# A robust sequencing assay of a thousand amplicons for the high-throughput population monitoring of Alpine ibex immunogenetics

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Running head: Targeted immunogenetics amplicon sequencing

**Abstract (~250 words)**

Genetic variation is a major factor determining susceptibility to diseases. Polymorphism at the major histocompatibility complex (MHC) and other immune function loci can underlie health and reproductive success of individuals. Endangered species of low population size could be severely compromised to evolve disease resistance due to reduced adaptive variation. A major impediment to screen adaptive genetic variation in wild species is the difficulty to comprehensively genotype immune-related loci based on low input material. Here, we design and validate a targeted amplicon sequencing assay to parallelize the analysis of a thousand loci of the MHC, other immunity-related genes, and genome-wide markers for the assessment of population structure. We apply the approach to Alpine ibex, one of the most successful examples of restoration of a large mammal in Europe. We used 51 whole genome sequenced individuals to select representative target SNPs. We integrated SNP call data from four related species for amplification robustness and genotyped 158 Alpine ibex individuals for validation. We show that the genome-wide markers perform equally well at resolving population structure as RAD-seq or low-coverage genome sequencing datasets with orders of magnitude more markers. The targeted amplicon sequencing assay is robust to >100-fold variation in input DNA quantity and generates useful genotype information from fecal samples. The amplicon marker set also identified recent species hybridization events with domestic goats. The immune loci show unexpectedly high degrees of differentiation within the species. Our assay strategy can realistically be implemented into population genetic surveys of a large range of species.

## Introduction

Biodiversity is currently undergoing a dramatic decline caused by ecological and anthropological pressures (Barnosky et al., 2011; G. Ceballos et al., 2015; Gerardo Ceballos et al., 2017; Wwf, 2018). Endangered species are particularly prone to genetic risks due to population bottlenecks and habitat fragmentation that lead to low genetic diversity, inbreeding, introgression from related species and deleterious mutation accumulation (Allendorf et al., 2010; Frankham, 2005). Low genetic diversity can affect fitness and survival, as shown for instance in cheetah, Florida panthers and Alpine ibex (Brambilla et al., 2015; O'Brien et al., 1983, 1985; Pimm et al., 2006; Reed & Frankham, 2003; Roelke et al., 1993). Loss of genetic diversity at adaptive immune loci is particularly problematic because populations will lack genetic variants conferring disease resistance, in particular infectious diseases, as it has been shown for example in amphibians (Kosch et al., 2019; Savage et al., 2011), Tasmanian devils (Siddle et al., 2007), giant panda (Zhu et al., 2020) and Alpine ibex (Brambilla et al., 2018). However, despite the importance for conservation management, the genetic underpinnings of disease susceptibility are largely unknown in most non-model species (Schoville et al., 2012). The major obstacles in investigating wildlife-immunogenetics are the challenge of collecting quantitatively and qualitatively adequate phenotypic data on diseases in wild populations, as well as the lack of genetic tools suitable in the conservation framework (Holderegger et al., 2019). Hence, there is a pressing need to establish effective genetic monitoring tools for many wild species (Acevedo-Whitehouse & Cunningham, 2006; Allendorf et al., 2010).

The major histocompatibility complex (MHC), a highly polymorphic region whose products are involved in foreign antigen recognition, is an important player for disease susceptibility in vertebrates. Co-evolution with pathogens often causes balancing selection on MHC polymorphism, maintaining high genetic diversity including deeply divergent alleles. Genetic variants under selection often encode the ability to present a wider range of antigens to T-cells and, thus, to recognize a greater variety of parasites. Consequently, heterozygotes often have elevated resistance and are favored by selection (Bernatchez & Landry, 2003). Species that underwent a strong bottleneck such as Alpine and Iberian

ibex (*Capra ibex and C. pyrenica*), Tasmanian devils, Cheetahs or Galapagos penguins (*Spheniscus mendiculus*), show strongly reduced genetic diversity at the MHC compared to related species (Angelone et al., 2018; Bollmer et al., 2007; Brambilla et al., 2018). Low genetic diversity in cheetahs is thought to have contributed to high mortality rates (O'Brien et al., 1983; 2017). High prevalence of a fatal facial cancer in Tasmanian devil is thought to, at least, partially stem from low MHC diversity (Siddle et al., 2007). Low levels of MHC diversity are consequently a major threat for endangered species. Alongside with the adaptive immune system, the innate immune system plays an important role in the defense against a wide range of pathogens. Genetic variation within some genes, *e.g.* Toll-like receptors (TLR), has been shown to be linked with variation in immune competence (Ammerdorffer et al., 2014; Tschirren et al., 2013). Yet the field of wildlife-immunogenetics is only emerging and immune-related genes outside of the MHC remain heavily understudied in wild species. The major challenge for monitoring non-model wild species is that inferring causal disease susceptibility from other species is only rarely possible and associations need to be established for each species – pathogen interaction (Acevedo-Whitehouse & Cunningham, 2006).

To make informed management decisions, the genetic health of populations should also be taken in account and assessed (Allendorf et al., 2010). Integrating information about immune-related polymorphisms and genetic diversity across the genome remains a major challenge. Conservation genetics studies largely rely on microsatellites (Allendorf et al., 2010; Brambilla et al., 2015; Ouborg et al., 2010; Witzenberger & Hochkirch, 2014), which are inexpensive and applicable for a wide range of input material. However, only in exceptional cases do microsatellites reveal adaptive genetic variation and are thus not applicable to most relevant immune-related polymorphism (Allendorf et al., 2010; Steiner et al., 2013). Similarly, modern conservation genomics methods based on restriction site associated DNA sequencing (e.g. RAD, ddRAD, GBS, Davey et al., 2011) intrinsically lack the power to target particular polymorphisms of interest in the genome (Garvin et al., 2010). Whole genome sequencing solves the issue of marker selection but is expensive and requires high-quality input material to work reliably (Davey et al., 2011). Conservation genetics monitoring requires targeted genotyping

97 assays that tolerate a wide range of input material, assess specific polymorphisms of interest and is cost
98 effective.
99
100 Here, we develop and validate a high-throughput amplicon sequencing array, which enables
101 immunogenetic monitoring of Alpine ibex. The species is an excellent model to assess the broad
102 applicability of novel genotyping assays for conservation because of a dramatic recent bottleneck
103 caused by a near extinction event in the 19th century (Grodinsky & Stüwe, 1987). The census size was
104 reduced to less than 100 individuals limited to one single population in Northern Italy (Gran Paradiso).
105 A captive breeding program restored the species to a current census of 53'000 across the European Alps
106 (Brambilla et al., 2020). The historic bottleneck left substantial genome-wide signatures of low
107 heterozygosity, in particular at MHC loci, which may threaten long-term population viability (Grossen
108 et al., 2014, 2018, 2020). Hybridization events with domestic goat produced introgression tracts at the
109 MHC re-establishing some genetic variation, which may have been lost due to the species bottleneck
110 (Grossen et al., 2014). Recently, concerns were raised over population declines as direct and indirect
111 consequences of epizootic disease outbreaks (*e.g.* sarcoptic mange, respiratory diseases, infectious
112 keratoconjunctivitis, brucellosis). One recent disease outbreak concerned cases of brucellosis in the area
113 of Bargy (France). Additional cases were identified in bovine and humans and were considered a
114 broader threat (Mick et al., 2014). As the ibex were seen as a reservoir for the brucellosis pathogen,
115 French authorities undertook a massive eradication program leading to the culling of more than 250
116 individuals (44% of the estimated population) within two years following the outbreak (Mick et al.,
117 2014; Quéméré et al., 2020). Hence, Alpine ibex present a model species where high-throughput
118 monitoring of inbreeding levels, potential hybridization events and immunogenetic diversity can
119 substantially improve genetic analyses and conservation management efforts.
120
121 We established a high-throughput assay of nearly a thousand loci covering largely genome-wide
122 (putatively neutral) polymorphisms, variants found at the MHC and other immune-related loci as well
123 as diagnostic variants useful to detect recent hybridization events with domestic goat. We used 51

whole-genome sequences of Alpine ibex and domestic goat to identify relevant SNPs to be targeted for the amplicons. Furthermore, we used whole genome sequences to mask polymorphism at primer sites to maximize amplification success. Based on highly parallel Illumina amplicon sequencing of 172 Alpine ibex, Iberian ibex and domestic goat samples, we assessed the accuracy and robustness of the assay across highly variable input sample quality. Finally, we compared the high-throughput assay with RAD-seq and low-coverage whole-genome sequencing datasets on the same populations.

## Materials and methods

*Collection of individuals and DNA extraction*

Genotyping was performed on 158 Alpine ibex samples, representing 8 populations with 19-20 individuals each. The samples included also an individual suspected to be an albino. Sample material consisted either of tissue or blood collected either by biopsy darting, during captures or legal hunting (Biebach & Keller, 2009; Willisch et al., 2012). We included four fecal samples collected in the Gran Paradiso National Park as well as six individuals representing suspected hybrids based on field observations, five domestic goats (*Capra hircus*) and three Iberian ibex individuals. Detailed information about the origin, collection method and sampling year of each sample is provided in Supplementary Table S1. All DNA extractions were carried out using the DNeasy Blood & Tissue kit (QIAGEN).

