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## Ximmer: A System for Improving Accuracy and Consistency of CNV Calling from Exome Data

--Manuscript Draft--

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<b>Abstract:</b>	<p>Motivation: While exome and targeted next generation DNA sequencing are primarily used for detecting single nucleotide changes and small indels, detection of copy number variants (CNVs) can provide highly valuable additional information from the data. Although there are dozens of exome CNV detection methods available, these are often difficult to use and accuracy varies unpredictably between and within data sets. This problem is exacerbated by a lack of mature software supporting CNV simulation, evaluation and quality control for exome based methods.</p> <p>Results: We present Ximmer, a tool which supports an end to end process for evaluating, tuning and running exome CNV detection tools. Ximmer includes a simulation framework, CNV detection analysis pipeline, and a visualisation and curation tool which together enable interactive exploration and quality control of CNV results. Using Ximmer, we comprehensively evaluate CNV detection on four data sets using four different detection methods. We show that application of Ximmer can improve accuracy and aid in quality control of CNV detection results. In addition, Ximmer can be used to run analyses and explore CNV results in exome data.</p> <p>Availability and Implementation: Ximmer is open source and freely available at <a href="http://ximmer.org">http://ximmer.org</a> (example results are viewable at <a href="http://example.ximmer.org">http://example.ximmer.org</a>).</p>	
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# Ximmer: A System for Improving Accuracy and Consistency of CNV Calling from Exome Data

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# Abstract

**Motivation:** While exome and targeted next generation DNA sequencing are primarily used for detecting single nucleotide changes and small indels, detection of copy number variants (CNVs) can provide highly valuable additional information from the data. Although there are dozens of exome CNV detection methods available, these are often difficult to use and accuracy varies unpredictably between and within data sets. This problem is exacerbated by a lack of mature software supporting CNV simulation, evaluation and quality control for exome based methods.

**Results:** We present Ximmer, a tool which supports an end to end process for evaluating, tuning and running exome CNV detection tools. Ximmer includes a simulation framework, CNV detection analysis pipeline, and a visualisation and curation tool which together enable interactive exploration and quality control of CNV results. Using Ximmer, we comprehensively evaluate CNV detection on four data sets using four different detection methods. We show that application of Ximmer can improve accuracy and aid in quality control of CNV detection results. In addition, Ximmer can be used to run analyses and explore CNV results in exome data.

**Availability and Implementation:** Ximmer is open source and freely available at <http://ximmer.org> (example results are viewable at <http://example.ximmer.org>).

# Background

In recent years, high throughput sequencing (HTS) of DNA has become an essential tool in biomedical science with a vast range of applications spanning both clinical and research investigations. In clinical settings, whole exome sequencing (WES) and custom targeted gene panels are especially important and have enabled significant improvements in the rate of diagnosis for genetically heterogeneous disorders (Stark *et al.*, 2016). WES has also had a profound impact on disease research, by allowing researchers to comprehensively search for protein altering genetic variation. As a result of these advances, the rate of discovery of new Mendelian disease genes has seen substantial improvements in recent years (X. Zhang 2014).

While WES has proven highly effective, this success has been based predominantly on the detection of single nucleotide variants (SNVs) and small insertions and deletions (indels). Larger variants, such as copy number variants (CNVs), are not routinely ascertained from WES data. Nonetheless, CNVs are frequently disease causing, both as the primary genetic lesion for disorders such as  $\alpha$ -thalassemia (Stankiewicz and Lupski 2010), Charcot-Marie-Tooth

