

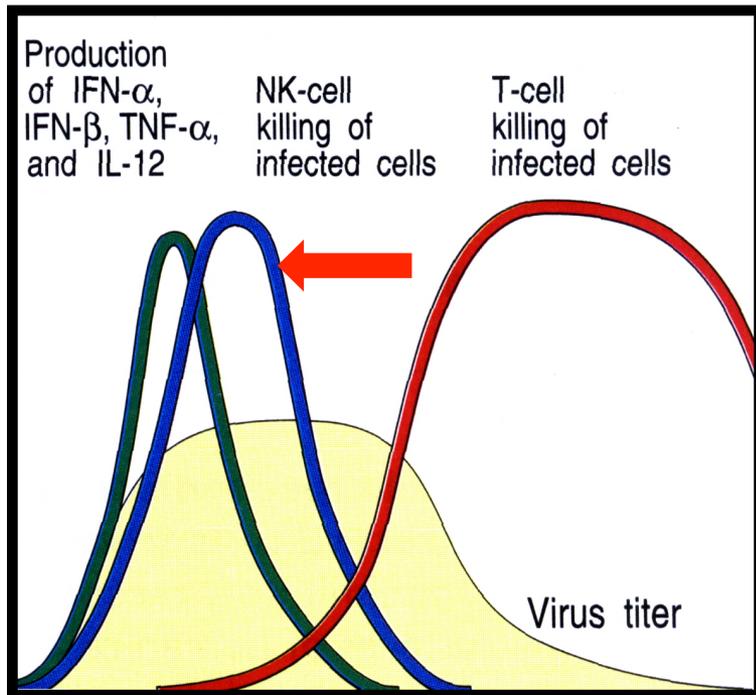


The unusual diversity of cattle natural killer cell receptors

NK cells are fundamental in the innate and adaptive responses to viral infection

The NK cell response contains infection

The T-cell response clears infection



Humans lacking functional NK cells succumb to overwhelming viral infection

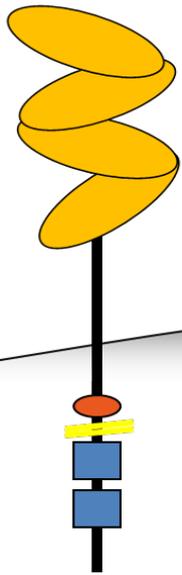
The NK response is now known to influence the magnitude and longevity of adaptive immune responses

NK cell function is controlled by highly variable receptors

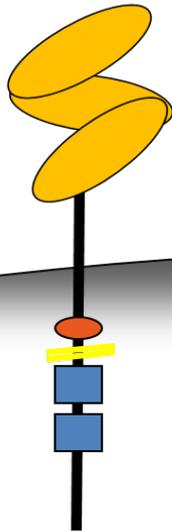
Mammalian NK cell receptors that all recognise **MHC class I ligands**



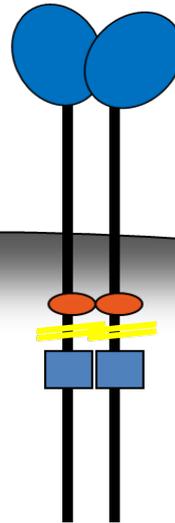
LILR
2-4 Ig domains



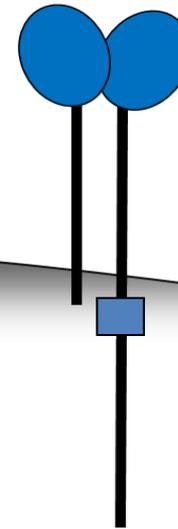
KIR
2-3 Ig domains



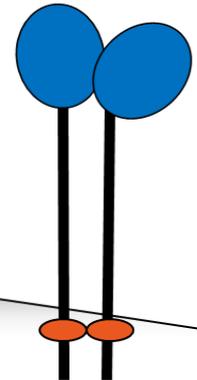
Ly49
homodimer



NKG2/CD94
heterodimer



NKG2D
homodimer



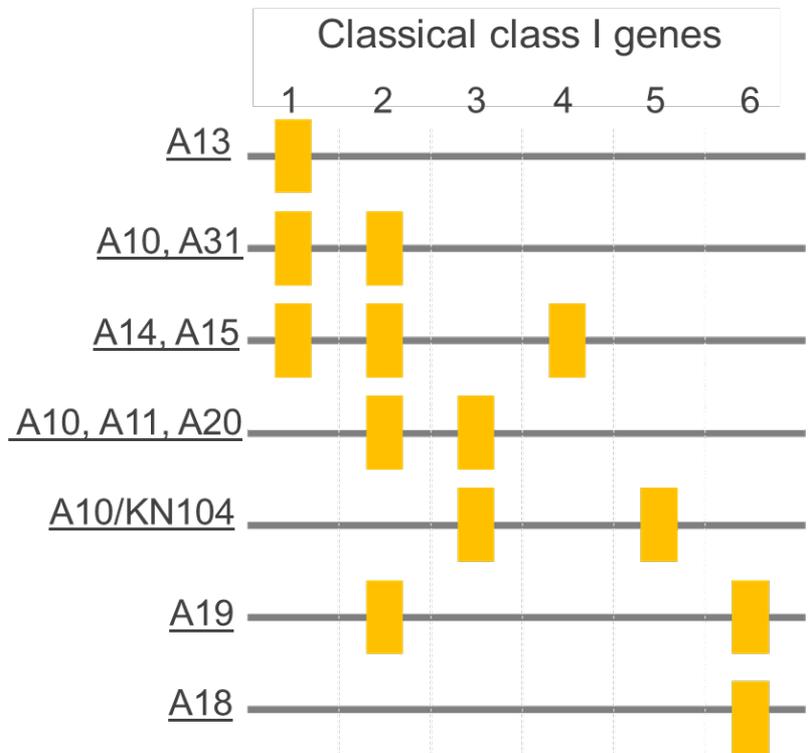
Leukocyte Receptor Complex

Natural Killer Complex

Human MHC class I is highly diverse but haplotypes do not vary in gene content

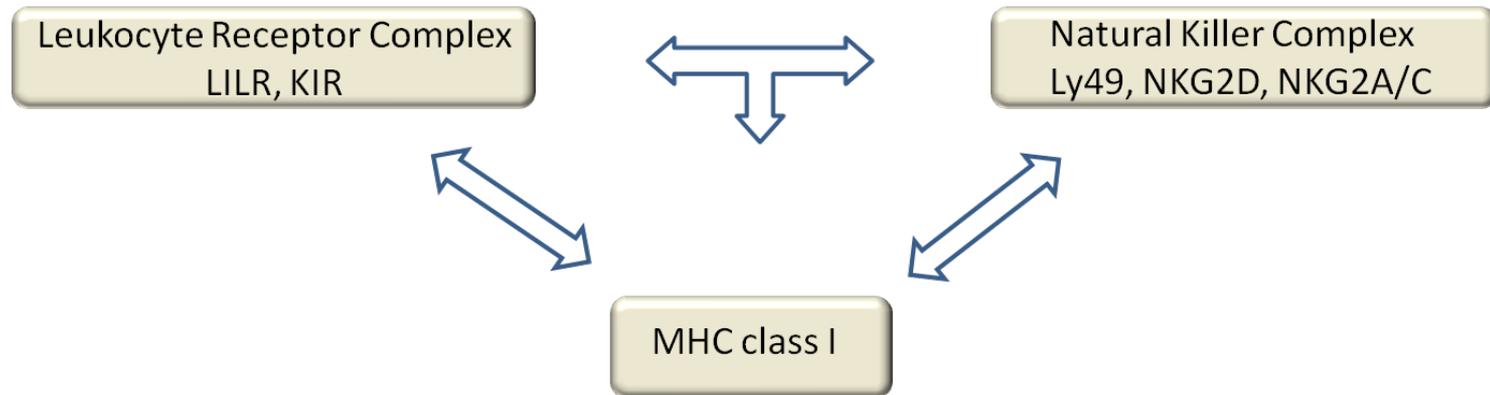


Gene	A	B	C	E	F	G
Alleles	2132	2798	1672	11	22	50
Proteins	1527	2110	1200	3	4	16
Nulls	102	92	44	0	0	2

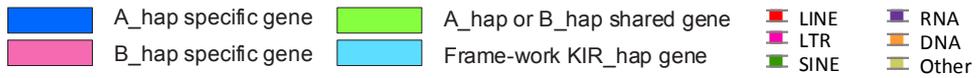


1. Very gene variable haplotypes
2. MHC genes 1-6 are polymorphic
3. Up to four other non-polymorphic genes

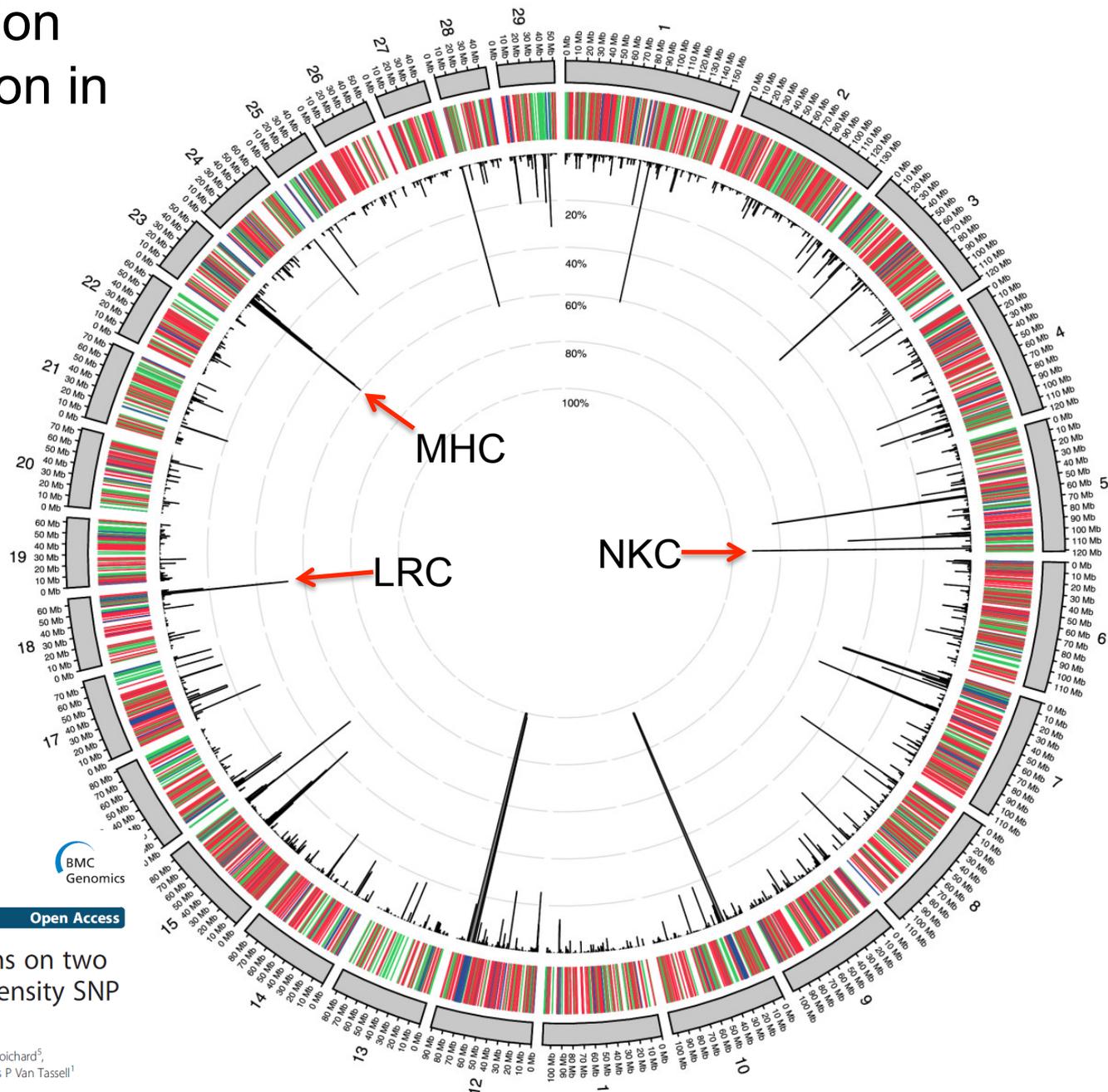
Innate immune variation: germ-line encoded NK cell receptors and MHC class I