*SNP discovery based on whole-genome sequenced individuals across species*

We used 29 Alpine ibex whole genome sequences (representing seven different populations) to identify segregating SNPs to design our target amplicons. 36 additional genome sequences (4 Iberian ibex, 16 domestic goats, 6 bezoar, 2 Siberian ibex, 2 Nubian ibex, 1 Markhor and 5 sheep, see (Grossen et al., 2020) for details) were used to detect diagnostic markers and to mask highly polymorphic regions (see below). Data from domestic goat, bezoar and sheep were produced by the NextSeq Consortium. Trimmed reads (Trimmomatic v.0.36, (Bolger et al., 2014) were mapped using bwa-mem (Li et al.,

2009) to the domestic goat reference genome (version ARS1, Bickhart et al., 2017) and duplicates were marked using MarkDuplicates from Picard (http://broadinstitute.github.io/picard, v.1.130). After genotype calling using HaplotypeCaller and GenotypeGVCF (McKenna et al., 2010; GATK, v.4.0.8.0, Van der Auwera et al., 2013), SNPs were removed using VariantFiltration of GATK if: QD <2.0, FS > 40.0, SOR > 5.0, MQ < 20.0, -3.0 > MQRandkSum > 3.0, -3.0 > ReadPosRankSum > 3.0 and AN < 46 (80% of all Alpine ibex individuals). We identified a total of 138 million SNPs segregating among all analyzed species with 5.3 million SNPs segregating among the 29 sequenced Alpine ibex. Genome-wide polymorphism was used in three ways to design amplicons for the high-throughput sequencing assay: (1) To discover SNPs variable in Alpine ibex to be targeted for amplification, (2) to discover SNPs differentially fixed between Alpine ibex and domestic goats (diagnostic markers). (3) to mask polymorphic sites near targeted SNPs to prevent designing primers on polymorphic sites potentially causing amplification drop-outs.

*SNP effect prediction and selection*

We annotated SNPs using SnpEff v4.3t 2017-11-24 (Cingolani et al., 2012) with gene annotations produced for the domestic goat genome ARS1 as reference (Bickhart et al., 2017). SNPs located in repeat-masked regions of the reference genome were excluded to avoid designing amplicons in repetitive regions. We designed four types of marker sets.

Marker type 1 - Genome-wide, putatively neutral markers: For the design of genome-wide markers, we focused on SNPs segregating only among Alpine ibex individuals from the Gran Paradiso population, the source of all current Alpine ibex populations (4 million SNPs). Our aim was to most accurately reflect segregating neutral polymorphism in the species. Hence, including non-source individuals could lead to ascertainment bias. Loci were selected with a genotyping rate of ≥70%, a minimal genotyping quality of 20 and at a minimal distance of 2.3 Mb between adjacent SNPs.

Marker type 2 - To detect recent hybridization events, we identified loci with fixed alleles distinguishing domestic goats and Alpine ibex. Specifically, we chose loci with fixed allele frequency differences between all Alpine ibex and all goats (both domestic goat and bezoar), with a minimal genotyping rate

of 80%, a minimal genotyping quality of 20 and a minimal distance between adjacent SNPs of 13 Mb. Markers in genes relevant to the immune system were selected for two subgroups (here called MHC region and Immune, outside MHC) with a minimal allele count of 1 among Alpine ibex.

Marker type 3 in MHC region: Because of the importance of the MHC region for immune functions and evidence for introgression from the domestic goat, we covered the entire MHC region on chromosome 23 (positions 20,892,916-23,588,623 bp). The chromosomal location of the MHC was identified using BLASTN v2.7.1+ (Altschul et al., 1990) based on gene sequences reported as belonging to the MHC in a previous goat genome assembly version (CHIR1, Dong et al., 2013, Supplementary Table S17). Additionally, we used gene ontology (GO) and gene homology to search for all MHC-related genes on chromosome 23. Matching gene sequences within 250 kb of the homologous MHC region of the CHIR1 assembly were considered as part of the MHC region for further analyses. We split the MHC region into 270 windows of 10 kb using bedtools v2.27.1 (Quinlan & Hall, 2010) to identify SNPs for the amplicon assay (Figure 1B). Evidence for introgression from domestic goat is particularly strong at the *DRB* exon 2 (Grossen et al., 2014). We hence designed the amplicon assay with more dense SNPs in the *DRB* region (positions 23,411,211-23,511,211, Figure 1B). The *DRB* was localized between positions 23,451,944-23,470,477 using BLASTN v2.7.1+ (Altschul et al., 1990) and the *DRB* sequence provided by (Dong et al., 2013). A buffer zone of 40'733 bp before and after the gene was added thereby also including gene ENSCHIG00000008942, which encodes immune-related functions (Figure 1B, lower panel). We divided the *DRB* region into 20 windows of 5 kb. We randomly selected a single SNP from every window in the defined MHC and *DRB* by prioritizing MODERATE impact mutations based on Snpeff and SNPs in coding regions. We manually selected a marker in the *DRB* exon 2 at position 23,460,796 bp.

Marker type 4: We targeted immune-related genes, outside of the MHC region on chromosome 23, based on a candidate gene approach using gene ontology (GO), gene homology, as well as literature reports (Acevedo-Whitehouse & Cunningham, 2006; Turner et al., 2011). We searched for all MHC-related GO terms on Ensembl Biomart in the goat genome. Then, we performed InterProScan v. 5.31-70.0 analyses and searched for protein domain annotations matching the term "histocompatibility". For

the lists by Acevedo-Whitehouse and Cunningham (2006) and Turner et al. (2011), we focused on 211 genes encoding important immune functions for mammals including interferons, interleukins, toll-like receptors (TLR) and MHC-related genes outside of the MHC region on chromosome 23. Each retained gene locus was targeted by a single SNP prioritizing SNPs in coding sequences or with a MODERATE impact annotation based on SnpEff (Supplementary Table S2).

*Amplicon design*

For each amplicon to be designed, we extracted a 1001 bp sequence from the domestic goat reference genome centered around the target SNP (IUPAC encoded) using bcftools v1.9 (https://www.htslib.org/). The extracted sequences had masked positions for all repetitive regions (masked reference genome) and positions which were polymorphic in Alpine ibex and/or with a minor allele count of three among all other *Capra* species (samtools v. 1.9 with option -m; (Li et al., 2009)). Sequences with more than 50% masked bases or sequences overlapping between different amplicon sets were excluded. We obtained a set of 1589 sequences for assay primer design by Fluidigm Inc. About 25% of the sequences were rejected by Fluidigm due to the lack of primer options matching the design criteria, leaving 1265 sequences for oligonucleotide primer synthesis (Figure 1). The targeted amplicon length was 200 bp.

*Targeted DNA sequencing library preparation and SNP calling*

We prepared libraries following the manufacturer protocol "Library Preparation with the LP 192.24 IFC" using the Fluidigm Inc. Juno system, except for the minimal concentration of genomic DNA. Due to very low reaction volumes, the manufacturer recommends ≥ 100-200ng/µl. As most samples wouldn't have reached that, we decided to use a minimal concentration of 50ng/µl. Samples also failing the concentration of 50ng/µl of genomic DNA, were concentrated using Ampure magnetic beads, whereas those, for which concentration to 50ng/µl was not possible due to low starting volumes, were used at the original concentration.

Barcoded and quality checked libraries were sequenced on a single lane of an Illumina NextSeq 500 in mid-output mode adding ~30% PhiX to avoid potential problems due to low sequence complexity. We demultiplexed raw read data using bcl2fastq v2.19.0.316 and used Trimmomatic v0.38 (Bolger et al., 2014) for quality trimming. Forward and reverse reads were merged using FLASH v1.2.11 (Magoč & Salzberg, 2011) and aligned to the goat reference genome ARS1 using bowtie2 v2.3.5 (Langmead & Salzberg, 2012). Read depths for each step were estimated with MultiQC v.1.7 (Ewels et al., 2016). We called SNPs using HaplotypeCaller, CombineGVCFs and GenotypeGVCFs from GATK v4.0.1 (McKenna et al., 2010; Van der Auwera et al., 2013). Variant sites were further filtered to meet the following conditions: QD < 5, MQ < 20, -2 > ReadPosRankSum > 2, -2 > MQRankSum > 2, -2 > BaseQRankSum > 2.

*Marker system performance on Alpine ibex populations*

To evaluate key performance metrics of the new high-throughput amplicon sequencing assay, we compared the outcome against two major classes of current population genomics sequencing approaches: RAD-seq and low-coverage whole genome sequencing. We used datasets reporting analyses on four populations also included in our study (Grossen et al., 2018, 2020; Leigh et al., 2018): the founder population of Gran Paradiso and the three Swiss populations Pleureur, Brienzer Rothorn and Albris. Because high-coverage whole genome sequencing is not feasible for large scale, practical applications, we generated realistic, low-coverage datasets at approximately 1x coverage. For this, we downsampled 15 whole genome Illumina sequencing datasets (Grossen et al., 2020). We used sambamba v.0.6.6 (Tarasov et al., 2015) to downsample individual bamfiles to a fraction of 0.05 (producing a final coverage of ~1x). We used the software ANGSD (Korneliussen et al., 2014) to calculate genotype likelihoods with the following options: -doGlf 2, -doMajorMinor 1, -doMaf 1, -minMaf 0.05, -SNP_pval 1e-6, -minMapQ 20, -minQ 20, -skipTriallelic 1, -uniqueOnly 1, -remove_bads 1, -only_proper_pairs 1. The resulting likelihoods were used to run PCAngsd (Meisner & Albrechtsen, n.d.) and NGSadmix (Skotte et al., 2013). For the RAD-seq dataset, we first trimmed reads using Trimmomatic v3.6 (Bolger et al., 2014) and performed read mapping using Hisat2 v.2.1 (Kim et

al., 2019) on the ARS1 reference genome. We de-duplicated bam files using Markduplicates from Picard v. v.2.5 (http://broadinstitute.github.io/picard/) and called SNPs on all autosomes (1-29) using the GATK v 4.1 pipeline with HaplotypeCaller, GenomicsDBimport and GenotypeGVCFs (McKenna et al., 2010). SNPs were flagged using the GATK VariantFiltration tool if any of the conditions were matched: QD <2.0, FS > 60.0, SOR > 3.0, MQ < 30.0, -12.5 > MQRandkSum > 12.5 and -8.0 > ReadPosRankSum. Next, we filtered for SNPs falling within 100 bp of a *SbfI* restriction cut site identified by *in silico* analyses with the ENSEMBL tool *restrict* (Yates et al., 2019). To compare genotyping performance between RAD-seq and the amplicon sequencing datasets, we filtered for a minimal individual genotyping rate of 0.5 and kept polymorphic sites only. We retained for the amplicon sequencing 892 SNPs genotyped in 75 individuals and for the RAD-seq 26,547 SNPs genotyped in 82 individuals. For all further analysis (population differentiation), we kept of the RAD seq dataset only bi-allelic SNPs with a minimal genotyping rate of 0.9, a minor allele frequency of 0.01 and a heterozygosity below 0.8 ($n$ = 8316 SNPs retained). For the amplicon sequencing, we only retained SNPs from the genome-wide (neutral) set and removed sites on the Xa/b sex chromosome ($n$ = 588 SNPs).

