# NMG Meeting 2011

**Scott D. Rose Ph.D., Director Of Molecular Genetics**

**Integrated DNA Technologies**

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# Objectives

1. Present the latest advances in qPCR assay design and implementation.

2. Educate the attendees on newly improved methodologies for genotyping

3. Illustrate new options for obtaining primers and probes from an ISO 13485:2003 certified Clinical and Commercial Manufacturing (CCM) facility.

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# Introducing PrimeTime Pre-designed qPCR Assays

- Pre-designed assays are available for human, mouse and rat transcriptomes (excluding miRNAs)
  - Designed using state of the art melting temperature prediction algorithms
  - Sequence information obtained from current Ref Seq build
  - Not a static design, updated as new Ref Seq builds are released
  - Assay designs archived and easily reordered by assay ID number
  - Probes incorporate new ZEN dual quenched configuration
  - Assay information includes details on common or unique transcripts detected, and whether the assay is susceptible to genomic DNA contamination
  - Full disclosure of assay details, including primer and probe sequences

- Assays are designed to
  - Avoid SNPs locations (Entrez SNIP database)
  - Eliminate off target amplification
  - Eliminate non-specific amplification due to primer interactions
  - Reduce impact from secondary structure formation

- Simple ordering tool
  - Filter to quickly view common or transcript specific assays
  - Sort by recommended assays
  - Select reaction size, dye-quencher, or primer to probe ratio
  - Group by species, gene symbol or refseq number for quick assay selection

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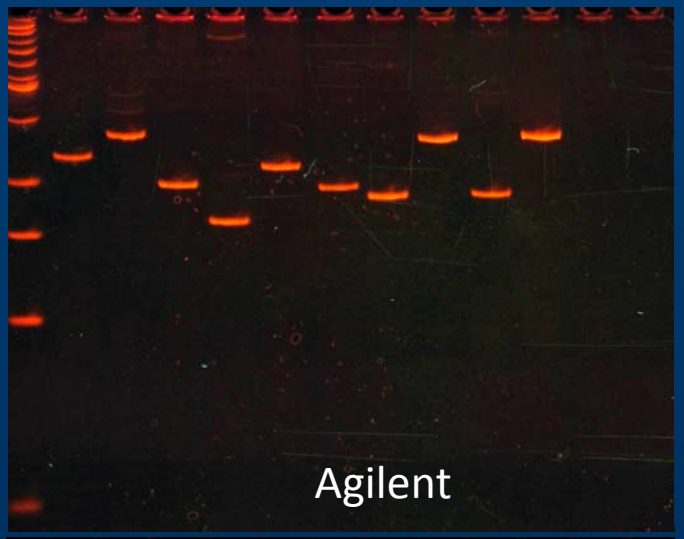
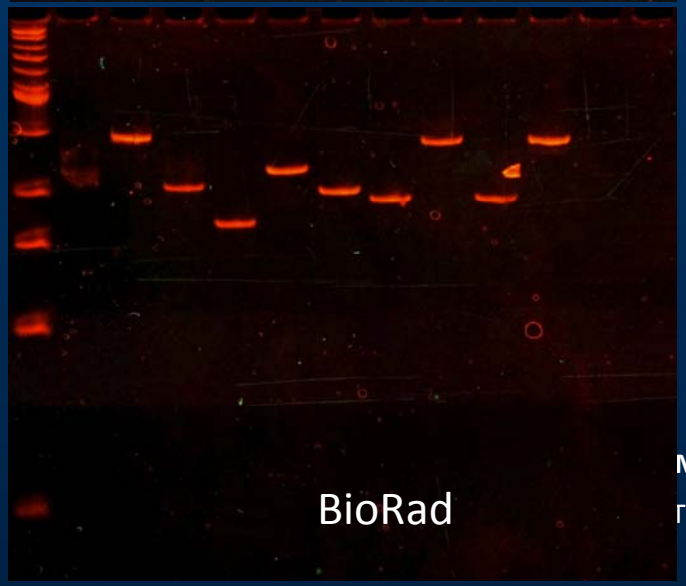
# Design Emphasis Is On Primers As Well As The Probe