3 independently segregating, highly polymorphic and synergistic gene complexes that influence the outcome of viral disease



Independent analysis of copy number variation confirms this variation in cattle



Hou et al. BMC Genomics 2012, 13:376
<http://www.biomedcentral.com/1471-2164/13/376>



RESEARCH ARTICLE Open Access

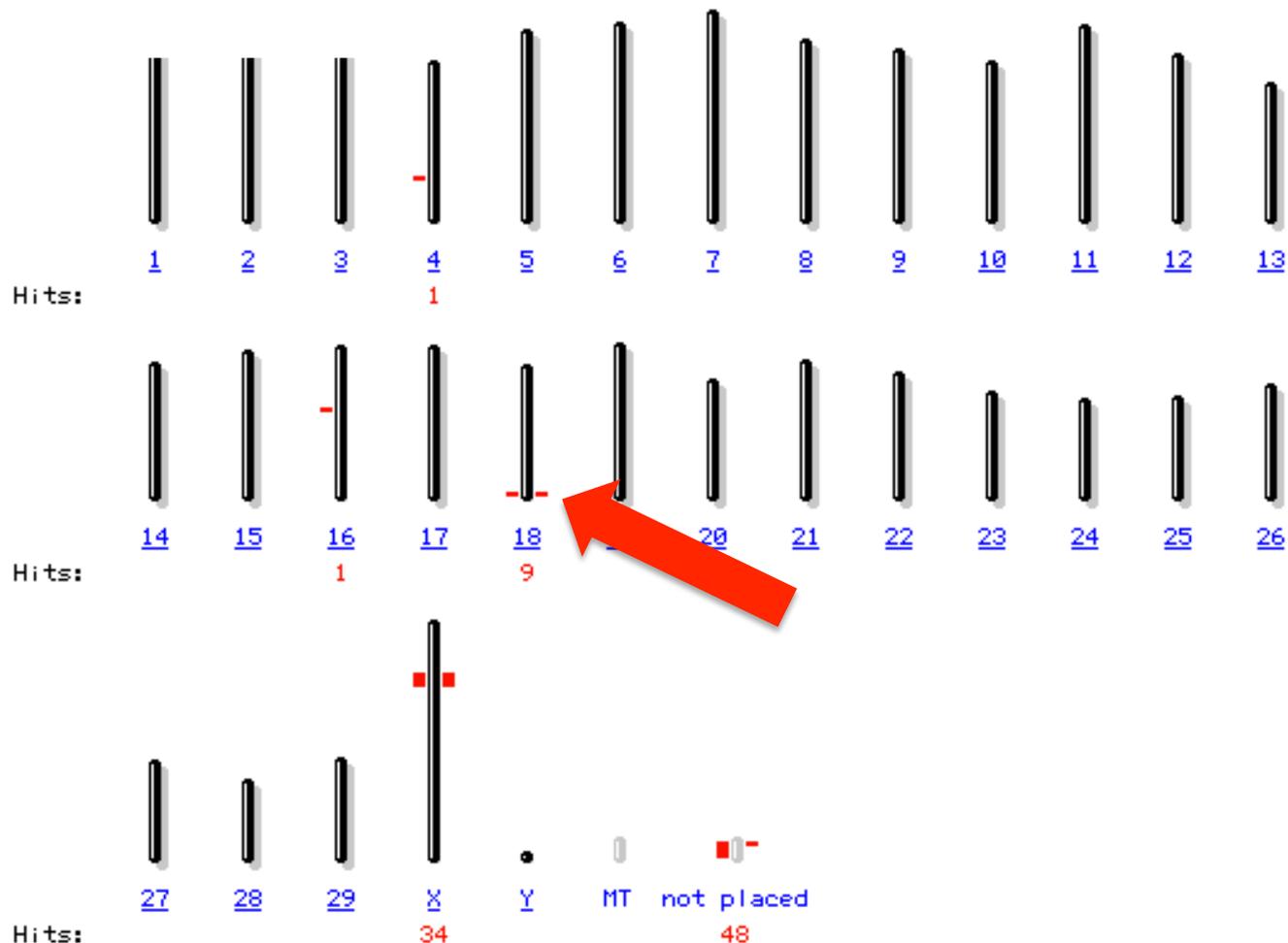
Fine mapping of copy number variations on two cattle genome assemblies using high density SNP array

Yali Hou^{1,2}, Derek M Bickhart¹, Miranda L Hvinden³, Congjun Li¹, Jiuzhou Song⁴, Didier A Boichard⁵, Sébastien Fritz⁶, André Eggen⁷, Sue DeNise¹, George R Wiggins⁸, Tad S Sonstegard¹, Curtis P Van Tassell¹ and George E Liu^{1*}

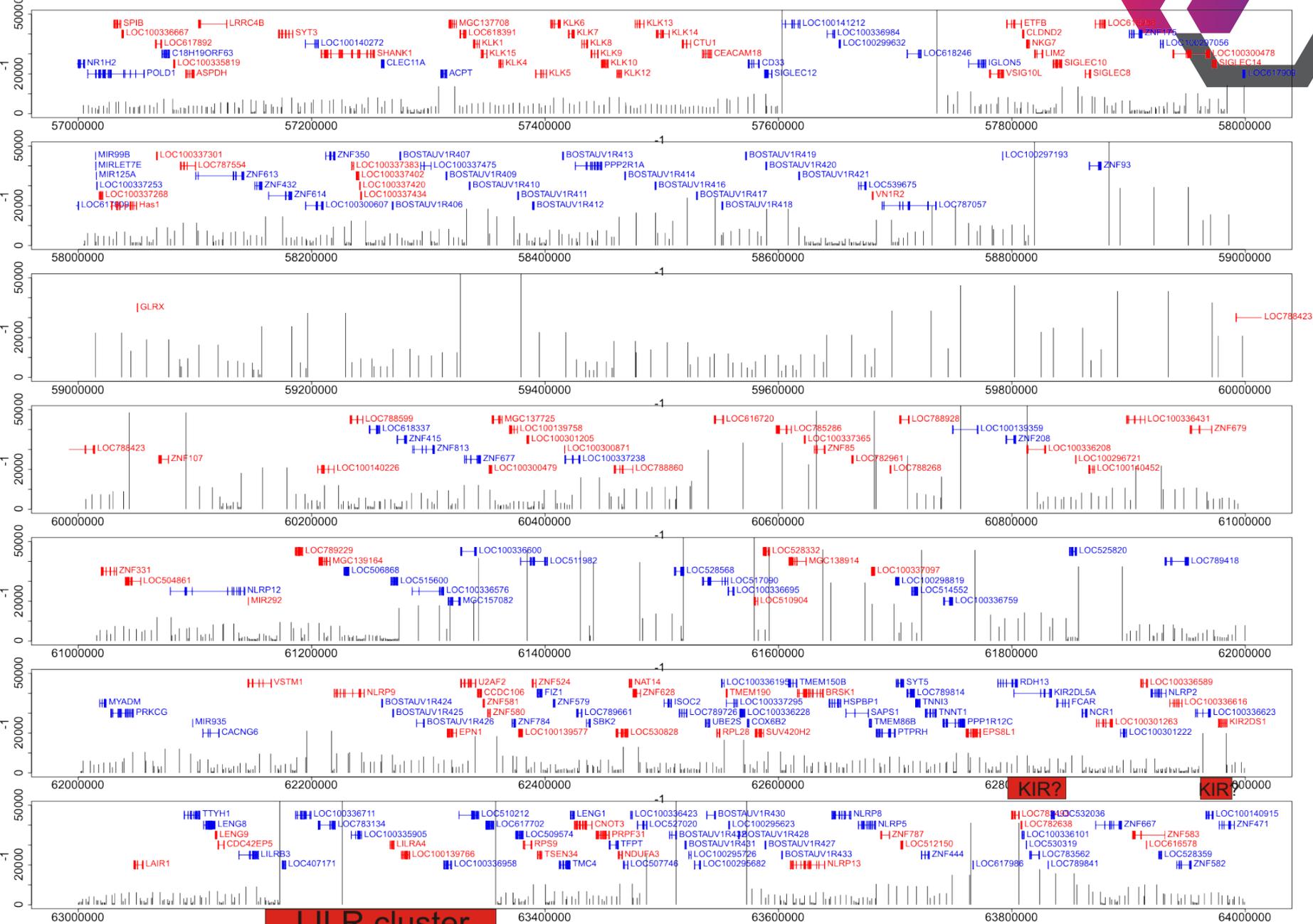
KIR genes in the cattle genome are currently mis-assembled and misplaced

Bos taurus (cattle) genome view

6.1 statistics [Switch to previous build](#)

