At Gene Link two type of 5’-phosphorylation reagents are used; mostly it is CPR-I for oligos that are not destined to be purified based on reverse phase requiring the presence of DMT (dimethoxy trityl) group. The DMT group present on the CPR-I is not retained after deprotection with ammonium hydroxide. CPR-II is used only for oligos that are requested by customers with 5’-phosphorylation DMT ON. These are assumed to be further purified by the customer using the DMT group that is retained after ammonium hydroxide deprotection. The coupling efficiency is nearly the same for both the phosphorylation reagents.

**Application** Oligonucleotides containing a 5’-phosphate group find use in molecular biology for a variety of purposes: e.g., as linkers and adapters, in cloning and gene construction, and in the ligase chain reaction. The venerable T4 polynucleotide kinase has served researchers well by phosphorylating the 5’-terminus using ATP as the phosphate source. A chemical alternative to kinase, the sulfonyl ethyl phosphoramidite, Chemical Phosphorylation Reagent has become increasingly popular over the years since it is convenient to use on the synthesizer and the yield of 5’-phosphate is generally much higher than with kinase. This reagent includes a dimethoxytrityl (DMT) protecting group which can be removed on the synthesizer to allow a determination of phosphorylation efficiency. However, the DMT protecting group can not be used for DMT-on purification. If the DMT group is intentionally left on the oligonucleotide, it is eliminated along with the sulfonyl ethyl group to produce the 5’-phosphate during the ammonium hydroxide deprotection.