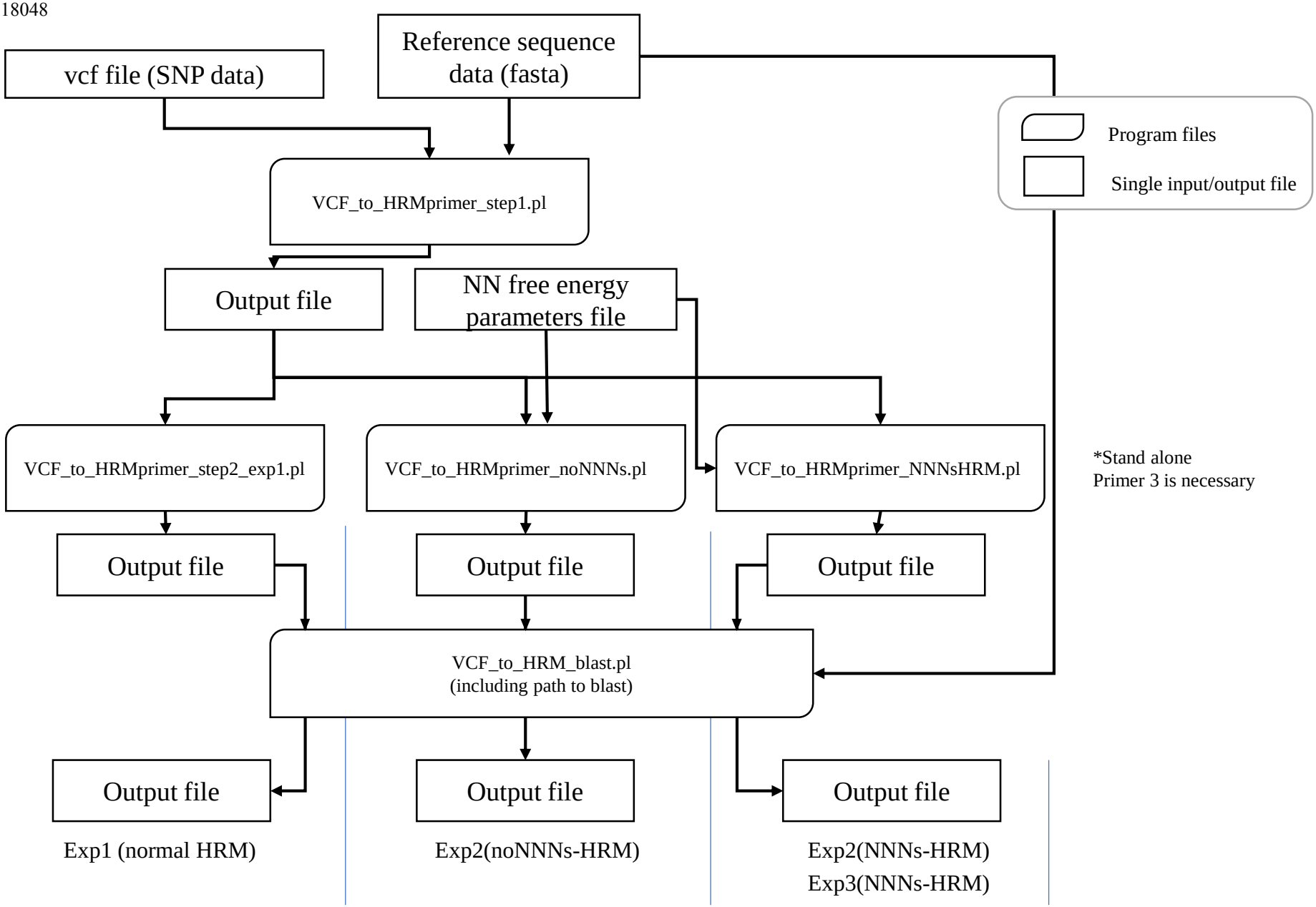
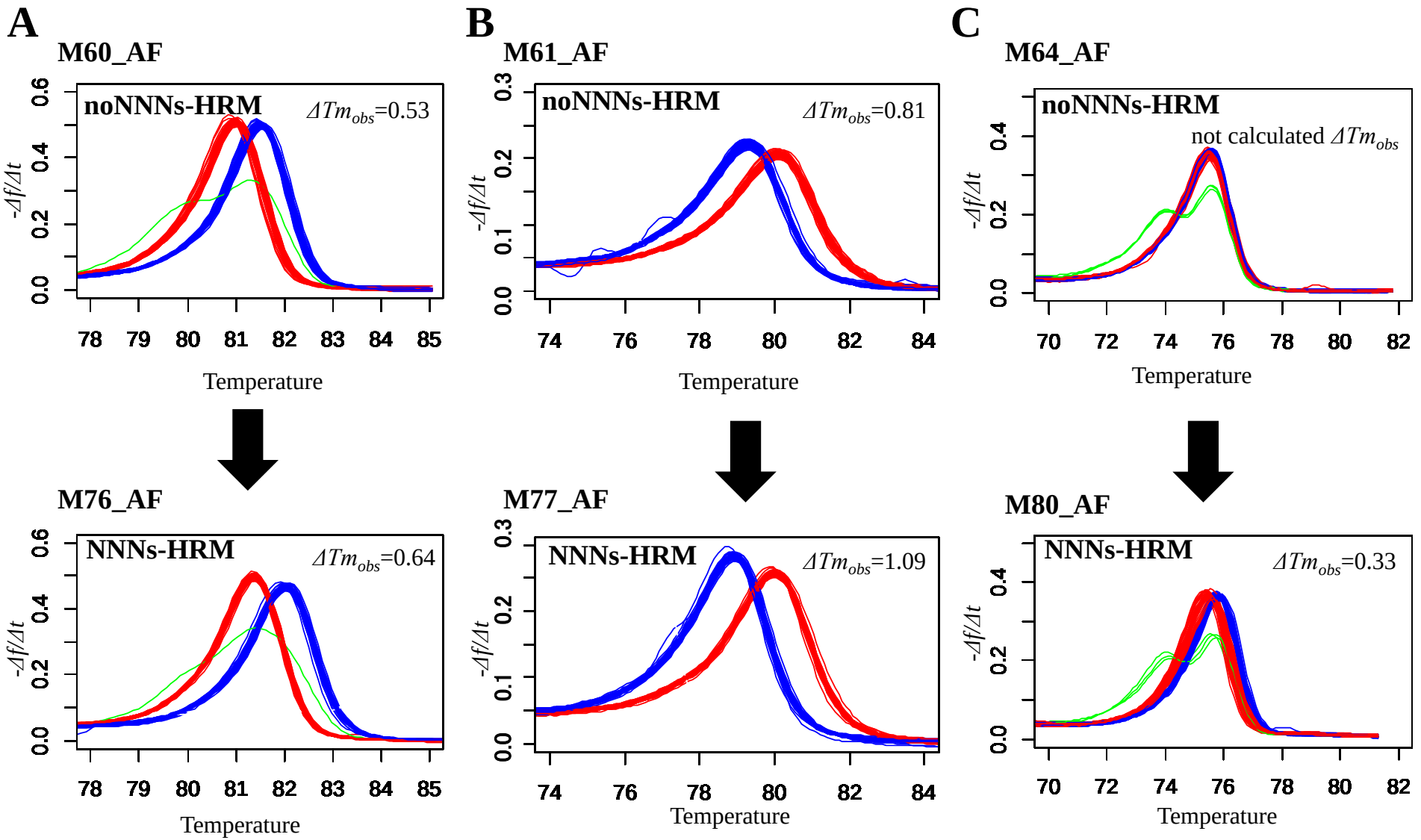
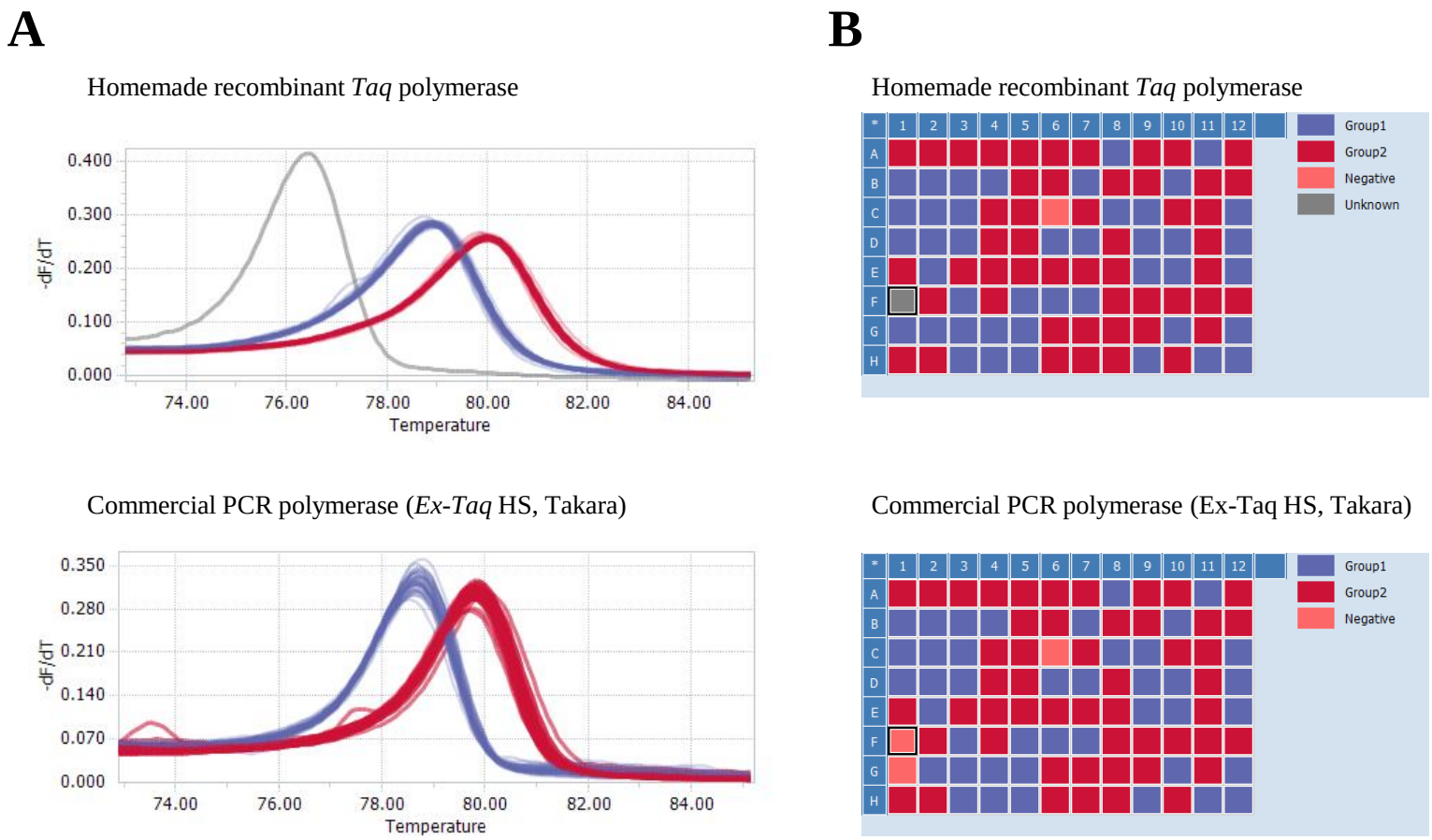


Supplemental Fig. 1. Processing and analysis of SNP data and the pipeline to design primers for HRM markers. Arrows indicate the file input. Programs include previously published software and custom Perl scripts developed in this study. The file describing NN free energy parameters and all Perl scripts shown in this figure are provided as supplemental text.



