



# Copy number variants in 23 Italian local chicken breeds

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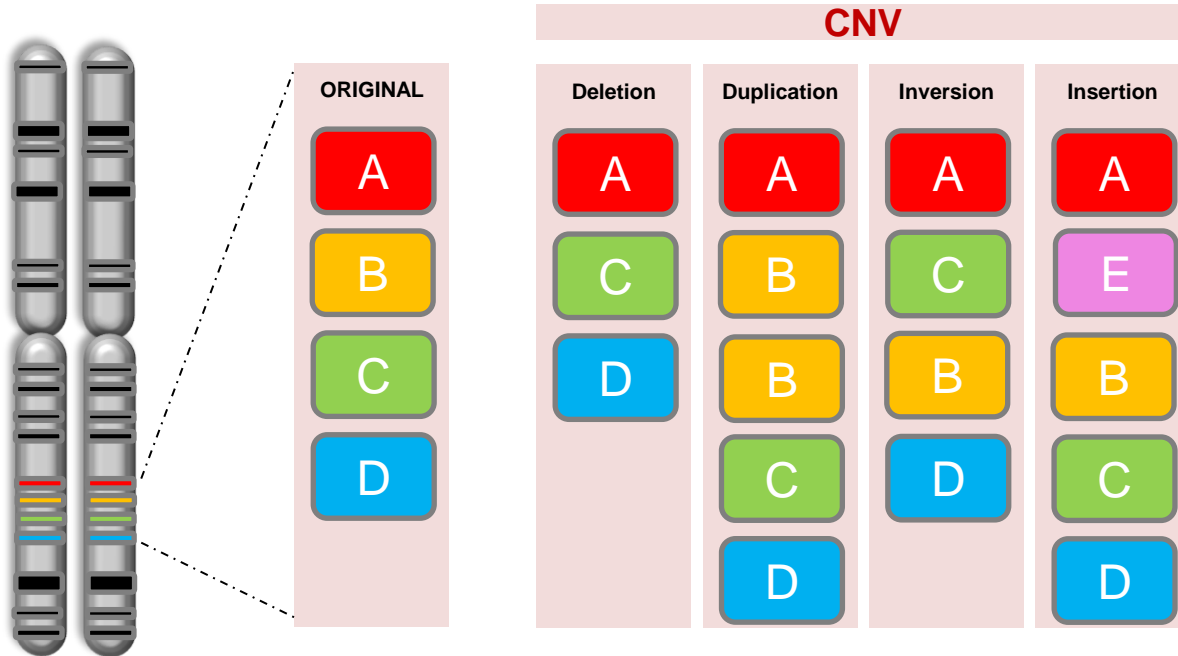
Progetto collettivo beneficiario per il Comparto Avicoli con il sostegno del  
Fondo Europeo Agricolo per lo Sviluppo Rurale (FEASR)  
[https://ec.europa.eu/agriculture/rural-development-2014-2020\\_it](https://ec.europa.eu/agriculture/rural-development-2014-2020_it)



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PER LO SVILUPPO RURALE:  
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# Copy number variants (CNV)

Copy number variants (CNV) refer to a circumstance in which the number of copies of a specific segment of DNA varies among genomes of different individuals. The individual variants may be short or include thousands of bases. These structural differences may have come about through duplications, deletions or other changes and can affect long stretches of DNA.



## Characteristics of the CNV and detection

1. Dislocated across the entire genome.
2. Could alter the gene structure and function.
3. Could be associated with a phenotypic trait or be expressionless.
4. The CNV dimensions change from 1 kb to several Mb.
5. They can involve several nucleotides.
6. Two classes were observed: *de novo* and hereditary.

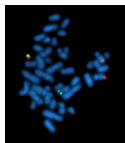


How to identify them?

