

Res_Adoc-1

Comparison of both annotations performed for the 23,926 exonic circRNAs

Classical annotation (CD + manual curation)	Exon	Minimal annotation
20,000	Ens-Ens	18,429
1,894	MSTRG-MSTRG	1,682
2,032	Mixed (Ens-MSTRG or MSTRG-Ens)	3,815
7,993	Number of parental genes	8,461

Res_Adoc-2

Overview across other_circRNAs detected in the different datasets and with alternative bioinformatic pipelines

Sample	circRNAs considered	Detected	with size < 160nt	
117T	All CD-other_circRNAs	36,215	8,285	22.88%
	<i>Defensin</i> region	3,159	403	12.80%
	Mitochondrial genome	487	235	48.30%
63T	All CD-other_circRNAs	17,956	3,966	22.10%
63m	All CD-other_circRNAs	4,341	2,129	(49,0%)
CIRI-117T	All CIRI-other_circRNAs	10,081	111	1.10%

Three comparisons of the number of other_circRNAs with small size were tested
 >> chisq_test with p-value < 2.2 10⁻¹⁶

Res_Adoc-3

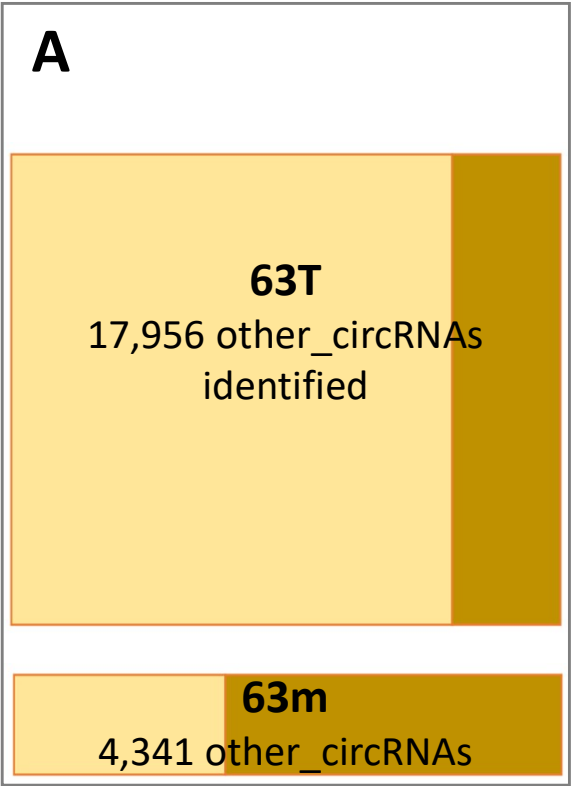
Comparison of features of other_circRNAs

	CD-other_circRNAs	CIRI-other_circRNAs
Two feet out of any exon	29.90%	18.00%
Only one foot in exonic sequences	7.70%	32.70%
Two feet in exonic sequences	62.40%	49.30%
The interval defined by the two feet contains a full exon	28.80%	74.00%
The size of the genomic interval is >500 kb	1%	0%*
The size of the genomic interval is <160 bp	22.88%	1.10%

