***Boechera* microsatellite scoring guide** – as discussed in Windham et al. (2016) and utilized in Beck et al. (2012); Alexander et al. (2015); and Windham et al. (2015) (cited therein).

– TERMINOLOGY –

main peak = a peak judged to represent an actual allele, i.e. not stutter

stutter peak = an erroneous peak, often lagging (see below) the main peak, resulting from PCR error; a stutter peak that is two bp shorter than the main peak would be noted as "-2bp stutter"

leading peak = the peak corresponding to the longer (in bp) fragment; a peak of 120 bp would be the leading peak relative to one of 118 bp
lagging peak = the peak corresponding to the shorter (in bp) fragment

larger peak = the peak exhibiting the higher relative fluorescence unit (RFU) value
smaller peak = the peak exhibiting the lower relative fluorescence unit value

small/large/medium = a pattern that emerges when two adjacent alleles (main peaks) both exhibit stutter, with the lagging peak's RFU value benefiting from the leading peak's stutter; therefore one observes (left to right, or lagging to leading) a relatively small peak (the lagging main peak's stutter); a relatively large peak (the lagging main peak + the leading main peak's stutter); and a medium peak (the leading main peak)

sawblade = a complex stutter pattern that appears as many (4+) adjacent peaks of varying intensity

– LOCI –

ICE3 (6-FAM labeled; CT repeat)

– Strong main peaks, but with consistent -2bp stutter that is much smaller (ca. 30% or less intensity) relative to the main peak. Heterozygotes of adjacent alleles appear as the small/large/medium pattern.
– Stutter becomes more pronounced at the large end of the marker (130-160 bp) and can be difficult to interpret.

ICE14 (HEX labeled; GAT repeat)

– Fairly consistent -1bp stutter of variable strength. In some genotyping runs the -1bp stutter peak is can be larger (higher RFU) than the leading, main peak. Be consistent regarding calling leading vs. lagging peaks.
– Note an allele number phase switch between alleles 223 and 227.
– Be aware that if used in a set with locus c8, the c8 alleles can cause misleading "pull-up" peaks at 225 bp+.

a1 (6-FAM labeled; GAT repeat)

– Consistent single main peaks, with only a slight -1bp stutter. Heterozygotes (relatively rare) appear as two peaks of approximately equal height in diploids (unequal in triploids).

a3 (6-FAM labeled; AG, AT repeat)
– Strong main peaks, generally very interpretable.
– Likely amplifying multiple loci.

b6 (6-FAM labeled; CT, GT repeat)
– Variable, sometimes with single main peak, but often with -1bp or (less commonly) with -2bp stutter peaks.
– The small/large/medium pattern is relatively common.

c8 (6-FAM labeled; CTT repeat)
– Strong main peaks with variable (and weak) -2bp, -4bp stutter.
– Potentially small main peaks and relatively large -1bp stutter peaks were observed around alleles 242 and 231. In general the small leading peak is viewed as erroneous, and the largest peak was therefore scored unless the leading peak was 50%+ the size of the lagging peak. In that case they were interpreted as heterozygotes (two alleles differing in size by 1 bp).

e9 (6-FAM labeled; CT repeat)
– Strong, clean main peak with very slight -1bp stutter.
– Numerous -1bp heterozygotes appear as the small/large/medium pattern.

BF3 (6-FAM labeled; GA repeat)
– Strong, clean main peaks usually with -2bp and -4bp stutter; but at times can be just the main peak.
– A very weak +2bp peak was seen at times and ignored.
– 2bp heterozygotes appear as the small/large/medium pattern, but if no stutter is present they can appear as 2 peaks of similar size only.
– Be aware that if used in a set with locus BF19, the BF19 alleles can potentially cause misleading "pull-up" peaks at the higher size end of this locus.

BF9 (6-FAM labeled; GA repeat)
– Strong main peaks with often strong -1bp, -2bp, -3bp (occasionally -2bp, -4bp) stutter. Note that in some plates the -1bp stutter peak can be larger than the leading, main peak)
– Numerous "off phase" (not in the expected 2bp interval) peaks were observed.
– Apparently little to no misleading "pull-up" peaks from locus BF18, which was used in the same locus set and which overlaps in size.
– Given the low fragment sizes of this locus some genotyping runs consistently ran "slower," i.e their peaks were consistently ca. 0.3 bp shorter.

BF11 (6-FAM labeled; GA repeat)
– Consistent -1bp stutter which at times can be larger than the main peak.
– A weak, wide, erroneous peak at 95-96 bp was consistently observed.

BF15 (6-FAM labeled; GA repeat)
– Typically a strong single main peak, but weak -2bp or (very small) +1bp stutter peaks

were observed at times.
– Sawblades were observed at times in the mid- to large size range of this locus. Missing data was scored if no clear strongest peak was discernable.
– Alleles at the higher fragment-size range of this locus can appear as very small but nevertheless scorable peaks, particularly in heterozygotes or triploids that exhibit three different alleles.
– Apparently little to no misleading "pull-up" peaks from locus Bdru266, which was used in the same locus set and which partially overlaps in fragment size.

BF18 (HEX-labeled; CT repeat)
– Strong main peaks with -1bp stutter of variable strength.
– -1bp heterozygotes are present and appear as the small/large/medium pattern. Note that in some plates the -1bp stutter peak can be larger than leading, main peak.

BF19 (HEX-labeled; GA repeat)
– Typically a strong main peak or with -2bp or -4bp stutter peaks. Very small leading peaks were also seen at times (didn't score).
– Likely amplifying multiple loci.
– Be aware that if used in a set with locus b3, the b3 alleles can potentially cause misleading "pull-up" peaks at the lower fragment size range (130 bp and less) of this locus.

BF20 (HEX-labeled; CT repeat)
– Consistent -2bp stutter that is less than 30% the strength of the main peak. Also a very weak -4bp stutter at times. Heterozygotes of adjacent alleles appear as two strong peaks.
– Be aware that if used in a set with locus a1, the a1 alleles can potentially cause misleading "pull-up" peaks at the higher fragment size range (230 bp+) of this locus.

Bdru266 (HEX-labeled; AT repeat)
– Generally strong main peaks with a variety of leading and lagging peaks (1bp, 2bp, etc.); generally scored largest (highest RFU) peak.
– In the case of 2 strong main peaks that were potentially -1bp heterozygotes, the lagging peak was scored unless the leading peak was larger (in this case both were scored). The frequency of this situation was plate-specific.
– Be aware of relatively small (low RFU) but nevertheless apparently real peaks.
– Be aware that if used in a set with locus BF15, the BF15 alleles can potentially cause misleading "pull-up" peaks at the lower fragment size range (110 bp and less) of this locus.

– NULL ALLELES –

Generally caused by mutational changes in the flanking regions that prevent primers from annealing to template DNA, null alleles occur sporadically at most of the loci analyzed in *Boechera*. They can be common (even diagnostic) at certain loci in particular taxa, which makes attempting to score them a worthwhile endeavor. Although undetectable based on standard analyses of peak diagrams, null alleles can be postulated to exist in homozygous state if there is no other reasonable explanation for the complete failure of a particular

amplification. This process is facilitated by the fact that each locus is multiplexed with two others. A null allele (0) is called if both of the following conditions are met: 1) both of the other multiplexed loci from that individual show strong expression (indicating good DNA quality and appropriate amplification), and 2) adjacent individuals in that run representing other taxa that lack null alleles show strong expression (indicating that the locus in question is amplifying properly).