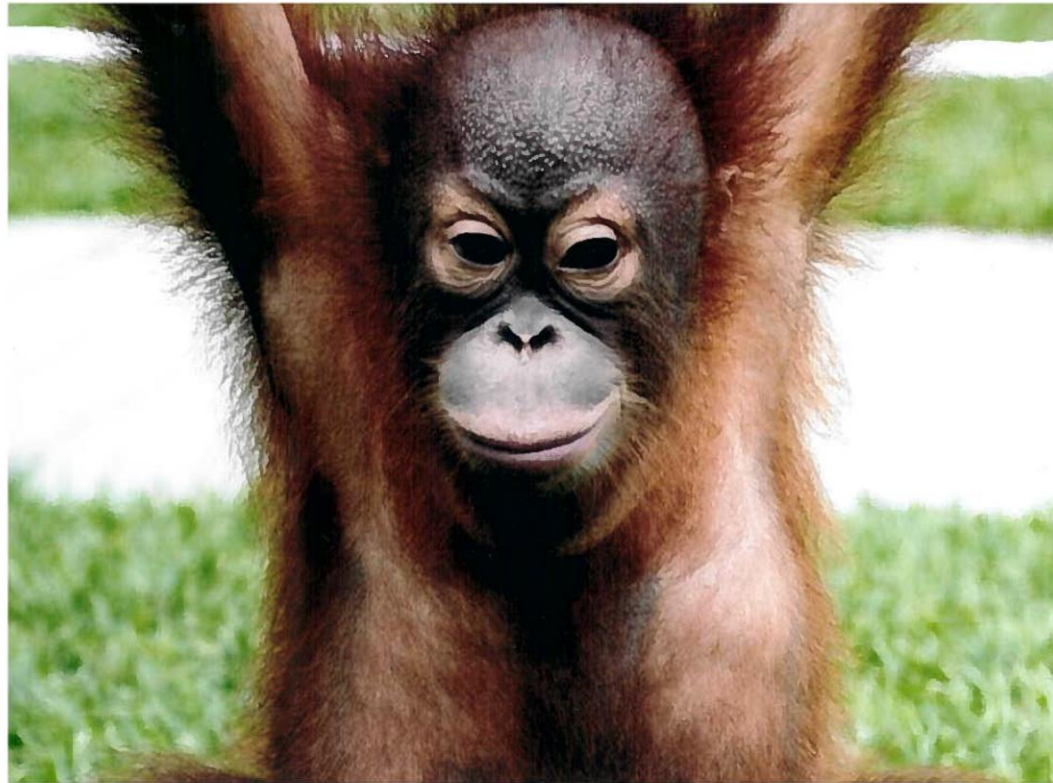


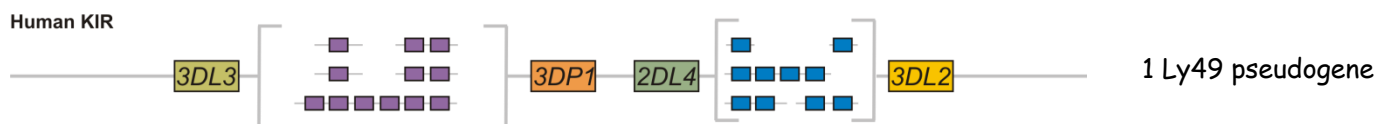
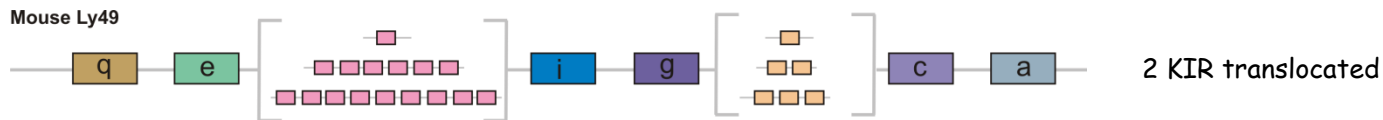
Genomic Architecture of the KIR and MHC-B and -C Regions in Orangutan



*Libby Guethlein
Parham Lab, Stanford University*

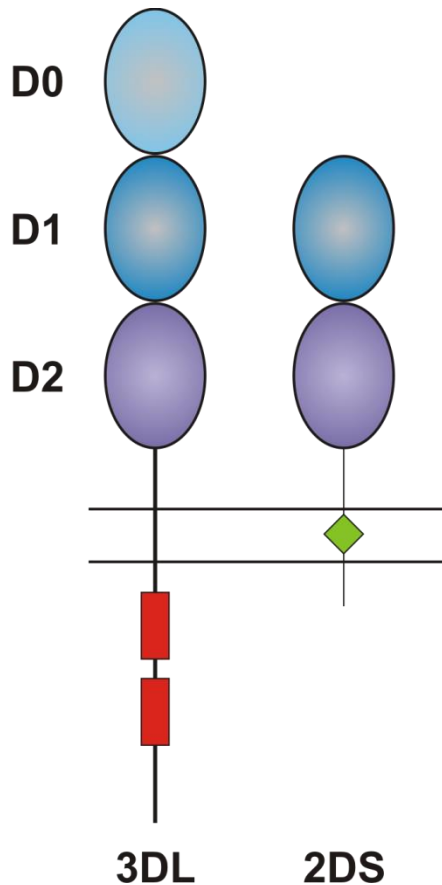
Natural killer cell function is modulated by families of cell surface receptors

- ❖ Receptor variation is germline encoded and not somatically altered
 - Gene content variation
 - Allelic diversity
 - Variegated expression
- ❖ Two broad structural groups of receptors
 - Immunoglobulin-like [KIR, LILR, MAIR]
 - Lectin-like [Ly49, CD94: NKG2]
- ❖ Both groups have MHC as a ligand
- ❖ Studied mammalian species appear to have expanded one group



KIR nomenclature, lineages, and ligands

- Killer cell Immunoglobulin-like Receptor
- Naming is done by number of extracellular domains and type of encoded tail
- Lineages were determined by phylogenetic analysis
- Ligands have been determined experimentally



Lineage	EC	Ligand	Epitope
I	2D	HLA-G	
II	3D	HLA-A and -B	A3/11 or Bw4
III	2D or 3D	HLA-C	C1(N80) or C2(K80)
V	3DL3	unknown	

