Dilution & Storage Protocol

~~Hair~~ was placed in 20 µL of Dilution Buffer containing 0.5 µL of DNA Release Additive. The samples were incubated at room temperature for 2 minutes and then at 98 °C for ~~2~~ 5 minutes. The samples were spun down and 1 µL of the supernatant was used as a template in a 20 µL reaction with Phire Tissue Direct PCR Master Mix and gene specific primers (Table 1). PCR was preformed as described in Table 2 and PCR products were loaded directly onto gels.

Table 1. PCR Reaction Setup.

Component	20 µL rxn	50 µL rxn	Final conc.
H_2O	add to 20 µL	add to 50 µL	
2X Phire Tissue Direct PCR Master Mix	10 µL	25 µL	1X
Primer A	X µL	X µL	0.5 µM
Primer B	X µL	X µL	0.5 µM
Sample			
Direct Protocol	–	0.5 mm punch of hair	
Dilution & Storage Protocol	1 µL	–	

Table 2. PCR Cycling Conditions.

Cycle step	2-step		3-step		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98°C	5 min	98°C	5 min	1
Denaturation	98°C	5 s	98°C	5 s	35
Annealing	–	–	X°C	5 s	
Extension	72°C	20 s ≤1 kb 20 s/kb >1 kb	72°C	20 s ≤1 kb 20 s/kb >1 kb	
Final Extension	72°C +4°C	1 min hold	72°C +4°C	1 min hold	1

Results and Discussion

The ability of Phire Tissue Direct PCR Master Mix to amplify target DNA from the hair samples was tested using mouse hair. Mouse hair samples were collected with tweezers from the animal's back, and only hair with follicles attached were used for PCR. Robust amplification of different length amplicons was achieved even from a single hair sample (Figure 1). Phire Tissue Direct PCR Master Mix can therefore be used for PCR based genotyping applications by using hair as a starting material.

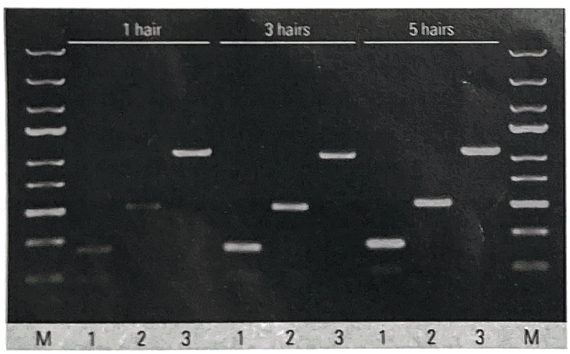

Figure 1. Different length amplicons were amplified using different number of mice hair and Phire Tissue Direct PCR Master Mix (direct protocol). Fragments sizes 1 – 237 bp, 2 – 515 bp, 3 – 1.1 kb. M – Thermo Scientific™ O'GeneRuler™ Express DNA Ladder.

Multiplex PCR amplification of five different length fragments was achieved using mouse hair and Phire Tissue Direct PCR Master Mix (Figure 2.). This approach can be used in genotyping applications that require simultaneous determination of several markers in one reaction.

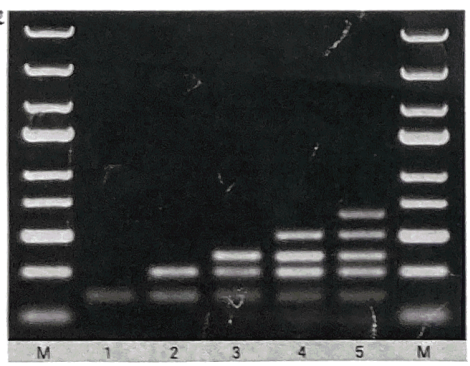

Figure 2. Five different length DNA amplicons, ranging from 185 to 650 bp, were amplified from mouse hair in 1-plex to 5-plex reaction with Phire Tissue Direct PCR Master Mix. Dilution & storage protocol and 5 hairs were used for the amplification. M – O'GeneRuler Express DNA Ladder.

The Phire DNA polymerase from Direct PCR Master Mix is optimized to achieve robust and sensitive amplification even from tiny amounts of tissue samples. The performance of Phire Direct PCR Master Mix was compared to that of corresponding kits from other vendors. Figure 3 shows that while other vendors' kits work

KO cells → RNA → qRT-PCR
→ protein → WB { co-IP / mass spectrumy. → batch 2. ch2p-seq
→ DNA → PCR. KO validation
→ NGS { RNA-seq.
ATAC-seq. 50K cells
ch2p-seq / cut&run { H3K27me3.
Ezh2
Jarid2
? PRC1/2. 1/2.
→ cell counting colony formation assay (2500 cells)
→ freezing cells for storage.
→ Xenograft. 4 weeks for mice ordering.
organoid { chip-seq
shRNA Jarid2

spatial { villus
patch
diff states
clustering

fetal
reprogram

cancer