preciseTAD: A machine learning framework for precise 3D domain boundary prediction at base-level resolution

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Abstract

High-throughput chromosome conformation capture technology (Hi-C) revealed extensive DNA looping and folding into discrete 3D domains. These include Topologically Associating Domains (TADs) and chromatin loops, the 3D domains critical for cellular processes like gene regulation and cell differentiation. The relatively low resolution of Hi-C data (regions of several kilobases in size) prevents precise mapping of domain boundaries. However, the high resolution of genomic annotations associated with boundaries, such as CTCF and members of cohesin complex, suggests they can inform the precise location of domain boundaries. Several methods attempted to leverage genome annotation data for predicting domain boundaries; however, they overlooked key characteristics of the data, such as spatial associations between an annotation and a boundary, and a much smaller number of boundaries than the rest of the genome (class imbalance).

We developed *preciseTAD*, an optimized random forest model to improve the location of domain boundaries. Trained on high-resolution genome annotation data and boundaries from low-resolution Hi-C data, the model predicts the location of boundaries at base-level resolution. We investigated several feature engineering and resampling techniques (random over- and undersampling, Synthetic Minority Over-sampling TEchnique (SMOTE)) to select the most optimal data characteristics and address class imbalance. Density-based clustering and scalable partitioning techniques were used to identify the precise location of boundary regions and summit points. We benchmarked our method against the Arrowhead domain caller and a novel chromatin loop prediction algorithm, Peakachu, on the two most annotated cell lines.

We found that spatial relationship (distance in the linear genome) between boundaries and annotations has the most predictive power. Transcription factor binding sites outperformed other genome annotation types. Random under-sampling significantly improved model performance. Boundaries predicted by *preciseTAD* were more enriched for CTCF, RAD21, SMC3, and ZNF143 signal and more conserved across cell lines, highlighting their higher biological significance. The model pre-trained in one cell line performs well in predicting boundaries in another cell line using only genomic annotations, enabling the detection of domain boundaries in cells without Hi-C data.

Our study implements the method and the pre-trained models for precise domain boundary prediction using genome annotation data. The precise identification of domain boundaries will improve our understanding of how genomic regulators are shaping the 3D structure of the genome. *preciseTAD* R package is available on https://dozmorovlab.github.io/preciseTAD/ and Bioconductor (submitted).

Introduction

The advent of chromosome conformation capture (3C) sequencing technologies, and its successor Hi-C, have revealed a hierarchy of the 3-dimensional (3D) structure of the human genome such as chromatin loops [1], Topologically Associating Domains (TADs) [2,3], and A/B compartments [4]. At the kilobase scale, chromatin loops connect gene promoters with distal enhancers, thereby regulating gene expression [5,6]. At the megabase scale, TADs represent regions on the linear genome, that are highly self-interacting. Disruption of boundaries demarcating TADs and loops promotes cancer [7,8] and other disorders [9–11]. Identifying the precise location of TAD and chromatin loop boundaries remains a top priority in our goal to fully understand the functionality of the human genome.

Several methods have been proposed to identify genomic coordinates of TADs (reviewed in [12,13]), and chromatin loops [1,14–16]. However, a key limitation among most of these TAD- and loop callers is that they depend on Hi-C data *resolution*. Resolution refers to the size of genomic regions (bins) used to segment the linear genome [17]. Lower resolution contribute to larger bin size, which leads to increased uncertainty in the location of boundaries. Due to high sequencing costs, most available Hi-C datasets have relatively low resolution, ranging from 25 kb to 100 kb. While these low-resolution Hi-C datasets can be used to probe higher-order genomic patterns such as A/B compartments, more refined resolutions are necessary to accurately define TAD and loop boundaries [18]. Because increasing the resolution of Hi-C data requires a quadratic increase in the total sequencing depth [19], obtaining high resolution remains difficult.

Another limitation among domain callers is that they disregard prior knowledge about functional genomic annotations associated with TAD and loop boundaries, referred hereafter as domain boundaries. The insulator binding protein, CTCF, and additional cofactors such as SMC3 and RAD21 have been identified as components of the loop extrusion model [18,20–24]. Furthermore, distinct patterns of histone modifications have also been shown to be present at boundaries [3,4,25]. These genomic annotations are obtained using chromatin immunoprecipitation followed by high-throughput DNA sequencing (ChIP-seq). The resolution of ChIP-seq experiments is typically on the order of tens to hundreds of bases [26], well below the resolution of Hi-C data (tens of kilobases; 750 bp is the highest resolution of Hi-C data to date [27]). Therefore, leveraging precisely mapped genomic annotations in a supervised machine learning framework provides the possibility of more precisely predicting the locations of domain boundaries.

We present *preciseTAD*, an optimally tuned machine learning framework for precise identification of domain boundaries using genome annotation data. Our method utilizes the random forest (RF) algorithm trained on high-resolution chromatin state data (BroadHMM), histone modifications (HM), and transcription factor binding sites (TFBS) data to predict low-resolution boundaries. We introduce a systematic pipeline for building the optimal boundary prediction classifier. We found that spatial associations (linear distance) between boundaries and annotations perform best, transcription factor binding sites further improve prediction accuracy, and a simple random undersampling technique effectively addresses the negative effect of class imbalance. Translated from Hi-C data resolution level to base level (annotating each base and predicting its boundary probability), *preciseTAD* employs density-based clustering (DBSCAN) and partitioning around medoids (PAM) to detect genome annotation-guided boundary regions and points at a base-level resolution. This approach circumvents resolution restrictions of Hi-C data, allowing for the precise detection of biologically meaningful boundaries. We demonstrate that *preciseTAD* predictions are more enriched for known molecular drivers of 3D chromatin, including CTCF, RAD21, SMC3, and ZNF143. Further, we show that *preciseTAD* boundaries are more conserved across cell lines, and the model trained in one cell line can predict boundaries in another cell line. This improved precision in the domain boundary location can provide insight into the association between genomic regulators and the 3D genome organization. The *preciseTAD* R package is freely available on https://dozmorovlab.github.io/preciseTAD/ and Bioconductor (submitted).

Methods

Data sources

Publicly available TAD and loop boundaries called by Arrowhead [28] and Peakachu [16] tools, respectively, were used for training. Arrowhead TAD boundaries were called from primary Hi-C data for the GM12878 and K562 cell lines [1]. The contact matrices were constructed from all read pairs that uniquely aligned to the genome (MAPQ>0). The default parameters for Arrowhead were used when extracting the autosomal genomic coordinates (GRCh37/hg19 human genome assembly) of TAD boundaries at 5 kb, 10 kb, 25 kb, 50 kb, and 100 kb resolutions (Additional File 1). Peakachu chromatin loop boundaries called at 10 kb for the GM12878 and K562 cell lines were downloaded from the Yue lab website (Supplementary Table 1). Unique boundaries were considered as the midpoints within the coordinate of each chromatin loop anchor. Chromosome 9 was excluded from all downstream analyses due to the sparsity of contact matrices at 5 kb and 10 kb resolutions for the K562 cell line. Cell-line-specific genomic annotations (BroadHMM chromatin states (BroadHMM), histone modifications (HM), and transcription factor binding sites (TFBS)) were obtained from the UCSC Genome Browser Database [29] (Supplementary Table 2).

Shifted-binning for binary classification

In Hi-C, each chromosome is binned into non-overlapping regions of length r, typically, 5 kb and above. The r parameter defines the resolution of Hi-C data. Here, we designed a strategy called *shifted binning* that partitions the genome into regions of the same length r, but with middle points corresponding to boundaries defined by the original binning.

To create shifted binning, the first shifted bin was set to start at half of the resolution r and continued in intervals of length r until the end of the chromosome ($mod\ r + r/2$). The shifted bins, referred hereafter as bins for simplicity, were then defined as boundary-containing regions ($Y = 1$) if they contained a TAD (or loop) boundary, and non-boundary regions ($Y = 0$) otherwise, thus establishing the binary response vector ($\mathbf{Y}$) used for classification (Figure 1A).

Feature engineering

Cell-line-specific genomic annotations were used to build the predictor space. Bins were annotated by one of either the number of overlaps with an annotation (*Overlap Count (OC)*), the percent of overlap between the bin and the total width of genomic annotation regions overlapping it (*Overlap Percent (OP)*), the average signal strength of the corresponding annotation (*Peak Signal Strength*), or the distance in bases from the center of the bin to the center of the nearest genomic annotation region (*Distance*) (Figure 1B). A ($log_2 + 1$)-transformation of distance was used to account for the skewness of the distance distributions. Models built using a *Peak Signal Strength* predictor space were only composed of histone modifications and transcription factor binding sites because BroadHMM chromatin states lack signal values.

