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Database issue

VISTA Enhancer browser: an updated database of tissue-specific developmental enhancers

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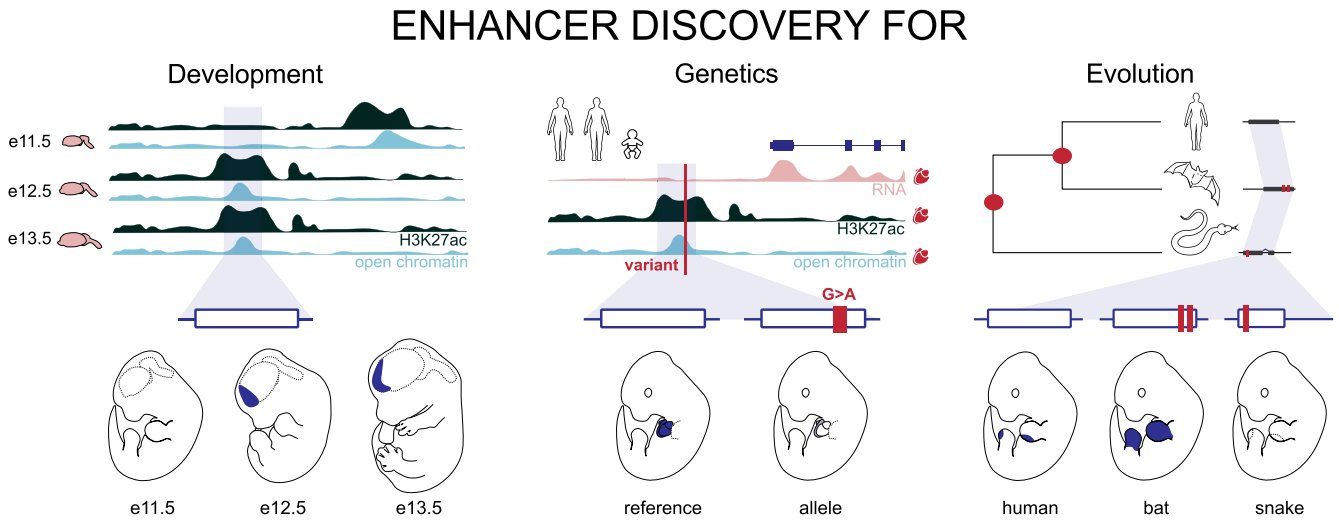
Abstract

Regulatory elements (enhancers) are major drivers of gene expression in mammals and harbor many genetic variants associated with human diseases. Here, we present an updated VISTA Enhancer Browser (https://enhancer.lbl.gov), a database of transgenic enhancer assays conducted in developing mouse embryos *in vivo*. Since the original publication in 2007, the database grew nearly 20-fold from 250 to over 4500 experiments and currently harbors over 23 500 images. The updated database provides structured information on experiments conducted at different stages of embryonic development, including enhancer activities of human pathogenic and synthetic variants and sequences derived from a variety of species. In addition to manually curated results of thousands of individual experiments, the new database also features hundreds of manually curated comparisons between alleles. The VISTA Enhancer Browser provides a crucial resource for study of human genetic variation, gene regulation and developmental biology.

Graphical abstract



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Introduction

Enhancers are typically defined as DNA sequences capable of driving gene expression at a distance. They often act in a cell type-specific fashion, have critical roles in regulating developmental processes, and changes in enhancer sequences have been central to vertebrate evolution (1,2). A majority of variants discovered in genome-wide association studies (GWAS) overlaps enhancers and numerous experimental studies demonstrate distal regulatory variation is crucial for normal and pathological human biology (3,4). Identification of active enhancers is thus fundamental to understanding of human diseases, embryonic development and evolution.

Genome-wide measurements of open chromatin status, histone modifications, and transcription factor binding are often used to discover potential regulatory elements, but are not sufficient to demonstrate that a given region is capable of driving gene expression. To elucidate the full *in vivo* function of a given enhancer conclusively, it is necessary to remove it from the genome or to silence its activity through genome engineering. However, these methods do not scale well at organismal level and are therefore predominantly applied to *in vitro* cultured cells, requiring prior assumptions about cell type specificity. Further, endogenous loci often harbor multiple regulatory elements, which may mask changes in target gene expression due to removal of an individual enhancer or introduction of a variant (5,6).