*Genetic data analyses*

Genetic data analyses were done using R 4.0.2 (R Core Team 2018). The R package {BioCircos} was used to generate the circular plot. Principal component analyses (PCAs) were performed using the glPCA function from the R package {adegenet}. The R package {hierfstat} was used for $F_{ST}$ calculations. The SNP intersection matrix was visualized with {UpSetR}. Genotype assignment plots were generated using sparse non-negative matrix factorization algorithms as implemented in the R package {LEA}. For each marker set, we ran 100 repetitions per K (K = 1-10) with entropy=TRUE to find the most likely number of clusters (*i.e.* K with the lowest entropy). Tajima's *D* estimates in coding sequences (all immune-related genes represented on the amplicon) were calculated using the 29 Alpine ibex whole-genome sequencing datasets and the R package {PopGenome}.

## Results

*Assessment of locus quality across the targeted sequencing assay*

We performed targeted amplicon sequencing of 1265 SNP loci covering genome-wide polymorphism and variants related to immune functions. The Fluidigm Inc. Juno microfluidics systems enables highly parallel amplification of all loci. We analyzed a total of 187 samples (representing 172 individuals) in a single run including 158 Alpine ibex, 5 domestic goats, 3 Iberian ibex and 6 suspected hybrids between Alpine ibex and domestic goats (Supplementary Table S1). HiSeq Illumina sequencing generated a total of 108 million read pairs after removal of PhiX spike-ins (32.4 Gbp total data). To assess genotyping quality across loci, we first focused only on samples that satisfied the manufacturer's recommended DNA concentration of ≥50 ng/µl. Furthermore, we required that each analyzed individual produced at least 100'000 mapped reads across all loci (Figure 2A). The 65 retained individuals represented 7 different Alpine ibex populations, 2 domestic goat breeds as well as 3 Iberian ibex. The number of mapped reads ranged from 230’801-1'576’615 reads (Supplementary Table S3). Based on these high-quality samples, we found that the median read depth per locus was high with nearly all loci having >20 reads (median across loci = 389 reads). We found 7 loci with a median read depth of 0. The highest median read depth was 1931 for a marker designed in the MHC. Next, we analyzed the genotyping rate across loci and found that 940 loci were genotyped in all high-quality samples and 989 loci were genotyped in more than 75% of samples (Figure 2C). We discarded 28 loci with a genotyping rate below 75% to prioritize loci providing the highest information content across individuals. We retained a total of 989 high-quality loci for further analysis (Figure 2D).

*Assessment of genotyping rate and accuracy*

The mean genotyping quality (GQ) across the 65 high-quality samples was on average 96 (Figure 3A). Taking advantage of four Alpine ibex individuals, which were both whole-genome sequenced and genotyped using our assay, we analyzed the overall accuracy of genotypes. Among the four individuals, a total of 62 loci were not assigned a genotype based on whole-genome sequencing. On average, 901

loci (range: 883-910, Figure 3B) showed perfectly matching genotypes and twenty loci (range: 17 - 27, Figure 3B) showed one mismatching allele (*i.e.* heterozygote vs. homozygote mismatch). The average genotype quality of the mismatched genotypes was 63 while it was 96 for matching genotypes. We found no complete allelic mismatch in any of the four individuals (*i.e.* homozygous calls for distinct alleles). An effective high-throughput genotyping assay should perform sufficiently well for low sample input quantity and quality. To establish a benchmark for input DNA sensitivity, we analyzed dilution series of input DNA. We found that genotyping rates >90% across loci are retained by diluting samples 25-fold from 100 ng/µl down to 4 ng/µl (Figure 3C). At 0.8 ng/µl, the genotyping rate was >75% for two out of three samples. The genotyping rate was >23% for 0.16 ng/µl of DNA (625-fold dilution). Challenging samples typically include fecal samples, which are contaminated with non-target DNA (*e.g.* plant and bacterial origin) and show elevated levels of overall DNA degradation. Therefore, we analyzed the performance of four DNA samples collected from Alpine ibex feces in the Gran Paradiso National Park. Three of the four fecal samples had a 25% genotyping rate (*i.e.* 248-270 genotyped loci). The fecal sample with the lowest quality had a 10.3% genotyping rate corresponding to 102 genotyped loci. Samples with low genotyping rates typically show inconsistently genotyped loci across samples. We found that this was indeed the case with 41-133 of the loci being genotyped in only one out of four fecal samples. A total of 41 loci were genotyped in at least three fecal samples (Figure 3D).

*High-resolution population structure*

A major reason for genotyping species of conservation concern is the identification of population subdivisions and admixture events. We expanded our genotyping assay to the full set of Alpine ibex samples (n = 158) spanning the extant distribution range across the Alps. Based on a PCA, we identified three major genotype clusters. The largest cluster was composed of the Gran Paradiso source population and populations reintroduced directly from Gran Paradiso to Italy or Switzerland (Figure 4A). A second cluster grouped the two French populations Haute Maurienne and Champsaur (the latter founded with individuals coming from the former). The third cluster was composed of the isolated Alpi Marittime population, which is thought to have only six effective founder individuals (Terrier & Rossi, 1994). The

identified population structure was also supported by individual ancestry coefficients using a sparse non-negative matrix factorization algorithm (K=3, Figure S1, Frichot et al. 2014). At the K with the lowest entropy (K=5, Figure S2), the analyses revealed a fine-scale population structure: all populations except for the two French populations Haute Maurienne and Champsaur, were clearly distinct (Figure 4C). The genotyping assay performed also well for population-level assignments of the low-input/quality samples. All four Gran Paradiso fecal samples had similar principal component values (Figure 4A) and structure population assignments (Figure 4C) as other, high quality samples from the same population. We also analyzed population differentiation of Alpine ibex using pairwise $F_{ST}$ (Fig. 4B). We found a consistent pattern separating Alpi Marittime from all other populations. Furthermore, the two French populations showed relatively low differentiation consistent with their foundation history: the Champsaur population was founded only 25 years ago (less than 4 ibex generations) with 31 individuals coming from Haute Maurienne. The three Swiss populations (Albris, Brienzer Rothorn and Pleureur) were only weakly differentiated from the Gran Paradiso source population as expected from former analysis based on microsatellites (Biebach & Keller, 2009).

*Performance contrasts among next-generation sequencing methods*

Next-generation sequencing methods for population monitoring can have distinct advantages or drawbacks. In contrast to the newly developed targeted amplicon sequencing for Alpine ibex, reduced representation (*e.g.* RAD-seq) and low-coverage whole genome sequencing have the potential for orders of magnitude larger numbers of scorable SNPs but of potentially lower genotyping quality and higher missingness. To objectively assess the performance of the newly developed assay, we analyzed genotyping outcomes of four core Alpine ibex populations (Gran Paradiso, Pleureur, Albris and Brienzer Rothorn; Figures 5, S2). After quality filtering of each dataset to ensure objective comparisons (see Methods), we retained 892 loci from targeted amplicon sequencing ($n$ = 75 individuals), 26,547 RAD-seq loci ($n$ = 82 individuals) and 3 Mio low-coverage whole genome sequencing loci ($n$ = 15 individuals). Overall, 97% of the individuals had a per-individual genotyping rate of ≥90% for targeted amplicon sequencing loci contrasting with 23% of the individuals genotyped at ≥90 % for RAD-seq

loci (Figure 5A). Enforcing a per-locus genotyping rate of ≥90% over all individuals, 96% of targeted amplicon sequencing loci but only 39% of RAD-seq loci were retained (Figure 5B). Locus and individual-level genotyping rates cannot meaningfully be retrieved from genotype likelihood-based analyses (low-coverage whole genome sequencing dataset). We performed comparative population differentiation analyses and found that the global $F_{ST}$ ranged between 0.071 (RAD-seq) and 0.077 (targeted amplicon sequencing). Pairwise $F_{ST}$ estimates were also similar among marker systems (Figure 5C). PCAs constructed from targeted amplicon sequencing and RAD-seq markers clearly resolved the four populations (Figure 5D, E). However, the low-coverage whole genome sequencing did not resolve Albris and Brienzer Rothorn populations (Figure 5F). The first and second principal component (PC) axes explained 6.2% and 4.9% for the targeted amplicon sequencing (Figure 5D), 5.2% and 4.2% for the RAD-seq (Figure 5E) and 23.8%-6.1% for the low-coverage whole genome sequencing (Figure 5F), respectively. Genotype assignments to clusters showed clear population differentiation for the targeted amplicon sequencing markers (Figure 5G) and slightly weaker resolution for RAD-seq markers (Figure 5H). Low-coverage whole genome sequencing genotyping clearly separated Gran Paradiso and Pleureur populations but again failed to resolve Albris and Brienzer Rothorn populations (Figure 5I).

