DNASCRIPT

SYNTAX system enables rapid probe synthesis and testing to accelerate custom qPCR assay development

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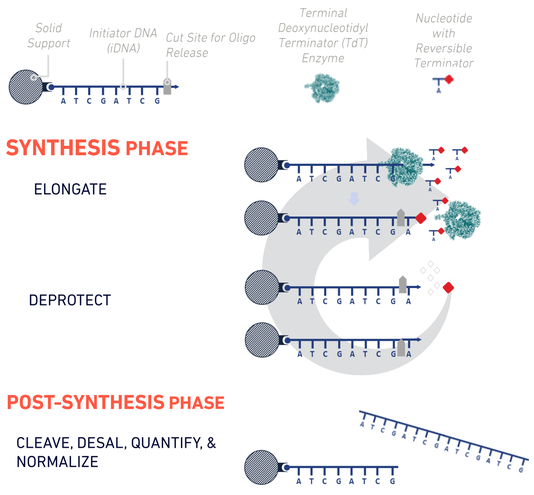
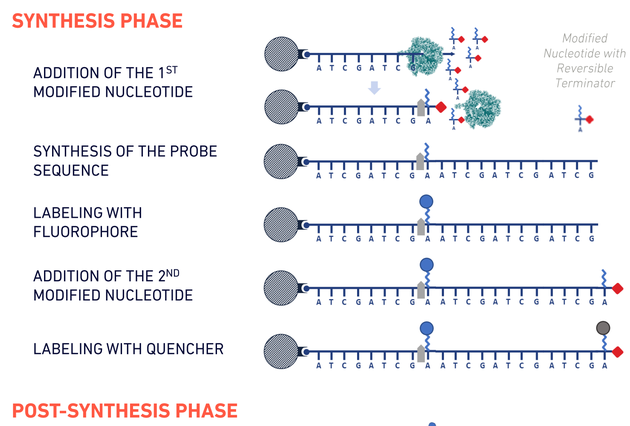
Overview of EDS Oligo Synthesis Process



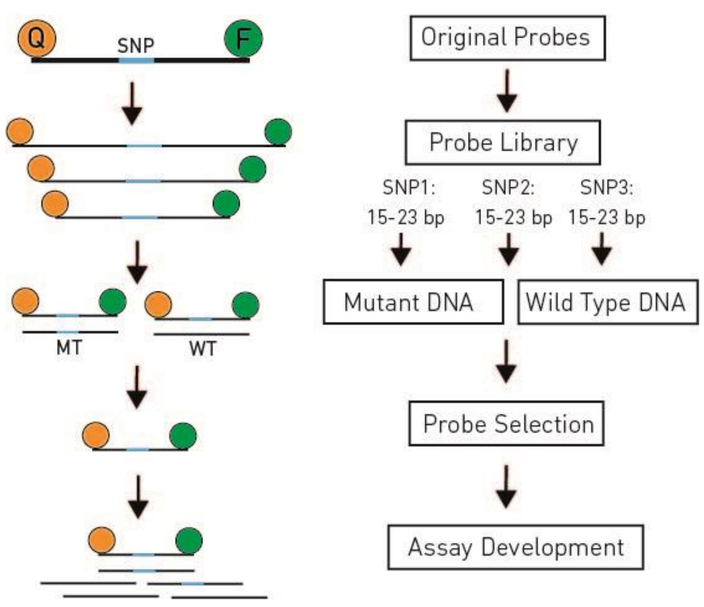
INTRODUCTION

- A dual-labelled probe consists of a small oligonucleotide with a fluorophore and quencher covalently attached to opposite ends of the probe
- Currently probes must be ordered from a commercial vendor with researchers often **waiting weeks to months** for their probes to arrive
- The SYNTAX system, powered by Enzymatic DNA Synthesis (**EDS**) technology, opens access to the **overnight** synthesis of **probes and primers** in the convenience of a researcher's labs
- Here we demonstrate the utility of the SYNTAX system to rapidly design, test and **improve probe performance**
- By providing the user with **complete control** over their oligo synthesis pipeline the SYNTAX system can ensure consistent productivity and **accelerate** the iterative design-build-test cycle