**PrimeTime predesigned assays focus on primer performance as well as a great probe**

**10 assays were randomly chosen and the primers were used with SYBR Green based qPCR mixes from two different manufacturers, and cDNA made from oligo dT/random hex primed human universal reference RNA. The PCR products were run out on a native acrylamide gel and were visualized with Gel Red nucleic acid gel stain.**

**Primer sets yielded single amplicons**

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# Improved Probe Performance-ZEN Dual Quenched Probes

## ZEN dual quenched probes are hydrolysis probes with improved performance

5’-Fluorophore --------- 3’-Quencher

Ways to improve quenching

1) Force reporter and quencher into proximity (Molecular Beacons, Scorpions, etc)
2) Make probes shorter (even with LNA or MGB, it is hard to get probes much shorter than 14-18 bases)

5’-Fluorophore --------- Internal Quencher

1) Place quencher internally on dT base (need a “T” nearby)
2) Place quencher between bases (destabilizing Tm effect?)

Internal quenching has many benefits, if the problem of destabilization can be solved

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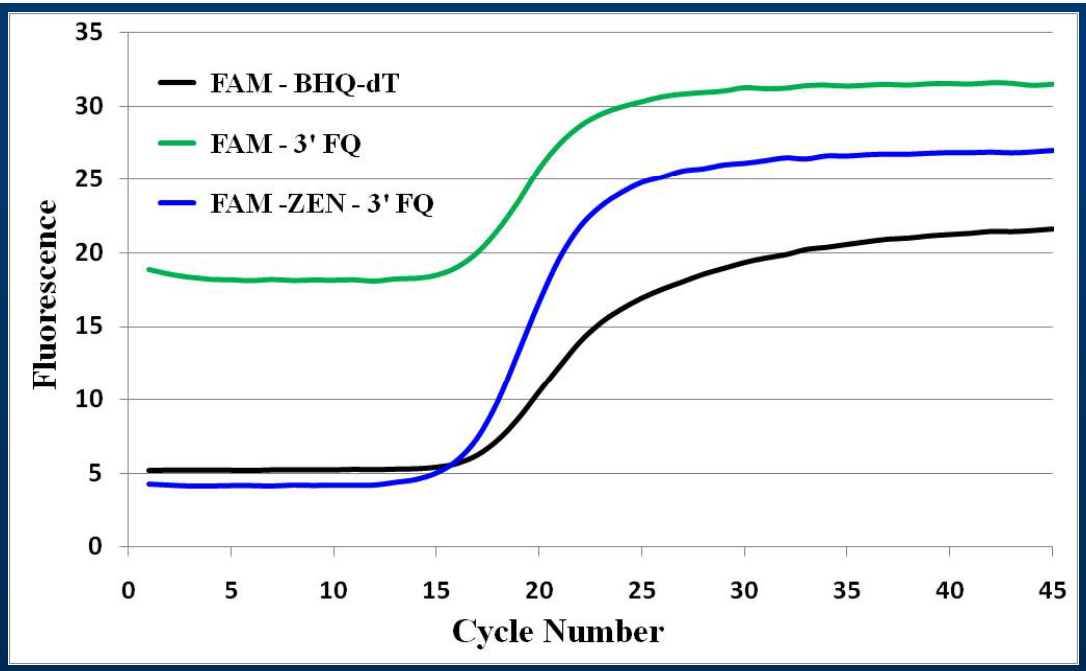
# ZEN-FQ Performance using CDC Swine-Flu Probe

FAM-CAGAATATACATCCAGTCACAATTGGAAAA-FQ
FAM-CAGAATATA/ZEN/CATCCAGTCACAATTGGAAAA-FQ
FAM-CAGAATATACA/BHQ-dT/CCAGTCACAATTGGAAAA-x




