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:

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 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**

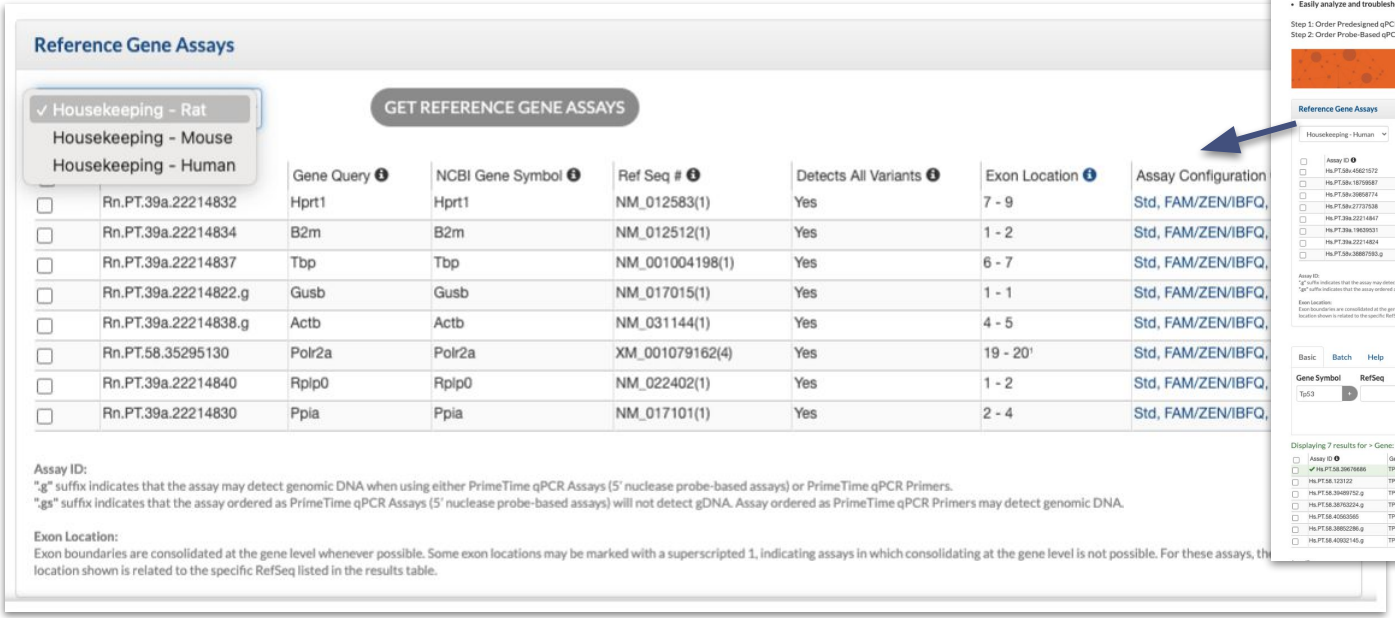
4. **Next Steps**

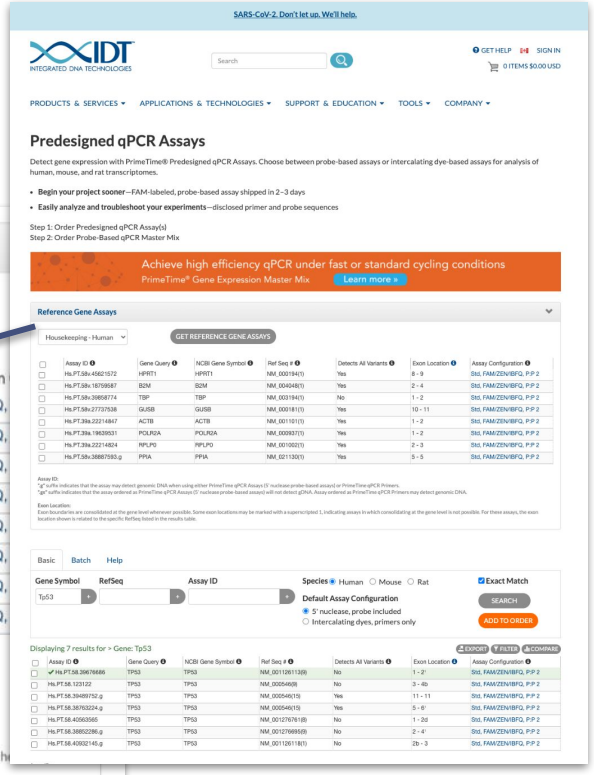
Analysis

1

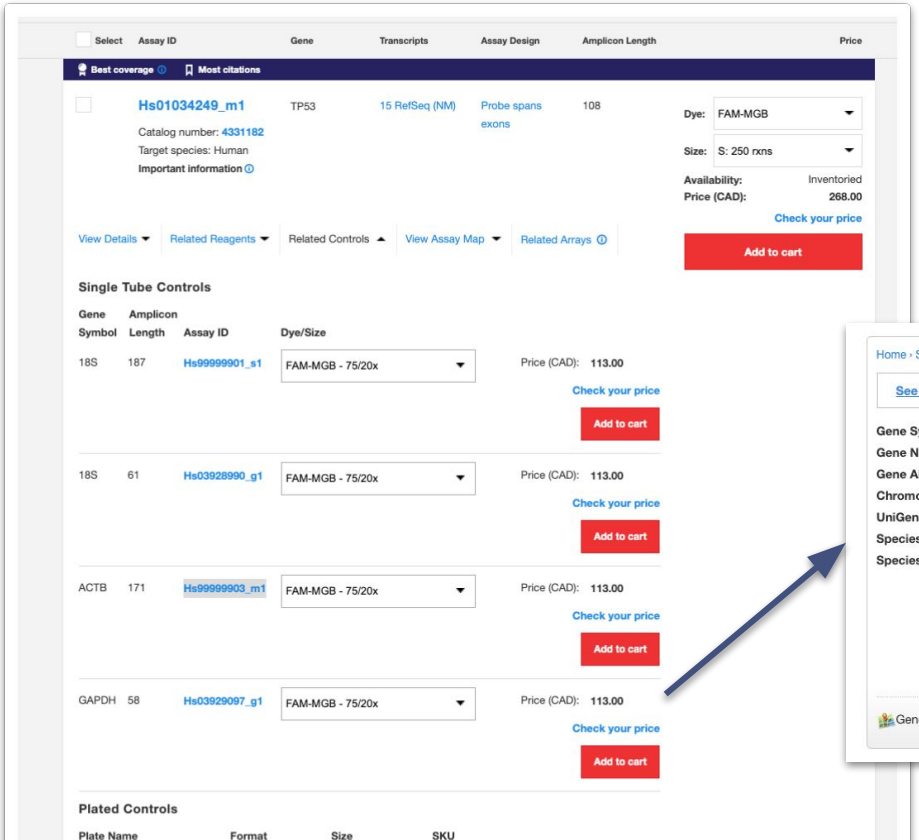
Selecting new control genes to use in a qPCR experiment when switching experimental context (ie: tissue, cell type)

