SNP Genotyping of DNA collected from RapiDri swab samples stored in warm, humid conditions

When collecting and transporting collected DNA to be used in genomic analysis, it is vitally important to ensure that the samples remain intact in a variety of climatic conditions. DNA samples can be vulnerable to degradation in hotter, particularly humid conditions, affecting the success of downstream analysis. In conditions such as this proper stabilisation of samples is vital.

One such stabilisation method is the Isohelix RapiDri integrated swab kit, which uses a specialised drying pouch to rapidly stabilise samples. This study aims to investigate the effectiveness of RapiDri in stabilising DNA in these conditions, and their suitability for use in downstream SNP genotyping.

Methods:

Four sets of two Rapi-Dri (RD-01) swab samples were collected and incubated at varying temperatures and relative humidities for up to 14 days. Two additional swabs were also collected and were isolated immediately to be used as Fresh (Day 0) reference control samples. The sets were as follows:

Set 1: 30°C @ 10% Relative Humidity

Set 2: 30°C @ 60% Relative Humidity

Set 3: 40°C @ 10% Relative Humidity

Set 4: 40°C @ 60% Relative Humidity

After 7 days incubation, one swab per set were isolated using the Isohelix Buccal-Prep Plus kit. Following 14 days the remainder of the swabs were also isolated. Sample purities and yields of the samples were quantified by Nanodrop and Qubit dsDNA BR assay, respectively. Following this, samples were diluted to a concentration of 2ng/uL and underwent KASP/Illumina genotyping analysis. Two genotyping systems were tested with the provided DNA, KASP SNP genotyping and Illumina Infinium.

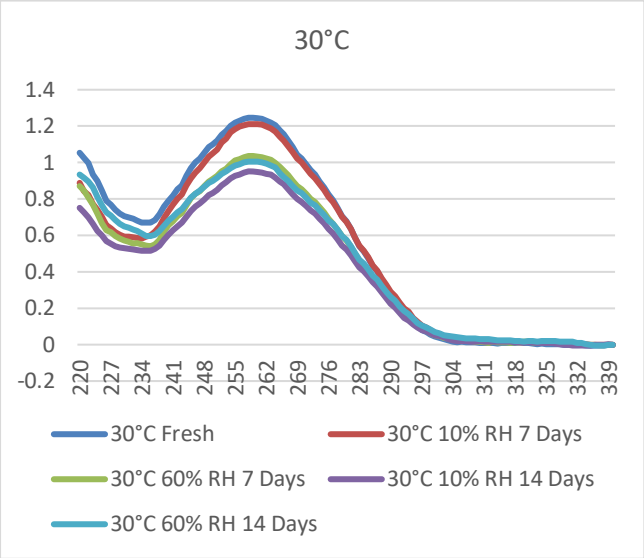
The following 25 markers were selected for genotyping with KASP:
rs1799883, rs1801282, Rs1042713, rs1042714, rs5082, rs9939609, Rs1801260, rs17782313, rs699, rs1799722, rs731236, rs4880, rs698, rs1001179, rs2395182, rs4988235, rs1801133, rs1544410, rs4680, rs174546, rs1260326, rs1595824, rs11121022, rs662799.

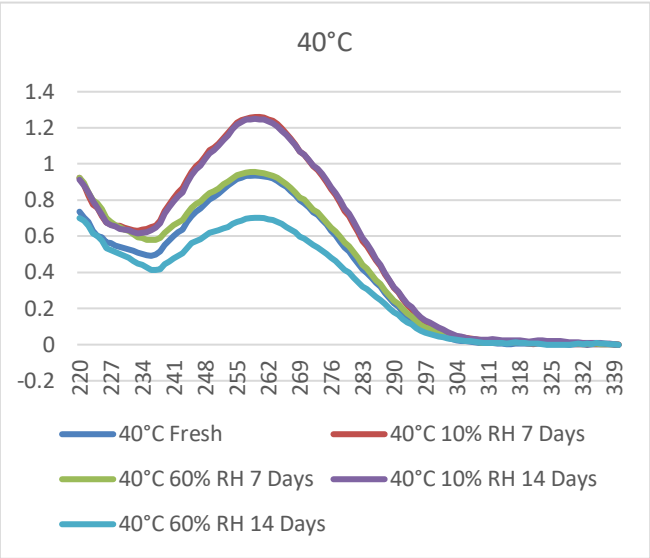
4uL of DNA was dried into the wells of a 384 well plate, and the KASP reactions carried out in 5uL KASP reaction mix (LGC). Plates were cycled for 30 cycles, then read in a QuantStudio5, and cycled for further rounds of 4 PCR cycles and re-read, until all the markers could be read. The Infinium HTS array contains over 4,000 loci, including the markers detailed above. The analysis of samples was carried out according to the manufacturer's recommendations.

Results:

Yield & Purity Analysis:

	Sample ID	Qubit ng/ul	260/280	260/230		Sample ID	Qubit ng/ul	260/280	260/230
A	*30°C Fresh*	*59.6*	*1.84*	*1.77*	**F**	*40°C Fresh*	*40.3*	*1.81*	*1.75*
B	30°C 10% RH 7 Days	59.6	1.80	2.04	**G**	40°C 10% RH 7 Days	53.7	1.77	1.94
C	30°C 60% RH 7 Days	50.0	1.81	1.81	**H**	40°C 60% RH 7 Days	42.3	1.75	1.49
D	30°C 10% RH 14 Days	46.8	1.82	1.80	**I**	40°C 10% RH 14 Days	57.1	1.72	1.96
E	30°C 60% RH 14 Days	44.4	1.75	1.55	**J**	40°C 60% RH 14 Days	32.1	1.76	1.43





All samples collected and purified showed good yields and purities throughout all conditions tested over the 14 day period, results which were comparable to the Fresh (Day 0) control samples. This indicates that results from buccal swab samples stabilized via RapiDri are consistent up to 14 days; even in hot, humid environments that would normally affect sample yield and purity.

KASP Analysis:

The samples were quantified using real time PCR (where no inhibitory effects on amplification were noted), and then normalized to a concentration of 1 ng/µL.

The KASP experiment was run, and all samples were amplifiable. Six datapoints could not be determined, two on E, one each on F and I. In general, this failure to read a datapoint was due to slow amplification of the sample with a particular marker. No marker had more than one undetermined sample. KASP is notoriously difficult to score accurately unless there is good representation of all genotype classes.

Illumina Analysis:

The supplied DNA was diluted 1:2. The samples were run in alongside >300 other samples. The call rate of the samples ranged from 96% to 98%, with the call rate for the 24 markers at 100%.

Below is the table with the approximate call rate for the 10 samples for all the SNPs present in the bead chips. 98% of the total samples in the run had call rate comprised within 0.95 and 0.99, which is considered good quality, while the remaining 2% had call rates below 0.95, however none of these were RapiDri samples.

Sample ID	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Average Call Rate	0.989	0.989	0.988	0.987	0.987	0.989	0.988	0.987	0.988	0.988

Conclusions:

- This study demonstrates that RapiDri self-drying buccal swabs maintain and protect the integrity of collected DNA in both dry and humid conditions at up to 40°C, making them suitable for sample storage and shipping in warmer climates for short to medium-term periods.

- Samples stored in this way provide consistent, high quality call rates on both KASP and Illumina methods of SNP analysis, demonstrating that samples maintain their integrity and suitability for downstream applications.