Transgenic mouse reporter assays are a sensitive, reproducible method for assessing *in vivo* enhancer activity at scale across all tissues. In a typical experiment, a putative enhancer is coupled to a minimal promoter and a LacZ reporter gene, inserted into the DNA of a fertilized mouse egg, and assessed for activity after a desired gestation period. Recent advancements in transgenic assay technology using CRISPR/Cas9 to direct the integration of transgenic construct to a safe harbor locus (enSERT method), have considerably improved the reproducibility and efficiency of this assay (7). No assumptions about tissue of activity are necessary, allowing discovery of elements active in multiple tissues and cell types. Crucially, effects of genetic variants on gene regulatory activity can be studied using these assays without the confounding of redundant elements at their endogenous loci (7,8). This is especially important for variants involved in common diseases, which often have low effect sizes.

Large catalogs of elements with confirmed regulatory activity *in vivo* are a crucial resource for genetic and genomic research. They serve as a golden standard for training and assessment of computational methods identifying regulatory elements and variants, as well as a substrate for studies on evolutionary innovation and tissue-specific gene regulation. Here, we present an update of the VISTA Enhancer Browser (9), which houses the results of over 4500 transgenic *in vivo* mouse experiments conducted in developing embryos. The structure of the new database allows easy access to and comparison of experiments performed at different embryonic stages, with different transgenic methods, using elements derived from different species or harboring human and synthetic variants. We anticipate the updated VISTA Enhancer Browser to provide value for the genomics, genetics, and developmental research communities.

Experimental workflow and database structure

The data model of the updated VISTA Enhancer Browser database encompasses a hierarchical, one-to-many structure consisting of loci, elements, experiments, and embryos (Figure 1), recapitulating the experimental workflow. These entities reflect the place in the genome from which the tested element originates (locus), its exact DNA sequence (element), the conditions under which it was tested (experiment) and the *in vivo* results of each independent transgenic insertion event (embryo; Figure 1A).

DNA sequences tested in the VISTA Enhancer Browser are often selected based on the presence of tissue- and embryonic stage-specific epigenomic marks correlating with transcriptional activity (H3K27ac histone mark measured by ChIP-seq, p300 protein binding measured by ChIP-seq), transcription factor binding (measured by ChIP-seq), open chromatin (measured by ATAC-seq or DHS-seq), or evolutionary conservation (Figure 1B). In many cases, more than one of these criteria are considered, and in many projects additional experimental data or pre-existing knowledge about a given locus is taken into consideration (9–17). Therefore, the content of the VISTA Enhancer Browser data should not be considered a random genomic sample, an important consideration when using this data for genome-wide statistical analyses.

The genomic region from which the tested DNA sequence is derived corresponds to the locus level in the database and is defined as a unique combination of a genomic assembly (hg38 for human loci, mm10 for mouse loci; Figure 1B) and genomic coordinates (e.g. chrX:123456–124567). Each locus is assigned an ID—an integer prefixed with 'hs' for human loci, 'mm' for mouse and 'x' for other species (e.g. hs200). Note that highly conserved enhancers from different species do *not* share a locus in this definition—links between them are established through comparative annotation (see Comparative experiment annotation below).

While DNA tested in the transgenic assay may correspond to locus reference sequence from a given genomic assembly, other sequences may be tested as well. Element level of the database corresponds to a specific DNA sequence from a given locus, including sequences containing human or synthetic variants, even larger deletions. Each element is identified by a unique VISTA ID, which is composed of the locus ID, appended by a number, for example hs200.2. Reference elements are typically assigned a zero, for example hs200.0 (Figure 1B). When locus ID is used without a qualifying number (e.g. hs200), it refers to the reference element.

Elements can be tested under a variety of conditions, which correspond to the experiment level of the database. An element is cloned into the desired reporter plasmid, typically one with a minimal promoter and a beta-galactosidase reporter (LacZ). The resulting DNA construct is then injected into mouse zygotes, along with Cas9 reagents (7), if targeted integration is desired (enSERT method, Figure 1C). The zygotes are transferred into pseudopregnant recipient mice and recovered as embryos at a chosen developmental stage (typically 11.5 days post-fertilization, E11.5, Figure 1D). A combination of element, minimal promoter, reporter, transgenic method, and developmental stage defines the experiment level in the database. It is also the level at which manual curation is performed, making it the central level of the database and the level displayed in the Browse tab on the website.

