as 15% of the reference C57BL/6J mouse genome may be found. A significant level of variation between strains, such that as much as 15% of the mouse genome may be found duplicated, or lost, in the genome of one individual or strain with respect to another. CNVs are thought to contribute significantly to phenotypic differences between mouse strains. In humans, CNVs have been causally linked to a range of disorders including schizophrenia (Moon et al., 2006), autism (Sebat et al., 2007) and birth defect syndromes (Lu et al., 2008). These studies have found a significant level of variation between strains, such that as much as 15% of the reference C57BL/6J mouse genome may be found as CNVs in another strain. While array CGH can be an effective way of identifying CNVs, aCGH studies are limited in resolution by the number of probes that can be placed on a microarray. The widespread adoption of short read sequencing platforms has led to a rapid decrease in the cost of whole-genome re-sequencing making it a viable alternative to array CGH (Xie and Tammi, 2009). Hidden Markov Models (HMM) have previously been used to detect copy number variation from array CGH data (Cahan et al., 2008; Fridlyand et al., 2004). We have developed a HMM to detect CNVs in inbred strains from the alignments of short read sequences to a reference genome.

1 INTRODUCTION

Copy number variants (CNVs) are segments of DNA that have been duplicated, or lost, in the genome of one individual or strain with respect to another. CNVs are thought to contribute significantly to phenotypic differences between mouse strains. In humans, CNVs have been causally linked to a range of disorders including schizophrenia (Moon et al., 2006), autism (Sebat et al., 2007) and birth defect syndromes (Lu et al., 2008). High-resolution surveys for CNVs have been performed in common laboratory strains of mice using array-comparative genomic hybridization (array CGH) (Cahan et al., 2009; Cutles et al., 2007; Gunibert et al., 2007; Hrnich et al., 2009; She et al., 2008). These studies have found a significant level of variation between strains, such that as much as 15% of the reference C57BL/6J mouse genome may be found as CNVs in another strain.

Our algorithm proceeds in three stages. First, the sequence reads are aligned to the mouse reference genome (build NCBI 37, Mouse Genome Sequencing Consortium, Waterston et al., 2002) using the MAQ aligner (Li et al., 2008). MAQ calls SNPs and classifies them as homozygous or heterozygous. Summary statistics are computed for the sequence read depth, the number of heterozygous SNPs and the average number of hits per read over 1 kb windows of the reference genome sequence. This triplet of data for each 1 kb region of the reference genome is input to the HMM which classifies each region as corresponding to a gain, loss or no change in copy number.
We evaluated our calls against a collection of previously published aCGH copy number variation data (Cahan et al., 2009; Cutler et al., 2007; Henrichsen et al., 2009; She et al., 2008).

Our algorithm called 22 copy number gains (1.38 Mb of sequence) and 21 losses (0.49 Mb) for the AJ data set (see Fig. 1 and Supplementary Fig. 6 for example regions). The gain regions overlap 38% of the regions identified by aCGH (36% by sequence, 1.1 Mb). Seventy-seven percent of the gains and losses were previously seen by aCGH. For CAST/EiJ, 45 gains (2.44 Mb of sequence) and 30 losses (1.16 Mb) were called. The gain regions overlap 76% of the gains and losses called by aCGH (79% by sequence, 1.3 Mb). Thirty-six percent of the gains found by aCGH were previously seen in the array CGH data set. This figure is much lower than that of AJ due to the fact that the CAST/EiJ strain was not used in the highest coverage aCGH study (Cahan et al., 2009). In both strains the regions of copy number loss called by our algorithm and aCGH differed widely (11% concordance by region for AJ and 52% for CAST/EiJ) owing to the relative difficulty of calling CNV losses compared to gains. We performed qPCR validation on a subset of both the gain calls that were novel to our algorithm (those not found by aCGH) and the novel gain calls found by aCGH. In total we attempted validation on 20 novel cnD gains, of which five were confirmed to be amplified relative to C57BL/6J. Of the 14 novel aCGH gains that we attempted to validate, one was confirmed to be a gain relative to C57BL/6J. Our concordance with array CGH and initial confirmation rates are similar to previously published copy number variation studies (Conrad et al., 2009; Redon et al., 2006; Scheret et al., 2007). Full details of the experimental validation are provided in the Supplementary Data.

2.1 The HMM

We developed a 10-state HMM of the copy number structure of the genome being sequenced. There are five major states of the model, representing normal sequence, a 2-fold increase in copy number, a 3-fold increase in copy number, a 2-fold decrease in copy number and zero copy number. In addition, each major state of the model has a sub-state corresponding to highly repetitive sequence, allowing the model to accommodate the frequent high-copy repeat elements dispersed throughout mammalian genomes. In all states expect for the repeat states the depth distribution is modeled by a normal distribution with the mean and variance reflecting the copy number of the state. For states representing a copy number gain, the heterozygous SNP rate is modeled by a negative binomial distribution. The heterozygous SNP rate model is modeled by a Poisson distribution in all other states. More information about the HMM and emission distributions is given in the supplemental material.

The parameters of the model are learned for each chromosome in the input data set by Viterbi training for both the transition probabilities and emission distribution parameters (Durbin et al., 1998). After the model parameters have been determined, the sequence of states is computed by a final application of the Viterbi algorithm. The output of the Viterbi algorithm is processed to extract contiguous regions of gain or loss. The minimum threshold for detection is the input window size, typically one kilobase. There is a final optional filtering step to remove calls below a minimum size threshold.

3 RESULTS

We tested our model on Illumina short read sequence data from chromosome 17 for the AJ and CAST/EiJ strains of mouse that were sequenced to 22- and 34-fold, respectively (ERA accession number ERA000077). The data sets were generated using 36-bp paired-end reads of 200-bp insert libraries. For this experiment, we set a minimum call size threshold of 10 kb (see Supplementary Data).

We evaluated our calls against a collection of previously published aCGH copy number variation data (Cahan et al., 2009; Cutler et al., 2007; Henrichsen et al., 2009; She et al., 2008).

Our algorithm called 22 copy number gains (1.38 Mb of sequence) and 21 losses (0.49 Mb) for the AJ data set (see Fig. 1 and Supplementary Fig. 6 for example regions). The gain regions overlap 38% of the regions identified by aCGH (36% by sequence, 1.1 Mb). Seventy-seven percent of the gains and losses were previously seen by aCGH. For CAST/EiJ, 45 gains (2.44 Mb of sequence) and 30 losses (1.16 Mb) were called. The gain regions overlap 76% of the gains and losses called by aCGH (79% by sequence, 1.3 Mb). Thirty-six percent of the gains found by aCGH were previously seen in the array CGH data set. This figure is much lower than that of AJ due to the fact that the CAST/EiJ strain was not used in the highest coverage aCGH study (Cahan et al., 2009). In both strains the regions of copy number loss called by our algorithm and aCGH differed widely (11% concordance by region for AJ and 52% for CAST/EiJ) owing to the relative difficulty of calling CNV losses compared to gains. We performed qPCR validation on a subset of both the gain calls that were novel to our algorithm (those not found by aCGH) and the novel gain calls found by aCGH. In total we attempted validation on 20 novel cnD gains, of which five were confirmed to be amplified relative to C57BL/6J. Of the 14 novel aCGH gains that we attempted to validate, one was confirmed to be a gain relative to C57BL/6J. Our concordance with array CGH and initial confirmation rates are similar to previously published copy number variation studies (Conrad et al., 2009; Redon et al., 2006; Scheret et al., 2007). Full details of the experimental validation are provided in the Supplementary Data.

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