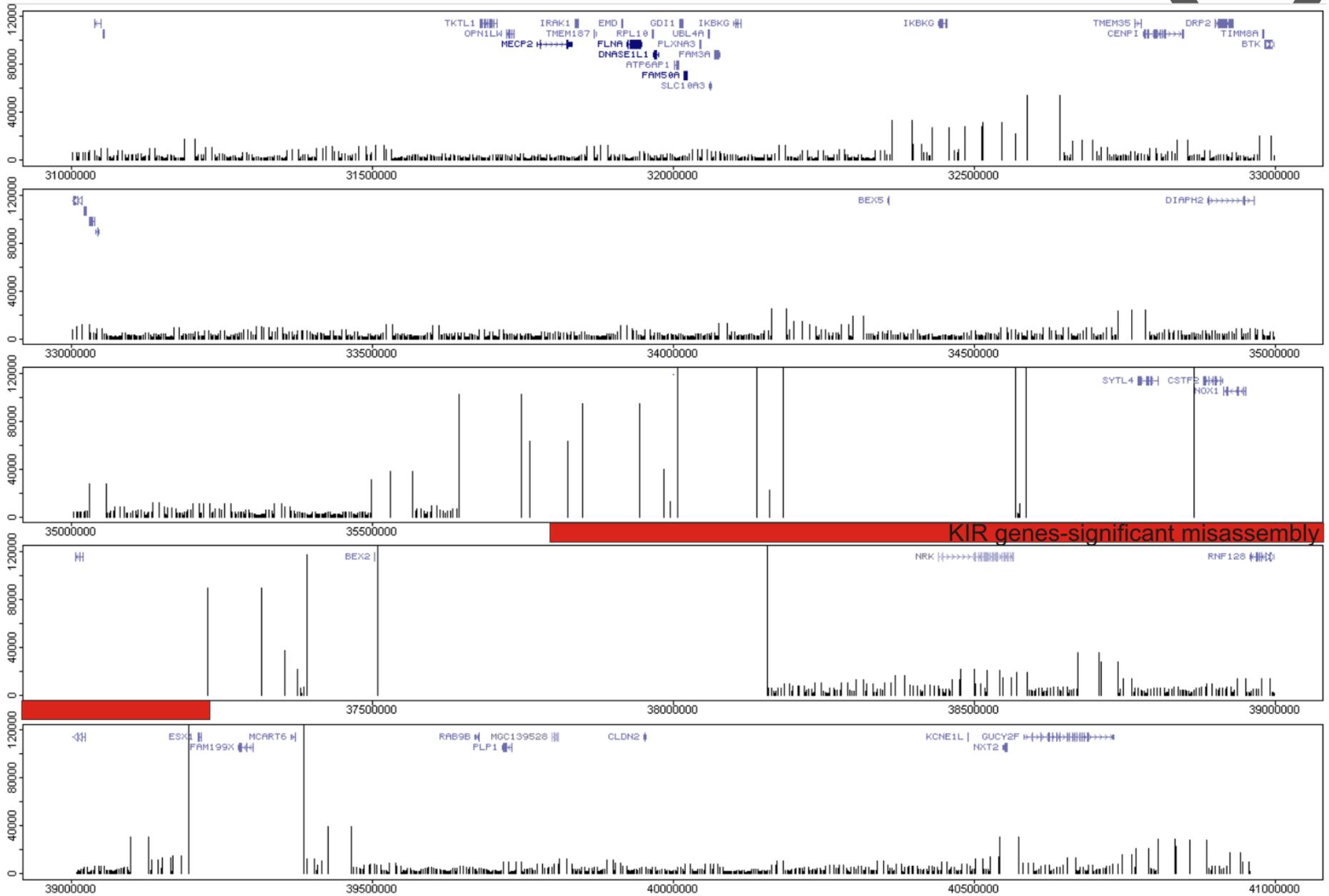


LRC2-Chr18



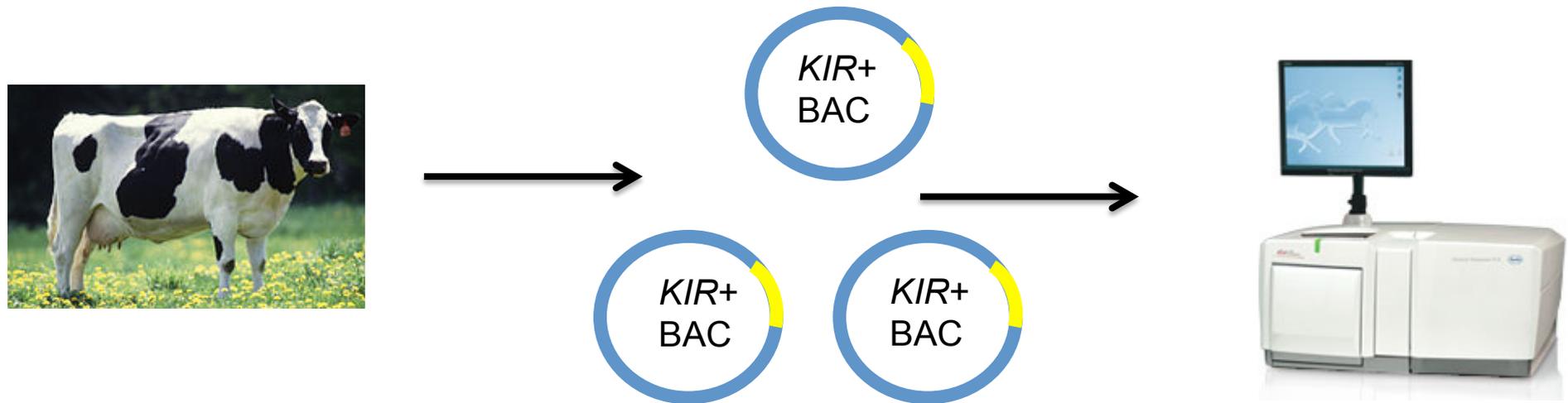
LILR cluster

LRC-ChrX

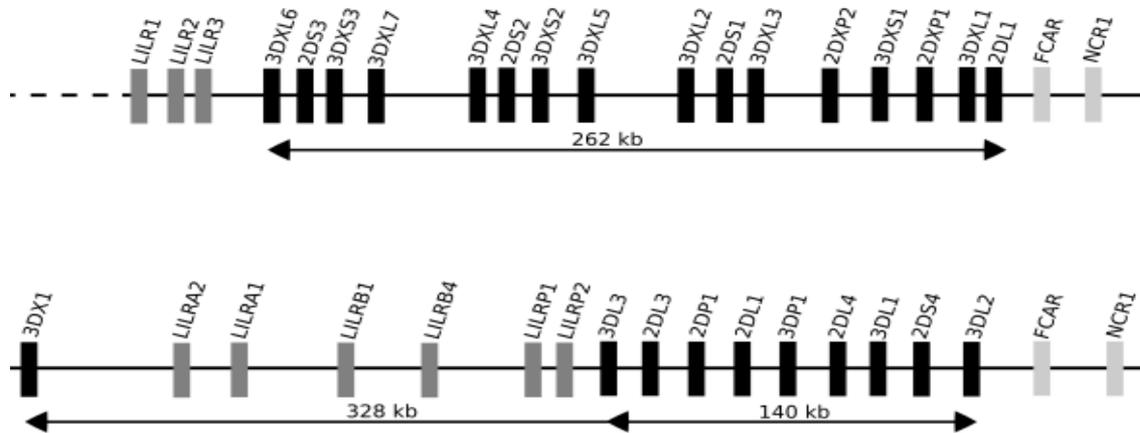
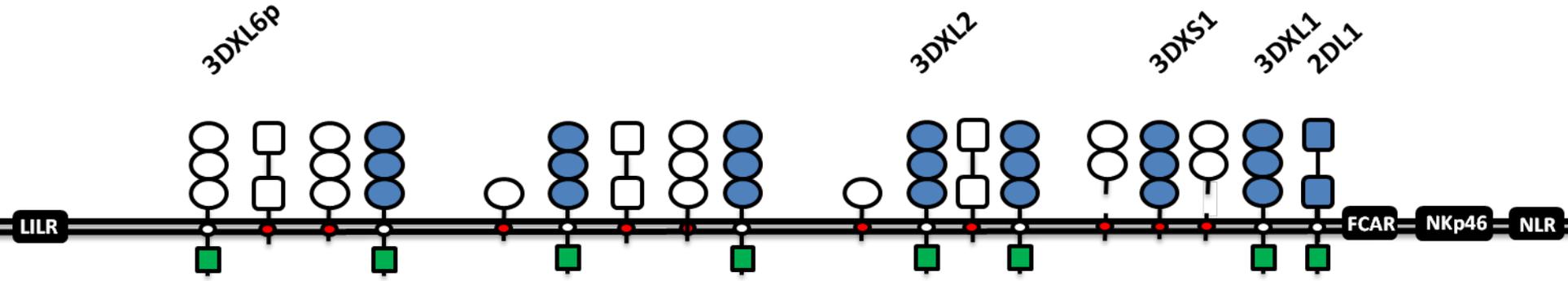


These regions of the genome are notoriously hard to assemble-used a mixed sequencing strategy with BAC clones

Generation	Tech name	Av read length	No reads/run	Total bases/run
First	Sanger	700-1200bp	1	700-1200bp
Second	454	400-700bp	1 million	0.8 Gb
Second	Illumina	35-150bp	3 billion	6 Gb/60 Gb
Third	PacBio	3kb	75,000	0.1 Gb

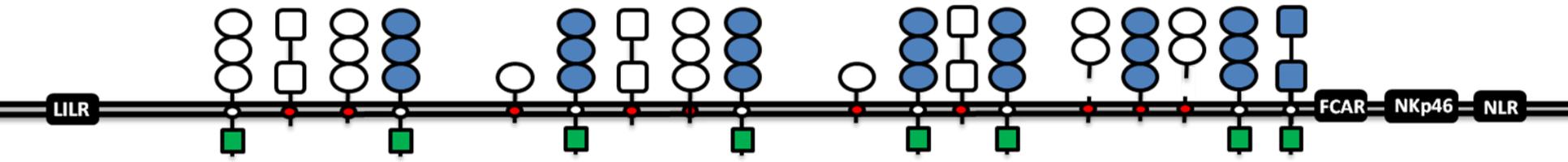


The first cattle *KIR* haplotype: Large and gene dense





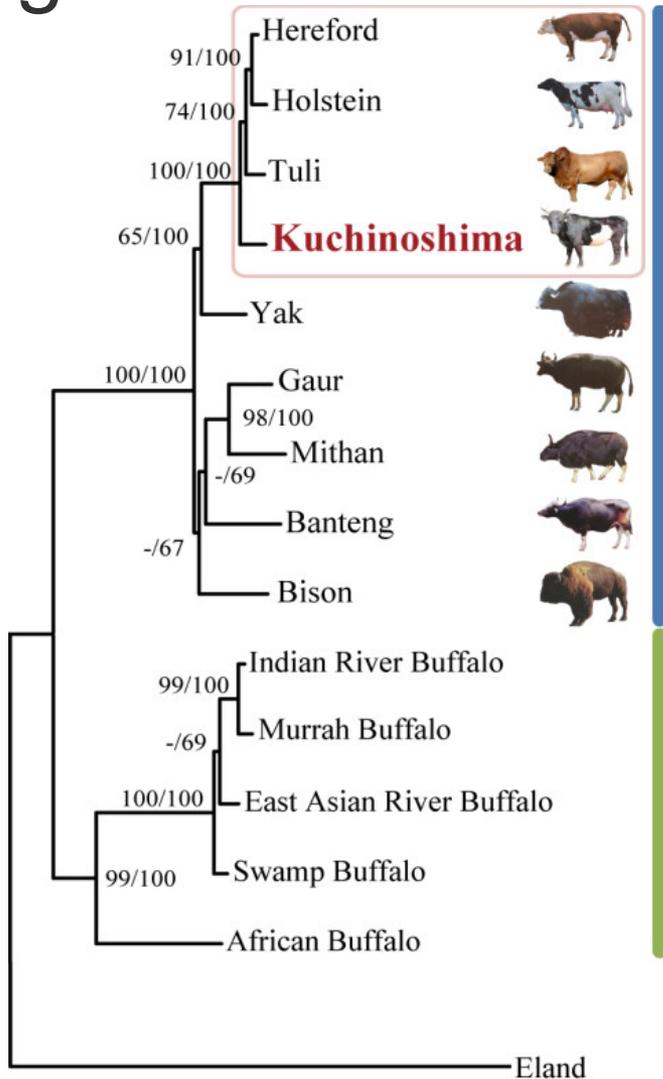
The defining characteristics of human KIR function are shared by cattle



