

# Competitive Analysis

PCR Reagents

# Introduction

Based on the insights from discovery interviews with 10 industry scientists and 10 academic/internal scientists, Product, Science, and RnD regrouped around the user challenges and prioritized the following 3 focus areas for launch ([raw workshop sheet](#)):

- 1. Selecting new control genes to use in a qPCR experiment when switching experimental context (ie: tissue, cell type)**
  - Most frequently observed challenge: experienced by 5/9 NVS interviewees
  - Is currently tackled using the published literature and/or trial and error
- 2. Identifying qPCR primers/probe products or sequences that have been used in the published literature for a gene, and in a particular cell type/tissue**
  - 3 scientists would turn to the literature to see what works best, and/or trial and error with custom design
- 3. Primer selection for conventional PCR applications**
  - While academics experienced challenges here, only 2 interviewees used conventional PCR and limited challenges were experienced by them - this does not mean there aren't any challenges in industry, but just that they haven't yet been uncovered

**This competitive analysis is therefore focused on the 3 areas identified.**

# Executive Summary

# Consolidated Takeaways

To see the full analysis, continue to the next slide. The following are quick links to the key takeaways for each of the identified focus areas, and the next steps:

1. [Selecting new control genes to use in a qPCR experiment when switching experimental context \(ie: tissue, cell type\)](#)
2. [Identifying qPCR primers/probe products or sequences that have been used in the published literature for a gene, and in a particular cell type/tissue](#)
3. [Primer selection for conventional PCR applications](#)
4. [Next Steps](#)

# Analysis



1

**Selecting new control genes to use in a qPCR experiment when switching experimental context (ie: tissue, cell type)**

In a search on IDT for pre-designed qPCR assays, IDT also provides a selection of reference gene assays for rat, mouse and human species. This could be helpful resource for scientists who are looking for housekeeping genes to use while purchasing qPCR probes and primers

## Reference Gene Assays

- ✓ Housekeeping - Rat
- Housekeeping - Mouse
- Housekeeping - Human

### GET REFERENCE GENE ASSAYS

Housekeeping - Human

	Gene Query <sup>1</sup>	NCBI Gene Symbol <sup>1</sup>	Ref Seq # <sup>1</sup>	Detects All Variants <sup>1</sup>	Exon Location <sup>1</sup>	Assay Configuration	
<input type="checkbox"/>	Rn.PT.39a.22214832	Hprt1	Hprt1	NM_012583(1)	Yes	7 - 9	Std, FAM/ZEN/IBFQ
<input type="checkbox"/>	Rn.PT.39a.22214834	B2m	B2m	NM_012512(1)	Yes	1 - 2	Std, FAM/ZEN/IBFQ
<input type="checkbox"/>	Rn.PT.39a.22214837	Tbp	Tbp	NM_001004198(1)	Yes	6 - 7	Std, FAM/ZEN/IBFQ
<input type="checkbox"/>	Rn.PT.39a.22214822.g	Gusb	Gusb	NM_017015(1)	Yes	1 - 1	Std, FAM/ZEN/IBFQ
<input type="checkbox"/>	Rn.PT.39a.22214838.g	Actb	Actb	NM_031144(1)	Yes	4 - 5	Std, FAM/ZEN/IBFQ
<input type="checkbox"/>	Rn.PT.58.35295130	Polr2a	Polr2a	XM_001079162(4)	Yes	19 - 20 <sup>1</sup>	Std, FAM/ZEN/IBFQ
<input type="checkbox"/>	Rn.PT.39a.22214840	Rplp0	Rplp0	NM_022402(1)	Yes	1 - 2	Std, FAM/ZEN/IBFQ
<input type="checkbox"/>	Rn.PT.39a.22214830	Ppia	Ppia	NM_017101(1)	Yes	2 - 4	Std, FAM/ZEN/IBFQ

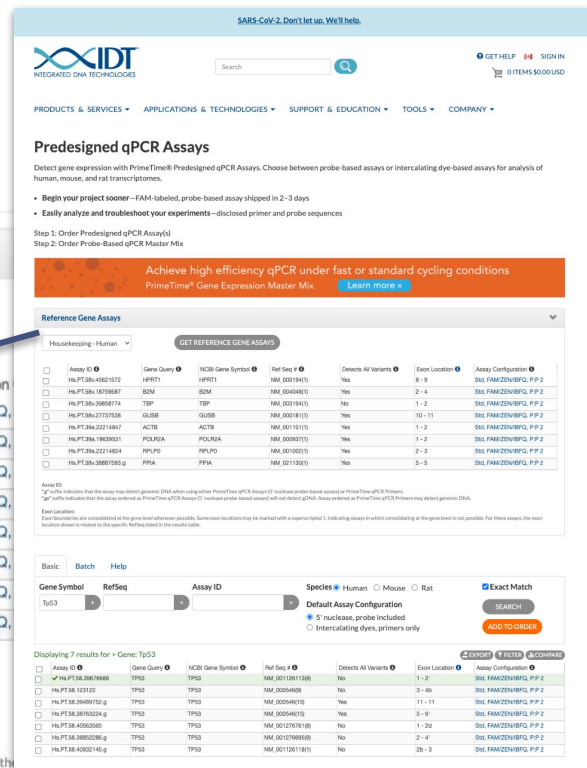
#### Assay ID:

\*g\* suffix indicates that the assay may detect genomic DNA when using either PrimeTime qPCR Assays (5' nuclease probe-based assays) or PrimeTime qPCR Primers.

\*gs\* suffix indicates that the assay ordered as PrimeTime qPCR Assays (5' nuclease probe-based assays) will not detect gDNA. Assay ordered as PrimeTime qPCR Primers may detect genomic DNA.

#### Exon Location:

Exon boundaries are consolidated at the gene level whenever possible. Some exon locations may be marked with a superscripted 1, indicating assays in which consolidating at the gene level is not possible. For these assays, the location shown is related to the specific RefSeq listed in the results table.



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### Predesigned qPCR Assays

Detect gene expression with PrimeTime® Predesigned qPCR Assays. Choose between probe-based assays or intercalating dye-based assays for analysis of human, mouse, and rat transcriptomes.

