Infinium® iSelect® Custom Genotyping Assays

Guidelines for using the DesignStudio™ Microarray Assay Designer software to create and order custom arrays.

Introduction

The Illumina Infinium Assay enables highly multiplexed, array-based genotyping. In addition to a broad portfolio of fixed content BeadArray™ products, Illumina offers custom and semicustom genotyping panels deployed on various BeadChip formats. Researchers can design their own content panels using the online DesignStudio Microarray Assay Designer (Microarray Designer) software. This online tool is compatible with Infinium iSelect high-definition (HD), iSelect high-throughput screening (HTS), and XT iSelect Custom BeadChips to target single-nucleotide polymorphisms (SNPs) and insertions/deletions (indels) in any species. Researchers can select which option best fits their experimental and throughput needs (Table 1). Infinium XT BeadChips are designed for production-scale genotyping and intended for annual throughputs of 100,000-1,000,000+ samples per year.

This technical note describes the process of designing, evaluating, and ordering a custom panel of markers. Microarray Designer software ensures successful custom panel assay development by evaluating lists of requested loci and selecting an initial panel of markers that are predicted to have a high likelihood of success. Metrics returned by Microarray Designer software provide predicted success information, Infinium validation status, and minor allele frequencies (MAFs) from published studies. Based on this evaluation, researchers can place an order for their custom panel design.

Table 1: Custom Panel Options

	Infinium iSelect HD	Infinium iSelect HTS	Infinium XT iSelect	Commercial+
Attempted Bead Types (ABTs)	3072-90,000	90,000-700,000	500-50,000	Varies
Number of Samples per BeadChip	24	24	96	Varies
Minimum Purchase Order (samples)	1152	1152	100,000	1152

Assay Design Considerations

Bead Types and Assay Design

Infinium BeadChips use 50-mer probes carefully designed to hybridize selectively to a locus, stopping 1 base before the SNP of interest. Marker specificity is conferred by enzymatic single-base extension to incorporate a labeled nucleotide. Subsequent dual-color fluorescent staining enables detection of the incorporated nucleotide by the HiScan® or iScan® System. Infinium BeadChips use 2 possible probe (or bead type) designs, depending on the type of SNP or marker being assayed.

Infinium II Probe Design

The Infinium II design uses only 1 probe per locus (ie, 1 bead type for both alleles). This probe design is suitable for noncomplimentary SNPs (eg, A to C or G) and enables genotyping of most loci in most organisms (eg, approximately 84% of known SNPs in the human genome).

Infinium I Probe Design

The Infinium I design is required for relatively less-common A/T and C/G SNPs. It uses 2 probes per SNP to determine the relative intensity ratio of the 2 possible target alleles for any given locus (ie, 2 bead types, 1 for each allele).

Bead Types Define Content Limit

The number of attempted bead types (ABTs), not the number of loci assayed, determines the maximum content limit for Infinium iSelect HD and HTS Custom BeadChips. If a project is exclusively limited to loci using Infinium II designs (A/G, A/C, T/G, T/C SNPs; and all indels), then the number of markers equals the number of ABTs. If more markers are ordered requiring Infinium I designs, then the total number of markers that can be attempted on a custom BeadChip decreases.

For example, a researcher designing a custom iSelect BeadChip containing 9500 Infinium II designs and 500 Infinium I designs would require a BeadChip with a total of 10,500 ABTs: 9500 Infinium II bead types (1 probe per marker) plus 1000 Infinium I bead types (2 probes per marker).

Bead Type Success Rate

Bead type success rate (ie, conversion rate) refers to the percentage of assay designs that result in successfully generated bead types. Customized Infinium BeadChip manufacturing guarantees a conversion rate of at least 80% of the attempted assays for iSelect HD and HTS BeadChips. Infinium XT iSelect Custom BeadChips have a guaranteed conversion rate of 95% to support screening many samples for a focused number of critical SNPs. Contact Illumina Technical Support if a project has specific conversion requirements for custom content. “Must-have” markers can be duplicated in the final design file to increase the likelihood of converting onto the final array. This significantly increases the ability of an assay to avoid a random exclusion from the final BeadChip product.

