**Supplemental Materials:**

**File S1.** Hop accessions used for association mapping including names, subspecies and origin were available.

**File S2.** Hop accessions used for marker validation.

**File S3.** Variant calling pipeline from unprocessed raw reads to variant call format file.

**File S4.** Filtered and imputed variant call format file used for to generate genotyping file for association mapping.

**File S5.** R script for association mapping and figure construction.

**File S6.** Genotype file for 765 accessions and 20,861 markers including marker name and allele calls for accessions as homozygous reference (0), heterozygous (1) and homozygous alternative (2).

**File S7.** Genetic map file containing 20,861 markers with chromosome position in relation to the cultivar Cascade reference assembly (Padgitt-Cobb et al., 2023).

**File S8.** Phenotype file for 765 hop accessions with male (0) and female (1) phenotypes.

**Figure S1.** Jitter plot of male and female distribution across reconstructed haplotypes of first and second significant markers. Haplotypes are constructed by assigning numeric values to unique phased genotypes for each marker (1-4) and concatenated (|) with the second phased marker (1-4). Females are shown in red on the top facet, whereas males are shown in blue on the bottom facet.

**Figure S2.** Scatter plot of fluorescence from the FAM fluorophore on the *x*-axis and HEX fluorophore on the *y*-axis for the primer boost optimization with FAM fluorophore boost and HEX fluorophore boost on the left and right facet, respectively.

**Figure S3.** Phylogenetic tree of all 765 hop accessions utilized within the association using 20,861 single nucleotide polymorphisms. The neinan genetic distance was calculated using the *aboot* command within *poppr* 2.9.4 (Kamvar *et al.* 2014) and plotted using the *ggtree* 3.9.0 (Yu 2020)extension of *ggplot2* 3.4.2 (Wickham 2016). Samples with correctly or incorrectly identified plant sex utilizing the SM1 diagnostic marker are highlighted with green and red nodes, respectively.