*Detection of recent hybrids and introgression tracts*

Introgression from domesticated animals is a major concern for a number of wild species. A set of SNP markers in the set of amplicons were specifically designed to detect introgression from domestic goats into Alpine ibex. To assess the power to discriminate genotypes suspected to be from hybrid individuals, we performed a PCA including all 158 Alpine ibex individuals, suspected hybrids based on field reports of unusual phenotypes ($n = 6$), domestic goats ($n = 5$) and Iberian ibex ($n = 3$; Figure 6A). As expected, the first PC clearly separated domestic goats, Iberian ibex, as well as Alpine ibex. The second PC differentiated the Alpi Marittime population from all other Alpine ibex. The suspected hybrid individual from Alpi Marittime (AM_H) clearly clustered with domestic goats. Two additional suspected hybrids (GR_ib1, GR_ib2), as well as a potential albino individual (FR_blanc has largely whitish fur, but no

393 red eyes) clustered with Alpine ibex. Three suspected hybrids (TI_ib, GPHB1 and GP_ib_V02_17)
394 were located near the mid-point between domestic goats and Alpine ibex matching expectations for
395 recent (F1 or backcross) hybrid genotypes.
396 Using the goat-Alpine ibex diagnostic marker set, we analyzed the recency of the hybridization event
397 by identifying contributions from each parental species (Figure 6B). Of the six suspected hybrids and
398 one potential albino, two individuals were confirmed to be Alpine ibex (FR_blanc, GR_ib1) and one a
399 domestic goat (AM_H). Our results confirm that the unusual phenotype of FR_blanc reported from the
400 field was not caused by domestic goat introgression. Individual GR_ib1 was suspected to be a hybrid
401 because it was behaving in very unusual ways, seeking proximity to buildings. Individual AM_H was
402 reported to resemble domestic goat but was living among Alpine ibex. Another suspected hybrid
403 (GR_ib2) with white hoofs showed a weak sign of domestic goat introgression (only one marker on
404 chromosome 10 was heterozygous) suggesting a potential backcross. Note that GR_ib2 showed no clear
405 differentiation from Alpine ibex based on the genome-wide marker PCA underlining the usefulness of
406 specifically designed diagnostic loci. Two individuals (GP_ib_V02_17 and GPHB) showed signs of
407 ~25% domestic goat introgression (*i.e.* likely F2 backcrosses). GP_ib_V02_17 had darker fur than
408 commonly seen in Alpine ibex and an unusual horn shape (no nodes and a triangular transverse section).
409 Chromosome painting showed one individual was being heterozygous for all diagnostic markers (TI_ib,
410 Figure 6C), hence representing very likely an F1 hybrid. This individual was observed going into a
411 stable following goats and had an unusual horn shape. The DNA from GPHB was from a small amount
412 of blood stored on FTA paper explaining the poor genotyping quality. Field reports suspected a F1
413 hybrid but both the contribution plot (Figure 6B) and the chromosomal painting (Figure 6C) suggest a
414 F2 hybrid. Our analyses show the power of highly discriminatory markers to detect recent hybridization
415 and introgression events in a pool of individuals with field-reported, suspected admixture.
416
417 *Immunogenetics of Alpine ibex populations*
418 The targeted amplicon sequencing specifically focused on 297 polymorphisms in immune-related genes
419 within and outside the MHC. Using whole-genome sequencing data for Alpine ibex ($n$ = 29), we found

that the Tajima's *D* in Alpine ibex ranged from -2.4 to 4.5 across all genes encoded in the MHC region and from -1.4 to 2.5 for all immune-related genes outside of the MHC region targeted by the amplicon sequencing. The median Tajima's *D* was lower in the MHC (0.45) compared to immune loci outside of the MHC (1.3, Figure 7A). The high Tajima's *D* values in some immune-related loci suggest long-term maintenance of alleles through balancing selection (Figure 7A). Comparing different sets of amplicon targets in our assay, we found that the genome-wide markers aimed at resolving population structure showed overall the highest average heterozygosity (Figure 7C). The lowest genome-wide heterozygosity was found in Alpi Marittime consistent with the severe founding bottleneck. Immune-related and MHC loci showed consistently lower levels of heterozygosity compared to genome-wide loci. With a notable exception of the Alpi Marittime population where the MHC showed higher levels of heterozygosity (0.25) than other immune-related and genome-wide markers (Figure 7C). The MHC of the Albris population showed a surprisingly low average heterozygosity (0.11) compared to genome-wide markers. On a PCA, the MHC in domestic goat and Iberian ibex showed a low degree of differentiation (Figure 7D). This is likely explained by the focus on segregating polymorphism in Alpine ibex only. MHC genotypes showed tight clusters among Alpine ibex individuals but only weak population signatures, which is in marked contrast to genome-wide markers (Figure 4A, 7D). Clusters of nearly identical Alpine ibex genotypes were generally composed of genotypes from multiple populations. The two French populations Haute Maurienne and Champsaur shared most MHC genotypes. Individuals from the Alpi Marittime were largely distinct from all other populations with the exception of a shared genotype with the Italian population Tarvisio. Some individuals from Alpi Marittime were translocated to Tarvisio in 1993, which may explain our finding.

## Discussion

Genetic monitoring is central to many population surveys and conservation efforts. However, there is currently no established implementation enabling both targeted and highly parallelized genotyping. Here, we developed an accurate and versatile tool based on whole-genome sequence derived SNP loci for the monitoring of Alpine ibex population health. The set of nearly a thousand loci enables the concurrent assessment of population structure, the detection of recent hybridization events and immune function related genotypes. We show that our new amplicon array performs well even with low-input samples. The amplicon sequencing also performs at least equally well as other widely used population genomic approaches while providing key implementation advantages.

Effective high-throughput genotyping relies on the consistent amplification of a large number of loci and robustness to variation in input DNA quality. We show that the targeted sequencing approach produces largely uniform coverage both across a thousand loci and nearly 200 samples of highly variable DNA quantity and quality. Integrating whole-genome sequencing derived SNPs from different species into the design of amplicons allowed us to amplify across Alpine ibex, Iberian ibex and the domestic goat. This extends the usability of the assay with the caveat that the potential ascertainment bias in other species should be considered because SNPs were selected based on polymorphism in Alpine ibex only. Furthermore, the identified genotypes were well-validated by cross-referencing with the whole-genome sequencing datasets. Uniform, high-accuracy genotyping with low input DNA has previously only been achieved through microsatellite marker analyses (Hodel et al., 2016) or SNP chips designed on related model species (Cronin et al., 2015; Pertoldi et al., 2009). Compared to amplicon sequencing, SNP chips often lack extensibility and provide no sequence information surrounding the genotyped loci. An application of SNP chip genotyping in Alpine ibex by (Grossen et al., 2014) was based on a 52 K Illumina Goat SNP Chip (Tosser-Klopp et al., 2015). Among 95 Alpine ibex individuals, the study recovered 677 polymorphic markers out of a total of ~52'000 markers known to be polymorphic among domestic goats. This shows how even SNP chips designed for closely related species can be largely unsuitable and may suffer from substantial ascertainment bias. High-throughput

methods such as RAD-seq and GBS sequencing are the most widely used next-generation sequencing approaches for non-model species often producing at least tens of thousands of SNPs. However, such restriction-based reduced representation approaches do not allow the targeting of specific loci (e.g. immune-relevant loci) and often produce highly uneven read depth across loci (Jiang et al., 2016). Furthermore, we show that replicating RAD-seq and low-coverage whole genome sequencing on the same set of populations analyzed in our targeted amplicon sequencing approach produces a highly similar resolution of the genetic structure. The comparatively low number of SNPs assayed in the targeted amplicon sequencing is most likely compensated by the highly consistent genotyping rates across loci and individuals.

We show that the microfluidics approach for amplicon sequencing produces consistent amplification across nearly all loci if minimum DNA requirements are fulfilled. We have set a minimum threshold of 50 ng/µl (or a total of 100 ng), which is usually achievable for well preserved tissue samples. We have also investigated the potential to recover genotypes from degraded and low-concentration samples such as fecal material. Fecal sampling is a widely used non-invasive sampling method and sometimes the only option for elusive species (Beja-Pereira et al., 2009). Fecal samples produce low quality (degradation) and low quantity DNA. In addition, fecal DNA samples can be heavily contaminated with bacteria, plant or prey DNA. Hence, the actual endogenous DNA is often much below the measured total DNA concentration. We analyzed fecal DNA quantities from 4-23 ng/µl but found that the proportion of successfully amplified loci was comparable to diluted non-fecal DNA at around 0.1 ng/µl. Consequently, non-targeted sequencing methods (*e.g.* RAD or whole-genome sequencing) would require very significant sequencing depth to adequately genotype endogenous DNA of such samples, because a large proportion of reads would be lost to non-endogenous DNA. We show that if analyses of fecal samples are combined with good quality samples, genotypes recovered from fecal DNA can be reliably assigned to individual populations. Furthermore, multiple amplifications of the same fecal samples could result in even better overall genotyping rates. The broad range of acceptable input DNA makes the targeted amplification of selected polymorphisms widely applicable across study systems

497 provided that genomic resources from a small number of individuals are available for the assay
498 development.
499
500 To assess the resolution of the genome-wide set of amplicon markers, we have genotyped a collection
501 of Alpine ibex samples representing all major reintroduction events. Alpine ibex were limited to the
502 Gran Paradiso National Park in Italy in the 19$^{th}$ century. Starting in the early 20$^{th}$ century, populations
503 were introduced independently from Gran Paradiso to Switzerland, France and Italy and from there
504 further populations were founded. The population structure of extant Alpine ibex populations are
505 dominated by signals of population reintroductions and translocations (Biebach & Keller, 2009;
506 Grossen et al., 2018). The Alpine ibex genotypes assessed by our targeted sequencing approach
507 confirmed all major aspects of the reintroduction history including placing Gran Paradiso at the center
508 of the extant genetic diversity. Populations reintroduced directly from Gran Paradiso were at the closest
509 genetic distances to the source population yet showed distinct clustering as expected from the strong
510 bottlenecks imposed by the translocation of few individuals. A major concern for wild species co-
511 occurring with closely related domestic animals is the potential for hybridization and introgression.
512 Alpine ibex populations across Europe are monitored for the presence of individuals with atypical
513 phenotypes (e.g. Giacometti et al., 2004; Steyer et al., 2016; Todesco et al., 2016). We assessed
514 genotypes of seven suspected hybrids (including one potential albino) and could show that only three
515 individuals are clearly identifiable as recent hybrids. One individual showed possible signs of past
516 introgression and three suspected hybrids clustered either with domestic goat or Alpine ibex genotypes
517 highlighting the importance of genetic monitoring of suspected hybridization events.
518
519 A major genetic factor for the long-term survival of endangered species is diversity at important
520 immune loci, in particular the MHC. We successfully amplified hundreds of loci involved in key
521 immune functions focusing on non-synonymous polymorphisms. In parallel, we amplified a dense array
522 of loci spanning the MHC. This 2-Mb locus on chromosome 23 recently received genetic material from
523 domestic goats regenerating heterozygosity at the DRB locus (Grossen et al., 2014). Genotypes across

