



common A/T and C/G SNPs and requires two probes (or bead types) per SNP because the probe stops at the base representing the SNP of interest (i.e., one probe for each of both alleles).

For ordering and pricing, custom BeadChip products are defined by their number of attempted bead types, not number of loci assayed. Only if a project is exclusively limited to loci using Infinium II designs (A/G, A/C, T/G, T/C SNPs; and all indels) are the number of markers equal to the number of bead types. The more markers ordered that require Infinium I design, the fewer total markers that can be attempted on a custom BeadChip.

**For example, a researcher designing a custom iSelect BeadChip containing 9,500 Infinium II assays and 500 Infinium I assays would require a beadchip with a total of 10,500 attempted bead types: 9,500 Infinium II bead types (one probe per marker) plus 1,000 Infinium I bead types (two probes per marker).**

### Bead Type Success Rate

Illumina guarantees a bead type success rate of at least 80%. The bead type success rate is the proportion of the total number of attempted assays present on the final product. Manufacturing a custom BeadChip encompasses many steps, including synthesizing all required oligonucleotide probes, attaching probes to silica beads, combining the various bead types into the final bead pool, loading the bead pools onto the BeadChip, and decoding the bead identities on the BeadChip. While the manufacturing steps required to build custom BeadChips have been optimized, no step is 100% efficient. The manifest file supplied with BeadChips will contain all successful assays (i.e., those present on the final product), not all of the ordered assays. Illumina does not guarantee analytical success rates due to the custom nature of these projects.

### Bead Pool and Product Shelf Life

Illumina guarantees the life of iSelect HD bead pools for 12 months from the date of manufacture. Illumina keeps custom bead pools in inventory for the entire 12 months to enable reorders of the same project. Once the 12 month shelf life has expired, any remaining bead pools are discarded. BeadChips are typically made to order and are not kept in inventory.

Illumina notifies customers of an impending bead pool expiration for product reorder of custom bead pools. In some cases, a bead pool may be kept past the 12 month expiration (by customer request), but the quality of the bead pool cannot be guaranteed, and Illumina reserves the right to not manufacture if the yield does not meet quality requirements.

### Preliminary Input Files

To submit preliminary input files, researchers can use five different file types corresponding to the five different methods for preliminary evaluation of custom SNP loci: Gene, Region, Identity, Sequence, and CNVRegion. Additionally, probe designs from a previously ordered iSelect product or current Illumina standard product use the Existing-Design file type. After preliminary evaluation, ADT generates a Score file that can be used as an input file in subsequent rounds of evaluation or ordering (Figure 1).

At this time, ADT returns only human sequences from Gene, Region, Identity, and CNVRegion input files. It is important to note that ADT only supports one build of the human genome at a time. Illumina keeps the supported version of the human genome current and gives users at least two weeks notice before switching to a new version. Illumina Technical Support Scientists can confirm which version of the human genome is currently supported.

ADT supports full duplicate and repeat region checking for human assays. When designs are created for other species supported by ADT, varying levels of support for duplicate and repeat region checking will be given, depending on the availability of public sequence data. If ADT does not support the species, no duplicate and repeat region checking will be performed and customers should ensure their assays target unique sequences. For non-human species, Illumina Technical Support Scientists can provide information about the level of duplicate and repeat region support provided by ADT.

Input files may be created or edited with any text editor or spreadsheet program. However, before submitting them to ADT, files must be saved in a comma-separated values (\*.csv) format. The examples provided in this document show files created in Microsoft Excel. Blank lines are not permitted in the data fields or between lines in the heading. The following formatting requirements must be followed precisely so ADT can properly evaluate your requests.

- **Format is comma-separated values with a \*.csv file extension. Since the input file format is comma-delimited, no commas may be used within the values.**
- **Each file type includes specific column headings for the data, as described below.**
- **File contains fewer than 1,000,000 markers or indels. If the number of markers exceeds this limit, the file must be split into smaller segments for serial ADT evaluation.**
- **If the file is submitted by email rather than on iCom, it must include a file header section. File header format is the same for all file types (Figure 8 and Table 7).**

### Gene

The Gene file type provides a method for querying all markers within a gene and in the regions upstream and downstream from the indicated gene. A Gene file allows for the interrogation of the currently supported build of the human genome using RefSeq NM accession ID (preferred) or HUGO identifiers. ADT maps these accession numbers

Figure 2: Gene File Format Example

	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

Example of properly formatted entries in a Gene file shown from Excel.