Key properties of <i>KIR</i> loci	Human <i>KIR</i>	Cattle <i>KIR</i>
Inhibitory and Activating	✓	✓
Activating genes disarmed	✓	✓
Functionally variable haplotypes	✓	? ✓
Polymorphic	✓	? ✓
Paired activating and inhibitory receptors	✓	✓



Bos taurus breeds with sequenced genomes



Fleckvieh
 – Illumina HiSeq

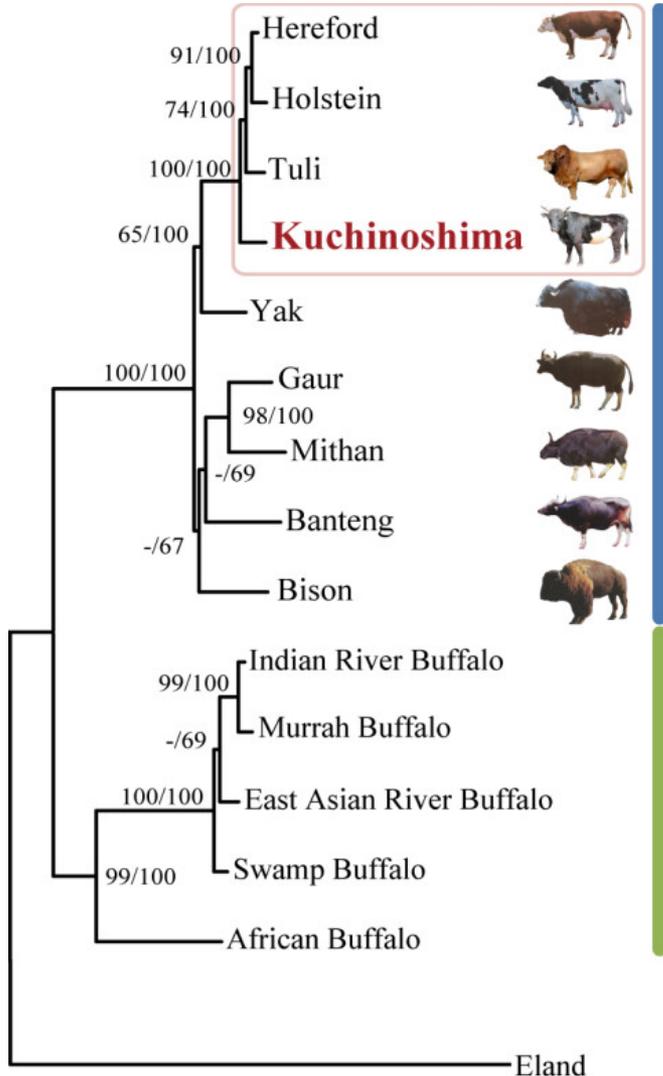
 Holstein “Goldwyn”
 – Abi SOLiD

 Angus
 – Illumina HiSeq

 Hereford
 – Sanger Genome



Related species to cattle with sequenced genomes



Sahiwal

22 individuals sequenced with Illumina
Av 82 bp
Total 55 Gb

Nellore

1 individual sequenced with Illumina
Av 75 bp
Total 46 Gb

Kuchinoshima

1 individual sequenced with Illumina
Av 69 bp
Total 71 Gb

Yak

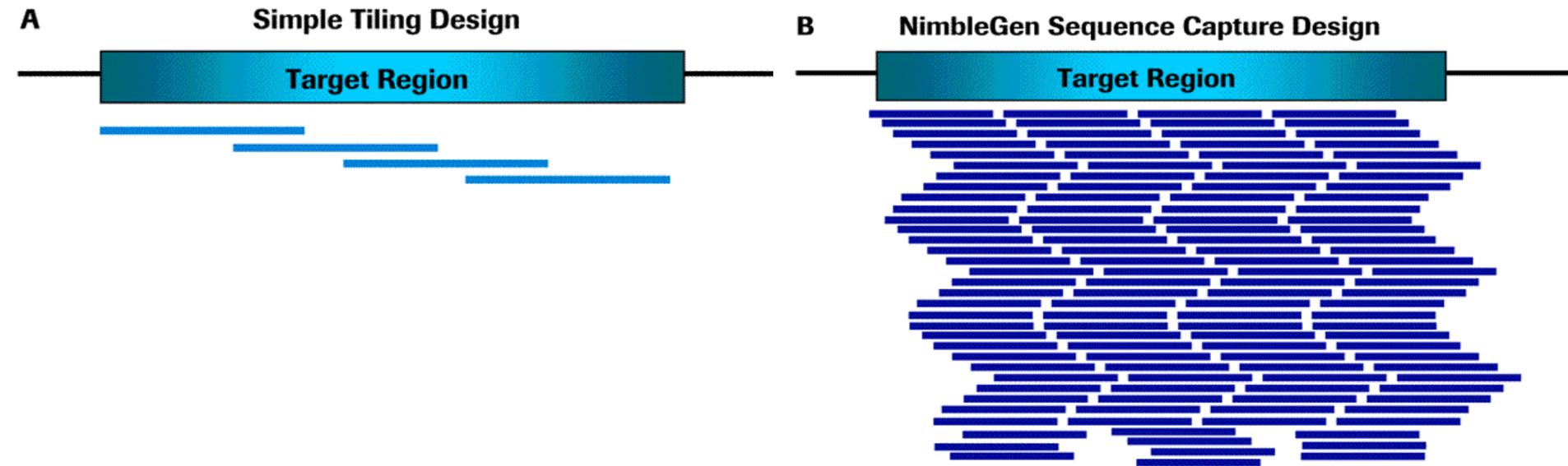
1 individual sequenced with Illumina
Av 108 bp
Total 220Gb

Water buffalo

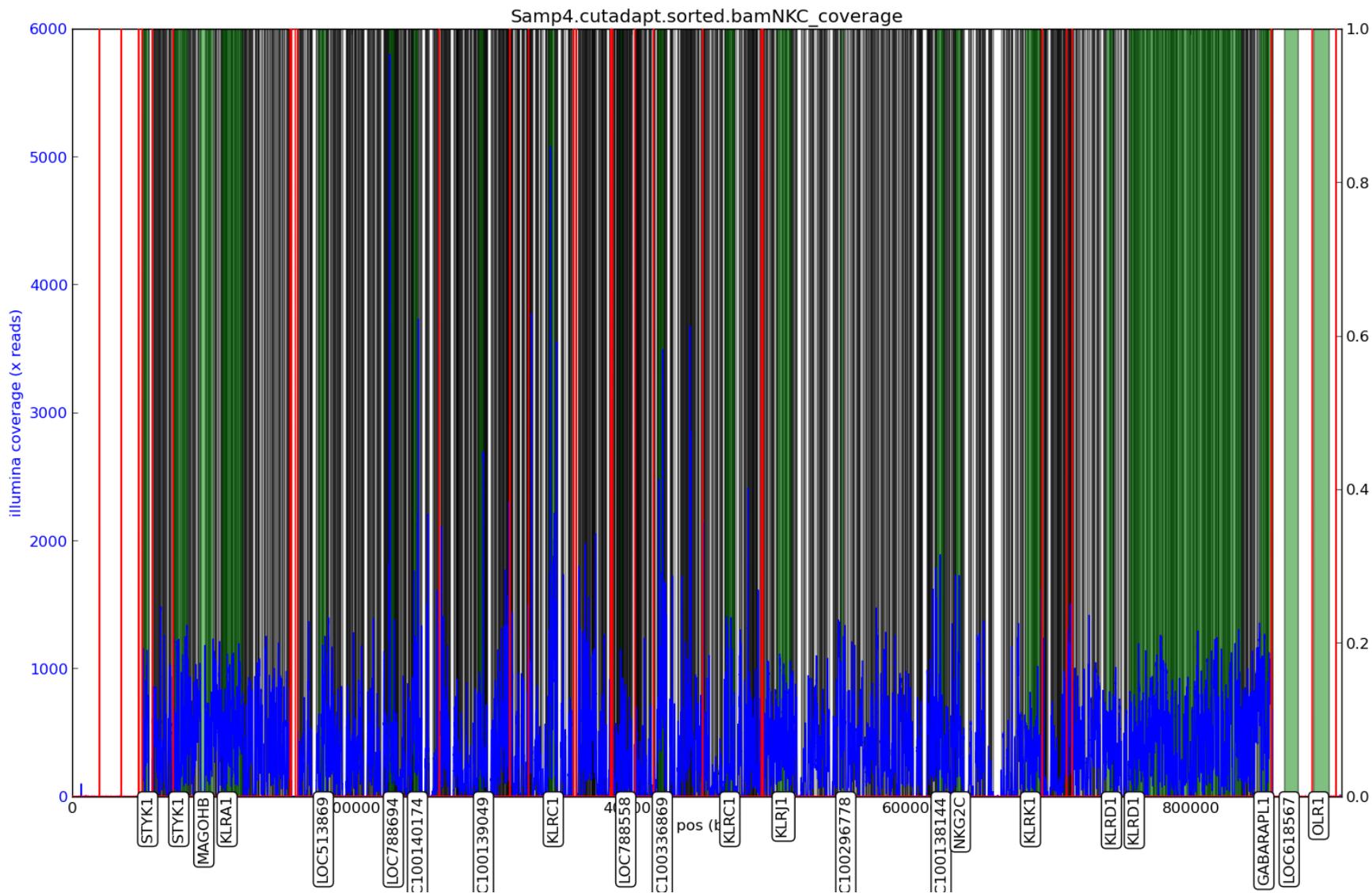
1 individual sequenced with Illumina
Av 73 bp
Total 117 Gb