Supplemental Fig. 2. Actual peak shifts obtained from negative derivative of fluorescence over temperature *(-df/dt)* for two combinations of non-NNNs-HRM and NNNs-HRM markers. Actual differences in Tm values (Δtm_{obs}) are shown. Three PCR fragments, homozygous for 'Aokimame' allele, heterozygous for 'Aokimame' and 'Fukuyutaka' alleles, and homozygous for 'Fukuyutaka' allele, are indicated by red, green, and blue lines, respectively. (A) Graphs show the changes of M60_AF (non-NNNs-HRM) and M76_AF (NNNs-HRM), (B) M61_AF (no NNNs-HRM), and M71_AF (NNNs-HRM), (C) M64_AF (no NNNs-HRM), and M80_AF (NNNs-HRM). The genotypes of M64_AF were predicted by that of M80_AF.



Supplemental Fig. 3. Evaluation of reproducibility of NNNs-HRM marker with different type of PCR polymerases. (A) Two –df/dT plots of AF_M77 with homemade recombinant *Taq* polymerase (top) and general commercial *Taq* polymerase (bottom) were displayed. (B) Heat maps displaying genotypes of each well of PCR plate were also shown. The genotypes of "Group 1" and "Group2" indicated homozygous allele for Fukuyutaka and Aokimame, respectively. Negative and Unknown wells indicated PCR frailer and non specific amplification samples.