Genome analysis

Chromosomal imbalances detected via RNA-sequencing in 28 cancers

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Abstract

Motivation: RNA-sequencing (RNA-seq) of tumor tissue is typically only used to measure gene expression. Here, we present a statistical approach that leverages existing RNA-seq data to also detect somatic copy number alterations (SCNAs), a pervasive phenomenon in human cancers, without a need to sequence the corresponding DNA.

Results: We present an analysis of 4942 participant samples from 28 cancers in The Cancer Genome Atlas (TCGA), demonstrating robust detection of SCNAs from RNA-seq. Using genotype imputation and haplotype information, our RNA-based method had a median sensitivity of 85% to detect SCNAs defined by DNA analysis, at high specificity (>95%). As an example of translational potential, we successfully replicated SCNA features associated with breast cancer subtypes. Our results credential haplotype-based inference based on RNA-seq to detect SCNAs in clinical and population-based settings.

Availability and implementation: The analyses presented use the data publicly available from TCGA Research Network (http://cancergenome.nih.gov/). See Methods for details regarding data downloads. hapLOHseq software is freely available under The MIT license and can be downloaded from http://scheet.org/software.html.

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Supplementary information: Supplementary data are available at Bioinformatics online.
further our understanding of disease. Yet, in many settings, particularly where tumor material or funding is limited, data exist from RNA-seq only. However, the extension of RNA-seq data into SCNA calling has not been as well developed. Inferring SCNAs from RNA-seq data is inherently difficult, since both regulation of expression and underlying DNA copy number will alter the observable quantities of mRNA. In addition, due to the non-uniform coverage of the genome from RNA-seq, it is challenging to differentiate between dynamically varying gene expression and SCNAs.

Recently the relative void of methods to detect SCNAs from RNA has been partially addressed. Most of these methods are exclusively tailored to single cell RNA-seq, such as HoneyBADGER (Fan et al., 2018), CopyKAT (Gao et al., 2021) and inferCNV (Fan et al., 2018; Gao et al., 2021; Tickle et al., 2019), while some can be applied to bulk RNA, such as CaSpER (Serin Harmanci et al., 2020) and SuperFreq (Flensburg et al., 2021). CaSpER integrates genome-wide total gene expression and allelic signals to detect and visualize SCNAs; SuperFreq also uses both read counts and BAF dispersions for SCNA inference, requiring referent samples to be available for normalization. Another approach for detection of SCNAs from bulk RNA profiling integrated coverage data and tumor-specific SCNA frequency patterns from public, external, data to identify chromosome-arm level aneuploidy, which was in turn assessed for association with prostate cancer outcomes (Stopsack et al., 2019). Yet, these methods do not utilize haplotype information (the genetic makeup of a single chromosome that is passed on from a parent), which has been shown to increase power for SCNA detection in studies with SNP microarray data (Baumberg et al., 2013; Loh et al., 2018; Swakumar et al., 2021; Vattathil and Scheet, 2013).

We sought to facilitate inference of SCNAs from RNA by applying an approach that utilizes haplotypes for SCNA detection from bulk RNA-seq, opening avenues for joint analysis of aneuploidy and expression from population-scale data. Consideration of haplotype structure implicitly models the signal at multiple genomic loci (or SNP markers) jointly, which not only offers an opportunity for increased power, but also requires the patterns to sustain beyond individual transcripts, which may be modulated by factors beyond SCNAs. Our approach enables robust detection of megabase-scale SCNAs that represent gain, loss or copy neutral loss of heterozygosity (cn-LOH) events. The strength of our approach derives from modeling the allelic imbalance (AI) at genomic regions affected by SCNAs. AI refers to a deviation from the expected 1:1 ratio of ‘A’ and ‘B’ alleles at germline heterozygous (genotype ‘AB’) loci. Alterations such as deletion (genotype: A– or B–, ratio: 1:0 or 0:1), duplication (genotype: AAB or ABB, ratio: 2:1 or 1:2) and cn-LOH (AA or BB, ratio: 2:0 or 0:2) are representative examples of AI.