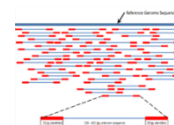


In the past

Fluorescence in situ hybridisation (FISH)



High throughput genomic sequencing



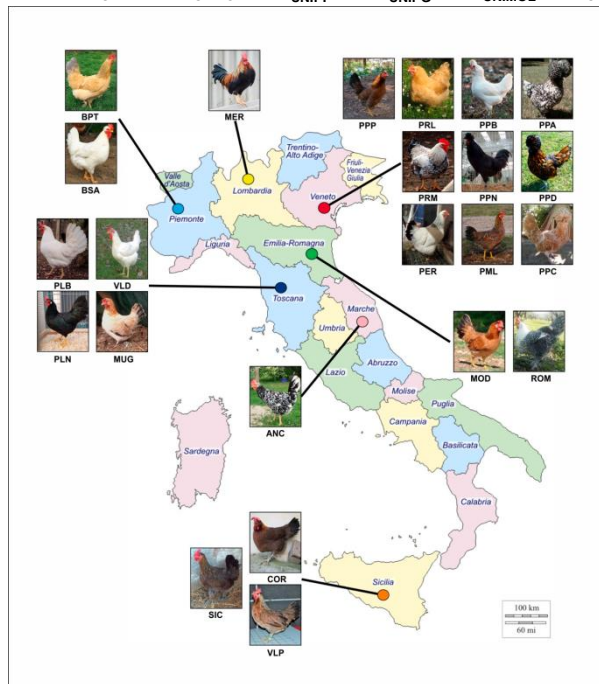
Today

Chip SNPs Array





## Local chicken breeds



**TUBAVI project**

<https://www.pollitaliani.it/>

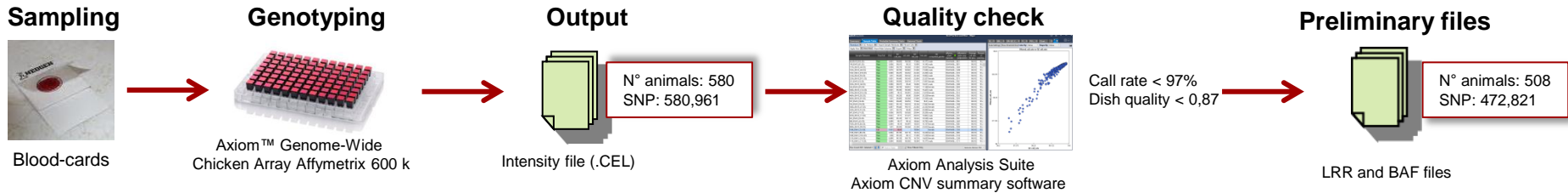
- Ancona (ANC)
- Bianca di Saluzzo (BSA)
- Bionda Piemontese (BPT)
- Cornuta di Caltanissetta (COR)
- Livorno Bianca (PLB)
- Livorno Nera (PLN)
- Mericanel della Brianza (MER)
- Modenese (MOD)
- Mugellese (MUG)
- Ermellinata di Rovigo (PER)
- Millefiori di Lonigo (PML)
- Padovana Argentata (PPA)
- Polverara Bianca (PPB)
- Padovana Camosciata (PPC)
- Padovana Dorata (PPD)
- Polverara Nera (PPN)
- Pepoi (PPP)
- Robusta Lionata (PRL)
- Robusta Maculata (PRM)
- Romagnola (ROM)
- Siciliana (SIC)
- Valdarnese (VLD)
- Valplatani (VLP)

### AIM

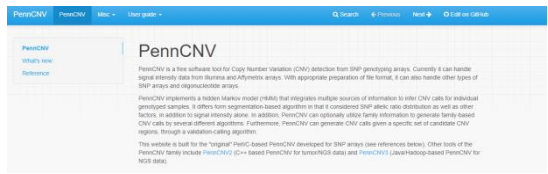
*To investigate and characterize the presence of CNV, copy number variation regions (CNVR) and candidate genes*