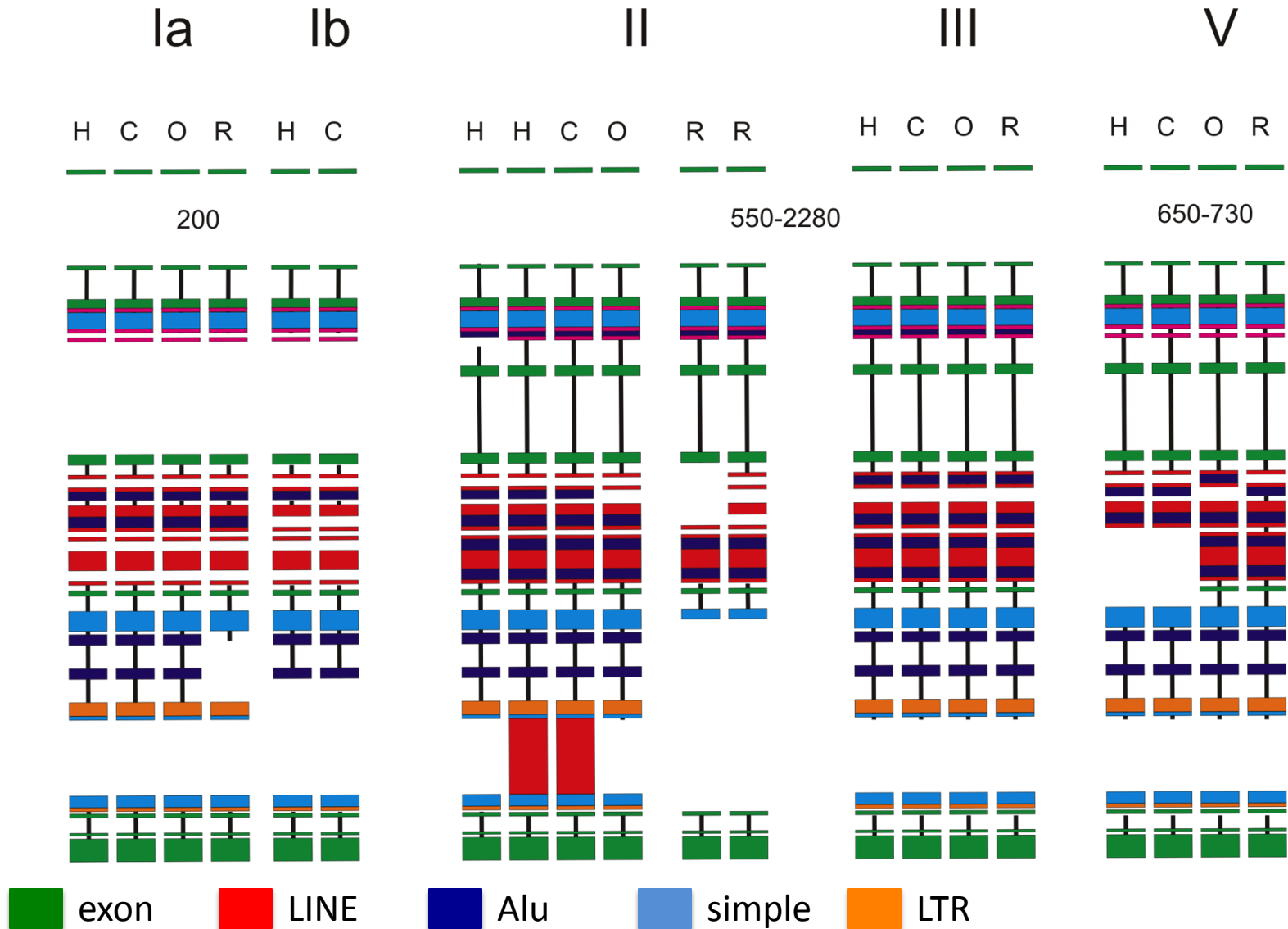
MHC nomenclature and background

- Major Histocompatibility Complex
- In humans HLA, in other species prefixed by genus/species, i.e. Popy for Pongo pygmaeus (orangutan)
- Most KIR ligands are Class I MHC
- Class I molecules have a polymorphic heavy chain complexed with $\beta 2m$
- Further divided into classical (polymorphic, broad tissue distribution) and non-classical (less polymorphic, restricted tissue distribution, sometimes specialized function)
- Along with their function as a KIR ligand they bind and present peptides to the immune system
- In humans HLA-A, -B, -C, -F, and -G can all act as KIR ligands
- HLA-C has emerged as the dominant ligand in humans and all allotypes are capable to act as a KIR ligand.

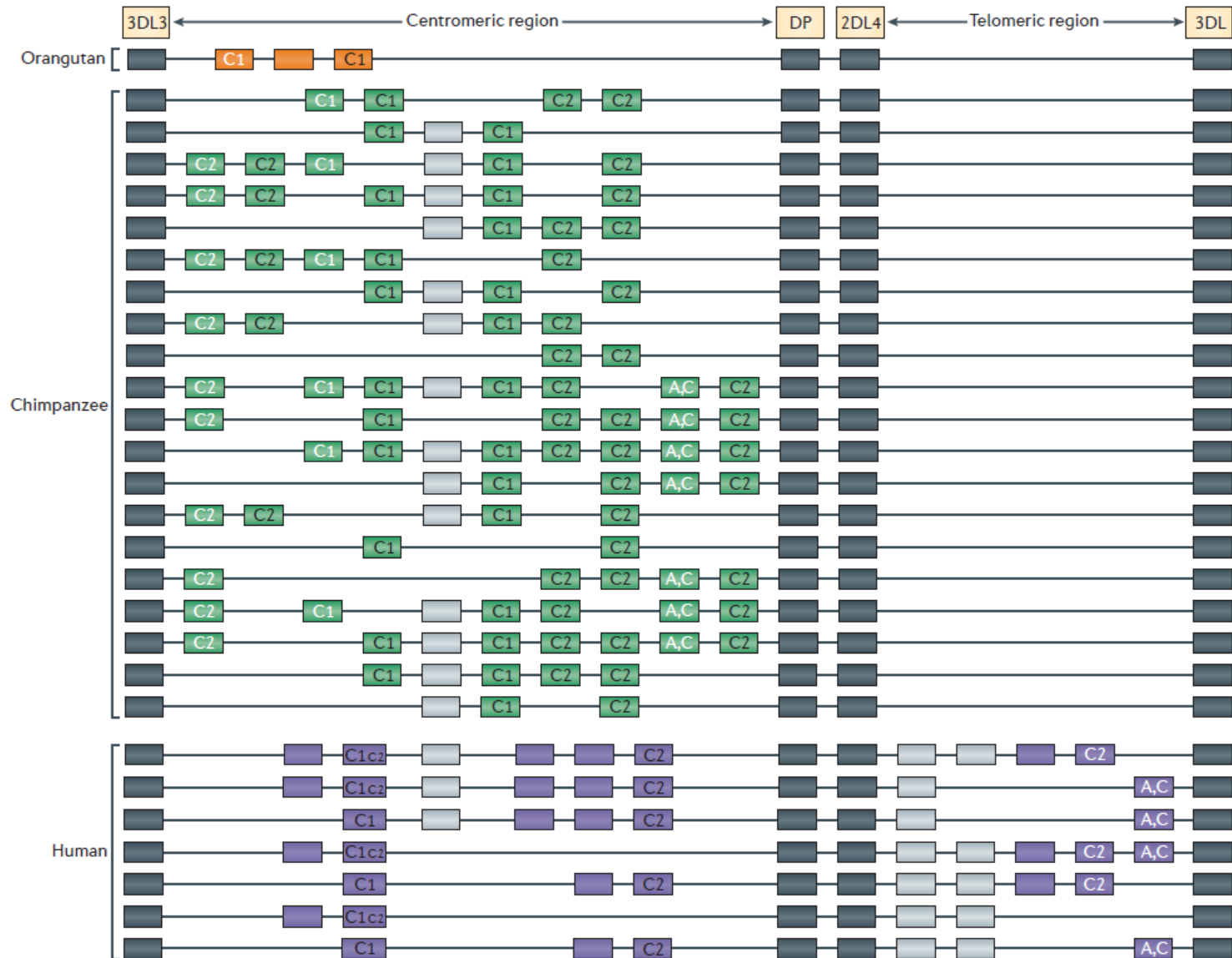
Orangutan is the most distant species to express homologs of all known human KIR ligands

