Application Note

Avoiding cross-contamination when using the Harris Uni-Core™ punch tool in Thermo Scientific Direct PCR protocols

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Abstract

The Thermo Scientific Direct PCR approach saves time and cost by allowing amplification of DNA directly from various source materials. With starting materials such as plant leaves, mouse ears or filter papers, the easiest way to produce samples of small and uniform size is to use the Harris Uni-Core Punch Tool and Harris Cutting Mat Technology. We found that 2 % sodium hypochlorite efficiently prevents cross-contamination of the samples from the tools.

Introduction

The Harris Uni-Core (Figure 1) tool is useful for obtaining small tissue samples for Direct PCR protocols. Since the same tool can be used several times, removing all traces of tissue and DNA between punches is critical to avoid cross-contamination of the PCR reactions. In this application note we have compared two commonly used substances, ethanol (EtOH) and sodium hypochlorite (NaClO), for their efficiency in cleaning the Harris Uni-Core tool and Cutting Mat when sampling a variety of source materials.

Materials and Methods

- Harris Uni-Core tool 0.50 mm
- Harris Cutting Mat
- 70 % Ethanol (EtOH)
- 2 % Sodium Hypochlorite (NaClO)
- Thermo Scientific Phire Hot Start DNA Polymerase
- Thermo Scientific Piko Thermal Cycler
- Thermo Scientific UTW (ultra-thin walled) Piko PCR Plates

Sampling

Sample materials: human blood stored on Whatman FTA® Elute cards, mouse ears (stored frozen), muscle tissue from brown bear (stored frozen in EtOH) and fresh plant leaves from various species.

The samples were placed on the Harris Cutting Mat, and the cutting edge of the Harris Uni-Core tool was pushed vertically through the sample material while rotating in opposite directions. After cutting, the plunger was used to eject the sample disc from the Harris Uni-Core Tool into a PCR reaction.

Figure 1. A Harris Uni-Core tool.

Cleaning of the Sampling Tools

The cutting edge of the Harris Uni-Core tool was cleaned between each sample by dipping it into either 70 % EtOH or 2 % NaClO solution, pumping the plunger several times, and then wiping with a clean paper towel. The Cutting Mat was also rinsed with the same solution after each sampling. After each cleaning, the punching tool was dipped into a PCR reaction to monitor the efficiency of cleaning method.

Results

70 % EtOH and 2 % NaClO solutions commonly used for cleaning the sampling tools were first tested with blood stored on a FTA Elute Card, mouse ear tissue, bear muscle tissue, and *Arabidopsis* leaves (Figure 2). For blood stored on FTA Elute cards and *Arabidopsis* leaves, both EtOH and NaClO treatments cleaned the punching tools adequately between samples. When using mouse or bear muscle tissue as starting material, EtOH cleaning was not sufficient as cross-contamination was seen with both tissues tested. In contrast, NaClO was efficient in preventing cross-contamination with all sample types tested.

Next, we tested plant species that were older and/or more fibrous than *Arabidopsis* leaves (Figure 3). With these samples EtOH was found to be less effective in cleaning the sampling tools than NaClO.

Finally, the cleaning method using NaClO effectively prevented cross contamination in an experiment using ear punches of transgenic mice as sample material (Figure 4). There was no carryover, and the method did not adversely affect subsequent PCR reactions.

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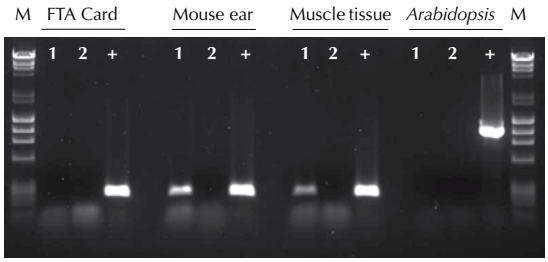
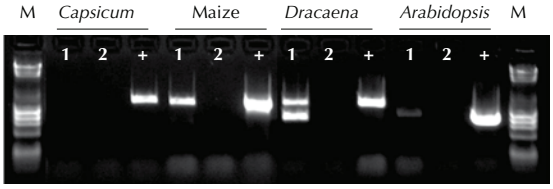


