

Genotyping with Juno

Frequently Asked Questions

ASSAYS

Can I use TaqMan Assays if the stock concentration is only 20X?

Yes. When preparing assays for use in Dynamic Array™ IFCs, first combine equal volumes of 20X SNP Genotyping Assay mix and 2X Assay Loading Reagent, then add the recommended volume of 50X ROX™ Reference Dye. For example, the volume per inlet with overage would include 2.5 µL of 20X SNP Genotyping Assay mix, 2.5 µL of 2X Assay Loading Reagent and 0.25 µL of 50X ROX, for a total volume of 5.25 µL. Although the resulting assay concentration will be less than 10X, the chemistry is sufficiently robust to tolerate a slight reduction in concentration of all three components in the assay mixture.

What Fluidigm instruments are required for SNP genotyping?

An IFC Controller, thermal cycler and fluorescence reader are required for Fluidigm SNP genotyping.

The type of IFC Controller that is needed depends on the genotyping IFC format:

IFC Controller	IFC Format
MX	48.48 GT
HX	96.96 GT and Flex Six™
RX	192.24 GT
WX	FR48.48

Thermal cycling can be performed using the stand-alone Fluidigm FC1™ cycler or the Biomark™ or Biomark HD system. For low concentrations of DNA, use the Juno™ system and the Juno 96.96 Genotyping IFC. The Juno system integrates the IFC Controller with the thermal cycler.

Fluorescence imaging can be performed using a Fluidigm EP1™ reader or the Biomark or Biomark HD system.

How do the SNP Type and TaqMan chemistries differ?

Both chemistries are robust methods for SNP genotyping, and both are compatible with specific target amplification protocols. Key differences between the chemistries are highlighted in the table below:

	TaqMan	SNP Type
Mechanism	Allele-specific hybridization	Allele-specific extension
Primers	Locus-specific (2)	Sequence-tagged, allele-specific (2), and locus-specific (1)
Probes	FAM™, VIC®	FAM, HEX
Labeling	Individual	Universal
Fluorescence generation	Probe displacement and cleavage	Fluorescent amplicon formation
Fluorescence detection	Real-time or endpoint	Endpoint

For more information regarding specific protocols and necessary reagent components, see the [SNP Genotyping User Guide](#) (PN 68000098) or the [Juno System User Guide](#) (PN 100-7070).

What genotyping chemistries can be used on the Fluidigm platform?

Fluidigm supports the use of its SNP Type™ assays and the TaqMan® SNP Genotyping Assays from Thermo Fisher Scientific. Although LGC Genomics offers a Fluidigm-specific KASP genotyping reagent for the Fluidigm platform, KASP genotyping is no longer supported on the platform.

What filters are included on the Biomark HD system?

The Biomark HD system comes with excitation sources and emission filters for FAM, VIC and ROX. In addition, a filter for Cy5® can be purchased for use with the Biomark HD system.

Can I use whole genome amplification (WGA) products as samples for genotyping?

Use of WGA samples is not recommended, because of the risk of allele dropout or imbalance resulting from the WGA method.

How many primer pairs can I include in a multiplex preamplification reaction?

The Fluidigm preamplification protocol has been validated for multiplex preamplification of up to 96 targets.

What is the minimum concentration of genomic DNA sample required for preamplification?

A minimum concentration of 10 ng/μL is recommended for human samples. As the size of the genome determines input template copy number, a relative concentration should be used for species of differing genome size.

Can I use any Fluidigm Sample Loading Reagent for my genotyping sample preparation?

No. Each of the available genotyping protocols has been optimized for best performance, and only the specified Sample Loading Reagent should be used. The 20X GT (Genotyping) Sample Loading Reagent, 20X Fast GT (Genotyping) Sample Loading Reagent and 20X SNP Type Sample Loading Reagent are different proprietary formulations.

If my gDNA is less than the minimum required concentration for genotyping, what can I do?

If the minimum gDNA concentration cannot be met, we recommend preamplification prior to genotyping. For more information, see the preamplification protocol in the [SNP Genotyping User Guide](#) (PN 68000098).

Note: Genomic DNA samples derived from blood, saliva, buccal swabs, laser capture microdissection or FFPE sources usually require preamplification.

What are SNP Type assays?

SNP Type assays are allele-specific PCR assays made by Fluidigm that use three primers and two universal probes to distinguish between two alleles. For more information, see the [SNP Genotyping User Guide](#) (PN 68000098).

What fluorophores are used for SNP Type genotyping? How do I know which fluorophore represents which allele?

