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Computational Prediction of Position Effects of Human Chromosome Rearrangements

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Abstract

Balanced and apparently balanced chromosome abnormalities (BCAs) have long been known to generate disease through position effects, either by altering local networks of gene regulation or positioning genes in architecturally different chromosome domains. Despite these observations, identification of distally affected genes by BCAs is oftentimes neglected, especially when predicted gene disruptions are found elsewhere in the genome. In this unit, we provide detailed instructions on how to run a computational pipeline that identifies relevant candidates of non-coding BCA position effects. This methodology facilitates quick identification of genes potentially involved in disease by non-coding BCAs and other types of rearrangements, and expands on the importance of considering the long-range consequences of genomic lesions.

Keywords

position effects; chromosome abnormality; cytogenetics; human genetics

Introduction

The compromised integrity of chromosome structure through translocations, inversions, insertions, deletion and duplications is associated with human disease through a variety of mechanisms (Kleinjan et al. 2001; Kleinjan and van Heyningen 2005; Zhang and Lupski 2015). Among the best characterized is the disruption of regulatory interactions between

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gene promoters and distal control elements such as enhancers, locus control regions, silencers, etc (Kleinjan et al. 2001; Kleinjan and van Heyningen 2005). In this context, even apparently balanced or balanced non-coding chromosome abnormalities (BCAs) may exert position effects associated with disease by inducing expression changes of neighboring genes without affecting their sequence. Well known examples of this phenomenon include translocations upstream and downstream of SOX9 linked to campomelic dysplasia (Velagaleti et al. 2005), and translocations downstream of PAX6, involved in congenital aniridia (Kleinjan et al. 2001; Velagaleti et al. 2005).

Because of the ever enlarging amount of sequencing information, the number of non-coding variants and rearrangements that need clinical interpretation has greatly increased (Ward and Kellis 2012; Zhang and Lupski 2015). As described in Ibn-Salem et al. 2014 and Zepeda-Mendoza et al. 2017 (Ibn-Salem et al. 2014; Zepeda-Mendoza et al. 2017), position effect analysis can in many cases enlighten the analysis of such variants. Zepeda-Mendoza et al. 2017 designed a computational pipeline that identifies and ranks potential position effect genes for chromosome rearrangements (either balanced or unbalanced) using publicly available enhancer, promoter and DNaseI hypersensitive sites (DHSs) datasets from the Encyclopedia of DNA Elements (ENCODE) project (2012), TAD boundaries (Dixon et al. 2012), predicted enhancer-promoter interactions (Thurman et al. 2012), as well as Human Phenotype Ontology (HPO) (Kohler et al. 2014) terms which tailors position effect gene ranking to those involved in a subject’s clinical phenotype. This unit provides a detailed method for running the position effect pipeline from Zepeda-Mendoza et al. 2017 to assess the contribution of distal genes in the generation of a clinical phenotype, and further allows for filtering of most relevant candidate genes. It offers examples and instructions that will provide clinical geneticists and researchers a quick and optimized way to identify additional genes in human disease.

Basic Protocol

The main purpose of this protocol is to identify potential position effect candidates related to particular clinical presentations in subjects with non-coding and apparently balanced chromosome rearrangements.

An example study case from Zepeda-Mendoza et al. 2017 is DGAP163 is a four-year-old boy with reported severe global developmental delay, absent speech, dysmorphic/distinctive facies, and hypospadias. As an infant he presented seizures, small left retinal coloboma, myopia, nystagmus, and he had a history of conductive hearing loss. He had normal fluorescence *in situ* hybridization (FISH) results for Smith Magenis syndrome (MIM#182290), DiGeorge syndrome (MIM#188400) and Velocardiofacial syndrome (MIM#192430), as well as a normal aCGH (1M Agilent array). Chromosome analysis revealed an apparently balanced translocation between chromosomes 2 and 14. His karyotype is 46,XY,t(2;14)(p23;q13)dn.arr(1–22)x2,(X,Y)x1 (Figure 1A). Genome sequencing revealed breakpoint locations to be chr2:39206240-39206242 and chr14:31717833-31717834.

To establish whether the translocation may generate position effects on neighboring genes that may be responsible for all or part of DGAP163's clinical phenotype, the position effect pipeline can be employed to determine the suitability of identified candidates (Figure 1B).

Necessary resources

Hardware

- Any standard workstation running iOS, Windows, or any Linux distribution with a minimum of 4Gb RAM can run the position effect pipeline.

Software

- The position effect prediction pipeline comprises a series of Perl, R and Java scripts that are run independently to categorize and rank different features of candidate genes. In order to run these scripts, it is recommended that users have access to a Linux-based operating system (i.e., iOS, Ubuntu, among others) or install the proper software additions. For more information on how to install Perl and R in Microsoft Windows refer to http://strawberryperl.com/, https://www.activestate.com/activeperl, and https://cran.r-project.org/bin/windows/base/rw-FAQ.html.

