## **What is KASP?**

* KASP stands for Kompetitive Allele-Specific PCR. You’re running two fluorescent primers in the same sample at the same time, then analyzing the levels of fluorescence to see which alleles are present in your sample. The main benefit of this strategy, and any type of quantitative PCR (qPCR) is that you don’t have to do gel electrophoresis afterwards to figure out what’s in the sample. KASP is especially cheap and has a high sensitivity, which is great for catching some of the SNP markers present in the literature.
* Here are a couple of videos from manufacturers giving a brief intro - dry, but useful.
  + [LGC How does it work?](https://www.youtube.com/watch?v=_S0m2PrwPdE)
  + [LGC Reaction details](https://www.youtube.com/watch?v=Uq9HhmzOqUQ)
  + [LGC Why you should use it](https://www.youtube.com/watch?v=GJbM7UbE7ZI)
* KASP is a proprietary name, so some manufacturers try to get around that by selling primers or mixes for [PCR Allele Competitive Extension, or PACE](https://sfvideo.blob.core.windows.net/sitefinity/docs/default-source/flyer/pace-snp-genotyping-assays.pdf?sfvrsn=daf11407_6#:~:text=PACE%20(PCR%20Allele%20Competitive%20Extension,allele%2Dspecific%20PCR%20genotyping%20technology.&text=KASP%2C%20on%20the%20other%20hand,%E2%84%A2%20Platform%20(Douglas%20Scientific).). It’s exactly the same process, though the formulation of the master mix may be formulated to work better with smaller or larger samples. In the literature, markers for this strategy are generally referred to as KASP markers, but when you order supplies they may be labeled PACE instead.

## **Ordering Primers and Master Mix**

* [Integrated DNA Technologies](https://www.idtdna.com/pages/products/qpcr-and-pcr/genotyping/pace-snp-genotyping-assays), or IDT, is where we’ll be ordering our specific primers from. They also sell the master mix, but for a higher price than we can find elsewhere.
  + Justine Alcaraz has some notes on our projects already - let her know if you’ve got questions on ordering or anything. jalcaraz@idtdna.com
* [3cr Bioscience](https://3crbio.com/pace-price-list-2021/) sells the master mix for less than I could find anywhere else. The 2.5 uL for 500 reactions should be fine to get started - if it works well, you can order a larger size and have it shipped out fairly quickly.
* When you’re ready to order some supplies, ask Henry or Valerio to show you how to purchase things through the McGill Marketplace.

## **Protocol**

**DNA Extraction**

* You should get some protocol notes from IDT or 3cr Biosciences when you make an order, but I’ll lay out the details here as well.
* DNA extraction notes from Andrea Corkal (former lab undergrad and all-around amazing person) are present on the lab github, but I put them in this folder under DNA Extraction Protocol because reorganizing any files in github breaks the links to them, and I find the whole thing kind of arcane.
  + DNA can be extracted from leaf tissue or seeds.
    - Leaf tissue tends to be easier, but waiting until the plants produce enough leaves can be time-consuming. Additionally, it tends to take more seeds, and some of the cultivars you’re working with might only have 20 seeds available at our lab, most of which need to be used for other purposes. There are leaf samples from all 31 cultivars used in the White Mold Nursery Greenhouse trial stored at -80C in the greenhouse basement. Ask Henry to show you where they are, it’s a big ole labeled box on the bottom shelf.
    - Seed chipping is another way to go about it. It tends to be more resource-efficient. Valerio experience with this method - read through some protocols and ask him any questions if you prefer to go about it this way.
      * You’ll likely be using the Geno Grinder for this method. Here are a few details and protocols for it.
        + [Comparison of DNA Isolation Methods from Soybean](https://www.spexsampleprep.com/knowledge-base/resources/application_notes/0627-102105-SP019%20-%20GG%20-%20Comparasion%20of%20Methods%20for%20the%20Isolation%20of%20DNA%20from%20Soybeans%202018.pdf)
        + [University of California Maize Seed Chipping Protocol](https://bio-protocol.org/bio101/e3553)
        + [Parmar et al. 2021 Seed Chipping in Groundnuts](https://www.mdpi.com/2073-4395/11/6/1226/pdf)