SNP Type genotyping assay probes are labeled with the FAM and HEX fluorophores. Allele-Specific Primer 1 (ASP1) yields a FAM-labeled, allele-specific amplicon, and Allele-Specific Primer 2 (ASP2) yields a HEX-labeled, allele-specific amplicon. SNP alleles and corresponding allele-specific primer sequences and fluorophores are provided by Fluidigm in a SNP Type assay design file.

Why can't I run real-time PCR with my SNP Type assays?

SNP Type genotyping reactions must be imaged at room temperature (25 °C) so that any unincorporated universal probes will be hybridized to their respective quencher oligos.

What PCR master mixes are supported for genotyping?

Fluidigm currently supports the use of the following master mixes:

- Biotium Fast Probe Master Mix (2X; Biotium PN 31005) for SNP Type genotyping
- TaqMan Universal PCR Master Mix (2X; Thermo Fisher Scientific PN 4304437) for standard TaqMan genotyping
- TaqMan GTXpress™ Master Mix (2X; Thermo Fisher Scientific PN 4403311) for fast TaqMan genotyping

Workflows

What if I don't have exactly 48 or 96 samples or assays? Can I load water into my empty inlets?

To maintain uniformity on the IFC, unused inlets must be filled. If sample and assay mixes are limited, unused inlets can be filled with water or buffer.

How much time can elapse between loading an IFC and thermal cycling?

Because IFCs are made of a gas-permeable material, it is best to start PCR on the Biomark HD system or FC1 cycler immediately to avoid IFC dehydration or reagent evaporation.

Can the IFCs be centrifuged to get rid of bubbles?

Do not centrifuge an IFC, as centrifugation might damage the IFC. To avoid introducing bubbles:

- Before adding sample and assay mixes to the inlets, check for bubbles in the source plates (such as any plate that is not an IFC). If there are bubbles in the wells, centrifuge the plate (but not the IFC) at a high speed for 30–60 seconds.
- When pipetting from a source plate to an IFC, confirm that there are no air gaps in pipette tips after aspiration.
- Pipet slowly, and only depress the pipette to the first stop to avoid introducing bubbles.
- After filling the sample and assay inlets, gently tap the IFC on a bench top to ensure that the sample and assay mixes cover the bottom of the inlets.

Can I use TaqMan Assays if the stock concentration is only 20X?

Yes. When preparing assays for use in Dynamic Array IFCs, first combine equal volumes of 20X SNP Genotyping Assay mix and 2X Assay Loading Reagent, then add the recommended volume of 50X ROX Reference Dye. For example, the volume per inlet with overage would include 2.5 µL of 20X SNP Genotyping Assay mix, 2.5 µL of 2X Assay Loading Reagent and 0.25 µL of 50X ROX, for a total volume of 5.25 µL. Although the resulting assay concentration

will be less than 10X, the chemistry is sufficiently robust to tolerate a slight reduction in concentration of all three components in the assay mixture.

Can I change the thermal cycling conditions for my genotyping reactions?

While it is possible to modify existing thermal protocols or create custom thermal protocols, Fluidigm does not support protocol variations other than those listed in the [SNP Genotyping User Guide](#) (PN 68000098), and any deviations are at the risk of the user.

For more information on modifying or creating thermal protocols, see the [FC1 Cyclor User Guide](#) (PN 100-1279), the [Biomark/EP1 Data Collection User Guide](#) (PN 68000127), the [Biomark HD Data Collection User Guide](#) (PN 100-2451) or the [Juno System User Guide](#) (PN 100-7070).

How should I store my assays?

As supplied, forward and reverse primers should be stored at -20°C . Diluted (10X) primer stocks can be stored at 4°C for one week, or longer at -20°C .

What filters are included on the Biomark HD system?

The Biomark HD system comes with excitation sources and emission filters for FAM, VIC and ROX. In addition, a filter for Cy5 can be purchased for use with the Biomark HD system.

What is the maximum number of preamplification cycles I can do?

No maximum number of preamplification cycles has been established. However, if there are major efficiency differences between the primer sets, increasing the number of cycles will exacerbate those differences in the preamplified material. Fluidigm recommends 18 preamplification cycles when using starting material as low as 10 pg of total RNA.

Can I use whole genome amplification (WGA) products as samples for genotyping?

Use of WGA samples is not recommended, because of the risk of allele dropout or imbalance resulting from the WGA method.

How many primer pairs can I include in a multiplex preamplification reaction?

The Fluidigm preamplification protocol has been validated for multiplex preamplification of up to 96 targets.

What is the minimum concentration of genomic DNA sample required for preamplification?

A minimum concentration of 10 ng/μL is recommended for human samples. As the size of the genome determines input template copy number, a relative concentration should be used for species of differing genome size.

Can I use any of the Fluidigm Sample Loading Reagents for my genotyping sample preparation?