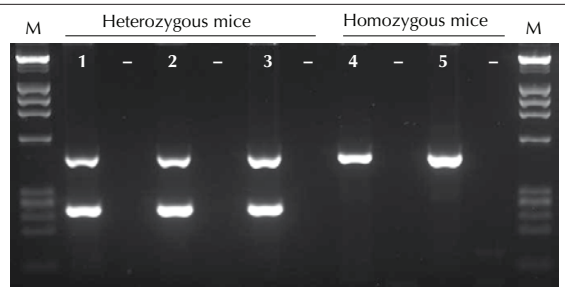
1 = EtOH, 2 = NaClO, + Positive control, M = Size Marker.

Figure 2. Cross-contamination test performed on 4 different sample types. Between each sampling, the Harris Uni-Core Tool and the Cutting Mat were cleaned with either 70 % EtOH (lane 1) or 2 % NaClO (lane 2) as described, and the punching tool was dipped in a PCR reaction. For animal samples, universal control primers amplifying a highly conserved 237 bp non-coding region upstream of SOX21 gene was used (Ref 1). For *Arabidopsis*, universal primers for plant mitochondrial DNA were used (Ref 2). The positive control (lane 3) in all cases was a tissue sample of 0.50 mm in diameter placed into a 20 µL PCR reaction. All amplifications were done with Phire Hot Start DNA Polymerase in Piko Thermal Cycler using UTW Piko PCR Plates.



1 = EtOH, 2 = NaClO, + Positive control, M = Size Marker.

Figure 3. Cross-contamination test performed on 4 different plant species. Between each sampling, the Harris Uni-Core Tool and the Cutting Mat were cleaned with either 70 % EtOH (lane 1) or 2 % NaClO (lane 2) as described and the punching tool was dipped in a PCR reaction. Universal primers for plant mitochondrial DNA (yielding products of different sizes depending on the species) were used (Ref 2). The positive control (lane 3) in all cases was a plant leaf sample of 0.50 mm in diameter placed into a 20 µL PCR reaction. All amplifications were done with Phire Hot Start DNA Polymerase in Piko Thermal Cycler using UTW Piko PCR Plates.



M = Size Marker, – = Negative control.

Figure 4. Cross-contamination test in mouse genotyping experiment. Five transgenic mice were genotyped using the Direct PCR approach with Phire Hot Start DNA Polymerase (20 µL reactions). The samples from mouse ears were taken with the Harris Uni-Core Tool 0.50 mm. After each sample the Harris Uni-Core Tool was cleaned with 2 % NaClO as described and dipped in a PCR reaction (every other well). Samples 1–3 are heterozygous for the transgene (larger PCR product), and samples 4–5 are homozygous. PCR reactions were performed in Piko Thermal Cycler using UTW Piko PCR Plates.

Conclusions

The Harris Uni-Core punch tool is an excellent tool for obtaining samples for Direct PCR protocols. Care must be taken, however, to carefully clean the tool between samples to avoid cross-contamination when taking multiple samples with a single tool. We have found that a 2 % solution of sodium hypochlorite (NaClO) prevents cross-contamination in all sample materials that we have tested, including blood dried onto storage cards, mouse tails and ears, and plant leaves. In many cases, 70 % ethanol was also effective but, for mouse tissues and several plant species, especially latex-containing plants in particular (data not shown), quite a bit of cross-contamination was seen. For any lab using these tools for sample collection for Direct PCR, we recommend using the 2 % NaClO solution for cleaning the sampling tools. It is also recommended to verify that there is no carryover by running a negative control reaction of the cleaned puncher in every PCR assay.

Notes

1. Never mix sodium hypochlorite with acids or ammonia-based cleaners.
2. More information on sampling and cleaning procedures, as well as further instructions for Direct PCR, are available at www.thermoscientific.com/directpcr

References

1. Woolfe A. *et al.* (2005) *PLoS Biology* 3: 116-130.
2. Demesure B. *et al.* (1995) *Molecular Ecology* 4: 129–131.

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