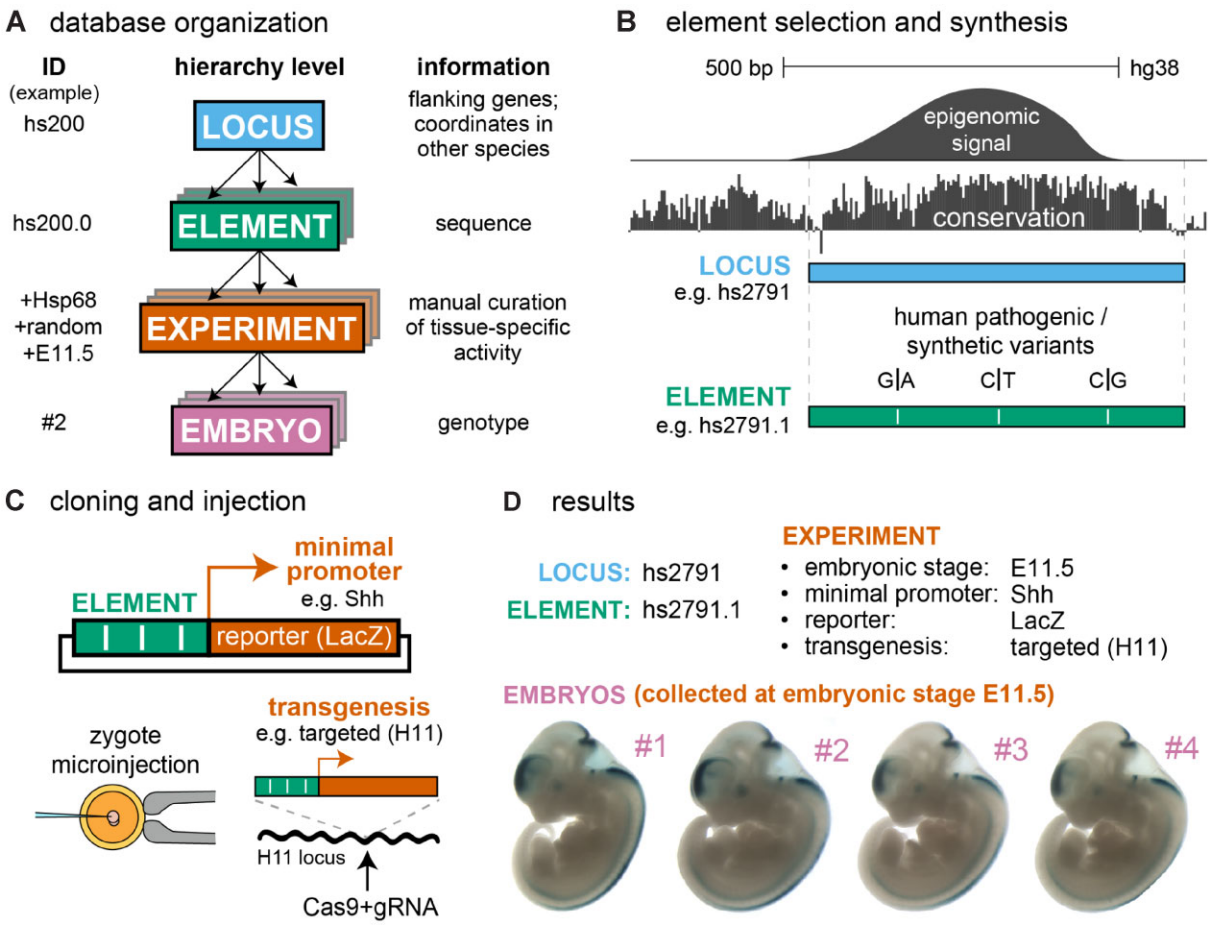

Figure 1. Database organization and experimental workflow. (**A**) Structure of VISTA Enhancer Browser data. (**B**) Selection of putative enhancers and introduction of variants. *Locus* and *element* hierarchy levels. (**C**) Cloning of selected enhancer into a reporter construct, zygote injection and targeted integration (enSERT method). *Experiment* hierarchy level. (**D**) Results. E11.5 = embryonic stage E11.5.