In this study, we demonstrate effective somatic chromosomal copy number alteration identification from RNA-seq, comparing results to those derived from a high-density SNP DNA microarray as a benchmark and so-called ‘gold standard’ for SCNA detection. We consider scenarios where data are available from RNA-seq only, as well as a complementary scenario where germline DNA data is available from another source such as routinely collected blood. We apply several novel techniques including using RNA-seq for inference of acquired AI and the incorporation of genotypes via an imputation step using publicly available large-scale genotype reference data, which improves our performance considerably by enhancing the quality of estimated genotypes and haplotypes. Our results demonstrate that comprehensive and robust inference of megabase-scale SCNAs is possible from bulk RNA-seq.

## 2 Materials and methods

### 2.1 Dataset

RNA-seq BAM files aligned against the human genome build hg38 (GRCh38) and the level 1 raw CEL files from Affymetrix Genome-Wide Human SNP Array 6.0 profiling of 4942 (primary solid) tumor samples across 28 cancer sites in The Cancer Genome Atlas (TCGA) were obtained from the Genomic Data Commons data portal along with BRCA clinical information. The level 1 raw CEL files of the matched-normal (blood) samples across these sites were also downloaded to perform genotype imputation. In addition, for a subset of 7 cancer sites (BRCA, COAD, GBM, LUAD, LUSC, PAAD and PRAD), WES BAM files of 888 (primary solid) tumor samples aligned against the GRCh38 were obtained for comparisons.

### 2.2 Processing of the tumor RNA-seq array data

Our method for the detection of SCNAs relies on the allele-specific signals at germline heterozygous sites. For the purpose of deriving germline genotypes, the sample can come from the tumor itself or from a matched-normal. We explored the utility of using two different sources of data for obtaining germline genotype calls: (i) tumor RNA-seq and (ii) imputed genotypes derived from SNP array data from a matched-normal, specifically blood for the samples to which we had access.

#### 2.2.1 Genotyping and phasing

##### 2.2.1.1 Approach 1: genotypes from tumor RNA-seq.

For this approach, using tumor RNA-seq, the genotypes were called at sites already known to be polymorphic from large-scale surveys of genetic variation. The Haplotype Reference Consortium (HRC; for individuals of European ancestry) was used as a reference and genotypes were called at these reference sites from the RNA data with the UnifiedGenotyper from Genome Analysis Toolkit (McKenna et al., 2010) (GATK; version 3.6). Subsequently, the genotypes were phased using the MacH software (Li et al., 2010) to reconstruct haplotypes using the set of individual-level genotypes as an internal reference. Singleton SNPs—heterozygous markers that were observed only in one sample at a particular SNP locus within a cancer site—were removed.

##### 2.2.1.2 Approach 2: genotypes imputed.

The accuracy of haplotype reconstruction increases with larger reference/internal sample size. Therefore, haplotype reconstruction accuracy is limited particularly for smaller cancer sites when using an internal reference as done in approach 1. For approach 2, we leveraged the available blood genotype data from SNP DNA microarrays, representing genotypes from the matched-normal samples of the TCGA resource (blood). After calling genotypes using the SNP array data of the matched-normal samples with the Birdsuite software (Korn et al., 2008), the genotypes were prepared for imputation. To assure the quality of the genotypes submitted for imputation, several quality control steps were performed. To filter out low-quality SNPs, we removed the SNPs that failed Hardy–Weinberg equilibrium test ($P$-value $< 1 \times 10^{-5}$), those with missing rate > 5% and excluded monomorphic sites. In addition, samples with greater than 5% missing genotype rate were removed from downstream analyses. Individuals of European ancestry were identified using principal component analysis [EIGENSTRAT (Price et al., 2006); using the genotype SNP at 1KG sites with. The cleaned, unphased genotypes from individuals of European ancestry were submitted to the Michigan Imputation Server (Das et al., 2016) (MIS), using hg19 (GRCh37) genome build, the HRC panel (Version r1.1 2016) as the reference, 0.1 $R^2$ cutoff and EUR for population. Consequently, the imputed genotypes and estimated haplotypes for 4942 TCGA samples of European ancestry from 28 cancer sites were downloaded from the MIS and markers with an $R^2 < 0.3$ were removed to remove poorly imputed markers.

