Complementary Analyses-1 (CA1)

Analysis of hemi-exonic circRNAs detected by CIRI2

When we re-analyzed the circRNAs identified by CIRI2 in 117 bovine samples, over 10,000 hemi-exonic circRNAs were detected relative to the bovine reference genome (ARS-UCD1.2) associated to the Ensembl annotation *(v-110). Through further annotation efforts (using a comprehensive second GTF file), we were able to annotate nearly half of these bovine circRNAs, suggesting that the half of these hemi-exonic circRNAs could be classified as exonic circRNAs.*

The bovine circRNAs identified in bovine tissues by CIRI2 were examined to highlight those with only one of their boundaries corresponding exactly to an exon boundary. From CIRI2 data, 58,373 circRNAs identified by two reads supporting a circular junction in at least one sample among 117 investigated (Robic et al. 2024 [13]), we retained 10,742 circRNAs (hemi-exonic circRNAs). Of the 8,155 SS-acceptors, only 3,789 were already known (Ensembl), while of the 8,451 distinct SS-donors, only 4,484 were already described.

> In this annotation, we considered 238,049 bovine exons, which defined 225,668 SS-acceptors and 227,041 SS-donors distinct boundaries. Among this set there was a notable prevalence of involvement of the SS acceptor (Pearson's Chi-squared test with Yates' continuity correction, p-value = 3.151 10^{-13}).

We can hypothesize the involvement of 4,366 new SS-acceptors and 3,967 new SS-donors, i.e. not yet known in the Ensembl annotation (v-103). Among them, 2,268 and 1,803 were currently described in the BovReg* annotation as SS-acceptors and SS-donors, respectively.

> The use of the BovReg annotation allows to re-equilibrate since we noted that 74% of initial SS-acceptors (6,057/8,155) and SS-donors (6,292/8,451) were now described.

	10,742 circRNAs	
8,155 unique SS-acceptor		8,451 unique SS-donor
3,789	described in Ensembl (v-103)	4,484
2,268	described in BovReg GTF	1,803
2,098	Currently unknown	2,164

From the 10,742 hemi-exonic circRNAs selected when we only considered the Ensembl annotation and after an annotation effort, 4,959 of them are now candidates for annotation as exonic circRNAs. To complete this analysis, it would be necessary to check that the two exons identified belong to the same gene and that they have already been associated in the same linear transcript.

CIRI2 detected in mRNA-seq 1.7% of circRNAs already detected in total-RNA-seq, but also an additional 1%, highlighting the need for careful interpretation of the CIRI2 filter on the backsplicing signature (canonical splicing sites).

Among the CIRI hemi-exonic circRNAs, 8.8% were also detected in mRNA-seq. For exonic circRNAs annotated with Ensembl+BovReg, the score is close to 0.5%.

* One of the aims of the European BovReg project (https://www.bovreg.eu/) was to generate a map of functionally active regulatory and structural elements in the bovine genome using a diverse catalog of at least 26 tissue types collected from individuals of both sexes and from divergent breeds/crosses.
The BovReg annotation consisted of a gtf_file defining 683,396 distinct exons (average length = 1,628 nt and median length = 226 nt). Only 235,049 were previously described by Ensembl v105 (average length = 308 nt and median length = 139 nt).