- Begin your project sooner—FAM-labeled, probe-based assay shipped in 2-3 days
- Easily analyze and troubleshoot your experiments—disclosed primer and probe sequences

Step 1: Order Predesigned qPCR Assay(s)  
Step 2: Order Probe-Based qPCR Master Mix

**Achieve high efficiency qPCR under fast or standard cycling conditions**  
PrimeTime® Gene Expression Master Mix [Learn more >](#)

#### Reference Gene Assays

Housekeeping - Human GET REFERENCE GENE ASSAYS

Assay ID	Gene Query	NCBI Gene Symbol	Ref Seq #	Detects All Variants	Exon Location	Assay Configuration
<input type="checkbox"/> HLPT.58.45821572	HPRT1	HPRT1	NM_000184(1)	Yes	8 - 9	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.1075957	ESM	ESM	NM_004848(1)	Yes	2 - 4	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.3880771	TBP	TBP	NM_003141(1)	Yes	1 - 2	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.27737338	GUSB	GUSB	NM_000181(1)	Yes	10 - 11	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.22214847	ACTB	ACTB	NM_001181(1)	Yes	1 - 2	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.1680511	POLR2A	POLR2A	NM_005810(1)	Yes	1 - 2	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.22214831	RPLP0	RPLP0	NM_001501(1)	Yes	2 - 3	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.38807933.g	PP1A	PP1A	NM_001133(1)	Yes	5 - 5	Std, FAM/ZEN/IBFQ, P.P.2

Assay ID:  
\*g\* suffix indicates that the assay may detect genomic DNA when using either PrimeTime qPCR Assays (5' nuclease probe-based assays) or PrimeTime qPCR Primers.  
\*gs\* suffix indicates that the assay ordered as PrimeTime qPCR Assays (5' nuclease probe-based assays) will not detect gDNA. Assay ordered as PrimeTime qPCR Primers may detect genomic DNA.

Exon Location:  
Exon boundaries are consolidated at the gene level whenever possible. Some exon locations may be marked with a superscripted 1, indicating assays in which consolidating at the gene level is not possible. For these assays, the exon location shown is related to the specific RefSeq listed in the results table.

Basic Batch Help

Gene Symbol RefSeq Assay ID Species Human Mouse Rat Exact Match SEARCH  
Tp53 Default Assay Configuration  
5' nuclease, probe included  
Intercalating dyes, primers only ADD TO CART

Displaying 7 results for > Gene: Tp53

Assay ID	Gene Query	NCBI Gene Symbol	Ref Seq #	Detects All Variants	Exon Location	Assay Configuration
<input type="checkbox"/> HLPT.58.38816868	TP53	TP53	NM_001128(1)	No	1 - 2 <sup>1</sup>	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.122122	TP53	TP53	NM_000468(1)	No	2 - 4b	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.38489792.g	TP53	TP53	NM_000468(1)	Yes	11 - 11	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.38703224.g	TP53	TP53	NM_000468(1)	Yes	5 - 6 <sup>1</sup>	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.4000365	TP53	TP53	NM_001179(1)	No	1 - 2b	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.38816286.g	TP53	TP53	NM_001179(1)	No	2 - 4	Std, FAM/ZEN/IBFQ, P.P.2
<input type="checkbox"/> HLPT.58.40001415.g	TP53	TP53	NM_001128(1)	No	2b - 3	Std, FAM/ZEN/IBFQ, P.P.2

Thermo Fisher also provides a similar feature when searching for TaqMan probes - in the product card for the probe, there is a section for “Related Controls” where a scientist can see possible control genes they could use, with an option to purchase the assay as well.

The screenshot shows a search results page for TaqMan probes. The top navigation bar includes tabs for Select, Assay ID, Gene, Transcripts, Assay Design, Amplicon Length, and Price. Below this, a table lists various assays. The first assay is Hs01034249\_m1, which targets TP53. It has a catalog number of 4331182 and is for Human target species. The assay design is 15 RefSeq (NM) and the amplicon length is 108. The dye is FAM-MGB and the size is S: 250 rxns. The availability is 'Inventoried' and the price is 268.00 CAD. There are links for 'View Details', 'Related Reagents', 'Related Controls', 'View Assay Map', and 'Related Arrays'. A red 'Add to cart' button is also present.

Below the table, there is a section for 'Single Tube Controls'. It lists several control genes with their respective assay IDs and prices. The first control is ACTB, with assay ID Hs99999903\_m1, priced at 113.00 CAD. The second control is GAPDH, with assay ID Hs03929097\_g1, priced at 113.00 CAD. Each control has a 'Check your price' link and an 'Add to cart' button.

At the bottom, there is a section for 'Plated Controls' with columns for Plate Name, Format, Size, and SKU.

The screenshot shows a detailed product card for the GAPDH assay. The top navigation bar includes links for Home, Search Tool, Search Results, and Hs03929097\_g1. Below this, there is a link to 'See other GAPDH GE Assays'. The main section contains the following information:

- Gene Symbol:** GAPDH
- Gene Name:** glyceraldehyde-3-phosphate dehydrogenase
- Gene Aliases:** G3PD, GAPD, HEL-S-162eP
- Chromosome Location:** Chr:12: 6534405 - 6538375 on Build GRCh38
- UniGene ID:** Hs.544577
- Species:** Human
- Species Specific ID (Flybase ID):** -

On the right side, there is a summary section with the following information:

- Assay ID:** Hs03929097\_g1
- Size:** FAM-MGB | S: 250 rxns
- Availability:** Inventoried
- Catalog #:** 4331182
- Price (CAD):** 268.00
- Check your price:** (link)
- Add To Cart:** (button)

At the bottom, there are links for Genomic Map, Assay Details, and More Information.





Currently, scientists often turn to search engines to determine housekeeping genes to use. Since the information is limited on vendor sites, often forums and literature help scientists find housekeeping genes they can use in their study of interest. We've heard scientists search for: cell line, cell type, "housekeeping gene", "qPCR", "PCR", tissue type, species, treatment (ie: compound/drug/cytokine), "heart transplant", "CAR-T"

Google hek293t housekeeping gene pcr

About 449,000 results (0.59 seconds)

Including results for **hek293** housekeeping gene pcr  
Search only for hek293t housekeeping gene pcr

Scholarly articles for **hek293t housekeeping gene pcr**

**Housekeeping gene** selection advisory: ... - Sikand - Cited by 76  
... human cell types based on **housekeeping gene** ... - Oyulu - Cited by 6  
... 23a~ 27a~ 24-2 cluster induced apoptosis in **HEK293T** ... - Chhabra - Cited by 70

<https://pubmed.ncbi.nlm.nih.gov> › ...  
**Normalization of Gene Expression by Quantitative RT-PCR in ...**  
by F Adeola · 2018 · Cited by 5 — Methods: In this study, twelve commonly used human **reference genes** were investigated in Human Embryonic Kidney Cell Lines (HEK293) using real-time ...