Material and  
methods

# Experimental design



## CNV calling



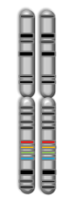
- Hidden Markov Model (Default parameters):**
- Standard deviation of LRR<0.30
  - BAF drift as 0.01
  - Waviness factor at 0.05
  - Minimum of 3 SNPs

## Validation

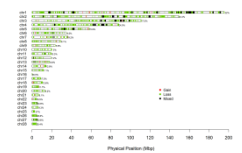
- agre.hmm
- affygw6.hmm
- hh550.hmm

Gorla et al., 2017

## CNV

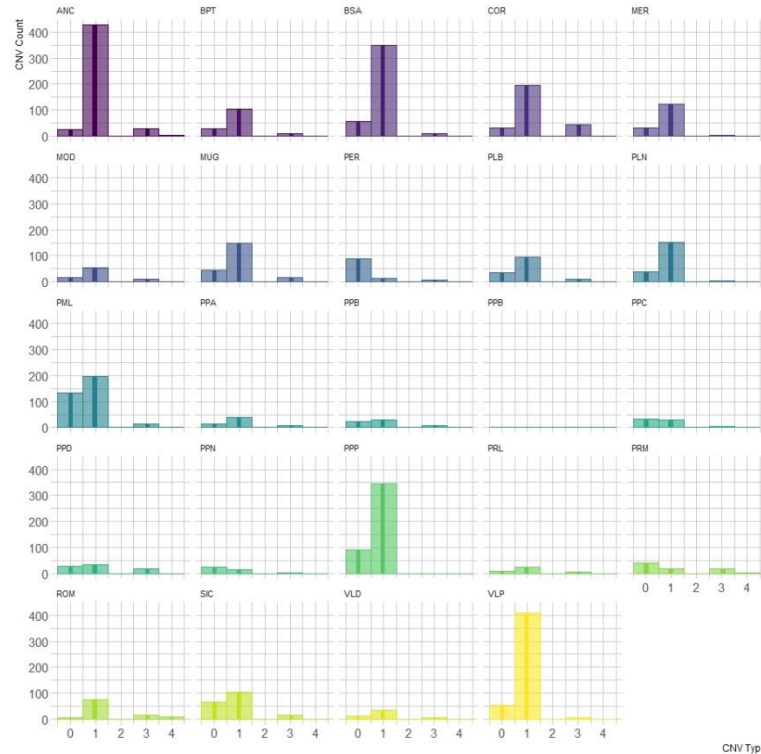


## CNVR and Genes



R package HandyCNV

# CNV



## CNV types:

- normal copy number is 2;
- 0 and 1 mean deletion;
- > 3 means duplication.

## General overview:

1. Deletion state is more present.
2. ANC, BSA, VLD and PPP show high number of CNV associated to deletions.
3. CNV associated to duplication events are detected in ANC and COR.