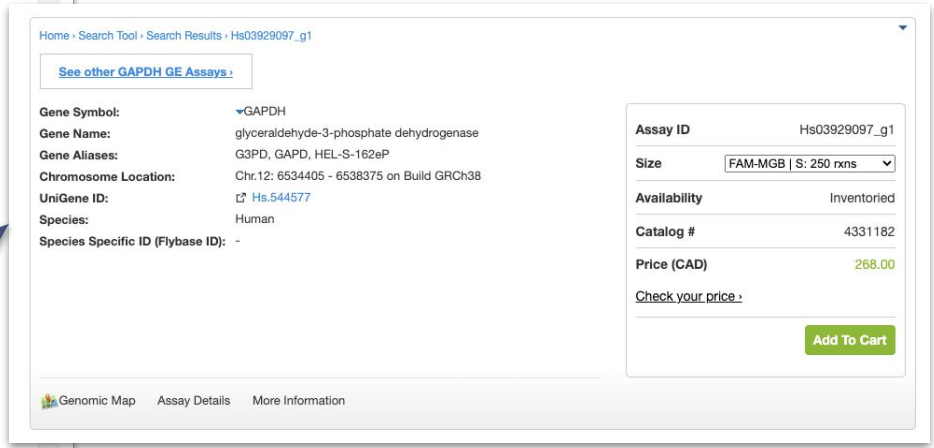
In a search on IDT for pre-designed qPCR assays, IDT also provides a selection of reference gene assays for rat, mouse and human species. This could be helpful resource for scientists who are looking for housekeeping genes to use while purchasing qPCR probes and primers





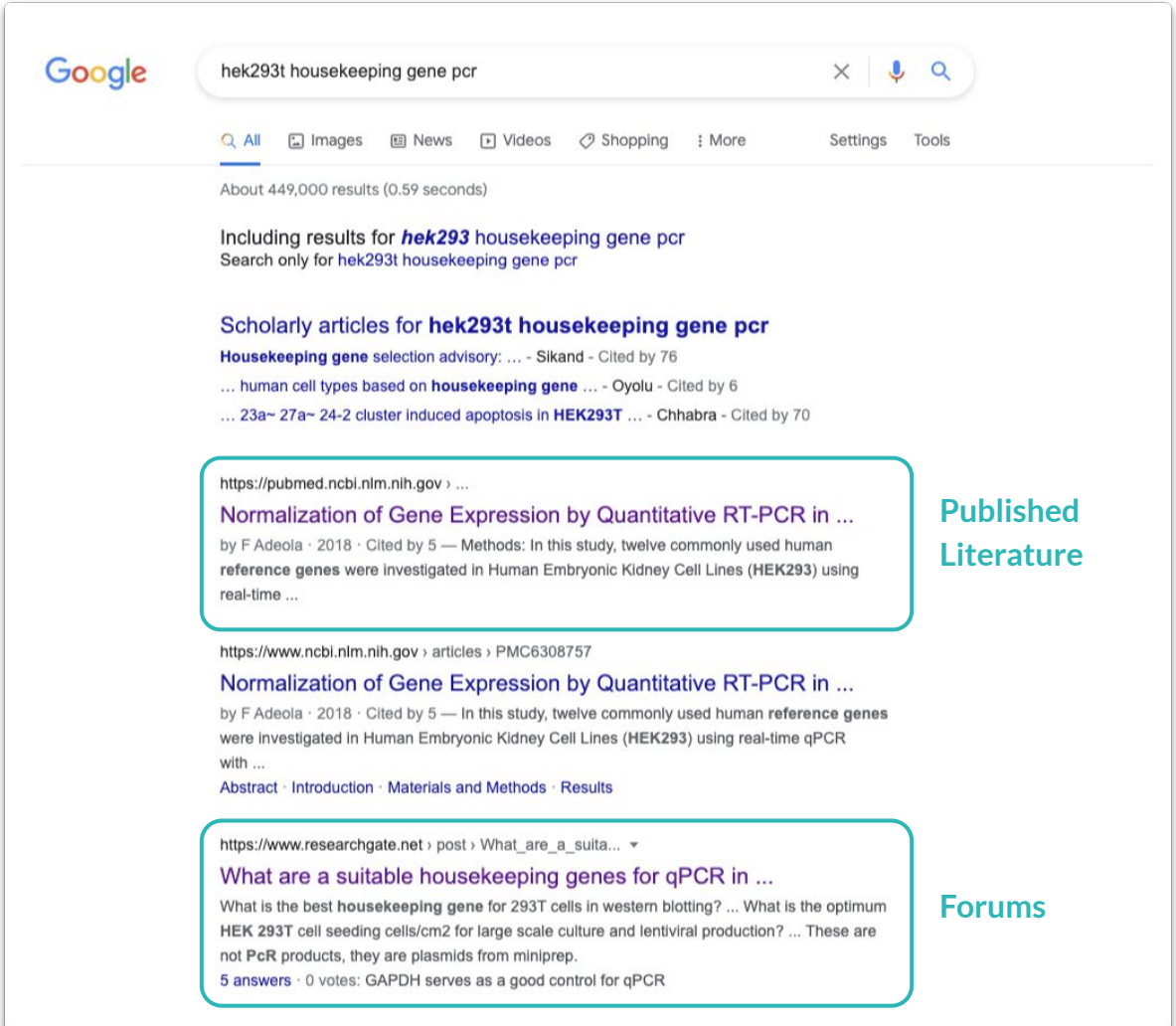
Thermo Fisher also provides a similar feature when searching for TaqMan probes - in the product card for the probe, there is a section for “Related Controls” where a scientist can see possible control genes they could use, with an option to purchase the assay as well.