1 neuropathy and Smith-Magenis-Syndrome, as well as a rare cause for a wide range of mendelian  
2 diseases. In particular, single copy deletions can be pathogenic for any disorder caused by  
3 haploinsufficiency. To detect CNVs, patients are often screened for CNVs using SNP or array-CGH  
4 microarrays prior to use of WES. However, affordable microarrays have limited resolution and  
5 add time, cost and complexity to the overall diagnostic workflow. There are consequently  
6 significant potential advantages if CNVs can be ascertained directly from WES.  
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11 CNVs can be detected from three primary signals in HTS data. These are: anomalous mapping of  
12 paired end reads that span CNV breakpoints (PE signals), the splitting of individual reads by CNV  
13 breakpoints (SR signals), and fluctuation in the coverage of reads falling in the body of a CNV  
14 (the read depth, or RD signal). While all of these signals are effective in whole genome data, the  
15 breakpoints of CNVs usually fall between the regions targeted by WES. Therefore only the RD  
16 signal is reliably observable in WES data. The RD signal has been shown to be informative due to  
17 a strong correlation of copy number with read coverage depth (Sathirapongsasuti *et al.*, 2011).  
18 However, detection of CNVs is confounded by a range of other factors that also influence read  
19 coverage depth. Therefore, these factors must be corrected, and failure to do so can result in  
20 significantly degraded accuracy.  
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30 Numerous methods have been developed to detect CNVs based on the RD signal. Examples  
31 include ExomeDepth (Plagnol *et al.*, 2012), ExomeCopy (Love *et al.*, 2011), XHMM (Fromer *et al.*,  
32 2012), cn.MOPS (Klambauer *et al.*, 2012), ExomeCNV (Sathirapongsasuti *et al.*, 2011),  
33 CoNVEX(Amarasinghe *et al.*, 2013), EXCAVATOR (Magi *et al.*, 2013), CoNIFER (Krumm *et al.*,  
34 2012), CANOES (Backenroth *et al.*, 2014), CODEX (Jiang *et al.*, 2015), and many others. The  
35 authors of these tools have often cited high sensitivity and specificity for their methods.  
36 However, independent comparisons frequently fail to replicate their findings. For example, Guo  
37 et al. reported ExomeDepth having sensitivity of only 19% (Guo *et al.*, 2013), while Ligt et al.  
38 observed a sensitivity of 35% (de Ligt *et al.*, 2013). In the same studies, sensitivity of CoNIFER  
39 was cited as 3% and 29% respectively, compared to the original evaluation estimate of 76%. In  
40 some contexts, high accuracy is reported. For example, Jo et al (Jo *et al.*, 2016), Ellingford et al  
41 (Ellingford *et al.*, 2017) and Feng et al (Feng *et al.*, 2014) all cited 100% sensitivity and high  
42 specificity for detection of larger CNVs encountered clinically, in each case using high coverage  
43 data. However, the circumstances in which high accuracy can be achieved are currently not well  
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57 Recent studies have compared performance across multiple data sets (Hong *et al.*, 2016; Tan *et*  
58 *al.*, 2014; Zare *et al.*, 2017), highlighting the problem of variability in the performance of CNV  
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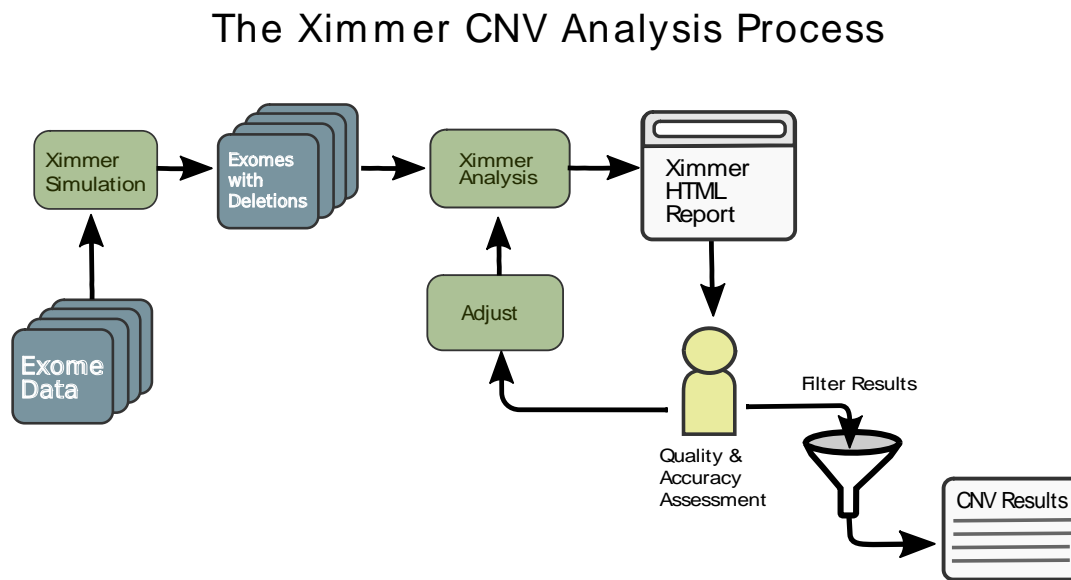
1 calling as well as high false positive rates (Samarakoon *et al.*, 2016). Some of the performance  
2 variability observed in these studies may be due to differences between the data sets and  
3 sequencing design such as the number of samples, read length, insert size, and mean read depth.  
4 Also of critical importance is the size and type of CNVs assessed. However, even when these  
5 known technical factors are controlled, significant variability is often still observed between data  
6 sets.  
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11 In this work we present Ximmer, a software tool that improves CNV calling reliability by  
12 enabling users of CNV detection tools to efficiently assess and tune performance. Ximmer  
13 contains three parts: a simulation method, an analysis pipeline, and a graphical report. First,  
14 Ximmer simulates synthetic single copy deletions in existing WES data. Then, the analysis  
15 pipeline automates detection of the deletions with up to 5 commonly used CNV detection  
16 methods. Finally, the graphical report shows the combined CNV calling results, including a suite  
17 of plots that give insight into the accuracy achieved and strategies for improving performance.  
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25 In this article we explain the implementation details of Ximmer, and demonstrate how using  
26 Ximmer improves CNV detection accuracy. We show results from four CNV callers on four  
27 datasets representing different exome capture kits and different sequencing depths. Our results  
28 concur with previous studies, finding that CNV detection performance is highly variable both  
29 within and between data sets. However, we show that using Ximmer to gain insight into the  
30 variability enables optimisation of the CNV calling, and improves detection of real CNVs. Ximmer  
31 offers an integrated framework that is easy to use and freely accessible, from <http://ximmer.org>.  
32 An example of Ximmer output is available at <http://example.ximmer.org>.  
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## Methods

The Ximmer process consists of a series of steps designed to optimise CNV detection performance. The steps consist of: (i) simulation of CNVs in the user's data, (ii) execution of CNV callers to find both real and simulated CNVs, (iii) quality and accuracy assessment to discover optimal settings for CNV calling, and finally, (iv) filtering of results to produce a curated CNV list. This process can be time consuming if conducted manually, however Ximmer automates all of the steps needed. The high level process is depicted in Figure 1.



**Figure 1** The Ximmer Process - Ximmer consists of three high level steps. In the first step, simulated CNVs are added to a set of sequence alignments in BAM or CRAM format. This creates new BAM files containing simulated CNVs which are passed to the integrated analysis pipeline. The analysis pipeline runs up to 5 different CNV detection methods and collates the results into a graphical report which generates insight into the performance of the tools and possible avenues for improvement. Finally, when the analysis is optimised provides an interface to filter CNVs, review and interpret them using the built in CNV curation tool.

## Simulation

Simulation is the first and most important element of the Ximmer method. By simulating CNVs in the user's own data, Ximmer generates both a prediction of the CNV calling performance, and also insights regarding how to improve performance. To simulate CNVs, Ximmer takes advantage of the exclusive use of the RD signal by WES based CNV detection methods. Specifically, Ximmer removes reads that overlap selected target regions, such that the RD signal is reduced to match the predicted level associated with a single copy deletion. Ximmer focuses