Addressing class imbalance

To assess the impact of class imbalance (CI), defined as the proportion of boundary regions to non-boundary regions, we evaluated three resampling techniques. These include *Random Over-Sampling (ROS)*, *Random Under-Sampling (RUS)*, and *Synthetic Minority Over-Sampling Technique (SMOTE)*. For ROS, the minority class was sampled with replacement to obtain the same number of data points in the majority class. For RUS, the majority class was sampled without replacement to obtain the same number of data points in the minority class. For SMOTE, under-sampling was performed without replacement from the majority class, while over-sampling was performed by creating new synthetic observations using the $k = 5$ minority class nearest neighbors [30] (implemented in the `DMwR` v.0.4.1 R package). We restricted the SMOTE algorithm to 100% over-sampling and 200% under-sampling, which creates perfectly balanced classes.

Establishing optimal data level characteristics for TAD boundary region prediction

Random forest (RF) classification models (the `caret` v.6.0 R package [31]) were built to compare model performances between combinations of data resolutions, feature engineering procedures, and resampling techniques. A *holdout chromosome* technique was used for model evaluation on each autosomal chromosome. The ith holdout chromosome was identified and a data matrix, $A_{N\times(p+1)}$, was constructed by combining the binned genome from the remaining chromosomes $(1, 2, \cdots, i-1, i+1, \cdots, 21, 22)$, where $N = [n_1 n_2 \cdots n_{21} n_{22}]'$ and n_k is the length of chromosome k after being binned into non-overlapping regions of resolution r, such that $k \neq i$. p, the number of annotations, together with the response vector $\mathbf{Y}$, defined the column-wise dimension of the matrix A. Re-sampling was then performed on A, and an RF classifier was trained using 3-fold cross-validation to tune for the number of annotations to consider at each node (*mtry*). The number of trees (*ntree*) that were aggregated for each RF model was set to 500. The minimum number of observations per root node (*nodesize*) was set to .01% of the rows in the data. The binned data for the holdout chromosome i was reserved for testing. Models were evaluated using Balanced Accuracy (BA), defined as the average of sensitivity and specificity:

$$BA = \frac{1}{2}\left(sensitivity + specificity\right) = \frac{1}{2}\left(\frac{TP}{TP+FN} + \frac{TN}{TN+FP}\right)$$

where TP refers to the number of bins correctly identified as containing a boundary (true positives), FP refers to the number of bins incorrectly identified as containing a boundary (false positives), TN refers to the number of bins correctly identified as not containing a boundary (true negatives), and FN refers to the number of bins incorrectly identified as not containing a boundary (false negatives). Each of these quantities is obtained from the confusion matrix created by validating the model on the test data. The process was repeated for each ith holdout chromosome, and performances were aggregated using mean and standard deviation.

Feature selection and predictive importance

Many genomic annotations, notably architectural proteins, tend to exhibit an extensive pattern of colocalization (correlation) [32]. To suitably reduce the predictor space and improve computational efficiency, while maintaining optimal performance, we implemented recursive feature elimination (RFE). In doing so, we estimated the near-optimal number of necessary features to include in the best fitting RF model. The best performing subset was identified, ranging from 2 to the maximum number of features incremented by the power of 2. We then aggregated the predictive importance of the union of the optimal set of features across chromosomes using the mean decrease in node impurity among permuted features in out-of-bag samples to determine the most common and top-ranked annotations for predicting boundary regions.

Evaluating performance across cell lines

When evaluating performance across cell lines, a random forest model was built using Arrowhead/Peakachu-detected boundaries ("ground truth") and genomic annotations from one cell line such that the data matrix was constructed using genomic bins from $n-1$ chromosomes. The test data was constructed from the binned data of the holdout chromosome with genomic annotations from the other cell line. The predicted boundaries were evaluated for overlap with the "ground truth" boundaries from the that other cell line. This process was repeated for each holdout chromosome. To evaluate performance, we constructed receiver operating characteristic (ROC) curves composed of the average sensitivities and specificities and reported the area under the curve (AUC).

Boundary prediction at the base-level resolution using *preciseTAD*

To investigate whether we could alleviate the resolution limitations of conventional domain calling tools, we developed *preciseTAD*. This algorithm leverages a random forest model in conjunction with density-based and partitioning techniques to predict boundaries at base resolution. A random forest model was built on the optimal combination of predictor type (predictor space), resampling technique (class balancing correction), and top-ranked annotations (most informative genomic elements) to maximally accurately predict boundaries at the *Hi-C resolution level*.

To precisely identify boundary locations, we first constructed a *base-level resolution* predictor space for a particular chromosome i. We then apply our optimally trained RF model on the base-level predictor space to extract the probability vector, p_{n_i}, denoting each base's probability of being a boundary. Here, n_i represents the length of chromosome i. A threshold t specifies the probability at which a base with $p_{n_i} \geq t$ is designated as boundary (the default $t = 1$). Next, density-based spatial clustering of applications with noise (DBSCAN; version 1.1-5) is applied to the matrix of pairwise genomic distances between boundary-annotated bases. The resulting clusters of spatially colocalized bases predicted as being boundaries were termed *preciseTAD boundary regions* (PTBR). To precisely identify a single base among each PTBR, *preciseTAD* then implements partitioning around medoids (PAM) on the sub distance matrix derived from each cluster. The corresponding cluster medoid was defined as a *preciseTAD boundary point* (PTBP), making it the most representative base coordinate within each clustered PTBR.

The DBSCAN algorithm has two parameters, *MinPts* and *eps* (ϵ). The *MinPts* parameter was set to the recommended value of 3, representing $1 + dim(data)$ [33]. To decide on the optimal value of t and ϵ in *preciseTAD*, we considered the normalized enrichment N_e of flanked predicted boundaries, defined as

$$N_e = \frac{1}{b}\Sigma_{i=1}^{n} e_i$$

where e_i is the number ChIP-seq peak regions that overlap with the ith predicted boundary, b, flanked by resolution r. We evaluated N_e for combinations of $t = \{0.95, 0.975, 0.99, 1.0\}$ and $\epsilon = \{5000, 10000, 15000, 20000, 25000\}$. The heuristic of ϵ is that density-reachable bases with genomic distances less than ϵ should occupy the same designated cluster.

Comparing called vs. predicted boundaries

We assessed the biological significance of our predicted boundaries by their association with several known molecular drivers of 3D chromatin architecture, including CTCF, RAD21, SMC3, and ZNF143 ChIP-Seq peaks. To do so, we used the *computeMatrix*, *plotProfile*, and *plotHeatmap* tools offered by deepTools (version 2.0) [34]. Additionally, we compared the median log_2 genomic distances between TAD boundaries and the same top predictive ChIP-seq annotations using Wilcoxon Rank-Sum tests. Furthermore, we compared the overlap between predicted and called boundaries in GM12878 and K562 cell lines. Boundaries were first flanked by resolution, r, and overlaps were visualized using Venn diagrams from the `Vennerable` R package (version 3.1.0). Overlaps were further quantified using the Jaccard index defined as

$$J_{(A,B)} = \frac{A \cap B}{A \cup B}$$

where A and B represent genomic regions created by flanked called and predicted boundaries. That is, between cell lines, the number of overlapping flanked boundaries divided by the total number of flanked boundaries. Wilcoxon Rank-Sum tests were used to compare chromosome-specific Jaccard indices across cell lines, between *preciseTAD* boundaries and both Arrowhead and Peakachu boundaries, respectively. All statistical analyses were performed in R version 4.0.1. The significance level was set to 0.05 for all statistical tests.

Results

Developing an ML framework for optimal TAD boundary prediction

We developed a machine learning (ML) framework aimed at determining the optimal set of data level characteristics necessary for precise prediction of boundaries of Topologically Associating Domains (TADs) and chromatin loops, collectively referred to as domain boundaries. We chose the random forest (RF) algorithm as our binary classification tool. The reason for it is two-fold: (1) to devise a tunable prediction rule in a supervised learning framework that is both robust to overfitting and able to handle multiple correlated predictors, and (2) to allow for an interpretable ranking of predictors [35]. We use published Arrowhead-called TAD boundaries [36] and Peakachu-predicted loop boundaries [16] as ground truth. Data from GM12878 and K565 cell lines at 5-100kb resolution (Arrowhead) and 10kb resolution (Peakachu) were used (Supplementary Table 1). We next developed *preciseTAD*, an R package that leverages an optimally built RF to precisely predict genomic coordinates of TAD and loop boundaries at base resolution from cell-type-specific genome annotation data.