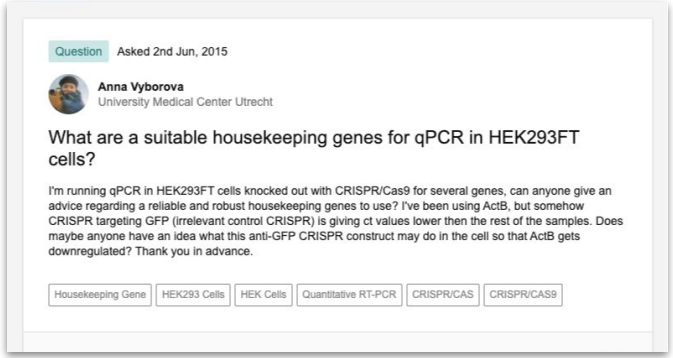


Google

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"



Forums



Question Asked 2nd Jun, 2015

Anna Vyborova
University Medical Center Utrecht

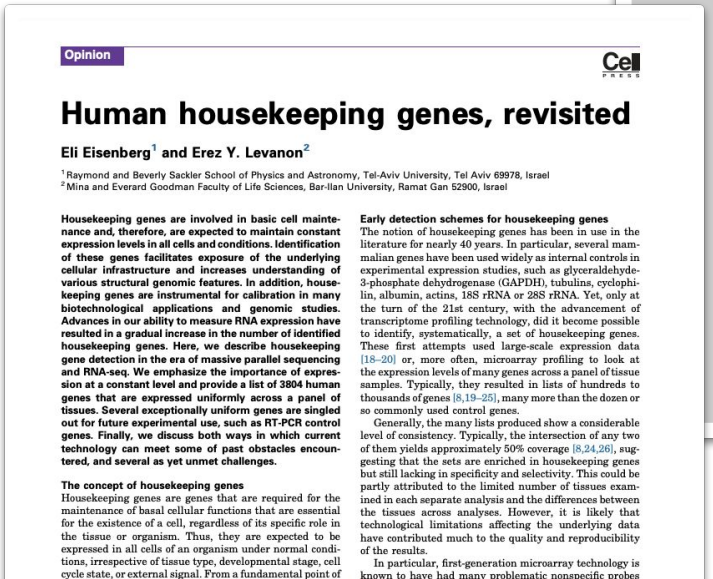
What are a suitable housekeeping genes for qPCR in HEK293FT cells?

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

Cell PRESS

Human housekeeping genes, revisited

Eli Eisenberg[1] **and Erez Y. Levanon**[2]

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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

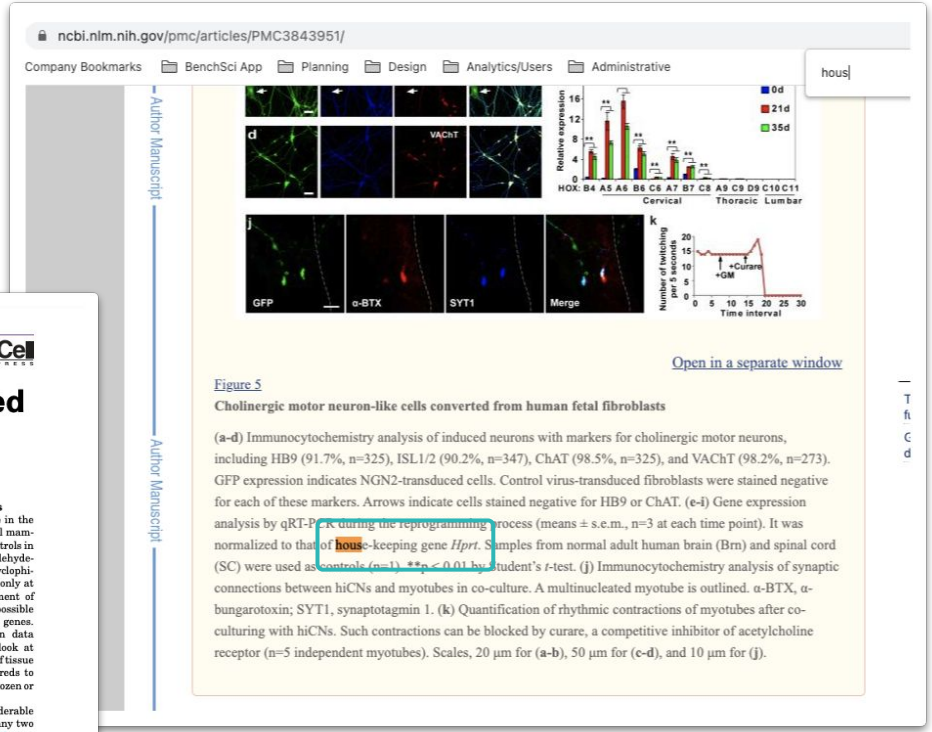
The notion of housekeeping genes has been in use in the literature for nearly 40 years. In particular, several mammalian genes have been used widely as internal controls in experimental expression studies, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), tubulins, cyclophilin, albumin, actins, 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



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

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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-i**) Gene expression analysis by qRT-PCR during the reprogramming process (means ± 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. (**j**) Immunocytochemistry analysis of synaptic connections between hiCNs and myotubes in co-culture. A multinucleated myotube is outlined. α-BTX, α-bungarotoxin; SYT1, synaptotagmin 1. (**k**) 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 μm for (**a-b**), 50 μm for (**c-d**), and 10 μm for (**j**).

