Depurination

Depurination of DNA occurs when a purine base (adenine (A) or guanine (G)) is removed from the DNA deoxyribose backbone via hydrolysis (Figure 1). This results in a deoxyribose lacking a purine base, which is known as an abasic or apurinic site. Depurination of sample DNA at the ligation sites of SALSA® MLPA® probes can affect probe hybridization to the sample DNA and can lead to the reduction or complete failure of a probe to ligate. Loss of probe hybridization/ligation also leads to variability in the obtained probe signals. Therefore, minimising depurination is important for the reproducibility and reliability of MLPA results. In most cases, depurination can be prevented by the presence of at least 5 mM Tris-HCl pH 8.0–8.5 in the DNA sample.

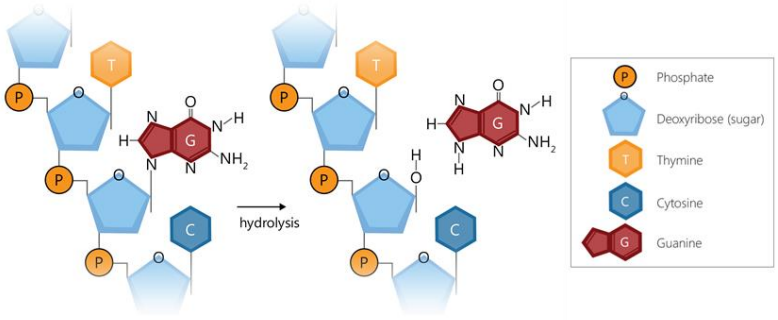

Figure 1. Depiction of DNA depurination. The DNA strand on the left shows an intact bond between the deoxyribose backbone and a guanine base. The arrow indicates the process of hydrolysis where the end result is a free guanine base and a deoxyribose backbone now lacking a base.

1. Depurination of sample DNA

During MLPA reactions depurination can occur during the initial five minute 98°C denaturation step if the sample DNA is in a solution with insufficient buffering capacity or a low pH (e.g. water[1]) (Figure 2). If the denaturation step is extended, the effect will be further exacerbated[2]. Sample treatments (e.g. prolonged heating, formalin fixation/paraffin embedding) can also result in depurination of your sample DNA, even with sufficient buffering capacity. Problems are more likely to arise when these factors are combined.

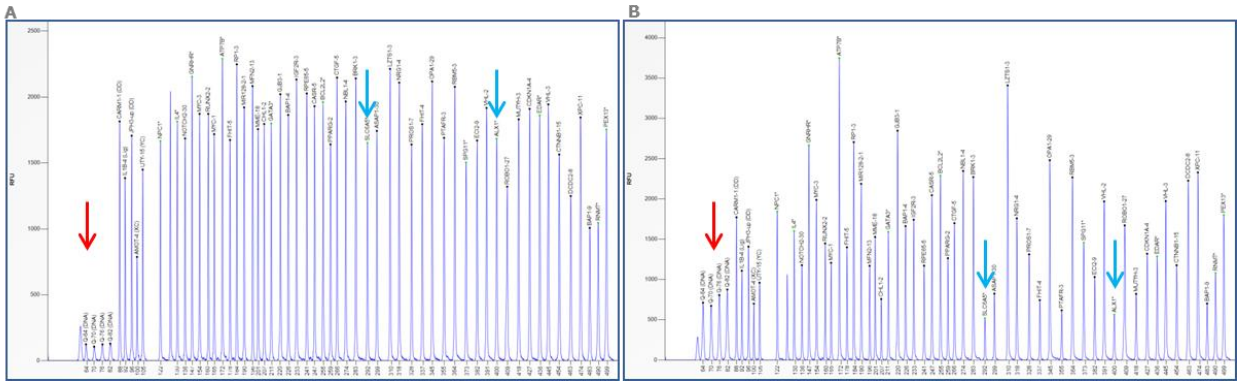

Figure 2. Electropherogram results from an MLPA experiment of the same sample DNA diluted in either (A) TE buffer or (B) water demonstrating the effects of sample DNA depurination (all other conditions were identical). (A) High quality MLPA results obtained from sample DNA diluted in TE. (B) An irregular peak pattern observed from sample DNA diluted in water. Probes show non-uniform heights as certain probe signals are diminished or increased. Probes targeting purine-rich ligation sites are the most severely affected (blue arrows). In addition, an increase in the signal intensity of the DNA quantity fragments (Q fragments, red arrows) is detected, signalling that less DNA template is available in the sample.

