From: Wilfred Vermerris

To: Seth C. Murray

Cc: Ana I Saballos; Bill Rooney; Stephen Kresovich; Jeff Pedersen; Martha Hamblin; sem30

Subject: Re: HIF Tissue for RNA - expression sequencing Date: Thursday, November 05, 2009 10:47:54 AM

Hi Seth,

Thanks for the update. My preference is that you ship all samples. With the constant improvements in sequencing technology we will likely be able to pool samples and use barcodes to distinguish the sample origins. The core facility at Cornell is, from what I understand, getting an upgrade to their Solexa system that would make that approach feasible.

You may want to prepare two separate shipments on dry ice to reduce the risk of mishaps during transit and ship at the beginning of the week. The boxes will need special stickers to indicate they have dry ice in them. Please email the tracking numbers so we can monitor. I have had to run out to the FedEx distribution center once or twice to rescue frozen items....

Let me know if you have any questions.

Thank you,

Wilfred

Seth C. Murray wrote:

> I finished the harvesting of tissue on Monday - given the cool temperatures the plants were in early hard dough stage and still had decent brix.

> For each plant that I harvested I collected two samples;

> Boot Time point: Flag Leaf and Internode 4

> Hard dough: Peduncle and Internode 4

> I took the center \sim 2 inches of internode 4 for RNA extraction and used each end of internode 4 in a handheld juice press to collect brix these two end values were then averaged. These values are reported in the attached spreadsheet.

> In preparing to ship these to Florida I have two main questions:

> 1. Handsqueezed brix values from a single internode are probably not reliable and full of error. However, in the samples I took from family 7, the handsqueezed brix value was higher for the allele line than the Rio allele. Should we cherry pick the samples that behaved as we expect (Choose samples with Rio allele having the highest handheld brix, samples with allele have lowest brix?). If so we could use Family 12 which behaves closer to what we expect but only has two samples in boot stage. Should we just ignore these handsqueeze values?

> 2. Should I ship all samples or a subset? There are probably three times more samples than we have money to analyze. If I ship a subset then if something happens we have backups I can reship.

> Any thoughts appreciated.

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> Ana: the hard dough samples (especially the peduncle) are dirty and should be surfaced washed

and/or cored to get the pith before RNA extraction if possible. I did not think about this until I was in the field with the liquid nitrogen and only a bandanna to wipe them off.

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> Thanks,

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> Seth

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