↩ Back to Consolidated Takeaways

Takeaways

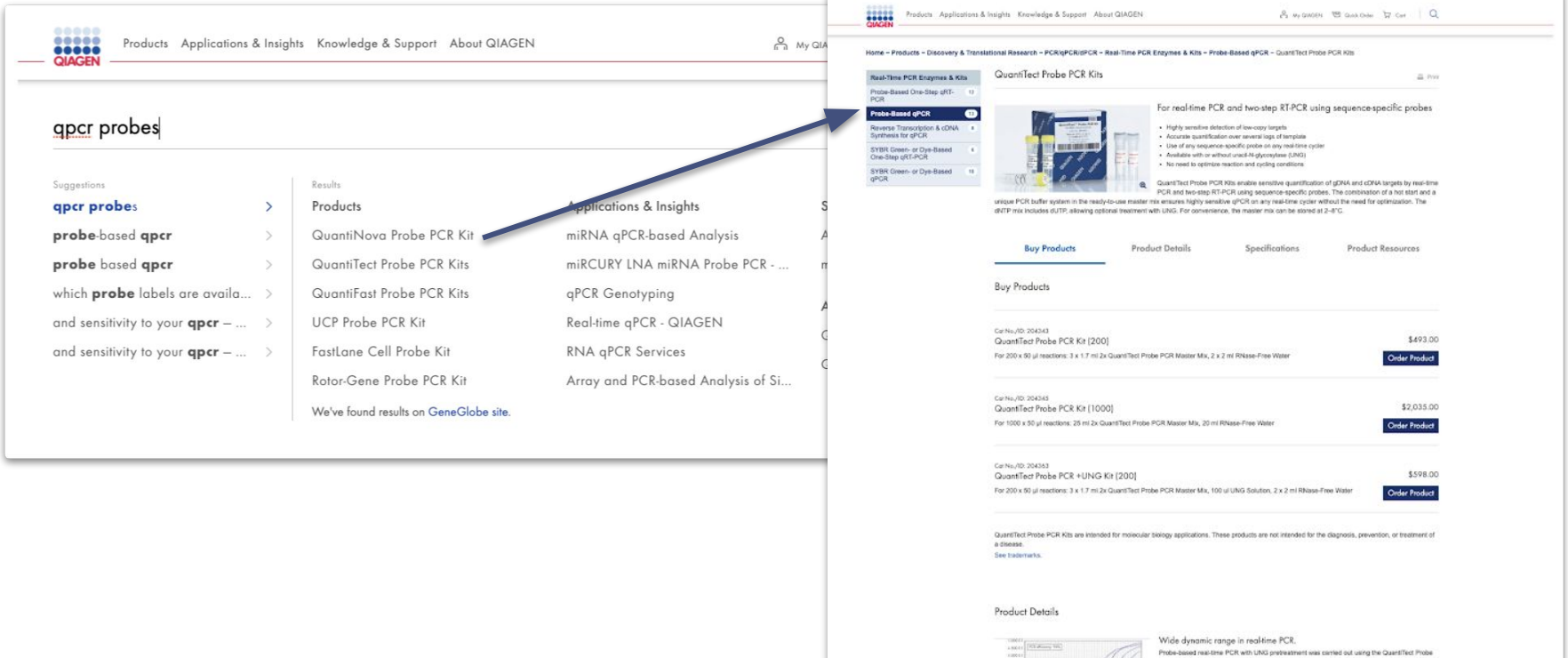
Currently, scientists either have to turn to Google to find suitable housekeeping genes via published literature or forums, or find limited information on vendor sites while purchasing qPCR probes/primers. We have an opportunity here on BenchSci to make this information more readily available, and specific 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

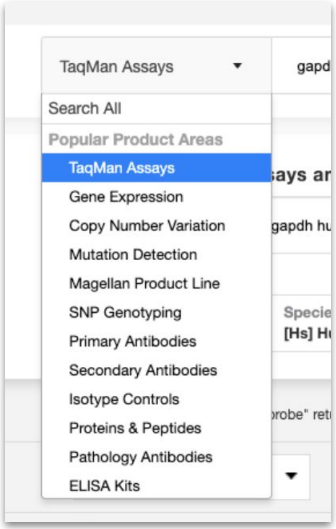
Identifying qPCR primers/probe products or sequences that have been used in the published literature for a gene, and in a particular cell type/tissue

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.