**As expected, internal quencher placement dramatically improves quenching in a long probe like this**

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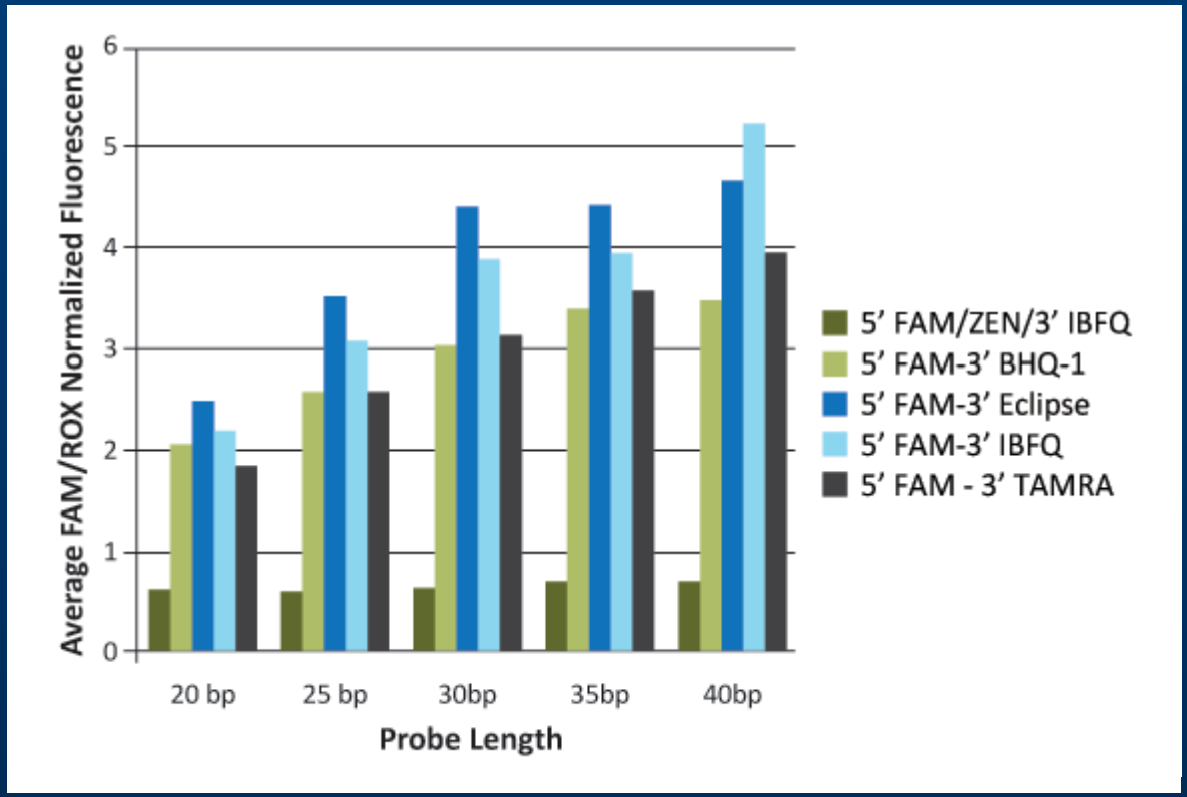
# ZEN Dual Quenched Probe Design Allows Use Of Longer Probes




**Using internal ZEN, the efficiency of quenching does not change as probes length increases. This allows for placement of the probe in A/T rich areas and still be able to achieve a sufficiently high Tm for qPCR.**

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# Improved Genotyping With LNA Fluorescent Probes in qPCR

**Designing qPCR assays that will discriminate between two sequences that vary by a single nucleotide can be challenging. One reason is a lack of availability of design algorithms that faithfully predict the energetics of the interactions between a DNA probe and its correct target versus a mismatch target.**

**Of the probe based detection methods (Taqman MGB, Molecular Beacons, or other linear based probes) IDT has developed an algorithm that utilizes Locked Nucleic Acid bases to design optimally discriminating primers or probes.**