In both approaches, the centromeric, human leukocyte antigen (HLA), VDJ and Database of Genomic Variants (DGV) regions were masked for exclusion of putative germline copy number changes. We examined the effects of not performing any masking step. This resulted in slightly higher sensitivities and slightly lower specificities. For example, in COAD, sensitivity is 85% (versus 79% masked), specificity is 96% (versus 97% unmasked), in LUAD, sensitivity is 86% (versus 84% masked), specificity is 87% (versus 90% masked), and in LUSC, sensitivity is 90% (versus 88% masked), specificity is 85% (versus 88% masked). As in our approach, we are not modeling explicitly the repeat-rich nature of these regions, we determined it was a better practice in general to exclude them up front.
Table 1. Gene-level performance assessment

|                      | BRCA Sens (%) | BRCA Spec (%) | COAD Sens (%) | COAD Spec (%) | GBM Sens (%) | GBM Spec (%) | LUAD Sens (%) | LUAD Spec (%) | LUSC Sens (%) | LUSC Spec (%) | PAAD Sens (%) | PAAD Spec (%) | PRAD Sens (%) | PRAD Spec (%) |
|----------------------|---------------|---------------|---------------|---------------|--------------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| hapLOHseq (RNA-seq)  | 71            | 94            | 47            | 99            | 79           | 93           | 74            | 91            | 73            | 89            | 69           | 97            | 45           | 97            |
| hapLOHseq (RNA-seq + imputed genotypes) | 84            | 92            | 79            | 97            | 89           | 92           | 84            | 90            | 88            | 88            | 80           | 95            | 66           | 94            |
| hapLOHseq (WES)      | 93            | 89            | 94            | 92            | 94           | 96           | 89            | 92            | 91            | 90            | 94           | 89           | 76           | 97            |

Note: We evaluated the method at the gene level by comparing SCNA status of genes between the RNA-seq-derived SCNAs and the gold standard (array-based analysis) for seven cohorts in the TCGA. Sens, sensitivity; the proportion of genes covered by an SCNA in the gold standard that were also identified by the listed approach. Spec, specificity; the proportion of genes that are not covered by an SCNA event in the gold standard that were also not inferred to be covered by an SCNA by the listed approach.

2.2.2 Detection of SCNAs
As noted earlier, we investigate two approaches that differ on how the germline haplotypes are obtained. While the first approach uses tumor RNA to statistically estimate haplotypes, in approach 2, the phased germline genotypes are obtained through genotype imputation performed on a matched-normal from SNP array. hapLOHseq (San Lucas et al., 2016) algorithm identifies the haplotype in excess and using the germline haplotypes, quantities phase concordance via switch accuracy to determine genomic regions that harbor SCNAs. hapLOHseq software (version 0.1.2) was applied using the default parameters in both approaches, except –end_param_event (0.9) and –event_prevalence (0.05). SCNA calls with <10 markers or those smaller than 2 Mb were excluded. Furthermore, SCNA calls that contain large (>10 Mb) genomic regions without any heterozygous sites were split up into multiple regions.

2.3 Processing of the tumor DNA WES data
To identify SCNAs from WES tumor data, the genotypes were called at the HRC sites with UnifiedGenotyper first. Second, the genotypes were phased with MaCH software using the set of individual-level genotypes as an internal reference. Third, hapLOHseq software was used with the default parameters to detect the SCNAs after masking the centromeric, HLA, VDJ and DGV regions and removing singleton SNPs. SCNA calls with <10 markers or those smaller than 2 Mb were excluded and the calls that contain large (>10 Mb) genomic regions without any heterozygous sites were split up into multiple regions.

2.4 Processing of the tumor SNP array data
To detect SCNAs from SNP array tumor data, first the genotypes were called using the Birdsuite software, second the genotypes were phased using MaCH software, and third hapLOH software was used with the default parameters to identify regions that harbor SCNAs. Prior to the third step, the markers were mapped from genome build hg19 to hg38 and the centromeric, HLA, VDJ and DGV regions were masked. The SCNA calls detected from the SNP array constitute a gold standard for assessing the performance.

To ensure the consistency in the way the samples were processed, SyQADA (Fowler et al., 2019) was used to automate the pipelines across the three platforms.