# CNV

Breed	Type					Length	Mean	Min Length	Max Length	Genome Coverage (%)
	0	1	3	4	Total					
ANC	24	427	27	2	480	30,895,153	64,365	2,378	918,621	2.71
BPT	27	104	8	0	139	9,930,942	71,446	5,580	1,862,447	0.87
BSA	58	348	11	0	417	44,933,947	107,755	6,464	803,891	3.94
COR	31	196	43	0	270	21,300,170	78,890	857	923,575	1.87
MER	32	124	4	0	160	15,414,093	96,338	5,157	900,960	1.35
MOD	15	54	9	0	78	2,324,960	29,807	4,494	100,019	0.20
MUG	46	150	15	0	211	10,431,587	49,439	2,726	506,421	0.91
PER	90	22	7	0	119	5,647,643	51,813	7,526	472,494	0.49
PLB	36	96	9	0	141	8,284,013	58,752	4,389	304,806	0.73
PLN	38	152	5	0	195	10,091,542	51,752	1,643	347,761	0.88
PML	134	198	13	0	345	24,815,055	71,928	4,431	412,846	2.17
PPA	15	39	8	0	62	3,095,619	49,929	9,035	153,514	0.27
PPB	24	31	8	1	64	1,847,570	28,868	3,023	115,628	0.16
PPC	34	39	5	0	78	2,284,986	33,603	3,023	221,819	0.20
PPD	27	35	18	0	80	2,276,698	28,459	3,023	221,819	0.20
PPN	23	26	2	0	51	527,861	12,875	3,023	45,506	0.05
PPP	91	344	0	0	435	75,993,667	174,698	4,904	2,863,848	6.66
PRL	9	25	6	0	40	941,152	23,529	3,614	68,519	0.08
PRM	40	30	17	1	88	2,075,529	27,310	2,378	69,072	0.18
ROM	6	74	16	8	104	4,068,436	39,120	6,264	217,674	0.36
SIC	65	104	15	0	184	8,853,192	48,115	5,105	256,437	0.78
VLD	13	33	7	0	53	4,929,325	94,795	3,499	1,059,613	0.43
VLP	52	409	7	0	468	79,647,292	170,187	3,249	2,849,628	6.98
<b>Total (*)</b>	930	3,060	260	12	4262	370,610,432	1,463,770	95,785	15,696,918	32

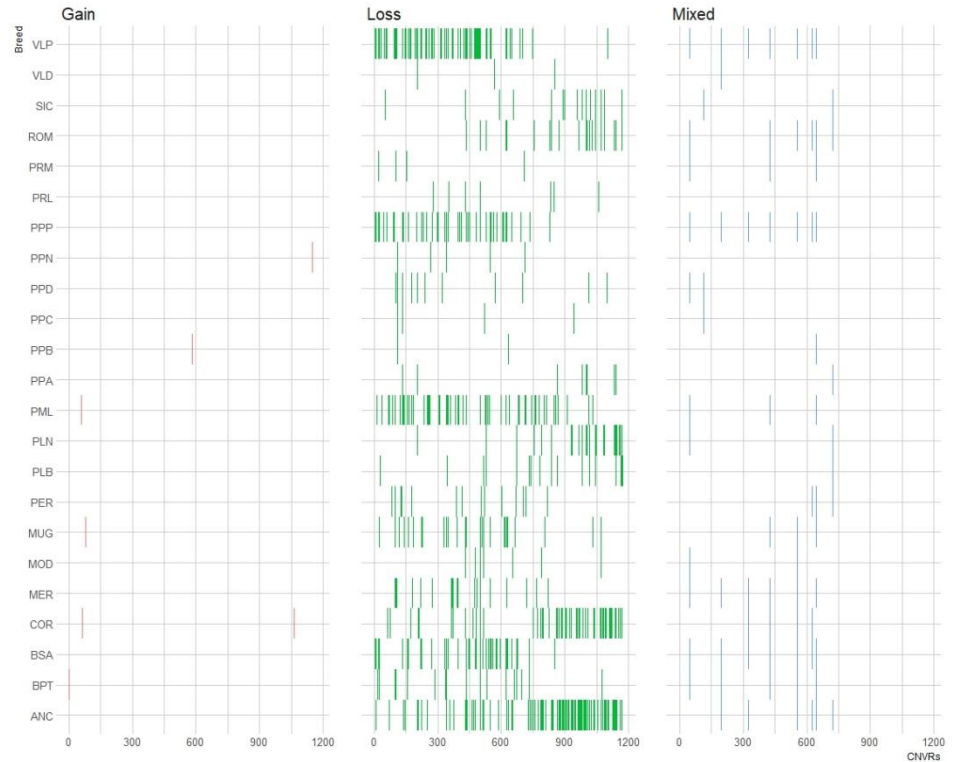
# CNVR

## CNVR types:

- **Loss:** loss of genetic material.
- **Gain:** gain of genetic material.
- **Mixed:** overlapping of loss and gain regions.

## General overview:

1. Loss of the genetic material occurs with high frequency.
2. ANC, PPP, PML COR and VLP breeds show high loss genomic regions.
3. Several CNVR among breeds overlap.