* CIRI2 does not retain circRNAs defining a genomic interval >200 kb

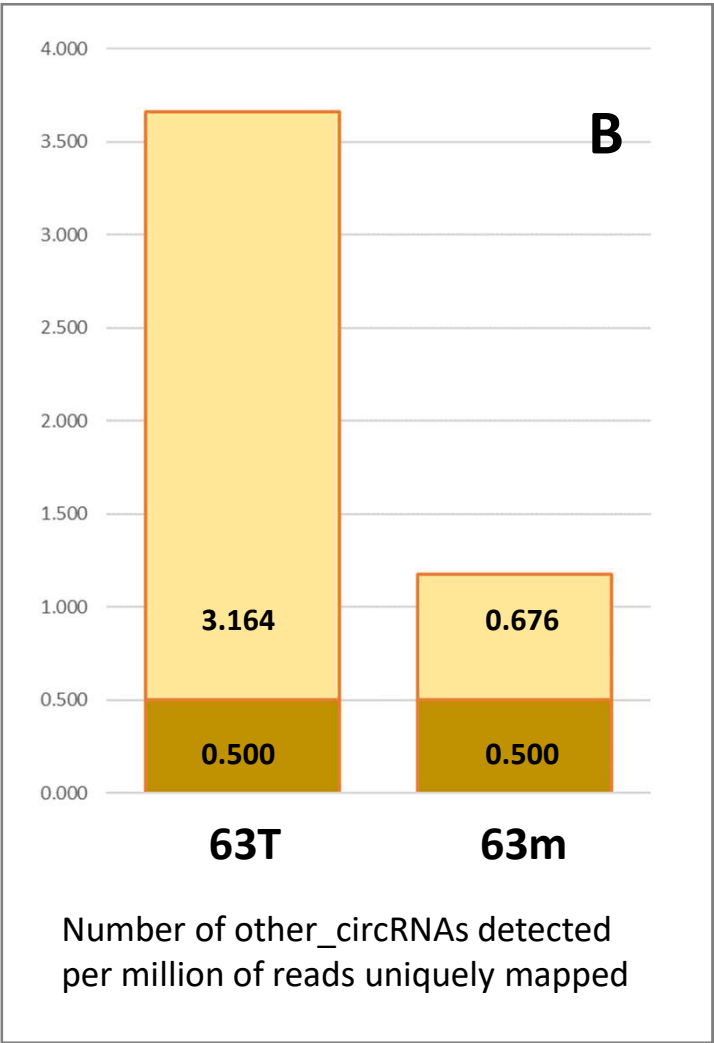
We defined the two ‘feet’ of a circRNA by the two genomic coordinates of its genomic boundaries.

Other_circRNAs in 63T/63m



Other_circRNAs

- Non-sub-exonic circRNAs
- Sub-exonic circRNAs from multi-exonic genes



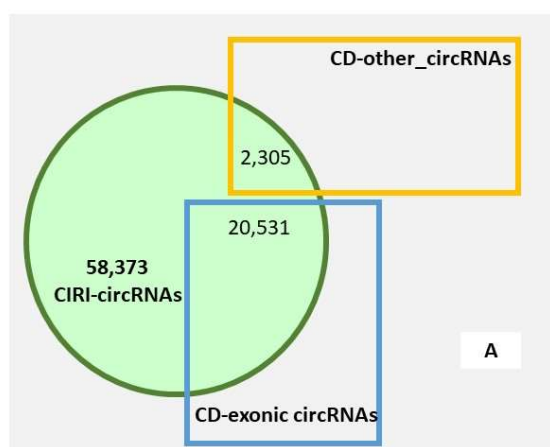
Res_Adoc-5

Other_circRNAs identified in this study

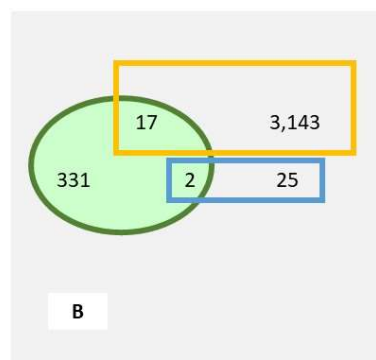
Number of other_circRNA identified		63m	63T	118T
Genome		4,341	17,956	36,245
	already known	1,529	17,956	36,245
	novel	2,812		
Albumin region		27	59	59
	already known	22	59	59
	novel	5		
Defensin region		7	1,671	3,160
BTA27: 6,21-6,23Mb	already known	4	1,671	3,160
	novel	3		
MT chromosome		556	319	487
	already known	98	319	487
	novel	458		