No. Each of the available genotyping protocols has been optimized for best performance, and only the specified Sample Loading Reagent should be used. The 20X GT (Genotyping) Sample Loading Reagent, 20X Fast GT (Genotyping) Sample Loading Reagent and 20X SNP Type Sample Loading Reagent are different proprietary formulations.

If my gDNA is less than the minimum required concentration for genotyping, what can I do?

If the minimum gDNA concentration cannot be met, we recommend preamplification prior to genotyping. For more information, see the preamplification protocol in the [SNP Genotyping User Guide](#) (PN 68000098).

Note: Genomic DNA samples derived from blood, saliva, buccal swabs, laser capture microdissection, or FFPE sources usually require preamplification.

How many Dynamic Array IFCs can I run with the provided assays?

At least 100 IFCs can be run with the provided assays. Refer to the following table for estimates with and without preamplification:

Number of IFCs

PREAMPLIFIED			96.96		48.48		192.24	
PN	Size	Vol. (μL)	YES	NO	YES	NO	YES	NO
ASY-GT-S	S	100	150	360	200	360	100	440
ASY-GT-M	M	200	300	720	400	720	200	880
ASY-GT-L	L	500	750	1,800	1,000	1,800	500	2,200

What are some things I can try to improve SNP Type assay performance?

- Preamplification prior to genotyping might help alleviate issues with samples that contain inhibitor carryover from extraction, are of low quality or low concentration, vary in concentration from other samples or come from a species whose genome size is large relative to the human genome. For a detailed preamplification protocol, see the [SNP Genotyping User Guide](#) (PN 68000098) or the [Juno System User Guide](#) (PN 100-7070).

- Some regions of DNA are intrinsically more difficult to amplify. If amplification is inefficient, adding five amplification cycles to the thermal protocol may help.
- If clusters are not sufficiently separated, increasing the EDTA concentration by diluting samples in TE buffer (10 mM Tris, 1 mM EDTA; pH 8.0) instead of DNA Suspension Buffer (10 mM Tris, 0.1 mM EDTA; pH 8.0; (TEKnova, PN T0221)) might increase stringency of the reaction and improve assay specificity. Note that too much EDTA will inhibit the reaction completely.
- Redesigning the assay to the opposite DNA strand might improve assay specificity.

What are the SNP Type assay performance specifications?

See the [SNP Type Assays Data Sheet](#) (PN 100-3464).

How should I store my SNP Type assays?

SNP Type assays are stable for up to six months at -20°C if 96-well plates remain sealed to avoid evaporation. Avoid multiple freeze-thaw cycles by aliquoting assays to separate plates.

Consumables

What is the shelf life for IFCs?

IFCs can be used for one year after they are received if they are unopened and stored at room temperature.

What if I don't have exactly 48 or 96 samples or assays? Can I load water into my empty inlets?

To maintain uniformity on the IFC, unused inlets must be filled. If sample and assay mixes are limited, unused inlets can be filled with water or buffer.

How much time can elapse between loading an IFC and thermal cycling?

Because IFCs are made of a gas-permeable material, it is best to start PCR on the Biomark HD system or FC1 cycler immediately to avoid IFC dehydration or reagent evaporation.

Can the IFCs be centrifuged to get rid of bubbles?

Do not centrifuge an IFC, as centrifugation might damage the IFC. To avoid introducing bubbles:

- Before adding sample and assay mixes to the inlets, check for bubbles in the source plates (such as any plate that is not an IFC). If there are bubbles in the wells, centrifuge the plate (but not the IFC) at a high speed for 30–60 seconds.

- When pipetting from a source plate to an IFC, confirm that there are no air gaps in pipette tips after aspiration.
- Pipet slowly, and only depress the pipette to the first stop to avoid introducing bubbles.
- After filling the sample and assay inlets, gently tap the IFC on a bench top to ensure that the sample and assay mixes cover the bottom of the inlets.

What is the difference between the Genotyping and Gene Expression IFCs?

Gene Expression and Genotyping IFCs are offered in the same formats (e.g., 48.48, 96.96, 192.24 and Flex Six) but do not necessarily have the same reaction chamber volumes. The Gene Expression and High-Precision Genotyping IFCs also undergo a more stringent quality control process than the standard Genotyping IFCs.

Can I reuse an IFC? Can I use part of an IFC, and then use the other part in another experiment?

All IFCs except the Flex Six IFC are single-use only, and the entire IFC must be filled with reagents when used. Flex Six IFCs (available in gene expression and genotyping formats) contain six single-use partitions within the same IFC. Individual partitions can be used on the same day or on different days within a three-month period, but each partition must be entirely filled with reagents when used.

How quickly do I need to use the IFC after the foil pouch is opened?