Bead Pool and Product Shelf Life

Illumina guarantees the shelf life of custom iSelect bead pools for up to 8 years from the date of manufacture. Notify Illumina at the time of purchase (before manufacturing) if a custom project is forecasted beyond the initial sample commitment to ensure that the bead pool is built at the appropriate scale and inventory is retained.

Custom Panel Design Workflow

Customers design and evaluate custom assays in Microarray Designer software and complete the ordering process from the MyIllumina site. The entire process is completed in 5 simple stages (Figure 1). Online support is available to provide detailed instructions for the design and ordering process.

1. **Start New Design**
   - Select Assay and Design Options
     Choose assay option: iSelect/iSelect+, XT iSelect/XT iSelect+, or Commercial+.
     Choose design option: Custom or Add on to Existing Design.
2. **Configure New Design**
   - Select Species for Assay
     Select 1 species for iSelect/iSelect+ and Commercial+ designs, and up to 4 species for XT iSelect/XT iSelect+ designs.
3. **Create and Add Input Target Files to Design**
   - Create Input Target Files
     Use templates in Microarray Designer to create Score, Sequence, Gene, Identity, and Region files.
   - Add Input Target Files
     Upload Input Target Files to Microarray Designer.
4. **Review Design**
   Review design details and summary in Score Results.
5. **Order Design**
   Finalize product name and complete order in MyIllumina.

Figure 1: iSelect Custom Microarray Designer Workflow–Microarray Designer software enables design, evaluation, and ordering of a custom genotyping panel in 5 simple stages.

Start a New Design

Select Assay and Design Options

In addition to building new custom panels (iSelect or XT iSelect), customers can augment previously designed iSelect BeadChips (iSelect+ or XT iSelect+) or commercial BeadChips (Commercial+) with newly discovered content. This content can originate from various sources, including genome-wide association studies (GWAS), whole-genome sequencing (WGS), and exome sequencing studies. Customers can add new content to a custom array after the initial design period is completed, enabling optimal customization. The maximum number of loci that can be supplemented depends on both the format of the BeadChip and the amount of base content already present.

To start a new custom panel design, log in to Microarray Designer software with MyIllumina credentials and select **Start Design** from the toolbar. Select **Help Me Choose** to open a wizard that will help determine which assay option best fits experimental needs. Otherwise, select the appropriate assay and design options for the current project.

- The **iSelect** assay and **Custom** design options create a full custom iSelect design.
- The **iSelect+** assay and **Add on to Existing Design** options add new custom content to an existing iSelect custom order. Select the base content to be augmented.
- The **XT iSelect** assay and **Custom** design options create a full XT iSelect design.
- The **XT iSelect+** and **Add on to Existing Design** options add new custom content an existing XT iSelect custom order. Select the base content to be augmented.
- The **Commercial+** option adds new custom content to a commercial BeadChip. Select the commercial base content to be augmented.

Configure New Design

Select Species for Assay

Configure the new design by selecting the species of interest from the **Select Your Species** field. Select 1 species for iSelect/iSelect+ and Commercial+ designs, and up to 4 species for XT iSelect/XT iSelect+ designs. If the species of interest is not listed, follow the assay-appropriate guidelines.

- For an iSelect/iSelect+ design, select **Other**. "Other" will be used as the species name in the Manifest file for that design.
- For XT iSelect/XT iSelect+ designs, select **Custom** if the species of interest is not listed, and provide the species name. The name entered will be used as the species name in the Manifest file for that design.

Microarray Designer software supports full duplicate and repeat region checking for assays where full genome reference support is available (eg, human, mouse, rat, and bovine species). When designs are created for other species supported by Microarray Designer software, varying levels of support for duplicate and repeat region checking will be given, depending on the availability of public sequence data. If Microarray Designer software does not list the species, no duplicate or repeat region checking will be performed. The Microarray Designer **Score Results** file includes the 50 bp probe sequences so that researchers can use their own reference data to confirm that these probes hybridize to unique locations in the genome.