# CNVR

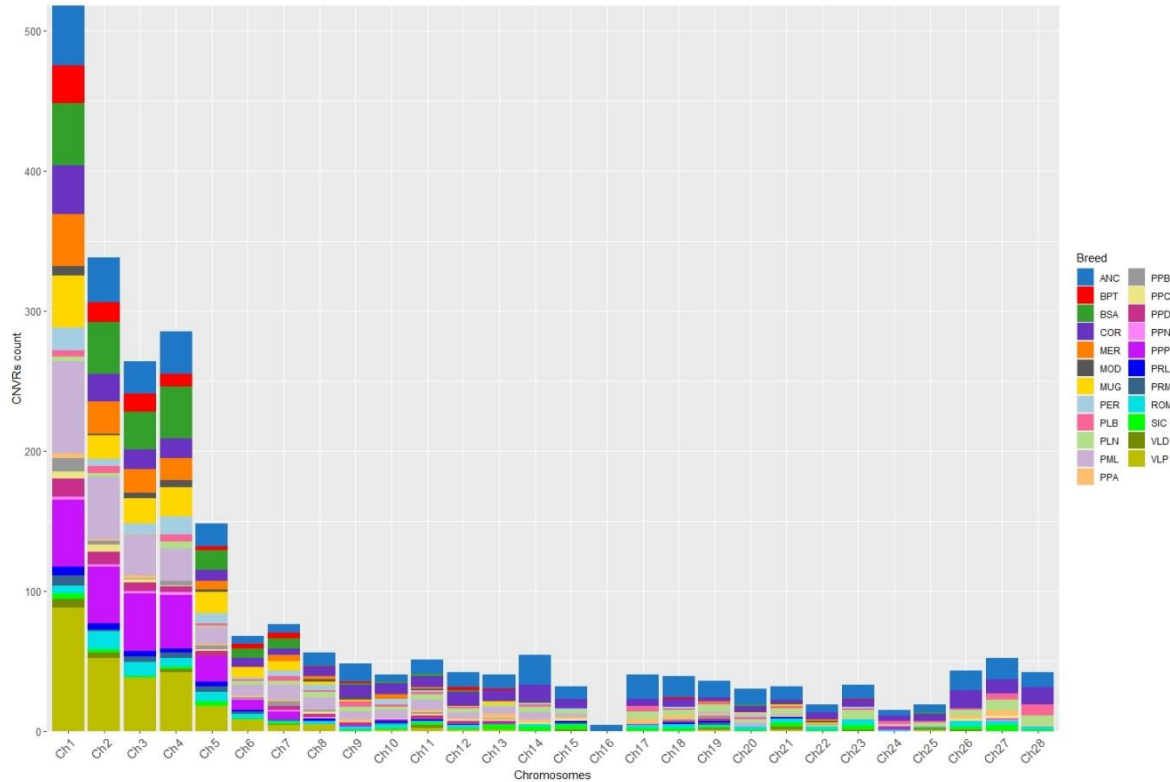
Breed	Type				Length	Mean	Min Length	Max Length	Genome Coverage (%)
	Loss	Gain	Mixed	Total					
ANC	345	7	28	380	23,529,525	61,920	2,378	565,429	2.06
BPT	54	4	22	80	5,317,732	66,472	5,580	1,862,447	0.47
BSA	137	3	40	180	13,727,398	76,263	6,464	391,642	1.20
COR	202	6	37	245	13,959,094	56,976	8,570	582,168	1.22
MER	84	0	28	112	9,360,221	83,573	5,157	814,780	0.82
MOD	20	3	4	27	648,977	24,036	4,494	77,226	0.06
MUG	99	6	26	131	6,214,065	47,436	2,726	246,916	0.54
PER	54	0	14	68	4,847,736	71,290	7,526	472,494	0.42
PLB	51	2	9	62	3,880,517	62,589	4,389	276,030	0.34
PLN	82	0	10	92	5,010,687	54,464	1,750	347,761	0.44
PML	217	5	22	244	17,840,778	73,118	4,431	333,470	1.56
PPA	30	2	6	38	2,408,448	63,380	9,035	153,514	0.21
PPB	19	6	5	30	757,175	25,239	3,023	110,042	0.07
PPC	19	3	5	27	1,343,003	49,741	3,023	221,819	0.12
PPD	39	7	8	54	1,796,233	33,264	3,023	221,819	0.16
PPN	9	2	1	12	192,241	16,020	3,023	45,506	0.02
PPP	167	0	36	203	50,708,742	249,797	4,904	2,863,848	4.44
PRL	27	2	2	31	689,461	22,241	3,614	66,137	0.06
PRM	18	3	6	27	692,116	25,634	2,378	69,072	0.06
ROM	41	4	5	50	3,862,446	44,396	6,264	160,389	0.34
SIC	40	1	8	49	2,430,370	49,599	5,105	256,437	0.21
VLD	16	3	3	22	1,488,845	67,675	3,499	1,059,613	0.13
VLP	213	5	45	263	30,904,092	117,506	3,249	1,148,469	2.71
<b>Total CNVR identified</b>	<b>1,084</b>	<b>36</b>	<b>54</b>	<b>1,172</b>	<b>155,598,588</b>	<b>317,459</b>	<b>1,750</b>	<b>2,929,354</b>	<b>13.64</b>

