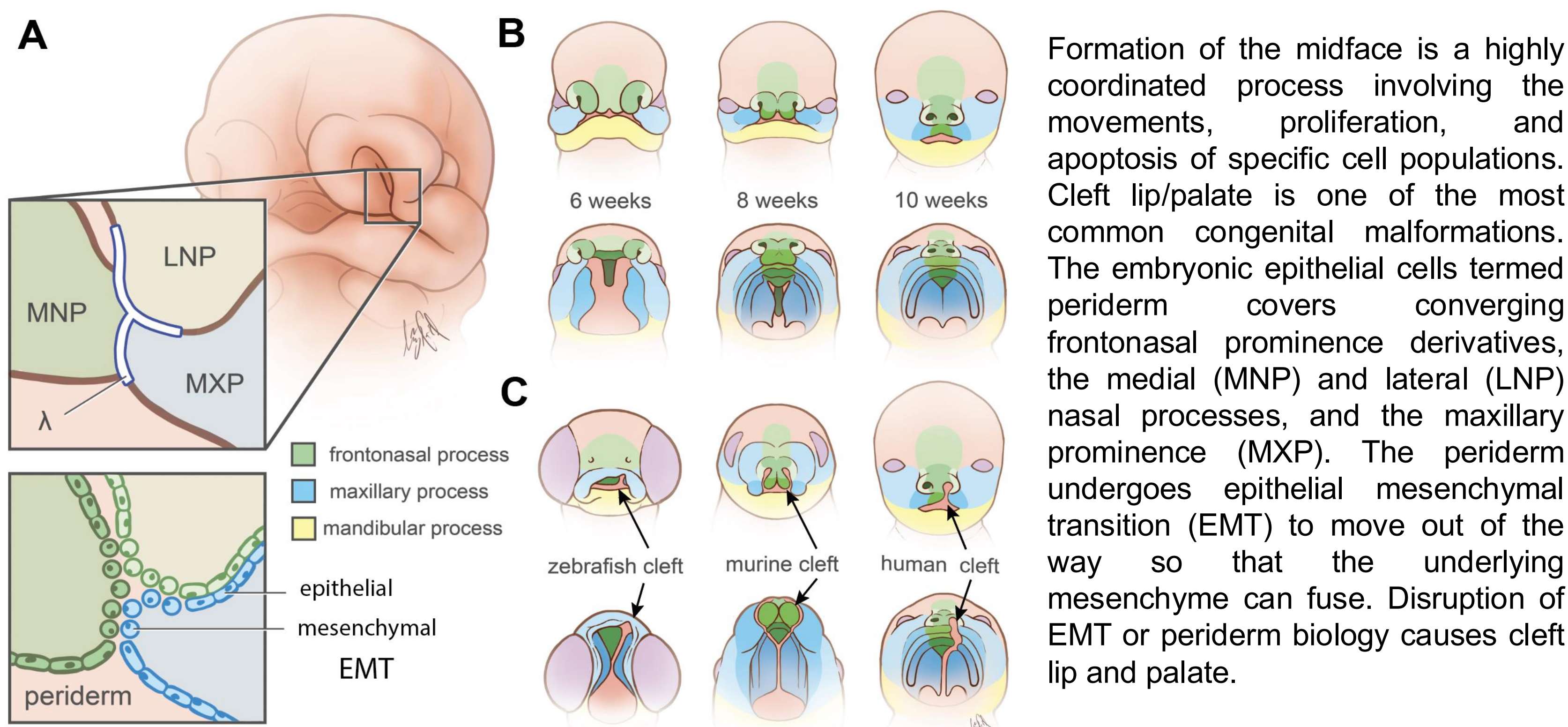