The information about the promoter, reporter and transgenic method can also be expressed in shorthand form as a 'backbone' - predominantly hZR (**h**sp68 minimal promoter, lac**Z** reporter, **R**andom transgenesis), for older experiments, or sZH (**s**hh minimal promoter, LacZ reporter, **H**11 enSERT targeted transgenesis), for more recent experiments. A specific experiment can thus be uniquely referred to as, for instance, 'element hs200.0 tested using sZH backbone at E11.5″ or 'element hs200.0 tested using enSERT with Shh minimal promoter at E11.5'. Experiments are also uniquely identified in the flat tables provided with the database using a 12-digit, base 36 hierarchy number, e.g. 002q00010015, to make joining of different tables easier. Currently, about 90% of experiments in the database are the single experiment for the corresponding element, in which case their VISTA ID is sufficient for their identification. Each experiment has its own unique page that can be directly accessed using the above mentioned information, e.g.: https://enhancer.lbl.gov/vista/element?vistaId=hs200&alleleId=0&backbone=sZH&stage=e11.5.

Embryos resulting from a transgenic mouse experiment are stained for LacZ activity, genotyped (see next section), imaged, and stored indefinitely for additional imaging or sectioning (Figure 1D). Their identifier in the database is an ordinal number given at collection. Since embryos that are not transgenic, transgenic embryos that do not show any reporter staining, and transgenic embryos that show predominantly non-reproducible ectopic staining may not be imaged or displayed, the numbering of embryos displayed in the database need not be consecutive. Once a sufficient number of transgenic embryos has been collected, the results of the experiment are manually curated and published on VISTA Enhancer Browser website (see next section).

The VISTA Enhancer Browser is implemented using React and TypeScript. Data are served through a dedicated Application Programming Interface (API), developed using the Slim Framework. For each experiment entry, links are provided to external resources, namely, the UCSC Genome Browser and NCBI RefSeq for genomic coordinates and flanking genes, respectively.

Genotyping and curation

Most of the experiments published in the VISTA Enhancer Browser are individually curated to confirm which activity patterns were reproducible. The criteria for assessing the reproducibility of activity in individual anatomical structures depend on the transgenesis method used. Older experiments performed using random transgenesis are more susceptible to ectopic staining in individual embryos due to position effects and therefore require a more stringent reproducibility threshold than experiments performing the newer targeted reporter integration approach (enSERT), which shows higher repro-

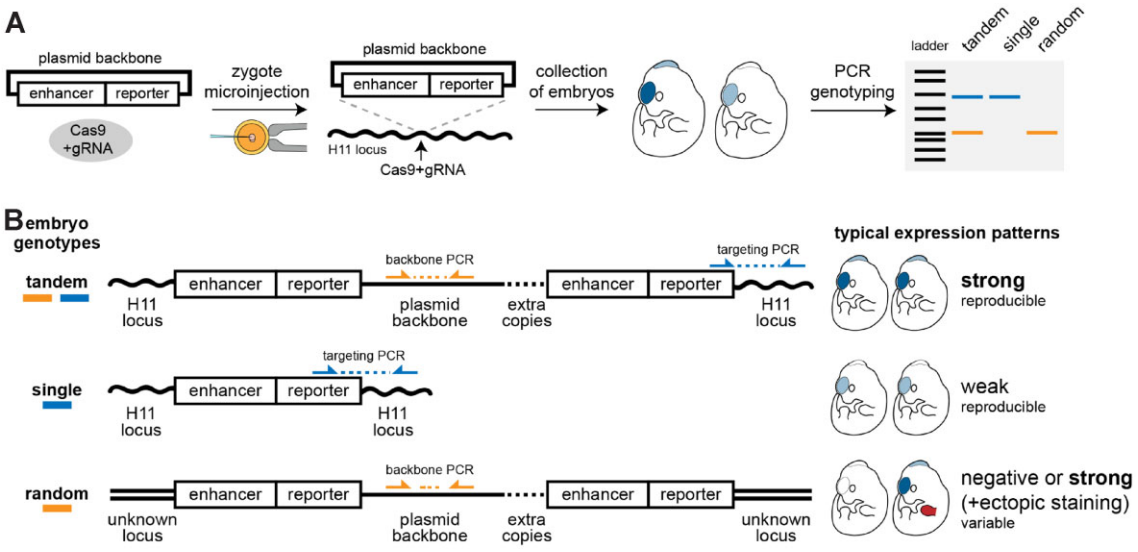

Figure 2. Genotyping. (**A**) Schematic of enSERT experimental workflow. (**B**) Interpretation of enSERT genotyping results.