Overview of Probe Synthesis Process



Experimental Overview



- Probe libraries and primers designed and printed over night
- Background fluorescence measurements are taken to determine **QUENCHING**
- Probes tested against mutant and wild type DNA to determine **SPECIFICITY**
- Optimal probe design selected based on **MAXIMUM QUENCHING and SPECIFICITY**

Probe Optimization: Quenching and Specificity

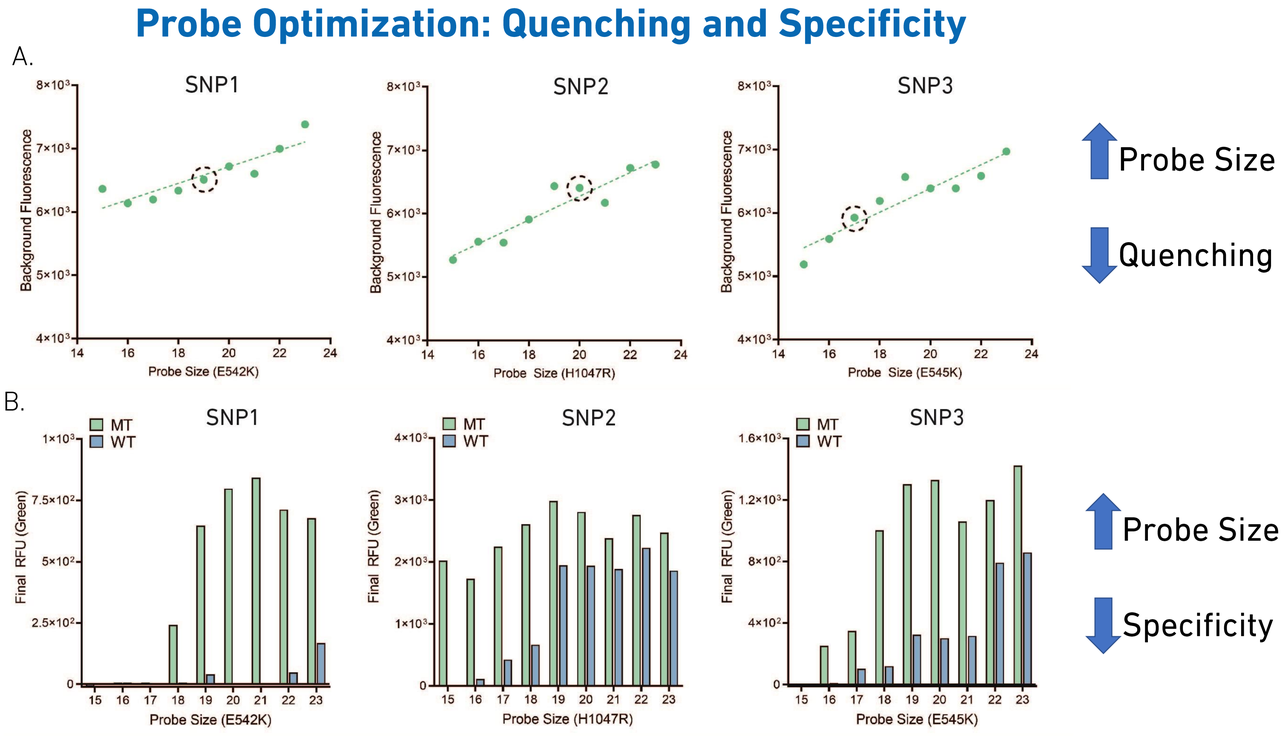
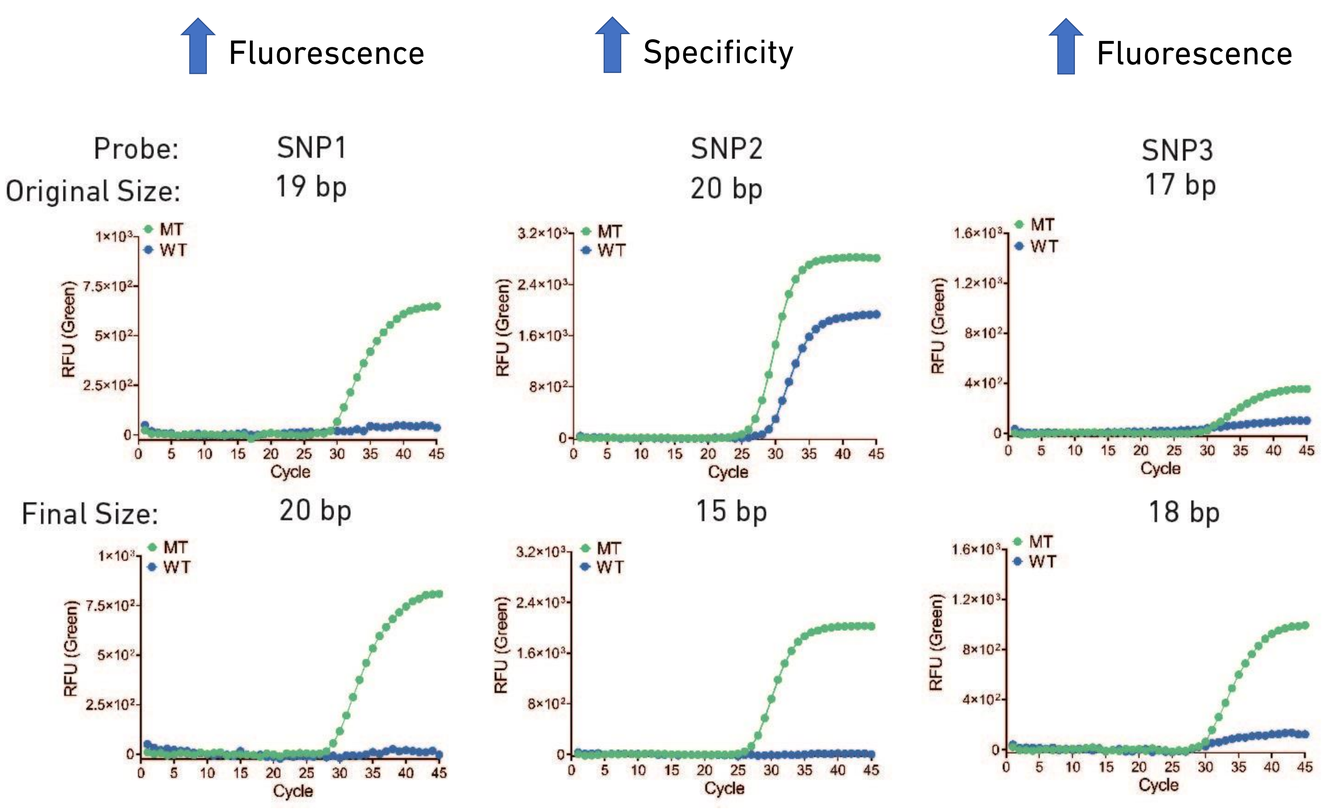