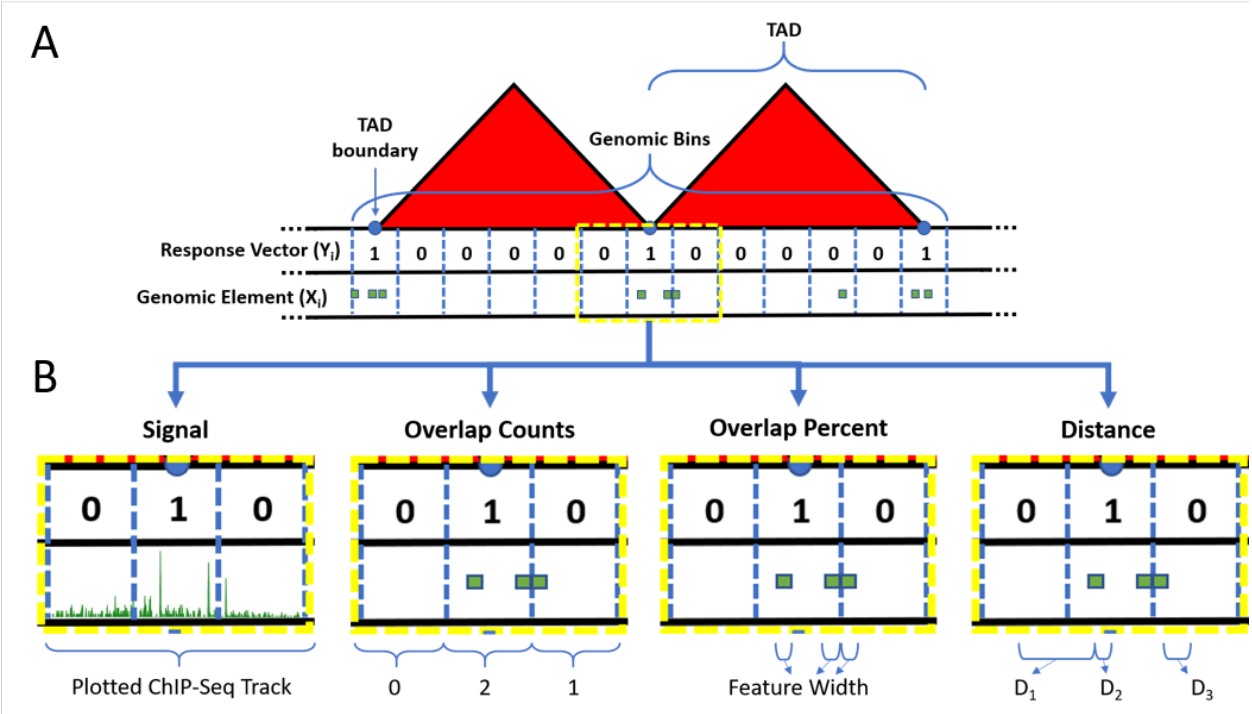

Figure 1: **Resolution-specific data construction and feature engineering used for downstream random forest modeling.** (A) The linear genome was binned into non-overlapping resolution-specific intervals (see Methods). The response vector **Y** was defined as 1/0 if a genomic bin overlapped/did not overlap with a TAD boundary. (B) Four types of associations between bins and genomic annotations (green shapes) were considered to build the predictor space including Average Peak Signal (Signal), Overlap Counts (OC), Overlap Percent (OP), and log_2 distance (Distance).

Boundary regions were defined as genomic bins containing a called boundary ($Y = 1$), while non-boundary regions were defined as bins that did not contain a called boundary ($Y = 0$) (Figure 1A, see Methods). The total number of called TADs, their unique boundaries, and the number of genomic bins expectedly decreased with the decreased resolution of Hi-C data (Table 1, Supplementary Table 3). The number of non-boundary regions highly outnumbered boundary regions. Such a disproportional presence of examples in one class is known as a "class imbalance" problem that negatively affects predictive modeling [37,38]. To address the class imbalance, we evaluated the effect of three resampling techniques. *Random over-sampling* (ROS) was

defined as sampling with replacement from the minority class (boundary regions). *Random under-sampling* (RUS) was defined as sampling with replacement from the majority class (non-boundary regions). Lastly, we tested *Synthetic minority over-sampling technique* (SMOTE), which is a combination of both random over- and under-sampling to create balanced classes [30] (see Methods).

Table 1: Domain boundary data and class imbalance summaries across resolutions for Arrowhead and Peakachu on the GM12878 cell line. Class imbalance is calculated as the ratio of total number of unique boundaries to the total number of genomic bins.

Tool	Resolution	Total number of called TADs/chromatin loops	Total number of unique TAD/chromatin loop boundaries	Total number of genomic bins	Class imbalance
Arrowhead	5 kb	8,052	15,468	535,363	0.03
Arrowhead	10 kb	7,676	14,253	267,682	0.05
Arrowhead	25 kb	4,670	8,363	107,073	0.08
Arrowhead	50 kb	2,349	4,224	53,537	0.08
Arrowhead	100 kb	1,031	1,883	26,768	0.07
Peakachu	10 kb	16,185	21,421	267,682	0.14

A total of 77 cell-line-specific genomic annotations were used to build the predictor space. These included BroadHMM chromatin state data, histone modifications (HM), and transcription factor binding sites (TFBS) (Supplementary Table 2). Four feature engineering procedures were developed to quantify the association between genomic annotations and bins (Figure 1B). These included signal strength association (Signal), direct (OC), proportional (OP), and spatial ($log_2 + 1$ Distance) relationships (see Methods, Supplementary Figure 1).

In total, we considered combinations of data from two cell lines L = *{GM12878, K562}*, five resolution R = *{5 kb, 10 kb, 25 kb, 50 kb, 100 kb}*, four types of predictor space P = *{Signal, OC, OP, Distance}*, and three re-sampling technique S = *{None, RUS, ROS, SMOTE}* (Figure 2). Once the model inputs were established, a random forest classifier was trained on $n - 1$ autosomal chromosomes, while reserving the ith chromosome for testing. Three-fold cross-validation was used to tune the *mtry* hyperparameter, while *ntree* and *nodesize* were fixed to default values of 500 and 1, respectively. Model performance was evaluated by aggregating the mean balanced accuracy (BA) across each holdout chromosome (see Methods). These strategies allowed us to select the best performing model in an unbiased manner.

Random under-sampling, distance-based predictors, and high-resolution Hi-C data provide optimal performance for boundary prediction

Expectedly, when using data with class imbalance present, that is, no resampling, the models exhibited low balanced accuracies, with minimal variability among different resolutions (Figure 3). Similarly, poor performances were found when using ROS. However, RUS and SMOTE re-sampling led to a drastic improvement in performance, especially at higher resolutions. We found that RUS marginally outperformed SMOTE as the optimal class balancing technique for all resolutions and predictor types when predicting TAD boundary regions.

Additionally, we found that using a distance-type predictor space yielded substantially higher balanced accuracy compared to the peak signal strength, overlap count, and overlap percent predictor types. As with class balancing techniques, this improvement was less evident at lower resolutions, with results consistent for K562 (Supplementary Figure 2). Random forest models built on Peakachu-defined loop boundaries with SMOTE resampling only marginally outperformed RUS for both GM12878 and K562 cell lines (Supplementary Figure 3). Furthermore, 5 kb resolution genomic bins led to the optimal prediction for both TAD