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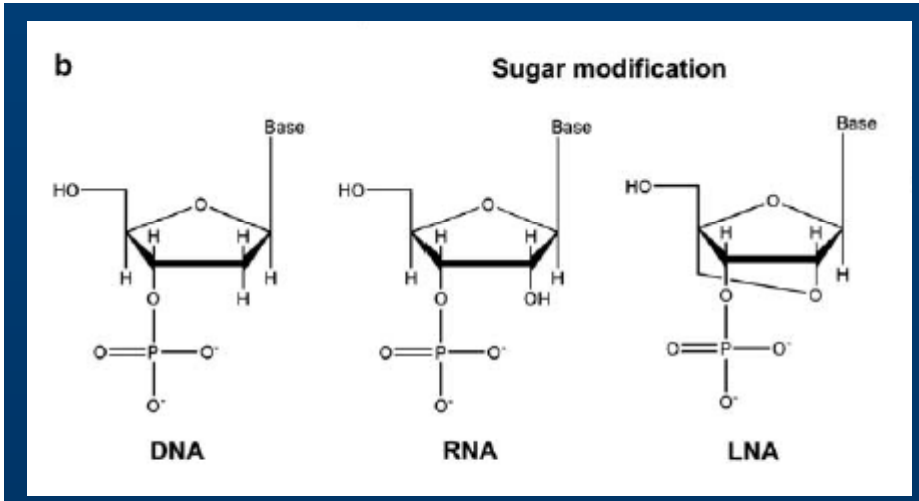
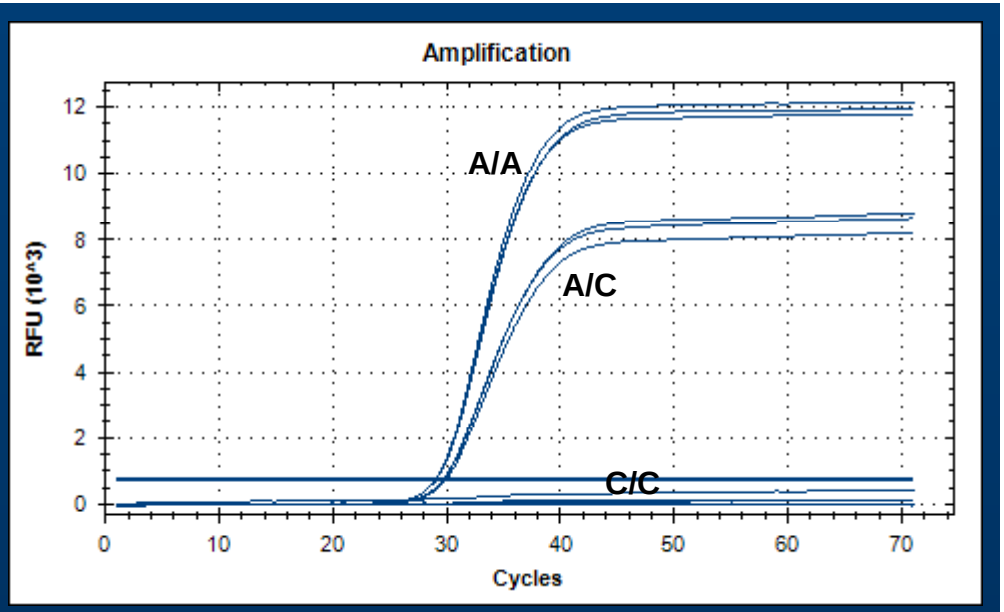
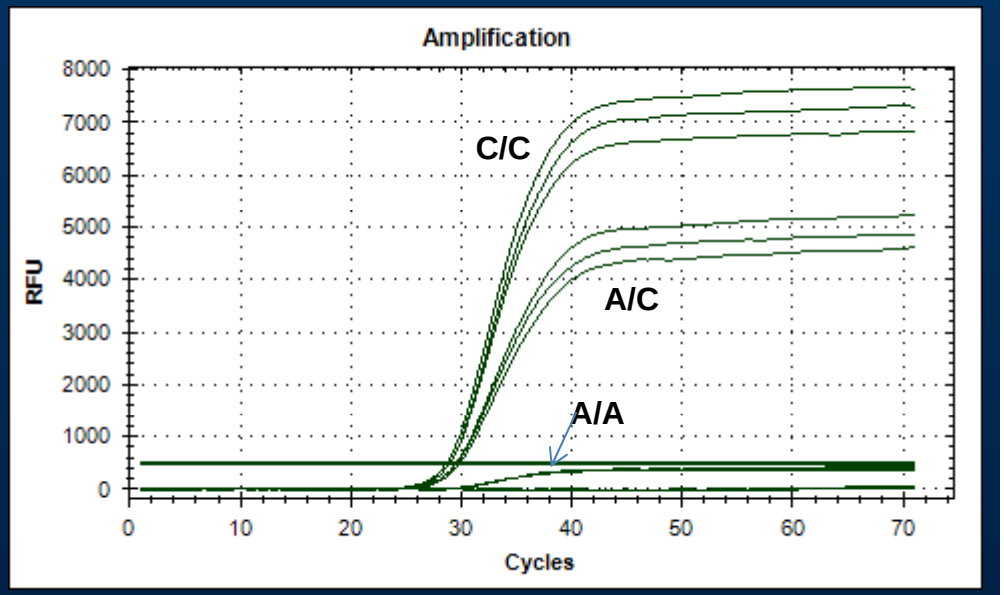
# Probe Specificity Using LNA Based Probes




