Service Profile

Expert Custom Assay Design Service for dPCR and qPCR

Answer your specific biological question. Thanks to the expert designed assays

We understand the struggle that researchers face when identifying faint genetic signals against a strong background while performing sensitive applications such as copy number variation and rare target detection. Digital PCR (dPCR) overcomes such challenges by partitioning the sample into a large number of individual reactions. To realize the full potential of dPCR you need high-quality multiplex custom assays to answer your specific biological question in the best possible way.

Partner to get expert assay design and unlock the full potential of your application

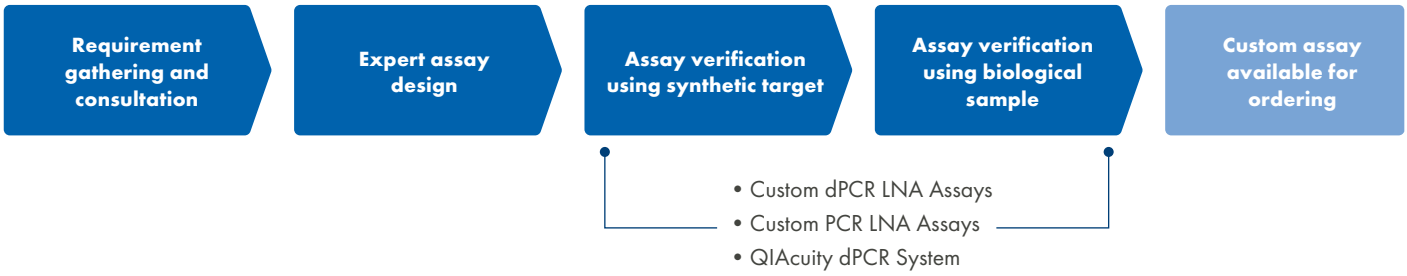

Figure 1. Expert assay design workflow.

Genomic services offers you custom assay design expertise at your fingertips. Our service provides:

- **Highly efficient assay design process:**
  A synergy between optimized internal design tools and highly skilled design experts with decades of experience in custom design.
- **Versatile assay design offering:**
  High level multiplexing across any type of species and application, such as mutation detection, CNV, gene expression and species detection.
- **Enhanced specificity and sensitivity:**
  Assay designs based on proprietary locked nucleic acid (LNA) technology.
- **Highly flexible service offering:**
  Tailored to fit your custom design needs. You can choose custom design only or in combination with an in-depth wet-lab verification service.

Verification service

Melting temperature (T_m) and specificity determination by qPCR

A two-step preliminary qPCR screening is performed. The first step is to determine the optimal melting temperature using a gradient format per assay. The second step involves a specificity assessment of the assays across various dilutions of individual targets with mix-match and mismatch targets as necessary. Results from this two-step preliminary screening is used to determine the metrics for the dPCR wet-lab validation process in a single-plex and multiplex format on the respective nanoplates.