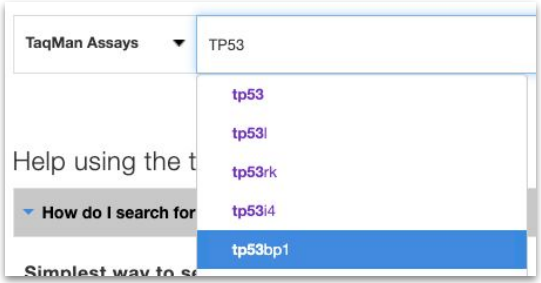


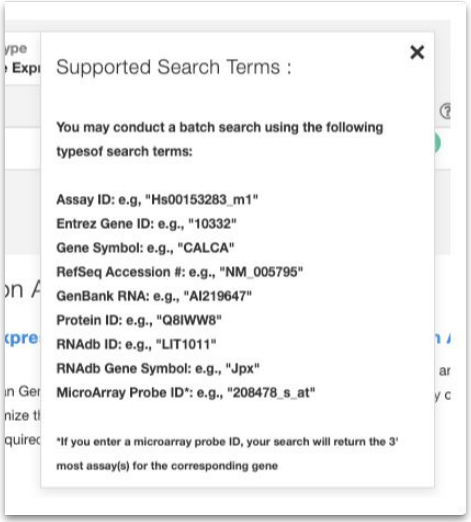
ThermoFisher SCIENTIFIC

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

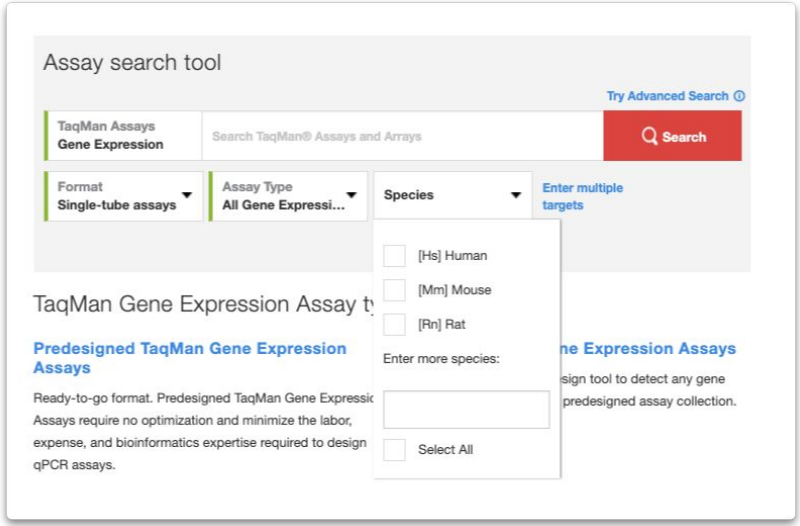


Basic Search has autocomplete available for genes

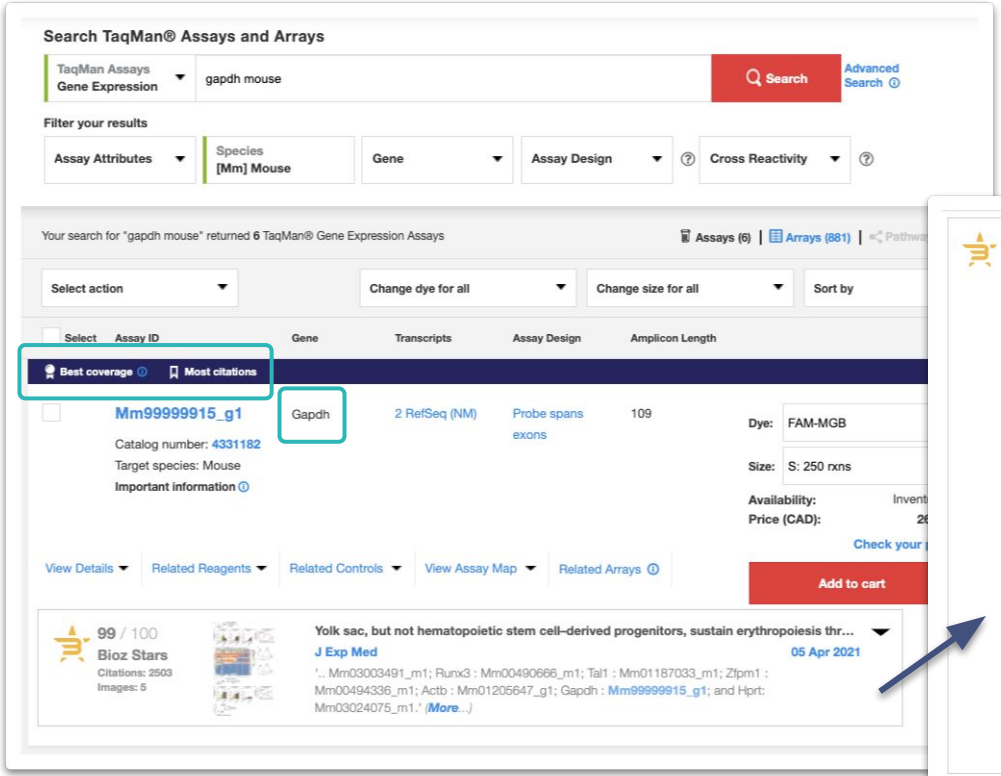




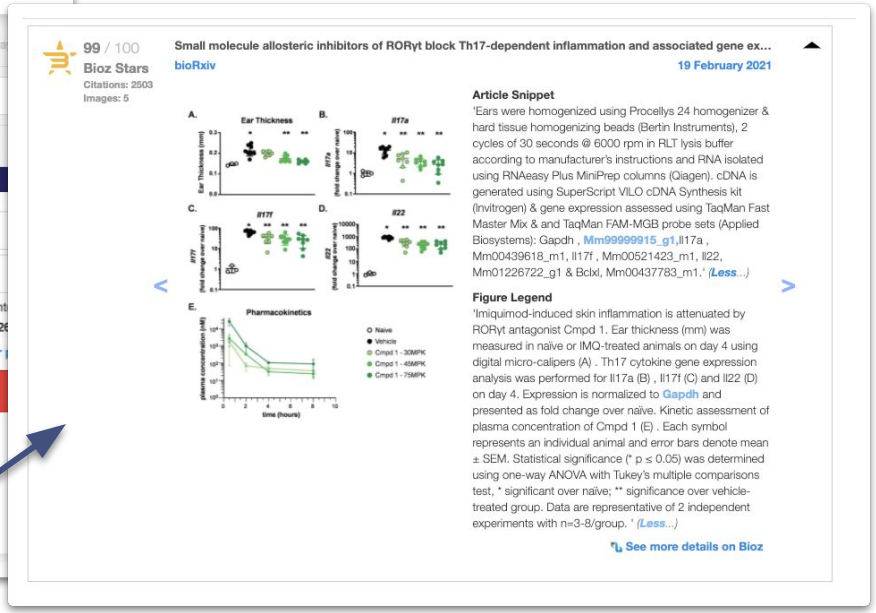
Advanced Search allows you to enter Format, Assay Type and/or Species



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.