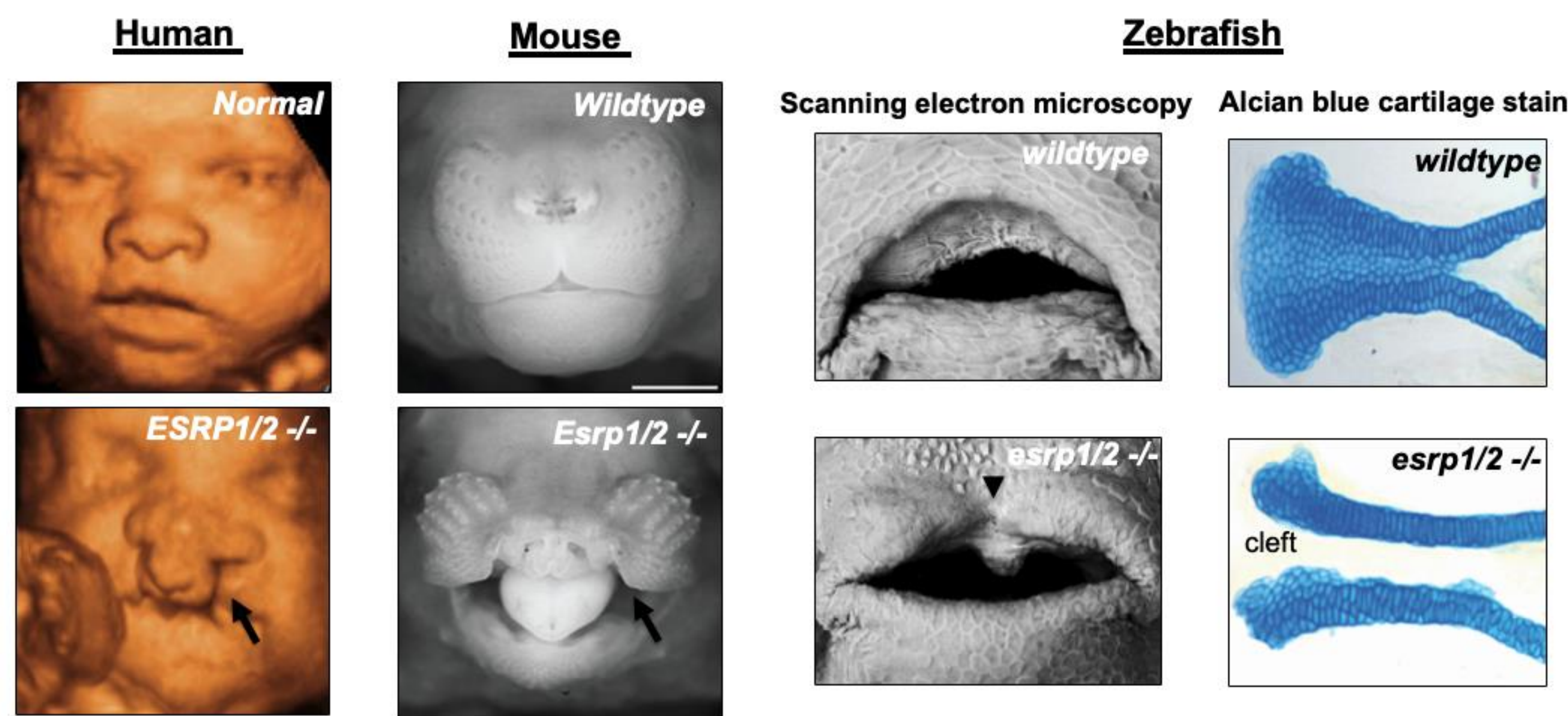