2.5 Performance assessment
We sought to assess the performance of our method for detection of SCNAs in tumors. To do so, we compared our set of SCNA calls to a gold standard set of SCNA calls from matched-tumor DNA samples processed using arrays, a gold standard set of calls. We contrasted the SCNA call sets at gene-, chromosome arm- and genome levels. At the gene level, we report sensitivity and specificity. Sensitivity represents the method’s power to detect true SCNAs that are identified by the gold standard, while specificity represents the method’s ability to correctly identify genes that do not fall within an SCNA region in the gold standard. Therefore, sensitivity (TPR) was calculated as TP/(TP + FN) and specificity (1 - FPR) was calculated as TN/(TN + FP) where TP is true positive, FN is false negative, TN is true negative and FP is false positive. For each sample, sensitivity and specificity were calculated individually, then median sensitivity and specificity for samples in each TCGA cohort were reported as cohort-level summary statistics. When assessing the method’s performance at the chromosome arm level, for each sample, we assessed presence or absence of a chromosome arm-level event, defining an arm-level event as present when at least 50% of the chromosome arm is affected by SCNAs. At the genome level, we calculated genomic burden for each sample, which reflects the percentage of a sample’s genome that exhibits SCNAs. For each cancer site independently, we calculated each sample’s genomic burden based on RNA-seq-derived SCNA calls and compared with the gold standard derived genomic burden.

2.6 Comparison to other methods
We followed the recommended workflow for SuperFreq of first applying VarScan2 (Koboldt et al., 2012) for variant identification, followed by SuperFreq itself which is in R. RNA-seq from two adjacent-to-tumor breast samples in the BRCA resource were supplied to SuperFreq for normalization. Results from the TP53 analysis of COAD samples were taken directly from their curated data in their GitLab page. Results from CaSpER were obtained directly from what they had curated previously, using their stored and available R data frames. We then summarized results by gene through direct tabulation or applying BEDTools intersect.

3 Results
To detect somatic (acquired) copy number alterations (SCNAs) using RNA, we applied a haplotype-based approach. In brief, hapLOHseq detects regions of the genome where the signal at heterozygous sites reflects one of the estimated haplotypes for that individual. A deviation from the expected 1:1 ratio of maternal to paternal DNA indicates a relative over-representation of one of the parental chromosomes, signaling the presence of an SCNA. This approach has been applied successfully to bulk DNA analyses of various tissues (Jakubek et al., 2020; Loh et al., 2018; Vattathil and Scheet, 2016).

Here, we explore the potential of this method for detection of large-scale SCNAs from NGS data of bulk RNA (RNA-seq). To do so, we obtained RNA-seq from seven large cancer sets in TCGA: Breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), glioblastoma multiforme (GBM), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD) and prostate adenocarcinoma (PRAD). To identify genomic regions that harbor SCNAs, we applied hapLOHseq. To assess the accuracy and potential of this approach, we compare SCNAs inferred from RNA-seq to high-confidence SCNA calls detected from DNA SNP microarray data, which have been documented previously (Sivakumar et al., 2021) with an estimated false-positive rate <3% (Vattathil and Scheet, 2016). For these purposes, since the RNA and DNA were derived from the same tissue (or tumor), we...
### Table 2. Gene-level performance summaries across 28 cancer sites