<https://www.ncbi.nlm.nih.gov> › articles › PMC6308757  
**Normalization of Gene Expression by Quantitative RT-PCR in ...**  
by F Adeola · 2018 · Cited by 5 — In this study, twelve commonly used human **reference genes** were investigated in Human Embryonic Kidney Cell Lines (HEK293) using real-time qPCR with ...  
[Abstract](#) · [Introduction](#) · [Materials and Methods](#) · [Results](#)

<https://www.researchgate.net> › post › What\_are\_a\_sulta...  
**What are a suitable housekeeping genes for qPCR in ...**  
What is the best **housekeeping gene** for 293T cells in western blotting? ... What is the optimum **HEK 293T** cell seeding cells/cm2 for large scale culture and lentiviral production? ... These are not **PcR** products, they are plasmids from miniprep.  
**5 answers** · 0 votes: GAPDH serves as a good control for qPCR

Published  
Literature

Forums

## Forums

Question Asked 2nd Jun, 2015



Anna Vyborova  
University Medical Center Utrecht

What are a suitable housekeeping genes for qPCR in HEK293FT cells?

I'm running qPCR in HEK293FT cells knocked out with CRISPR/Cas9 for several genes, can anyone give an advice regarding a reliable and robust housekeeping genes to use? I've been using ActB, but somehow CRISPR targeting GFP (irrelevant control CRISPR) is giving ct values lower then the rest of the samples. Does maybe anyone have an idea what this anti-GFP CRISPR construct may do in the cell so that ActB gets downregulated? Thank you in advance.

Housekeeping Gene HEK293 Cells HEK Cells Quantitative RT-PCR CRISPR/CAS CRISPR/CAS9

## Articles

There are also helpful articles/resources available, not necessarily in the form of published literature

Opinion

## Human housekeeping genes, revisited

Eli Eisenberg<sup>1</sup> and Erez Y. Levanon<sup>2</sup>

<sup>1</sup> Raymond and Beverly Sackler School of Physics and Astronomy, Tel-Aviv University, Tel Aviv 69978, Israel  
<sup>2</sup> Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel

Housekeeping genes are involved in basic cell maintenance and, therefore, are expected to maintain constant expression levels in all cells and conditions. Identification of these genes facilitates exposure of the underlying cellular infrastructure and increases understanding of various structural genomic features. In addition, housekeeping genes are instrumental for calibration in many biotechnological applications and genomic studies. Advances in our ability to measure RNA expression have resulted in a gradual increase in the number of identified housekeeping genes. Here, we describe housekeeping gene detection in the era of massive parallel sequencing and RNA-seq. We emphasize the importance of expression at a constant level and provide a list of 3804 human genes that are expressed uniformly across a panel of tissues. Several exceptionally uniform genes are singled out for future experimental use, such as RT-PCR control genes. Finally, we discuss both ways in which current technology can meet some of past obstacles encountered, and several as yet unmet challenges.

### The concept of housekeeping genes

Housekeeping genes are genes that are required for the maintenance of basal cellular functions that are essential for the existence of a cell, regardless of its specific role in the tissue or organism. Thus, they are expected to be expressed in all cells of an organism under normal conditions, irrespective of tissue type, developmental stage, cell cycle state, or external signal. From a fundamental point of

### Early detection schemes for housekeeping genes

The notion of housekeeping genes has been in use in the literature for nearly 40 years. In particular, several mammalian genes have been used widely as internal controls in experimental expression studies, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), tubulin, cyclophilin, albumin, actin, 18S rRNA or 28S rRNA. Yet, only at the turn of the 21st century, with the advancement of transcriptome profiling technology, did it become possible to identify, systematically, a set of housekeeping genes. These first attempts used large-scale expression data [18–20] or, more often, microarray profiling to look at the expression levels of many genes across a panel of tissue samples. Typically, they resulted in lists of hundreds to thousands of genes [8,19–25], many more than the dozen or so commonly used control genes.

Generally, the many lists produced show a considerable level of consistency. Typically, the intersection of any two of them yields approximately 50% coverage [8,24,26], suggesting that the sets are enriched in housekeeping genes but still lacking in specificity and selectivity. This could be partly attributed to the limited number of tissues examined in each separate analysis and the differences between the tissues across analyses. However, it is likely that technological limitations affecting the underlying data have contributed much to the quality and reproducibility of the results.

In particular, first-generation microarray technology is known to have had many problematic nonspecific probes

## Published Literature

Scientists are able to find literature related to their study of interest and search for housekeeping genes used

ncbi.nlm.nih.gov/pmc/articles/PMC3843951/

Company Bookmarks BenchSci App Planning Design Analytics/Users Administrative

hous

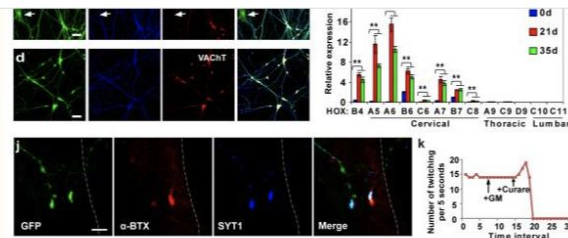


Figure 5

Cholinergic motor neuron-like cells converted from human fetal fibroblasts

(a–d) Immunocytochemistry analysis of induced neurons with markers for cholinergic motor neurons, including HB9 (91.7%, n=325), ISL1/2 (90.2%, n=347), ChAT (98.5%, n=325), and VACHT (98.2%, n=273). GFP expression indicates NGN2-transduced cells. Control virus-transduced fibroblasts were stained negative for each of these markers. Arrows indicate cells stained negative for HB9 or ChAT. (e–f) Gene expression analysis by qRT-PCR during the reprogramming process (means  $\pm$  s.e.m., n=3 at each time point). It was normalized to that of house-keeping gene *Hprt*. Samples from normal adult human brain (Brn) and spinal cord (SC) were used as controls (n=1). \*\*p < 0.01 by Student's t-test. (g) Immunocytochemistry analysis of synaptic connections between hiCNs and myotubes in co-culture. A multinucleated myotube is outlined.  $\alpha$ -BTX,  $\alpha$ -bungarotoxin; SYT1, synaptotagmin 1. (h) Quantification of rhythmic contractions of myotubes after co-culturing with hiCNs. Such contractions can be blocked by curare, a competitive inhibitor of acetylcholine receptor (n=5 independent myotubes). Scales, 20  $\mu$ m for (a–b), 50  $\mu$ m for (c–d), and 10  $\mu$ m for (j).

