

# Ask Frank

by Fred and Hank



FRED HYDE



HANK DAUM

## Questions about the BuccalAmp™ DNA Extraction Kit, Catch-All™ Sample Collection Swabs, and the MasterPure™ DNA and RNA Purification Kits

Introducing Fred and Hank, EPICENTRE's helpful Technical Service Scientists, often known collectively as "Frank". As a new feature in the Forum, they will answer some Frequently Asked Questions about EPICENTRE's products. But first, here is a little background on Fred and Hank.

Fred Hyde received bachelor's degrees in Microbiology and Zoology (with a minor in organic chemistry) from Michigan State University, and a PhD in Microbiology from the University of Minnesota. His project focused on the molecular biology of the Lyme disease spirochete, *Borrelia burgdorferi*, and relapsing fever spirochetes. He did postdoctoral work at 3M Company, where he developed immunoassay technologies for Lyme disease and periodontal disease. After 7 years in 3M's corporate research laboratories, Fred started his own contract manufacturing organization, producing antibodies and proteins for bioresearch. He has been with EPICENTRE for 6 years as a marketing product manager and technical product manager/technical consultant. Reach Fred at 1-800-284-8474 extension 6119.

Hank Daum received a BS in Chemistry from Augsburg College, Minneapolis, MN and an MS in Biochemistry from the University of Minnesota, Minneapolis, with additional training in gene expression and molecular biology from the University of Mississippi Medical Center, Jackson, and Louisiana State University, Shreveport. He moved to the Northeast, and into industry at FMC BioProducts, Rockland, ME for 10 years, as a research scientist and then a technical consultant. After a brief foray into marketing at National Diagnostics, Atlanta GA, and then at EPICENTRE, Hank returned to his true love for technical consulting. Hank has been with EPICENTRE for 6 years and can be reached at 1-800-284-8474 extension 6120.

### QUESTION

What is the best way to quantitate DNA that was isolated from buccal cells using the BuccalAmp™ DNA Extraction Kit?

### ANSWER

We rarely quantitate the DNA extracted from the sample; we simply use 5 µl of the DNA directly in a PCR reaction. The range of DNA obtained from BuccalAmp samples is about 1-7 µg, and depends upon how many buccal cells the subject "sheds". If you need to quantitate the DNA, DO NOT USE A<sub>260</sub> optical density (OD) readings. Residual NTPs from RNA degradation will give an artificially high OD reading. Use fluorimetry with a DNA-specific dye (Hoechst 33258 or bis benzimide). For more information see EPICENTRE Forum (1998) 5(4),1 at [http://www.epicentre.com/f5\\_4yeast.asp](http://www.epicentre.com/f5_4yeast.asp).

### QUESTION

How stable is the buccal cell sample on a Catch-All™ Sample Collection Swab?

### ANSWER

Stability of buccal cells on the Catch-All™ Sample Collection Swab depends upon the way the swab is stored. After a brief drying period (by propping the tip of the swab against the plastic shipping tube for 15 minutes), samples are stable for at least two weeks at 37°C, 6 weeks at room temperature, "months" at 4°C and "years" at -20°C.

### QUESTION

How stable is the DNA after extraction using the BuccalAmp™ DNA Extraction Kit?

### ANSWER

After extraction, the DNA obtained using the BuccalAmp DNA Extraction Kit is stable for years when stored at -20°C. The final 98°C heating step inactivates remaining nucleases in the samples.

### QUESTION

How are the MasterPure™ DNA and RNA Purification Kits different from other nucleic acid purification kits?

### ANSWER

Many nucleic acid purification kits are based on spin column technology. Spin columns do offer some convenience, but at a higher cost per sample. In addition, limitations on the sample size make spin column methods much less flexible than liquid-based purification systems. Spin columns use an ion-exchange resin format, while the MasterPure™ DNA and RNA Purification Kits use a non-toxic, liquid-based purification technology. Liquid-based systems offer flexibility in sample size, improved recoveries, both in the quality and quantity of nucleic acids, and cost savings per sample.

### QUESTION

Do I need to use RNase inhibitors with the MasterPure™ RNA Purification Kit?

### ANSWER

The addition of RNase inhibitors is not necessary with the MasterPure™ RNA Purification Kit. The combination of Proteinase K digestion and the MPC Protein Precipitation Reagent, which removes cell debris, does an excellent job of removing RNases (and are probably the best RNase inhibitors). However, for rare samples or RNA purified from a heavily contaminated source (such as pancreatic tissues), make sure the sample is well-protected, with a chemical or enzymatic RNase inhibitor, BEFORE using the MasterPure RNA Purification Kit. After the purification, the RNA is very stable.