ducibility across embryos (7,18). The reproducibility criteria for both methods were established empirically, based on the results observed across thousands of transgenic experiments. Both methods require a minimum number of transgenic embryos to make a curation call.

When using random transgenesis, presence of any LacZ staining is considered conclusive evidence of successful transgenesis of a given embryo. With this method, elements without intrinsic activity often yield embryos with variable ectopic staining due to positional effects, i.e. activation of the reporter construct by enhancers or promoters present near the genomic insertion site. PCR genotyping to identify additional transgenic, unstained embryos would only be performed only if the number of stained embryos is insufficient to make a conclusive call. When the minimum number of transgenics is obtained, the experiment can be curated as:

- **POSITIVE** if a similar staining pattern is observed for any anatomical structure in at least three embryos
- **NEGATIVE** if at least five transgenic embryos were obtained and positive criteria were not fulfilled

With the site-directed enSERT method (7), elements without intrinsic activity typically yield transgenic embryos without staining (Figure 2A). Further, embryos injected with elements that do drive reporter activity often differ in overall staining intensity based on the copy number of integration events. Thus, to establish that a sufficient number of transgenics has been obtained for negative elements, and to enable correct interpretation of results for positive elements, PCR genotyping is required.

All embryos generated using the enSERT method are genotyped using two PCR reactions: one assessing correct targeting at the H11 locus and one assessing the presence of the reporter plasmid backbone (Figure 2A, B). Embryos positive for both PCRs are labeled *tandem*, indicating that at least two concatenated copies of the vector are inserted correctly at the targeted H11 locus. Embryos only positive for the targeting PCR are labeled *single*, indicating insertion of a single copy of the vector at the H11 locus. Embryos only positive for backbone PCR are labeled *random*, indicating insertion of at least two concatenated copies of the vector outside of the H11 locus. These labels match observed activity patterns. Staining intensity of *tandem* embryos is usually greater than that of *single* embryos, with both *tandem* and *single* embryos exhibiting very reproducible activity patterns. *Random* embryos tend to either be negative, likely due to integration in repressive chromatin, or to have variable, ectopic staining, due to bystander activation. These genotypes are therefore an important component of our annotation protocol (Figure 2B).

Thanks to the additional layer of information provided by genotyping and due to overall higher reproducibility, experiments using site-directed enSERT transgenesis require fewer transgenic embryos to make a call than experiments using random transgenesis. Experiments are curated as:

- **POSITIVE** if a similar pattern in the same structure is observed in at least two transgenic embryos, at least one of which was correctly targeted at the safe harbor locus (i.e. had a *single* or *tandem* genotype)
- **POSITIVE** if a similar pattern in the same structure is observed in at least three embryos of any genotype (same as for random transgenesis)
- **NEGATIVE** if two *tandem* embryos without staining were observed
- **NEGATIVE** if no reproducible expression was observed in five *random* or *tandem* transgenic embryos (similar to random transgenesis criterion)

PCR genotype and the observed patterns do not always coincide, since routine genotyping does not directly assess the genomic location of all insertions or their copy number. In particular, an estimated 14% of embryos with an intrinsically active enhancer and a *tandem* genotype exhibit divergent patterns. These include variable ectopic staining, *single*-like activity pattern and no activity. This is likely due to true *tandem* insertion at H11 locus being indistinguishable co-occurence of a targeted insertion at H11 locus (low or high copy number) with a random genomic insertion, which can be silenced or ectopically activated. *Single* genotypes are more reliable. Less than 2% of embryos with an intrinsically active enhancer and a *single* genotype exhibit divergent patterns, virtually all of which are *tandem*-like.