Open in a separate window

# Takeaways

Currently, scientists either have to turn to Google to find suitable housekeeping genes via published literature or forums, or find limited information on vendor sites while purchasing qPCR probes/primers. We have an opportunity here on BenchSci to make this information more readily available, and specific to a scientist's study of interest. The following are some things we can do to have a competitive advantage:

- We should ensure our catalogue captures the "related controls" assays from the vendors
- Make housekeeping gene info easily findable for the scientist's experimental context
  - This could be in the form of a side filter, where the scientist can see the most often used housekeeping gene (design solution TBD)
  - Ensure housekeeping genes can be searched by context **tissue**, **cell line**, **cell type**, and **species** (these are terms scientists will type into the search bar)
    - Being able to filter for context will be really helpful so scientists don't have to dig into the specs (like the do on the vendor sites) to see this
  - Handle the term "housekeeping gene" in the search bar (exact solution TBD, dependent on data structure and design)
    - Perhaps other terms like "control gene" should also be handled
  - Handle terms like "PCR" or "qPCR" in the search bar as well (likely solution will be to apply the product type PCR Reagents and the Application PCR or qPCR)



2

**Identifying qPCR primers/probe products or sequences that have been used in the published literature for a gene, and in a particular cell type/tissue**



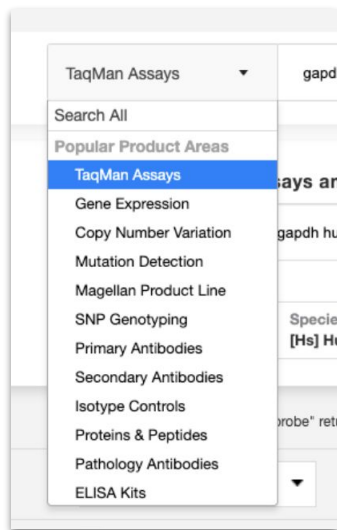
Qiagen's search finds matches to any of the words, not necessarily the whole phrase. They seem to search across the whole site, broken down into categories; products being one of them. Clicking a product link takes you directly to a product page. Product pages consist of: graphs from PCR experiments using the product, Procedure, Applications, Specifications, MSDS, FAQ's, Brochures and Guides, Kit Handbooks. The products don't appear to have citations.

The screenshot shows the Qiagen website's search results for the query "qpcr probes". The search bar at the top left contains the text "qpcr probes". Below the search bar, there are two columns of results. The left column, titled "Suggestions", lists several search suggestions: "qpcr probes", "probe-based qpcr", "probe based qpcr", "which probe labels are availa...", "and sensitivity to your qpcr - ...", and "and sensitivity to your qpcr - ...". The right column, titled "Results", lists various products and applications: "Products", "miRNA qPCR-based Analysis", "miRCURY LNA miRNA Probe PCR - ...", "qPCR Genotyping", "Real-time qPCR - QIAGEN", "RNA qPCR Services", "Array and PCR-based Analysis of Si...", "We've found results on GeneGlobe site.", "QuantINova Probe PCR Kit", "QuantiTect Probe PCR Kits", "Quantifast Probe PCR Kits", "UCP Probe PCR Kit", "FastLane Cell Probe Kit", and "Rotor-Gene Probe PCR Kit". A blue arrow points from the "Applications & Insights" link in the "Results" column to the "QuantiTect Probe PCR Kits" product page.

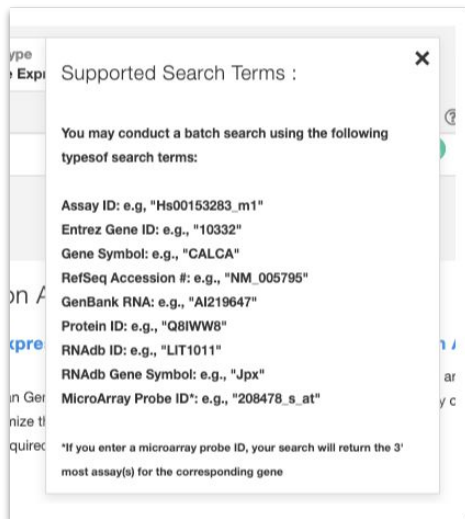
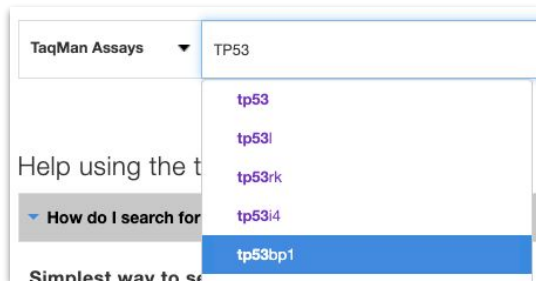
The screenshot shows the Qiagen website's product page for "QuantiTect Probe PCR Kits". The page is titled "QuantiTect Probe PCR Kits" and features a large image of the product packaging. Below the image, there is a description of the product: "For real-time PCR and two-step RT-PCR using sequence-specific probes". The description highlights several key features: "Highly sensitive detection of low-copy targets", "Accurate quantification over several logs of template", "Use of any sequence-specific probe in any real-time cycle", and "Available with or without uracil-N-glycosylase (UNG)". The page also includes a "Buy Products" section with a table of products and their prices. The table lists three products: "QuantiTect Probe PCR Kit [200]", "QuantiTect Probe PCR Kit [1000]", and "QuantiTect Probe PCR Kit +UNG Kit [200]". The prices are \$493.00, \$2,035.00, and \$598.00, respectively. The page also includes a "Product Details" section and a "Specifications" section.

Product	Price
QuantiTect Probe PCR Kit [200]	\$493.00
QuantiTect Probe PCR Kit [1000]	\$2,035.00
QuantiTect Probe PCR Kit +UNG Kit [200]	\$598.00

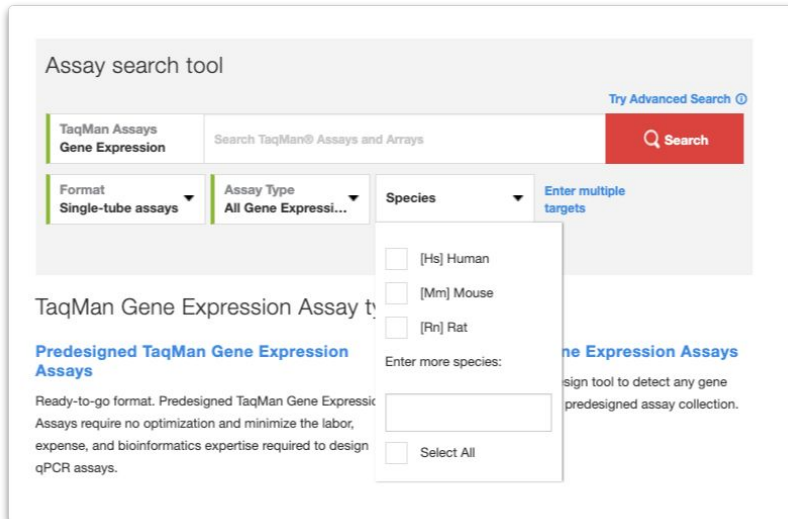
Thermo Fisher has a page dedicated to searching specifically for TaqMan Assays - it's also considered its own product type.