1 on deletions because depleting reads is significantly simpler than realistically synthesising and  
2 adding new reads. While the approach is strictly limited to deletions, the inferences derived are  
3 still likely to apply to other CNV states, because in most CNV calling tools the same underlying  
4 statistical principles are applied regardless of the number of copies being searched for.  
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7 The simulation process begins by randomly selecting the genomic region to become the deletion  
8 “target”. The reads mapping to these locations can then be depleted using two alternate  
9 methods, referred to as “Downsampling” and “X-Replacement”. The downsampling method  
10 randomly removes each read overlapping the deletion target with probability of 0.5, based on an  
11 assumption that the relationship between copy number and read depth is linear. By contrast, the  
12 X-Replacement method avoids this assumption. The X-Replacement method replaces reads  
13 mapping to X chromosome deletion target regions in a female sample with an adjusted number  
14 of reads from the same genomic regions in a male sample. This method exploits the true  
15 difference in copy number between male and female X chromosomes to avoid the assumption of  
16 linearity implied by downsampling. The X-Replacement method also ensures that other aspects  
17 of the reads are preserved in a realistic manner, such as the zygosity and phasing of overlapping  
18 SNVs and indels. Further details of the simulation implementation are provided in the  
19 supplementary methods (S-1). The result of the simulation step is a new set of alignments (BAM  
20 files) for the whole exome, but with deletions simulated in selected regions.  
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## 31 **CNV Analysis Pipeline**

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34 The second step in the Ximmer process is to analyse the data containing simulated CNVs to  
35 produce CNV calls. Ximmer provides a built in analysis pipeline that automatically installs,  
36 configures and runs 5 commonly used CNV detection methods. These tools are: ExomeDepth,  
37 XHMM, cn.MOPS, CoNIFER and CODEX. The analysis pipeline is constructed using Bpipe  
38 (Sadedin *et al.*, 2012), a framework for creating bioinformatic workflows. In addition to running  
39 the CNV detection tools, Ximmer performs any necessary pre-processing required by the tools  
40 and also post processes the results to merge and annotate the resulting CNV calls. Additional  
41 CNV callers can be added to Ximmer with only a small effort through the extensible Bpipe  
42 framework.  
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50 The analysis produces a report in HTML format that contains a full summary of all the simulated  
51 deletions, along with a range of plots and tables to highlight CNV calling performance and  
52 potential quality issues.  
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## Results Assessment

Once CNV analysis has been performed, the next step in the method is to critically review the HTML report to assess performance of the CNV callers for detecting the simulated deletions, and to evaluate options for improving the results.

### *Quality Assessment*

Three plots are of particular relevance in understanding potential quality issues. These are the Sample Counts, Genome Distribution and Quality Score Calibration plots.

The Sample Counts plot (Figure 2A) shows the distribution of the number of CNV calls among the samples, separately for each CNV caller. In most studies we expect the number of CNV calls to be similar for each sample. If some samples contain a disproportionate fraction of the total CNV calls, it is likely that there is a problem with the sample quality. It may be desirable to either remove the samples from the CNV calling altogether, to adjust the caller settings to compensate, or to isolate poor quality samples from use in normalising other samples.

The Genome Distribution plot (Figure 2B) divides the genome into 5 megabase bins and displays the number of CNVs overlapping each bin. Clicking on a particular region displays an enlarged plot encompassing that region for more detailed inspection. If particular regions contain very large numbers of CNV calls, it may be desirable to remove these from the target regions used for calling, as their presence may distort quality statistics and degrade overall calling accuracy.

The Quality Score Calibration plot (Figure 2C) assists in interpreting the confidence measures (or quality scores) assigned to CNVs by the CNV callers. For each caller, Ximmer groups the whole CNV call set into approximately 5 quality score bins that collectively span the full range of values assigned by the caller. Ximmer then calculates the fraction of calls categorised as true positives in each bin as an estimate of the precision. The estimates are plotted as a line to illustrate the empirical relationship between precision and quality score for each CNV caller.

When quality scores are well behaved it is expected that the precision should increase monotonically as quality score increases. Failure to observe this relationship suggests the caller may produce high confidence false positives, in which case filtering by quality score alone may be insufficient to reduce the false positive rate. As with the Sample Counts plot, it may be appropriate to review normalisation settings for methods if quality scores assigned by tools are not well behaved.

### *Accuracy Assessment*

After reviewing the quality assessment the next step in the Ximmer process is to review the accuracy estimate. This is presented using a plot designed to mimic a traditional “Receiver

Operator Characteristic” curve, but displayed using absolute measures to better accommodate the unknown positions of true negatives in CNV calling. Instead, the ROC-style plots show how the detection of simulated true positives (Y-axis) changes with the number of detections not part of the simulation (false positives, X-axis) as results are progressively filtered to lower significance levels. It should be noted that false positives are defined as regions that are not simulated to be deletions but they could actually be true positives from the sample itself. Unlike comparisons of absolute sensitivity and precision, this method primarily compares the ranking of true and false positives, and thus takes into account the utility of confidence measures output by tools for filtering the results. For the CNV calling tools included in Ximmer, the confidence measure used for ranking results was chosen in each case by consulting the documentation or by discussion with the tool author (Supplementary Methods, Table S1).