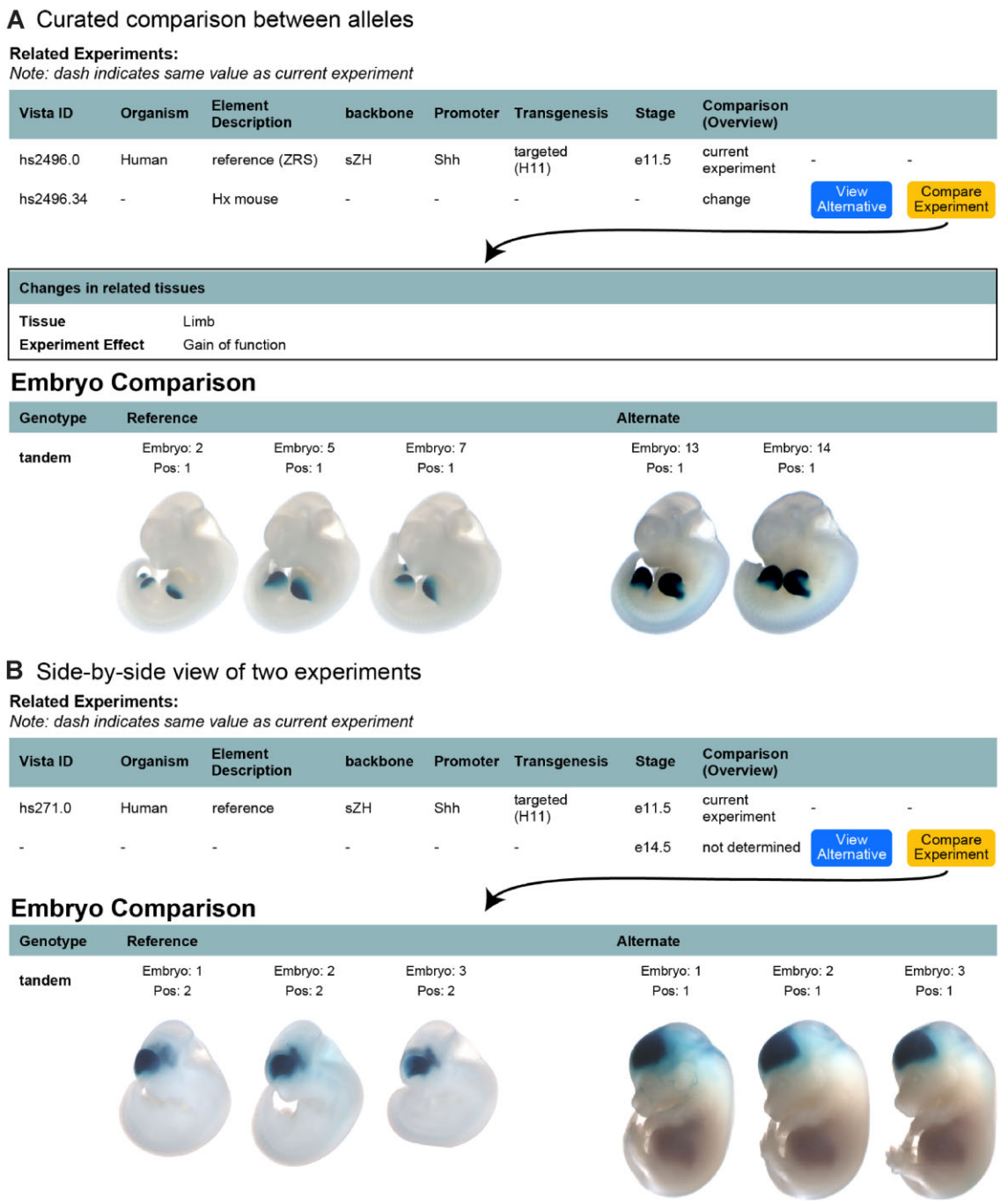

Figure 3. Experiment comparison. (**A**) Example of a manually curated comparison between alleles. Shown are the reference ZRS allele experiment (hs2496.0 sZH E11.5) and an experiment using single basepair ZRS mutation associated with polydactyly in hemimelic extra-toe mouse (hs2496.34 sZH E11.5). Introduction of the variant resulted in additional activity in the limb, as described in the boxed panel. Top panel is a fragment of Experiment page for reference experiment, bottom is a fragment of Experiment Comparison page. (**B**) Example of a side-by-side view of two experiments. The same element (hs271.0) was tested at two embryonic timepoints (E11.5 and E14.5). Similar forebrain-specific activity was observed at both timepoints.