Basic Search has autocomplete available for genes



Advanced Search allows you to enter Format, Assay Type and/or Species



Results returned show "Best Coverage" product first. There is another label titled "Most Citations" on products. Products with citations show the rating as well as link out to the publications. To see more details, user has to navigate to the external site. TaqMan probes are mapped to genes.

**Search TaqMan® Assays and Arrays**

TaqMan Assays  
Gene Expression

gapdh mouse

**Search** [Advanced Search](#)

**Filter your results**

Assay Attributes Species [Mm] Mouse Gene Assay Design Cross Reactivity

Your search for "gapdh mouse" returned 6 TaqMan® Gene Expression Assays

Assays (6) | Arrays (881) | Pathways

Select action Change dye for all Change size for all Sort by

Select	Assay ID	Gene	Transcripts	Assay Design	Amplicon Length	Dye	Size	Availability	Price (CAD)
<input type="checkbox"/>	<b>Mm99999915_g1</b>	Gapdh	2 RefSeq (NM)	Probe spans exons	109	FAM-MGB	S: 250 rxns	Available	26

**Best coverage** **Most citations**

Catalog number: 4331182  
Target species: Mouse  
[Important information](#)

[View Details](#) [Related Reagents](#) [Related Controls](#) [View Assay Map](#) [Related Arrays](#)

**Add to cart**

**99 / 100 Bioz Stars**  
Citations: 2503  
Images: 5

**Yolk sac, but not hematopoietic stem cell-derived progenitors, sustain erythropoiesis thr...**  
**J Exp Med**  
05 Apr 2021  
... Mm03003491\_m1; Runx3: Mm00490666\_m1; Tal1: Mm01187033\_m1; Zfp1: Mm00494336\_m1; Actb: Mm01205647\_g1; Gapdh: **Mm99999915\_g1**; and Hprt: Mm03024075\_m1. [\(More...\)](#)

Quotes from the articles can be expanded, as well as the figure legend

**99 / 100 Bioz Stars**  
Citations: 2503  
Images: 5

**Small molecule allosteric inhibitors of RORγt block Th17-dependent inflammation and associated gene ex...**  
19 February 2021

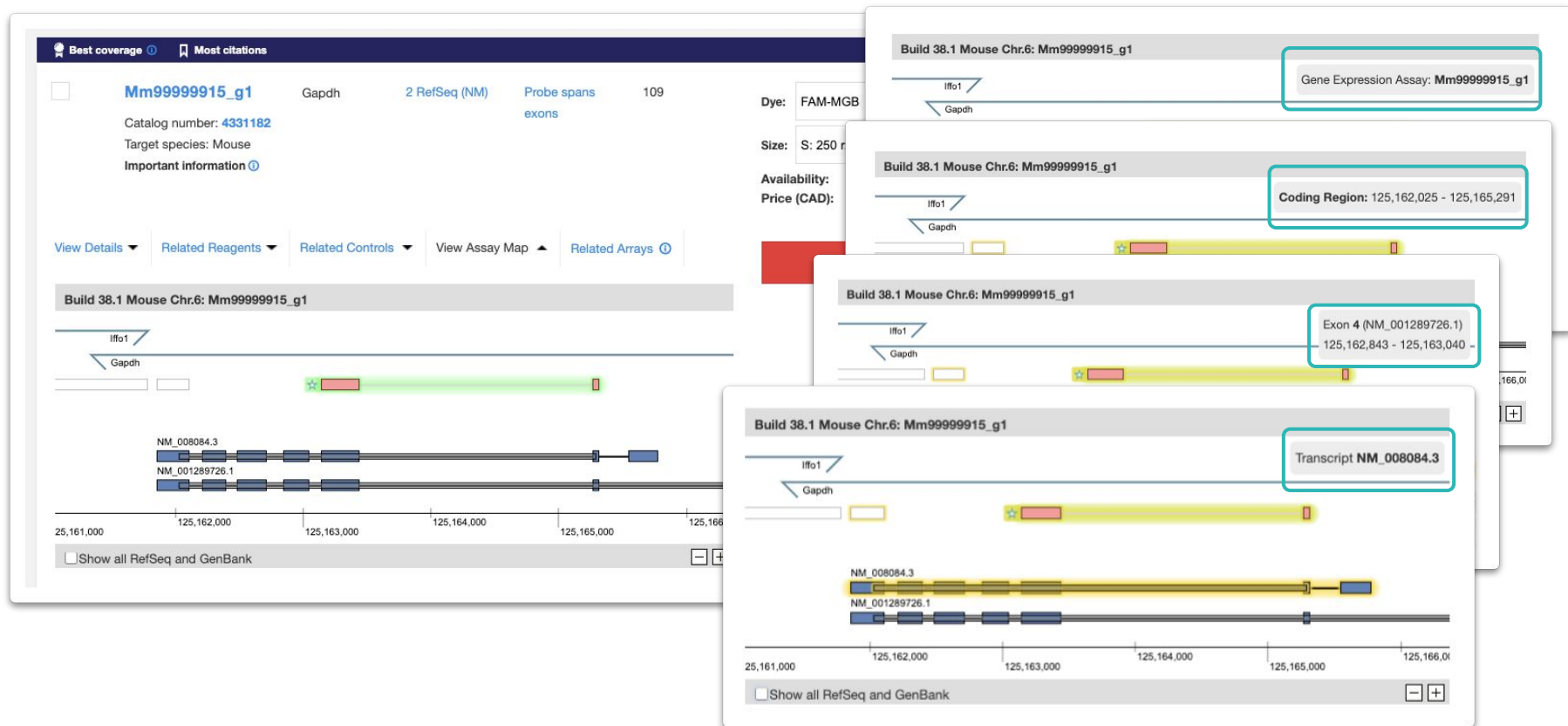
**Article Snippet**  
'Ears were homogenized using Procellis 24 homogenizer & hard tissue homogenizing beads (Bertin Instruments), 2 cycles of 30 seconds @ 6000 rpm in RLT lysis buffer according to manufacturer's instructions and RNA isolated using RNeasy Plus MiniPrep columns (Qiagen). cDNA is generated using SuperScript VIL0 cDNA Synthesis kit (Invitrogen) & gene expression assessed using TaqMan Fast Master Mix & TaqMan FAM-MGB probe sets (Applied Biosystems): Gapdh, **Mm99999915\_g1**, Il17a, Mm00439618\_m1, Il17f, Mm00521423\_m1, Il22, Mm01226722\_g1 & Bclxl, Mm00437783\_m1.' [\(Less...\)](#)

