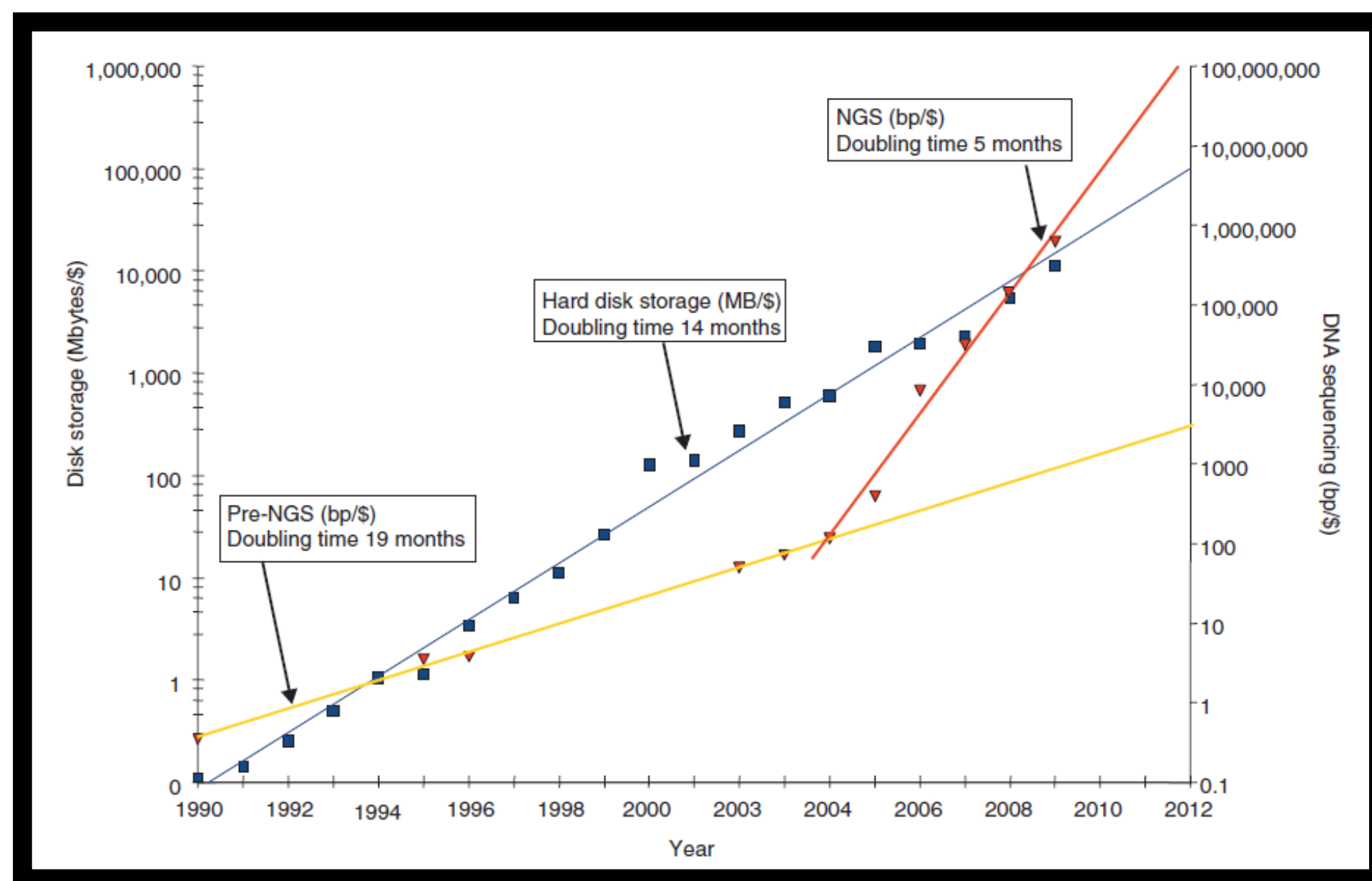


## Introduction

- Existing genotyping methods are too expensive for breeding use
- Data yield per dollar from DNA sequencing is increasing exponentially (Stein, 2010-see figure below).
- DNA sequencing may soon provide a cost-effective genotyping method



## Research Objectives

- Develop cost-effective genotyping for pine
- Genotype samples of parents and progeny from 7 provenances of loblolly pine, growing at 2 different sites
- Test for interactions of genetic variants and climate factors

## Sample Collection

- 1600 tissue samples collected in Georgia
- 1600 more samples will be collected from a northern site.



## Sample Processing

- DNA extraction was adapted from a published protocol (Ivanova et al, 2008).