Comparative experiment annotation

The VISTA Enhancer Browser allows for direct comparison of differences between any two experiments. Related experiments, such as those testing testing different alleles of the same element (Figure 3A), the same element at different embryonic stages (Figure 3B) and the same element with different transgenic methods are automatically linked in the database to enable convenient side-by-side visual comparison (see example in Figure 3B). In addition, selected comparisons, in particular those between different alleles of the same element or between evolutionarily related elements, have been manually curated (see example in Figure 3A). The results of manual comparative curation are reported for each tissue as *no change*, a *gain of function*, a *loss of function*, a *partial loss of function or complex*. For *complex* results, a description of the change in activity within the tissue is also provided, e.g. 'loss of original dorsal forebrain staining, ventral gain'. Manually curated comparisons also explicitly mention which

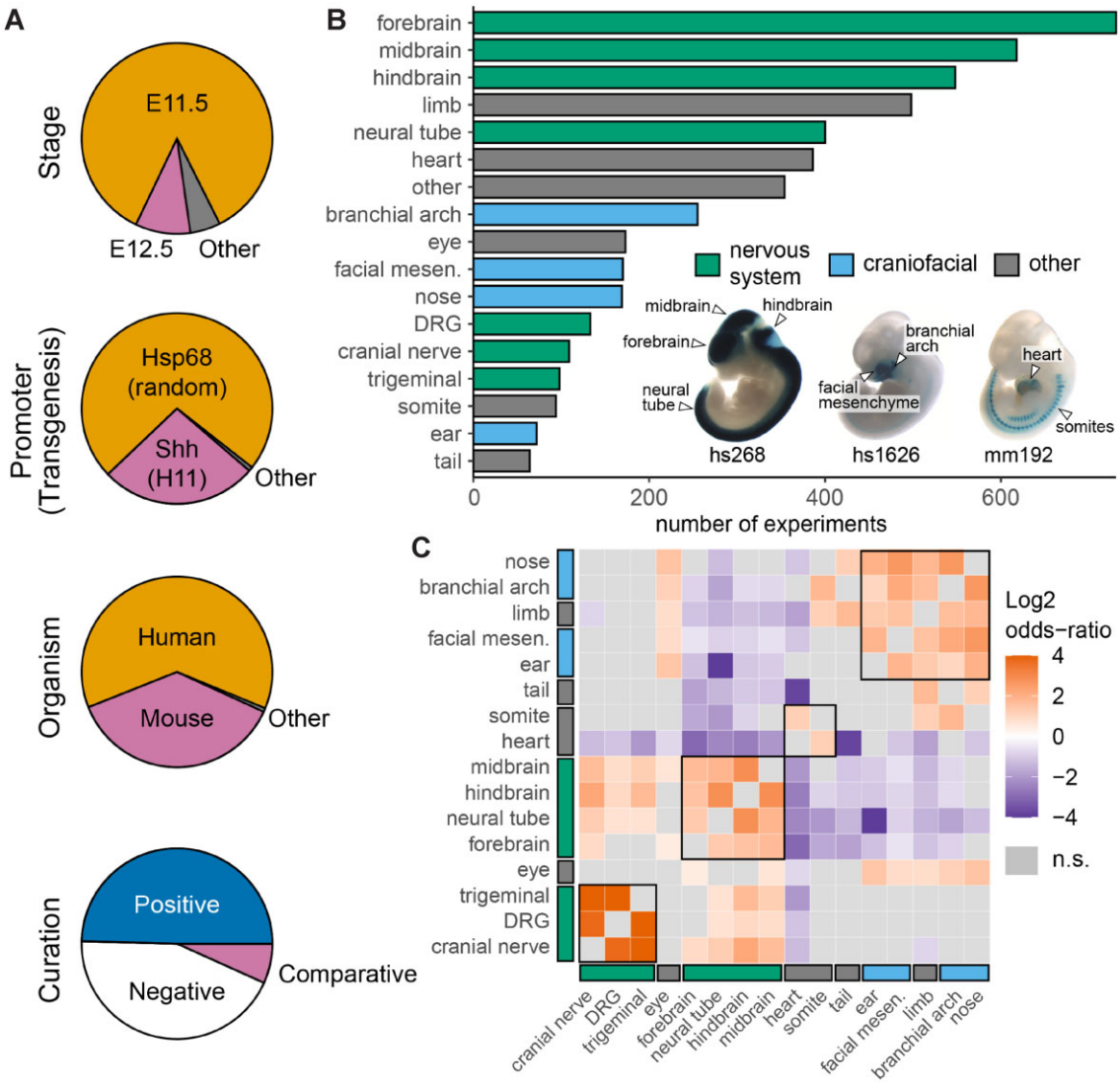

Figure 4. Database contents. (**A**) Distribution of experiments in terms of embryonic stage, promoter and transgenic method, organism from which the element was derived and the result of manual curation. 'Comparative' refers to experiments which were not curated individually, but only in relationship to other experiments (typically alleles). (**B**) Distribution of experiments that were curated positive in terms of tissue. (**C**) Correlation between tissues observed in the same experiment. n.s. = not significant (Fisher's Exact test, FDR ≥ 0.05). Blocks of similar tissues highlighted. DRG = dorsal root ganglia. facial mesen. = facial mesenchyme.

genotypes were taken into account when making the call—all transgenics, tandems and singles in combination, or tandems alone.

Curation calls on experiments involving suspected human pathogenic variants are made through blind annotation involving at least four reviewers, unless noted otherwise. For more details on any particular comparison, refer to cited studies or inquire with the VISTA Enhancer Browser team. Changes associated with synthetic variants and non-allelic comparisons are typically called in a non-blinded fashion.

The database currently contains records for 315 manually curated allelic comparisons, ∼62% of which are annotated as resulting in a change of function. The large majority of manually curated allelic comparisons stem from studies on limb loss in snakes due to ZRS mutations, on human pathogenic mutations in ZRS enhancers or on sensitivity of ultraconserved brain enhancers (7,8,19).