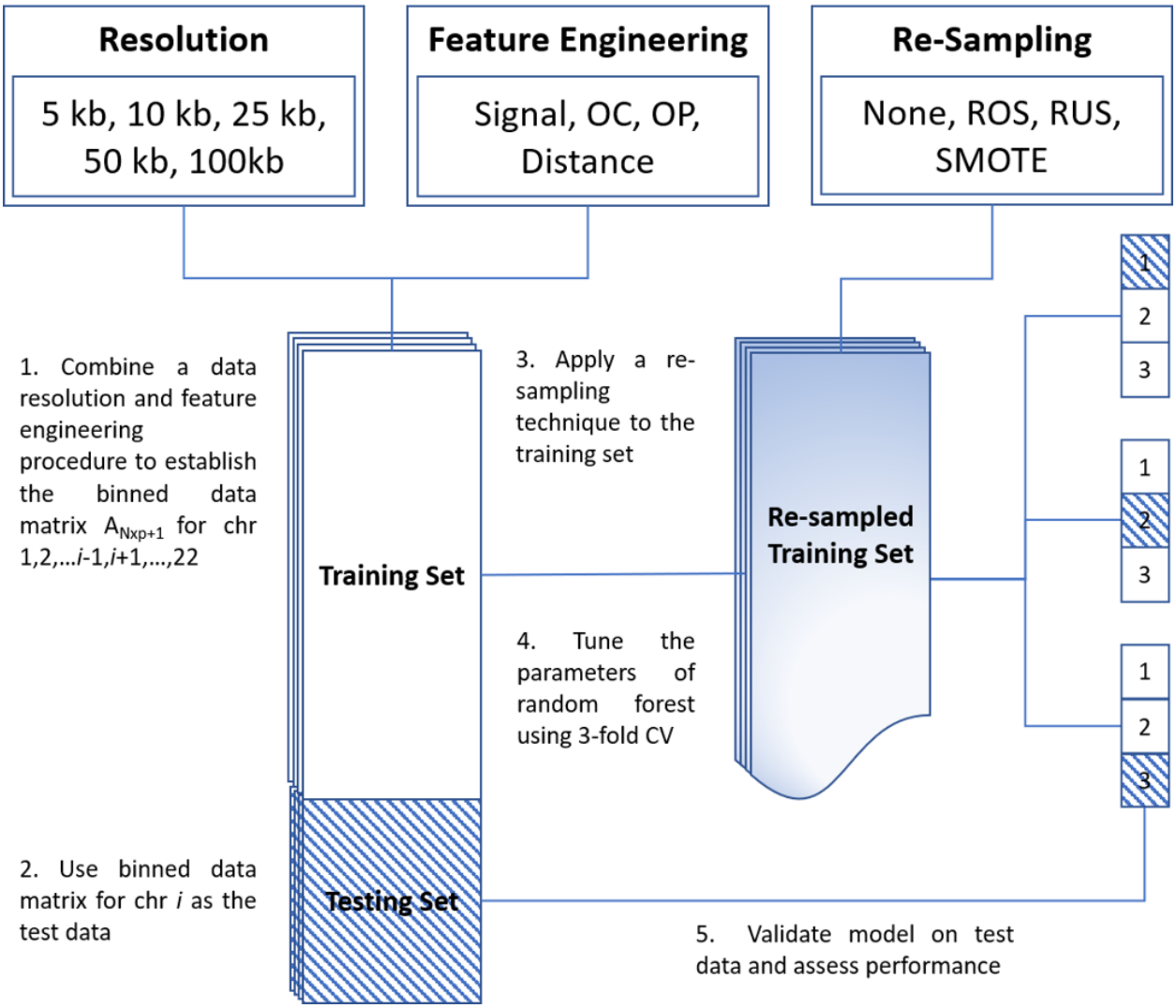

Figure 2: **A machine learning framework for building domain boundary region prediction models.** Step 1 employs a range of feature engineering techniques to define defines the predictor matrix $A_{N\times(p)}$, where N is the number of genomic bins, p is the number of genomic annotations, i is a holdout chromosome. The response vector Y_N is defined as a boundary region ($Y = 1$) if it overlaps with a genomic bin (else $Y = 0$). Step 2 is reserves the predictor-response matrix for the holdout chromosome i as the test data. Step 3 applies a resampling technique to the training data to address the class imbalance. Step 4 trains the random forest model and performs 3-fold cross-validation to tune the mtry parameter. Finally, step 5 validates the model on the separate test data composed of the binned data from the holdout chromosome i and to evaluate model performance using balanced accuracy (BA).

and loop boundaries, on both cell lines. Our results indicate that random under-sampling, distance-type predictors, and high-resolution Hi-C data provide the optimal set of data level characteristics for both TAD and chromatin loop boundary prediction.

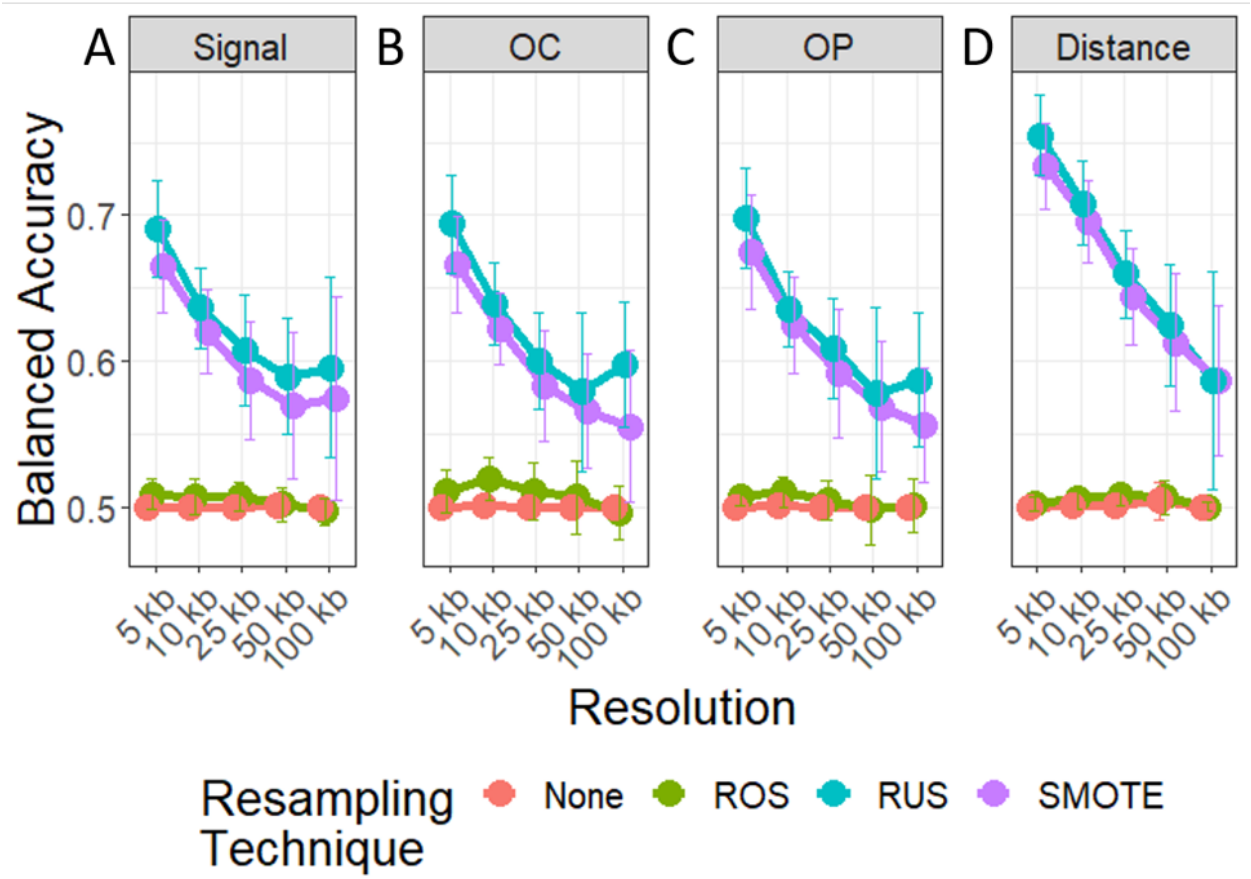

Figure 3: **Distance-type predictors combined with random under-sampling at 5 kb resolution genomic bins yielded optimal TAD boundary region predictive performance for GM12878 Arrowhead TAD boundaries.** Averaged balanced accuracies are compared across resolution, within each predictor-type: (A) Signal, (B) OC, (C) OP, and (D) Distance, and across resampling techniques: no resampling (None; red), random over-sampling (ROS; green), random under-sampling (RUS; blue), and synthetic minority over-sampling (SMOTE; purple). Error bars indicate standard deviation away from the mean performance across each holdout chromosome used for testing.

Transcription factor binding sites outperform histone- and chromatin state-specific models

We hypothesized that the type of genomic annotations may also affect predictive performance. Using the established optimal settings (RUS, Distance, 5 kb bins), we built separate random forest models using BroadHMM chromatin state data, histone modifications (HM), and transcription factor binding sites (TFBS). We found that models built on TFBSs outperformed other annotation-specific models, with results consistent for loop boundaries, on both cell lines (Figure 4A; Supplementary Figures 4A). These results suggest that TFBS are the primary drivers of TAD and loop boundary formation in both GM12878 and K562.