[1] Due to their lack in buffering capacity, DNA samples eluted from a purification column with water are especially vulnerable to depurination, and can easily give suboptimal MLPA results.
[2] If you are experiencing denaturation problems please visit support.mlpa.com for suggestions; do not extend the denaturation time.

2. Effects of sample depurination

Depurination at the ligation site of a probe, especially those with a large number of pyrimidines (cytosine (C), or thymine (T)), will result in inefficient probe hybridization and subsequent limited or failed probe ligation, as these pair with the purines (G, A) that are lost during depurination (Figure 3). As a consequence, the probe signal will be reduced or even absent.

MLPA probes that consist of three oligonucleotides (an LPO, RPO, and a spanning oligo) are also more likely to be affected by depurination. This is due to these "three part probes" having two ligation sites (as opposed to one), making them especially vulnerable to ligation failure in suboptimal conditions.

While depurination near the probe ligation site is the most detrimental, depurination that occurs elsewhere in the probe hybridizing sequence may also result in probe binding destabilisation and a reduced probe signal. MLPA probes are designed to work optimally when all bases of the probe oligos bind during the hybridization step, and any deviation from this binding can have adverse effects on the resulting probe signals.

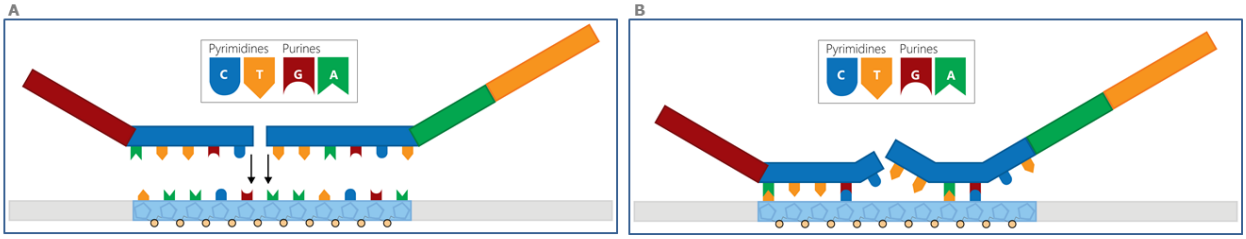

Figure 3. Schematic representation of an MLPA probe with high pyrimidine content around the ligation site (i.e. purines present in the target DNA). (A) The probe hybridises successfully to the sample DNA, which in this case is unaffected by depurination. (B) The same probe hybridised to sample DNA that is heavily affected by depurination: the guanines and adenines of the target on the sample DNA have all been lost. The result is a very poor probe hybridization, leading to an inefficient or failed ligation of the probe oligonucleotides, ultimately causing a reduced or even absent probe signal.

3. Preventing sample DNA depurination

To prevent sample depurination it is essential that sample DNAs contain sufficient buffering capacity. The presence of at least 5 mM Tris-HCl pH 8.0–8.5 in the sample DNA is sufficient to prevent depurination during the five-minute 98°C denaturation step. If it is not known what a sample DNA is dissolved in we recommend that 1 µl of 50 mM Tris-HCl pH 8.5 is added to 4 µl of sample DNA. With sufficient sample DNA buffering, optimal MLPA results are obtained with a five-minute denaturation step at 98°C as stated in the MLPA general protocol. Denaturation time should never be altered.

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