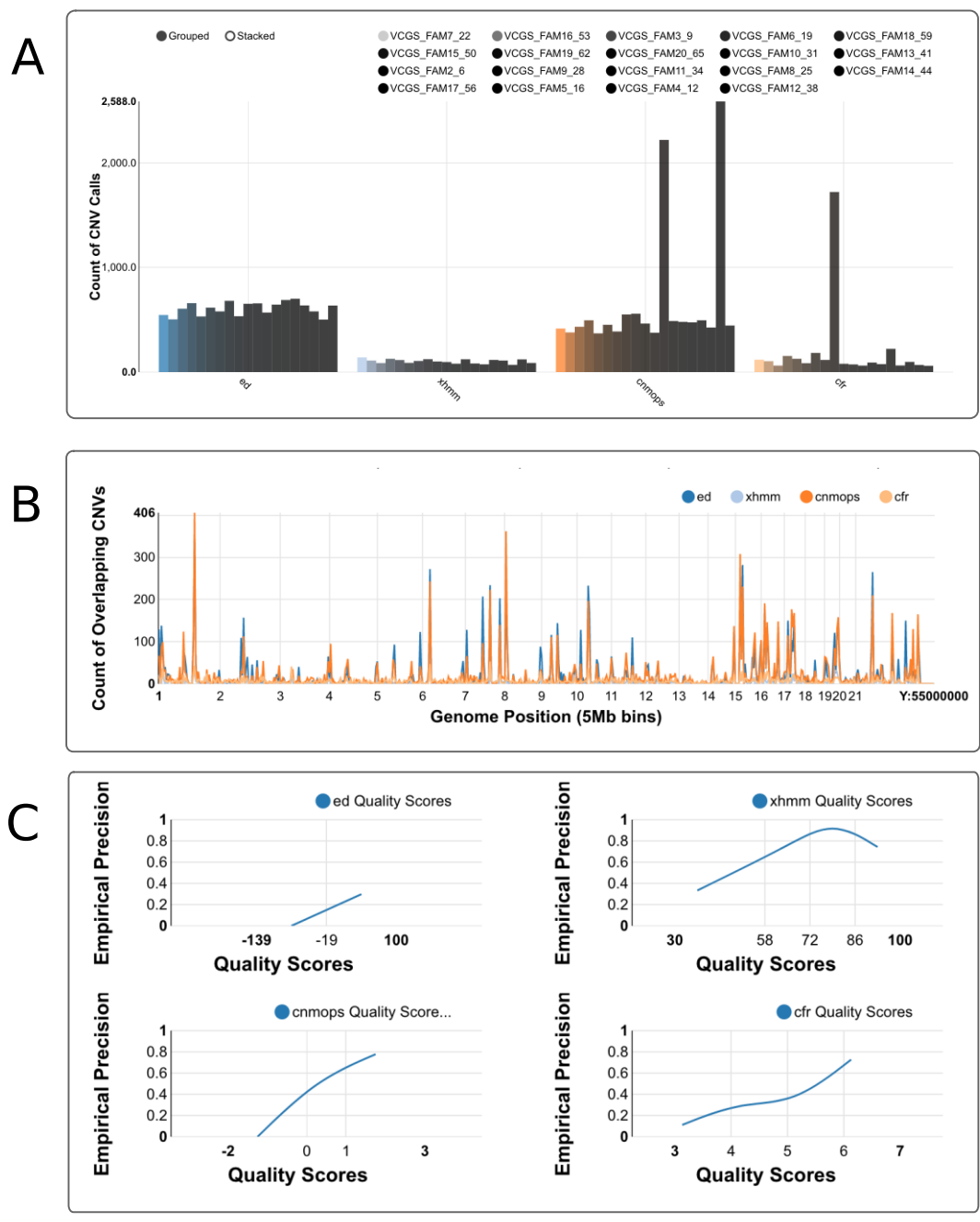
The initial display of the ROC-style curve shows the accuracy for the whole set of simulated deletions. As a first step this can suggest an appropriate level at which to filter results so that the optimal level of sensitivity and specificity is achieved. However, the plot can be interactively adjusted, to show performance of a subset of CNVs within specific size ranges.

### ***CNV Discovery***

Once the performance of the CNV callers is well understood, the final step in the Ximmer process is to filter the CNV calls according to the decided quality filtering thresholds. The remaining CNVs may then be manually reviewed in Ximmer’s CNV curation interface. The interface includes a range of annotations to support interpretation of the likelihood that a CNV is real, and whether the CNV is potentially of functional interest. The annotations include overlapping genes, population frequency of relevant CNVs from the Database of Genomic Variants (DGV (Zhang *et al.*, 2006)), overlapping single nucleotide variants (SNVs) or indels, and a pictorial diagram of the read depth deviation over the CNV region.

If desired, the discovery of real CNVs can be from the same analysis result set containing simulated CNVs. This approach relies on an assumption that simulated and real CNVs of interest are unlikely to overlap. Alternatively, Ximmer can be re-executed on the original raw data with simulation disabled to derive a stand-alone result set.

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**Figure 2** Screen shots of Ximmer Quality and Accuracy Plots. **A.** Sample counts plot, showing the number of CNV calls for each sample, by each CNV caller. **B.** Genome distribution plot, showing frequency of CNV calls along the genome. **C.** Quality score calibration plot, showing relationship of empirical precision to quality score.

## Data Sets

To demonstrate the application of Ximmer, we applied it to four data sets representing different Illumina sequencing platforms, exome captures, read configurations and sequencing depths (Table 1).

**Table 1** Data sets analysed with Ximmer

Capture	Samples	Capture Size (Mb)	Read Length	Mean Read Depth
SureSelect v5	16 Male, 14 Female	51.2 Mb	2 x 100	30
Nextera 1.2	24 Female, 28 Male	45.3Mb	2 x 100	120
Nimblegen v2	19 Female, 19 Male	47Mb	2 x 75	60
TruSeq / Custom Broad Capture	19 Female 16 Male	37.5Mb	2 x 150	90

The SureSelect data set was produced as part of an unrelated research program, the Nextera data was created as part of the Melbourne Genomics Health Alliance demonstration project (<https://www.melbournegenomics.org.au/>) and the TruSeq data was created by the Broad Institute, Center for Mendelian Genomics. The NimbleGen data set was downloaded from the Sequence Read Archive (SRA) from a previous study as part of the Simons Foundation Research Autism Initiative (Sanders *et al.*, 2012).

The SureSelect, Nextera and Nimblegen data sets were analysed in house to produce alignment files in BAM format using Cpipe (Sadedin *et al.* 2015). The TruSeq data set was produced and analysed at the Broad Institute using the institute's standard analysis pipeline, also based on GATK.