Figure 5: Sequence File Format Example

1	Locus_Name	Target_Type	Sequence	Chromosome	Coordinate	Genome_Build	Version	Source	Source_Version	Sequence_Orientation	Plus_Minus
2	seq-rs10403552	SNP	ATTCTCTCCAGGGCT	19	59900243	36		dbSNP	126	Forward	Plus
3	seq-rs13343438	SNP	cagtggcccaatcagctca	19	59751810	36		dbSNP	126	Forward	Plus
4	seq-rs11671249	SNP	tagaggtcattagcctaagtc	19	59814896	36		dbSNP	126	Forward	Plus
5	seq-rs34785303	SNP	GAAC CAGGCTGCTAA	19	59976309	36		dbSNP	126	Forward	Plus
6	seq-rs35835276	SNP	CCGACCTGGGTTTT	19	59937631	36		dbSNP	126	Forward	Plus

Example of properly formatted entries in a Sequence file shown from Excel.

### Score Output File

If preliminary input files are submitted to ADT via iCom, an email notification is sent when scoring is complete, often within an hour. The results are returned as a Score file that can be downloaded from iCom on the Prelim Assay Design files page. If an input file is submitted via email to Technical Support, an Illumina scientist will submit the file to ADT for processing. ADT generates the Score output file, which is returned to the customer by email or secure FTP in 1–2 days. The Score file provides information for filtering and selecting their marker lists, as described in the following section.

The Score file header section includes additional summary information, such as the total number of markers in the file. This is further broken down into numbers of markers in each of three Normalization\_Bins: A, B, and C. Bin C assays are Infinium II designs. Bin A and B assays are Infinium I designs. Infinium I assays are classified into one of these two bins based on the sequence context (the base pair after the locus) and strand that the assay is designed against. For a final order, if there are any loci in a given normalization bin, there must be at least 100 loci in that bin to ensure normalization functions as expected. If Bin A or Bin B needs to be supplemented to reach 100, submit additional A/T or C/G SNPs to ADT for scoring. ADT will report whether each of these SNPs are in Bin A or Bin B. The appropriate assays can then be added to the original design as needed.

The Number of Bead Types value is important for ordering, since iSelect BeadChip orders are priced based on the number of attempted

bead types. The number of bead types may be different than the number of assays because Infinium I assays require two bead types per markers and Infinium II assays only require one bead type per marker.

Following the Score file header section, detailed information for each marker is listed in the data section. All Score column headers are described in Table 8. Important performance values are also presented for each SNP. The Final\_Score indicates the expected success for designing a given assay, and may be supplemented with Failure\_Codes for further information. 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, minor allele frequencies (MAFs) in several populations are provided for SNPs when available from dbSNP. MAF from the largest study is reported, and is qualified based on peer-reviewed publication, study design and size, and verified results.

### Filtering and Selecting Custom List

In addition to being an output file format, Score files can be used as input files to ADT. Thus, users can create a filtered or edited output file (with markers removed or added) for iterative ADT analysis while determining optimal loci. Markers identified using more than one input search method (e.g., Gene, Region, Identity, Sequence, CNVRegion, or ExistingDesign) can be combined as one Score file and resubmitted to ADT as an input file for evaluation as a single product.

Illumina recommends applying the following criteria for discriminating

Figure 6: CNVRegion File Format Example

1	Chromosome	Start_Coordinate	End_Coordinate	Probes_Per_Region
2	17	58898166	58962935	5
3	17	37266705	37338798	4
4	1	54776489	54883005	5
5	3	188033157	188068946	5
6	1	201166576	201204034	5

Example of properly formatted entries in a CNVRegion file shown from Excel.

Figure 7: Existing Design File Format Example

1	Illum_ID
2	rs2175797-126_T_F_IFB1169569408:0
3	rs2715434-100_T_F_IFB1169518617:0
4	rs3803476-126_T_R_IFB1169502135:0
5	rs8043155-116_T_F_IFB1169494767:0

Example of properly formatted entries in a Region file shown from Excel.

marker lists to create a successful product that achieves the scientific aims of the experiment and has the highest chances of generating meaningful results.