- Phloem disk halves were rolled flat and placed in 96-well plates for extraction



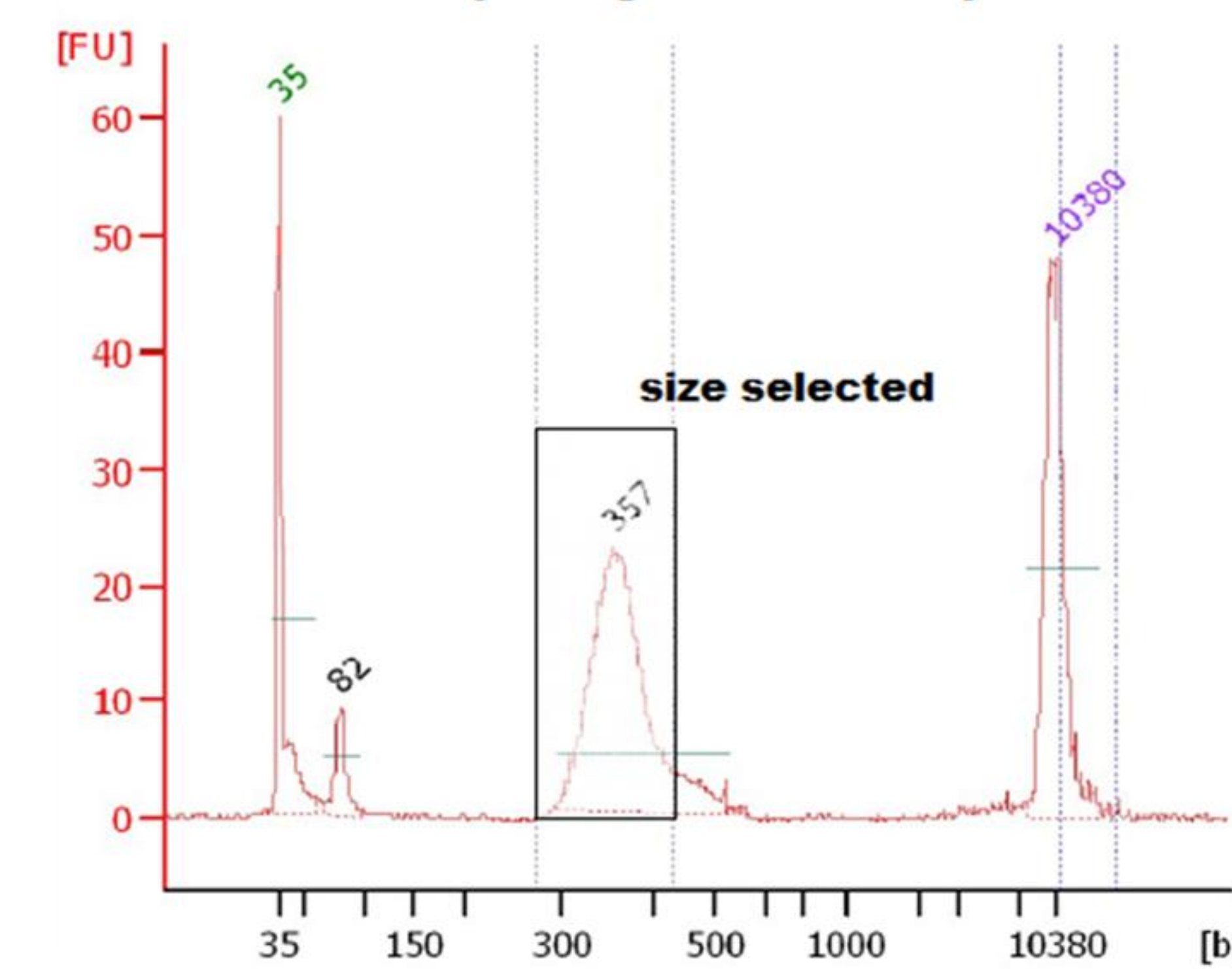
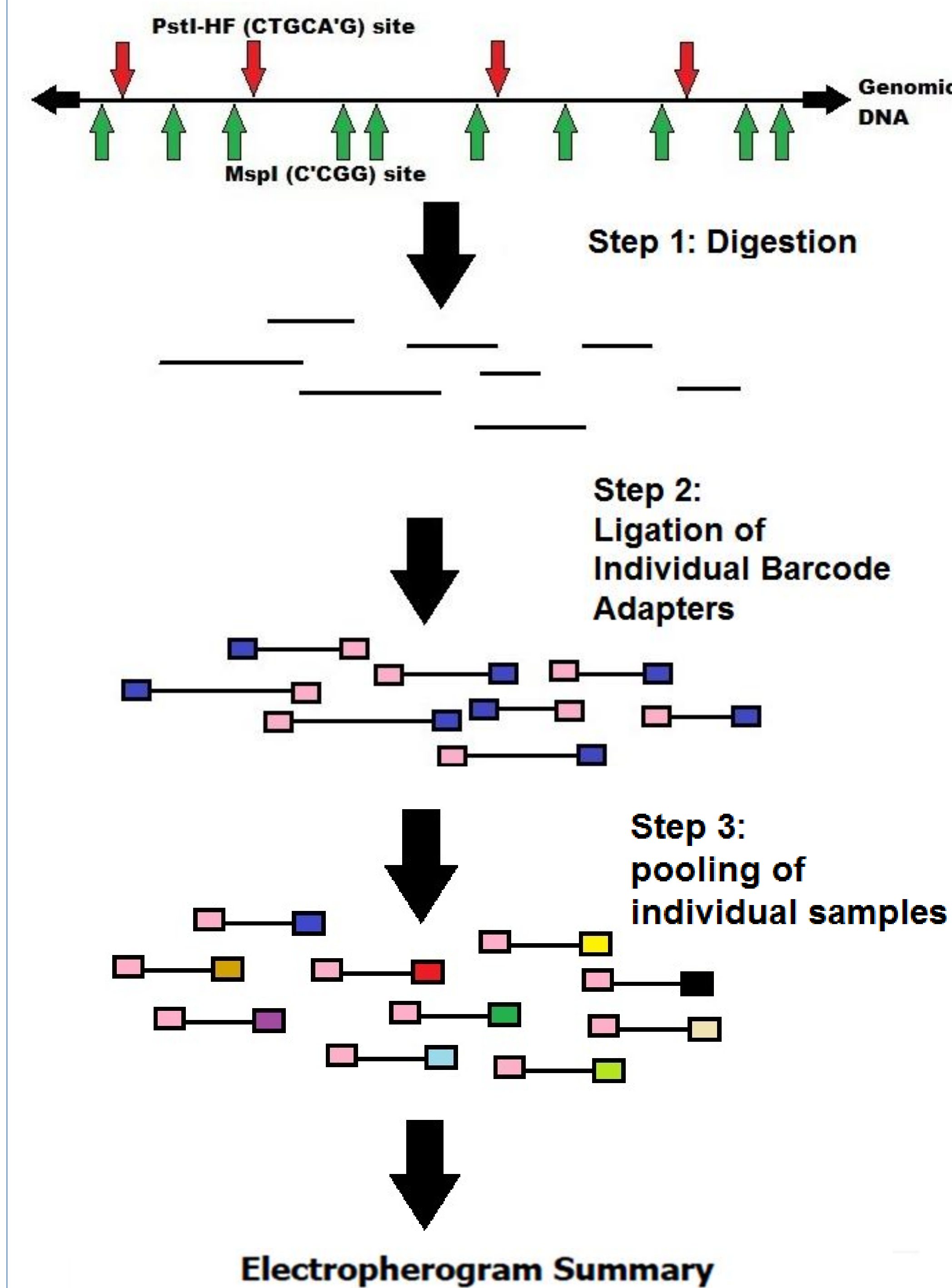
## GBS

- First described for maize and barley by Elshire et al. (2011), and for wheat and oats by Poland et al. (2012).
- Peterson et al. (2012) included size-selection step and use of an index primer set to increase the number of individual samples.

GBS 96-well plate format; image from Elshire et al (2011). Colors represent different bar-coded adapter sequences.



## GBS continued



**Step 4:** samples are size-selected at ~350 basepairs (bp) on a PippinPrep automated instrument (Sage Biosciences)

## Preliminary Results

- Preliminary experiment without size-selection
- DNA of a single loblolly pine parent tree and 90 haploid DNAs from seeds of that parent.
- About 15% of single-end sequence tags (64-bp sequences) show 1:1 segregation and map to a single contig in the v 0.6 draft assembly of the pine genome sequence.
- Further analysis is underway using new software that allows analysis of sequences from both ends of the DNA fragment.

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## References

- Elshire RJ, et al. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6:e19379.
- Ivanova et al. 2008 Semi-automated, membrane-based protocol for DNA isolation from plants. Plant Mol. Biol. Rep 26:186-198.
- Peterson BK, et al. 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE 7(5): e37135.
- Poland JA, et al. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7:e32253.
- Stein LD. 2010. The case for cloud computing in genome informatics. Genome Biol 11(5):207.