Results and  
discussion



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DI PADOVA

# CNVR for each chromosome



## Considerations:

Chr 1: highest number of CNVR.

Detection of CNVR on Chromosome 16 only in ANC breed.

# Gene annotation

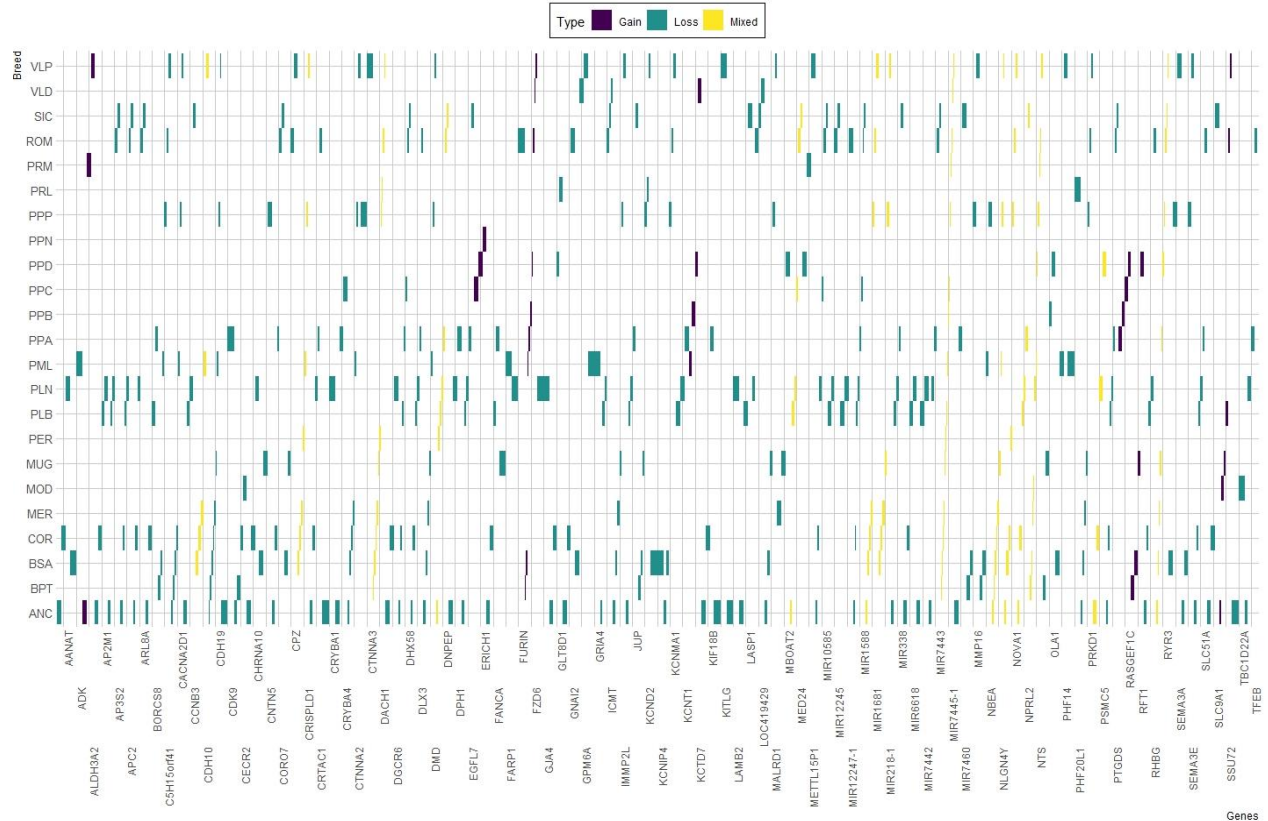
## Identified genes:

