Genome-Wide Mapping of Human Craniofacial *in vivo* Enhancers

Cailyn Spurrell[1], Yoko Yuzawa[1], Iros Barozzi[1], Han Wu[1], Ingrid Plajzer-Frick[1], Catherine Pickle[1], Brandon Mannion[1], Veena Afzal[1], Jennifer Akiyama[1], Diane Dickel[1], Len Pennacchio[1,2], and Axel Visel[1,2,3]

[1]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley CA, [2]US Department of Energy Joint Genome Institute, Walnut Creek, CA, [3]School of Natural Sciences, University of California Merced, Merced, CA

Genetic studies show that non-coding sequences contribute to the susceptibility for craniofacial birth defects, as well as general facial variation in humans. Distant-acting enhancers are a major category of non-coding elements with important roles in development and disease, but they are notoriously difficult to annotate in a systematic way because of their highly specific spatial and temporal *in vivo* activity patterns, many of which are difficult or impossible to recapitulate in cell culture models. While many enhancers are conserved across vertebrate species, a significant fraction of human enhancers do not have orthologs in experimentally accessible model organisms. To enable the functional interpretation of genetic studies of craniofacial development and disorders, we generated comprehensive annotations of enhancers active at different stages of human and mouse craniofacial development. We performed ChIP-seq directly on a series of mouse and human craniofacial tissues using marks of active and repressive chromatin (H3K4me1, H3K27ac, and H3K27me3). We generated data from five biological replicates of Carnegie Stage 18 (CS18) human prenatal craniofacial tissue. In mouse, we produced enhancer catalogues from the mandibular, maxillary, and nose subregions in e13.5 embryos, the developmental equivalent of CS18. In total, we identified more than 15,000 predicted enhancers from human samples, of which only 45% were conserved to mouse. Of the more than 8,000 regions that were not conserved, 90% had a clear murine ortholog but no ChIP signal in mouse, whereas 10% of sequences were deleted altogether. Validation of subsets of human-specific enhancers in transgenic mouse reporter assays, as well as comparative analysis of enhancers with predicted activity differences between species is in progress. Our results provide a comprehensive collection of enhancers active during human craniofacial development that are expected to enable and facilitate the interpretation of genetic studies of non-coding sequences involved in craniofacial biology and birth defects.