Primate species	MHC class I genes					KIR	Divergence time from human (million years)
	E	G	A	B	C		
Prosimians						one pseudogene	58-69
New World monkeys						expansion of a novel lineage	40-45
Old World monkeys		Inactive		Bw6 Bw4		expansion of lineage II	28-30
Gibbons				Bw6 Bw4		contraction of KIR locus	19-24
Orangutans				Bw6 Bw4	C1	first expansion of lineage III	14-18
Gorillas				Bw6 Bw4	(C1) C2	further expansion of lineage III	10-12
Chimpanzees				Bw6 Bw4	C1 C2		7-10
Human				Bw6 Bw4	C1 C2	elaboration of group A and B haplotypes	
Cognate receptor in human	CD94: NKG2	lineage I KIR	lineage II KIR	lineage II and III KIR	lineage III KIR		

KIR genes are organized head-to-tail with gene lengths of approx. 15kb and 2 kb between loci

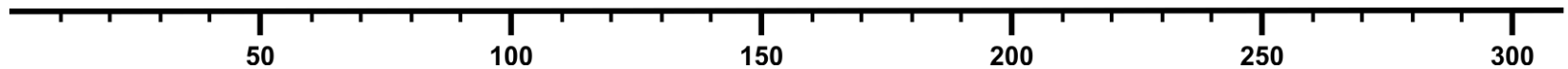


KIR haplotypes vary in gene content



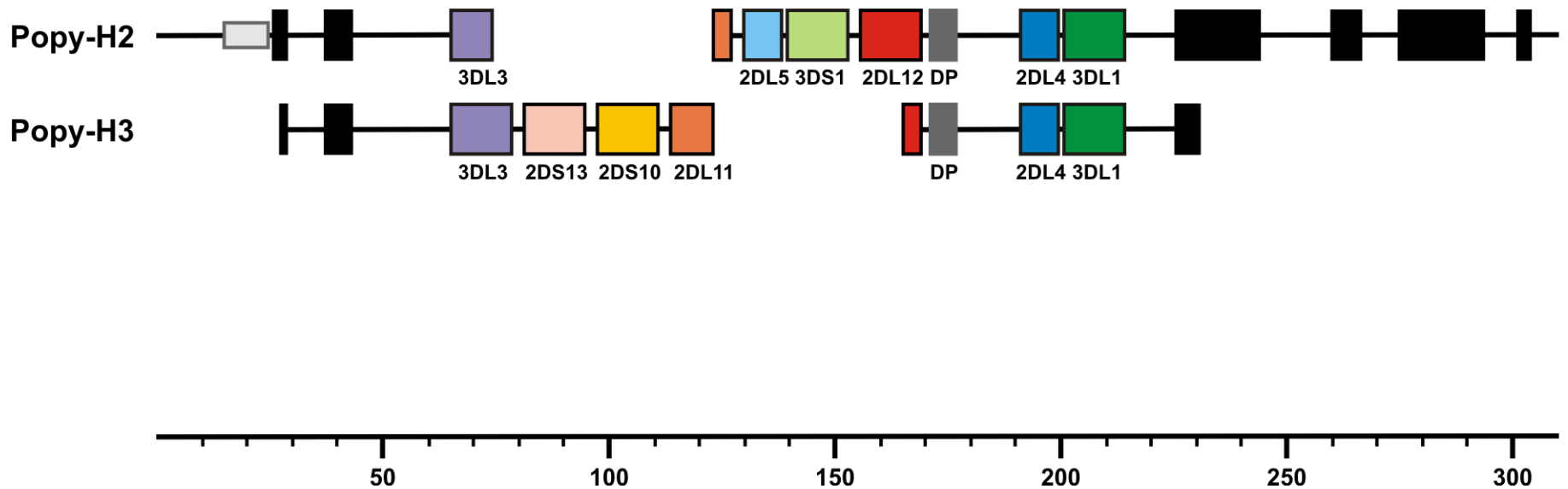
Initial orangutan haplotype determined by sequencing overlapping cosmids

Popy-H3



Cosmids were sequenced by generating two subclone libraries [BamHI and EcoRI] that were then used to determine the complete sequence
Strategy chosen to ensure proper assembly of closely related genes.

