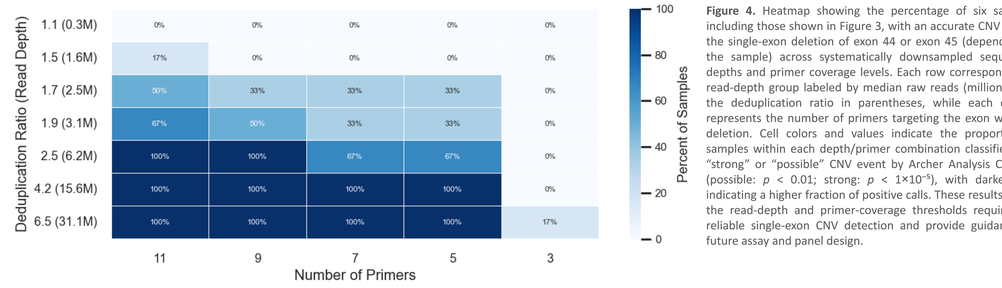


Figure 4. Heatmap showing the percentage of six samples, including those shown in Figure 3, with an accurate CNV call for the single-exon deletion of exon 44 or exon 45 (depending on the sample) across systematically downsampled sequencing depths and primer coverage levels. Each row corresponds to a read-depth group labeled by median raw reads (millions) with the deduplication ratio in parentheses, while each column represents the number of primers targeting the exon with the deletion. Cell colors and values indicate the proportion of samples within each depth/primer combination classified as a "strong" or "possible" CNV event by Archer Analysis CNV 2.0 (possible: $p < 0.01$; strong: $p < 1\times10^{-5}$), with darker blue indicating a higher fraction of positive calls. These results define the read-depth and primer-coverage thresholds required for reliable single-exon CNV detection and provide guidance for future assay and panel design.

Conclusions

The Archer Analysis cohortless CNV method eliminates the need for matched normal samples or panels of normals through intra-sample normalization, simplifying CNV analysis while avoiding variability introduced by external reference cohorts. Across diverse sample types, including FFPE tumor samples, cell lines, and reference materials, this method demonstrated robust performance for a range of panel sizes and CNV event types. High concordance with orthogonal ddPCR measurements (R^2 of 0.83 across 108 inputs) supports the accuracy of copy-number fold-change estimation (Figure 1), while evaluation using the Seraseq CNV Copy Loss DNA Mix demonstrates detection of multiple concurrent CNV loss events across clinically relevant tumor suppressor genes (Figure 2). Enhanced interactive visualizations improve data interpretation and facilitate identification of various types of copy-number alterations (Figure 1-3). Finally, in combination with targeted custom primer spike-ins, the cohortless CNV algorithm enables reliable detection of challenging single-exon deletions (Figure 3 and 4). By eliminating the need to establish, validate, sequence, and maintain matched normal samples or panels of normals, this approach reduces the time, cost, and logistical burden associated with CNV workflow implementation. Together, these results demonstrate accurate and scalable CNV detection with exon-level resolution, expanding the utility of targeted NGS without the complexity of matched-normal or panel-of-normal workflows.

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