| Tumor site (abbreviation) | Sample size | Sensitivity (%) | Specificity (%) |
|---------------------------|-------------|----------------|-----------------|
| Adrenocortical carcinoma (ACC) (n = 58) | 89 | 93 |
| Bladder urothelial carcinoma (BCG) (n = 261) | 83 | 90 |
| Breast invasive carcinoma (BRCA) (n = 641) | 84 | 92 |
| Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) (n = 150) | 88 | 94 |
| Cholangiocarcinoma (CHOL) (n = 26) | 85 | 95 |
| Colon adenocarcinoma (COAD) (n = 256) | 79 | 97 |
| Esophageal carcinoma (ESCA) (n = 54) | 91 | 79 |
| Glioblastoma multiforme (GBM) (n = 99) | 89 | 92 |
| Head and neck squamous cell carcinoma (HNSC) (n = 346) | 86 | 94 |
| Kidney chromophobe (KICH) (n = 6) | 94 | 95 |
| Kidney renal clear cell carcinoma (KIRC) (n = 56) | 90 | 94 |
| Kidney renal papillary cell carcinoma (KIRP) (n = 143) | 92 | 95 |
| Brain lower grade glioma (LGG) (n = 388) | 79 | 96 |
| Liver hepatocellular carcinoma (LIHC) (n = 115) | 82 | 95 |
| Lung adenocarcinoma (LUAD) (n = 312) | 84 | 90 |
| Lung squamous cell carcinoma (LUSC) (n = 221) | 88 | 88 |
| Mesothelioma (MESTO) (n = 76) | 87 | 95 |
| Ovarian serous cystadenocarcinoma (OV) (n = 249) | 87 | 87 |
| Pancreatic adenocarcinoma (PAAD) (n = 124) | 80 | 95 |
| Pheochromocytoma and paraganglioma (PCPG) (n = 133) | 89 | 96 |
| Prostate adenocarcinoma (PRAD) (n = 317) | 66 | 94 |
| Rectal adenocarcinoma (READ) (n = 133) | 80 | 96 |
| Skin cutaneous melanoma (SKCM) (n = 87) | 86 | 94 |
| Stomach adenocarcinoma (STAD) (n = 190) | 86 | 87 |
| Testicular germ cell tumors (TGCT) (n = 116) | 88 | 93 |
| Thyroid carcinoma (THCA) (n = 276) | 85 | 94 |
| Uterine carcinosarcoma (UCS) (n = 34) | 85 | 90 |
| Uveal melanoma (UV) (n = 75) | 87 | 98 |

Note: For each cancer site, the study abbreviation, number of samples analyzed in the cohort and median gene level sensitivity and specificity are shown.

3.1 SCNA detection from RNA-seq

We compared and contrasted the RNA-seq derived SCNAs with the gold standard SCNAs with units of analysis as gene, chromosome arm and entire genome (i.e. burden) (Supplementary Figs S1 and S2), focusing here on gene-level summaries. Table 1 [first row: ‘hapLOHseq (RNA-seq)’] shows the sensitivity and specificity for SCNA detection solely using tumor RNA for seven cancer sites. Sensitivity is the method’s power to detect true SCNAs (defined as those identified by the gold standard) and specificity measures the method’s ability to correctly identify genomic regions where there is no SCNA detected by the gold standard. We obtained a generally high concordance between the events called from RNA-seq with those called from SNP arrays at a gene level, and this held for the other units of analysis as well. Our SCNA detection rate (sensitivity) from RNA-seq was highest for the GBM data at 79%, whereas the sensitivity for PRAD was markedly lower at 45%. We observed from RNA-seq was highest for the GBM data at 79%, whereas the other units of analysis as well. Our SCNA detection rate (sensitivity) from RNA-seq was highest for the PRAD cohort, we further investigated the samples with the highest specificities for all seven cancer sites, ranging between 89% and 99%.

To assess which factors drive the lower than average performance in the PRAD cohort, we further investigated the samples with the highest sensitivities. We observed that the samples with the highest quartile of sensitivity had the highest median number of heterozygous sites and the highest haplotype accuracies (Supplementary Fig. S3). After grouping the samples into quartiles based on their sensitivity, we statistically compared the groups with the Kruskal–Wallis test and noted that the groups were significantly different when compared both by the number of heterozygous sites (P-value < 1e-8) and phase accuracy (P-value = 0.008).

3.2 SCNA detection from RNA-seq and imputation-based haplotype inference

We hypothesized that the standard genotyping (variant calling) pipelines for NGS, along with modest reference sizes for haplotype phasing, were holding back the potential of our approach. To address this, we imputed germline genotypes and haplotypes from large-scale reference data using the optimized workflows in the Michigan Imputation Server (MIS). We leveraged the genotype data from a matched-blood sample, available for most participants in TCGA. While this sample does not provide any direct information about SCNAs of the tumor, it does provide more accurate identification of heterozygous sites and estimated haplotypes, central to our approach, but without the need to extract DNA from the tumor. We then applied hapLOHseq for detection of tumor SCNAs using RNA-seq signal combined with the more accurate haplotype information, as detailed in the methods.