524 the MHC clustered tightly among populations revealing that Alpine ibex were genetically impoverished
525 across the entire MHC. Comparative analyses with domestic goat breeds will enable a refinement of
526 our understanding of historic and potentially ongoing introgression into the Alpine ibex gene pool. Our
527 findings of low diversity at the MHC are also in accordance with previous studies based on
528 microsatellite and RAD genotyping (*e.g.* (e.g. Alasaad et al., 2012; Brambilla et al., 2018; Grossen et
529 al., 2014). Underlining the relevance of performing large-scale targeted amplicon sequencing of
530 immune loci are recent findings that high heterozygosity at the MHC was correlated with higher
531 resistance to infectious keratoconjunctivitis, a major disease factor in some Alpine ibex populations
532 (Brambilla et al., 2018). Surveillance of disease susceptibility through genetic analyses of immune loci
533 will also significantly improve conservation strategies by informing the choice of founder individuals
534 prior to reintroductions.
535
536 In conclusion, our highly parallel targeted amplicon assay demonstrates how next-generation
537 sequencing techniques can be adapted for the needs of population genetic surveys and conservation
538 monitoring. The WGS-informed approach enabled selecting a highly specific set of loci to
539 simultaneously address questions of population structure, recent hybridization events and how
540 polymorphism is shaped across major components of the immune system. Efficient and precise
541 characterization of individual genotypes can be translated into recommendations on how to prioritize
542 translocation events and replenish genetic diversity at immune loci. The versatility to amplify the same
543 loci across related species enables also powerful screens for recent introgression events. Our study
544 shows the relevance of bridging population genomic investigations with assays that can be realistically
545 implemented into population genetic surveys and decision-making for conservation management.
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NextGen consortium; Next generation methods to preserve farm animal biodiversity NEXTGEN; 2014; NCBI SRA; ERR470105, ERR470101, ERR313212, ERR313211, ERR313204, ERR297229, ERR313206, ERR405774, ERR405778, ERR315778, ERR318768, ERR246140, ERR340429, ERR246152, ERR345976, ERR340334, ERR340340, ERR340333, ERR340331, ERR340335, ERR340348, ERR157945, ERR299288, ERR157938, ERR454948, SRR501898

778 Data accessibility: The raw amplicon sequencing data produced for this project was deposited at the
779 NSBI Short Read Archive under the Bioproject Accession number PRJNA669599. The raw whole-
780 genome sequencing data produced for this project was deposited at the NCBI Short Read Archive under
781 the Accession nos. SAMN10736122–SAMN10736160 (BioProject PRJNA514886
782 [https://www.ncbi.nlm.nih.gov/sra/PRJNA514886]). The whole-genome data produced by the NexGen
783 Consortium (Capra hircus accessions: ERR470105, ERR470101, ERR313212, ERR313211,
784 ERR313204, ERR297229, ERR313206, ERR405774, ERR405778, ERR315778, ERR318768,
785 ERR246140, ERR340429, ERR246152, ERR345976, Capra aegagrus accessions: ERR340334,
786 ERR340340, ERR340333, ERR340331, ERR340335, ERR340348; Ovis aries accessions: ERR157945,
787 ERR299288; Ovis orientalis : ERR157938; Ovis vignei: ERR454948; Ovis canadensis: SRR501898)
788 was downloaded from [ftp://ftp.sra.ebi.ac.uk/vol1/fastq].
789
790 Author contributions: CK, CG and DC conceived the study, CK, DW, GC, IB and CG performed
791 analyses, AB contributed samples, DL contributed datasets, CK, CG and DC wrote the manuscript
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# Figures

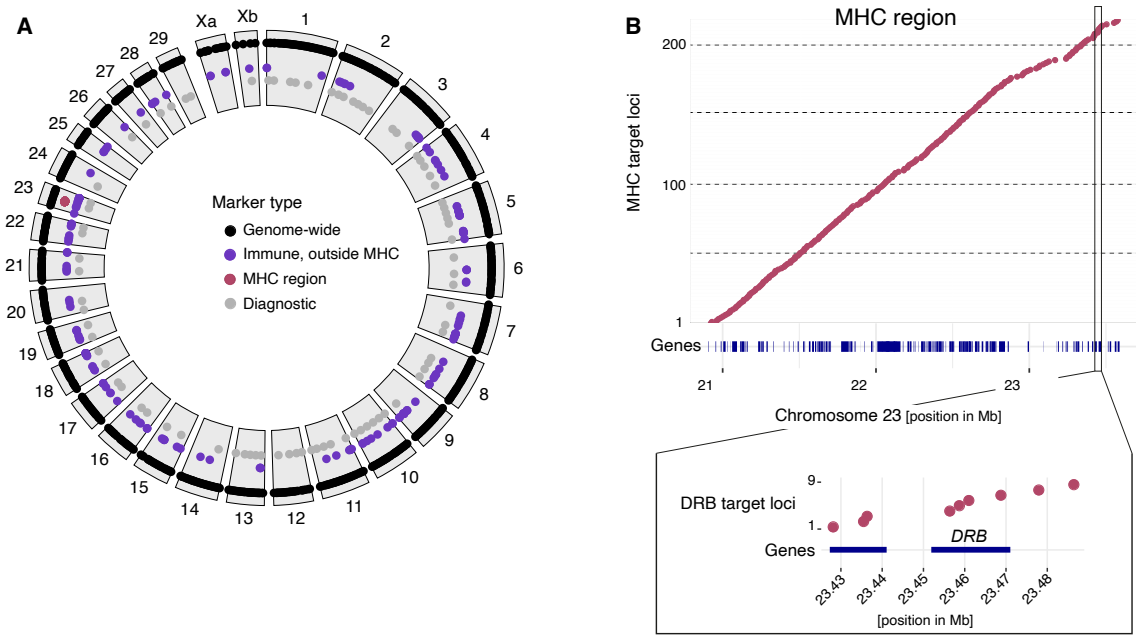



**Figure 1: Design and distribution of targeted amplicon sequencing loci**. A) Distribution along chromosomes for each marker category (genome-wide, immune loci, MHC region, diagnostic); B) Regularly spaced loci across the MHC region on chromosome 23 (20.89-23.59 Mb). Blue segments identify coding sequences in the MHC. The *DRB* gene region of the MHC (zoom view) was targeted by a denser array of loci.