IFCs must be used within 24 hours of opening the sealed foil pouch. The exception is the Flex Six IFC, which has a 90-day lifetime. However, the lifetime of a Flex Six IFC will decrease if it is not handled according to Fluidigm guidelines. For example, postponing the post-PCR script or altering thermal cycling conditions shortens the expected lifetime of a Flex Six IFC.

Why can't I load the samples in the assay inlets and the assays in the sample inlets?

The sample and assay chambers inside the IFC are different sizes. For this reason, the sample mix is prepared at a 1X concentration, whereas the assay mix is prepared at a 10X or 20X concentration, depending on the IFC used. Pipetting the sample and assay preparations into the incorrect inlets will result in incorrect concentrations for both in the final reaction.

What Fluidigm instruments are required for SNP genotyping?

An IFC Controller, thermal cycler and fluorescence reader are required for Fluidigm SNP genotyping.

The type of IFC Controller that is needed depends on the genotyping IFC format:

IFC Controller	IFC Format
MX	48.48 GT
HX	96.96 GT and Flex Six
RX	192.24 GT
WX	FR48.48

Thermal cycling can be performed using the stand-alone Fluidigm FC1 cycler or the Biomark or Biomark HD system. For low concentrations of DNA, use the Juno system and the Juno 96.96 Genotyping IFC. The Juno system integrates the IFC Controller with the thermal cycler.

Fluorescence imaging can be performed using a Fluidigm EP1 reader or the Biomark or Biomark HD system.

How should I store my assays?

As supplied, forward and reverse primers should be stored at -20°C . Diluted (10X) primer stocks can be stored at 4°C for one week, or longer at -20°C .

How long does it take to prime, load and run each IFC?

IFC Type	Prime	Load-Mix	Thermal Cycling	Protocol
48.48 Gene Expression	10 min	55 min	30 min	Fast TaqMan
			1.5 hr	Standard TaqMan
			30 min	EvaGreen®
48.48 Genotyping	10 min	55 min	30 min	Fast TaqMan
			1.5 hr	Standard TaqMan
			1 hr	SNP Type
			1.25 hr	Fast TaqMan
96.96 Gene Expression	20 min	1.5 hr	2.25 hr	Standard TaqMan
			1.25 hr	EvaGreen
			1.25 hr	Fast TaqMan
96.96 Genotyping	20 min	1.5 hr	2.25 hr	Standard TaqMan
			1.75 hr	SNP Type
			30 min	Fast TaqMan
			1.5 hr	Standard TaqMan
192.24 Gene Expression	N/A	30 min	30 min	EvaGreen
			30 min	Fast TaqMan
			1 hr	SNP Type
192.24 Genotyping	N/A	30 min	2 hr	Fast TaqMan
			3 hr	Standard TaqMan
			2 hr	SNP Type
			2 hr	Fast TaqMan
Flex Six Gene Expression	15 min	50 min	3 hr	Standard TaqMan
			2 hr	SNP Type
			2 hr	Fast TaqMan
Flex Six Genotyping	15 min	50 min	3 hr	Standard TaqMan

IFC Type	Prime	Load-Mix	Thermal Cycling	Protocol
FR48.48	5 min	30 min	2.5 hr	SNP Type
			30 min	Fast TaqMan
			1 hr	SNP Type
12.765 Digital 48.770 Digital/37K™ qdPCR	5 min	41 min	1.5 hr	
	12 min	40 min	1.5 hr	
C1	10 min	30–60 min	5.5–8 hr depending on application, IFC type	

What are the volumes for the chambers inside each of the IFCs?

IFC	Chamber Type	Volume (nL)
48.48 Gene Expression	Sample	9.1
	Assay	1
48.48 Genotyping	Sample	6.75
	Assay	0.6
96.96 Gene Expression	Sample	6.7
	Assay	0.49
96.96 Genotyping	Sample	6.7
	Assay	0.49
192.24 Gene Expression	Sample	8
	Assay	1
192.24 Genotyping	Sample	8
	Assay	1
Flex Six Gene Expression	Sample	7.9
	Assay	0.97
Flex Six Genotyping	Sample	7.9
	Assay	0.97
12.765 Digital	Chamber	6.075
48.770 Digital/37K qdPCR	Chamber	0.75
C1		4.5 + 9 + 9 +
		135 + 135

Software

What is the difference between SNP Type Normalization and no template control (NTC) normalization?

SNP Type Normalization is the default normalization method for Fluidigm SNP Type assays. It helps guide automatic calling of genotypes even when nonspecific signal is observed in the

NTC ("floating NTC"). SNP Type Normalization determines a global NTC position based on the majority position of NTCs across the entire IFC run.