## Results

### Ximmer Simulations

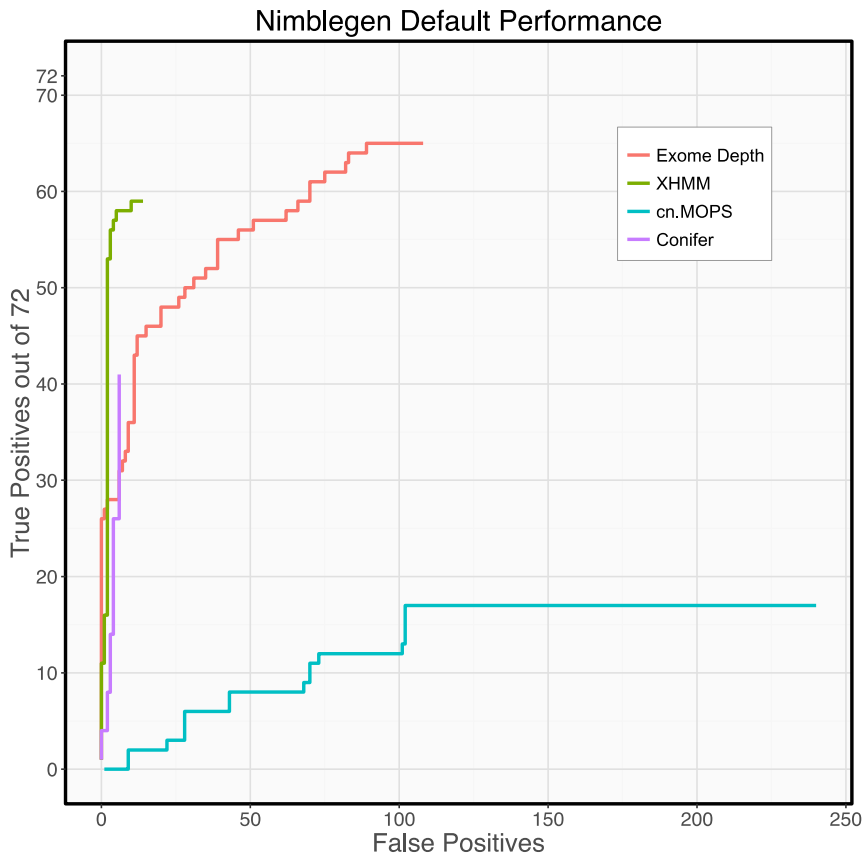
In order to demonstrate Ximmer we applied it to four different exome datasets with a variety of different properties (Table 2). We configured Ximmer to simulate between 2 and 10 deletions

1 per sample using the X-replacement method in each of the four datasets. As the X-replacement  
2 method was employed, deletions were simulated only in the X chromosome of female samples  
3 from each respective data set. The number of simulated CNVs ranged from 72 - 144 for each  
4 dataset (see supplementary material). The simulated deletions spanned between 100bp and  
5 6.9kbp of targeted bases, equating to genomic spans of between 471bp and 4.3Mbp.  
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## 8 9 **Comparison of CNV Detection Methods with Default Settings**

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11 First we used Ximmer to compare the accuracy of the four different CNV detection methods.  
12 Parameters for each tool were set to their defaults, except for cases where the tool setting was  
13 clearly misaligned to the simulated data. Specifically, the cn.MOPs minimum CNV width was  
14 lowered to 1, and XHMM mean number of targets were lowered to 3 to better match the  
15 generally smaller size of deletions included in the simulation.  
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19 In the Nimblegen data set, we observe that there were significant differences between the  
20 performance of the different CNV callers (Figure 3). ExomeDepth achieved substantially better  
21 absolute sensitivity than any other tool, finding 88% of all the simulated deletions compared to  
22 XHMM finding 57%. However, the precision of ExomeDepth was poor (54%) compared to  
23 XHMM (93%). A substantial difference in precision persisted even when ExomeDepth results  
24 were filtered to equivalent sensitivity as XHMM. Therefore in this case the optimal caller is a  
25 trade off between sensitivity and specificity. Both cn.MOPs and Conifer performed poorly in  
26 terms of sensitivity, each finding less than 30% of simulated deletions. cn.MOPs has very poor  
27 precision in this data set (0.5%), and appears to output many very high confidence calls that are  
28 ranked higher than the true positives it detects.  
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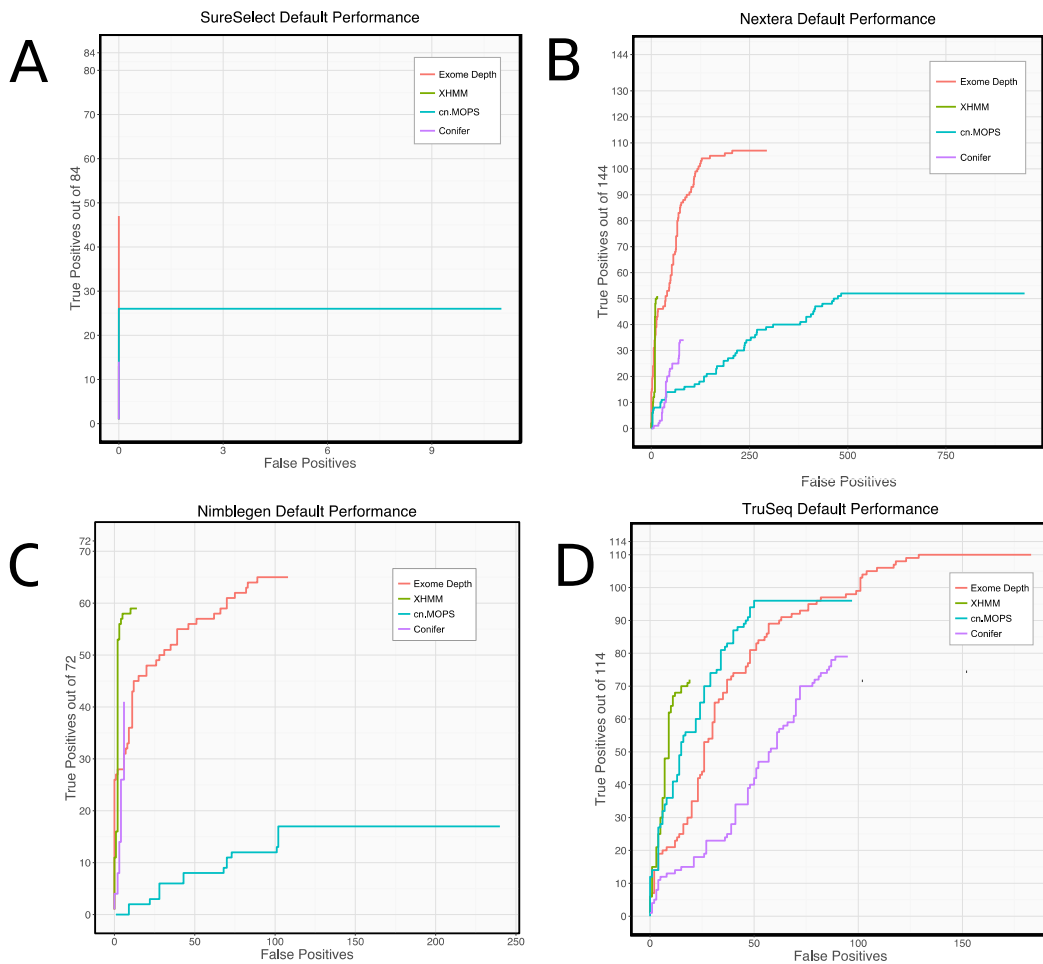
**Figure 3:** Performance of CNV callers on Nimblegen data with default settings. Performance differs greatly between callers. ExomeDepth has significantly higher sensitivity than any other caller, while Conifer and XHMM have significantly better precision.