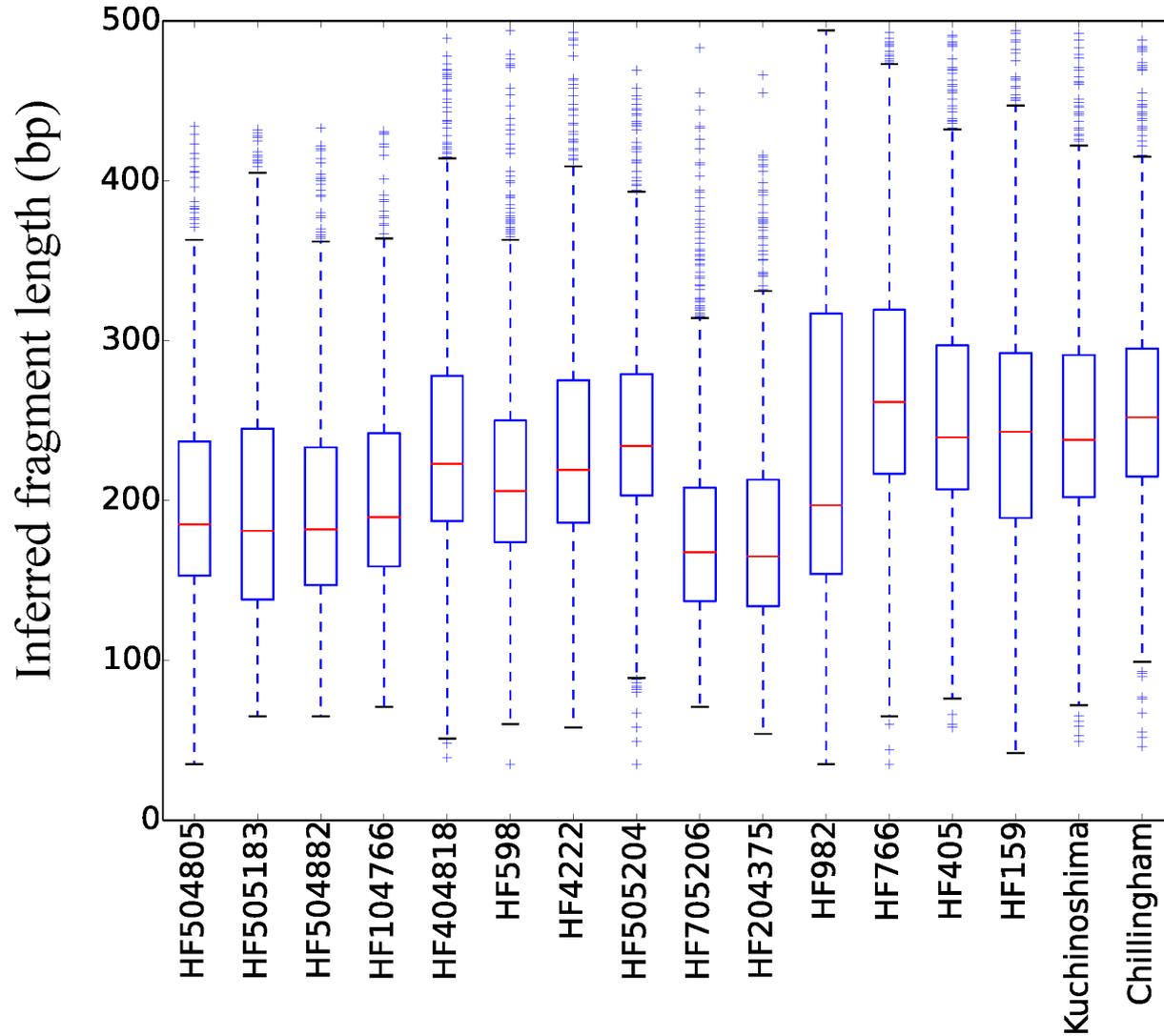
Genome enrichment using the Roche (Nimblegen) SeqCap EZ system to target regions of the genome



Pulled down sequence is then massively parallel sequenced and mapped back to the target region



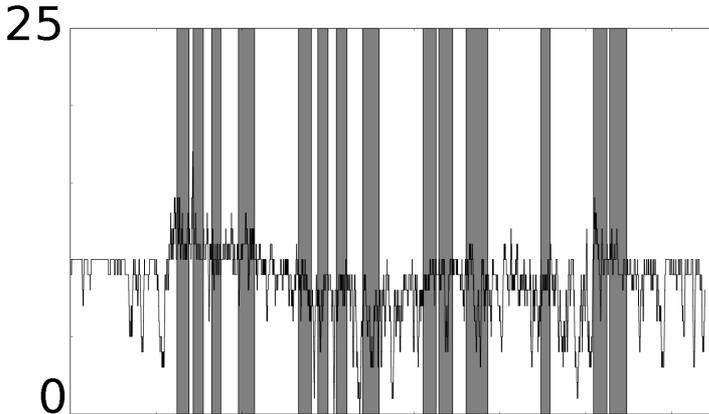
Increasing the fragment size fills gaps



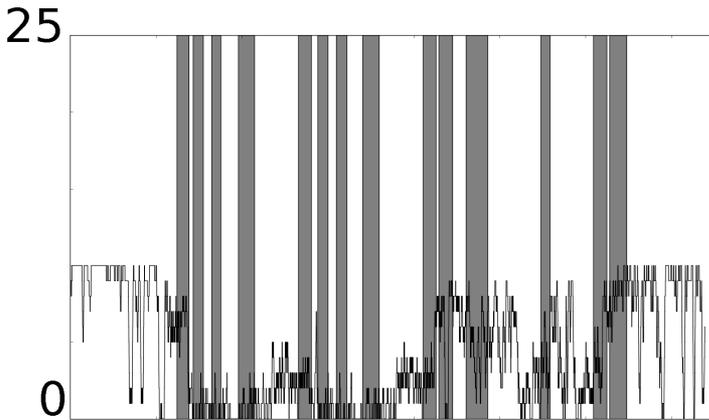
Haplotype coverage was as expected with very high resolution



