Shelf-Life testing on GeneFix buffer over 10 years and DNA stability testing over 5 years by an accelerated ageing study at 50^{0}C.

Method for Shelf-Life testing:

Accelerated ageing studies are widely used to reliably determine the stability of buffers using the Arrhenius equation to calculate the equivalent storage time at room temperature for a buffer held at an elevated temperature. In this case the elevated temperature used was 50^{0}C and the equivalent stability time at 21^{0}C was calculated.

10ml samples of 2 different batches of GeneFix buffer (LYS4) were placed in GeneFix tubes in a dry-block heater at 50^{0}C on 11/08/2016. LYS4 BN L006 was manufactured on 29/07/2014 and was 2 years old when the study was set up, LYS4 BN L049 was the most recently manufactured batch made on 01/06/2016. For the Day 0 samples, a fresh saliva sample was collected, vortexed to ensure homogeneity, then 500µl added to an equal volume of both batches of GeneFix buffer. The DNA was isolated through the GeneFix Saliva-Prep 2 kit according to the manufacturer's instructions, resuspending in 100µl TE buffer, before storing at -20^{0}C. Further 500µl samples were removed from each tube at time points equivalent to 2 years, 3 years, 4 years, 5 years, 8.5 years and 10 years, and used to isolate freshly collected saliva samples in the same way as the day 0 samples.

On completion of the study, all 7 samples for both batches of buffer were analysed by UV spectrophotometry for A260/280 and A260/230 ratios and yield, and DNA integrity checked by running whole DNA on a 1% agarose gel.

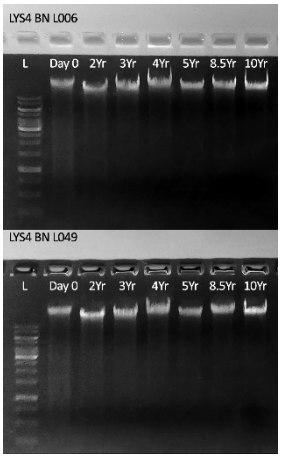
Method for DNA stability testing:

2 x 4ml samples of both batches of GeneFix buffer was placed into 10ml GeneFix tubes. 3 saliva samples were collected, pooled and vortexed to ensure homogeneity, then 4ml added to each of the tubes containing the GeneFix buffer. The tubes were vortexed again and a 500µl sample removed for DNA isolation through the Saliva-Prep 2 kit according to the manufacturer's instructions to give Day 0 samples for the DNA stability testing. Further 500µl samples were removed from each tube at time points equivalent to 2 years, 3 years, 4 years and 5 years and isolated through Saliva-Prep 2 in the same way as the Day 0 samples.

On completion of the study, all 5 samples for both batches of buffer were analysed by running whole DNA on a 1% agarose gel to demonstrate DNA integrity.

Results for Shelf-Life testing:

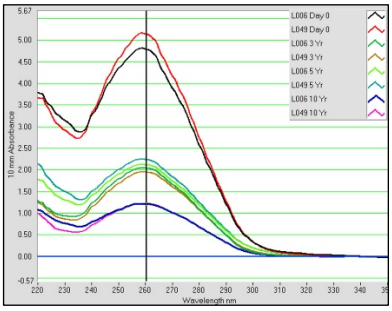
Whole DNA run on a 1% agarose gel against a 1Kb DNA ladder



- All samples contain intact non-degraded high molecular weight genomic DNA >10Kb in size.
- The DNA quality from buffers stored for extended periods of time before use, remains consistently high, with no evidence of reduced performance.
- The 2 batches of buffer which differed in age by 2 years at the beginning of the study perform consistently with each other.
- Observed shelf-life is well in excess of that stated.
- The shelf-life demonstrated here allows for both the 40 months shelf-life given from the date of manufacture plus the stability period post-sampling of 5 years.

AutoGen is the North American supplier for the GeneFiX. Please contact Rob Osborn at 508.395.8161 or at ROsborn@autogen.com for more information.

A260/280 and A260/230 UV absorbance ratios, and DNA concentration



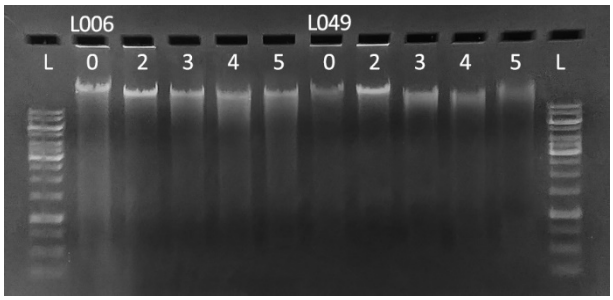
- Over the entire study period, the mean A260/280 for L006 = 1.82, and the mean A260/280 for L049 = 1.81
- Over the entire study period, the mean A260/230 for L006 = 1.71, and the mean A260/230 for L049 = 1.84
- Purity of samples is consistently high over the entire study period.
- Purity of DNA isolated with both batches of buffer at any one time point are both high, and consistent with each other.

Sample ID	ng/ul	A260	A280	260/280	260/230	340 raw
L006 Day 0	239.60	4.792	2.575	1.86	1.52	0.382
L049 Day 0	257.17	5.143	2.742	1.88	1.76	0.227
L006 2 Yr	118.96	2.379	1.309	1.82	1.86	0.165
L049 2 Yr	102.35	2.047	1.144	1.79	1.96	0.095
L006 3 Yr	102.02	2.040	1.088	1.88	2.18	0.063
L049 3 Yr	97.34	1.947	1.072	1.82	2.26	0.063
L006 4 Yr	143.70	2.874	1.548	1.86	1.86	0.203
L049 4 Yr	153.74	3.075	1.708	1.80	1.87	0.227
L006 5 Yr	105.85	2.117	1.184	1.79	1.63	0.278
L049 5 Yr	111.93	2.239	1.243	1.80	1.49	0.195
L006 8.5 Yr	116.35	2.327	1.319	1.76	1.30	0.418
L049 8.5 Yr	143.89	2.878	1.644	1.75	1.48	0.404
L006 10 Yr	60.83	1.217	0.679	1.79	1.59	0.024
L049 10 Yr	60.84	1.217	0.676	1.80	2.07	0.052

- Over the entire study period, the mean concentration for L006 = 126.76ng/ul, and the mean concentration for L049 = 132.47ng/ul
- Sample yield depends on the individual fresh saliva sample used at each time point, so will vary between time points, however the DNA concentration for both batches of buffer is consistent with each other for any individual time

Results for DNA stability testing:

Whole DNA run on a 1% agarose gel against a 1Kb DNA ladder



- All samples contain intact high molecular weight genomic DNA >10Kb in size.
- There is no significant difference in the appearance of the DNA over time.
- The DNA from both batches of buffer looks to be of the same high quality.
- Looking at band brightness, there is no obvious drop in yield over the time period of the study.

Conclusions:

- The 2 batches of GeneFix buffer which differed in age by 2 years at the start of the study performed consistently with each other over the period of the shelf-life study, equivalent to 10 years at room temperature.
- No loss of performance was seen from either batch of buffer over the study period.
- The length of the study allows for both the 40 months shelf-life given from the date of manufacture, plus the 5 year stability period post-sampling.
- The performance of the GeneFix buffer is maintained for at least 12 years from the date of manufacture.
- DNA stability is maintained for 5 years in GeneFix buffer which was 2 years old when the saliva sample was added, no difference was seen in ability to stabilise DNA long-term between a 2 year old buffer and a fresh batch.
- GeneFix tubes used at the end of their shelf-life are able to stabilise DNA for the full stability period quoted for the product.