### Comparison between Data Sets

We next compared Ximmer results using the four CNV callers with default settings on all four data sets. Our results (Figure 4) show that individual methods have marked differences in performance on different data sets. For example, all callers exhibited a low false positive rate when applied to the SureSelect data (fewer than 10 false positive calls for any caller), but showed much higher false positive rates on Nextera data (ExomeDepth and cn.MOPs both having more than 200 false positive calls). cn.MOPs performed poorly on the SureSelect, Nimblegen and Nextera data, detecting very few true and many false CNVs. However cn.MOPs is arguably the best caller for the TruSeq data, having a lower false positive rate than ExomeDepth and Conifer, but higher sensitivity than XHMM. These differences suggest that some data sets are better suited to the algorithms or default settings of particular calling methods.

Despite the differences, some aspects of individual caller performance were consistent across all datasets: XHMM consistently achieved the lowest false positive rate for a given sensitivity, and conversely ExomeDepth consistently achieved higher total sensitivity than any other caller.

In some respects differences between datasets are consistent between callers. With SureSelect data (30x mean coverage), no caller could achieve more than 60% sensitivity. However with TruSeq data (90x mean coverage), all callers found more than 60% of the simulated deletions, and ExomeDepth found nearly all deletions (96%).



**Figure 4** ROC-style Curve with Default Parameters - Count of true positives vs false positives as ranked results are filtered by varying quality score threshold, when the four CNV calling methods are executed on four different data sets with their default parameters. Performance is highly variable both between different methods on the same data set, and between the same method on different data sets

It is likely that homogeneity of the data is an important factor in determining these characteristics: data sets having very low inter-sample variation with few significant batch effects may work well with callers that apply relatively little normalisation or are flexible in their normalisation approach.

Overall our results suggest that each data set has individual characteristics that affect the performance of each CNV caller differently. Consequently, there is no single best CNV detection tool for all data sets. Depending on the priorities of the investigation, and the particular data set

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2 in question, a different tool or combination of tools may be more appropriate. Therefore users  
3 should assess their own data and choose CNV detection methods using Ximmer.

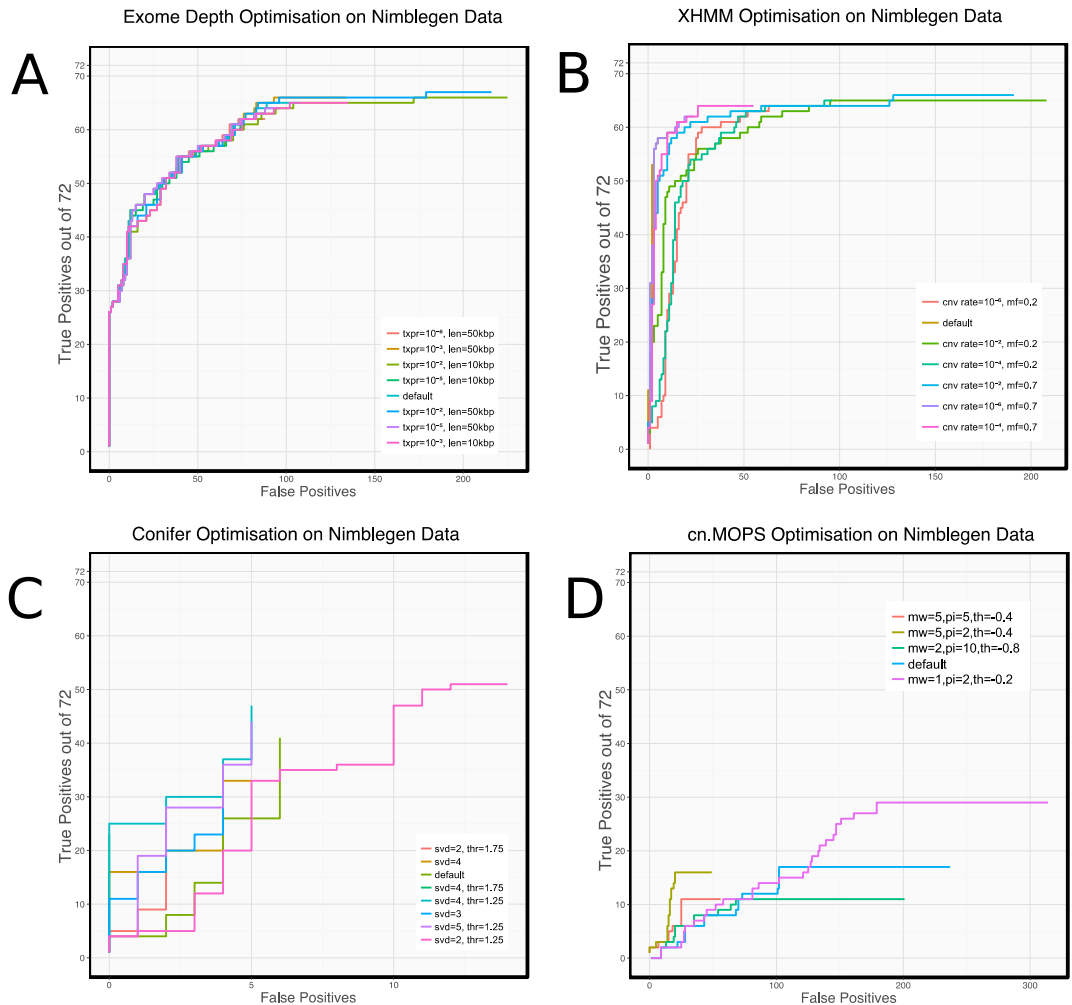
## 4 **CNV Calling performance can be improved with parameter optimisation**