Create and Add Input Target Files to Design

Create Input Target Files

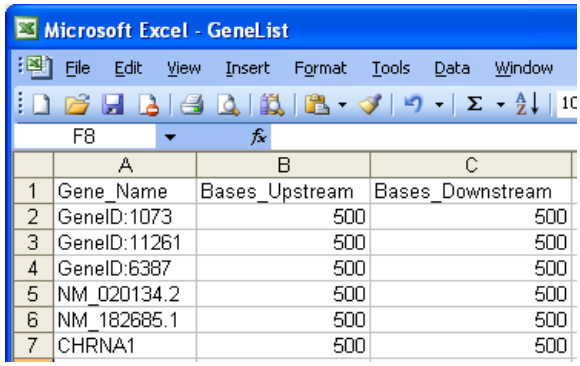
Researchers must submit their custom panel of requested loci as Input Target Files using 1 of 4 different file types corresponding to the different evaluation methods of custom SNP loci: **Gene**, **Region**, **Identity**, and **Sequence**. For SNPs previously scored by Microarray Designer software, **Existing Design** and **SNP Score** are also acceptable input formats. This technical note describes each of the Input Target File format options for Microarray Designer software and includes examples for each option. Template files for each input type can be downloaded from Microarray Designer software.

Users can create or edit Input Target Files with a text editor or spreadsheet program. To be submitted to Microarray Designer software, files must be saved in a comma-separated values (*.csv) format. This technical note provides example files created in Microsoft Excel. Blank lines are not permitted in the data fields. Files must meet the following formatting specifications.

- Input Target File format is *.csv. Therefore, commas must not be used within values in the data fields.
- Input Target File types include specific column headings for the data that must be used (Table 2).
- Input Target Files must contain no more than 1M markers or indels for iSelect HD or HTS Custom BeadChips, and 50K for XT iSelect Custom BeadChips. If the number of markers exceeds these limits, the Input Target File must be split into smaller files with the appropriate maximum number of lines.

Gene List

The **Gene List** file type returns designs on all markers within a gene and in the regions upstream and downstream from a gene in the current human genome build (Table 3). A **Gene List** requires input using RefSeq NM (mRNA) accession IDs (preferred) or Human Genome Organisation (HUGO) identifiers. Microarray Designer software maps these accession numbers to the human genome to identify gene regions and return all SNPs in those regions. The size of upstream and downstream bases is customizable via the **Gene List** input file format (Figure 2). Markers in overlapping gene regions will be listed in the **Score Results** output file only 1 time, but will be annotated as being present in both regions in the design output **Region_Description** field. Marker limits apply to the **Gene List** file. Generally, a **Gene List** with up to 600 genes and a range of 10,000 bases upstream and downstream will be within the marker limit.

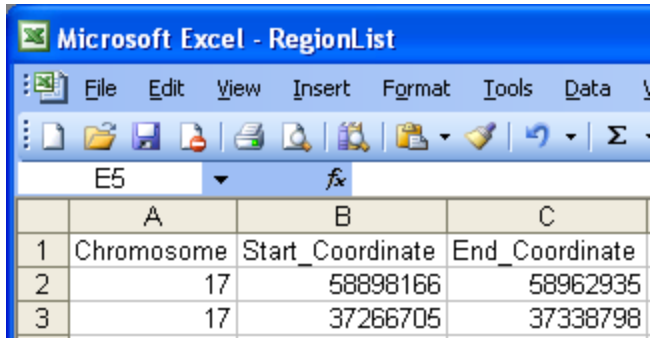


	A	B	C
1	Gene_Name	Bases_Upstream	Bases_Downstream
2	GeneID:1073	500	500
3	GeneID:11261	500	500
4	GeneID:6387	500	500
5	NM_020134.2	500	500
6	NM_182685.1	500	500
7	CHRNA1	500	500

Figure 2: Gene List Format Example–Example of properly formatted entires in a Gene List, suitable for upload in Microarray Designer software.

