The general understanding of nucleic acid chemistry is mostly dominated by the view of molecular biologists, and, in this respect, the chemistry of DNA degradation in solution is well understood [61, 62, 73, 138, 139]. However, mechanistic studies describing DNA storage stability in the dried state are scarce. Indeed, storing DNA-based formulations in a solid state may be highly desirable not only to reduce the requirement for costly cold storage (e.g., −80 °C, −20 °C [60, 140]) but most importantly to preserve product quality for prolonged storage at ambient conditions. From a historical perspective, the interest of lyophilizing naked DNA began a long time back. Sixty years ago, in April of 1953, Watson and Crick made history describing the double-helix structure of DNA [141]. Although a year prior to this significant achievement, investigators had already isolated and handled dried DNA for research purposes [142]. However, early results in the literature on lyophilized DNA are conflicting in terms of the effect of dehydration on the stability of DNA. For instance, some initial dehydration studies reported a stabilizing effect [142, 143], whereas other investigators found that DNA was readily damaged during lyophilization [96, 144, 145]. Of note, DNA purity was uncertain during those early years of nucleic acid lyophilization, and its effect on stability may explain these contradictory findings. In fact, 60 years ago, scientists did not possess the sophisticated technology that is available nowadays in terms of DNA isolation at high purity [59, 137, 146–149]. Furthermore, it has been recently shown that impurities have a detrimental effect on plasmid quality and gene delivery performance [150].

The lyophilization process has been shown to alter the hydration shells around DNA molecules and, ultimately, influence its conformational stability [91]. Indeed, DNA structure is intrinsically dynamic and plasmid DNA under certain conditions (e.g., humidity driven) can assume conformational changes. Dehydration (e.g., lyophilization) has a remarkable influence on the B- (most commonly found DNA conformation under normal physiological conditions) to A-DNA (found under low-hydration conditions) conformational transition [151, 152]. Typically, A- and B-DNA vary in the slope of base pairs to the helical axis, major- and minor-groove proportions, crystal packing, and deoxyribose ring conformation [153–155]. Studies have reported that during lyophilization, DNA loses its native helical structure [91]. An observation that appears to be different for RNA molecules which preserve an A-like structure independently of their local water activity [156]. Although this well-defined DNA B-to-A transition promoted by removal of water is likely reversible after rehydration [80], recent studies have demonstrated that sugars (e.g., trehalose) when employed completely stabilized DNA formulations during the lyophilization process [82, 91, 96]. Furthermore, our recent unpublished studies monitoring the phosphate antisymmetric stretching vibration (i.e., wave number redshift from 1240 to 1223 $\mathrm{cm}^{-1}$) of dried DNA formulations in trehalose through Fourier transform infrared (FTIR) analysis demonstrate that the right-handed B form could be maintained during lyophilization in the presence of sugars. For instance, when DNA is lyophilized in the presence of sufficient amounts of trehalose (i.e., 5 % trehalose to DNA ratio, w/w), we clearly have observed that dry DNA persists in the B-conformation after acute lyophilization, and it is comparable to the hydrated state (Fig. 1, unpublished observations). This finding is consistent with a previous work