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7 We next configured Ximmer to re-analyse the Nimblegen data while varying several configurable  
8 parameters of each CNV caller. The parameters varied were chosen by reviewing the  
9 documentation and experimenting to find those having the largest direct effect on sensitivity  
10 (Table S2).  
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13 We found that adjusting two parameters (the exome-wide CNV rate to  $10^{-4}$  and the  
14 normalisation factor to 0.2), increasedXHMM sensitivity (Figure 5A) by 21% (67% to 88%) with  
15 an acceptable loss of precision (81% to 55%). Similarly, we evaluated alternative values for the  
16 SVD number and calling threshold for Conifer (Figure 5C), and found that, by adjusting the  
17 calling threshold parameter from 1.5 down to 1.25, sensitivity could be improved from 25% to  
18 40% with only a small sacrifice in precision. cn.MOPs adjustments were able to improve  
19 sensitivity from 19% to 36% by adjusting the prior impact parameter from 5 to 2, and the calling  
20 threshold upwards from -0.8 to -0.4. Although we tried varying two parameters (transition  
21 probability and expected CNV length), ExomeDepth appeared to have nearly optimal parameters  
22 as its defaults for this data set.  
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32 This analysis demonstrates that tuning parameter settings should be considered an important  
33 element of usage of CNV detection tools, and can lead to significantly improved accuracy. Many  
34 previous comparison studies (Hong *et al.*, 2016; Tan *et al.*, 2014) have been evaluated without  
35 rigorous optimisation of parameters. Our results suggest that the discrepancies in the results  
36 from these studies may have been reduced if calling parameters were optimised.  
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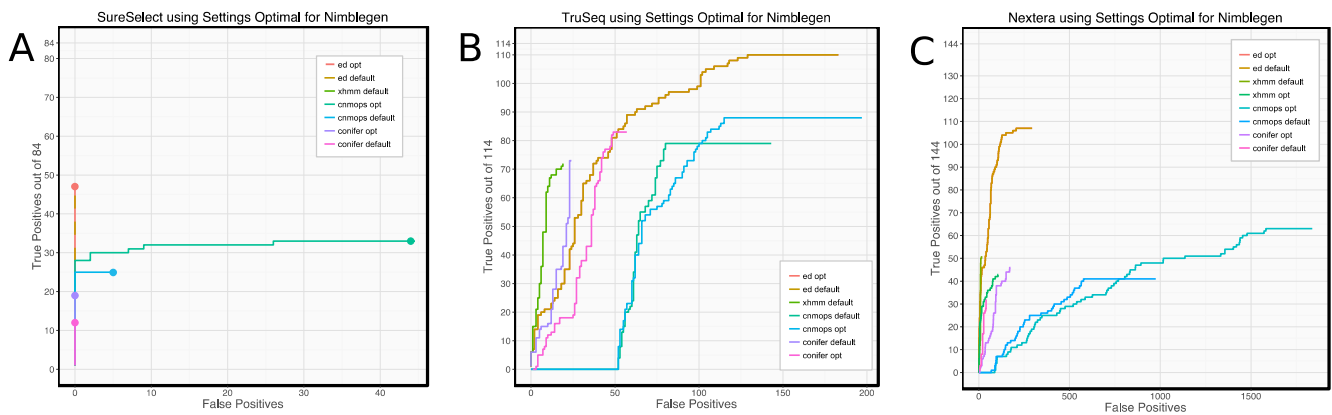
**Figure 5** Results of adjusting CNV calling parameters on ROC-style curves. XHMM, Conifer and cn.MOPS all have configurations where sensitivity or precision can be substantially improved: reducing Conifer calling threshold to 1.25 increases sensitivity from 25% to 40%; increasing the exome-wide CNV rate to  $10^{-4}$  and reducing the normalisation factor from 0.7 to 0.2 increases XHMM sensitivity from 67% to 88%; Reducing the cn.MOPS prior impact factor to 2 and raising the calling threshold to -0.4 allowed sensitivity to nearly double (from 24% to 36%), however these settings caused a substantial reduction in precision.

## Optimisation of Parameters across Data Sets

We applied the optimised settings derived from simulation performance on Nimblegen data to the analysis of the other three data sets. However, we observed that these settings are not optimal for every other data set. For example, on the SureSelect and TruSeq data (Figure 6A, 6B), XHMM achieves both high sensitivity (85%) and precision (80%) with the default settings, but produces no calls at all with the optimised settings. The optimisations increase sensitivity in cn.MOPS, however, the marginal increase (69% to 77%) is much less significant than for Nimblegen data, and causes a substantially higher number of false positive calls. Similarly

Conifer also shows a much smaller proportional increase in sensitivity (64% to 73%) and experiences a significant fall in precision (75% to 59%).

We conclude that optimisation needs to be performed on each data set or data type separately. Ximmer supports this process efficiently and easily through modifying simple configuration settings.



**Figure 6** Performance of other data sets (A: SureSelect, B: TruSeq, C: Nextera) when analysed using parameters optimal for Nimblegen data (opt), compared to default settings (default). Nimblegen-optimised parameters are frequently unsuitable on other datasets. XHMM is severely compromised by the Nimblegen settings on all datasets: SureSelect and TruSeq data, produce no XHMM CNV calls, while both sensitivity and precision are poorer in Nextera data. Conifer and cn.MOPs both gain in sensitivity, but by a much smaller proportion and with a larger inflation of false positive calls than with Nimblegen data.