**Figure Legend**  
'Imiquimod-induced skin inflammation is attenuated by RORγt antagonist Cmpd 1. Ear thickness (mm) was measured in naïve or IMQ-treated animals on day 4 using digital micro-calipers (A). Th17 cytokine gene expression analysis was performed for Il17a (B), Il17f (C) and Il22 (D) on day 4. Expression is normalized to **Gapdh** and presented as fold change over naïve. Kinetic assessment of plasma concentration of Cmpd 1 (E). Each symbol represents an individual animal and error bars denote mean ± SEM. Statistical significance (\* p < 0.05) was determined using one-way ANOVA with Tukey's multiple comparisons test, \* significant over naïve; \*\* significance over vehicle-treated group. Data are representative of 2 independent experiments with n=3-8/group.' [\(Less...\)](#)

**See more details on Bioz**



An interactive assay map is provided for each probe. A scientist can hover over sections to see: gene expression assay, transcript, exon, coding regions.





Scientists can filter by gene, assay design, as well as cross-reactivity - and species, if not already specified

**Search TaqMan® Assays and Arrays**

TaqMan Assays  
Gene Expression

TP53 human

**Filter your results**

Assay Attributes

Species [Hs] Human

Gene

Assay Design

**Cross Reactivity**

Select all that apply:

- ☐ [Mm] Mouse
- ☐ [Rn] Rat
- ☐ [Bt] Bovine
- ☐ [Cf] Dog
- ☐ Select all

Your search for "TP53 human" returned 97 TaqMan® Gene Expression Assays

Select action

Change dye for all

Change size for all

Select	Assay ID	Gene	Transcripts	Assay Design	Amplicon Length	Price
<input type="checkbox"/>	Hs01034249_m1	TP53	15 RefSeq (NM)	Probe spans exons	108	Dye: FAM-MOD Size: S: 250 rxns Availability: Inventoried

Catalog number: 4331182

Target species: Human

Important information

Transcript is also searchable and returns results for probes

TaqMan Assays  
Gene Expression

NM\_008084.3

**Search**

Select action

Change dye for all

Change size for all

Select

Assay ID

Gene

Transcripts

Assay Design

Amplicon Length

View Details

Related Reagents

Related Controls

View Assay Map

Related Arrays

Build 38.1 Mouse Chr.6: Mm99999915\_g1

Transcript NM\_008084.3

NM\_008084.3

NM\_001289726.1

25,161,000

125,162,000

125,163,000

125,164,000

125,165,000

125,166,000

Show all RefSeq and GenBank

Not all splice variants for a particular gene are available, however the ones that are can be searched for. For example, **VEGFA165** and **VEGFA189** could be searched for among the [common isoforms](#) of VEGFA ( VEGFA121, VEGFA165, VEGFA189, and VEGFA206).

### Search TaqMan® Assays and Arrays

TaqMan Assays

Gene Expression

VEGFA

Filter your results

Assay Attributes

vegfa

vegfaa

vegfab

vegfa189

vegfa165

Your search for "tp53" returned 0 TaqMan® Gene Expression Assays

Showing 146 results filtered by Gene

TaqMan Assays

Gene Expression

vegfa165

Search

Advanced Search

View filters

Select action

Change dye for all

Change size for all

Sort by

Select	Assay ID	Gene	Transcripts	Assay Design	Amplicon Length	Price
<b>Best coverage</b>						
<input type="checkbox"/>	<b>Ch04653812_m1</b>	VEGFA	2 RefSeq (NM) 2 RefSeq (XM)	Probe spans exons	59	
	Catalog number: 4351372					
	Target species: Goat					

Dye: FAM-MGB

Size: S: 360 rxns

Availability: Made to order

Price (CAD): 424.00

Check your price

Add to cart

Home › TaqMan® Gene Expression Assays › Search Tool › Search Results › Ch04653812\_m1

[See other VEGFA GE Assays](#)

Gene Symbol:

▼ VEGFA

Gene Name:

vascular endothelial growth factor A

Gene Aliases:

VEGF, VEGFA165, VEGFA189

Chromosome Location:

Chr.23: 31541136 - 31557205 on Build ARS1

UniGene ID:

Chi.8260

Species:

Goat

Species Specific ID (Flybase ID):

-

M) Probe spans

M) exons

60

Dye: FAM-MGB

Size: S: 360 rxns



Availability: Made to order


Price (CAD): 424.00

Results returned don't display the variant searched for in the "Gene" column, however, the variant does show in the "Gene Aliases" section on the product details page.

Search accepts: Gene, RefSeq (transcript) and Assay ID, Species and Assay Configuration (two options). The exon location specified for each pre-designed primer, and whether it detects all variants (isoforms)

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## Predesigned qPCR Assays


Detect gene expression with PrimeTime® Predesigned qPCR Assays. Choose between probe-based assays or intercalating dye-based assays for any human, mouse, and rat transcriptomes.

- **Begin your project sooner**—FAM-labeled, probe-based assay shipped in 2–3 days
- **Easily analyze and troubleshoot your experiments**—disclosed primer and probe sequences

Step 1: Order Predesigned qPCR Assay(s)  
Step 2: Order Probe-Based qPCR Master Mix

**Achieve high efficiency qPCR under fast or standard cycling conditions**  
PrimeTime® Gene Expression Master Mix [Learn more »](#)

**Reference Gene Assays**

Housekeeping - Rat  [GET REFERENCE GENE ASSAYS](#)

Basic Batch Help

Gene Symbol RefSeq Assay ID Species ☒ Human ☐ Mouse ☐ Rat ☒ Exact Match

HPRT1 + NM\_000194 + Hs.PT.58.2145446 + [SEARCH](#)

Default Assay Configuration

☒ 5' nuclease, probe included  
☐ Intercalating dyes, primers only

Basic Batch Help

Gene Symbol RefSeq Assay ID Species ☒ Human ☐ Mouse ☐ Rat ☒ Exact Match

GapDH + + [SEARCH](#) [ADD TO ORDER](#)