Database contents

As of August 2024, the database contained data from over 4500 experiments, including over 23 500 images of over 13 500 transgenic embryos. The majority of experiments were assessed at embryonic stage E11.5 (>85%), using random transgenesis with Hsp68 minimal promoter (>70%) or enSERT (>25%; targeted H11 transgenesis with Shh minimal promoter), testing a human or mouse element (>60% and >35%, respectively) with a positive result (∼50%; Figure 4A). Of the positive experiments, nearly 80% showed activity in the brain, limb or heart (Figure 4B), reflecting the main tissue groups investigated by this lab and the propensity of evolutionarily conserved elements to be positive for brain activity (9–17).

As an example of an analysis that can be performed using this data, we investigated the co-occurence of activity across all pairs of tissues with more than 50 experiments (Figure 4C). We found an expected, strong correlation between activity in

dorsal root ganglia, the trigeminal nerve and other cranial nerves, between different parts of the brain and the neural tube, between heart and somites, as well as between craniofacial tissues (branchial arches, facial mesenchyme, nose, ear). Interestingly, there was also a strong correlation between craniofacial and limb activity, likely reflecting a common evolutionary origin of these structures (20).

The VISTA Enhancer Browser with its enhanced features for display of time series, allelic comparisons, and major expansion in content will continue to provide a rich resource for enhancer studies in the context of developmental biology, gene regulation, and human variation.

Data availability

All data is available on enhancer.lbl.gov and on gitlab.com/egsb-mfgl/vista-data.

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Author contributions: M.K. supervised the project, designed the database, performed data integration and cleanup, wrote the manuscript. F.A.B. designed and implemented the external facing website. G.K., J.B. and Y.O. designed and implemented the internal interface. L.E.C. helped with data cleanup and designed the graphical abstract. D.E.D. designed the database and supervised the project. G.A.P. supervised F.A.B. and obtained funding. L.A.P. supervised the project and obtained the funding. A.V. supervised the project, obtained the funding and wrote the manuscript.

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Conflict of interest statement

None declared.

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