The inclusion of imputed genotype calls and high-quality haplotypes provided a substantial improvement in overall SCNA detection [Table 1; second row: ‘hapLOHseq’ (RNA-seq + imputed]
Chromosomal imbalances detected via RNA-sequencing in 28 cancers

Fig. 1. Chromosome arm-level concordance assessment summaries across 28 cancer sites. We identified chromosome arms that were spanned by SCNAs (≥50%) and for each arm, we evaluated the concordance between RNA-seq and gold standard. The distribution of the non-acrocentric autosomal chromosome arms (n = 39) across the cancer sites is shown. Each site, a stacked bar plot of the number of samples with concordance-specific chromosome arm-level SCNAs are shown in Table 2.

Table 2 contains per cancer site gene-level summaries of sensitivity and specificity for 28 cancer sites, comprising 4942 samples in the TCGA, after applying the imputation workflow. At the gene level, our imputation-based approach achieved an 85% median sensitivity and 94% median specificity (all genes, all samples). Across the sites, median sensitivities ranged from 66% to 94%, with median specificities between 79% and 98%. With the exception of ESCA, specificity was always greater than or equal to the sensitivity for a given site. At 79%, 79% and 66%, COAD, brain lower grade glioma (LGG) and PRAD (respectively) were the only cancer sites with sensitivity below 80%. Interestingly, kidney cancers (kidney chromophobe: KICH; kidney renal clear cell carcinoma: KIRC; and kidney renal papillary cell carcinoma: KIRP) were the three cohorts that we observed the best performances for with 94%, 90% and 92% sensitivity and 95%, 94% and 95% specificity. We evaluated the method at the chromosome arm level as well (Fig. 1). For each chromosome arm, we assessed the concordance with the gold standard calls and the results indicate that the majority of the true arm-level SCNAs were inferred correctly across all cancer sites. However, several, such as 5p, 9p, 13q and 21q were missed with RNA consistently across the cohorts. We also note that the majority of the chromosome arm-level SCNAs inferred from RNA-seq in the THCA cohort were not present in the gold standard set.

To evaluate the SCNA patterns at a whole genome level, we calculated ‘genomic burden’ – the proportion of a sample’s genome exhibiting SCNAs. Marginally, we observed a median 0.28 genomic burden across all samples across all cancer sites, compared with 0.31 from DNA microarrays. Further, we investigated the patterns of genomic burden per cancer site. The highest genomic burden was observed in ESCA (0.63), followed by OV (0.53) and TGCT (0.53). THCA was the lowest (0.05), followed by PRAD (0.09). Median genomic burden for all cancer sites along with the corresponding sensitivity and specificity remained similar. In comparison to WES, the imputation approach has lower sensitivity with similar specificity. For instance, for the BRCA cohort, the imputation approach’s sensitivity is 9% lower while specificity is 3% higher. These results indicated the potential for inference of SCNAs purely from RNA-seq, so long as there exist sufficiently informative germline genotypes. To comprehensively characterize the potential for SCNA inference from RNA-seq across a range of tissue types, we applied this approach to the remaining cancer sites from the TCGA for which data existed. Study abbreviations for all 28 TCGA cohorts investigated in this study are shown in Table 2.

Across all cancer sites, the median SCNA size was 31.28 Mb, approximately equal to the median SCNA size (31.31 Mb) detected by the gold standard. The median number of SCNA events detected per sample was 20 (gold standard: 19). The highest frequency of SCNA calls per sample were observed in esophageal carcinoma (ESCA), ovarian serous cystadenocarcinoma (OV), LUSC, uterine carcinosarcoma (UCS) and bladder urothelial carcinoma (BLCA) with each with a median of 30 SCNAs or more per sample, consistent with the gold standard-derived genomic burden at a sample level. The majority of the true arm-level SCNAs were inferred correctly across all cancer sites, ranging from 10% to 32% in absolute increase, i.e. BRCA (13%), COAD (32%), GBM (10%), LUAD (10%), LUSC (15%), PAAD (11%) and PRAD (21%), while high specificities remained similar. In comparison to WES, the imputation approach has lower sensitivity with similar specificity. For instance, for the BRCA cohort, the imputation approach’s sensitivity is 9% lower while specificity is 3% higher. These results indicated the potential for inference of SCNAs purely from RNA-seq, so long as there exist sufficiently informative germline genotypes. To comprehensively characterize the potential for SCNA inference from RNA-seq across a range of tissue types, we applied this approach to the remaining cancer sites from the TCGA for which data existed. Study abbreviations for all 28 TCGA cohorts investigated in this study are shown in Table 2.