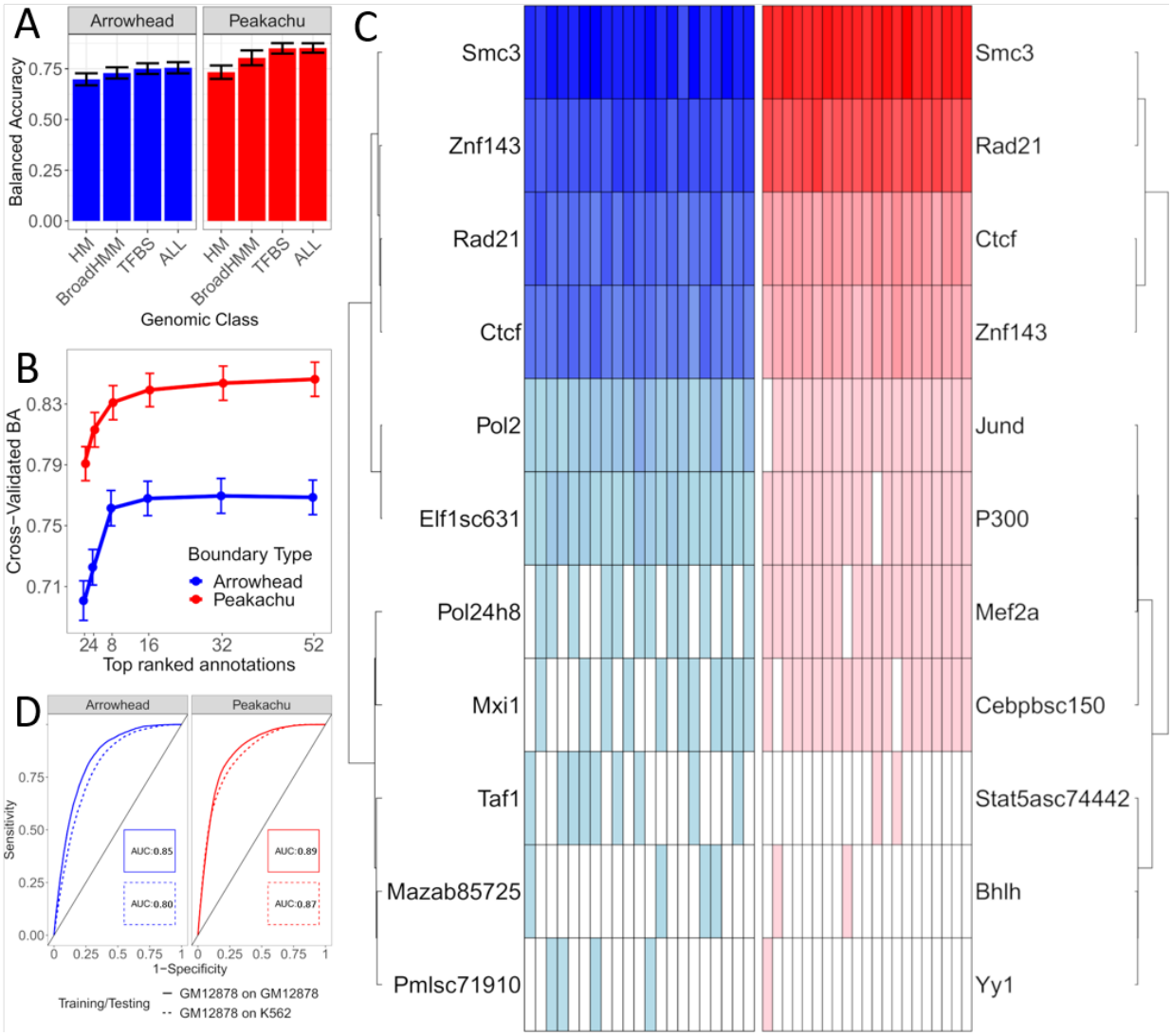

Figure 4: **Binding of SMC3, RAD21, CTCF, and ZNF143 transcription factors accurately predicts TAD and loop boundaries in GM12878 cell line.** (A) Barplots comparing performances of TAD (Arrowhead) and loop (Peakachu) boundary prediction models using histone modifications (HM), chromatin states (BroadHMM), transcription factor binding sites (TFBS), in addition to a model containing all three classes (ALL). (B) Recursive feature elimination (RFE) analysis used to select the optimal number of predictors. Error bars represent 1 standard deviation from the cross-validated accuracy across each holdout chromosome. (C) Clustered heatmap of the predictive importance for the union of the top 8 most predictive chromosome-specific TFBSs. The columns represent the holdout chromosome excluded from the training data. (D) Receiver operating characteristic (ROC) curves and subsequent area under the curves (AUCs) when training and testing on the same cell line versus across cell lines. The curves represent the average sensitivities and specificities across each holdout chromosome.

Predictive importances confirmed the biological role of CTCF, RAD21, SMC3, and ZNF143 for boundary formation

We sought to further optimize our boundary region prediction models by recursive feature elimination to avoid overfitting and selecting only the most influential TFBS across all autosomal chromosomes. We were able to obtain near-optimal performance using approximately eight TFBS (Figure 4B; Supplementary Figures 4B). However, given that we trained our model on chromosome-specific data, the most significant annotations varied for each chromosome set. To determine transcription factors most important for boundary prediction across all chromosomes, we clustered the predictive importance (mean decrease in accuracy) of the top eight significant TFs across chromosomes. We found four transcription factors, CTCF, RAD21, SMC3, and ZNF143, being consistently predictive of TAD and loop boundaries (Figure 4C; Supplementary Figures 4C). We optimized our model by only considering these top four TFBS when building the random forest model, thereby decreasing computational burden while maintaining high predictive performance. In summary, our model was able to yield the known molecular drivers of the loop extrusion model [18,20–24].

Training in one cell line accurately predicts boundary regions in other cell lines

Having demonstrated the optimal performance in our framework's ability to predict boundary regions, we next attempted to predict boundary regions in one cell line using the model pre-trained on data from another another cell line (See Methods). We found that training and testing using Arrowhead ground truth TAD boundaries and genomic annotation data from GM12878 cell line resulted in an average AUC=0.845 (Figure 4D). The performance of this model only slightly decreased when evaluated using K562-specific Arrowhead boundaries and genomic annotation data (AUC = 0.803). Likewise, the performance of the model trained using Peakachu boundaries and genomic annotation data from GM12878 cell line was comparable when evaluated using K562-specific Peakachu boundaries and genomic annotations (AUC=0.885 and 0.868, respectively). These results were consistent when training on K562 and testing on GM12878 data (Supplementary Figure 4D). In summary, our observations suggest that a model trained on data from one cell line performs well when using the data from another cell line.

preciseTAD identifies precise and biologically relevant domain boundaries

Using our optimally built random forest model trained on Arrowhead/Peakachu boundaries, we attempted to predict boundaries at base-level resolution. Intuitively, instead of bin-level annotations, the predictor-response space was built on a base-level, that is, each base was associated with a genomic annotation. The model trained on a bin-level space was then applied on a base-level space to predict each base's probability of being a boundary. Our method, referred to as *preciseTAD*, uses density-based spatial clustering (DBSCAN) and partitioning around medoids (PAM) to predict the location of boundary regions and summit points (highest boundary probability) at base-level resolution (see Methods).

When trained using Arrowhead and Peakachu ground truth boundaries at 5 kb and 10 kb, respectively, the *preciseTAD* model predicted a total of 7,846 TAD and 15,838 chromatin loop boundaries in GM12878, as well as 8,883 TAD and 11,016 chromatin loop boundaries in K562 cell line (Supplementary Table 4). We reported less predicted TAD boundaries at 5 kb than Arrowhead on both cell lines (Table 1, Supplementary Table 3). This can be attributed to Arrowhead's inflation of called TADs, that, when visualized, often do not correspond to domains enriched in internal interactions (Figure 5A) and signal of known drivers of domain boundaries (Supplementary Figure 5). *preciseTAD* also predicted fewer chromatin loop boundaries than Peakachu (Table 1, Supplementary Table 3). This can be attributed to the fact that *preciseTAD* leverages four known drivers of 3D chromatin, including CTCF, RAD21, SMC3, and ZNF143. This is in contrast to Peakachu, which considers only CTCF sites to mark the predicted boundaries as true positives. These results suggest that genome annotation-driven predicted boundaries may be more biologically relevant than those detected from Hi-C data alone.