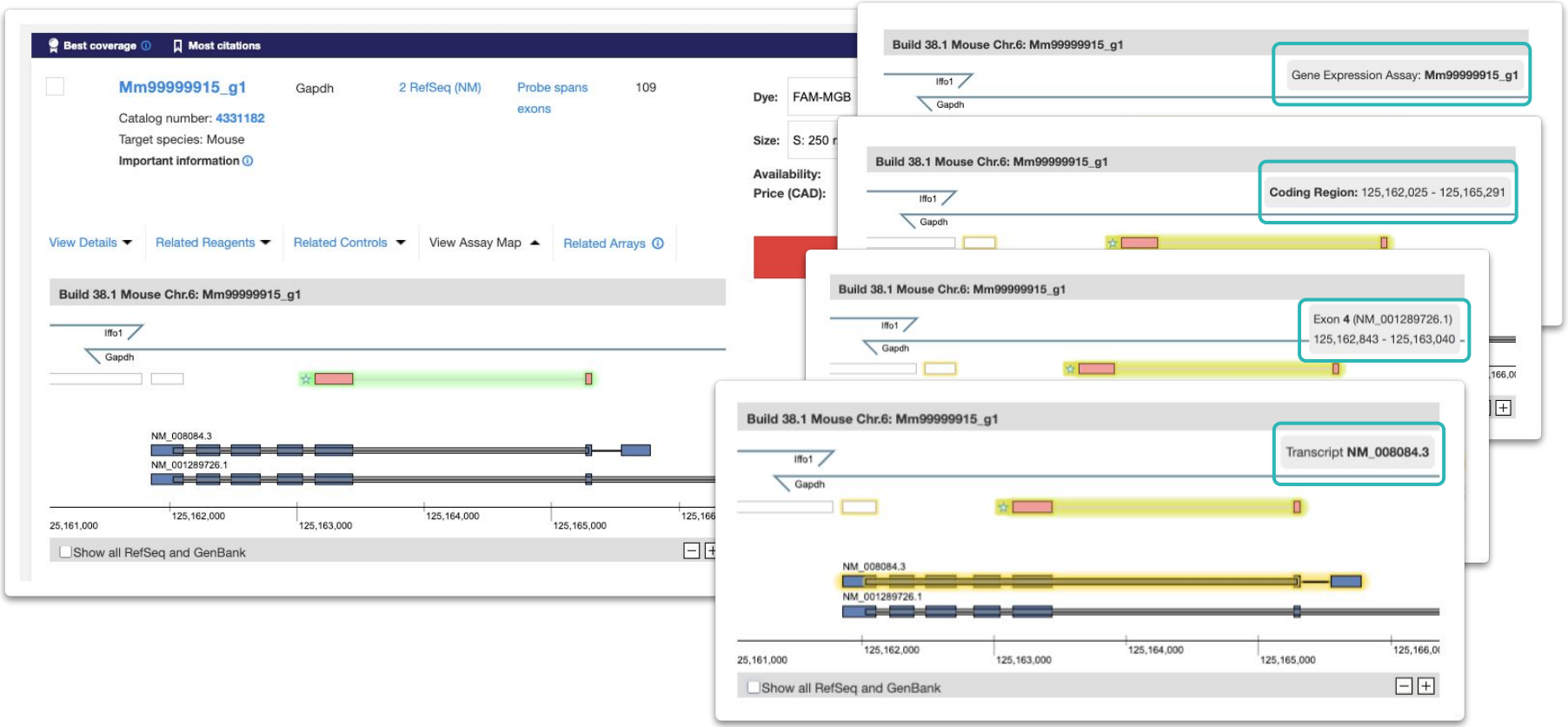


Quotes from the articles can be expanded, as well as the figure legend



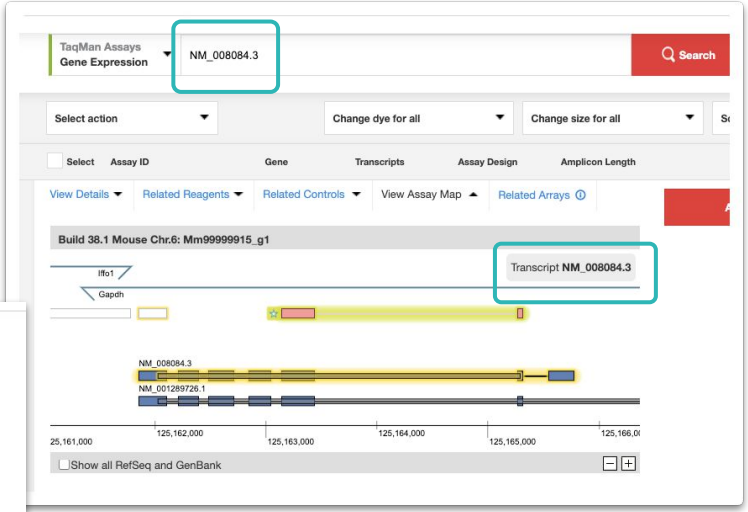
ThermoFisher SCIENTIFIC

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

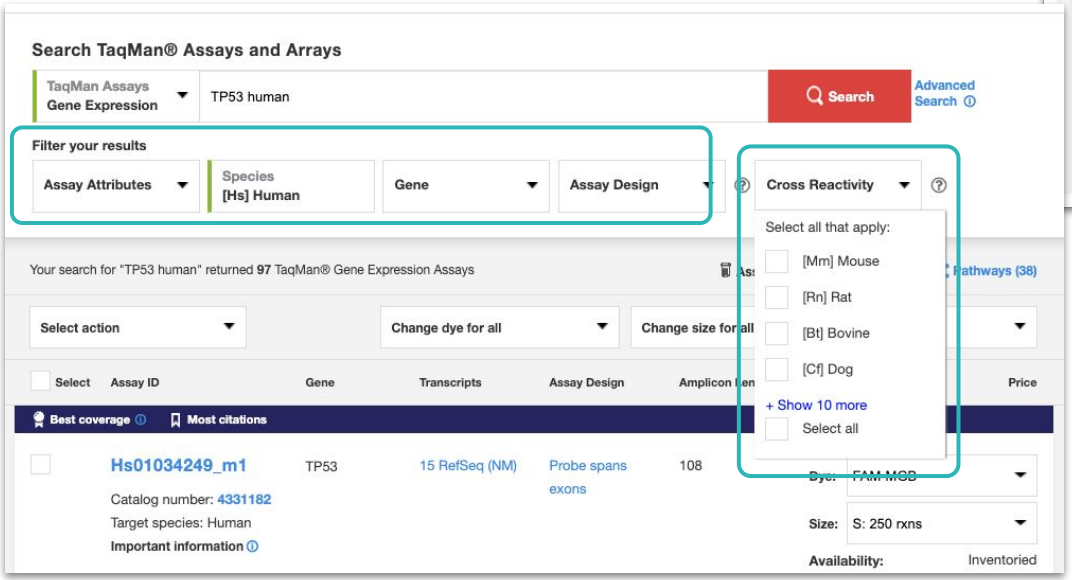


ThermoFisher SCIENTIFIC

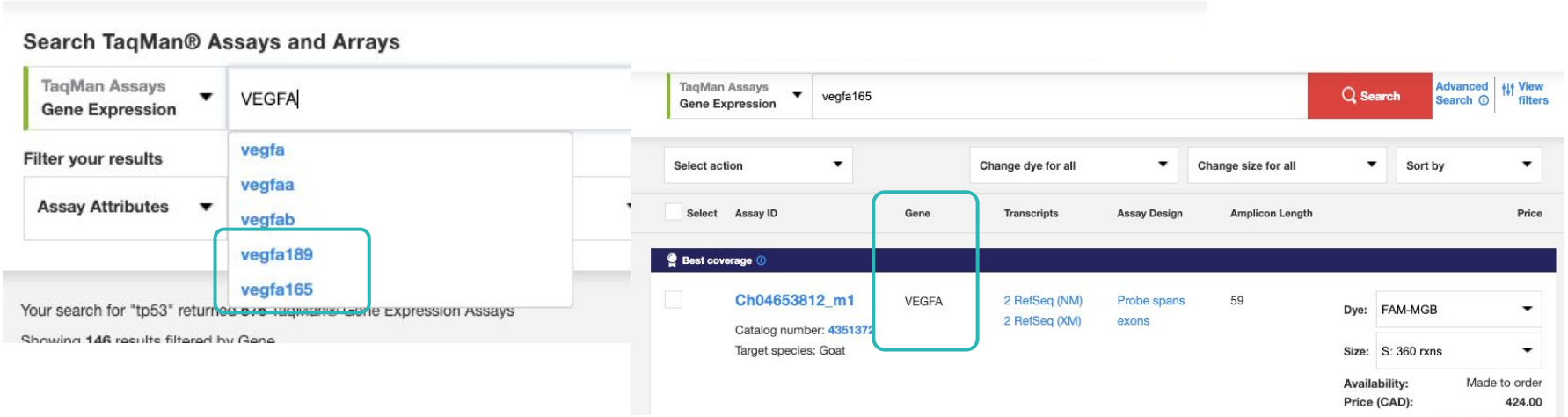
Transcript is also searchable and returns results for probes



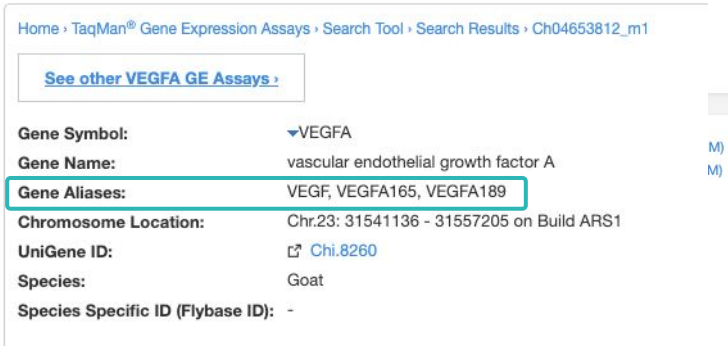