Singleplex assay setup

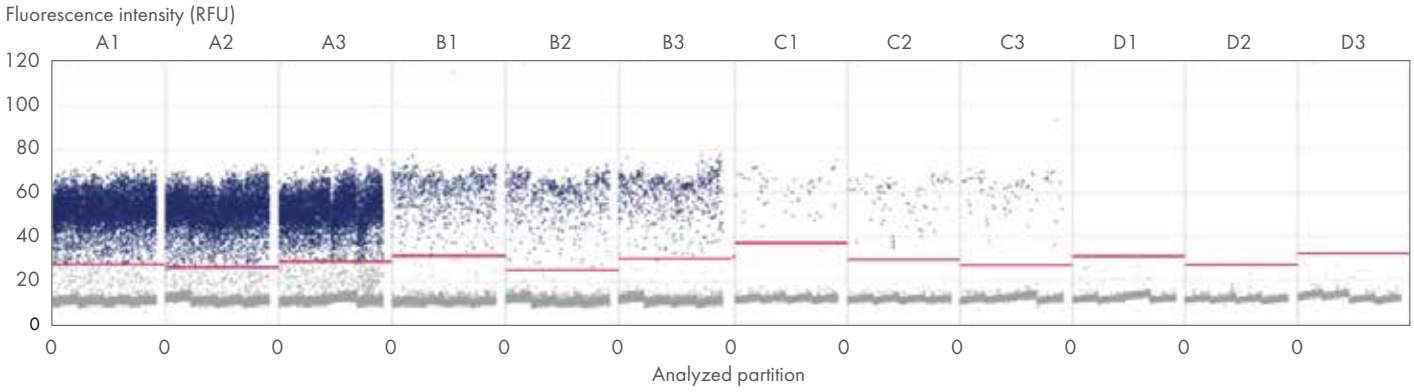

Figure 2. 1D scatterplot graphs.
Representing assays tested in a singleplex setup on QIAcuity. Each assay is tested on synthetic targets in replicates of 4 dilutions for evaluating signal intensity and signal-to-noise- separation and linearity of dilution.

Sample/NTC	Conc. (copy/µl)	CI -95%	Valid partitions	Positive partitions	Negative partitions	Threshold	Positive/ valid
Assay 1 ($1x10^6$)	Infinity	n,a,	25346	25346	0	50.25	100.00%
Assay 1 ($1x10^5$)	1812.9	1.00%	24921	19225	5696	50.25	77.14%
Assay 1 ($1x10^2$)	1	42.70%	25334	22	25312	50.25	0.09%
Assay 1 ($1x10^1$)	0.235	95.70%	25353	5	25348	50.25	0.02%
Assay 1 ($2,5x10^5$ mixmatch)	4260.2	0.70%	24897	24121	776	50.25	96.88%
Assay 1 ($2,5x10^4$ mixmatch)	449	2.00%	25356	7933	17423	50.25	31.29%
Assay 1 ($2,5x10^1$ mixmatch)	0.658	54.20%	25405	14	25391	50.25	0.06%
Assay 1 (2,5 mixmatch)	0.197	109.10%	24905	4	24901	50.25	0.02%
Assay 1 ($1x10^6$ mismatch)	0.151	130.00%	25455	3	25452	50.25	0.01%
Assay 1 $1x10^5$ mismatch)	0.107	168.60%	25421	2	25419	50.25	0.01%
Assay 1 $1x10^2$ mismatch)	0.255	95.70%	25466	5	25461	50.25	0.02%
Assay 1 $1x10^1$ mismatch)	0.151	130%	25464	3	25461	50.25	0.01%
Assay 1 (NTC)	0.204	109.20%	25435	4	25431	50.25	0.02%
Assay 2 ($1x10^6$)	788.1	1.60%	25349	11734	13615	69.63	46.29%
Assay 2 ($1x10^5$)	174	3.40%	25464	3202	22262	69.63	12.57%
Assay 2 ($1x10^2$)	0.248	95.70%	25466	5	25461	69.63	0.02%
Assay 2 ($1x10^1$)	0.05	274.40%	25465	1	25464	69.63	0.00%

Sample/NTC	Conc. (copy/µl)	CI -95%	Valid partitions	Positive partitions	Negative partitions	Threshold	Positive/ valid
Assay 2 (2,5x10 mixmatch)	702.5	1.70%	25468	10666	14802	69.63	41.88%
Assay 2 (2,5x10^4 mixmatch)	74.1	5.10%	25484	1454	24030	69.63	5.71%
Assay 2 (2,5x10^1 mixmatch)	0.199	109.10%	25476	4	25472	71.03	0.02%
Assay 2 (2,5 mixmatch)	0.051	274.40%	25474	1	25473	71.03	0.00%
Assay 2 (1x10^6 mismatch)	4.4	21.20%	25465	86	25379	69.63	0.34%
Assay 2 (1x10^5 mismatch)	0.43	73.30%	25448	8	25440	69.63	0.03%
Assay 2 (1x10^2 mismatch)	0	–	25475	0	25475	69.63	0.00%
Assay 2 (1x10^1 mismatch)	0	–	25460	0	25460	69.63	0.00%
Assay 2 (NTC)	0	–	25480	0	25480	71.03	0.00%

Table 1. Quantitative analysis.
Positive and valid partitions are used to calculate the copies per µl in match/mixmatch/mismatch detection setup to determine robustness (recovery in added background i.e mixmatch) and specificity (lack of signal from off-target i.e. mismatch) of the singleplex assay.