Region List

A **Region List** file contains a list of regions in the human genome, identified by chromosomal coordinates. Microarray Designer software will search and evaluate from among cataloged and curated markers from the Single Nucleotide Polymorphism Database (dbSNP).[1] Multinucleotide repeats (MNPs), microsatellites (simple sequence repeats or SSRs), or markers with ambiguous or multiple locations are not supported. Markers in overlapping regions will be listed in the **Score Results** output file only 1 time, but will be annotated as being present in both regions in the **Region_Description** field. Due to marker limits, submit a properly formatted **Region List** file that is ≤ 60 Mb (Figure 3).



	A	B	C
1	Chromosome	Start_Coordinate	End_Coordinate
2	17	58898166	58962935
3	17	37266705	37338798

Figure 3: Region List Format Example–Example of properly formatted entries in a Region List, suitable for upload in Microarray Designer software.

Identity List

Known markers from the current version of dbSNP for the human reference genome can be requested specifically using the **Identity List**. The dbSNP is the source for rs marker and flanking sequence data. The column heading **Locus_Name** is the only input field in a properly formatted **Identity List** that is suitable for upload in Microarray Designer (Figure 4). An error output file will be generated to indicate any merged SNP IDs or unsupported molecule (eg, RNA) or marker types (eg, tri-allelic markers).

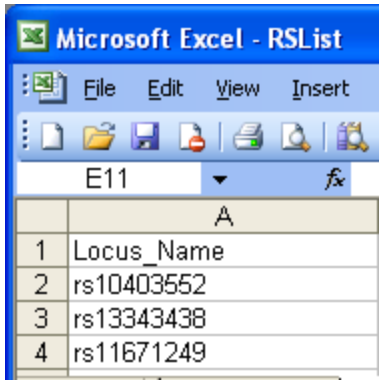


Figure 4: Identity List Format Example–Example of properly formatted entries in an Identity List, suitable for upload in Microarray Designer software.

Sequence List

The **Sequence List** allows researchers to evaluate markers from private databases or other sources, including any species. The **Locus_Name** field is used to name sequences for easy identification. **Locus_Name** entries contained in this file must not begin with "rs" because that prefix designates rsIDs in the internal Illumina dbSNP database and will trigger a database search. A **Sequence List** file must be formatted correctly with appropriate column headers and data fields (Table 4).

Any rs-ID sequences from dbSNP within compatible adjacent polymorphisms can be adjusted to be designable. Replace an adjacent polymorphism with a known major allele in the population, if the adjacent polymorphism is greater than 10 bases from the target SNP (20 bases recommended). Submit such designs as a sequence file by adding a prefix (eg, adj_rs1234).

To specify SNPs, put brackets around a polymorphic locus in the submitted sequence, and separate the 2 alleles with a "forward slash" (eg, TGC[A/C]CCG). Similarly, to specify an indel, use a "forward slash" between a single minus sign (indicating the deletion) and bases representing the insertion (eg, TGC[-/AT]CCG). A minimum of 50 bp of sequence on either side of the SNP is ideal for evaluating both strands for the best design. Microarray Designer software also accepts International Union of Pure and Applied Chemistry (IUPAC)[3] codes for degenerate bases in the flanking sequence, and avoids placement of probes over these polymorphisms adjacent to the targeted SNP.

If the **Lowercase_Weighting** checkbox in Microarray Designer Upload Targets is checked, lowercase nucleotides will have penalized final scores reflecting suboptimal probe placement over these lowercase regions. Because lowercasing in public databases is not standard for indicating repetitive or duplicated regions, best practice is to clear the **Lowercase_Weighting** checkbox by default.

Existing Designs

Illumina provides a convenient method for ordering assays that were designed and ordered as a previous iSelect or Illumina Commercial product. Use an **Existing Design** file, which contains a list of the Illumina IDs (Ilmn_Ids) from the original design or bead pool manifest file, to order.