Figure 1. Probe design and selection overview. Three probes targeting three SNP's of the PI3K gene were taken from Keraite et al. 2020. Additional nucleotides were added/subtracted from the original probe design to create a probe library. The three probe libraries were then printed overnight and each probe was tested against mutant and wild type DNA (positive and negative controls, respectively). SNP1 = E→K mutation at position 542 (E542K), SNP2 = H→R mutation at position 1047 (H1047R) and SNP3 = E→K mutation at position 545 (E545K). Q=Quencher (orange circle), F = fluorophore (green circle) and the light blue line represent the target SNP.

Figure 2. A. Background probe fluorescence. Green dots indicate the mean fluorescent value of replicate qPCR reactions (n=3) with green dotted lines indicating the line of best fit as determined by linear regression analysis. R^2 = 0.34, 0.67, 0.79 for mutational targets E542K, H1047R, and E545K, respectively. The original probe size from Keraite et al. 2020 is indicated by the dotted black circle. B. Probe specificity (Final fluorescence). Final RFU values, target SNP (MT, green) and wild-type DNA (WT, blue), for each probe following 45 cycles of qPCR cycling. Each bar represents the mean RFU value of replicate qPCR reactions (n=4).

Improved Performance vs. Original Probe Design



Choose Your Fluorophore!

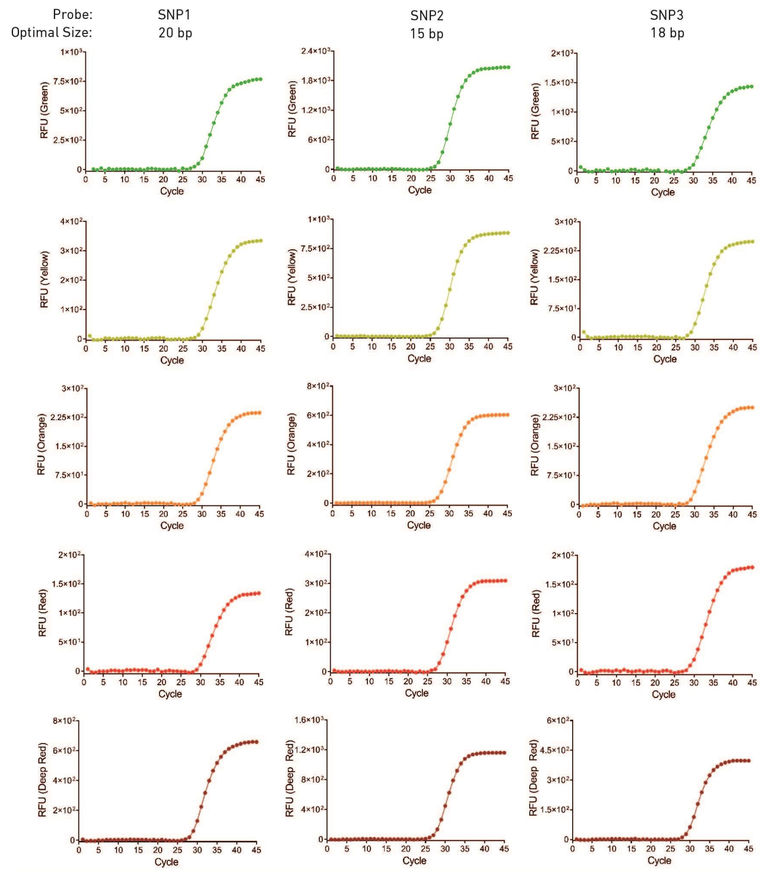


Figure 3. Probe specificity (Amplification curves). Amplification curves were generated from qPCR reactions, SNP (MT, green) and wild-type DNA (WT, blue), containing the original probe size (upper panel) and final probe size (lower panel) are displayed. Each dot represents the mean RFU value of replicate qPCR reactions (n=4).

Figure 4. Performance with different fluorophores. Amplification curves for qPCR reactions with mutant template DNA containing the target SNP of interest with optimal probe size, for each of the 3 target mutations, using probes with 5 different fluorophores. Each dot represents the mean RFU value of replicate qPCR reactions (n=4).

CONCLUSIONS

- The starting point of any custom assay development pipeline is the identification of a probe (or set of probes) exhibiting optimal performance within a specific set of experimental conditions
- Here we have demonstrated how the SYNTAX system supports the rapid design, testing and improvement of probe performance for future assay development
- By implementing modifications on the SYNTAX System, we enable fully automated printing of custom labelled probes as a benchtop lab solution giving any research or diagnosis lab overnight access to their probes

- Fully automated, walk-away synthesis
- Plug-and-play integration
- Flexibility for up to 96 oligos per run
- Modify oligos with fluorophores, quenchers, and biotin
- 15-minute setup time per run
- Same-day synthesis of 15 - 80 nt oligos
- Synthesize oligos overnight for next-day use
- Custom iDNA length: 1 - 45 nt
- Default 5'-phosphate
- Up to 600 pmol (no mods), up to 400 pmol (with mods)
- 5-7 µM (no mods), ≥ 5 µM (with mods)