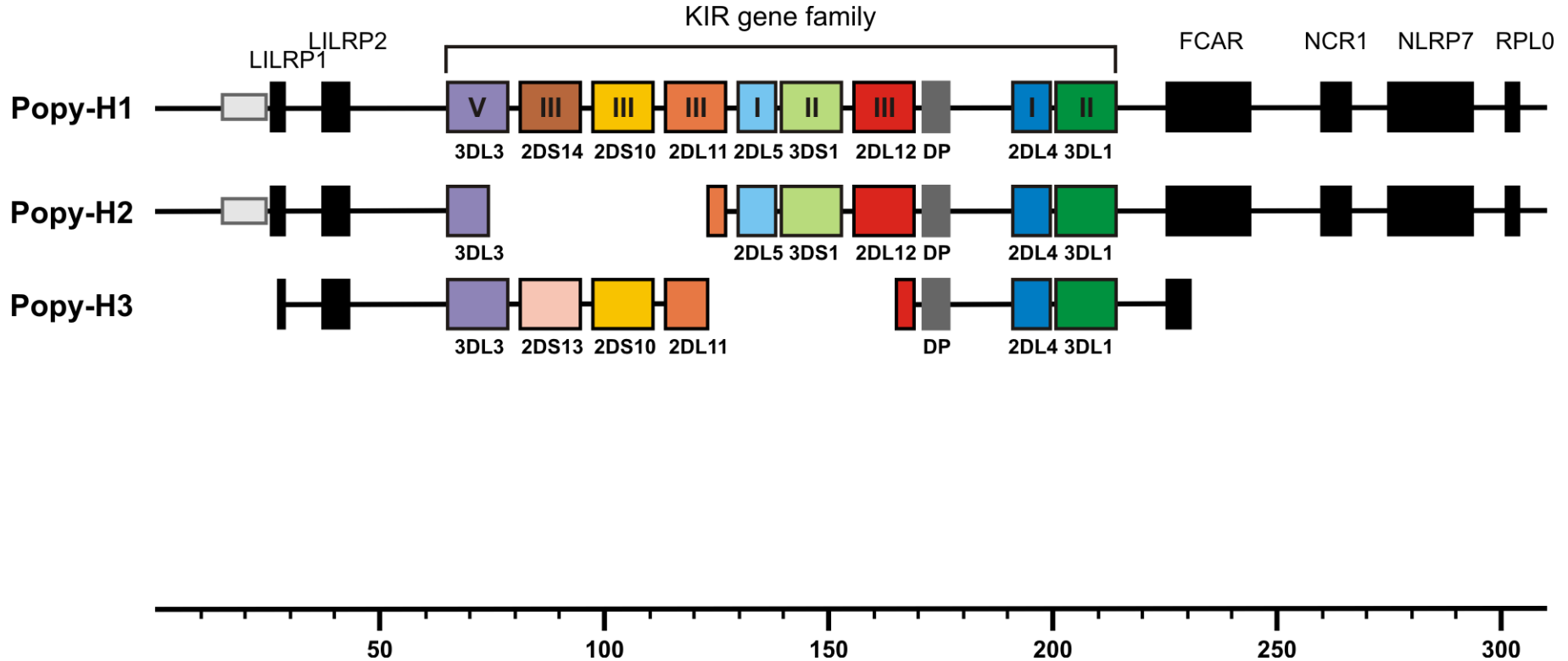
Subsequent sequencing by the Oragutan Genome Project revealed a second haplotype



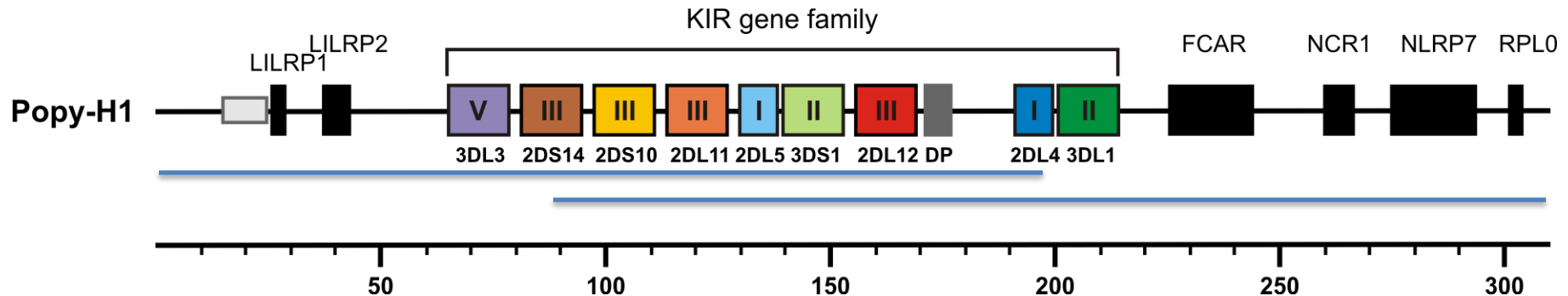
Popy-H2 was the result of complete sequencing of a single BAC

Analysis of the read archives has identified Popy-H3 as the other haplotype present in the individual sequenced.

A third haplotype structure identified through sequencing of two overlapping BACs

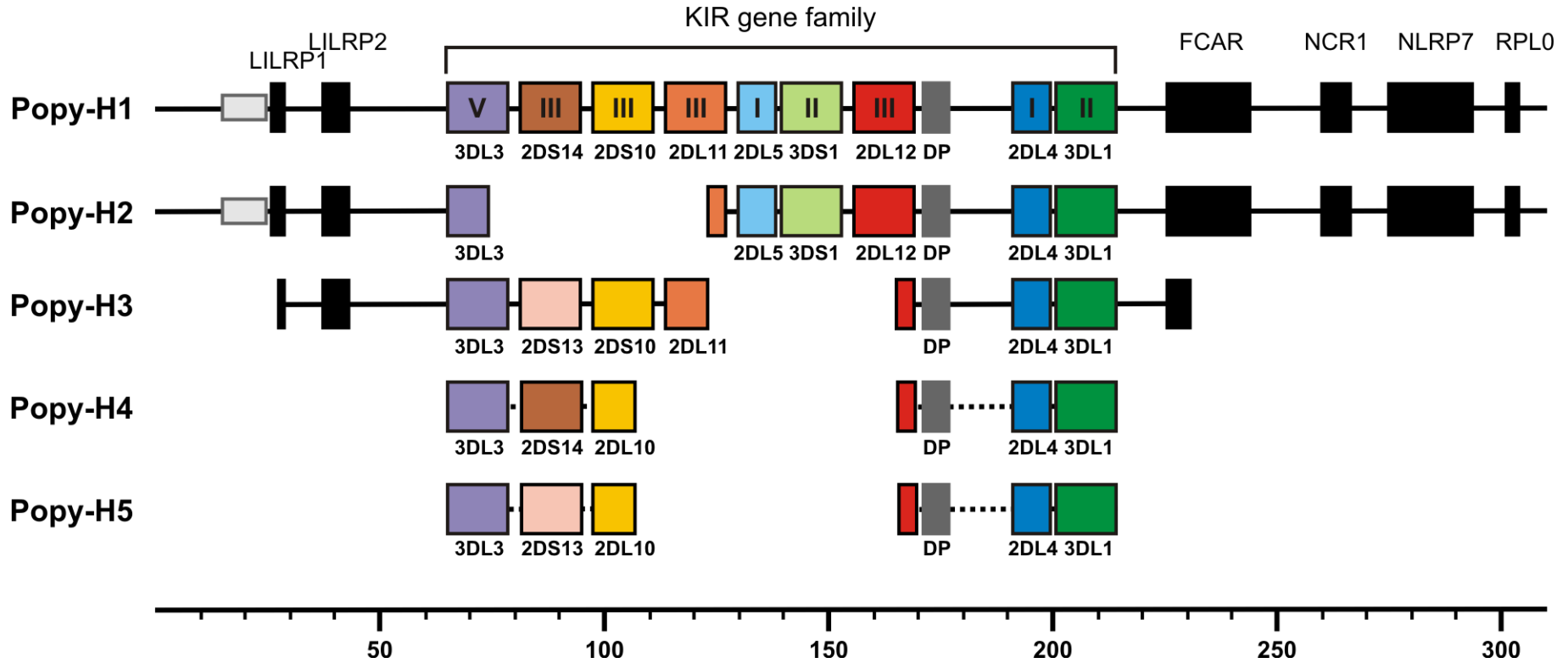


A third haplotype structure identified through sequencing of two overlapping BACs