Next, we evaluated the biological significance of *preciseTAD* boundaries. Peak regions associated with known molecular drivers of 3D chromatin, including CTCF, RAD21, SMC3, and ZNF143, colocalized more

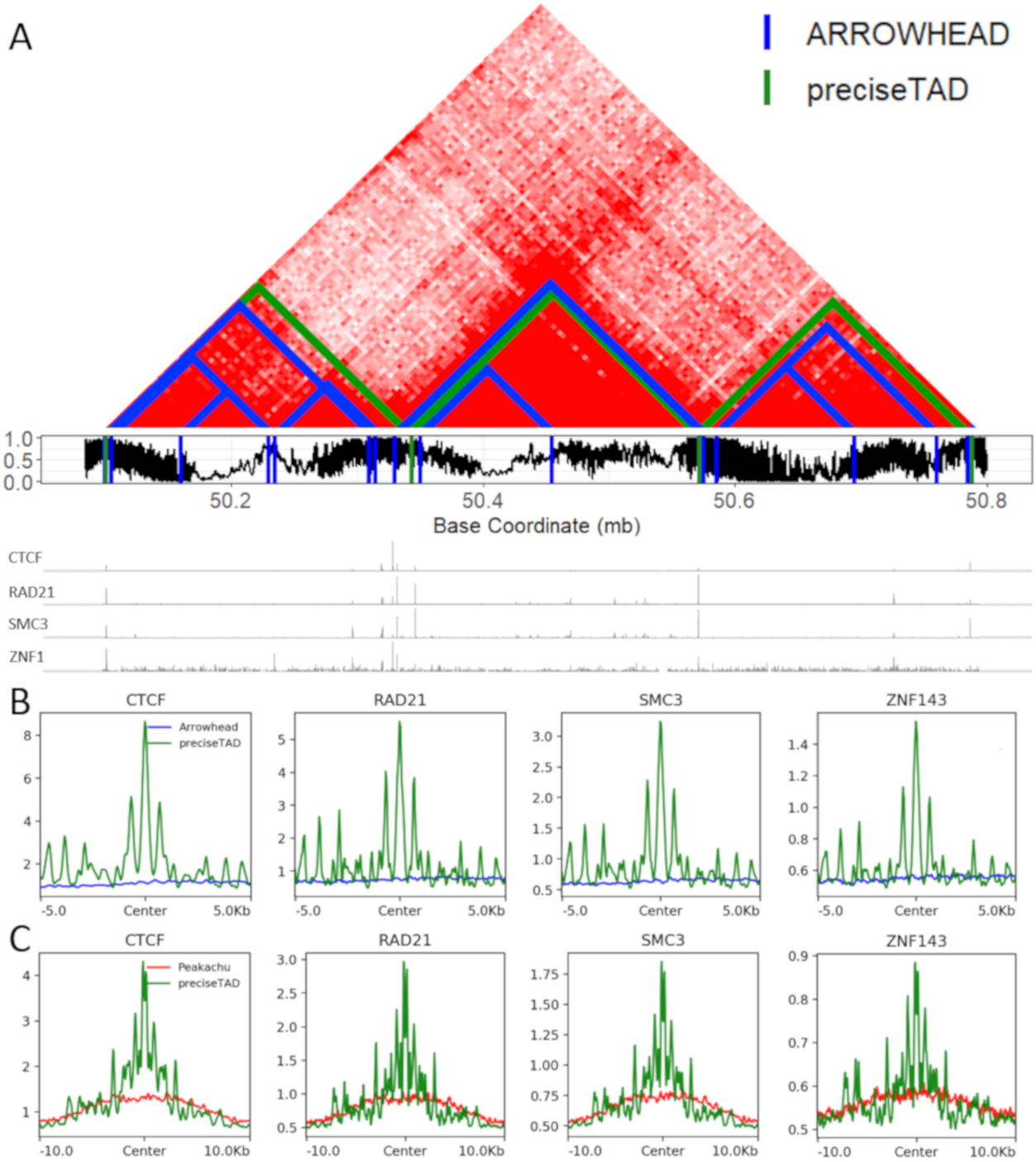

Figure 5: **preciseTAD boundaries are enriched for known molecular drivers of 3D chromatin.** (A) The location of Arrowhead-called TAD boundaries (blue) vs. *preciseTAD*-predicted TAD boundaries (green) on GM12878 data (chr14:50085000-50800000). The black line represents the predicted probability of each base being a TAD boundary. (B, C) Profile plots comparing the signal strength of CTCF, RAD21, SMC3, and ZNF143 around Arrowhead-called boundaries (blue, B), Peakachu loop boundaries (red, C) vs. *preciseTAD*-predicted boundaries (green).

frequently around *preciseTAD*-predicted TAD and loop boundaries compared to Arrowhead-called TAD and Peakachu loop boundaries respectively (Figure 5B, C; Supplementary Figure 5A, B). Surprised by the poor signal distribution at the Arrowhead boundaries, we compared signals centered on boundaries called by Arrowhead, Peakachu, and our SpectralTAD [39] TAD caller. We confirmed the poor signal distribution around Arrowhead boundaries, in contrast to the well-performing Peakachu- and SpectralTAD-called boundaries (Supplementary Figure 5C, D). Signal heatmaps indicated that *preciseTAD*-predicted boundaries were more enriched for the same genomic annotations than either Arrowhead or Peakachu boundaries alone (Supplementary Figures 6-7). *preciseTAD* boundaries were statistically significantly closer to the top-ranked TFBSs (Wilcoxon p-value < 0.001 versus Arrowhead and Peakachu boundaries, respectively, Figure 6A, Supplementary Figure 8). These results indicate that *preciseTAD*-predicted boundaries better reflected the known biology of boundary formation.

preciseTAD boundaries are more conserved across cell lines

Previous studies suggest that TAD boundaries are conserved across cell lines [2,3,40,41]. To assess the level of cross-cell-line conservation, we evaluated the overlap between cell line-specific boundaries detected by *preciseTAD*, Arrowhead, and Peakachu. Boundaries involving Arrowhead were flanked by 5 kb, while boundaries involving Peakachu were flanked by 10 kb. We found that 46%/63% of *preciseTAD*-predicted domain boundaries using models trained on Arrowhead/Peakachu data, respectively, were conserved between GM12878 and K562 cell lines. This is in contrast to only 26% and 63% for Arrowhead and Peakachu boundaries themselves (Figure 6B). The better conservation of *preciseTAD*-predicted boundaries further supports the notion of their higher biological relevance.

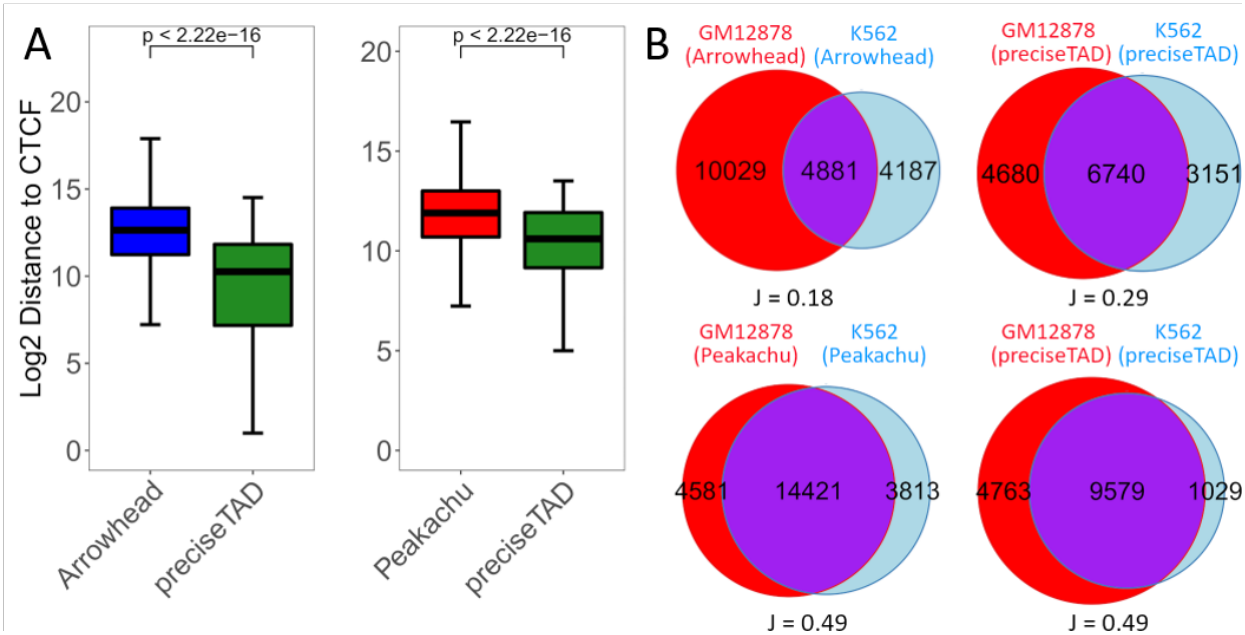

Figure 6: **preciseTAD-predicted boundaries are both statistically significantly closer to CTCF sites and more conserved across cell lines**. (A) Log2 genomic distances from called and predicted boundaries to nearest CTCF sites. The p-values are from the Wilcoxon Rank Sum test. (B) Venn diagrams illustrating the levels of conservation between domain boundaries for GM12878 (red) and K562 (blue) cell lines identified by Arrowhead-trained *preciseTAD* model (top left), Arrowhead boundaries themselves (top right), Peakachu-trained *preciseTAD* model (bottom left), and Peakachu boundaries themselves (bottom right). J - the Jaccard Index evaluating the similarity between each set of boundaries flanked by resolution.