CIRI2-117T

detection performed with at least two reads spanning the circular junction



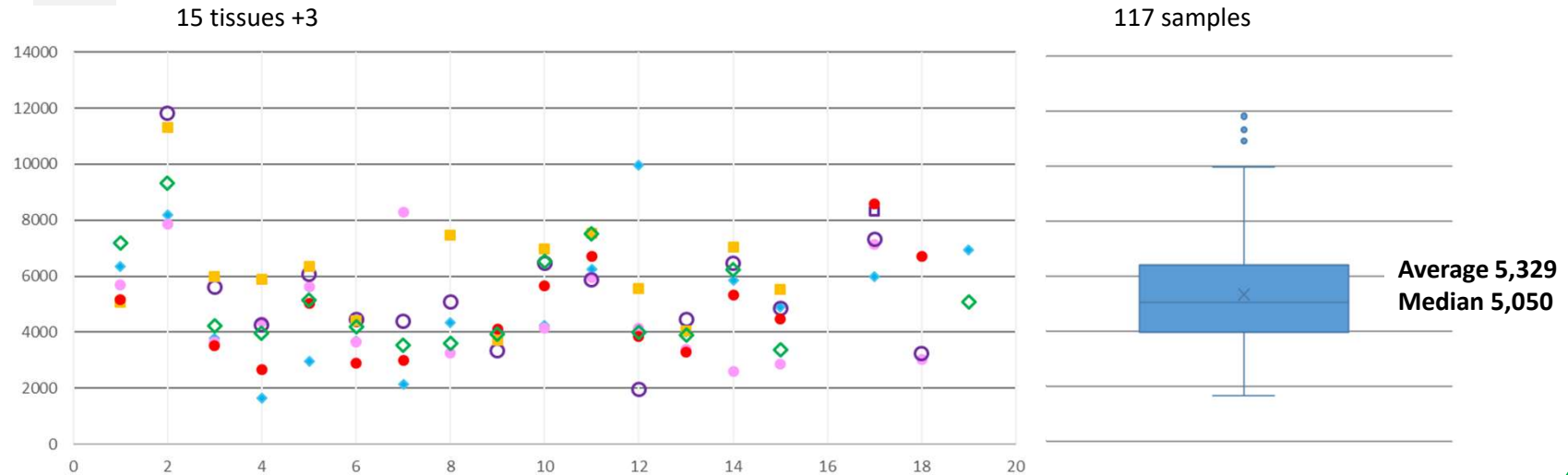
Identification of the 58,373 CIRI-circRNAs



Identification of the CIRI-circRNAs from the *Defensin* region

A

Number of exonic circRNAs with a non null expression



◆ NN-male ● NN-female ○ Juvenile-female ■ Juvenile-male castrated ● adult-female ◇ adult-male

15 tissues +3

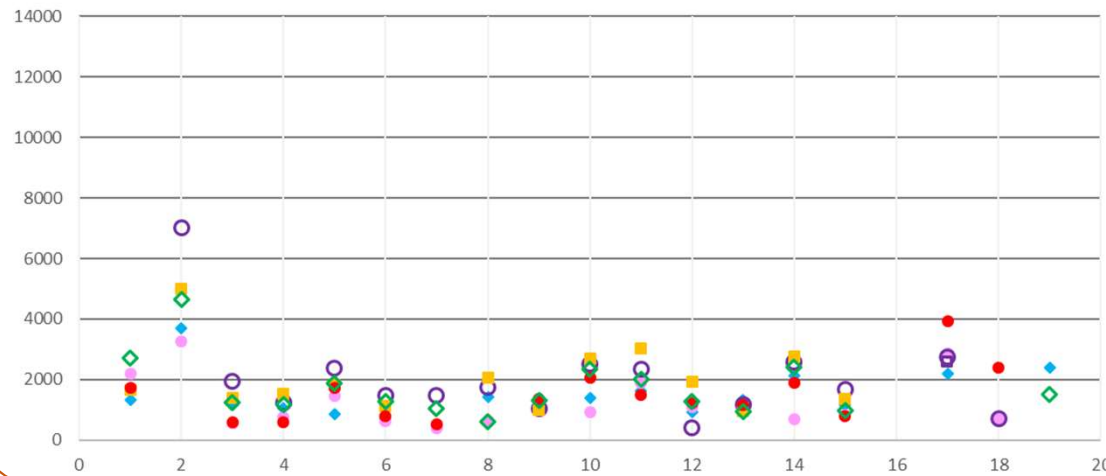
Adrenal gl.	1	Lung	10
Cerebellum	2	S. Muscle	11
Colon	3	Pituitary gl.	12
Duodenum	4	Rumen	13
Heart	5	Spleen	14
Ileum	6	S. fat	15
Jejunum	7		
Kidney	8	Cerebral cortex	17
Liver	9	Ovary	18
		Testis	19