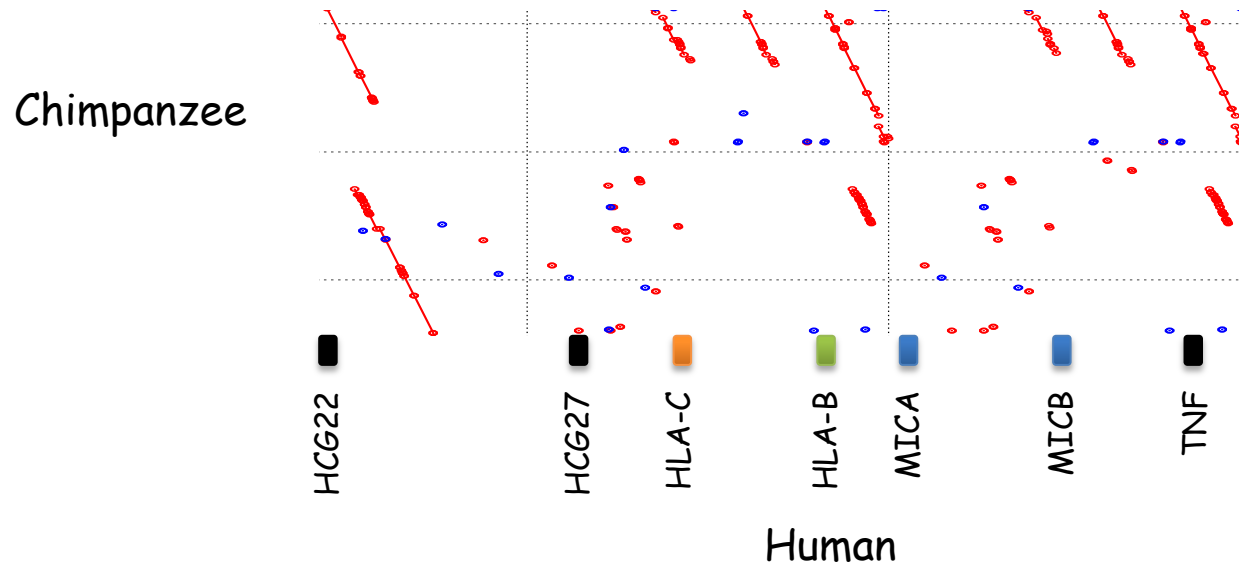


- Initial sequencing with 454
 - ✓ de novo assembly in MIRA with each BAC individually
 - ✓ manual editing
 - ✓ assessed gene content
 - ✓ construction of reference from two known haplotypes
 - ✓ hybrid reference/de novo assembly in MIRA with combined BACs
 - ✓ manual editing
 - ✓ finishing and check of assembly
- Confirmatory sequencing with PacBio
 - ✓ de novo assembly confirmed the 454 assembly and finished areas that had not been completed

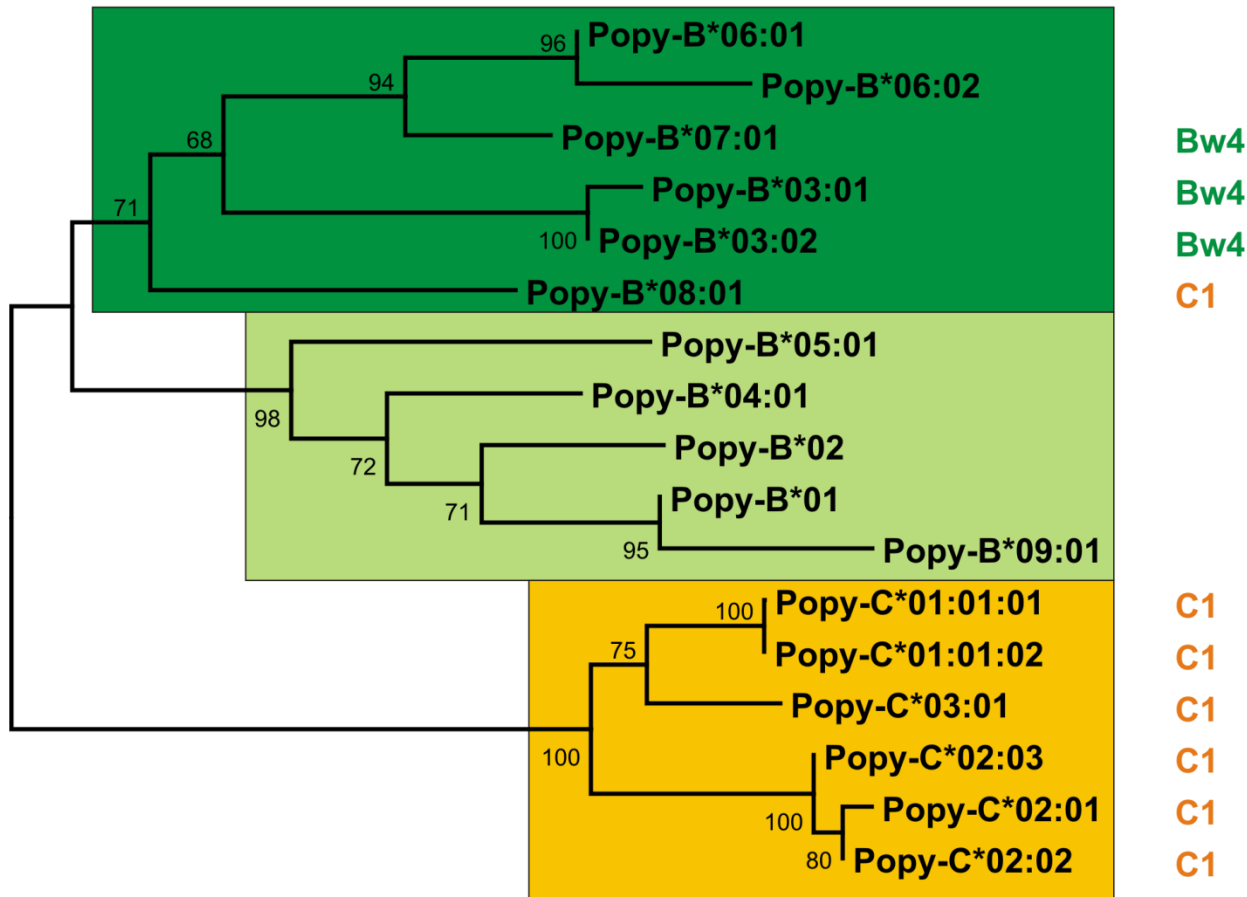
Sequenced KIR haplotypes reveal expansion of lineage III KIR and diversification through tail swap