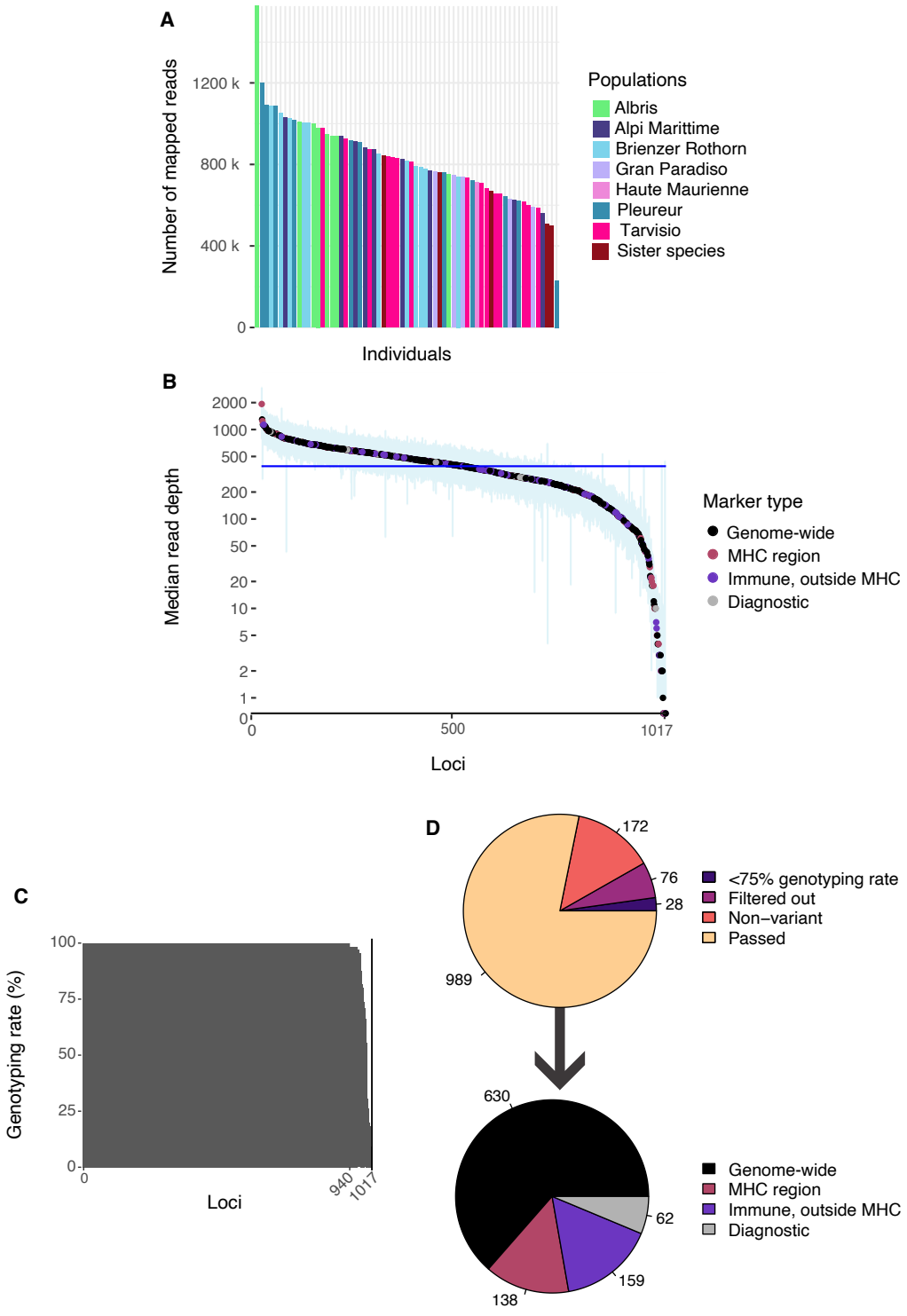



**Figure 2: Assessment of amplification consistency and genotyping rates.** A) Number of mapped reads per individual sample, colored by population *(N=65)*. Seven Alpine ibex populations are shown in addition to domestic goats (*N=2*) and Iberian ibex (*N=3*). Low-quality input DNA samples (fecal samples, etc.) were excluded. B) Median read depth per locus across 1017 amplicon loci. Markers are colored according to their category. The blue line represents the median (389) and the blue area represents the 95% confidence interval for each marker. C) Variation in genotyping rates across the 1017 loci. D) Outcomes of the different filtering stages. A total of 989 loci were retained for further analyses. The marker category of the retained markers is shown below.

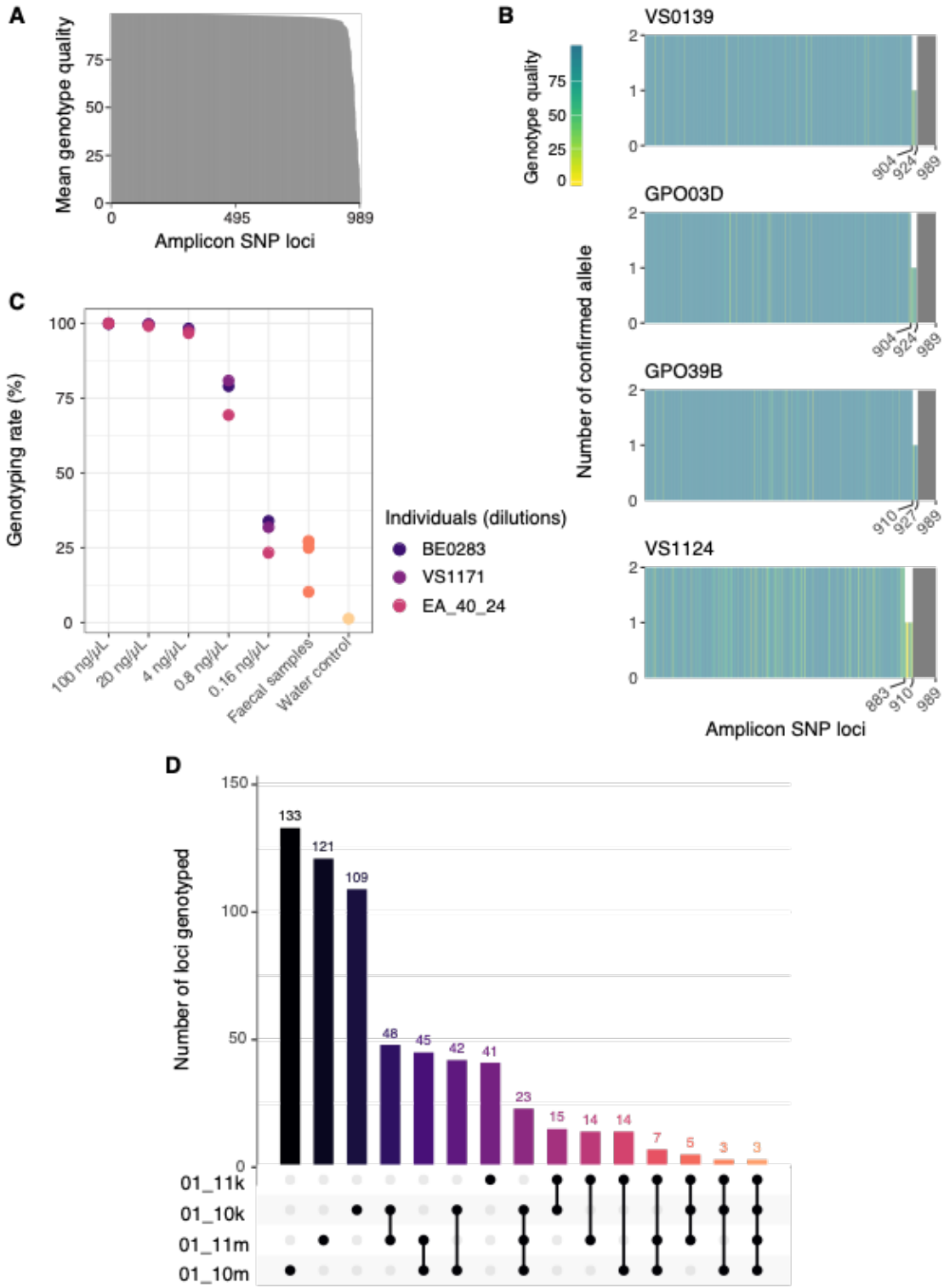



**Figure 3: Genotyping accuracy and performance with low-quality or low-quantity input DNA.** A) Mean genotyping quality (GQ) for each high-quality locus across the 65 high-quality DNA samples. B) Genotype accuracy assessed by matching recovered alleles from the targeted amplicon sequencing and whole-genome sequencing of the same four individuals. C) Assessment of genotyping rates for serial dilutions of three individuals and four fecal samples. D) Analysis of genotyped loci shared among the four fecal samples. The overlaps show recovered genotypes out of a total of 989 loci.

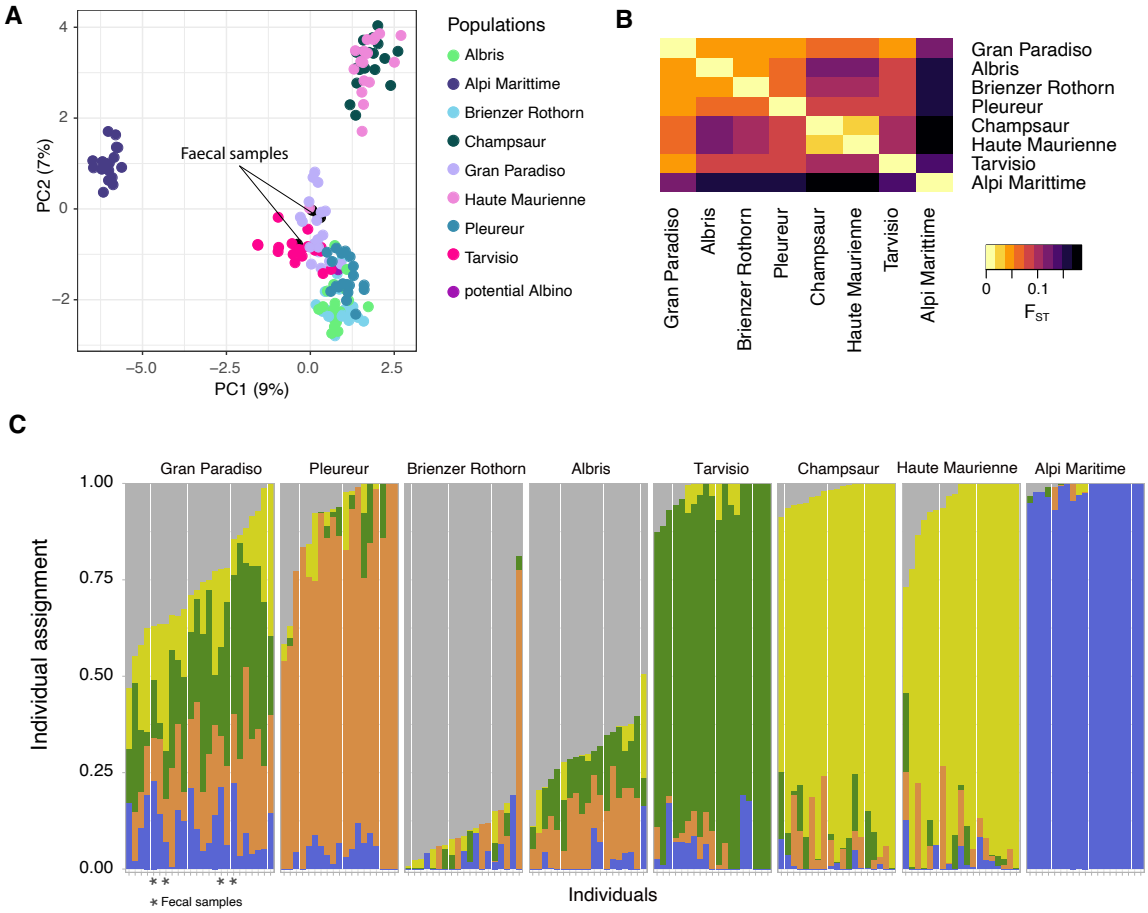



**Figure 4: Population genetic analyses of Alpine ibex.** A) Principal component analysis of 158 Alpine ibex individuals based on 617 genome-wide SNP loci designed for capturing genome-wide population differentiation. B) Pairwise $F_{ST}$ matrix of all Alpine ibex populations based on genome-wide SNPs. Gran Paradiso was the only population surviving the near extinction and hence is the source population of all existing Alpine ibex populations. The Swiss populations Albris, Pleureur, Brienzer Rothorn and the Italian population Alpi Marittime were founded in early 1900 from Gran Paradiso individuals. The populations Tarvisio, Champsaur and Haute Maurienne were later founded independently from Gran Paradiso individuals. C) Structure-like analysis (based on sparse non-negative matrix factorization algorithms) of all Alpine ibex with K=5. *) fecal samples.