Scientists can filter by gene, assay design, as well as cross-reactivity - and species, if not already specified



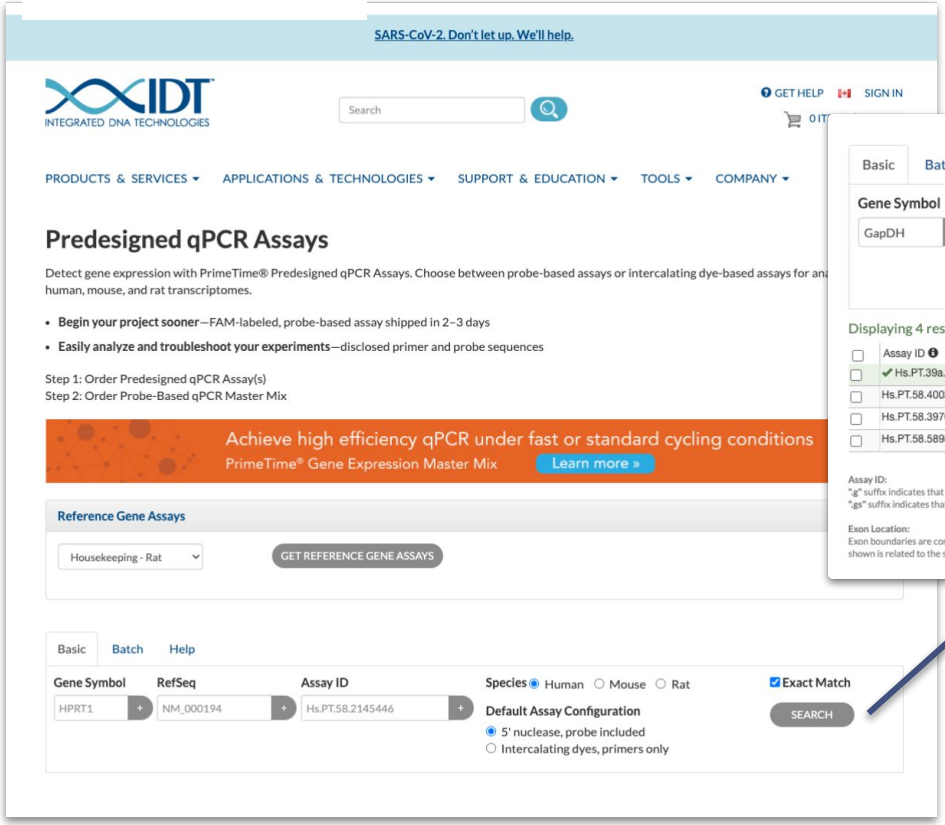
Not all splice variants for a particular gene are available, however the ones that are can be searched for. For example, **VEGFA165** and **VEGFA189** could be searched for among the common isoforms of VEGFA (VEGFA121, VEGFA165, VEGFA189, and VEGFA206).