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SCNA calls discovered by RNA-seq that were not detected in the DNA (gold standard) are putative false positives in our analysis. However, some appear to be false negatives in the gold standard set. To attempt to discriminate between these, we examined in more detail the COAD data, where these putative calls made up 6% of all SCNAs discovered in RNA-seq. We explicitly tested the genomic regions of the putative false positive SCNAs in the corresponding SNP array data with a specific binomial test leveraging phase concordance. Among the 6%, we found that approximately one-fourth (23%) of the calls were validated in the SNP array data (at a P-value < 0.05),
indicative that the reported false positive rates are modestly overestimated.

We investigated the method’s performance for different SCNA categories (Supplementary Table S2). We observed that ‘undetermined’ events—subtle events in bulk samples with low mutant cell fraction that cannot be classified as gain, loss or cn-LOH—in the gold standard were the SCNA category that was most frequently missed by RNA-based detection. The difference was most striking in the PAAD and PRAD sites. Exclusion of these undetermined (low mutant cell fraction) events results naturally in higher overall sensitivity rates. Perhaps not surprisingly, the cn-LOH category consistently had the best performance, presumably due to greater BAF perturbation for this SCNA type.

### 3.3 Comparison to other methods for bulk RNA-seq

While most methods for SCNA inference from RNA-seq have been designed for single cell data, we were able to conduct a detailed comparison with one state-of-the-art method for bulk RNA. Table 3 contains a summary of results from our method, hapLOHseq and CaSpER, for BRCA and GBM, sites analyzed in the original paper for CaSpER. We compared both methods to the gold standard. Compared with the gold standard calls at the gene level, for both cancers, hapLOHseq offered a superior performance, with a substantial increase in sensitivities with absolute gains of 30% and 42%. Against CaSpER’s own benchmark, the methods appeared more similar, with an edge to hapLOHseq in detection but at some cost in specificity (Supplementary Table S3).

We were also able to successfully run SuperFreq on a subset of the BRCA samples. From analysis of 12 samples where we had results from both SuperFreq and hapLOHseq, the sensitivity for hapLOHseq was 85% versus 77% for SuperFreq (specificities were 95% and 98%, respectively). The authors of SuperFreq demonstrated high sensitivities analyzing TP53 alterations in high mutant cell fraction settings for COAD. We were able to detect SCNAs in TP53 in this set with hapLOHseq at an equivalent rate but without the need for additional RNA samples for normalization.

### 3.4 Translational/prognostic use

Finally, to demonstrate a translational potential, we examined the portability of conclusions from others’ analyses of DNA to ours from RNA-seq. In an example in breast cancer, we recapitulated the distinct genomic burden distributions across different subtypes previously observed in TCGA BRCA data (Cancer Genome Atlas Network, 2012), demonstrating that those of a basal subtype are characterized by high genomic burden in comparison to the others (Fig. 3A). We observed that the samples in the basal subtype that have more than 40% of their genome altered comprise 84% of all basal samples, consistent with the previous report. Furthermore, analyzing the RNA-seq, we were also powered to observe that chromosome arm 5q is more frequently altered in the basal subtype, whereas chromosome arm 1q and 16q are more frequently altered in the luminal subtypes, consistent with the previous findings from analyzing DNA directly (Cancer Genome Atlas Network, 2012) (Fig. 3B). Finally, we specifically investigated the concordance...
between hapLOHseq and gold standard results for five genes that are frequently affected by CNA events for the BRCA cohort, i.e. BRCA1/2, PTEN, RB1 and TP53. We showed that hapLOHseq obtained promising results that have a potential clinical use with 93% sensitivity and 85% specificity for BRCA1, 78% sensitivity and 95% specificity for BRCA2, 85% sensitivity and 88% specificity for PTEN, 87% sensitivity and 94% specificity for RB1 and 90% sensitivity and 86% specificity for TP53. Sample-level concordance assessment for each of the genes is shown in Figure 3C.