Files

- All the necessary files can be downloaded from the following GitHub repository: https://github.com/ibn-salem/position_effect

Download and unzip the prediction effect pipeline scripts

1 The pipeline is available for download at https://github.com/ibn-salem/position_effect. The downloaded file, position_effect-master.zip, needs to be decompressed and stored in a user-defined folder. To unzip the file, write the following command in a terminal:

```
unzip position_effect-master.zip -d destination_folder
```

You will also need to unzip the data folder contained within the position_effect-master file. This folder contains the GRCh37 annotation files that will be used throughout the protocol, including happloinsufficiency scores, chromatin contacts, gene annotations, among others. To unzip the data folder, move to your destination folder and run in a terminal the following command:

```
unzip data/data_files.zip -d data/
```

Formatting of rearrangement breakpoint positions

2 The nucleotide positions of the BCAs or other rearrangements subjected to position effect analysis must be formatted in .bed style. In the case of

DGAP163, translocation breakpoints were mapped at chr2(39206240-39206242) and chr14(31717833-31717834). Thus for this subject, open a text editor, create a new document, and write down the following lines using tabulator to separate column values:

```
chr2	39206240	39206242	DGAP163_A
chr14	31717833	31717834	DGAP163_B
```

These lines indicate the presence of two breakpoints in chromosomes 2 and 14, as well as their nucleotide positions and an identifier (breakpoint DGAP163_A and breakpoint DGAP163_B). Save this file in the analysis folder with the name DGAP163_positions.bed.

For additional bed formatting questions, the University of California Santa Cruz genome browser has a comprehensive guide that can be accessed at https://genome.ucsc.edu/FAQ/FAQformat.html

Selection of analysis window size

3 It is important to determine an analysis window size for the prediction of position effects. From our experience, the optimal window size for candidate gene discovery is +/− 1Mb upstream and downstream of the rearrangement breakpoints, as determined from analyzing 57 cases with known pathogenic and likely-pathogenic position effect variants. Results contained within the +/− 1Mb window are automatically included in the final candidate report (Step 9 of this protocol). Users are free to select smaller or larger analysis windows to suit their needs. The maximum recommended distance for predicting position effects is +/− 3Mb (i.e., 6Mb window size), according to reported distances of architectural loops within TADs (Krijger and de Laat 2016). For the rest of this protocol we will use a +/− 3Mb window to exemplify how many candidates could be obtained at the recommended maximal range of analysis.

Analysis of neighboring genes haploinsufficiency and TAD overlaps

4 Gene happloinsufficiency is one of the main pathogenicity criteria used in the position effect prediction of this pipeline. Moreover, inclusion of a known or predicted haploinsufficient gene within the same TAD as the rearrangement breakpoints additionally strengthens its value as a candidate. This assessment is derived from the hypothesis that the rearrangement disrupts the native regulatory environment of the TAD; such disruption could lead to haploinsufficient gene misregulation and disease. The haploinsufficiency analysis and assessment of breakpoint-mediated TAD disruption is performed by running the dgap_features_check.pl script, which utilizes the following parameters:

```
perl dgap_features_check.pl \
  -f GENOMIC_REGIONS \
  -i HI_SCORES_FILE \
  -g ENSEMBL_GENE_FILE_SUMMARY \
  -c HIC_DOMAINS \
  -n WINDOW_BP_SIZE \
  -o OUTPUT_FOLDER_PATH
```

The GENOMIC_REGIONS file is the .bed file generated in Step 2 of this protocol. The HI_SCORES_FILE is the genomic haploinsufficiency score list published by Huang et al. 2010 (Huang et al. 2010), the ENSEMBL_GENE_FILE_SUMMARY is a gene annotation file derived from Ensembl BioMart (output columns selected include: Ensembl Gene ID, Chromosome Name, Gene Start (bp), Gene End (bp), HGNC ID(s), HGNC symbol, Gene type, WikiGene Description, Description, Phenotype description),and the HiC domains file is a processed bed file containing the TAD positions reported by (Dixon et al. 2012). All of these files are included in the data folder unzipped in Step 1 of this protocol WINDOW_BP_SIZE is the analysis window size selected in Step 3 of this protocol, and finally OUTPUT_FOLDER_PATH is the folder where the output analysis files will be saved. Included in the example data folder downloaded from the pipeline GitHub repository, the following files are used to run the script:

```
perl perl/dgap_features_check.pl \
  -f DGAP163_positions.bed \
  -i data/HI_Predictions_Version3.bed \
  -g data/GRCh37.p13_ensembl_genes.txt \
  -c data/hESC_hg37_domains.bed \
  -n 3000000 \
  -o.
```

This script generates the files HiC_list_DGAP.txt and HI_list_DGAP.txt. The first file contains a list of overlapped Hi-C domains by the rearrangement breakpoints, while the second file is a summary of breakpoint-overlapped Hi-C domains and genes with happloinsufficiency information within the selected analysis window. The output column structure for HI_list_DGAP.txt is shown in Table 1. For DGAP163, the output HiC_list_DGAP.txt should have two rows while HI_list_DGAP.txt has 46 rows of results. You can compare your output files to those included in the Supplementary Materials for this protocol.