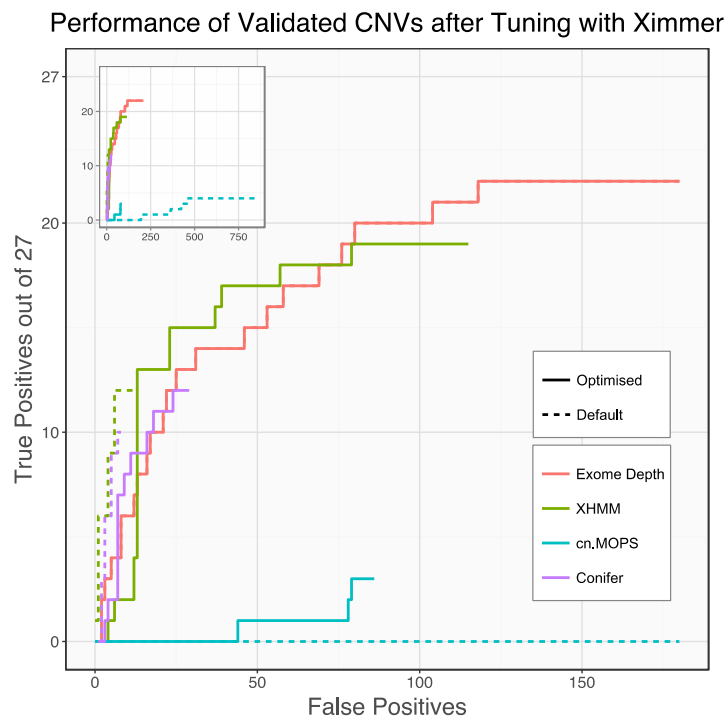
## Application of Ximmer to a set of Validated CNVs

We extracted a set of validated CNVs for the samples that were captured in the Nimblegen data set from a previous study by N. Krumm et al. (2015). After filtering to include only CNVs overlapping autosomal target regions of the exome capture, 25 validated CNVs remained. We then tested detection of these CNVs from the exome data, first using default parameters as described above for each of the four CNV callers. With the exception of XHMM, the sensitivity estimated by Ximmer using simulation approximately reflected the sensitivity observed on the validated CNVs (Table 2). In the case of XHMM we suspect that differences in the composition of the CNV sizes between the simulation and the validated CNV set may partially account for the discrepancy. Precision is harder to evaluate as predictions of CNVs in regions not in our validated set could be true deletions. However, the number of total detections varied greatly between callers (Conifer and XHMM having fewer than 12, compared to ExomeDepth and cn.MOPS having more than 200), as predicted by Ximmer.

**Table 2** Estimated, actual and improved sensitivity for validated CNVs from Krumm et al. (2015). The predicted sensitivities reflect the actual sensitivity well for all callers exception XHMM.

Caller	Predicted Sensitivity	Actual Sensitivity	Improved Sensitivity
ExomeDepth	88%	90%	90% (0%)
XHMM	82%	48%	76% (+28%)
Conifer	57%	40%	48% (+8%)
cn.MOPS	24%	16%	12% (-3%)

We next applied the optimised settings identified previously through simulation to improve sensitivity for ExomeDepth, XHMM and Conifer. Due to the poor precision observed with the default settings for cn.MOPS, we chose to improve precision rather than sensitivity. By reviewing the sample counts plot, we identified that a significant fraction of the putative false positive calls were concentrated in just 3 out of 20 samples (Supplementary Figure S7). Therefore we excluded these samples from the analysis for cn.MOPS.



**Figure 7** Comparison before and after applying adjusted parameters to improve performance. XHMM and Conifer sensitivity are substantially improved (+28%, +8%) with minimal loss of precision, while no improvement was possible for ExomeDepth. cn.MOPS precision was substantially improved by excluding poor quality samples identified by Ximmer's sample counts plot

1 The results after incorporating parameter adjustments suggested by Ximmer show substantially  
2 improved performance of several methods (Figure 7). For example, sensitivity was improved in  
3 XHMM (+28%) and Conifer (+8%). Conversely, removing the three poor quality samples from  
4 cn.MOPs slightly lowered sensitivity, but removed 90% (856) of the false positive CNV calls.  
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## 9 **Conclusion**

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11 While there is great utility in detecting CNVs from WES data, adoption of CNV detection methods  
12 in practice has met with significant challenges. These are primarily centred around highly  
13 unpredictable performance and lack of reproducibility between data sets. We have addressed  
14 these challenges by creating Ximmer, a tool that facilitates efficiently assessing and improving  
15 the accuracy of WES-based CNV detection methods. Our comparison of four different data sets  
16 analysed by four different CNV calling methods represents one of the most comprehensive  
17 evaluations to date. Our results show, consistent with previous studies, that there is significant  
18 variability in performance of CNV detection between tools and between data sets. We conclude  
19 that to effectively use these methods, attention must be applied to understand and optimise  
20 their behavior on each individual data set. Ximmer can be used to automate these procedures,  
21 avoiding a significant burden. In addition, we have demonstrated that Ximmer can produce  
22 valuable insights into the quality of data sets for CNV calling and the behavior of CNV detection  
23 tools. As the first tool offering combined simulation, evaluation, tuning and interpretation of  
24 results from CNV detection methods, we believe Ximmer will assist increasing practical adoption  
25 of CNV detection methods for exome data. Ximmer is open source and available at  
26 <http://ximmer.org>. An example Ximmer report can be viewed online at  
27 <http://example.ximmer.org>.  
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## 43 **Competing Interests**

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46 The authors declare no competing interests.  
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## 51 **Ethics, consent and permissions**

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54 Not applicable.  
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## Availability of Data and Material

The SureSelect, Nimblegen and Nextera datasets are available from SRA under accessions SRP132744, SRP010920 and SRP148622 respectively. The NimbleGen dataset is available from dbGaP under accession phs001272.

## Authors' Contributions

SS conceived of and implemented the method, performed simulation work and drafted the manuscript. AO advised on design, implementation and interpretation of results, and edited the manuscript. SM and JE collected samples, extracted DNA, arranged sequencing and provided feedback on the manuscript. All authors approved the final manuscript.

## Additional Files

### Additional File 1

A set of figures, tables and supplementary methods supporting the results. Details of the simulation method. Description of methodology for optimising tuning parameters of data sets. **Table S1.** Description of confidence measures used to rank CNV calls. **Table S2.** Parameters selected for optimisation of CNV detection methods. **Figures S3 – S5.** ROC-style curves for optimisation of CNV detection methods. **Figure S7.** Sample count QC plot showing frequency of CNV calls for each sample across different cn.MOPs configurations.

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**Supplementary Material**

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