Rough estimates of the number of loci one can expect to recover from various combinations of restriction digests and size selection can be generated from experimental runs of various enzyme combinations run on an Agilent Bioanalyzer HS-DNA assay. A few replicate good-quality traces for a given double digest should be evaluated in parallel to get a sense for noise in the estimate, but the basic protocol is as follows:

1) perform a collection of double digests in replicates (a good start for the “flex” condition described in the ddRAD protocol would be SphI-EcoRI ; SphI-MluCI ; NlaIII-EcoRI ; NlaIII-MluCI -- note that this last condition will give MANY loci, with low specificity and should only be used for experiments intending to sample a substantial fraction of the source genome). Also perform the requisite single digests for the same source DNA.

2) run these on a Bioanalyzer HS-DNA assay.

3) After confirming high-quality data, use the bioanalyzer software to create a series of regions in the region tool (region table) corresponding to candidate size selection regimes. A good place to start might be 200+/-20; 300+/-30; 400+/-40 and elaborate from there. Record the “% of total” estimate for each region.

4) for the single-digest conditions create a single region encompassing the entire distribution of fragments (50bp-10000bp or more, for example). From this, record mean fragment size.

5) copy the [Locus count estimate from bioanalyzer](https://docs.google.com/spreadsheet/ccc?key=0AnHEwF1NpAxDdGI4RFpIdno0bHV1bFZzOXh6ZS1fclE) spreadsheet to your google docs account and edit the fields in green to contain the values described above. Duplicate column B for additional potential digest conditions.