NTC normalization is the default normalization method for TaqMan Assays. Each TaqMan SNP Genotyping assay contains two dually labeled probes, which may differ with respect to background fluorescence. NTC normalization takes into account these differences and attempts to position each NTC at a 0.1, 0.1 position on the scatter plot.

For more information, see the [SNP Genotyping User Guide](#) (PN 68000098).

What is the difference between NO CALL and an Invalid call in the Fluidigm Genotyping Analysis software?

A NO CALL is assigned by the genotype-calling algorithm when, based on the confidence threshold set by the user, it cannot associate a given datapoint with a cluster. Although no genotype call has been assigned to the datapoint, a NO CALL remains part of the analyzed dataset. An Invalid call can only be manually assigned by a reviewer and should be used to remove datapoints from the analysis.

If data is reanalyzed after invalidating datapoints, the software will reanalyze the entire IFC, excluding the invalid datapoints. This may change automatic calls of datapoints, but any manually edited calls will not change upon reanalysis.

When should I use the All SNP Scatter Plot View option in the SNP Genotyping Analysis software?

The All SNP Scatter Plot View option displays all SNP data from the IFC in a single scatter plot. It is used as a quick, global assessment of a run and can be helpful for identifying outlier datapoints that may affect the display or scale of individual SNP scatter plots. Any manual genotype editing should be done within the individual SNP scatter plots.

How does the Fluidigm SNP Genotyping Analysis software determine the genotype call for a sample?

Allele discrimination is primarily based on ratios from the two normalized probe measurements.

The Fluidigm SNP Genotyping Analysis software uses the k-means clustering analysis method to automatically call genotypes. The algorithm starts by initializing a number of cluster centers (k), first assuming all three clusters are present (k=3). It is followed by an iterative process of assigning datapoints to clusters based on the proximity to the cluster center and updating cluster centers based on the means of the datapoints within each cluster.

If the algorithm fails to identify three clusters, then a k of two clusters is tried. Clusters must be sufficiently far apart from each other. If they are not, clusters may receive the same genotype assignments.

A review of automatic genotype calls in individual scatter plots is recommended after cluster analysis.

What can I do to help improve call rates?

Genotype call quality can vary when the amount or the quality of the input DNA is not ideal, potentially leading to either an incorrect genotype call or a NO CALL for a particular SNP. Preamplification can be used prior to genotyping to increase the input DNA copy number.

Design

What species and targets do you design assays to?

Delta Gene™ assays are designed to human and mouse species for targets with an associated RefSeq ID, gene name, target sequence or genome coordinate. (See the [D3 Assay Design User Guide](#), PN 100-6812.) Submit requests for other species to the Assay Design Group.

Primers are designed to cross an intron wherever possible to avoid amplifying genomic DNA. They are also designed to detect all known isoforms when possible.

Do you have any advice on primer design?

See these documents:

- [Delta Gene Assays Data Sheet](#) (PN 100-3035)
- [D3 Assay Design User Guide](#) (PN 100-6812)

How do I design and order SNP Type assays?

SNP Type assays can be designed and ordered through the D3™ assay design on the Fluidigm website. For more information, see D3 assay design on the Fluidigm website or the [D3 Assay Design User Guide](#) (PN 100-6812).

What is provided with my order of SNP Type assays?

SNP Type assays are provided in three separate oligo plates per 96 assays. All oligos are in nuclease-free water. An order contains:

- Allele-Specific Primers (ASP1 and ASP2): Each primer is mixed in equal molar ratios to a final concentration of 100 µM (per primer) in 100 µL for small; 200 µL for medium; 500 µL for large.
- Preamplification Primer: Each primer is normalized to 100 µM (per primer) in 100 µL for small; 200 µL for medium; 500 µL for large.
- Locus-Specific Primer (LSP): Each primer is normalized to 100 µM (per primer) in 100 µL for small; 200 µL for medium; 500 µL for large.
- Customer Informatics Packet

Can SNP Type assays be wet-lab tested?

No, SNP Type assays cannot be ordered wet-lab tested.

What types of SNPs can be targeted with D3 assay design?

We currently offer designs for bi-allelic SNPs only.

Can SNP Type assays be designed to targets with adjacent SNPs?

Target SNPs that contain adjacent SNPs within 20 base pairs on either side will not be designed. Fluidigm recommends removal of an adjacent SNP (from the context sequence submitted) if it is not pertinent within the study population.

How many bases upstream and downstream of target SNPs do I need to provide when submitting a D3 assay design request?

A minimum of 60 base pairs (bp) upstream and 60 bp downstream of the targeted SNP is recommended. Up to 250 bp can be included to increase the likelihood of a successful design.