**rs1042044 (A/C) snp genotyping using a FAM or HEX labeled LNA containing probe and Coriell genomic DNAs.**

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# Tools For Calculating Tm Of Match Vs Mismatch For LNA Containing Probes

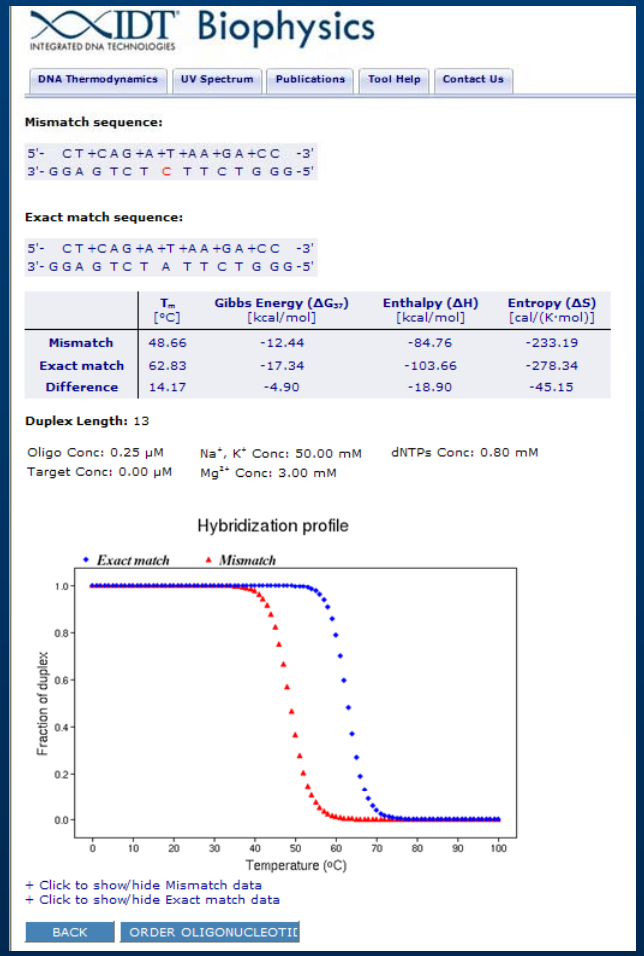
**LNA containing probes (antisense strand)**

| | Match | Mismatch | ΔTm (°C) |
|---|---|---|---|
| rs1042044 FAM A<br>CT+CAG+A+T+AA+GA+CC | 62.8 | 48.7 | 14.2 |
| rs1042044 HEX C<br>T+CAG+A+G+AA+GA+CC | 62.3 | 43.8 | 18.5 |

(+ indicates LNA base)

http://biophysics.idtdna.com/

Additional links
http://www.owczarzy.net/biodata.htm

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BIOCHEMISTRY
including biophysical chemistry & molecular biology

Article

pubs.acs.org/biochemistry

# Stability and Mismatch Discrimination of Locked Nucleic Acid–DNA Duplexes

Richard Owczarzy,* Yong You, Christopher L. Groth, and Andrey V. Tataurov

Department of Molecular Genetics and Biophysics, Integrated DNA Technologies, 1710 Commercial Park, Coralville, Iowa 52241, United States