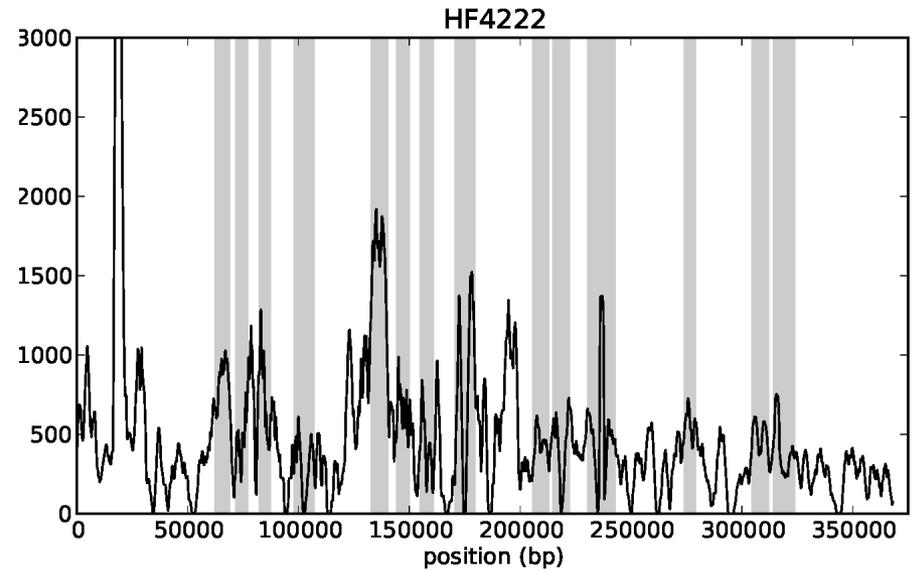
Simulated data



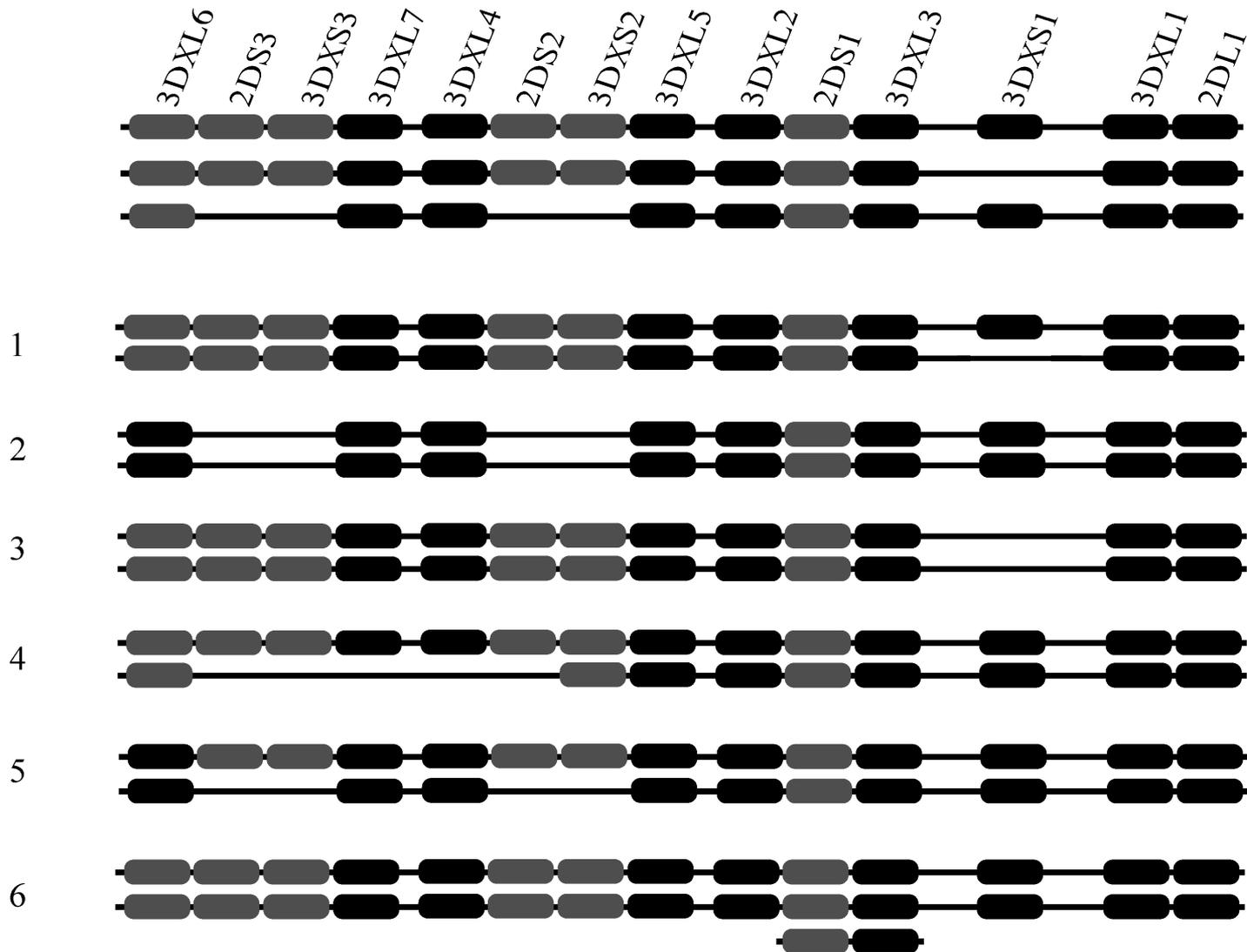
Normal coverage



Unique coverage

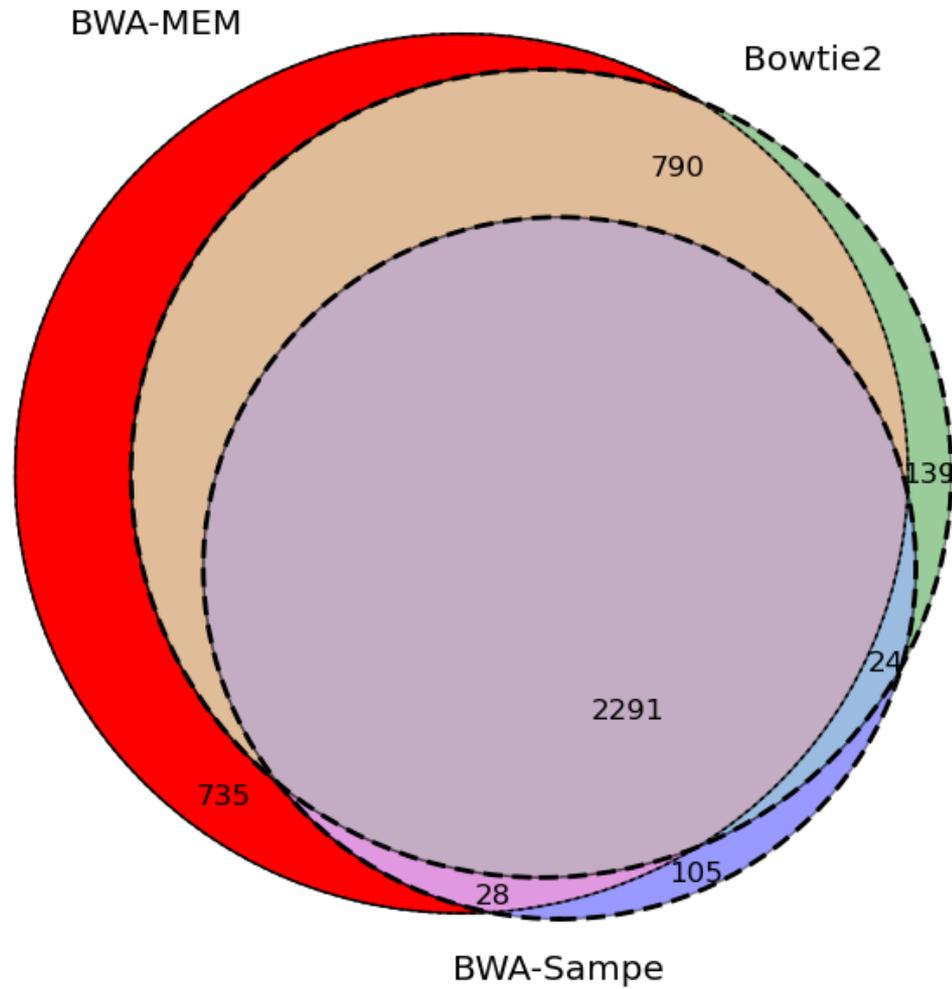


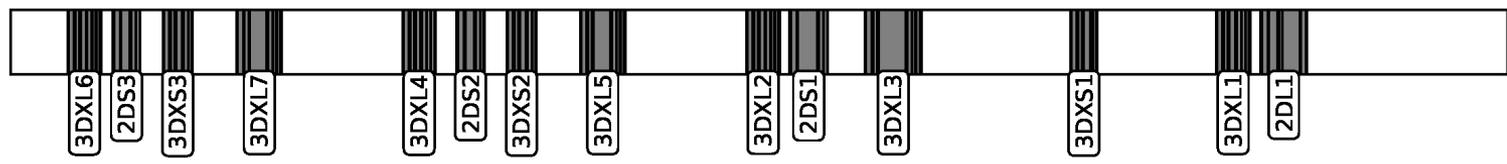
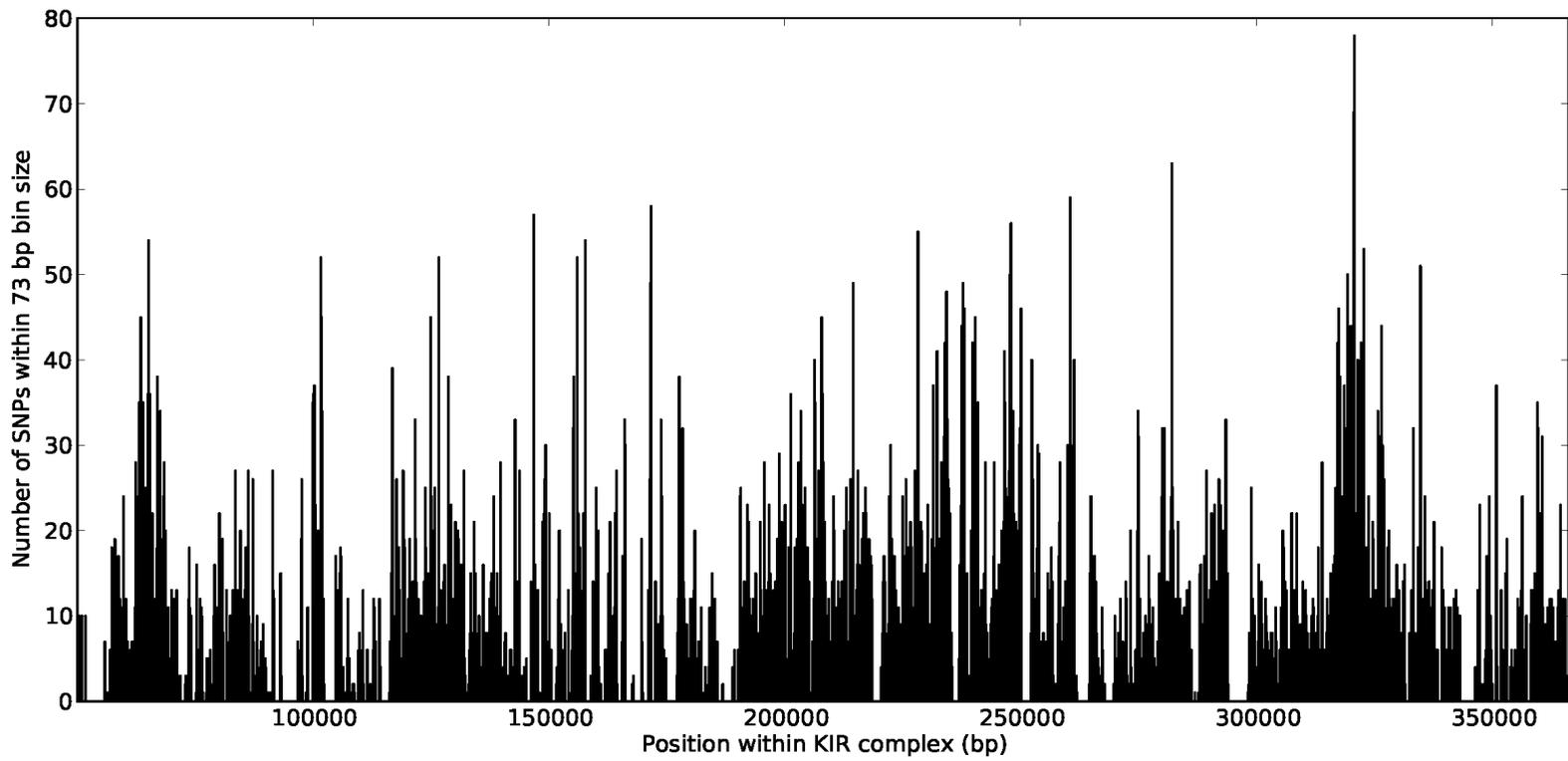
KIR haplotype diversity is limited in cattle

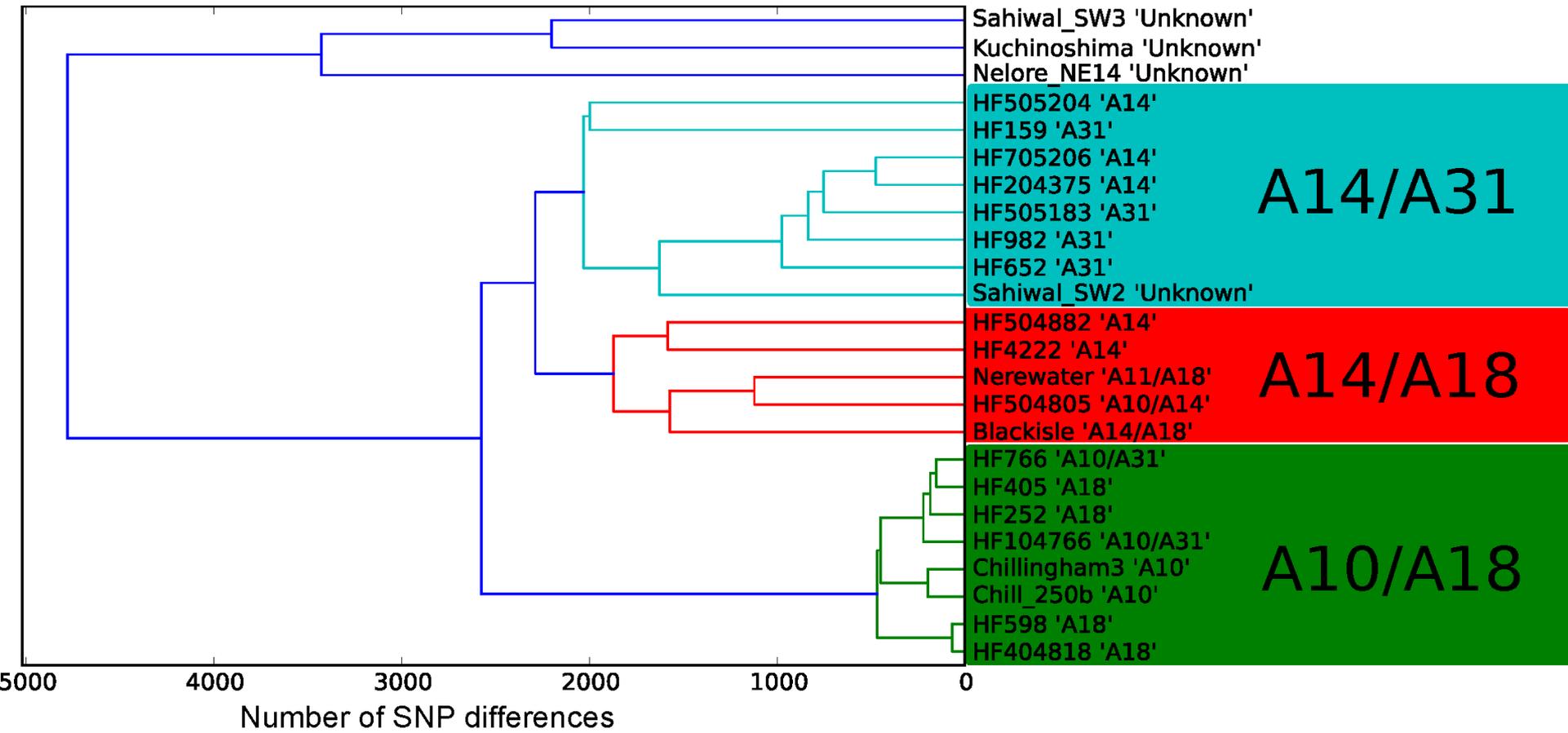


High resolution allowed stringent SNP calling

Aligner SNP comparison - 'uniquely mapped'