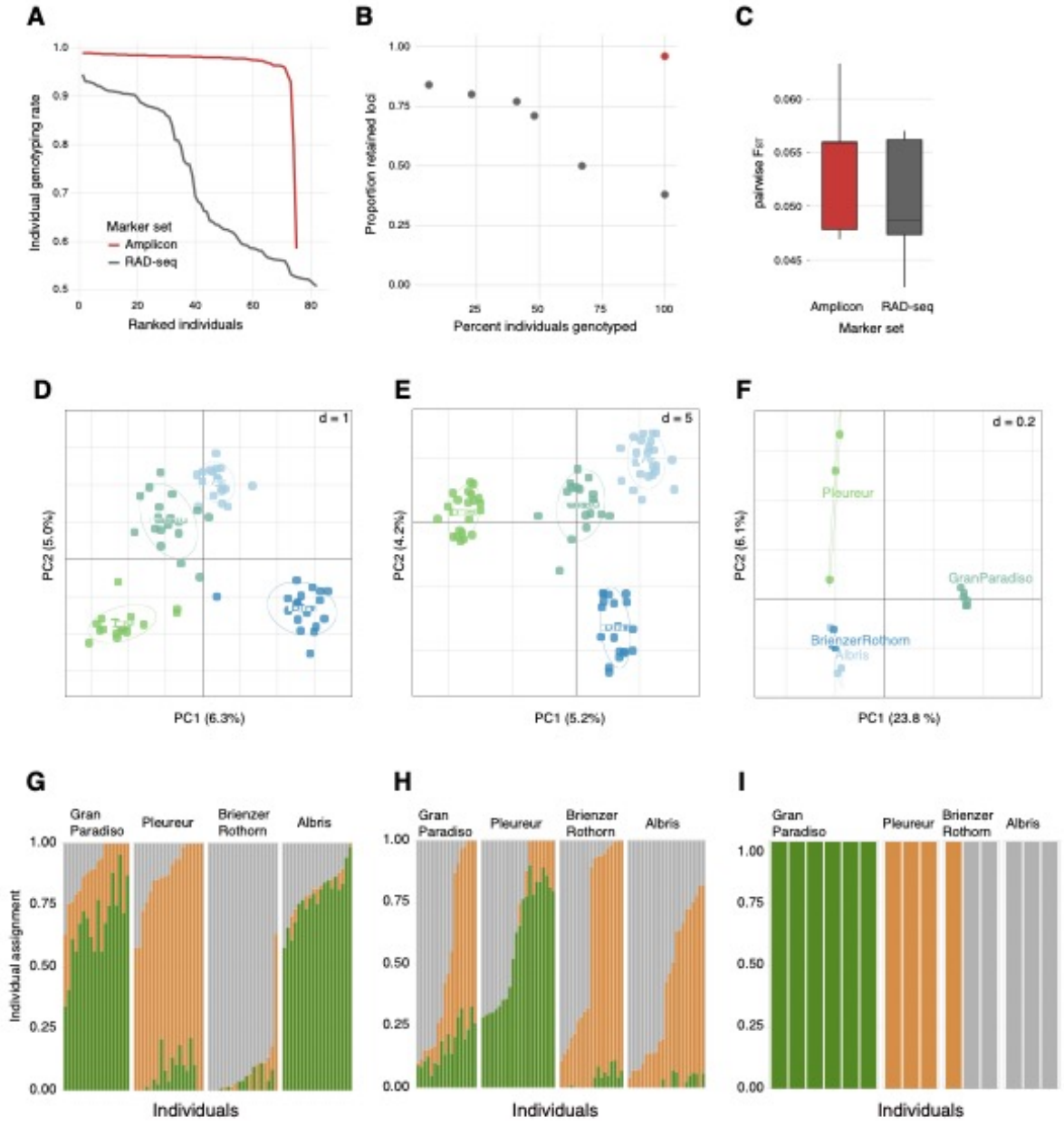



**Figure 5: Performance comparisons between targeted amplicon sequencing, RAD-seq and low-coverage whole genome sequencing on four Alpine ibex populations.** A) Individual genotyping rate ranked by individual for amplicon sequencing and RAD-seq. B) Proportion of SNP loci retained at a genotyping rate of 90% as a function of the percent of individuals included. The comparison is only meaningful for amplicon sequencing and RAD-seq. C) Boxplot of all pairwise $F_{ST}$ estimates among populations for the amplicon sequencing and RAD-seq. D-F) Principal component analysis of population differentiation for (D) the amplicon sequencing (plotted are -PC1 against -PC2), (E) RAD-seq and (F) low-coverage whole-genome sequencing. Structure-like analysis (based on sparse non-negative matrix factorization algorithms) of (G) the amplicon sequencing, (H) RAD-seq and (I) low-coverage whole-genome sequencing datasets. I) is based on a NGSadmix analysis (Skotte et al., 2013).

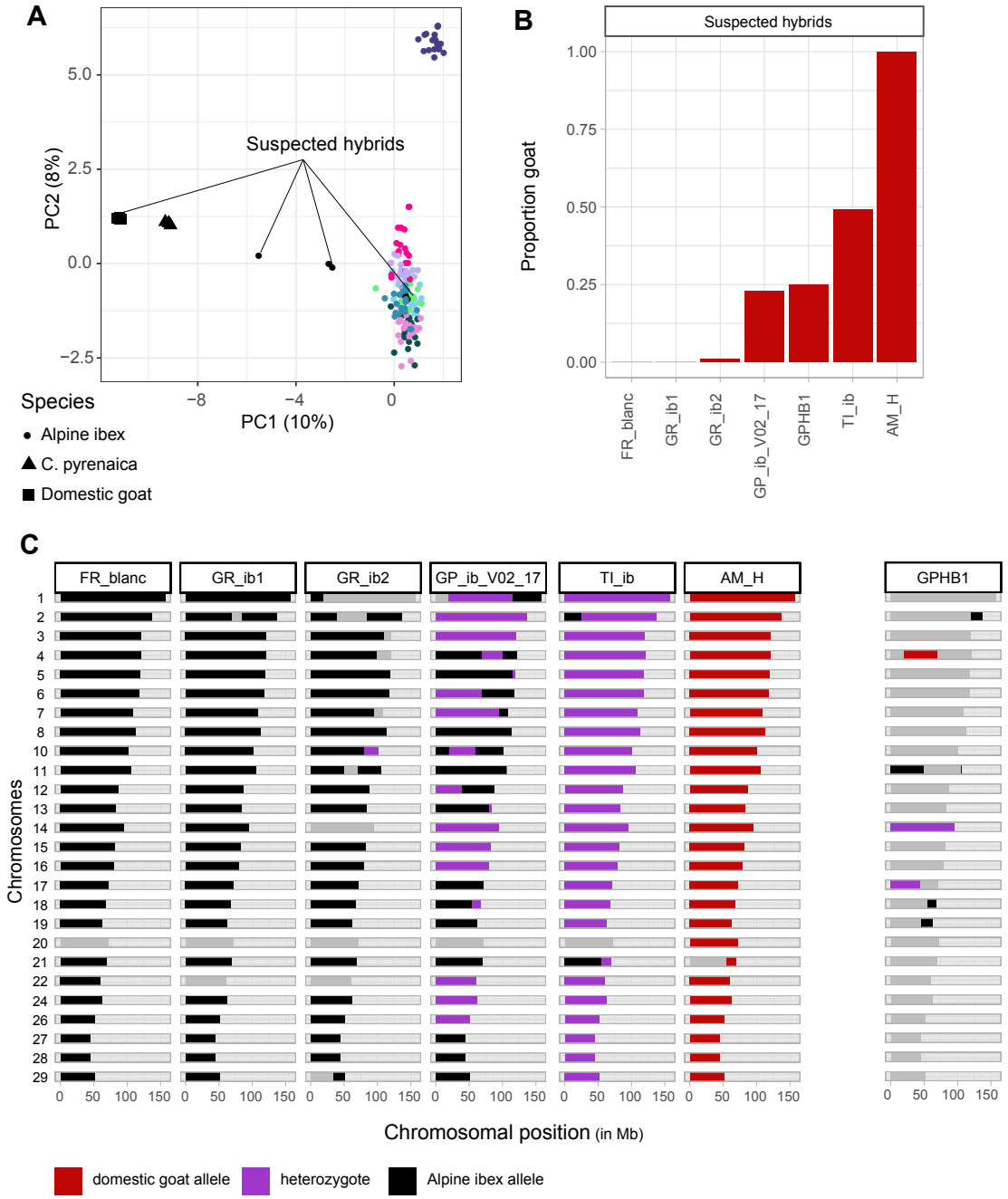



**Figure 6: Performance of targeted amplicon sequencing to detect recent hybridization events.** A) Principal component analysis of 168 individuals (excluding fecal samples) including domestic goat and Iberian ibex (N=5 and 3) and suspected hybrids based on phenotypic observations (N=6). The PCA was performed based on 617 genome-wide SNP loci designed for capturing putatively neutral population differentiation. B) Analysis of diagnostic markers for the detection of hybrids for 6 suspected hybrids and one potential albino. The proportion of diagnostic markers assigned to domestic goat are shown, where the proportion is calculated as (1*homozyogous goat genotype + ½*heterozygous genotype)/(total genotypes at diagnostic markers). C) Chromosome painting using diagnostic markers along the 29 autosomes. Colors identify Alpine ibex (blue), heterozygous (purple) and domestic goat (red) genotypes. Sample GPHB1 was of low DNA quality and quantity (drop of blood conserved on FTA filter paper; see Methods).