Multiplex assay setup

Channel	Sample/NTC/ Control	Conc. (copy/µl)	CI -95%	Valid partitions	Positive partitions	Negative partitions	Threshold	Positive/ valid
Assay 1	Template 4 1x10^6	0.747	54.10%	25464	14	25450	35.14	0.05%
Assay 2		0.053	274.40%	25432	1	25431	64.08	0.00%
Assay 3		0.32	86.20%	25450	6	25444	28.93	0.02%
Assay 4		11899.7	0.40%	25465	25461	4	28.71	99.98%
Assay 1	Template 4 1x10^5	0.153	130.00%	25470	3	25467	35.14	0.01%
Assay 2		0.051	274.50%	25468	1	25467	64.08	0.00%
Assay 3		0.051	274.50%	25470	1	25469	28.93	0.00%
Assay 4		1954.4	1.00%	25471	19817	5654	28.71	77.80%
Assay 1	Template 4 1x10^2	0.404	73.30%	25456	8	25448	35.14	0.03%
Assay 2		0	–	25464	0	25464	64.08	0.00%
Assay 3		0	–	25464	0	25464	28.93	0.00%
Assay 4		1.5	37.00%	25460	29	25431	28.71	0.11%
Assay 1	Template 4 1x10^1	0.267	95.70%	25462	5	25457	35.14	0.02%
Assay 2		0.053	274.40%	25449	1	25448	64.08	0.00%
Assay 3		0.053	274%	25462	1	25461	28.93	0.00%
Assay 4		0.053	274.40%	25442	1	25441	28.71	0.00%

Table 2. Quantitative analysis.
Positive and valid partitions are used to calculate the copies per µl in multiplex detection setup to determine robustness and multiplex performance.

Service specification

Consultation

Get a free consultation with an expert to define a custom assay design approach based on your specific assay requirements.

Expert Assay design

Custom assays will be designed based on specific design criteria to answer your biological question, while using proprietary design tools featuring LNA technology. Number of designs will be defined based on your project requirements and assay complexity.

Known mutations	Homo sapiens - Genome Build 38: COSMIC ID (COSV+8 digits) or Gene Globe ID required
Known fusions	Homo sapiens - Genome Build 38: COSMIC ID (COSF)
Novel mutation targets	The type and the mutation site should be clearly identified in the sequence (100 nt upstream and 100 nt downstream of mutation site are required). Example: SNV: (+100nt)ccccgaccacatgaa(g>C)cagcacgacttcttca(+100 nt)
Copy number variation	Homo sapiens - Genome build 38: Ensembl Gene ID required
Gene expression	Homo sapiens - Genome build 38: Ensembl Transcript ID required
Species level detection	Species name and NCBI Taxonomy ID required

Deliverables:

- Assay specific cat. no. for probes and primers.

Wet lab assay verification service (optional)

Get sensitivity and specificty testing with our custom dPCR and qPCR assays. We will test primers, probe, synthetic target and corresponding relevant close off-target(s) (when applicable) in dPCR and qPCR reactions over 4 dilutions of the target. Assays will be tested individually in a singleplex setup with one round of iteration of potential mix and match swapping primers/probes and/or fine-tuning the cycling conditions when appropriate and necessary.

When applicable, assays will be tested in a multiplex setup for a minimum of 4 dilutions of the target in defined (off target) background. Assays will be tested with two rounds of iteration for any potential mix and match swapping primers /probes/ assays and/or fine-tuning the cycling conditions when appropriate and necessary.

Experimental setup for wet lab verification will be designed depending on project requirements and assay complexity.

Deliverables:

dPCR

- Assay Design Project Report
- Raw data: assay conditions, Ct values /well, QC positive and negative controls, assay data on 4 serial dilutions.
- Analyzed data: serial dilution curves, 1d scatter plots, copies/µl, % mutant fraction /copy numbers.

qPCR

- Assay Design Project Report
- Raw data: Ct values/well, QC positive and negative controls, assay data on 4 serial dilutions.

Note: Service specifications can be tailored to your project needs.

How can we accelerate your research?

Our expert team is looking forward to learning about your research project and designing your customized service with QIAGEN.

Tell us about your project

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