Sogand Schafer, MD, Feng Wang, PhD, Yongjun Li, PhD, Yi Xing, PhD, Eric C. Liao, MD, PhD. Children's Hospital of Philadelphia, Philadelphia, PA, USA.

Orofacial clefts result from impaired epithelial mesenchymal transition during embryogenesis

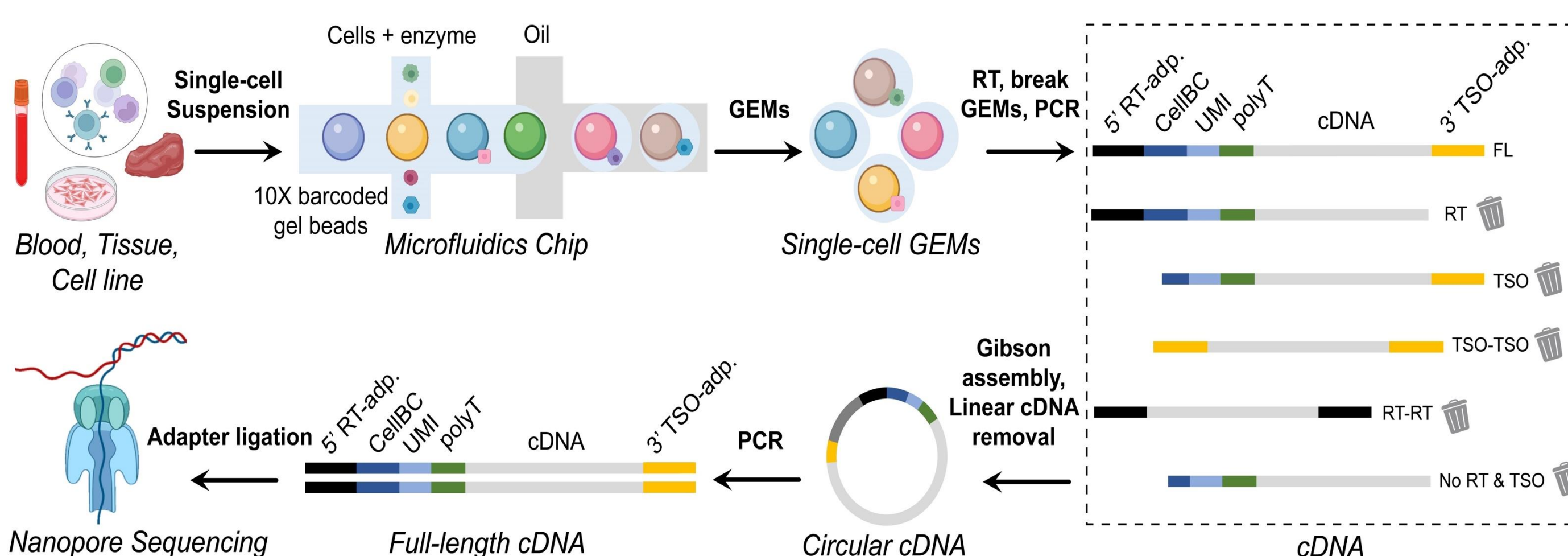


Most (90%) human genes undergo alternative splicing, regulated by ESRP1/2 in periderm