Unlike KIR genomic architecture in the MHC is more conserved

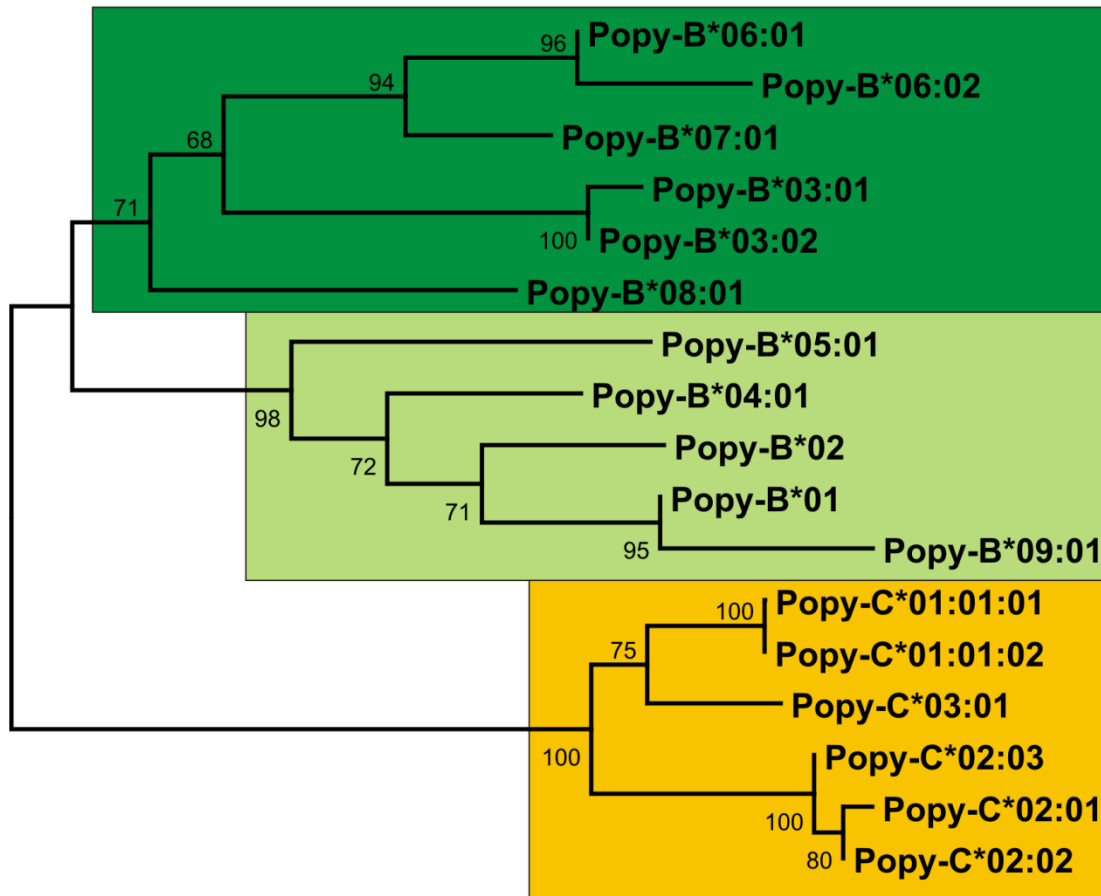


All Popy-C alleles encode C1 epitopes and Popy-B alleles can be broadly divided into two subgroups one of which contains alleles encoding Bw4 or C1 epitopes

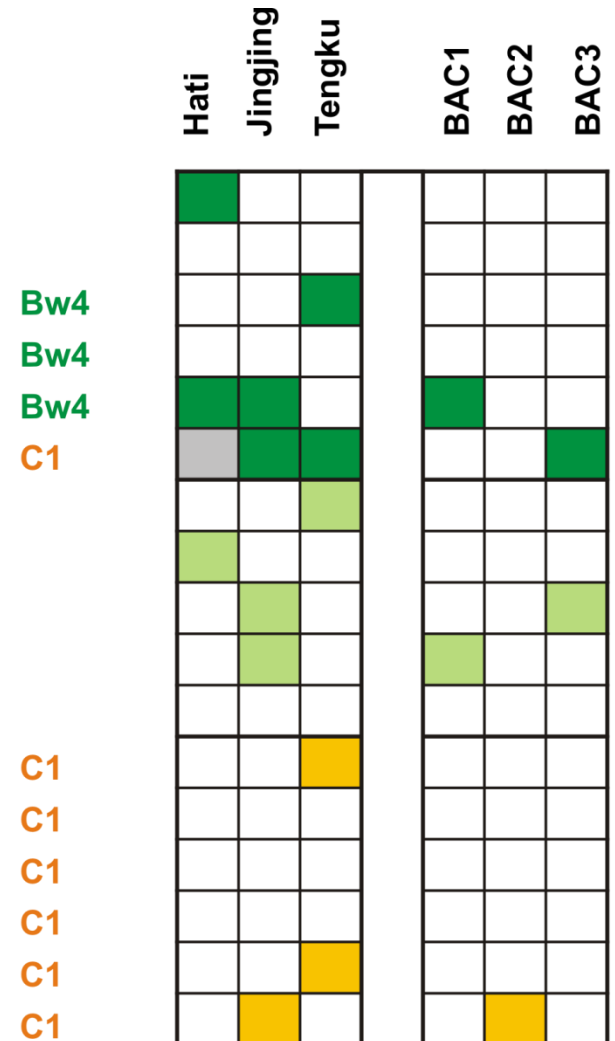


0.02

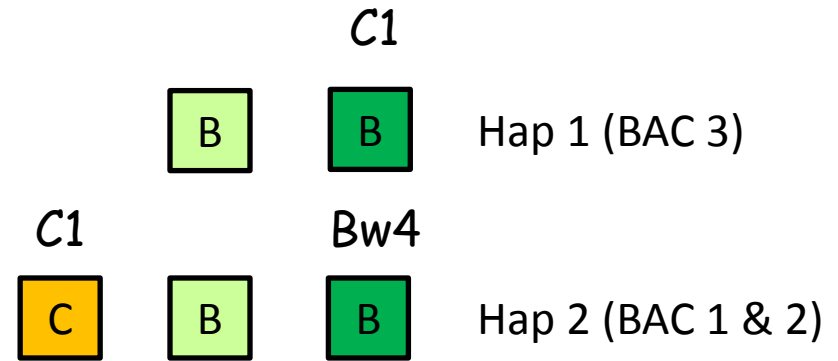
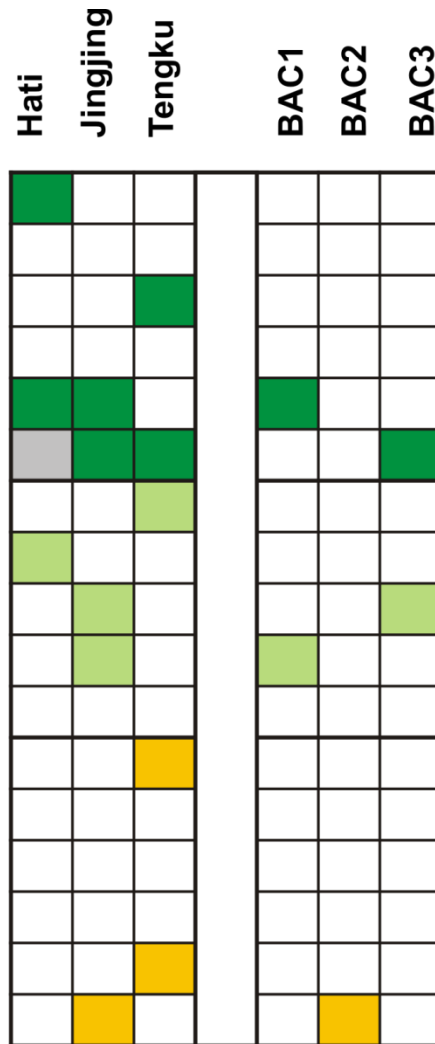
Initial typing results and BACs are consistent with a model of a single Popy-C locus and two Popy-B loci