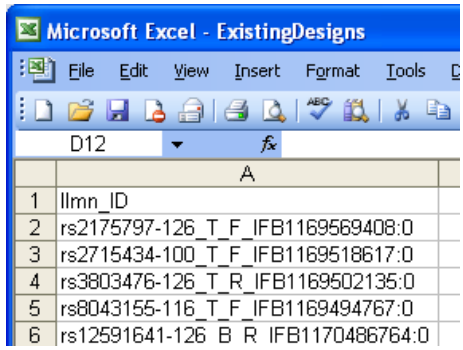


Figure 5: Existing Design Input Example–Example of properly formatted entries in an Existing Design List, suitable for upload in Microarray Designer software.

Guidelines for Human Sequences

Currently, Microarray Designer software returns **Gene**, **Region**, and **Identity** Input Target Files for Human only. Microarray Designer software only supports 1 build of the human genome at a time. Illumina staff maintain the currently supported version of the human genome and notify users at least 2 weeks before switching to a new version. Microarray Designer software returns the version and build of the current reference database in **Score Results**.

Add Input Target File

After choosing, creating, and formatting any type of Input Target File, upload the file using the **Upload Targets** screen in Microarray Designer software. Follow onscreen prompts and click **Submit for Design** to submit the Input Target File for evaluation. Microarray Designer software will complete scoring typically within a few minutes to several hours, or possibly up to 2 days. Customers are notified via email after scoring is complete. Evaluation results in 1 of 3 possible outcomes:

1. Failed design
2. Successful design but not orderable
3. Successful design and orderable

Review Design

Download and review the **Score Results**. If an error occurs during Microarray Designer evaluation, it can be reported in **Score Results** as an **Unknown** Error, **Unable to Design** error, or a more specific error description. Edit the list based on error and warning codes, design scores, and research requirements. Refer to the DesignStudio Microarray Assay Designer User Guide for more information on common errors and troubleshooting guidelines.

Score Results Output File

Microarray Designer file submission results are returned as a **Score Results** file for review and revision, or for input into a **Final** file submission for product ordering.

The **Score Results** file header section includes additional summary information, such as the total number of markers in the file. This summary information is provided in the **Review and Order Design** page in Microarray Designer software. Summary information is further broken down into numbers of markers in each of 3 Normalization_Bins: A, B, and C. Bin C assays include all Infinium II designs requiring a single bead type. Bin A and B assays are Infinium I designs in the red and green channels, respectively, and are classified into 1 of these 2 bins based on the color channel required to detect the target alleles across the 2 bead types used in the Infinium I assay.