Default Assay Configuration

☒ 5' nuclease, probe included  
☐ Intercalating dyes, primers only

Displaying 4 results for > Gene: GapDH [EXPORT](#) [FILTER](#) [COMPARE](#)

<input type="checkbox"/> Assay ID <sup>1</sup>	Gene Query <sup>1</sup>	NCBI Gene Symbol <sup>1</sup>	Ref Seq # <sup>1</sup>	Detects All Variants <sup>1</sup>	Exon Location <sup>1</sup>	Assay Configuration <sup>1</sup>
<input checked="" type="checkbox"/> Hs.PT.398.22214836	GAPDH	GAPDH	NM_002046(1)	Yes	2 - 3	Std, FAM/ZEN/IBFQ, P-P 2
<input type="checkbox"/> Hs.PT.58.40035104	GAPDH	GAPDH	NM_002046(1)	No	1 - 4	Std, FAM/ZEN/IBFQ, P-P 2
<input type="checkbox"/> Hs.PT.58.39769835.g	GAPDH	GAPDH	NM_001256799(1)	No	3 - 4	Std, FAM/ZEN/IBFQ, P-P 2
<input type="checkbox"/> Hs.PT.58.589810.g	GAPDH	GAPDH	NM_001256799(2)	Yes	10 - 10	Std, FAM/ZEN/IBFQ, P-P 2

**Assay ID:**  
 "g" suffix indicates that the assay may detect genomic DNA when using either PrimeTime qPCR Assays (5' nuclease probe-based assays) or PrimeTime qPCR Primers.  
 "gs" suffix indicates that the assay ordered as PrimeTime qPCR Assays (5' nuclease probe-based assays) will not detect gDNA. Assay ordered as PrimeTime qPCR Primers may detect genomic DNA.

**Exon Location:**  
 Exon boundaries are consolidated at the gene level whenever possible. Some exon locations may be marked with a superscripted 1, indicating assays in which consolidating at the gene level is not possible. For these assays, the exon location shown is related to the specific RefSeq listed in the results table.

# Takeaways

## Search/Filter

- Handle the terms “probe” and “primer” in the search bar (exact solution TBD depending on organization of subtypes - likely solution will be to apply the product type PCR Reagents and the subtype probe or primer)
- Similarly with housekeeping genes, handle terms like “PCR” or “qPCR” in the search bar as well (likely solution will be to apply the product type PCR Reagents and the Application PCR or qPCR)
- Capture cross-reactivity as a spec (i.e. Reactivity)
- Provide a way to search and/or filter for isoforms (solution requires coordination with Science)

## Use Cases

- Capture which probes are used with which primers and housekeeping genes, and list them in the same figure, so scientists can see which PCR reagents were used together
  - This would also apply to DNA/RNA Isolation kits, which we are likely adding shortly after launch
  - Capture housekeeping genes in the use cases when a probe/primer is used (mapping the probe/primer to the housekeeping gene)

## Other

- Determine how to classify TaqMan probes - should it be a separate sub-type, or a specific brand, but grouped together with the other probes of other brands?
- We are unlikely to compete with the assay map visualization, but if can capture the probes, scientists can access the information by navigating to the TF site



3

## Primer selection for conventional PCR applications

Thermo Fisher has a small selection of pre-designed primers (16 on this page), but they don't appear to be paired with another primer (listed separately)

## Thermo Scientific Pre-Designed Primers

Thermo Scientific Reverse Transcription

Maxima Reverse Transcriptases

**Thermo Scientific Pre-Designed Primers**



Primers for sequencing cloned DNA, cDNA synthesis and other molecular biology applications

We offer a range of pre-designed oligos for a variety of applications. Random Hexamer and Oligo dT Primers are suitable for first-strand cDNA synthesis with a reverse transcriptase. Sequencing Primers are complementary to common cloning vectors elements and allow for sequencing of cloned DNA fragments. Exonuclease-Resistant Random Primer is a mixture of single-stranded random oligonucleotides used for highly efficient random priming of various DNA

synthesis reactions.

- [See primers for cDNA synthesis](#)
- [See primers for sequencing of cloned DNA inserts](#)
- [See exo-resistant primers](#)

### Custom DNA oligonucleotides

Thermo Scientific Custom DNA HPLC-purified Oligonucleotides offer a range of modifications, synthesis scales and purity grades for your research and application requirements. Oligonucleotides are synthesized by solid phase synthesis using phosphoramidite chemistry and purified by reverse phase HPLC. This standard production method combined with our experienced production team and fully enforced ISO9001 certification enables us to consistently provide high-quality custom DNA oligos.

### Primers for cDNA synthesis

Catalog #	Name	Size	Price (CAD)	Qty
SO131	<a href="#">Oligo(dT)18 Primer</a>	60 µL	105.00	<input type="text"/>
SO132	<a href="#">Oligo(dT)18 Primer</a>	120 µL	186.00	<input type="text"/>
SO142	<a href="#">Random Hexamer Primer</a>	120 µL	108.00	<input type="text"/>
				<a href="#">Add to cart</a>


### Primers for sequencing of cloned DNA inserts


Catalog #	Name	Size	Price (CAD)	Qty
SO501	<a href="#">pJET1.2 Forward Sequencing Primer, 23-mer</a>	10 µM	53.25	<input type="text"/>


IDT also has a few pre-designed primers available for selection. There is no special searching or filtering because of the limited selection; a user could review them all. These also are not sold in pairs, but are grouped.



Citations linked from another company at the bottom of the page


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## ReadyMade™ Primers

HPLC purified, inventoried oligonucleotides for sample preparation, sequencing, and gene expression analysis of common genes

ReadyMade Primers include random hexamers, T7 promoter/terminator, M13 primers, 16S rRNA primers, and varieties of oligo dT that are available for same-day shipping.

- Routine purity >90%
- Sequence-verified via ESI-mass spectrometry\*
- Obtain 10 µg of dry product—enough for 100 sequencing reactions

\*With the exception of mixed base oligos, which could potentially represent multiple sequences and therefore cannot be accurately evaluated by ESI mass spectrometry.

### Ordering ▾


#### ReadyMade™ Primers

10 µg of >95% pure primer as established by CE. Identity confirmed by mass spec.\*

\*With the exception of mixed base oligos, which could potentially represent multiple sequences and therefore cannot be accurately evaluated by ESI mass spectrometry.