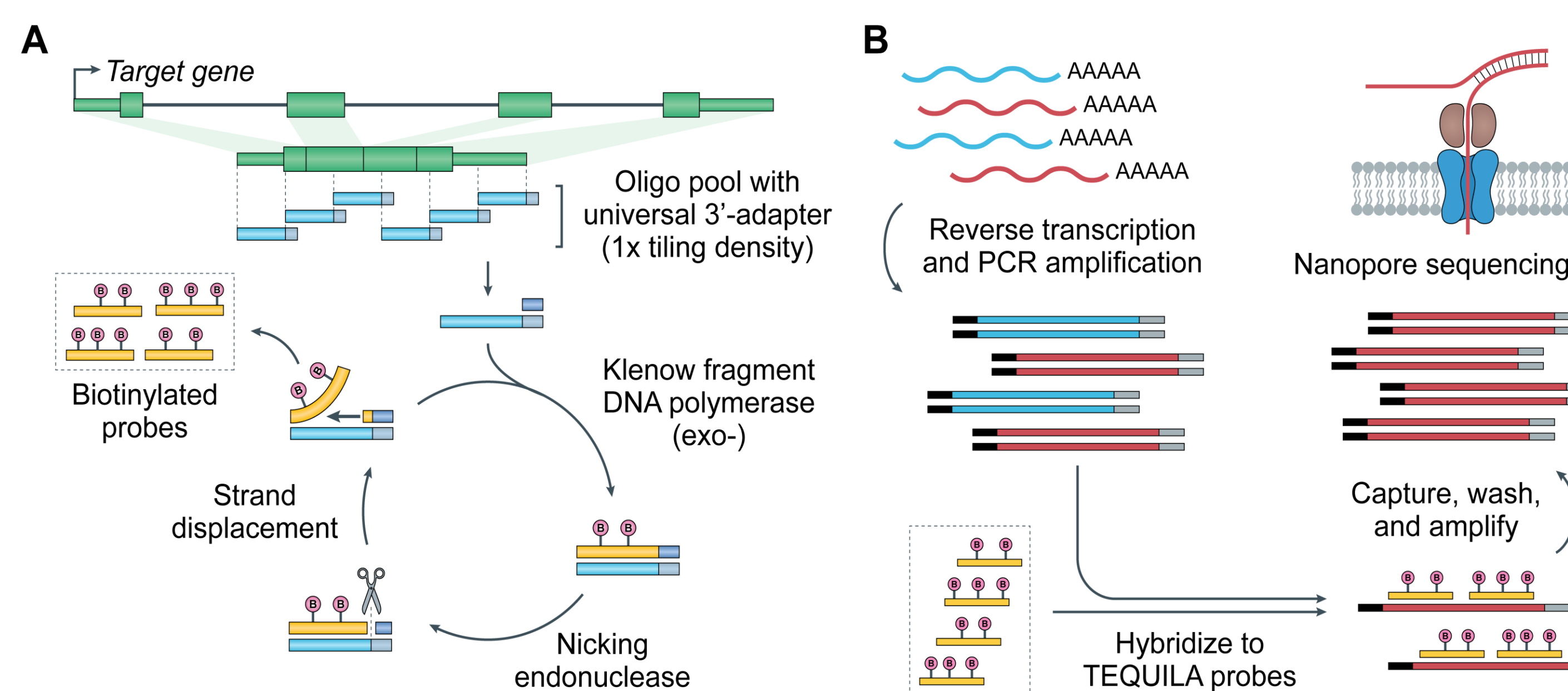
We previously identified epithelial splicing regulatory proteins ESRP1 and ESRP2 as regulators of an epithelial-specific splicing program that maintains epithelial state and is reverted during EMT. ESRP1/2 are among the most downregulated genes in multiple EMT model systems. Disruption of ESRP1/2 in human, mouse, and zebrafish abrogates cell fate of embryonic epithelium, and leads to dramatic orofacial cleft phenotypes.

Long read RNAseq enabled us to comprehensively identify isoforms genome wide during EMT