Res_Adoc-6A

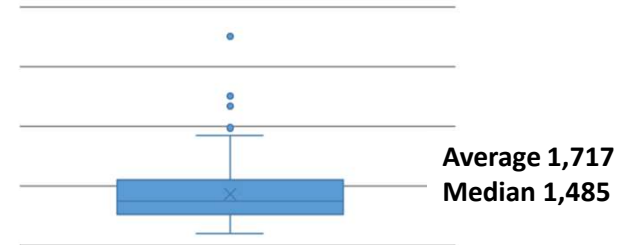
B**Number of exonic circRNAs with a notable expression***

15 tissues +3

117 samples



* **Notable expression: Exp > 0.05**
CCR per million of reads uniquely mapped



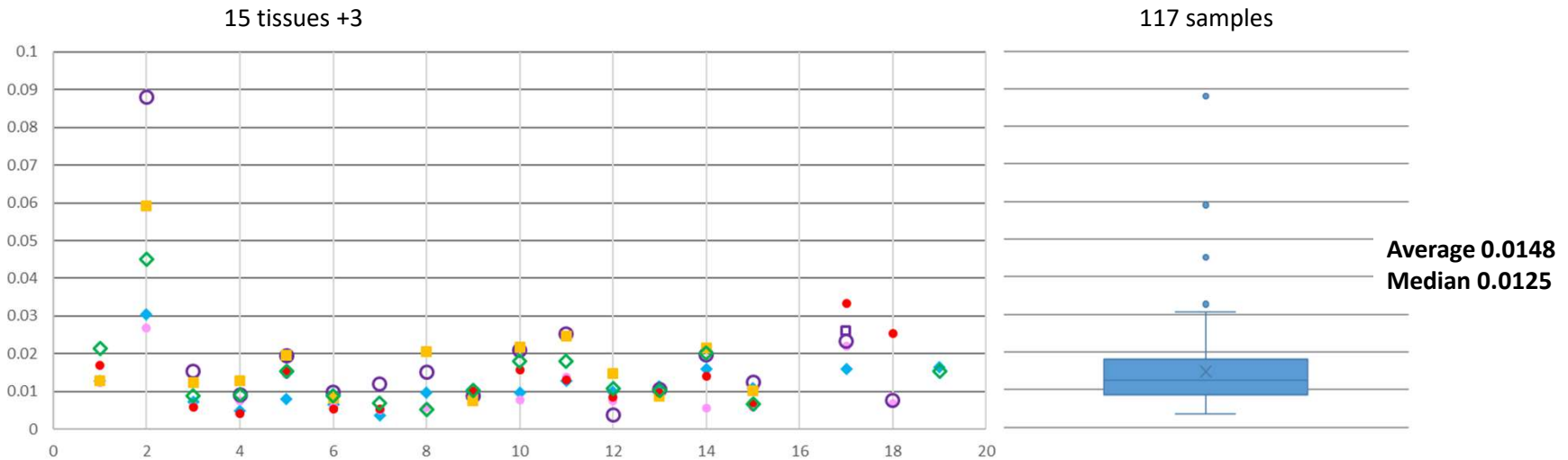
◆ NN-male ● NN-female ○ Juvenile-female ■ Juvenile-male castrated ● adult-female ◇ adult-male

15 tissues +3

Adrenal gl.	1	Lung	10
Cerebellum	2	S. Muscle	11
Colon	3	Pituitary gl.	12
Duodenum	4	Rumen	13
Heart	5	Spleen	14
Ileum	6	S. fat	15
Jejunum	7		
Kidney	8	Cerebral cortex	17
Liver	9	Ovary	18
		Testis	19

Res_Adoc-6B

Average expression of exonic circRNAs



◆ NN-male ● NN-female ○ Juvenile-female ■ Juvenile-male castrated ● adult-female ◇ adult-male

15 tissues +3

Adrenal gl.	1	Lung	10
Cerebellum	2	S. Muscle	11
Colon	3	Pituitary gl.	12
Duodenum	4	Rumen	13
Heart	5	Spleen	14
Ileum	6	S. fat	15
Jejunum	7		
Kidney	8	Cerebral cortex	17
Liver	9	Ovary	18
		Testis	19