The files HI_SCORES_FILE, ENSEMBL_GENE_FILE_SUMMARY, and HIC_DOMAINS can be replaced with more recent versions or other custom files as long as the column structure is maintained.

Analysis of predicted enhancer/promoter interactions disrupted by the rearrangement breakpoints

5 The analysis pipeline predicts position effects based on haploinsufficiency information as well as disruption of chromosome organization. The alteration of enhancer-promoter interactions combines both criteria and further refines the list of candidate neighboring genes contributing to a clinical phenotype. The script enh_promoter_disruption_checker_DGAP.pl assesses the number of disrupted predicted DHS enhancer/promoter interactions (Thurman et al. 2012) within the analysis window. The script uses the following parameters:

```
perl enh_promoter_disruption_checker_DGAP.pl \
  -f GENOMIC_REGIONS \
  -d DHS_ENH_PROMOTER_FILE \
  -a WINDOW_BP_SIZE \
  -o OUTPUT_FILENAME
```

To run the analysis on DGAP163, use the DGAP163_positions.bed file from Step 2 of this protocol, the WINDOW_BP_SIZE selected in Step 3, as well as the DHS_ENH_PROMOTER_FILE from the data folder downloaded from the pipeline GitHub repository:

```
perl perl/enh_promoter_disruption_checker_DGAP.pl \
  -f DGAP163_positions.bed \
  -d
data/
genomewideCorrs_above0.7_promoterPlusMinus500kb_withGeneNames_32celltypeCateg
ories.bed8 \
  -a 3000000 \
  -o DHS_promoter_broken_DGAP163.txt
```

This script will output the number and location of the predicted DHS enhancer/promoter interactions affected by the rearrangement breakpoints. The file used in this analysis was obtained from (Thurman et al. 2012), but additional regulatory interaction datasets may be used provided their column structures follow the one used in the DHS_ENH_PROMOTER_FILE.

Conversion of clinical phenotype to HPO terms

6 To further refine the selection of candidate position effect genes, a “phenomatch score” calculation is performed to compare the similarity of the subject’s clinical presentation and the potential neighboring position effect genes. Before calculating phenomatch scores (see next step in the protocol), users need to convert the clinical descriptions of their cases to Human Phenotype Ontology (HPO) terms (Kohler et al. 2014). The HPO project has curated over 11,000 terms, with the goal of facilitating the large scale computational analysis of human phenotypic annotations. Users can convert their clinical descriptions to HPO terms using Phenomizer (http://human-phenotype-ontology.github.io/tools.html), Phenotips (https://phenotips.org/), and the HPO Browser (http://human-phenotype-ontology.github.io/).

The file structure of the phenotype description for DGAP163 is as follows:

ID	HPO
DGAP163	HP:0000047
DGAP163	HP:0000480
DGAP163	HP:0000545
DGAP163	HP:0000639
DGAP163	HP:0001250
DGAP163	HP:0001270
DGAP163	HP:0001344
DGAP163	HP:0001763
DGAP163	HP:0008598
DGAP163	HP:0011344

Save this information in a file named DGAP163_phenotype.txt. Notice how all of the HPO IDs correspond to the clinical description provided in the beginning of the protocol (i.e., HP: 0000047 is hypospadias, HP:0000480 is retinal coloboma, etc.) The phenomatch score calculation script uses a combined file with “chr”, “start”, “end”, “Breakpoint_ID”, “HPO” column. This file can be easily made by running the script combine_breakpoints_and_phenotypes.R. The parameters used are as follows:

```
Rscript combine_breakpoints_and_phenotypes.R \
  SUBJECT_ PHENOTYPES \
  GENOMIC_REGIONS \
  WINDOW_SIZE \
  OUTPUT_FILE
```

The SUBJECT_ PHENOTYPES file contains the case ID and the associated HPO terms, just as constructed for DGAP163_phenotype.txt. GENOMIC_REGIONS is the DGAP163_positions.bed file built in Step 2 of this protocol. The WINDOW_SIZE for this program will be the selected window size selected in Step 3, but multiplied by 2 (i.e., if 500Kb was selected for Step 3 of the protocol, 1Mb (1,000,000bp) will be used to run this script.) For DGAP163 run the script in the following manner:

```
Rscript R/combine_breakpoints_and_phenotypes.R DGAP163_phenotype.txt
DGAP163_positions.bed
  6000000 \
  DGAP163_breakpoint_window_with_HPO.6MB_win.bed
```

