# Ligand Controlled Hybridization

**Normalization • Accuracy • Sensitivity**

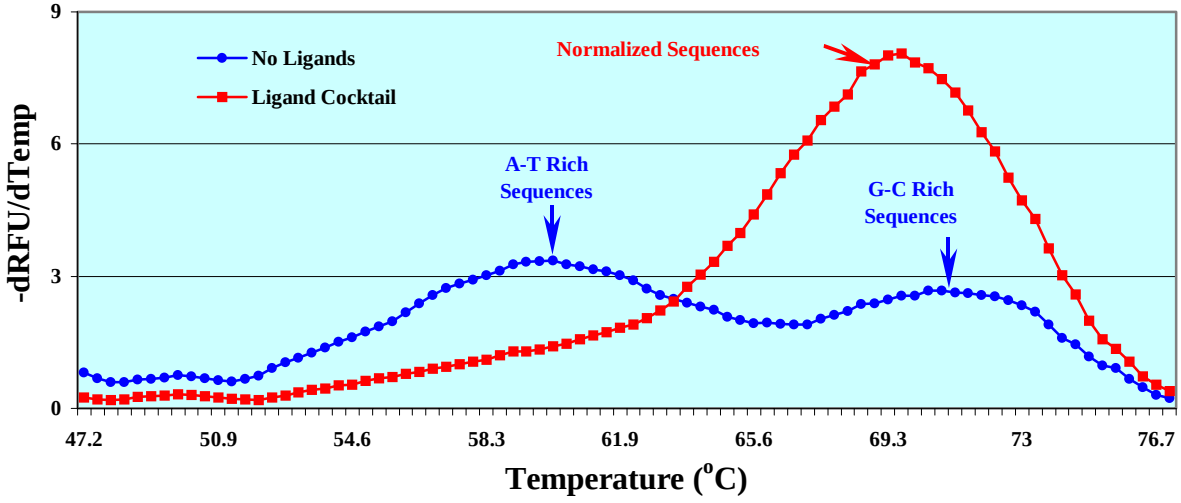
## Introduction

A DNA binding ligand can be defined as any DNA interacting drug or compound which acts to change or modulate the behavior of the DNA with which it comes into contact. Ligands can include DNA binding drugs, such as distamycin, salts of various types (*i.e.*, ammonium ions) or organic solvents such as dimethylsulfoxide (DMSO). TMC's Ligand Controlled Hybridization (LCH) technology uses unique ligands in various experimentally determined combinations to modulate specific hybridization reactions, that is the precise and predictable association of the DNA strands, in many assay formats. These include solution phase procedures such as the polymerase chain reaction (PCR) and solid phase procedures performed in microwells and/or on microarray formats. Particularly, universally designed proprietary ligand cocktails enable the fine control of multiplexed hybridizations essentially normalizing the window in which the nucleic acids bind their cognate sequences. This becomes essential, for example, as the number of probe sequences on array platforms increases. Since the complexity of complementary target mixtures also increases dramatically, the potential for mismatched hybridization (i.e. false positives) becomes a likely possibility. This is evident in mixed populations where differences in A-T and G-C content cause severely compromised optimal hybridization temperatures.

## Normalization of Complex Hybridizations using Ligand Mixtures

Tm's ligand mixtures are composed of proprietary DNA binding compounds which bind to and stabilize relatively unstable stretches of A-T sequences allowing them to behave similar to the more stable G-C rich sequences. A simple illustrative example of this "normalization" effect is shown in **Figure 1**. In the absence of ligands the A-T duplex will melt at a lower temperature relative to a G-C rich duplex. By implementing a ligand cocktail the two peaks merge and melt as one.

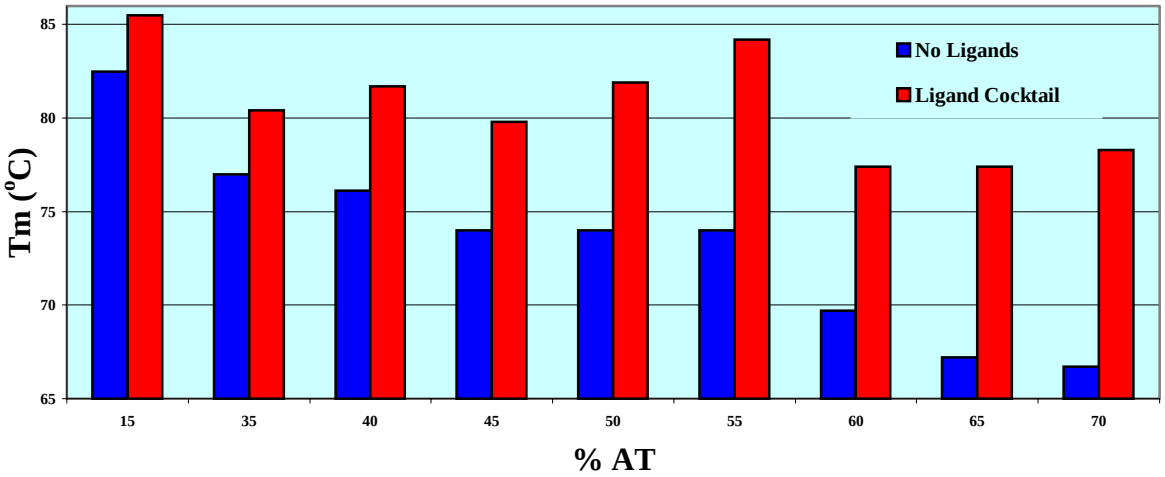
**Figure 1. Effect of Ligand Cocktail on AT and GC Primers**



## Normalization of p53 Gene Probes Using Ligand Mixtures

In a second example nine duplex primer sequences spanning the tumor suppressor -p53- cDNA sequence were randomly selected whose A-T content ranged from 15-70%. The sequences were subjected to thermal dissociation in the presence and absence of ligands and the Tm for each duplex was ascertained from the first derivative values of the melting curve. The compiled data is shown in **Figure 2**.

**Figure 2. Ligand Cocktail Effect on Native p53 Duplexes**




As shown above, the Tm in the absence of ligands ranged from 66.7 to 82.5 (span = 15.8 $^{o}$C), whereas in the presence of ligands the melting temperature ranged from 77.4 to 85.5 $^{o}$C (span = 8.1$^{o}$ C). These data illustrate that ligands can effectively tighten the thermal dissociation window of complex mixtures of nucleic acids thereby enabling hybridization reactions to occur under more uniform conditions. An added benefit of ligand modulated hybridization is the apparent elevated Tm of the duplexes. This allows for higher hybridization temperatures to be used, thereby ensuring stringency control.

### Conclusions

Ligand cocktails allow for uniformly modulated hybridization performance of complex nucleic acid mixtures. This allows for thermodynamically enhanced hybridization specificity and sensitivity. Moreover, optimal ligand cocktails have the added ability to minimize cross talk or the likelihood of generating false positive signals. With these distinctive characteristics, TMC's ligand modulated hybridization technology provides a powerful solution to problems related to accuracy, consistency and reliability in data obtained from DNA array and non-array platforms.
Other ligand cocktails available from Tm Bioscience Corporation include mismatch discrimination mixtures and mismatch normalization mixtures.

### Status

This technology is covered in part in the following United States patents: No. 5,593,834, 6,027,884 and 6,221,589. Additional patents are pending. Companies interested in licensing this technology or discussing the development of custom ligand cocktails should contact:
Dr. Jeremy Bridge Cook, VP Business Development at (416)-593-4323 ext. 229 or by e-mail at bridgecook@tmbioscience.com.