4 Discussion

In this study, we detect and characterize the genomic landscapes of SCNAS from tumor bulk RNA-seq using a haplotype-aware statistical method. We proposed two approaches that differ in the way germline genotypes are obtained for subsequent analysis of B allele frequencies (BAFs). While the first approach solely uses RNA-seq from tumor to estimate the haplotypes, the second approach leverages available or potentially collectible SNP array (or equivalent) data from a matched-normal sample to achieve higher accuracies in genotyping and haplotype reconstruction through a popular imputation pipeline.

In an analysis of 28 cancer sites from TCGA, our method achieved high sensitivity for SCNA detection with the imputation approach (85% versus 68%), retaining high specificity as well (~95%). Summaries of SCNA genomic burden were sufficiently high as to potentially obviate the need for analyzing DNA. In sites with lower sensitivities, such as PRAD, analyses of DNA exome sequencing reflected difficulties as well, indicating challenges for such sites more specific to targeted sequencing data.

Our imputation approach addresses difficulties associated with genotype calling from RNA-seq, e.g. due to non-uniform coverage, which in turn provides highly accurate and phased genotypes. Indeed, we explored these factors as direct contributors to improved performance in PRAD (Supplementary Fig. S3). Our germline heterozygote identification could improve other methods for bulk RNA analysis, as well, such as CaSpER and SuperFreq. In different statistical implementations, each of these combine information from not only total read counts but also BAF dispersion at heterozygotes. Whereas SuperFreq relies on external data for normalization (e.g. paired normal RNA samples), CaSpER’s approach works on a sample-by-sample basis, as does hapLOHseq. We conducted a detailed comparison to CaSpER, observing higher sensitivities with our haplotype-based approach. Ultimately, getting the absolute performance characteristics will depend on improved gold standard datasets. We note that these methods use information orthogonal to that leveraged by hapLOHseq and thus may offer improvements when applied in combination, or integrated for joint analyses, an area of future study.

Blood, buccal or adjacent normal samples can serve as representative of the germline. The first two are non-surgical and more easily collected, whereas the third may be available for some specimens. Array-based genotyping of these samples presents an economical approach for improved tumor SCNA characterization. This is feasible for existing clinical cohorts with banked patient blood samples or biobanks with existing genotype data. In our exhibition, we focused on individuals of European ancestry to assess the performance of our method in a ‘best case’ scenario given current resources. However, efforts such as TOPMed (Taliun et al., 2021) will generate high-density genotype panels for individuals of non-European ancestry. Our approach further highlights the need for genetic panels of high diversity in biomedical research.

Our approach, as we have demonstrated here, does not attempt to detect balanced duplications, i.e. those where maternal and paternal chromosome segments are present in equal ratios. This may be overcome through integration of coverage data with our existing approach, which would be feasible for large balanced duplications as shown previously (Serin Harmanci et al., 2020; Stopsack et al., 2019). While any method for RNA-seq will have natural limitations...
in detecting alterations that do not span expressed genes, over larger regions limiting factors will be mitigated or averaged toward genome levels. Further, molecular alterations caused by focal SCNAs of key cancer drivers may be detectable through traditional RNA analyses of altered gene expression, including at the pathway level, and/or identification of specific transcripts.

In summary, we show that the proposed haplotype-based approach for RNA-derived SCNA calls is robust for detection of megabase-scale somatic mutations. Overall, detection rates were generally higher than 85% at specificities high enough for de novo discoveries and assessments of genomic associations with clinical phenotypes, across malignancies. Indeed, we successfully recapitulated SCNA features associated with clinical subtypes of breast cancer. Our findings show that our method can be used to increase the utility of bulk RNA-seq by allowing for a more comprehensive molecular profiling of tumors in settings where DNA analysis is impractical due to limited tissue sample availability or financial constraints and enables secondary analyses of existing data from high-value clinical cohorts.

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