The combine_breakpoints_and_phenotypes.R script requires the stringr and readr packages. For more information about how to install packages in R, visit https://www.r-bloggers.com/installing-r-packages/

Phenomatch scores calculation

7 The phenomatch score metric was created by (Ibn-Salem et al. 2014), and provides a quantitative measure of phenotypic similarity between two datasets of

HPO terms. Comparing the similarity of the subject's clinical presentation and the neighboring position effect genes is one of the most important pieces of information to rank position effect candidates, as genes without pathogenic consequences related to those of the cases we analyze are excluded. Phenomatch scores are calculated by running the phenomatch.jar script with the following parameters:

```
java -jar phenomatch.jar \
  -i INPUT_FILE \
  -g GENES \
  -O PHENOTYPE_ONTOLOGY
  -a ANNOTATION_FILE \
  -o OUTPUT_FILE
```

The INPUT_FILE is the file generated in Step 6, GENES is a list of annotated genes with a format of chr, start, end, entrez_id, strand; PHENOTYPE_ONTOLOGY is the obo file derived from HPO which contains all the ontological relationships between terms; ANNOTATION_FILE contains all annotated terms to each gene in the human genome, and is also obtained from the HPO webpage; finally, OUTPUT_FILE specifies the name of the file where the phenomatch scores of all breakpoint neighboring genes within the analysis window will be written.

For the DGAP163 example:

```
java -jar bin/phenomatch.jar \
  -i DGAP163_breakpoint_window_with_HPO.6MB_win.bed \
  -g data/knownGene.txt.entrez_id.tab.unique \
  -O data/hp.obo \
  -a data/ALL_SOURCES_TYPICAL_FEATURES_genes_to_phenotype.txt \
  -o DGAP163_breakpoint_window_with_HPO.6MB_win.bed.phenomatch
```

More recent versions of HPO and gene phenotype annotations can be downloaded from: http://human-phenotype-ontology.github.io/downloads.html

Ranking of phenomatch scores

8 Once all phenomatch scores of breakpoint neighboring genes are calculated, a ranking system will prioritize the most likely candidates over those with no apparent contribution. This is performed by sorting the genes with the get_percentiles_DGAP_all.r script, which calculates the percentile rank for the phenomatch and max_phenomatch scores calculated in Step 7 of this protocol. The program is run using the following input files:

```
Rscript --vanilla get_percentiles_DGAP_all.r PHENO_FILE > OUTPUT_FILENAME
```

For DGAP163:

```
Rscript --vanilla R/get_percentiles_DGAP_all.r \
  DGAP163_breakpoint_window_with_HPO.6MB_win.bed.phenomatch \
  > percentiles_6Mb_pheno_maxpheno.txt
```

Summarizing results

9 9. The last step in the position effect analysis is putting together all of the results for a final ranking of the candidates. The script dgap_final_table_maker.pl will pull together all data and is run using the parameters:

```
perl dgap_final_table_maker.pl \
  -f TAD_ANALYSIS_FEATURES_FILE \
  -c HIC_DOMAINS\
  -d DHS_ENH_PROMOTER_DISRUPTED_CONTACTS_FILE \
  -h CLINGEN_HAPLO_FILE \
  -t CLINGEN_TRIPLO_FILE \
  -m PHENOMATCH_PERCENTILES_FILE \
  -o OUTPUT_FILENAME
```

The TAD_ANALYSIS_FEATURES_FILE is the HI_list_DGAP.txt file that was obtained in Step 1. The file HIC_DOMAINS is the TAD information file used in Step 1. The DHS_ENH_PROMOTER_DISRUPTED_CONTACTS_FILE is the output file of Step 2 that is not the summary. CLINGEN_HAPLO_FILE and CLINGEN_TRIPLO_FILE are haploinsufficiency and triplosensitivity files derived from ClinGen Dosage Sensitivity Map (Rehm et al. 2015). Both of these are in bed format and contain the chr, start, end, gene HUGO name, dosage score. PHENOMATCH_PERCENTILES_FILE is the processed file obtained from Step 8. OUTPUT_FILENAME is the name of the file where the summary will be written.

Running this script will produce an additional section from the features analyzed in Step 1, and analyzes the genes' inclusion in the initially selected size windows (Step 3), the +/ − 1Mb (2Mb) window, the gene inclusion within the breakpoint TAD, whether or not predicted enhancer/promoter contacts were disrupted for the gene, and finally a summary of the genes phenomatch and max_phenomatch scores with their corresponding percentile values within the analyzed dataset (output of Step 4). Values of 0 and 1 indicate inclusion within the analyzed regions (Step 3 Mb windows, 2Mb window, TADs) or presence of disrupted contacts (enhancer/promoter).