1172 CNVR in which **711** (60.7%) did not involve any genes, while **461** (39.3%) encompassed one or more genes.

- 406 (88.0%) were protein-coding genes;
- 45 (9.8%) were miRNAs;
- 10 (2.2%) were genes of uncertain function (LOC).

## Filter

Genes present in at least 3 animals among breeds were considered, in order to evaluate those with higher incidence in the whole population of animals. **94 genes** were identified.



Genes

# Gene annotation

Chromosome	Gene	Function	Breed
1	<i>CACNA2D1</i>	Muscle activity	ANC, BPT, BSA, COR, PML, PPP, VLD
	<i>DACH1</i>	Skeletal development	BPT, BSA, COR, <u>MER</u> , <u>MUG</u> , PER, <u>PPP</u> , PRL, ROM, VLD
	<i>DMD</i>	Muscle development and tissue stability	ANC, BSA, <u>MER</u> , <u>MUG</u> , PML, <u>PPP</u> , VLD
	<i>MIR7445-1</i>	Conserve among species as regulatory factor	BPT, BSA, COR, MER, MUG, PER, PLB, PML, PPB, PPC, PPP, PRM, ROM, VLD, VLP
2	<i>CRISPLD1</i>	Thermal stress resistance	BSA, <u>COR</u> , MER, MUG, PML, PPP, VLD
	<i>PHF14</i>	Immune response	BSA, PML, VLD
4	<i>CCNB3</i>		ANC, <u>PLB</u> , <u>PLN</u> , SIC
5	<i>RYR3</i>		ANC, BSA, MOD, MUG, <u>PPA</u> , <u>PPD</u> , PPP, ROM, SIC, VLD
7	<i>DNPEP</i>	Muscle development and tissue-specific biological processes in muscle	ANC, PER, <u>PLB</u> , <u>PLN</u> , PPA, ROM, SIC
9	<i>SLC51A</i>		ANC, COR, <u>PLB</u> , <u>PLN</u> , PPA, ROM
28	<i>APC</i>		ANC, COR, <u>PLB</u> , <u>PLN</u> , ROM, SIC

## Conclusions

Some CNV markers were mapped in chromosomal regions where crucial functional genes are annotated, indicating potential functional associations between CNV and genes



Genetic variability and diversity within and between 23 Italian local chicken breeds using CNV markers were assessed



Results provide a foundation for the recognition of Italian chicken population as an important reservoir of genetic diversity



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UNIFI

# Thank you all for your attention



Progetto collettivo beneficiario per il Comparto Avicoli con il sostegno del Fondo Europeo Agricolo per lo Sviluppo Rurale (FEASR)  
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