Table 2: Score Output File Column Headers

Column	Description
Locus_Name	rsID or unique name (customer-defined).
Sequence	The bracketed site identified by the **Locus_Name** with ≥ 50 bases of flanking sequence.
Genome_Build_Version	Genome build. Contact Illumina Technical Support for the currently supported build.
Chromosome	Chromosome on which the marker is located. Must be a valid chromosome for the species being analyzed.
Coordinate	Chromosomal coordinate of marker.
Source	Source of the sequence and annotation data.
Source_Version	Source version number.
Sequence_Orientation	Must contain 1 of the following 3 values: **Forward**, **Reverse**, or **Unknown** (not case-sensitive). A score file resulting from a sequence input and field **Sequence_Orientation** is customer-supplied and not validated.
Region_Description	Description of the region of interest.
Final_Score	Final scores are based on a proprietary algorithm and can range from 0–1 with higher values reflecting their "probe-ability," or likelihood of success for a custom assay design. A final score of 1.1 indicates that Illumina has validated the SNP as a successful design for Infinium assays.
Failure_Codes	If applicable, reasons why a successful assay at this marker locus is unlikely (see Table 5).
Validation_Class	Numerical representation of Validation_Bin (see Table 6).
Validation_Bin	Manner in which designed assays have been validated (see Table 6).
MAF_(Population)	MAF from the largest peer-reviewed study conducted in the indicated population, the study size in terms of number of chromosomes, and the study type. Data are retrieved from dbSNP for each of the following human genome populations. Score output resulting from Sequence input files will be blank for these fields.
Chr_Count_(Population)	
Study_Name_(Population)	Caucasian, African, African-American, Han Chinese, Japanese, Unknown.
Normalization_Bin	A, B, or C.
Bead_Types/Assay	1 for Infinium I or 2 for Infinium II.
Assay_Type	Infinium I or Infinium II.
ILMN_ID	Unique identifier assigned by MIcroarray Designer for the designed assay.
Gene_ID	Gene ID from NCBI.
Gene_symbol	HUGO identifier.
Accession	RefSeq accession number.
Location	Structural location of the marker: intron, coding, flanking_5UTR, flanking_3UTR, 5UTR, 3UTR, UTR.
Probe Sequences	50 bp probe sequence designed by Illumina.
Location_relative_to_gene	If the marker does not fall within an exon, the value is the actual base pair distance from gene start. The absolute value of this number is the distance to the closest transcript. The negative sign is a formatting symbol and is not meant to imply strand or direction. If the SNP is within an exon, 2 values separated by a '/ ' represent distances to the exon-intron boundaries. Information in this column can be used to identify potential splice site variants.
Coding_status	**NONSYN** or **SYNON**. If the marker falls within an exon, this field notes a synonymous or nonsynonymous amino acid change.
Amino_acid_change	If the marker falls within an exon, this field notes the actual change to the amino acid, followed by the GenBank protein sequence used in numbering the change.
Id_with_mouse	Ratio of identical bases within 60 bp of flanking sequence compared to mouse sequence that has been aligned with the homologous human sequence and covers the marker in question.
Phast_conservation	Metric used by the UCSC Genome Browser to identify highly conserved markers among species.
Design_Date	Date of design.
Species	The species used in the assay (customer-defined). Available for XT iSelect only.

Table 3: Gene List Column Descriptions

Column	Description
Gene_Name	Customer-supplied gene name. Can be a RefSeq accession ID or HUGO gene symbol.
Bases_Upstream	Number of bases to search upstream of the gene starting coordinate.
Bases_Downstream	Number of bases to search downstream of the gene starting coordinate.

For any normalization bin that contains loci, there is a minimum requirement of 100 loci in that bin to ensure normalization of the intensity data during scanning. If a bin has fewer than 100 loci, submit additional A/T SNPs (Bin A) or C/G SNPs (Bin B) in Microarray Designer software for scoring, or change the Force_Infinium_I column in the input file (Bin C) to increase representation in Bin A and/or Bin B. Microarray Designer software will report if the SNPs are in Bin A or Bin B in the **Score Results** file. Add the appropriate assays to the original design as needed.

Following the **Score Results** file header section, detailed information for each marker is listed in the data section. Important performance values are presented for each SNP. The **Final_Score** indicates the expected success for designing a given assay, and can be supplemented with **Failure_Codes** for further information (Table 5). Validation status is also indicated to provide even greater confidence in design success. To assist researchers in ordering the most applicable markers for their studies, MAFs in several populations are provided for SNPs when available from dbSNP. MAF from the largest study is reported and qualified based on peer-reviewed publication, study design, study size, and verified results.

Filtering and Selecting Custom Lists

In addition to being an output file format, **Score Results** files can be used as Input Target Files in Microarray Designer software. Users can create a filtered or edited **Score Results** file (with markers removed or added) for iterative Microarray Designer analysis during final SNP selection. Markers identified using more than one input search method (eg, **Gene**, **Region**, **Identity**, **Sequence**, or **Existing Design**) can be combined as one **Score Results** file. Users can resubmit this file to Microarray Designer software using the **Upload New Targets** functionality on the **Review and Order Design** page.

Apply the following criteria for discriminating marker lists to create a successful product that achieves the scientific aims of the experiment, and has the highest probability of generating meaningful results.