For DGAP163:

```
perl perl/dgap_final_table_maker.pl \
  -f HI_list_DGAP.txt \
  -c data/hESC_hg37_domains.bed \
  -d DHS_promoter_broken_DGAP163.txt \
  -h data/ClinGen_haploinsufficiency_gene.bed \
  -t data/ClinGen_triplosensitivity_gene.bed \
  -m percentiles_6Mb_pheno_maxpheno.txt \
  -o DGAP163_table_summary.txt
```

The output file has columns as described in Table 2. ClinGen haplo/triplo-sensitivity scores are indicated with ranges from 0 (no evidence) to 30 (known). Users need to establish their cut-off criteria for the selection of follow-up candidates. For example, to consider only genes with emerging evidence suggesting dosage sensitivity associated with the clinical phenotype, select ClinGen haplo/triplo-sensitivity scores from 2 and above and give those regions a final table value of 1. This can be done in the ClinGen file by substituting the desired scores with 1 and making everything else a 0, or by adding an extra column to the excel file and making this change with the IF selection formula. The same applies to the phenomatch and max_phenomatch percentiles (i.e., to include only the top quartile, assign a 1 to everything with >=0.75 percentile value).

For the final candidate selection and ranking, add the 0 and 1 values from different fields. Based on our experience, adding the following columns gives the best candidate ranking: PERC+DHS+TAD+HAPLO+TRIPLO or PERC+DHS+2Mb+HAPLO+TRIPLO. When sorting by these values, the first few rows of results should look similar to the ones in the table in Figure 3. From this table, we conclude that there are two important candidates which may explain part or all of DGAP163's observed phenotypic characteristics. The first candidate is SOS Ras/Rac guanine nucleotide exchange factor 1 (*SOS1*, MIM#182530), whose potential misregulation could be involved DGAP163's developmental delay and neurological problems. A quick look at *SOS1* in chr2 reveals how the rearrangement disrupts several long-range interactions present in the region, potentially causing misregulation of this gene (Figure 2). The second candidate, cochlin (*COCH*, MIM#603196) could be related to the hearing loss present in DGAP163. It is important to remark that while these candidates represent an initial step in understanding DGAP163's clinical features, functional experiments should be performed to unveil the pathogenic mechanisms by which they may contribute to the phenotype, especially since DGAP163 does not present with the full clinical spectrum of Noonan syndrome (MIM#610733).

The dosage sensitivity map can be downloaded from https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/

The full list of files generated throughout the protocol can be downloaded from https://drive.google.com/open?id=1PRkdQHxagzR6HpwnoHDf2de1p3QX0qzw

Commentary

Recent studies in diverse cell lines and tissues have revealed an extensive and intrinsic modular organization of the human genome into topologically associating domains (TADs) (Dekker and Heard 2015; Nora et al. 2012). TADs are defined as DNA segments, usually

~1Mb in size, that have a higher number of chromatin interactions contained within the region compared to neighboring segments. At the functional level, TADs have been associated with the establishment and regulation of cell developmental programs (Beagan et al. 2016; Gorkin et al. 2014; Phillips-Cremins et al. 2013) and genome replication (Thurman et al. 2012). The high degree of correlation between genomic function and chromatin organization makes evident the importance of studying potential clinical pathogenic consequences. Chromosome rearrangements, even when not directly disrupting a gene's coding sequence, have been shown to generate disease by altering long-range associations between gene promoters and their regulatory elements (Giorgio et al. 2015; Groschel et al. 2014; Ibn-Salem et al. 2014; Lupianez et al. 2015; Roussos et al. 2014; Visser et al. 2012). Therefore, even apparently balanced or balanced non-coding chromosome abnormalities (BCAs) may exert position effects which could be further associated with disease, such as those seen in translocations nearby *SOX9* linked to campomelic dysplasia (Velagaleti et al. 2005), and translocations downstream of *PAX6*, in congenital aniridia (Kleinjan et al. 2001).The computational method described in this protocol provides a streamlined pipeline that assesses the contribution of distal genes in the generation of a clinical phenotype. It is important to highlight that position effect analyses are not typically included in studies of balanced or unbalanced chromosomal abnormalities, thus biasing for negative results in the case of non-coding breakpoints.

With the availability of a computational method to automate such tasks, clinicians and researchers are now able to provide a quick overview of the region beyond the rearrangement location, and thus focus their functional characterizations towards relevant candidate genes.

All types of chromosome rearrangements are amenable to position effect studies (see paper by Ibn-Salem et al. 2014 regarding the study of position effects in copy number variants (CNVs) and Zepeda-Mendoza et al. 2017 for non-coding BCAs); however, careful attention must be paid in the interpretation of chromosome abnormalities that disrupt coding sequences. In such cases, the detection of position effects may complement the diagnosis or explain features not fully accounted for by the disrupted gene sequence(s).