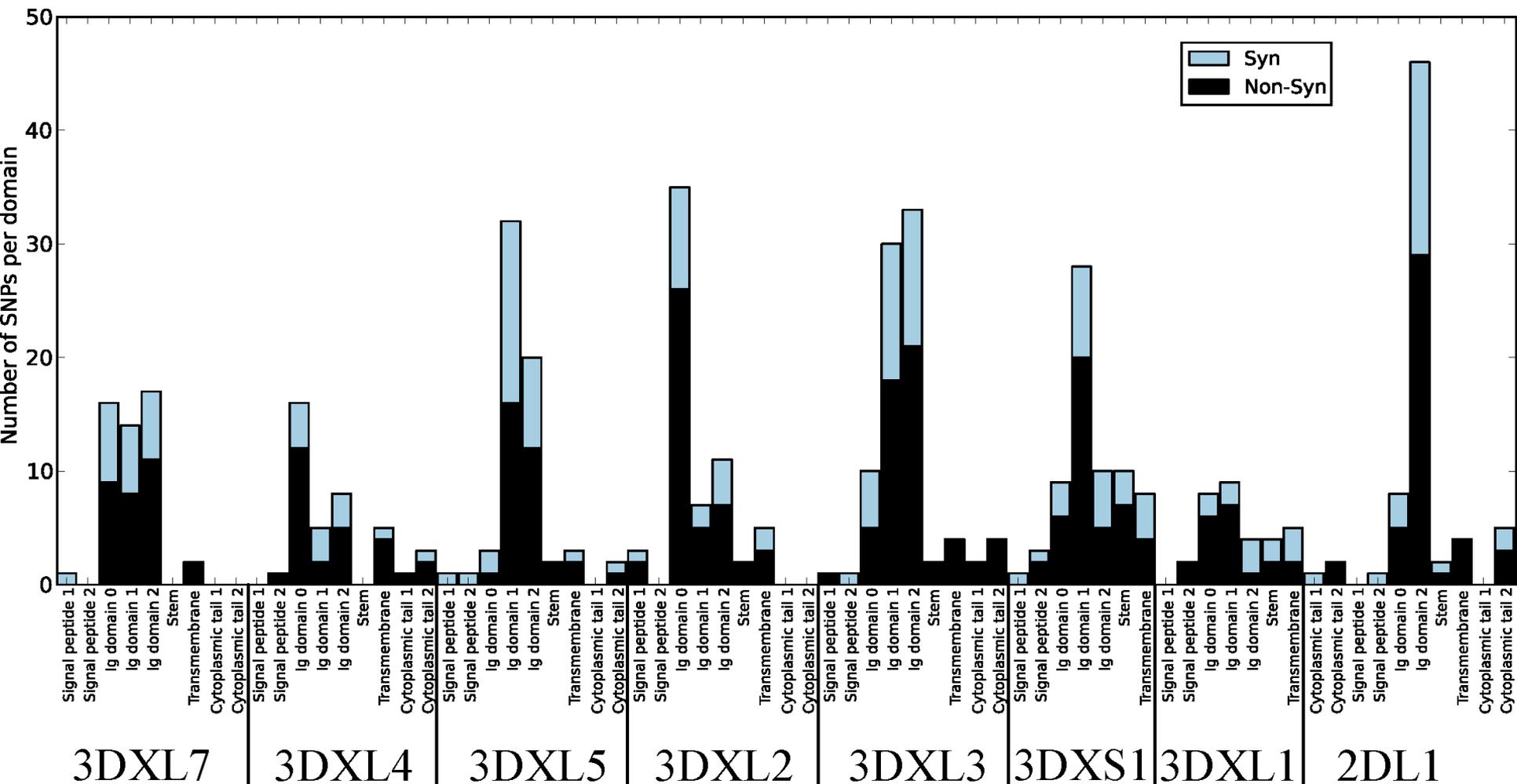




A14/A31

A14/A18

A10/A18





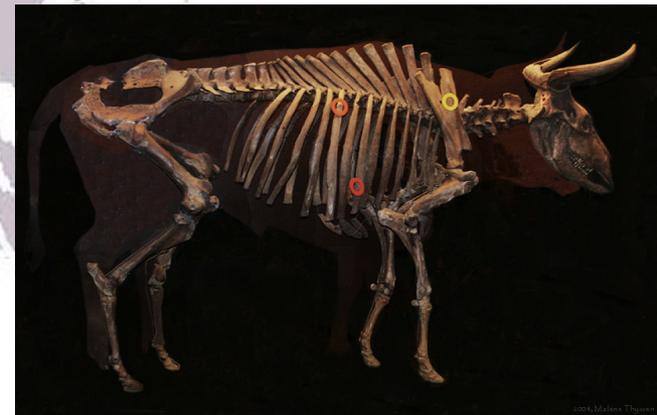
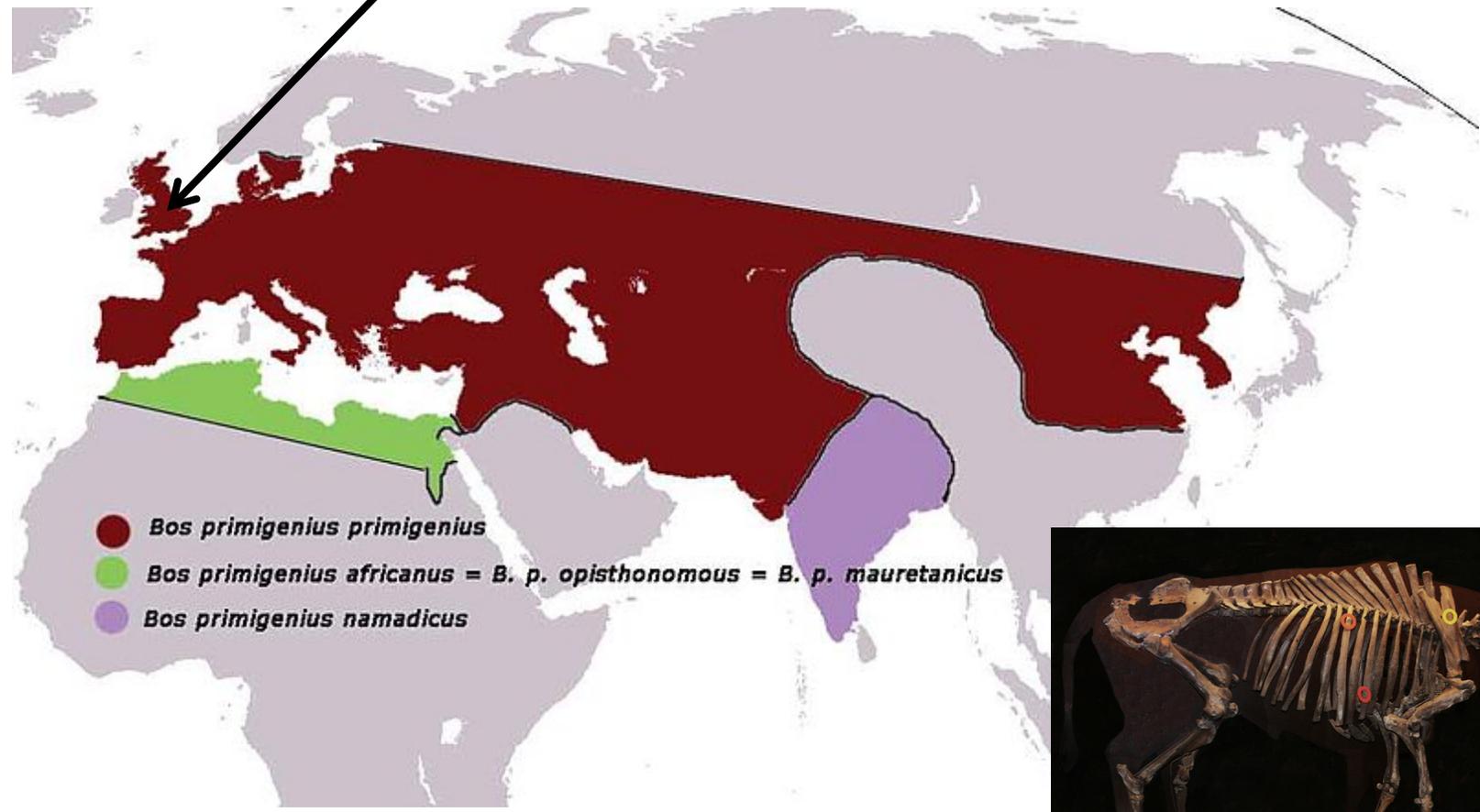
Does domestication impact on rapidly evolving genes of the immune system?

Has KIR gene duplication been driven by natural selection?

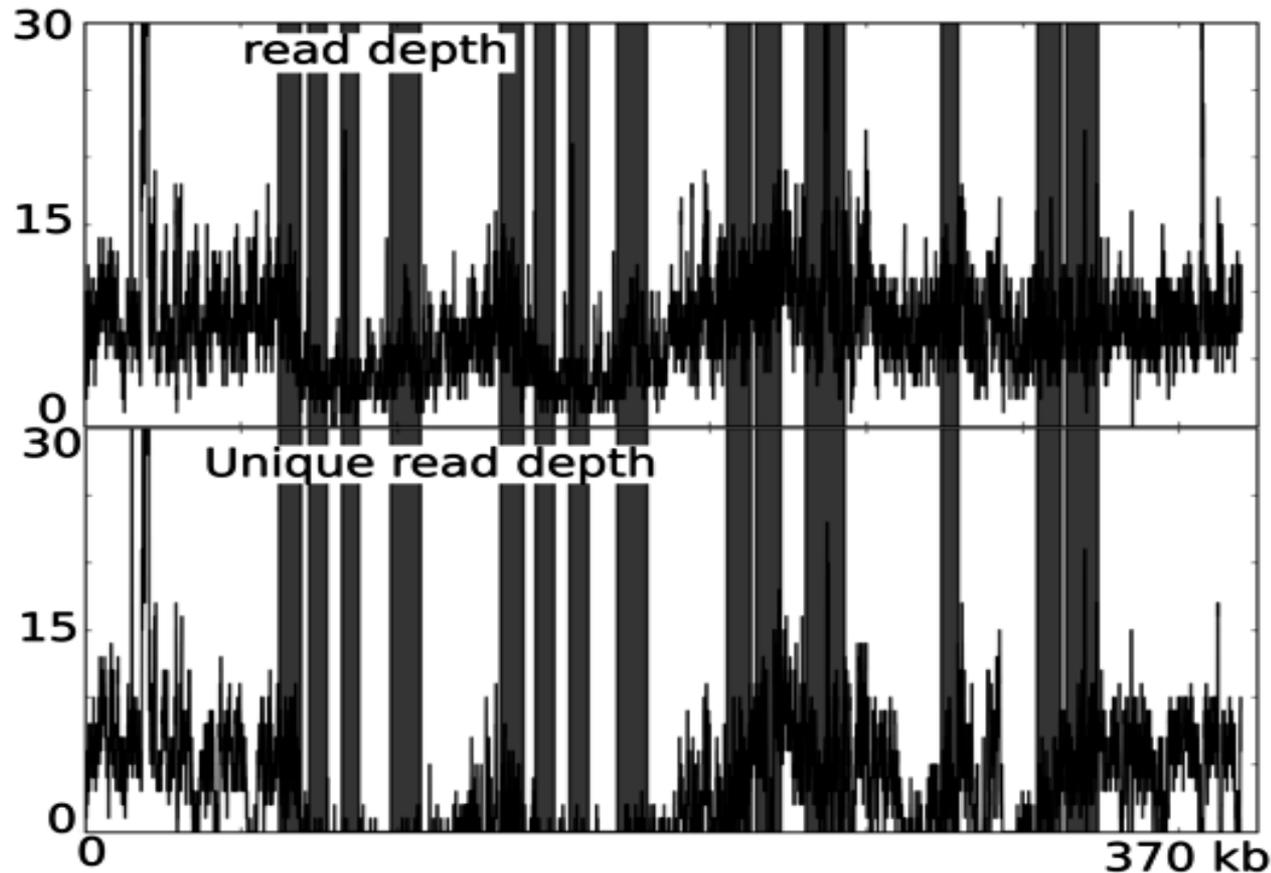
Has a reduction of natural selection led to the disruption of activating KIR?