Res_Adoc-6C

A	Nb of exonic circRNAs included in the considered panel		96 samples		56 samples		40 samples	
				15 tissues X 3-4 animals (juveniles or adults)		20 tissues X 2 animals (neonates)		
			Data normalized by log-binary method					
top-30	353	1.5%	Cerebral cortex / Sub. Fat / Pancreas	top-30		top-30		
top-40	456	1.9%	<div>Clustering OK</div> <div>For 9 tissues (5 or 6 animals)</div> <div>Kidney Cerebellum Muscle Heart</div> <div>Liver Lung Spleen Adrenal gland</div> <div>Rumen</div> <div>For 2 tissues (4 old animals)</div> <div>Sub. fat Pituitary gland</div> <div>For 3 tissues (2 young animals)</div> <div>Cerebral cortex Thyroid Pancreas</div>	top-40	Rumen	top-40	Cortex/Rumen/Pancreas/Spleen/Ileum	
top-60	679	2.9%		top-60		top-60	Pancreas	
top-80	912	3.8%		top-80		top-80	+ Ileum	
top-100	1124	4.7%		top-100	<div>Clustering OK for 11 tissues (3 or 4 animals)</div> <div>Kidney Cerebellum Muscle Heart</div> <div>Liver Lung Spleen Adrenal gland</div> <div>Rumen</div> <div>Sub. Fat Pituitary gland</div>	top-100	<div>Clustering OK for 12 tissues (2 animals)</div> <div>Kidney Cerebellum Muscle Heart</div> <div>Liver Lung Spleen Adrenal gland</div> <div>Rumen</div> <div>Thyroid Cerebral cortex Pancreas</div>	
top-150	1749	7.4%	<div>shown in Fig 7</div>	top-150		top-150		
top-200	2430	10.2%		top-200		top-200	Cortex/Rumen/Pancreas/Spleen/Ileum	
top-250	3126	13.2%		top-250		top-250	Cortex/Rumen/Pancreas/Spleen	
top-300	3834	16.1%		top-300		top-300	Cortex/Rumen/Pancreas/Spleen Ileum	
top-400	5398	22.7%		top-400		top-400		
top-450	6173	26.1%		top-450		top-450		
top-1200	16555	69.7%	Rumen	top-1200		top-1200	Rumen/Pancreas/Spleen	
Fraction (in %) of the 23.737 exonic circRNAs								

B	Nb of exonic circRNAs included in the considered panel		56 samples										40 samples						
			96 samples					15 tissues X 3-4 animals (juveniles or adults)					20 tissues X 2 animals (neonates)						
			Data normalized by standard score method																
top-30	353	1.5%	Spleen Lung Rumen					top-30	Spleen					top-30					
top-40	456	1.9%	+ Rumen					top-40	Spleen					top-40					
top-60	679	2.9%	For 9 tissues (5 or 6 animals) Kidney Cerebellum Muscle Heart Liver Lung Spleen Adrenal gland					top-60	Spleen					top-60					
top-80	912	3.8%						top-80	Spleen					top-80					
top-100	1124	4.7%						top-100	Clustering OK for 10 tissues (3 or 4 animals) Kidney Cerebellum Muscle Heart Liver Lung Spleen Adrenal gland Sub. Fat Pituitary gland					top-100	Clustering OK for 11 tissues (2 animals) Kidney Cerebellum Muscle Heart Liver Lung Spleen Adrenal gland Rumen Thyroid Cerebral cortex				
top-150	1749	7.4%						top-150											top-150
			For 1 tissue (4 old animals) Sub. fat																
			For 2 tissues (2 young animals) Cerebral cortex Thyroid																
top-200	2430	10.2%						top-200						top-200					
top-250	3126	13.2%						top-250						top-250					
top-300	3834	16.1%						top-300						top-300	Spleen Ileum				
top-400	5398	22.7%						top-400						top-400	Spleen Ileum				
top-450	6173	26.1%						top-450						top-450	Spleen Ileum				
top-1200	16555	69.7%						top-1200						top-1200	Spleen Ileum				
Fraction (in %) of the 23.737 exonic circRNAs																			

All HCAs were built using the "ward" agglomeration method and Pearson correlations as distance. Results of all HCAs built using different panels of exonic circRNAs with data normalized by the log-binary method (B) or by the standard score (C) were summarized. Only the observations that differed from the respective reference results (HCA obtained with top-150) were reported, From left to right, 96 samples from 20 tissues were considered, only the 56 samples from oldest animals (J and A) were considered, and only the 40 samples from neonatal animals (N) were considered.