Finally, we would like to mention that the accuracy of the position effect prediction method is directly dependent upon the curation of public databases. Major efforts are being carried out by projects such as ClinGen (Rehm et al. 2015) and DECIPHER (Firth et al. 2009) to advance the annotation of the clinical genome. The results of a position effect prediction analysis may differ between different annotations as additional information becomes available about gene function.

Troubleshooting

It is important to note that the position effect analysis requires the mapping of rearrangement breakpoints, either through next-generation sequencing and/or Sanger sequencing. Positions derived from standard or high resolution cytogenetic karyotypes or estimated regions by fluorescence *in situ* hybridization (FISH) probes comprise thousands of base pairs containing several regulatory regions and interactions, and are thus not specific or amenable to this analysis.

In addition to having base-pair resolution mapped breakpoints, because the protocol relies on comparing specific rearrangement breakpoint coordinates to annotated genes and regulatory element interactions it is essential to use the same reference genome. For example, hg19 was used throughout this protocol. Other reference genome versions can be used as long as regulatory genomic features are reported in said genome version. Some difficulty may arise when formatting new and updated files downloaded from public databases such as Ensembl (Yates et al. 2016) and UCSC (Kent et al. 2002). To address this problem, it is important to ensure that the downloaded file structure matches the ones described in this protocol. In this regard, Ensembl's BioMart tool has the option to output desired data annotation columns, while UCSC has extensive documentation regarding file column descriptions. For UCSC files, the described position effect prediction protocol does not require further formatting.

Anticipated Results

The position effect analysis pipeline described in this protocol provides users with a ranked list of genes that may contribute in the phenotype of a subject with balanced or apparently balanced chromosome abnormalities. The number of highest ranking candidate genes depends on the specificity and availability of phenotypic information per subject, as well as the annotation of the neighboring genes in public clinical databases. Users should expect one or more high ranking candidates; however, certain cases do not reach a significance ratio. We recommend not pursuing functional validation of candidate genes with less than two different lines of evidence supporting their inclusion.

Time considerations

Analysis times usually take less than an hour for 20 breakpoints or less, using a computer with 4Gb RAM memory. For a higher number of breakpoints, consider partitioning your files for a faster analysis run.

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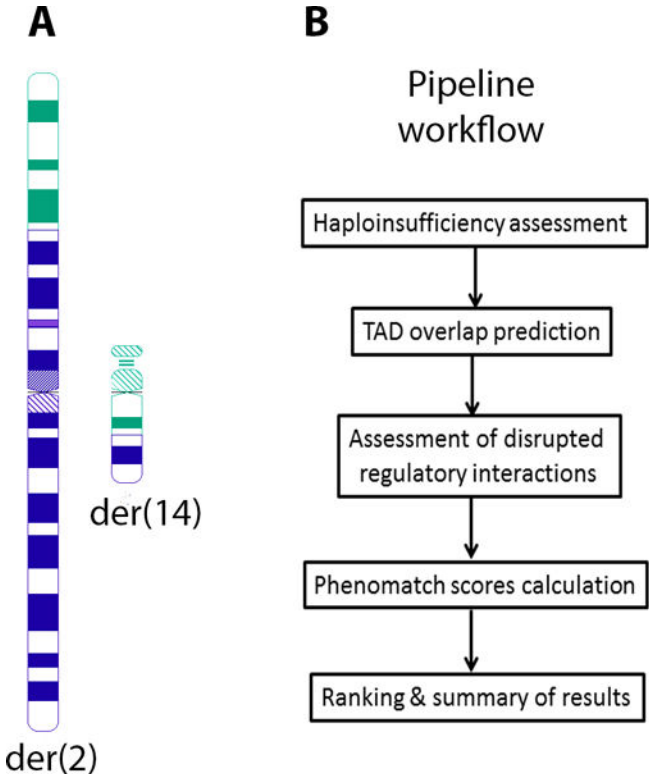

A
der(14)
der(2)
B
Pipeline workflow
Haploinsufficiency assessment
TAD overlap prediction
Assessment of disrupted regulatory interactions
Phenomatch scores calculation
Ranking & summary of results

Figure 1.

A) Ideogram of DGAP163's t(2;14)(p23;q13). Chromosome 2 is depicted in blue while chromosome 14 is depicted in green. B) Analysis steps of the computational pipeline described in this protocol. A full analysis of chromatin interactions, haploinsufficiency and phenotypic relatedness is assembled and distal candidate genes are ranked based on their functional evidence.