0.02



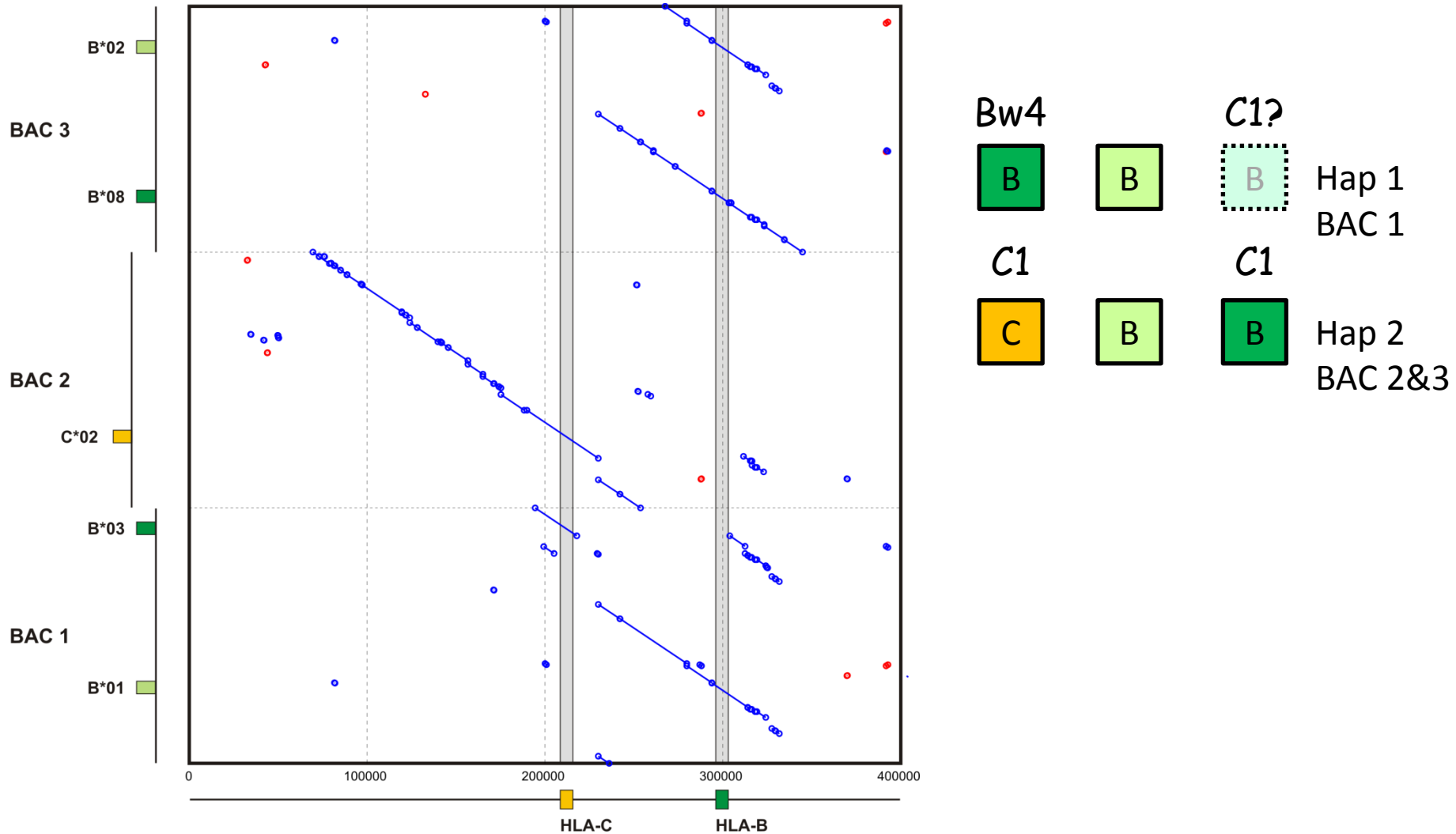
Proposed haplotype structure based primarily on the typing results with some sequence overlap



- Initial sequencing with 454
 - ✓ de novo assembly in MIRA with each BAC individually
 - ✓ manual editing
 - ✓ Many hybrid contigs with two alleles mixed in
 - ✓ Phasing was not clear for much of the polymorphism