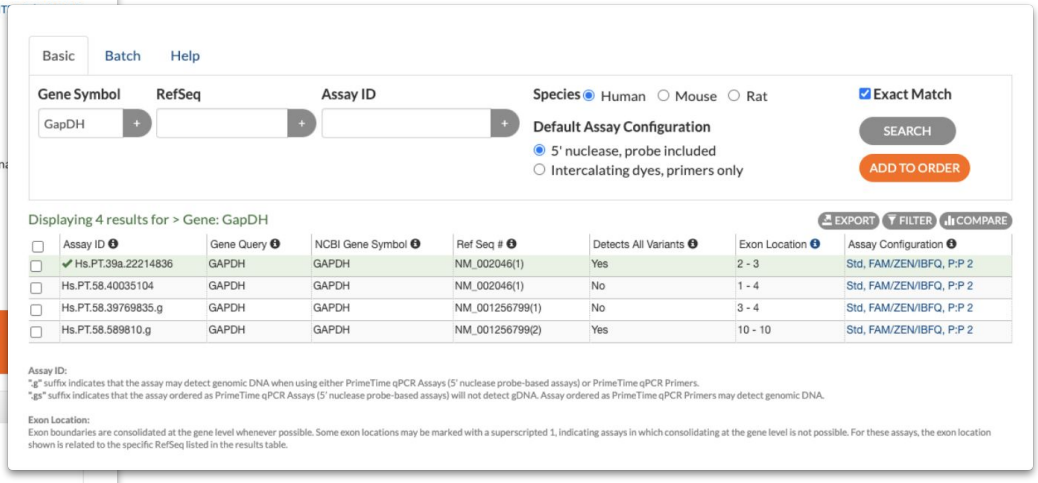


Results returned don't display the variant searched for in the "Gene" column, however, the variant does show in the "Gene Aliases" section on the product details page.



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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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

ThermoFisher SCIENTIFIC

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

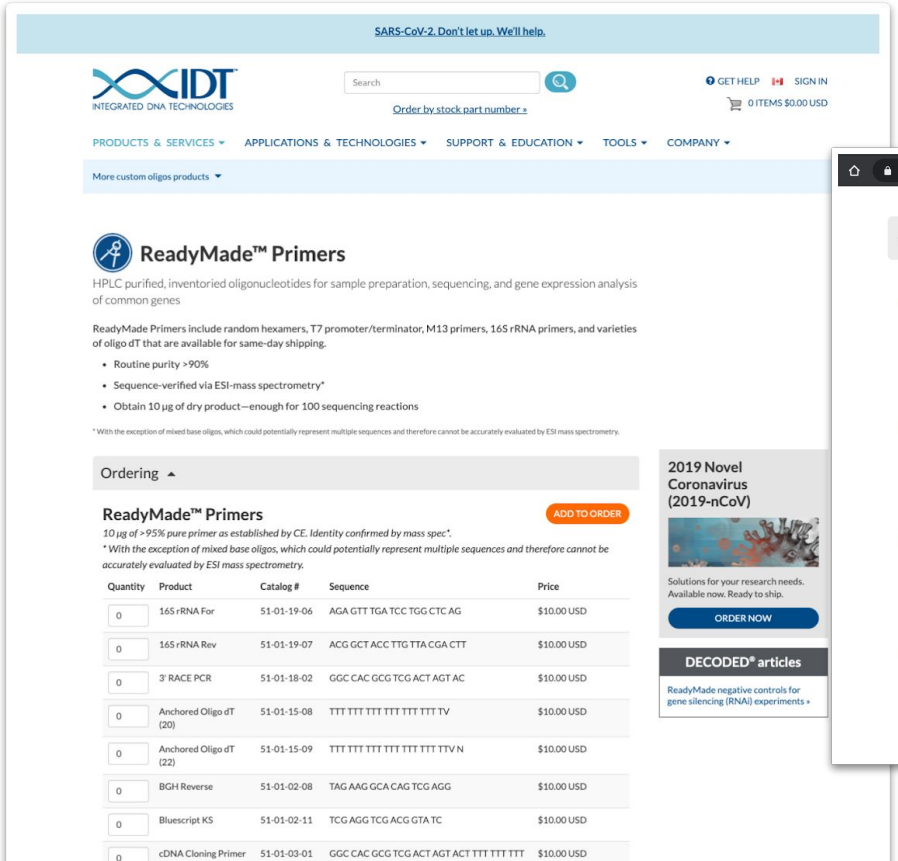
Catalog #	Name	Size	Price (CAD)	Qty
SO131	Oligo(dT)18 Primer	60 µL	105.00	
SO132	Oligo(dT)18 Primer	120 µL	186.00	
SO142	Random Hexamer Primer	120 µL	108.00	

Add to cart

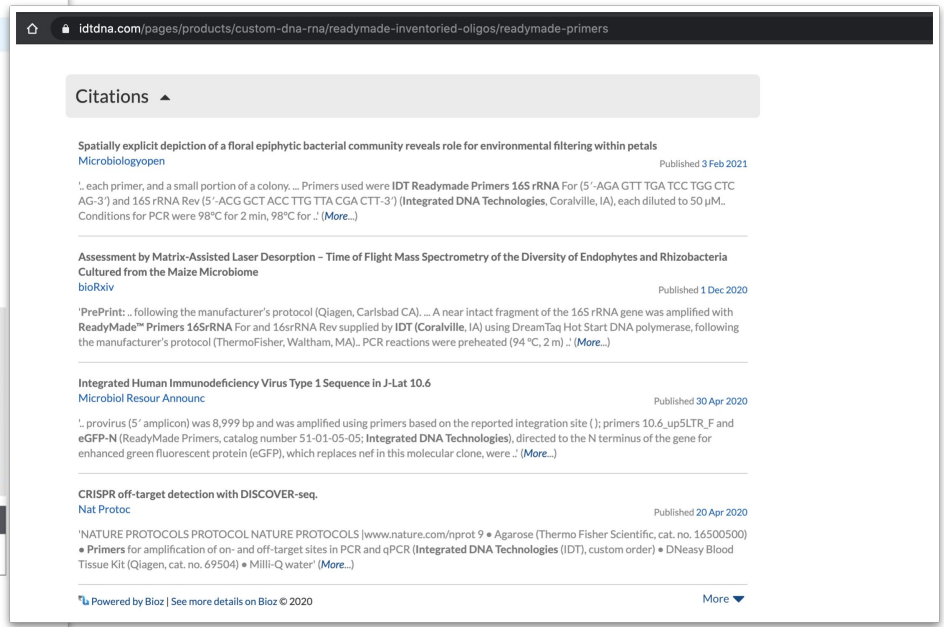
Primers for sequencing of cloned DNA inserts

Catalog #	Name	Size	Price (CAD)	Qty
SO501	pJET1.2 Forward Sequencing Primer, 23-mer	10 µM	53.25	

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



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

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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

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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.)