- Remove markers that cannot be ordered (critical failure codes in the 101-110 range, see Table 5).
- Consider research requirements (eg, tags, spacing, or MAF).
- Favor Infinium-validated markers when possible, because they have successfully converted to functional assays for any species.
- Use TwoHitValidated markers, Validation Class 110 (Table 6) based on the **Validation_Bin** field. Higher **Final_Scores** are preferred.
- Submit a number of bead types and corresponding SNPs to Microarray Designer software that are at least 20% over the targeted number for final design. It is easier to remove SNPs from the design file than to add more SNPs and reevaluate with Microarray Designer software. For example, the Infinium OmniExpress-24+ BeadChip supports up to 30,000 ABTs; therefore, submit up to 36,000 SNPs for consideration in the final design.

Order Design

Ordering a New Bead Pool or Product

If the design is successful and passes evaluation in Microarray Designer software, place an order through MyIllumina.

1. Finalize product name and select **Proceed to Order** to navigate from Microarray Designer software to MyIllumina.
2. Provide ordering parameters and select **Get Pricing and Package Details** to view product pricing.
3. Select **Add to Cart** to enter the order into the Illumina manufacturing process.

Reordering an Existing Bead Pool or Product

To place an order based on an existing product or custom beadpool, click **My Chips/Reorder**. Reorders have a minimum order requirement of 288 samples, and must be ordered in multiples of 48. Only bead pools in inventory within acceptable QC metrics are available for reordering. Illumina Customer Service will receive a notification to confirm that inventory is available before processing the online order. If a design file has been used to place a BeadChip order, that design file is never removed.

Completing an Order

After all desired orders are added to the shopping cart, click **Continue to Checkout**. Click **View Cart** to display the shopping cart. During the checkout process, shipping and payment information are required. An initial shipping date and partial shipment request can be entered before final submission. After clicking **Submit**, the order will be sent to Illumina Customer Service for review. If needed, modify an order by emailing orders@illumina.com during a 48-hour review period immediately following submission.

Table 4: Sequence List Column Descriptions

Column	Description
Locus_Name	Supply a unique locus name (cannot begin with rs or cg). Name must not contain any of the following characters: " % / = \ ? ` , @ ; ', or spaces. Duplicate probe names are not allowed. Underscores are discouraged and have the risk of interfering with the assignment of Illumina IDs (Ilmn_IDs) in the Illumina database.
Target_Type	Must be **SNP** or **INDEL** (not case-sensitive).
Sequence	Limited to 10,000 bases. May only contain one bracketed SNP or indel. Output will be ≤ 122 bases per line.
Chromosome	Chromosome on which the marker is located. Must be a valid chromosome for the species being analyzed. Enter **0** if unknown. Contig numbers are not accepted and may result in a corrupt manifest.
Coordinate	Chromosomal coordinate of marker. Enter **0** if unknown.
Genome_Build_Version	Enter genome version number. Otherwise, enter **0**.
Source	Identify the source of the sequence and annotation data. Must be completed. Enter **unknown** if no information is available.
Source_Version	Source version number. Enter **0** if unknown.
Sequence_Orientation	Must be either **Forward**, **Reverse**, or **Unknown** (not case-sensitive). For consistency and cross-platform data compatibility, Forward and Reverse strand should be based upon the public database definition of strand, if available.
Plus_Minus	Must be either **Plus**, **Minus**, or **Unknown** (not case-sensitive). For consistency and cross-platform data compatibility, Plus and Minus strands should be based upon the public database definition of strand, if available.
Force_Infinium_I	Must be **TRUE** or **FALSE**; enables the ability to force an Infinium I design, which may be needed to fulfill a normalization bin.
Species	Select species from drop-down list. For iSelect/iSelect+ designs, if species is not listed, select **Other**. For XT iSelect/XT iSelect+ designs, if the species is not listed, select **Custom** and provide the species name.