- Sequencing with PacBio
 - ✓ de novo assembly

Orangutans have a minimum of two Popy-B loci and one 'hybrid' Popy-B/C locus



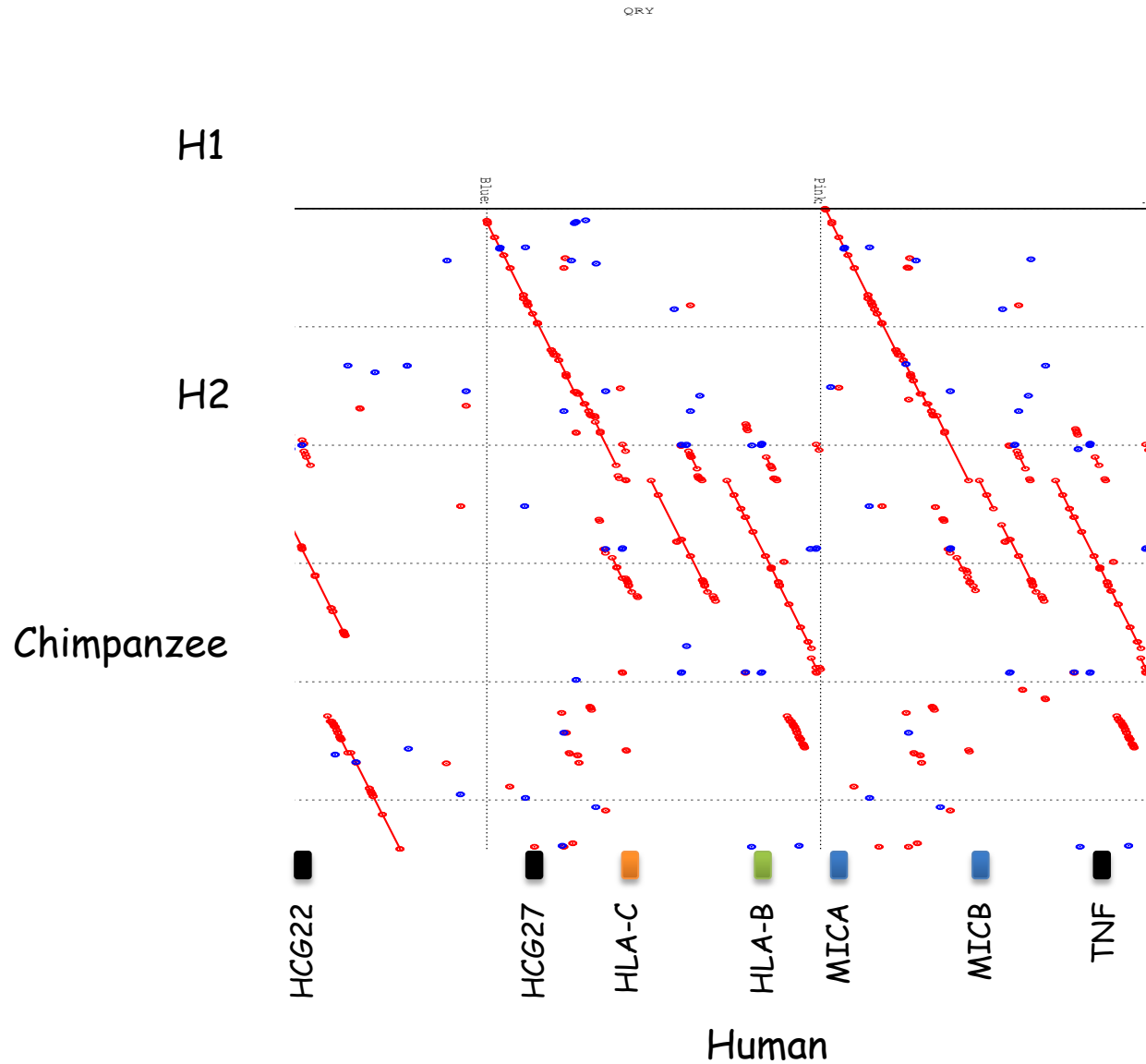
Additional BACs identified by searching BAC ends

13 BACs found by in silico probing the BAC end library with full length BAC sequence

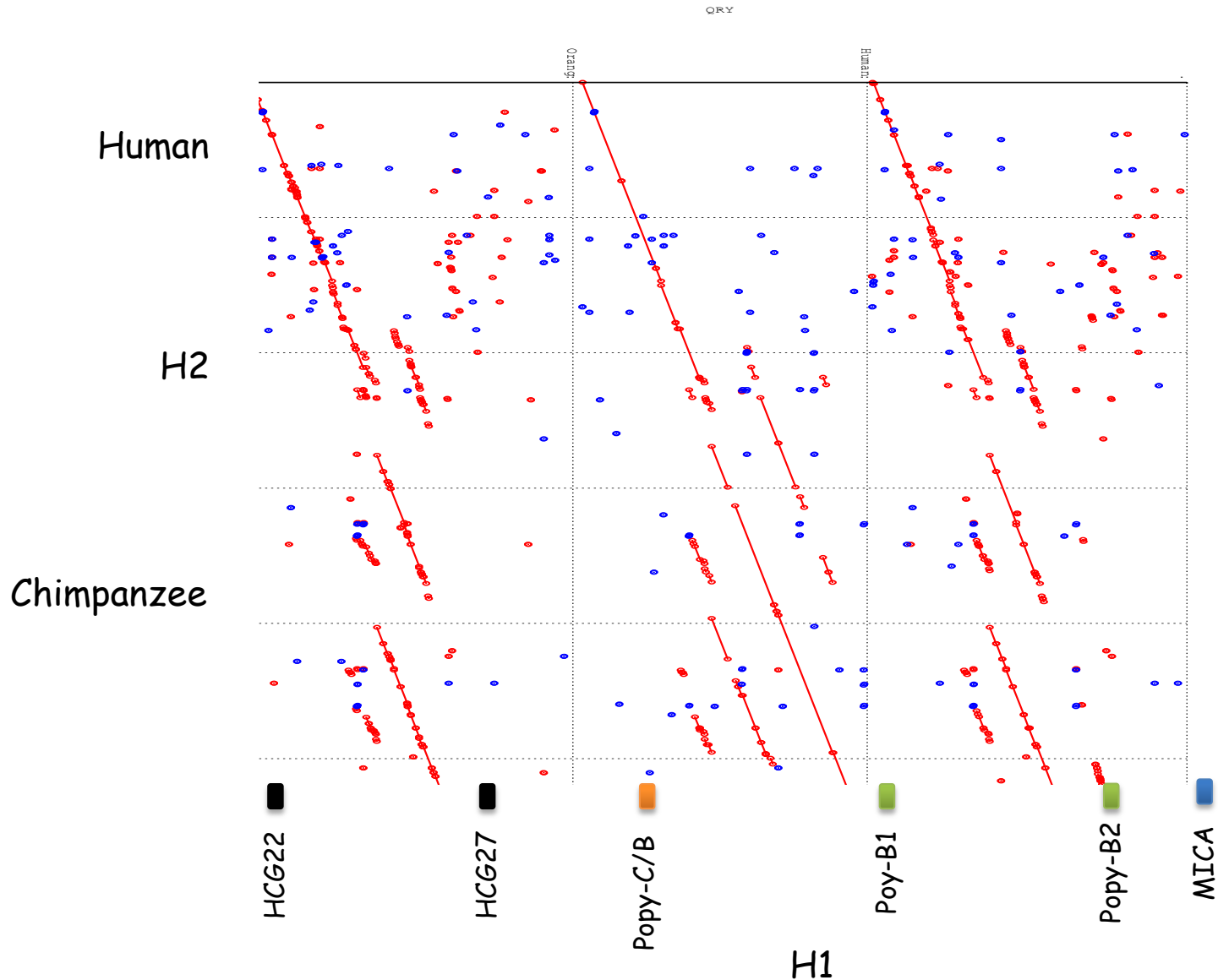
10 overlapped the existing clones

Prepped and sequenced with PacBio

The additional BACs have extended both haplotypes and 'anchored' the ends



The additional BACs have extended both haplotypes and 'anchored' the ends



Summary

- ❖ Orangutan KIR haplotypes have diversified by duplication and recombination
 - Many of the events involve swapping of tail encoding exons
- ❖ Diversification of Popy-KIR has occurred after speciation
- ❖ Popy-B can be divided into two broad groups only one of which encodes potential KIR ligands and these appear to occupy separate loci
- ❖ Popy-C and Popy-B*03:02 occupy the same locus

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