Quantity	Product	Catalog #	Sequence	Price
0	16S rRNA For	51-01-19-06	AGA GTT TGA TCC TGG CTC AG	\$10.00 USD
0	16S rRNA Rev	51-01-19-07	ACG GCT ACC TTG TTA CGA CTT	\$10.00 USD
0	3' RACE PCR	51-01-18-02	GGC CAC GCG TCG ACT AGT AC	\$10.00 USD
0	Anchored Oligo dT (20)	51-01-15-08	TTT TTT TTT TTT TTT TTT TV	\$10.00 USD
0	Anchored Oligo dT (22)	51-01-15-09	TTT TTT TTT TTT TTT TTT TVN	\$10.00 USD
0	BGH Reverse	51-01-02-08	TAG AAG CCA CAG TCG AGG	\$10.00 USD
0	Bluescript KS	51-01-02-11	TCG AGG TCG ACG GTA TC	\$10.00 USD
0	cDNA Cloning Primer	51-01-03-01	GGC CAC GCG TCG ACT AGT ACT TTT TTT TTT	\$10.00 USD


**2019 Novel Coronavirus (2019-nCoV)**



Solutions for your research needs. Available now. Ready to ship.

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**DECODED® articles**

ReadyMade negative controls for gene silencing (RNAi) experiments 

idtdna.com/pages/products/custom-dna-rna/readymade-inventoried-oligos/readymade-primers

### Citations ▴

**Spatially explicit depiction of a floral epiphytic bacterial community reveals role for environmental filtering within petals**  
Microbiologyopen  
Published 3 Feb 2021

Each primer, and a small portion of a colony. ... Primers used were IDT Readymade Primers 16S rRNA For (5'-AGA GTT TGA TCC TGG CTC AG-3') and 16S rRNA Rev (5'-ACG GCT ACC TTG TTA CGA CTT-3') (Integrated DNA Technologies, Coralville, IA), each diluted to 50 µM. Conditions for PCR were 98°C for 2 min, 98°C for 10 s. (More...)

**Assessment by Matrix-Assisted Laser Desorption - Time of Flight Mass Spectrometry of the Diversity of Endophytes and Rhizobacteria Cultured from the Maize Microbiome**  
bioRxiv  
Published 1 Dec 2020


\*PrePrint: ... following the manufacturer's protocol (Qiagen, Carlsbad CA). ... A near intact fragment of the 16S rRNA gene was amplified with ReadyMade™ Primers 16SrRNA For and 16SrRNA Rev supplied by IDT (Coralville, IA) using DreamTaq Hot Start DNA polymerase, following the manufacturer's protocol (ThermoFisher, Waltham, MA). PCR reactions were preheated (94 °C, 2 min). (More...)

**Integrated Human Immunodeficiency Virus Type 1 Sequence in J-Lat 10.6**  
Microbiol Resour Annu  
Published 30 Apr 2020

... provirus (5' amplicon) was 8,999 bp and was amplified using primers based on the reported integration site (1); primers 10.6\_up5LTR\_F and eGFP-N (ReadyMade Primers, catalog number 51-01-05-05; Integrated DNA Technologies), directed to the N terminus of the gene for enhanced green fluorescent protein (eGFP), which replaces nef in this molecular clone, were. (More...)

**CRISPR off-target detection with DISCOVER-seq**  
Nat Protoc  
Published 20 Apr 2020

\*NATURE PROTOCOLS PROTOCOL NATURE PROTOCOLS | www.nature.com/nprot 9 • Agarose (Thermo Fisher Scientific, cat. no. 16500500) • Primers for amplification of on- and off-target sites in PCR and qPCR (Integrated DNA Technologies (IDT), custom order) • DNeasy Blood Tissue Kit (Qiagen, cat. no. 69504) • Milli-Q water (More...)

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## Custom Primer Design

It's very cheap to make your own - IDT lets you design and order your own. The primers IDT supports designing in pairs.



### RxnReady® Primer Pools

Premixed primer pairs supplied ready to use

All PCR and qPCR primers are produced with industry-leading coupling efficiencies, resulting in higher quality DNA products. Our specialized platforms allow us to deliver the purest primers for your research needs.

#### Ordering ▲

#### RxnReady Primer Pools

[ORDER NOW](#)

2 oligos premixed in a single tube. Shipped dry, or resuspended to your specifications.

Product	Length	DNA bases
RxnReady® Oligos, 25 nmol	15 - 60 bases	\$0.37 CAD / base
RxnReady® Oligos, 100 nmol	10 - 90 bases	\$0.55 CAD / base
RxnReady® Oligos, 250 nmol	5 - 100 bases	\$1.05 CAD / base

The following mixing fee will be applied to each pool: \$2.75 CAD

### RxnReady™ Primer Pools

☐ Select All
 ACTIONS: ▾
 # of Items: 1 GO
 [BULK INPUT](#)

# 1 Item Name \*

Scale ⓘ

RxnReady™ Oligos, 25 nmol ▾

Purification

Standard Desalting ▾

Sequence 1 \* (5' → 3')

5' MOD ▾ INTERNAL ▾ 3' MOD ▾ BASES ▾

# Bases: 0 (Min:15 Max:60) [Convert to reverse complement](#)

GC: % Tm: °C [DeltaG](#): kcal/mole

Sequence 2 \* (5' → 3')

5' MOD ▾ INTERNAL ▾ 3' MOD ▾ BASES ▾

# Bases: 0 (Min:15 Max:60) [Convert to reverse complement](#)

GC: % Tm: °C [DeltaG](#): kcal/mole

Formulation

☒ Quantity only
 ☐ Quantity and volume

Quantity (nmol)

0

Please enter a nmol amount between 1 and 10



# Takeaways

- Capture and link primer pairs if possible, for both custom and pre-designed primers
  - Primers not paired together on all vendor sites, we offer advantage here if we can identify pairs that were used together in the literature
- Capture all the publications that cite the SKUs, since the selection seems small for pre-designed primers, so we have figures for them
  - This will ensure we at least have all of the publications cited on the vendor sites
- We are not replacing custom design tools for primers; we should ensure we extract out primer sequences so scientists can use the sequences to order their own custom primers

# Next Steps

- Connect with Science and R&D teams on identified takeaways
  - Work closely to coordinate the data structure and ensure design solutions can be supported
    - Detecting primer pairs
    - How to display housekeeping genes and help scientists find the data
    - Capturing NoSKU primer and probe sequences
    - Determine how isoforms will be captured and displayed
  - Obtain sample catalogue from Science to determine product type/sub-type IA
    - Use specs to make decisions on product types and sub-types
    - Ensure our catalog captures the "related controls" assays from the vendors
- Further define search logic to handle identified terms (i.e. "housekeeping gene", "PCR", etc.)