Boundaries predicted by *preciseTAD* models trained on TAD and loop boundaries are highly overlapping

The majority of boundaries predicted by the Arrowhead-trained *preciseTAD* model represented a subset of the larger group of boundaries predicted by the Peakachu-trained model. We found that 86.8% and 92.6% of our predicted TAD boundaries were overlapped by predicted loop boundaries for GM12878 and K562, respectively (Supplementary Figure 9). This is expected as loop boundaries (Peakachu) are more abundant, while TAD boundaries (Arrowhead) represent the narrower level of the 3D chromatin organization. Together with the results in Figure 4, the high overlap between boundaries predicted by Arrowhead- and Peakachu-trained models suggests that TAD and loop boundaries may be driven by similar molecular mechanisms.

Discussion

We present *preciseTAD*, a machine learning approach for precise prediction of TAD or loop boundaries from functional genomic annotations. *preciseTAD* leverages a random forest (RF) classification model built on low-resolution domain boundaries obtained from domain calling tools, and high-resolution genomic annotations as the predictor space. *preciseTAD* predicts the probability of each base being a boundary, and identifies the precise location of boundary regions and the most likely boundary points. *preciseTAD* was benchmarked against two boundary calling tools, Arrowhead [28], an established TAD-caller, and Peakachu [16] a recently published algorithm for predicting chromatin loops. We first optimized our RF model by systematically comparing different combinations of genome binning (resolution), feature engineering procedures, and resampling techniques. Our results demonstrate that genomic distance between boundary regions and genomic annotations coupled with random under-sampling results in best model performance. We show that binding of four transcription factors (SMC3, RAD21, CTCF, ZNF143) is sufficient for accurate boundary predictions. *preciseTAD* is implemented as an R package, provides the pre-trained models, and allows for precise prediction of boundaries for any cell type for which the genomic annotations are available.

Our machine learning framework yielded several interesting observations. We first demonstrated that RF models built using *distance*-type predictors outperformed models built on previously published feature engineering techniques, including signal strength, overlap counts, and overlap percents [42–45]. We further demonstrated that class imbalance hinders boundary prediction, but can be effectively addressed by a simple random under-sampling technique, an aspect of boundary prediction unaddressed in previous studies [42–44]. We showed that information about only four transcription factors (CTCF, SMC3, RAD21, ZNF143) is necessary and sufficient for accurate boundary prediction, outperforming histone modification- and BroadHMM-built models [43,44]. These are known components of the loop extrusion model, an established theory of how loops are made by a ring-shaped adenosine triphosphatase-driven complex called cohesin [18,20–24]. Interestingly, the same transcription factors accurately predicted both TAD and loop boundaries, suggesting a similarity of the mechanisms of TAD and loop formation. This suggested that the random forest model, when tuned and feature engineered correctly, is highly effective in predicting biologically relevant domain boundaries.

Guided by both low-resolution Hi-C data and high-resolution genome annotation data, *preciseTAD* predicts base-level resolution boundaries, alleviating resolution limitations of Hi-C data. We show that, unlike Arrowhead, *preciseTAD* does not inflate the number of predicted boundaries, providing only the most biologically meaningful boundaries that demarcate regions of high inter-chromosomal interactions. *preciseTAD* boundaries were enriched for known architectural transcription factors including CTCF, RAD21, SMC3, and ZNF143, supporting recent observations that TADs and loops may be generated by similar mechanisms [20]. Likewise, *preciseTAD* boundaries were more conserved between GM12878 and K562 cell lines, a known feature among the 3D architecture of the human genome, further highlighting their biological significance.

preciseTAD offers flexibility in controlling both the number of predicted boundaries and the distance between them. The two primary parameters are the probability threshold t and ϵ (referred to as eps; parameter of DBSCAN). The combination of these two quantities changes the resulting number of predicted boundaries from *preciseTAD*. Lower values of t and ϵ will result in more clusters of bases, and therefore, more boundaries.

As a heuristic, we evaluated the pairwise combination of four different thresholds ($t = (0.95, 0.975, 0.99, 1.0)$) and four different *eps* values ($\epsilon = (r, 2r, 3r, 4r, 5r)$)), where $r = 5000$, the optimal resolution. We found that the normalized overlaps - calculated as the total number of ChIP-seq peaks that overlapped within a given flanked boundary, divided by the number of boundaries - between top TFBS sites and flanked *preciseTAD* boundaries were maximized for combinations of $t = 1.0$ and $eps = r$ (Supplementary Figure 10).

A limitation of *preciseTAD* is that it is dependent on the "ground truth" boundaries provided by a domain caller. Given the wide variety of domain callers and their variable performance [12,13], defining "ground truth" boundaries is challenging. Ultra-deep Hi-C sequencing [27] and newer technologies for precise mapping of chromatin interactions (e.g., Micro-C [46]), coupled with more precise technologies for genomic annotation profiling (e.g., CUT&RUN for precise mapping of transcription factor binding sites) will help to refine the location and the genomic signatures of the "ground truth" boundaries. In the current work, we feel that the total number of domain boundaries is sufficient to guide learning of the association between genomic annotations and boundaries for precise boundary predictions. Indeed, models trained on the larger number of Peakachu-predicted boundaries performed better than those trained on Arrowhead boundaries. Although we provide models trained on both boundary types, we recommend Peakachu-trained models for the base-level prediction of domain boundaries.

Another limitation of *preciseTAD* is that it does not distinguish boundary types. The hierarchical nature of TAD boundaries [39,47,48] is not considered by *preciseTAD* due to the lack of gold standard of TAD hierarchy. *preciseTAD* also does not consider the directionality of CTCF binding [49] as it predicts individual boundaries in contrast to pairs of convergent CTCF motifs marking individual domains. Recent research distinguishes CTCF-associated boundaries, CTCF-negative YY1-enriched boundaries, CTCF- and YY1-depleted promoter boundaries, and the fourth class of weak boundaries largely depleted of all three features [46]. Furthermore, actively transcribed regions can serve as TAD boundaries themselves, independently of CTCF binding [49]. This may lead to some TAD boundaries being undetected by *preciseTAD* despite being detected by domain callers. Our future work will involve incorporating the directionality of CTCF binding in predictive modeling, including additional predictor types, and defining separate models trained on different boundary types.

Using the provided pre-trained models, *preciseTAD* is able to identify precise domain boundaries using cell-type-specific genome annotation information of only four transcription factors, CTCF, SMC3, RAD21, ZNF143. Although the ENCODE, NIH Roadmap Epigenomics, FANTOM5, BLUEPRINT, and other members of the International Human Epigenome Consortium (IHEC) [50] have been actively cataloging cell-type-specific genome annotation datasets, these data remain very sparse. To date (September 2020), the ENCODE project has only five cell lines (GM12878, K562, H1-hESC, HeLa-S3, HepG2) with all four transcription factor genomic annotations. Efforts to impute missing cell-type-specific genome annotation information [51] started to appear. We envision the broader availability of cell-type-specific genomic annotations will enable a more systematic analysis of domain boundaries using *preciseTAD*.