Does Fluidigm design to insertions or deletions (indels)?

As a nonstandard option, D3 assay design can be used for indels ≤ 10 base pairs. Indels > 10 bp should not be included.

What is the turnaround time for D3 assay design?

D3 assay design makes designs available for review within 72 hours of submission. Fluidigm will deliver primers within four weeks.

What defines difficult genomic regions, and can D3 assay design be used for those regions?

Regions bearing greater than 65% guanine-cytosine content can be difficult to design against. Sequences are filtered based on GC content. If an assay bearing less than 65% GC content cannot be derived, this is reported as a "no design." Successfully designed sequences that have greater than 65% GC content will be assigned a medium design rank automatically.

How do I determine the status of D3 assay design requests?

The Fluidigm D3 assay design tool shows you the status of your assay design requests, and they are updated automatically as your status changes:

Status	Notification
DESIGN	Created: An empty project has been created without targets.
	In Progress: Targets have been added and/or targets have been submitted for design.
	Completed: Designs are completed and ready for your review.
ORDER	Quote Requested Submitted
	Quote Requested Received
	P.O. Processed
	Shipped

You are also welcome to contact Sales for an update on your order.

To contact a Sales Representative:

North America | +1 650 266 6170 | info-us@fluidigm.com

Europe/EMEA | +33 1 60 92 42 40 | info-europe@fluidigm.com

Japan | +81 3 3662 2150 | info-japan@fluidigm.com

Asia | +1 650 266 6000 | info-asia@fluidigm.com

Latin America | +1 650 266 6000 | info-latinamerica@fluidigm.com

Does Fluidigm design SNP Type assays for nonhuman species?

Yes. Please specify species type and provide sequences when using the D3 assay design service. Note that assay specificity checks and masking options are available only for “supported” genomes (currently, human and mouse). Designs to other genomes rely only on the sequence provided by the customer, so it is important to (1) avoid submitting sequences for duplicated or repetitive genomic regions and (2) indicate any additional nontargeted SNPs using IUPAC codes.

How many Dynamic Array IFCs can I run with the provided assays?

At least 100 IFCs can be run with the provided assays. Refer to the following table for estimates with and without preamplification:

Number of IFCs

Preamplified PN	Size	Vol. (µL)	96.96		48.48		192.24	
			Yes	No	Yes	No	Yes	No
ASY-GT-S	S	100	150	360	200	360	100	440
ASY-GT-M	M	200	300	720	400	720	200	880
ASY-GT-L	L	500	750	1,800	1,000	1,800	500	2,200

What is specific target preamplification (STA)?

Specific target amplification (STA) takes place as a multiplex PCR reaction that uses a low molar concentration of each primer pair (50 nM) and limited cycles (typically 10–18) to provide sufficient template molecules for use on Fluidigm IFCs with lower-quality or limited samples. This allows for unbiased target enrichment prior to performing targeted gene expression on the Biomark HD system.

Orders

What is included in the Fluidigm Installation Qualification/Operation Qualification (IQOQ) services?

The Fluidigm IQOQ service is a reliable, secure and convenient solution for customers using Fluidigm equipment in GLP/GMP environments. Installation Qualification verifies that an instrument is installed properly to Fluidigm specifications and that the customer's lab environment is suitable for operation. Operation Qualification is a performance test that, at the time of testing, verifies the system functions according to Fluidigm operational specifications where the instrument is installed.

Fluidigm can help you meet regulatory compliance by qualifying the installation and operation of your Biomark, Biomark HD or EP1 system and by certifying your Fluidigm thermal cyclers, Juno or Access Array™ instruments and IFC Controllers.

How do I place orders?

Find a sales representative.

Locate a distributor.

Phone: 866 359 4354, extension 2

Fax: 650 589 2548

Email: sales.admin@fluidigm.com

What are the delivery options?

Standard, noncustomized consumable orders typically ship within three to five business days. Shipping charges will be 2% of your shopping cart subtotal (\$50.00 minimum charge), with an additional \$20.00 handling fee for consumable reagent orders requiring dry ice.

Your products will ship via FedEx® Overnight®, selected as the best way to maintain quality delivery and product integrity.

If you have questions about shipping, please **contact us**.

For information about shipping outside the United States, contact your **regional Fluidigm representative**.

What currencies do you accept?

For now, online ordering is available only to organizations in the United States and its territories. We do business online only in US dollars (USD). To place international orders, email us at sales.admin@fluidigm.com or contact your local sales representative or distributor.

How do I find my local distributor?

Online ordering is not currently offered outside the United States. You can contact your country's regional distributor to purchase Fluidigm products directly. **Locate your local distributor here**.

How do I request a quote?