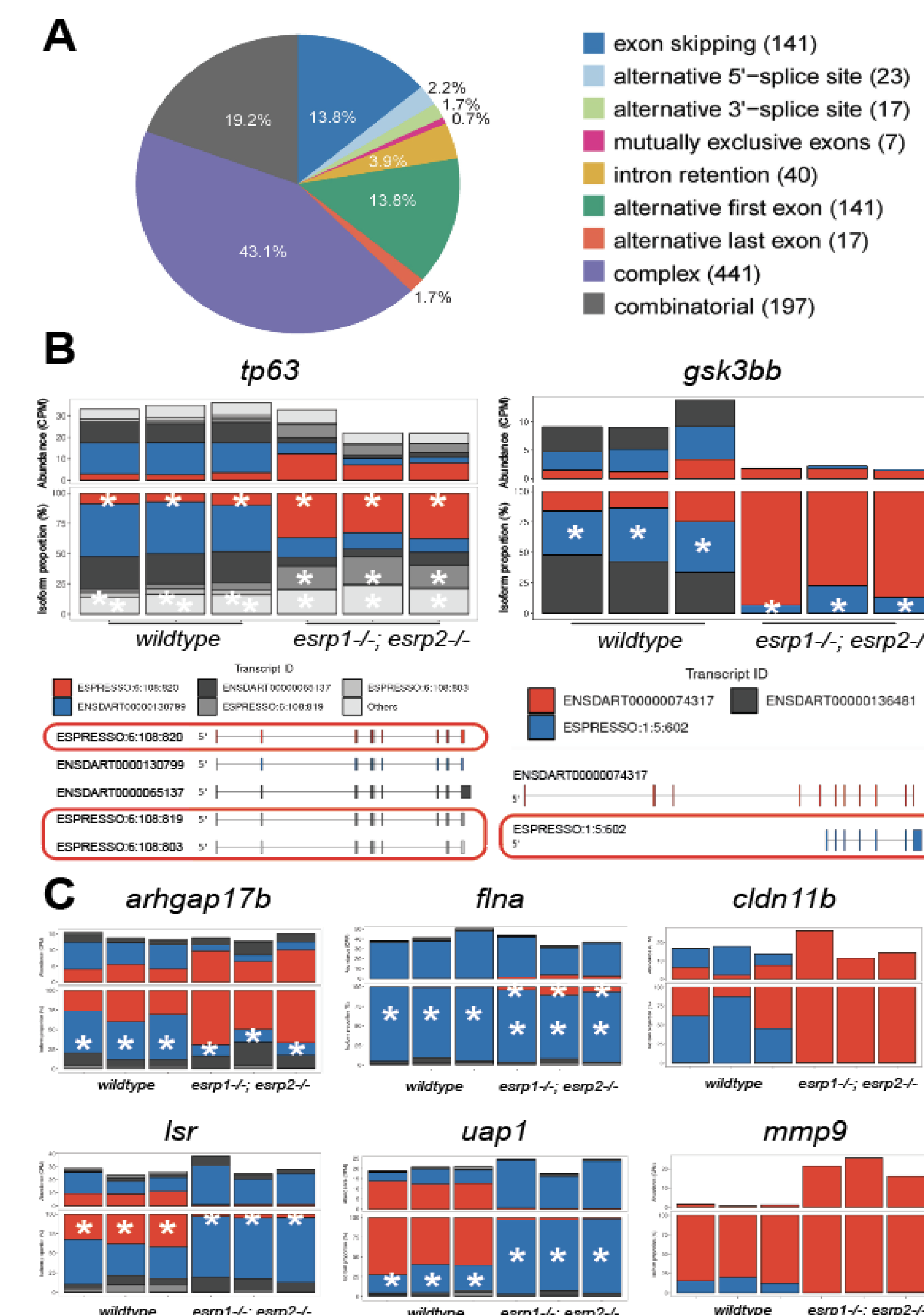
We developed a new approach called scCirRL-seq (single-cell Circularized Re-Linearized sequencing) Here, full-length cDNA is converted into circular cDNA using Gibson assembly with a DNA splint. 10X library artifacts that lack the full-length cDNA structure with 5' and 3' end adaptors and thus cannot be circularized are digested. The circular cDNAs are then re-linearized by a limited-cycle PCR using primers against the DNA splint and then subjected to nanopore sequencing. This long read technology enabled us to identify actual isoforms apart from prior short read sequences that computational methods could not distinguish isoform from artifact.

TEQUILA-seq (Transcript Enrichment and Quantification Utilizing Isothermally Linear-Amplified probes in conjunction with long-read sequencing)