In summary, we demonstrate that domain boundary prediction is a multi-faceted problem requiring consideration of multiple statistical and biological properties of genomic data. Simply considering the properties of Hi-C contact matrices ignores the fundamental roles of known molecular drivers of the 3D chromatin structures. Instead, we propose *preciseTAD*, a supervised machine learning framework that leverages both Hi-C contact matrix information and genomic annotations. Our method introduces three concepts - *shifted binning*, *distance*-type predictors, and *random undersampling* - which we use to build random forest classification models for predicting boundary regions. Our method can bridge the resolution gap between 1D ChIP-seq annotations and 3D Hi-C sequencing data for more precise and biologically meaningful boundary identification. We introduce *preciseTAD*, an open source R package for leveraging random forests to predict domain boundaries at base-level resolution. We hope that *preciseTAD* will serve as an efficient and easy-to-use tool to further explore the genome's 3D organization.

Supplementary Legends

Additional File 1. An example script for applying Arrowhead to in situ Hi-C data (HIC001-HIC018) to obtain chromosome-specific TAD boundaries on the GM12878 cell line at 5kb, 10kb, 25kb, 50kb, 100kb resolutions.

Supplementary Table 1. Data source for Hi-C matrices used to call topologically associating domains with Arrowhead, as well as loop boundaries obtained by Peakachu.

Supplementary Table 2. A complete list of genomic annotations used to build the predictor space for all downstream models. The GRCh37/hg19 human genome assembly was used. "Genomic Class" - broad category of genomic features, "Element" - names of genomic features, "Cell Type-Specific Source" - download URL specific to the cell line (not all annotations were provided by the same institutions).

Supplementary Table 3. Domain boundary data and class imbalance summaries across resolutions for Arrowhead and Peakachu on the K562 cell line. Class imbalance is calculated as the ratio of the total number of unique boundaries to the total number of genomic bins.

Supplementary Table 4. Genomic coordinates of *preciseTAD*-predicted boundary regions (PTBR) and points (PTBP) for GM12878 and K562, using Arrowhead TAD and Peakachu chromatin loop boundary coordinates as ground truth. For PTBRs, the start and end coordinates define the clusters of spatially proximal bases with the probability of being a boundary equal to 1. For PTBPs, the start and end (start+1) coordinates define the uniquely identified boundary coordinate within each PTBR. All coordinates are in hg19/GRCh37 reference genome. (Work in progress)

Supplementary Figure 1. Density curves of distances before (red) and after (blue) performing a log_2 transformation across 10 kb, 25 kb, 50 kb, and 100 kb data resolutions for both the (A) GM12878 and (B) K562 cell lines, respectively. Distances are measured as the number of bases from the center of a genomic bin to the nearest genomic annotation center. Each density curve represents an individual genomic annotation (77 total).

Supplementary Figure 2. Distance-type predictors combined with random under-sampling at 5 kb resolution genomic bins yielded optimal TAD boundary region predictive performance for K562 Arrowhead TAD boundaries. Averaged balanced accuracies are compared across resolution, within each predictor-type: (A) Signal, (B) OC, (C) OP, and (D) Distance, and across resampling techniques: no resampling (None; red), random over-sampling (ROS; green), random under-sampling (RUS; blue), and synthetic minority over-sampling (SMOTE; purple). Error bars indicate standard deviation away from the mean performance across each holdout chromosome used for testing.

Supplementary Figure 3. Distance-type predictors combined with random under-sampling or SMOTE re-sampling yielded optimal predictive performance Peakachu loop boundaries at 10 kb resolution. Averaged balanced accuracies are compared for A) GM12878 and B) K562 within each predictor-type: Signal, OC, OP, and Distance, and across resampling technique: no resampling (None; red), random over-sampling (ROS; green), random under-sampling (RUS; blue), and synthetic minority over-sampling (SMOTE; purple). Error bars indicate standard deviation away from the mean performance across each holdout chromosome used for testing.

Supplementary Figure 4. Binding of SMC3, RAD21, CTCF, and ZNF143 transcription factors accurately predicts TAD and loop boundaries in K562 cell line. (A) Barplots comparing performances of TAD (Arrowhead) and loop (Peakachu) boundary prediction models using histone modifications (HM), chromatin states (BroadHMM), transcription factor binding sites (TFBS), in addition to a model containing all three classes (ALL). (B) Recursive feature elimination (RFE) analysis used to select the optimal number of predictors. Error bars represent 1 standard deviation from the cross-validated accuracy across each holdout chromosome. (C) Clustered heatmap of the predictive importance for the union of the top 8 most predictive chromosome-specific TFBSs. The columns represent the holdout chromosome excluded from the training data. (D) Receiver operating characteristic (ROC) curves and subsequent area under the curves (AUCs) when training and testing on the same cell line versus across cell lines. The curves represent the average sensitivities and specificities across each holdout chromosome.

Supplementary Figure 5. Profile plots comparing the signal strength of top TFBS around flanked (A) Arrowhead called TAD boundaries (blue) and *preciseTAD*-predicted TAD boundaries (green), (B) Peakachu chromatin loop boundaries (red) and preciseTAD predicted loop boundaries (green) on K562, (C) Arrowhead called TAD boundaries (blue), Peakachu chromatin loop boundaries (red), and SpectralTAD called TAD boundaries (green) on GM12878 and (D) on K562.

preciseTAD boundaries are enriched for known molecular drivers of 3D chromatin. Profile plots comparing the signal strength of CTCF, RAD21, SMC3, and ZNF143 around Arrowhead-called boundaries (blue, A), Peakachu loop boundaries (red, B) vs. *preciseTAD*-predicted boundaries (green) on K562 data (chr14:50085000-50800000).

Supplementary Figure 6. *preciseTAD* boundaries are more enriched for known molecular drivers of 3D chromatin. Enriched heatmaps comparing the enrichment of (A) Arrowhead called TAD boundaries vs *preciseTAD*-predicted TAD boundaries, and (B) Peakachu loop boundaries vs *preciseTAD*-predicted loop boundaries around key GM12878-specific transcription factor binding sites known to contribute to the 3D chromatin architecture.

Supplementary Figure 7. *preciseTAD* boundaries are more enriched for known molecular drivers of 3D chromatin. Enriched heatmaps comparing the enrichment of (A) Arrowhead called TAD boundaries vs *preciseTAD*-predicted TAD boundaries, and (B) Peakachu loop boundaries vs preciseTAD predicted loop boundaries around key K562-specific transcription factor binding sites known to contribute to the 3D chromatin architecture.

Supplementary Figure 8. *preciseTAD* boundaries are spatially closer to known molecular drivers of 3D chromatin. Boxplots comparing the log2 genomic distances from predicted and called boundaries to the nearest (A) GM12878-specific and (B) K562-specific CTCF, RAD21, SMC3, and ZNF143 transcription factor binding sites. The p-value is derived from the Wilcoxon Rank Sum test.

Supplementary Figure 9. *preciseTAD*-predicted TAD boundaries using Arrowhead are nested within *preciseTAD*-predicted chromatin loop boundaries using Peakachu. Venn diagrams illustrating the agreement between boundaries predicted using Arrowhead- and Peakachu-trained models for (A) GM12878 and (B) K562. Domain boundaries were flanked by resolution (5 kb for models using Arrowhead and 10 kb for Peakachu).

Supplementary Figure 10. Linecharts illustrating the normalized overlap between known molecular drivers of 3D chromatin and resolution-flanked *preciseTAD*-predicted boundaries (trained on Arrowhead boundaries) for different combinations of thresholds (t) and epsilon parameter values (eps). Data from GM12878 cell line, chromosome 22, at 5kb resolution was used. Normalized overlap was calculated as the total number of ChIP-seq peaks that overlapped within a given flanked boundary, divided by the number of boundaries that were predicted.

Abbreviations

AUC - Area Under the Curve; BA - Balance Accuracy; bp - base pair; BroadHMM - BroadHMM chromatin state data; ChIP-seq - Chromatin Immunoprecipitation followed by high-throughput DNA sequencing; CI - Class Imbalance; DBSCAN - Density-Based Spatial Clustering of Applications with Noise; FN - False Negatives; FP - False Positives; Hi-C - High-throughput chromosome conformation capture technology; HM - Histone Modifications; kb - kilobase; ML - Machine Learning; OC - Overlap Counts; OP - Overlap Percents; PAM - Partitioning Around Medoids; PTBP - preciseTAD Boundary Point; PTBR - preciseTAD Boundary Region; RF - Random Forest; RFE - Recursive Feature Elimination; ROC - Receiver Operating Characteristic; ROS - Random Over-Sampling; RUS Random Under-Sampling; SMOTE - Synthetic Minority Over-sampling Technique; TAD - Topologically Associating Domains; TFBS - Transcription Factor Binding Sites; TN - True Negatives; TP - True Positives

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Conflict of Interest. None.

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