To request a sales quote online:

- For Fluidigm systems: Select Inquire to request a quote from your local sales representative.
- For general consumables: Select Inquire to send a quote request.
- For offline quotes: Contact your local sales representative or dial 650 266 6170.

How do I cancel, add, or modify orders?

We process and ship orders quickly, so there is limited time to change or cancel them. To make changes to an online order, contact our sales administration team immediately at sales.admin@fluidigm.com or 866 359 4354, extension 2, and we'll do our best to assist you.

How do I sign in to my account?

Click the **Menu** icon to the left of the Search field to get the login prompt. Sign in with your email address and password. You can update account settings, manage other account details and see previous online orders and support requests.

What information is provided in the Customer Informatics Packet?

An informatics packet is emailed at the time of product shipment. It includes primer sequences, plate layout and instructions on where to download supporting documents such as user guides and quick references. Informatics packets are also available online with your D3 assay design orders.

The information that is provided in the Customer Informatics Packet for Delta Gene assays:

- The Design File, which is a Microsoft® Excel® file that includes assay name, Fluidigm Assay ID, primer sequences, representative RefSeq, gene symbol, gene aliases, gene full name and gene ontology: function, process and component
- A plate layout file containing positions of the assays in 96-well plate format
- A PDF assay report that details the assay names, NCBI Reference Sequence ID (RefSeq), image of primer binding positions, amplicon length and gene ontology information
- Assay performance report (for wet-lab tested assays only) including mean delta Ct slope for each assay

How do I design and order SNP Type assays?

SNP Type assays can be designed and ordered through the D3 assay design on the Fluidigm website. For more information, see D3 assay design on the Fluidigm website or the [D3 Assay Design User Guide](#) (PN 100-6812).

What is provided with my order of SNP Type assays?

SNP Type assays are provided in three separate oligo plates per 96 assays. All oligos are in nuclease-free water. An order contains:

- Allele-Specific Primers (ASP1 and ASP2): Each primer is mixed in equal molar ratios to a final concentration of 100 μ M (per primer) in 100 μ L for small; 200 μ L for medium; 500 μ L for large.
- Preamplification Primer: Each primer is normalized to 100 μ M (per primer) in 100 μ L for small; 200 μ L for medium; 500 μ L for large.
- Locus-Specific Primer (LSP): Each primer is normalized to 100 μ M (per primer) in 100 μ L for small; 200 μ L for medium; 500 μ L for large.
- Customer Informatics Packet

Can SNP Type assays be wet-lab tested?

No, SNP Type assays cannot be ordered wet-lab tested.

What types of SNPs can be targeted with D3 assay design?

We currently offer designs for bi-allelic SNPs only.

Can SNP Type assays be designed to targets with adjacent SNPs?

Target SNPs that contain adjacent SNPs within 20 base pairs on either side will not be designed. Fluidigm recommends removal of an adjacent SNP (from the context sequence submitted) if it is not pertinent within the study population.

How many bases upstream and downstream of target SNPs do I need to provide when submitting a D3 assay design request?

A minimum of 60 base pairs (bp) upstream and 60 bp downstream of the targeted SNP is recommended. Up to 250 bp can be included to increase the likelihood of a successful design.

Does Fluidigm design to insertions or deletions (indels)?

As a nonstandard option, D3 assay design can be used for indels ≤ 10 base pairs. Indels > 10 bp should not be included.

What is the turnaround time for D3 assay design?

D3 assay design makes designs available for review within 72 hours of submission. Fluidigm will deliver primers within four weeks.

What defines difficult genomic regions, and can D3 assay design be used for those regions?

Regions bearing greater than 65% guanine-cytosine content can be difficult to design against. Sequences are filtered based on GC content. If an assay bearing less than 65% GC content cannot be derived, this is reported as a "no design." Successfully designed sequences that have greater than 65% GC content will be assigned a medium design rank automatically.

What information is provided in the Customer Informatics Packet?

- The Design File, which is a Microsoft Excel file that includes all ASP sequences (without tag sequences), preamplification of specific targeted sequences, and LSP sequences and their location on the assay plates.
- A README PDF file, which contains instructions on where to download supporting documents such as user guides and Quick-References.

How do I determine the status of D3 assay design requests?

The Fluidigm D3 assay design tool shows you the status of your assay design requests, and they are updated automatically as your status changes:

Status	Notification
DESIGN	Created: An empty project has been created without targets.
	In Progress: Targets have been added and/or targets have been submitted for design.
	Completed: Designs are completed and ready for your review.

ORDER Quote Requested Submitted
 Quote Requested Received
 P.O. Processed
 Shipped

You are also welcome to contact Sales for an update on your order.