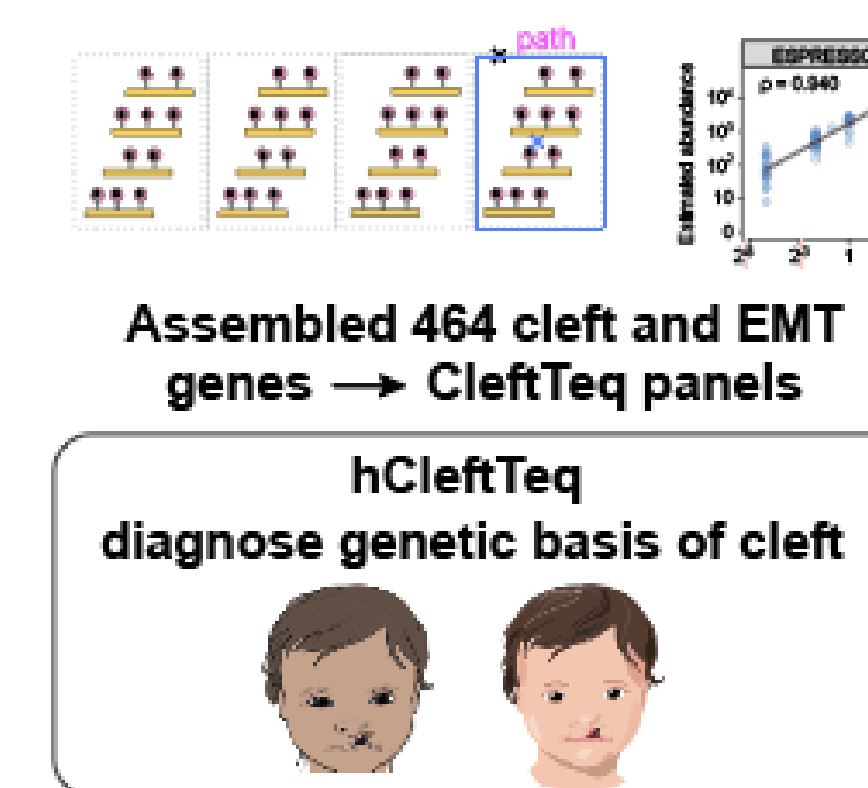
A) Single-stranded DNA (ssDNA) oligos (120 nt) are designed to tile across all annotated exons of target genes and synthesized using array-based DNA synthesis technology. TEQUILA probes are amplified from ssDNA oligo templates in a single pool using nickase-triggered strand displacement amplification with universal primers and biotin-dUTPs. **B)** Full-length cDNAs are synthesized from poly(A)⁺ RNAs by reverse transcription PCR. TEQUILA probes are then hybridized to cDNAs. Upon capture and washing, captured cDNAs are amplified by PCR and subjected to nanopore cDNA library preparation and sequencing.

Esrp-regulated genes prioritized for mechanistic analysis



Underscoring the advantage of the long-read RNA-seq approach, >60% of isoform switches identified by rMATS-long involved complex or combinatorial AS patterns, which are challenging to analyze and often overlooked by standard short-read RNA-seq. We also identified numerous novel isoforms, that were absent from Ensembl annotations of zebrafish transcripts, including in genes with well-established functions in EMT (red brackets and white asterisks). For the top Esrp-regulated EMT genes we identified to exhibit isoform switches between wildtype and esrp1/2 mutant, 6 of the 8 genes involved switching of novel isoforms. For example, we identified novel isoforms of *tp63* and *gsk3bb* that were not previously annotated. Further, the altered proportion of these isoforms between wildtype and *esrp1/2* mutant across 3 biological replicates of each genotype suggests that these isoforms are regulated by *esrp1/2*. This preliminary result validates the critical unmet need of existing publicly available isoform annotation and demonstrates the ability of long-read RNA-seq technology to identify novel isoforms as well as isoform switches involving complex or combinatorial AS patterns, where short-read RNA-seq and conventional methods fall short.

RNA-first diagnosis of cleft anomalies – fast, accurate and cost effective



We are carrying out comprehensive and systematic mechanistic analysis of EMT and cleft isoforms. We will use single cell long-read RNA-seq and ESPRESSO workflow to identify Esrp-regulated isoforms from murine and zebrafish models, and functionally study isoforms using *in vitro* and *in vivo* assays rapid and cost-effective isoform identification and quantification of 1200 cleft and EMT genes across experimental models and RNA-guided diagnosis of orofacial cleft. We will share CleftTeq panel and scRNAseq data atlases online with the community.

SCHAFERS1@CHOP.EDU

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