Utilising the Aurochs genome (*Bos primigenius primigenius*)

Bone dates to ~7000 years ago; 800 years before the Neolithic



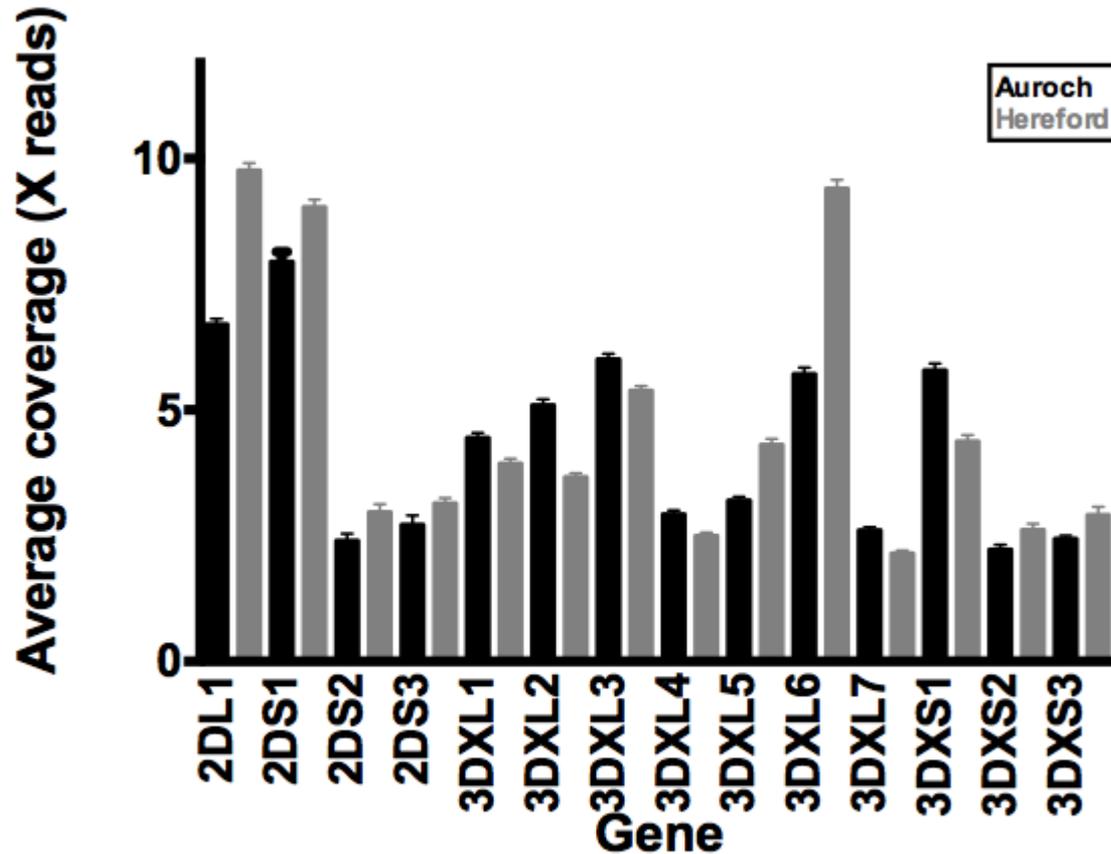
Aurochs KIR are within the genome but highly identical genes within the blocks are hard to identify



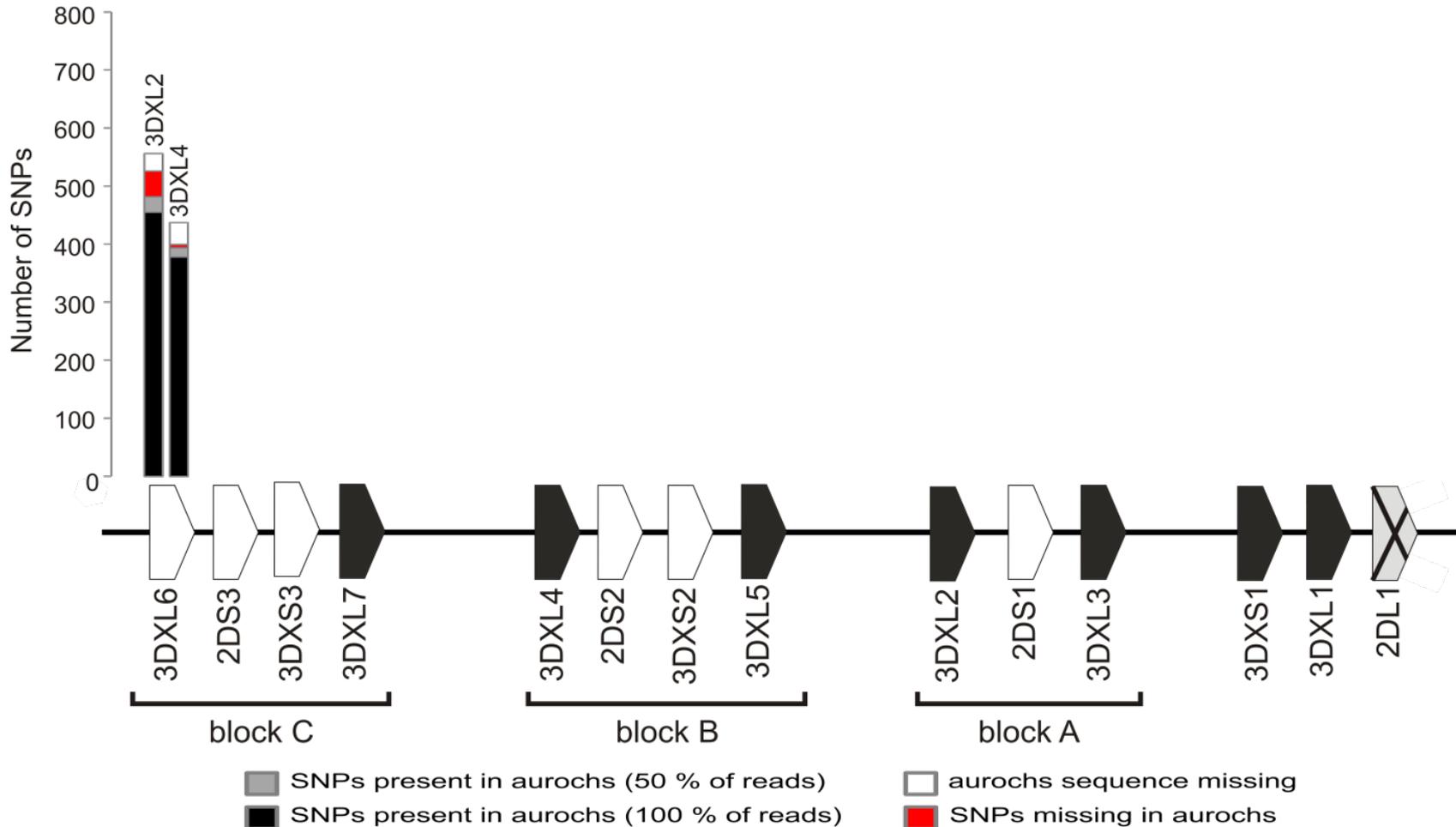
Good average read coverage over all the KIR exons after filtering



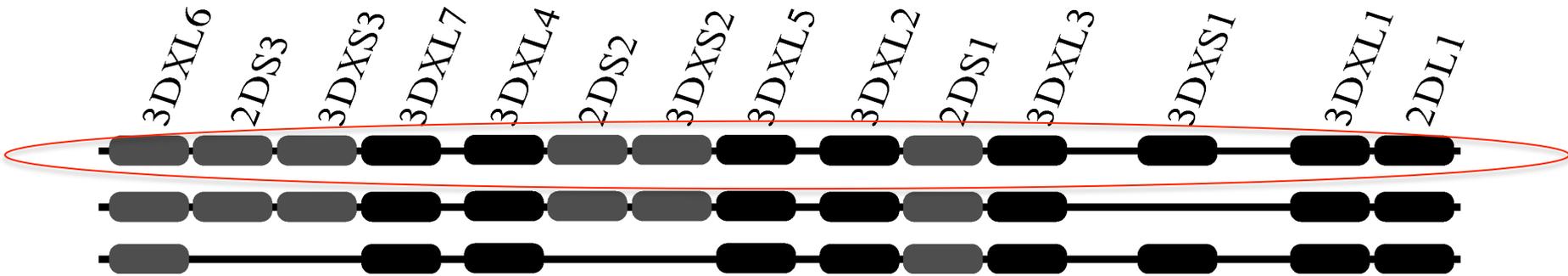
Average Coverage of KIR exons



Gene defining SNPs are present in the aurochs



The aurochs has the most common cattle haplotype





All the KIR genes in a modern cattle are present in the aurochs

The coding and signalling potential of each KIR gene is identical between Holstein-Friesian and aurochs.

Although there is polymorphism between aurochs and Holstein-Friesian cattle, domestication has not altered the major functional properties of the KIR genes.

Summary

The cattle (ruminant) NK cell receptor system is diverse and has evolved under positive selection

A key function of these systems in primates and rodents is to recognise and control intracellular pathogen infection and **enhance subsequent immune functions**

We now need to understand this diversity in terms of ligand recognition and evolutionary history to help decipher function

This is a great opportunity to exploit natural genetic variation to improve disease resistance and vaccine efficacy

Acknowledgments



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Michael Watson



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