To contact a Sales Representative:

North America | +1 650 266 6170 | info-us@fluidigm.com

Europe/EMEA | +33 1 60 92 42 40 | info-europe@fluidigm.com

Japan | +81 3 3662 2150 | info-japan@fluidigm.com

Asia | +1 650 266 6000 | info-asia@fluidigm.com

Latin America | +1 650 266 6000 | info-latinamerica@fluidigm.com

Does Fluidigm design SNP Type assays for nonhuman species?

Yes. Please specify species type and provide sequences when using the D3 assay design service. Note that assay specificity checks and masking options are available only for “supported” genomes (currently, human and mouse). Designs to other genomes rely only on the sequence provided by the customer, so it is important to (1) avoid submitting sequences for duplicated or repetitive genomic regions and (2) indicate any additional nontargeted SNPs using IUPAC codes.

How many Dynamic Array IFCs can I run with the provided assays?

At least 100 IFCs can be run with the provided assays. Refer to the following table for estimates with and without preamplification:

Number of IFCs

			96.96		48.48		192.24	
Preamplified			Yes	No	Yes	No	Yes	No
PN	Size	Vol. (µL)						
ASY-GT-S	S	100	150	360	200	360	100	440
ASY-GT-M	M	200	300	720	400	720	200	880
ASY-GT-L	L	500	750	1,800	1,000	1,800	500	2,200

How do I supply my sales tax exemption?

If your institution benefits from a sales tax exemption, please send your Sales Tax Exempt Certificate to credit@fluidigm.com with “TAX” in the attention field. We charge applicable sales tax unless we receive a Sales Tax Exempt Certificate.

How can I track shipments?

To check your order status or track your shipment, you can request a FedEx tracking number by emailing sales.admin@fluidigm.com and including your sales order number. If you do not have your sales order number, please provide other details of the order, including the date, the name of your organization, contact name and purchase order number (if applicable).

How can I request a copy of my invoice?

You can request a copy of your invoice from our accounts receivable team by emailing accountsreceivable@fluidigm.com. Please include your sales order number, the name of your organization and contact name. Invoices are issued after items have been shipped.

How do I view my online order history?

You can view online order history by selecting Orders. Orders are listed in chronological order. To get a record of your offline orders, including those placed by email, fax or phone, email your request to sales.admin@fluidigm.com.

How do you manage security?

For online payments we use the Authorize.Net Payment Gateway, which provides the infrastructure and security needed to send transaction data reliably and securely. All secure logins and checkouts use the latest Secure Sockets Layer (SSL) technology to encrypt your credit card and password information. To protect your financial information, Fluidigm keeps no record of your credit card information. You do not have to re-enter your credit card for each new order because it is stored securely using Payment Card Industry standards.

Can I order for multiple labs?

Yes, you can. If you are ordering for separate labs with different shipping addresses, you must enter them individually. If they use a single central receiving address, you can order for more than one lab at a time on the same purchase order or purchasing card.

I have a quote from my sales representative. Can I use that quote online?

Quotes from sales representatives cannot be redeemed online at this time, but we're working to allow that in the future.

What is Secure Sockets Layer (SSL)?

The Secure Sockets Layer uses encryption enabled by our server's SSL Certificate. When your browser points to our secure domain, an SSL handshake authenticates our server and your browser, establishing an encryption method and a unique session key. You then have a secure session, with your privacy and message integrity protected.

We routinely schedule security audits of our code and data exchange procedures to ensure that all of our processes are secure and compliant with frequently published security standards.

For technical support visit fluidigm.com/support.

North America +1 650 266 6100 | Toll-free: +1 866 358 4354 in the US | support.northamerica@fluidigm.com **Europe** +33 1 60 92 42 40 | support.europe@fluidigm.com

China (excluding Hong Kong) +86 21 3255 8368 | techsupportchina@fluidigm.com **Japan** +81 3 3662 2150 | techsupportjapan@fluidigm.com

All other Asian countries +1 650 266 6100 | techsupportasia@fluidigm.com **Central and South America** +1 650 266 6100 | techsupportlatam@fluidigm.com

For Research Use Only. Not for use in diagnostic procedures.

Information in this publication is subject to change without notice. **Safety data sheet information:** fluidigm.com/sds. **Patent and license information:** fluidigm.com/legalnotices. **EU's WEEE directive information:** fluidigm.com/compliance. Fluidigm, the Fluidigm logo, Access Array, Biomark, D3, Delta Gene, Dynamic Array, EP1, FC1, Flex Six, Juno, and SNP Type are trademarks or registered trademarks of Fluidigm Corporation in the United States and/or other countries. All other trademarks are the sole property of their respective owners. © 2016 Fluidigm Corporation. All rights reserved. 03/2016