1. Remove markers that cannot be ordered (error codes in the 101–199 range).
2. Consider research requirements (e.g., tags, spacing, or MAF).
3. When appropriate, favor Infinium-validated markers, since they have the highest chance of converting into functional assays.
4. Use two-hit validated markers based on Final\_Score. Higher Final\_Scores are preferred.
5. Do not be hindered by proximity limits for Infinium assays. Markers can be close to each other without potential interference.
6. Submit a number of bead types that is within the required range. Human OmniExpress+ require 1,000 to 200,000 attempted bead types. iSelect HD BeadChips can accommodate 3,000 to 1,000,000 attempted bead types.

### Final Score File

After selecting SNPs that meet your research criteria and the ADT criteria, a final Score file must be created to place an order. A preliminary Score file is converted to a final Score file by the completion of four existing header rows: Design\_Iteration, Scale (Number\_of\_Tubes), Purchase\_Order\_Number, and Product\_Name. It is important to ensure that the Number of Bead Types (not SNPs) value in the final file matches the number on the corresponding quotation or contract.

**Figure 8: Examples of the File Header Section Required Only For Email Submission**

	A	B	C	D	E
1	[heading]				
2	Customer_Name	Jane Doe			
3	Company_Name	BigBio Inc.			
4	Company_Address1	1234 Biotech Way			
5	Company_Address2				
6	City	San Diego			
7	State/Province	CA			
8	Postal_Code	92121			
9	Country	USA			
10	Phone_Number	858-202-1234			
11	Email_Address	jdoe@illumina.com			
12	Order_Description	SNPsforProjectX1			
13	Order_Comments	This comment isnt used by any ILMN software			
14	Platform_Type	WGGT			
15	Lowercase_Weighting	0			
16	Format_Type	Score			

Examples of properly formatted entries in the header of a Score file shown from Excel. This header is only required for email submissions, and is formatted the same for any preliminary file format.

### Ordering

To place an order, submit the final Score file on the iCom website (<https://iCom.illumina.com>, Figure 9). Options for reordering an existing iSelect panel or obtaining customized project pricing information before placing an order are also available on iCom.

### Upload iSelect HD Design Files

To upload files to iCom, follow these steps:

1. On the iCom welcome page, select **Orders | Upload iSelect HD Files**.
2. Click **Upload Final Score File**.
3. Select the **File Type** (Score or ExistingDesign).
4. Select the **Assay Type** (WGGT).
5. Select the **Species**.
6. Enter the **Number of Assays**, which is the number of different markers or variants (e.g., SNPs or CNVs) being interrogated in the custom project, not the number of bead types.
7. Enter the **Description** (user-defined and optional).
8. Enter the location and file name of the target file or click **Browse** to navigate to the file.
9. Click **Upload** and **Continue with Validation**. The uploaded file will then be listed in the iSelect Files table, with an initial status of "In Scoring." Within two days, ADT scoring will be completed, and an email notification will be sent. At that point, the file status will be updated to "Ready to Order" or "Warning." See Table 9 for a description of possible warning codes.
10. After a file is uploaded and successfully validated, it can be used for product order placement by selecting the checkbox next to the file and clicking **Continue to Project Pricing**.

### iSelect HD Project Pricing Calculator

The Project Pricing Calculator can be used at any time. It opens automatically when you select **Continue to Project Pricing** for a design file as described above. To get project pricing information at any other time, go to the iCom web page and select **Tools | iSelect Project Pricing Calculator** or **Order Now**. On the next screen, click **Infinium Genotyping | Project Wizard**.

1. Select the **Product Type**: iSelect, iSelect+, or HumanOmniExpress+ (Figure 10).
2. If ordering iSelect+, select the BeadChip you wish to supplement in the **Existing Content** field.
3. Enter the number of samples between 1,152 and 116,352 in multiples of 48.
4. Do one of the following:
  - To determine pricing based on a design file, select the appropriate file in the **Final iSelect File** section.