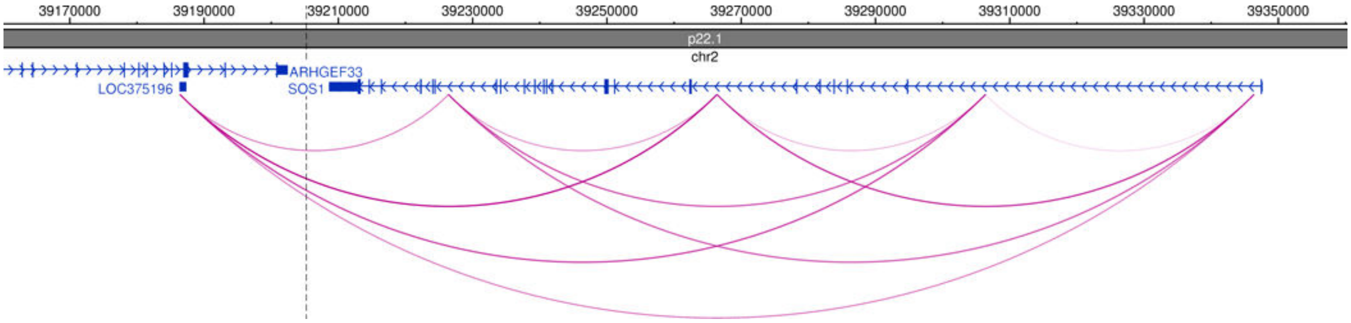
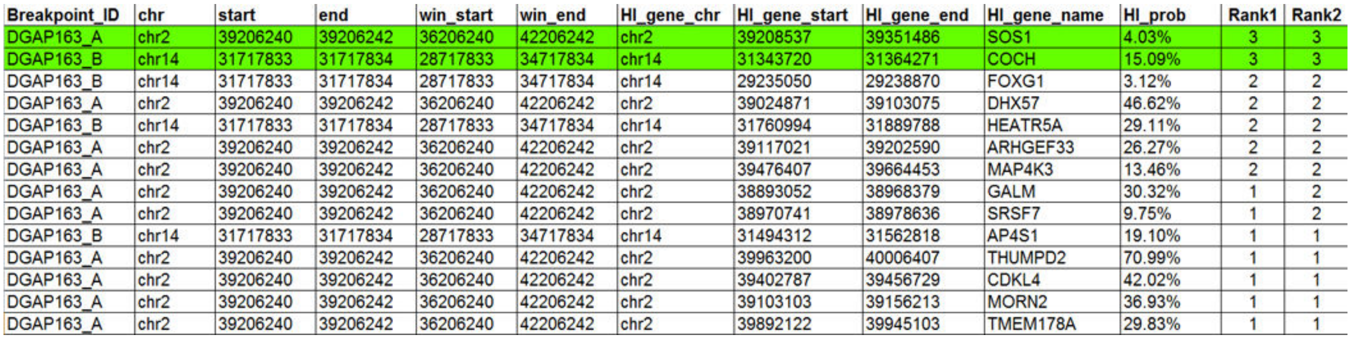


Figure 2.

SOS1 region in chromosome 2. Genes present in the region are depicted in blue, including SOS1. The translocation breakpoint is indicated in a dashed black vertical line. Chromatin interactions in H1-hESC cells at 40Kb resolution are depicted in pink arcs for this region. This image was obtained through the WashU Epigenome Browser (http://epigenomegateway.wustl.edu/browser/), an excellent resource for the quick visualization of chromatin interactions and regulatory element datasets (Zhou and Wang 2012).



Breakpoint_ID	chr	start	end	win_start	win_end	HI_gene_chr	HI_gene_start	HI_gene_end	HI_gene_name	HI_prob	Rank1	Rank2
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	39208537	39351486	SOS1	4.03%	3	3
DGAP163_B	chr14	31717833	31717834	28717833	34717834	chr14	31343720	31364271	COCH	15.09%	3	3
DGAP163_B	chr14	31717833	31717834	28717833	34717834	chr14	29235050	29238870	FOXG1	3.12%	2	2
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	39024871	39103075	DHX57	46.62%	2	2
DGAP163_B	chr14	31717833	31717834	28717833	34717834	chr14	31760994	31889788	HEATR5A	29.11%	2	2
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	39117021	39202590	ARHGEF33	26.27%	2	2
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	39476407	39664453	MAP4K3	13.46%	2	2
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	38893052	38968379	GALM	30.32%	1	2
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	38970741	38978636	SRSF7	9.75%	1	2
DGAP163_B	chr14	31717833	31717834	28717833	34717834	chr14	31494312	31562818	AP4S1	19.10%	1	1
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	39963200	40006407	THUMPD2	70.99%	1	1
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	39402787	39456729	CDKL4	42.02%	1	1
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	39103103	39156213	MORN2	36.93%	1	1
DGAP163_A	chr2	39206240	39206242	36206240	42206242	chr2	39892122	39945103	TMEM178A	29.83%	1	1

Figure 3.