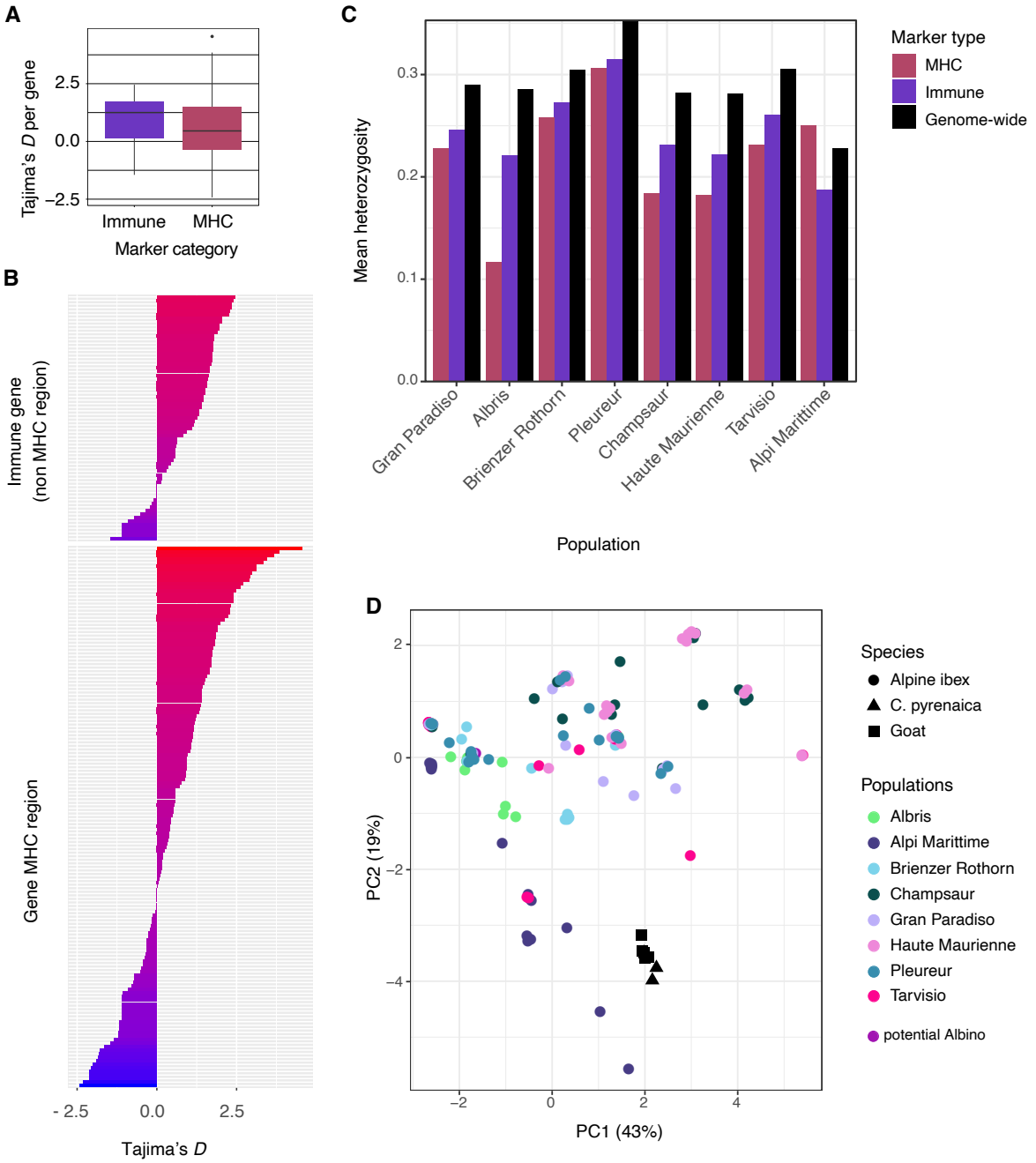



**Figure 7: Genome-wide immunogenetics survey of Alpine ibex populations.** A) Boxplot of Tajima's *D* estimates based on whole-genome sequencing of 29 Alpine ibex of immune-related loci covered by the targeted amplicon sequencing assay. The MHC region is shown separately from other immune-related loci. B) Tajima's *D* estimates shown per each gene represented by immune-related loci covered by the targeted amplicon sequencing assay. C) Targeted amplicon sequencing analyses of heterozygosity across Alpine ibex populations (*N=154*, excluding fecal samples). Heterozygosity is shown separately for genome-wide SNPs, the MHC region and other immune-related SNP loci. D) Principal component analysis of Alpine ibex and sister species based on 138 SNPs genotyped in the MHC region using targeted amplicon sequencing.

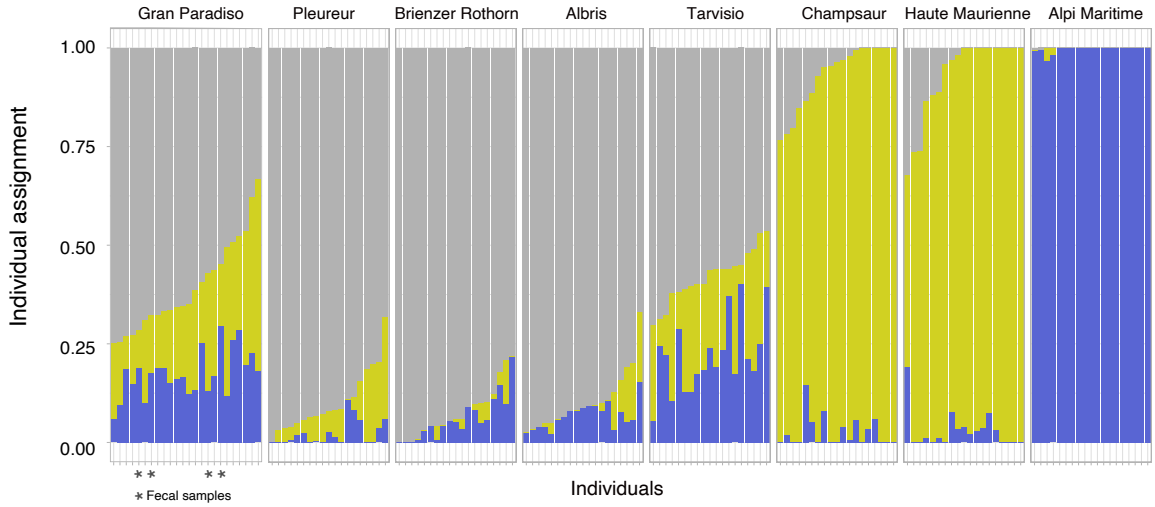
# Supplementary Information

(SupplementaryTables.xlsx)

**Supplementary Table S1: Overview of individual sampling.** Overview of all sequenced individuals analysed in this study including information of species, population, inclusion in high-quality samples and sample type.

**Supplementary Table S2: Overview of SNP positions included in the marker design.** Shown are marker type (Genome-wide, Diagnostic, MHC region, Immune), Batch (1st or 2nd submission to Fluidigm for marker design), Exclusion (N: Kept in final marker set, Y: Yes, excluded due to quality checks), Excluded_by (N_percentage: contained >50% masked positions in the sequence surrounding the focal SNP; Fluidigm: marker did not pass Fluidigm design principles; VariantFiltration: marker failed GATK hard filtering; Non_variant: marker was invariant; <75%: genotyping rate was below 75% among individuals), functional annotation is based on SnpEff.

**Supplementary Table S3: Individual read numbers**. Given are read numbers per each individual before and after quality trimming, merging and genome alignment.

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881 **Supplementary Figure 1: Population genetic analyses of Alpine ibex.** Structure-like visualization
882 (based on sparse non-negative matrix factorization algorithms) of all Alpine ibex with *K*=3.

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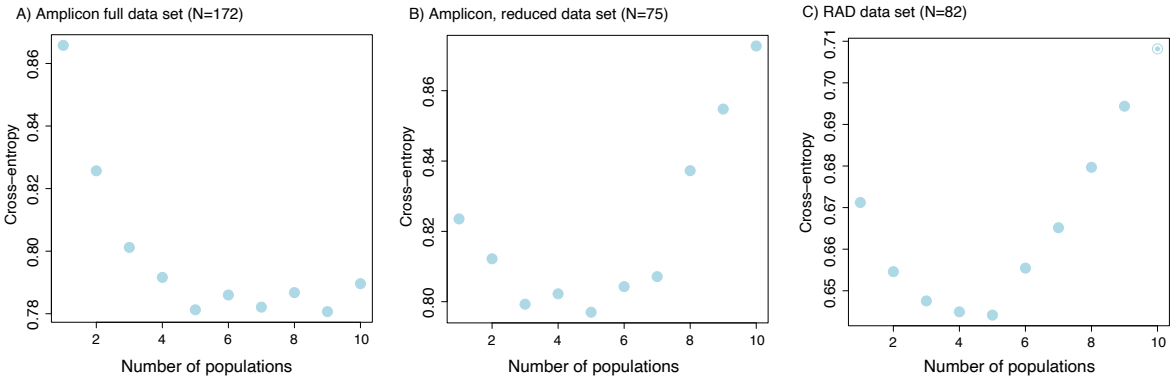


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889 **Supplementary Figure 2: Structure-like analysis of Alpine ibex.** Shown is the distribution of entropy
890 for K varied from 1 to 10 (100 replicates each) for the Structure-like analysis (sparse non-negative
891 matrix factorization algorithms) based on (A) the full Alpine ibex amplicon data set, (B) the reduced
892 amplicon data (for methods comparison) and (C) the RAD data.