Published online 10-10-2011

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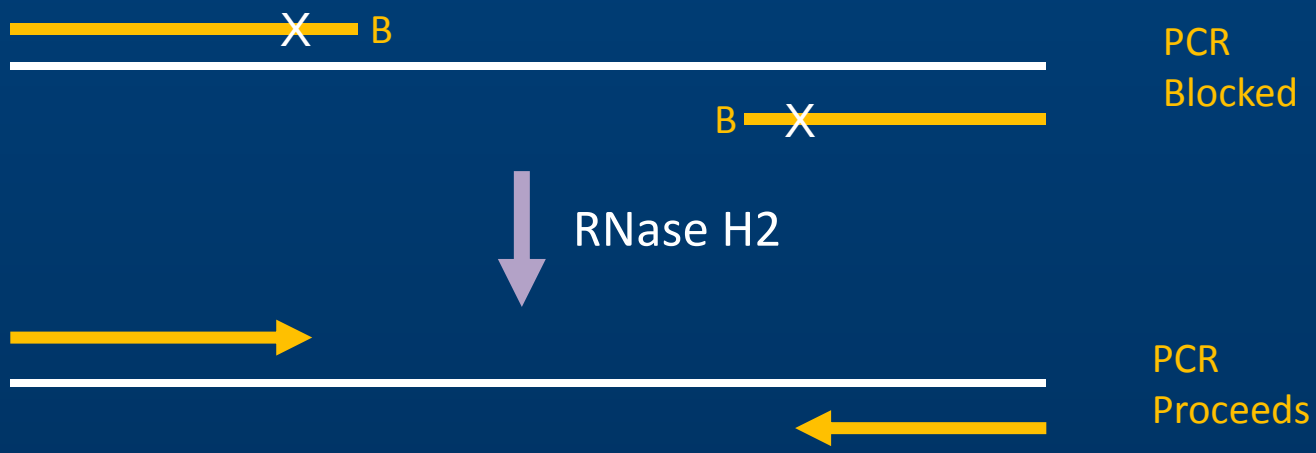
# Novel RNase H2 Based rhPCR




1) Primer deblocking is required for PCR, which in turn requires that primers be annealed to the target DNA sequence.

2) The deblocking enzyme is inactive at low temperature. "Hot start" is achieved without need for a modified "hot start" polymerase.

3) The enzymatic deblocking cleavage event is sensitive to base mismatch and confers added specificity to the ensuing PCR reaction.

4) Primer-dimer formation is prevented.

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Dobosy *et al. BMC Biotechnology* 2011, **11**:80
http://www.biomedcentral.com/1472-6750/11/80

BMC Biotechnology

METHODOLOGY ARTICLE Open Access

# RNase H-dependent PCR (rhPCR): improved specificity and single nucleotide polymorphism detection using blocked cleavable primers

Joseph R Dobosy, Scott D Rose, Kristin R Beltz, Susan M Rupp, Kristy M Powers, Mark A Behlke* and Joseph A Walder

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# The CCM Overview

- **IDT's ISO 13485:2003 certified Clinical and Commercial Manufacturing (CCM) suite:**
  - **Parallels IDT's proven research line (a facility within a facility) and positions IDT to better serve both our current and future clinical/ medical device customers**
  - **Specializes in oligonucleotides for *clinical* applications, and *commercial* products requiring customer-defined and controlled processes and deliverables (e.g., yields, purities, packaging and labeling, certificates of analysis).**
  - **Registered with US FDA a contract manufacturer for for In-vitro diagnostic devices (IVD / ASR) & nucleic acid tests (NAT)**
- **CCM service includes dedicated ordering, production, final-fill, and shipping systems that meet the elevated quality standards required for the products' use in clinical or mission-critical commercial applications .**

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## The CCM Infrastructure/Facility

- 3100 $Ft^2$ suite including CCM Production, 2 final-fill labs, and an analytical lab
- Production, processing and final fill areas are ISO 14644 Class 8 or lower
- DNase & RNase–free in all four labs
- Restricted card-key access to CCM Suites
- Customer end use awareness training for all personnel involved in manufacture of CCM oligos
- Validated equipment and software with electronic batch record capability
- Extensive line clearance consideration
- Retain lot and stability programs available
- Company–wide template contamination mitigation plan

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# CCM Production/Capacity

- Capability to provide 10nmole to 1 gram final deliverable utilizing dedicated production equipment
- Opportunity to optimize the production process specific to customers' needs and specifications
- Standard Desalt or HPLC purification available
- Freedom Dye™ program: Royalty- & license-free fluorescent dye labeled oligos for use in:
  - Kits or oligos for resale
  - Diagnostic/clinical products
  - Commercial services
  - Non-human in-vivo applications
- Multiple final-fill options and OEM solutions provider with services including:
  - Full range of options for third parties, including: customer specified formulation, packaging, third party labeling, stability testing and functional QC.

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# Contact Information

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