ELF for PacBio library fragment size selection

Yes, we are currently using the ELF for all PacBio library size selection protocols, including the 20 Kb libraries. This is my feedback based on your questions:

In our experience, the ELF in general works very well for separation and elution of fragments for the range required for 20 Kb libraries. We used the conditions suggested by SageScience for the highest fragment size range (I.e., ~3kb-19 Kb, we specify 19 kb for well #1). We use the 0.75% Agarose (Native) Gel Cassettes v2 (Cat# ELD7510), specified for 1-18 kb fragments. Fortuitously, these cassettes appear to actually separate and resolve library fragments under these conditions in a range higher than specified by SageScience. Of course, these "differences" may in part be due to the fact that we use the Agilent TapeStation for sizing right out of the elution step. A fairly typical result looks like this:

Well 2: ~23-26 kb fragments
Well 3: ~20-23 Kb fragments
Well 4: ~18-19 Kb fragments
Well 5: ~14-16 Kb fragments

The nice thing about it is that the fragments are very tight. Even in the 15-16 kb fraction, there is no visible fragments below 10 kb. A huge advantage as compared to the BluePippin is that we don't throw away any of the fractions. So, we usually try to sequence first the 26 kb library fraction, and if we need more runs we use the smaller size fractions. We've taken a look at fragments in wells 2 and 3 and if there were large fragments in the library prep, they will be there. However, our sizing accuracy in this range is less reliable.

For some reason, we see occasional variation in well location of the fragment sizes. We think that this may be due to potential variation in the gel cassettes manufacture or perhaps due to strange sample behavior. Also, the amount of material you load on the gel cassette seems to be important for reproducibility. Things appear to separate reproducibly in the 500 ng to 3 microgram range.

For IsoSeq we use the same cassettes, except that we specify 1.5 Kb (or 2.5 Kb) fragments to well 10, as recommended by PacBio. Under these conditions the fragment sizes match very closely what the SageScience table says one should expect.

We start by sequencing the library fragment in well 2 (~23-26 kb as indicated by TapeStation runs). If we need more runs from a given prep we sequence the lower size fractions. We normally don't pool fractions, unless the yield is so low that we need it for having sufficient library for the run set up.

The recovery in each fraction depends on the distributions of the fragment sizes in the library prep. From our library construction protocols we typically recover at least 50% of the input library mass if we add the mass of the fragments in wells 2-5. This is much better and reproducible than our experience ever was with the BluePippin.