Table 5: Microarray Designer Failure Codes

Critical Failures (undesignable)		Possible Troubleshooting
101	Flanking sequence is too short.	
102	Polymorphism or sequence formatting error. Possible causes: Check polymorphism format: SNP => [X/Y], INDEL => [-/XYZ], CpG => [CG]. More than 1 set of brackets in sequence. Missing brackets around polymorphism. SNP alleles not separated by a "/". Spaces found in submitted sequence.	Fix the formatting issue and resubmit the Input Target File.
103	TOP/BOT strand cannot be determined. A possible cause is low sequence complexity.	
104	Variant is not appropriate for Illumina platform. Possible causes: Variant is not biallelic. Contains characters other than A, G, C, or T. Non-DNA molecule type that is not supported (eg, RNA).	
105	Polymorphism is in the mitochondrial genome. Using mitochondrial polymorphisms for oligo pools is not recommended.	Remove any mitochondrial polymorphisms and resubmit the Input Target File.
106	Degenerate nucleotides in assay design region (eg, W, R, S, N).	
107	SNP sequence not found.	
108	The final score fails below the assay limit.	
109	Indels not supported for Infinium I assay.	
110	Locus name duplicated in the base commercial content.	Rename the locus name that is flagged as a duplicate.
Warnings (designable)		Possible Troubleshooting
301	Polymorphism in duplicated/repetitve region.	
302	Melting temperature (Tm) outside assay limits.	
304	There are known SNPs within the probe region. See Underlying_SNP column for details.	
311	SNP inappropriate for Infinium I assay.	
340	Another polymorphism in this list is ≤ 60 nucleotides away.	
360	Low score warning.	Remove any targets with a low design score and resubmit the Input Target File.
399	Multiple contributing issues.	
601	Potentially nonspecific against the genome.	
602	Probe sequence is duplicated in the base commercial content.	Remove the probes with duplicated sequence and resubmit the Input Target File.
603	Probe sequence is duplicated in the custom content.	

Table 6: Validation Status Descriptions

Validation_Bin	Validation_ Class	Description
NonValidated	1	Locus has been seen in only 1 study or population. Even if it has a high design score, there is a chance that it is monomorphic.
OneKGenomeValidated	100	Locus has been sequenced in the 1000 Genomes Project.
TwoHitValidated	110	Both alleles have been seen in 2 independent studies and populations.
HapMapValidated	120	Locus has been genotyped by the HapMap Project.
TwoHit_ OneKGenomeValidated	200	Both alleles have been seen in 2 independent studies and populations. Locus has been sequenced in the 1000 Genomes Project.
HapMap_ OneKGenomeValidated	210	Locus has been genotyped by the HapMap Project. Locus has been sequenced in the 1000 Genomes Project.
TwoHit_HapMapValidated	220	Locus has been genotyped by the HapMap Project. Both alleles have been seen in 2 independent studies and populations.
TwoHit_HapMap_ OneKGenomeValidated	300	Locus has been genotyped by the HapMap Project. Both alleles have been seen in 2 independent studies and populations. Locus has been sequenced in the 1000 Genomes Project.
InfiniumValidated	910	This is a previous design that has successfully generated polymorphic results using the Infinium assay.

Shipping Schedule

Shipping schedules are defined after an order is placed through MyIllumina. Otherwise, orders ship complete or according to a default schedule. The first shipment of custom BeadChips will typically arrive within 8-12 weeks after order confirmation.

Ordering by Email

To place an order by email, submit the **Final Score** output file and a purchase order to orders@illumina.com. Illumina Customer Service will send confirmation of a successful order or contact you if essential information is missing, or if the submission file contains unorderable designs.

Summary

Custom Illumina Infinium iSelect products allow researchers to create assays tailored to their specific needs for focused, high-throughput genotyping or fine-mapping of candidate disease association regions. Microarray Designer software is a simple and powerful tool for evaluating individual loci and creating successful custom BeadChips for genotyping. By following the guidelines in this technical note, researchers can make sure that their orders are designed and placed quickly and easily.

Learn More

For more information about iSelect Custom Infinium products, visit www.illumina.com/techniques/popular-applications/genotyping/custom-genotyping.html.

References

1. Single Nucleotide Polymorphism Database (dbSNP). www.ncbi.nlm.nih.gov/SNP/index.html. Accessed August 2016.
2. International Union of Pure and Applied Chemistry. iupac.org/. Accessed October 2016.

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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

illumina®