First 14 rows of the results file obtained in Step 9 of this protocol. Notice that several data columns were omitted in order to highlight the top candidate gene names and their ranking. The Rank1 column corresponds to the PERC+DHS+TAD+HAPLO+TRIPLO addition, while Rank2 represents the PERC+DHS+2Mb+HAPLO+TRIPLO sum. The best two candidates are highlighted in green. Notice how the top candidates have equal Rank1 and Rank2 values (3).

Table 1

HI_list_DGAP.txt column descriptions from Step 4 of this protocol.

Column name	Description
Breakpoint_ID	Identification number of the studied rearrangement breakpoints
chr	Rearrangement breakpoint chromosome location
start	Rearrangement breakpoint start position
end	Rearrangement breakpoint end position
win_start	Analysis window start position
win_end	Analysis window end position
Hi_domain	TAD coordinates that contain the rearrangement breakpoint
HiC_chr	TAD chromosome location
HiC_start	TAD start position
HiC_end	TAD end position
HI_gene_chr	Chromosome location for gene contained within the analysis window
HI_gene_start	Position start for gene contained within the analysis window
HI_gene_end	Position end for gene contained within the analysis window
HI_gene_name	HUGO name for gene contained within the analysis window
LOD	Log-odds score derived from Huang et al., 2010 for gene haploinsufficiency
HI_prob	Calculated probability of a gene being haploinsufficient
HI_ensembl_gene_ID	Ensembl ID of gene contained within the analysis window
HI_chr	Ensembl chromosome location of gene contained within the analysis window
Gene_start	Ensembl reported start position of gene contained within the analysis window
gene_end	Ensembl reported end position of gene contained within the analysis window
HGNC_ID	HUGO Gene Nomenclature Committee identifier for gene contained within the analysis window
HGNC_symbol	HUGO Gene Nomenclature Committee symbol for gene contained within the analysis window
Gene_type	Ensembl reported type of gene contained within the analysis window
WikiGene_Description	WikiGene description of gene contained within the analysis window
Description	Gene description
Phenotype_description	Ensembl reported phenotypic associations of gene contained within the analysis window

Table 2

Column descriptions for the summary file generated in Step 9 of this protocol.

Column name	Description
Breakpoint_ID	Identification number of the studied rearrangement breakpoints
chr	Rearrangement breakpoint chromosome location
start	Rearrangement breakpoint start position
end	Rearrangement breakpoint end position
win_start	Analysis window start position
win_end	Analysis window end position
Hi_domain	TAD coordinates that contain the rearrangement breakpoint
HiC_chr	TAD chromosome location
HiC_start	TAD start position
HiC_end	TAD end position
HI_gene_chr	Chromosome location for gene contained within the analysis window
HI_gene_start	Position start for gene contained within the analysis window
HI_gene_end	Position end for gene contained within the analysis window
HI_gene_name	HUGO name for gene contained within the analysis window
LOD	Log-odds score derived from Huang et al., 2010 for gene haploinsufficiency
HI_prob	Calculated probability of a gene being haploinsufficient
HI_ensembl_gene_ID	Ensembl ID of gene contained within the analysis window
HI_chr	Ensembl chromosome location of gene contained within the analysis window
Gene_start	Ensembl reported start position of gene contained within the analysis window
gene_end	Ensembl reported end position of gene contained within the analysis window
HGNC_ID	HUGO Gene Nomenclature Committee identifier for gene contained within the analysis window
HGNC_symbol	HUGO Gene Nomenclature Committee symbol for gene contained within the analysis window
Gene_type	Ensembl reported type of gene contained within the analysis window
WikiGene_Description	WikiGene description of gene contained within the analysis window
Description	Functional gene description
Phenotype_description	Ensembl reported phenotypic associations of gene contained within the analysis window
SelectedWindowMb	Location of gene within the selected analysis window. 1 indicates presence, 0 is absence. By default, this column should always be 1
2Mb	Location of gene within a 2Mb analysis window. 1 indicates presence, 0 is absence
TAD	Location of gene within TAD analysis window. 1 indicates presence, 0 is absence
DHS_promoter	Presence of disrupted enhancer-promoter interactions. 1 indicates presence, 0 is absence
Haploinsufficiency_score	ClinGen reported haploinsufficiency score for the gene
Triplosensitivity_score	ClinGen reported triplosensitivity score for the gene
Phenomatch_score	Phenomatch score for the gene
MaxPhenoScore	Max Phenomatch score for the gene
Pheno_percentile	Percentile of phenomatch score for the gene within the analysis window
MaxPheno_percentile	Percentile of max phenomatch score for the gene within the analysis window