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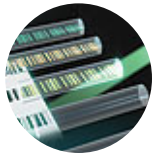
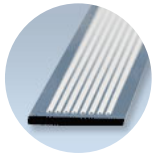
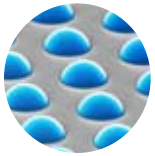
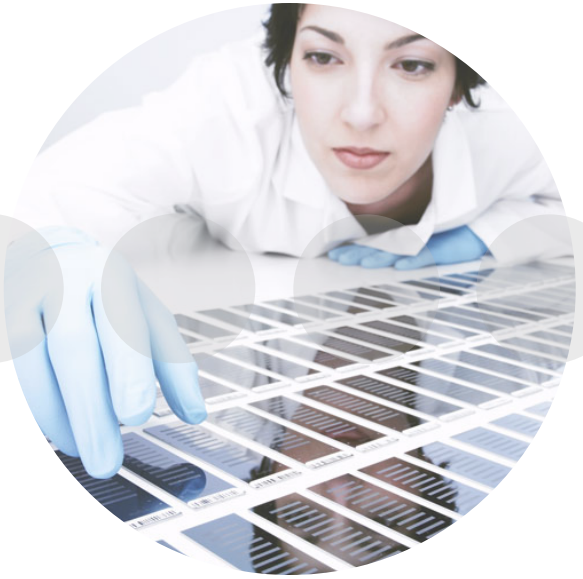
making sense out of life









Custom GoldenGate Genotyping Using the Illumina® Assay Design Tool

The Illumina® Assay Design Tool (ADT)

- All files (preliminary and final) for custom OPA design are being processed with the Illumina® Assay Design Tool (ADT)
- All template files for submission of Preliminary and Final Files are available on the web. Navigate to www.illumina.com, Support Tab, Downloads. Or, directly at:

http://www.illumina.com/pagesnrn.ilmn?ID=75

- .pdf files of this training presentation are available from the same link
- If you are having difficulty retrieving information from the web site, please contact TechSupport@illumina.com and they can provide all materials via e-mail

Benefits Features of ADT

- Begin from RS list, Sequence list, Regions (by coordinate), or Gene List
- Full annotation as part of standard results—including design score, design rank, minor allele frequency (MAF) and validation status
- Information from the most up to date sources for both genome build and dbSNP version
- New, more descriptive failure codes
- Improved Assay Design algorithms
- Future plans for self-service through our e-commerce program

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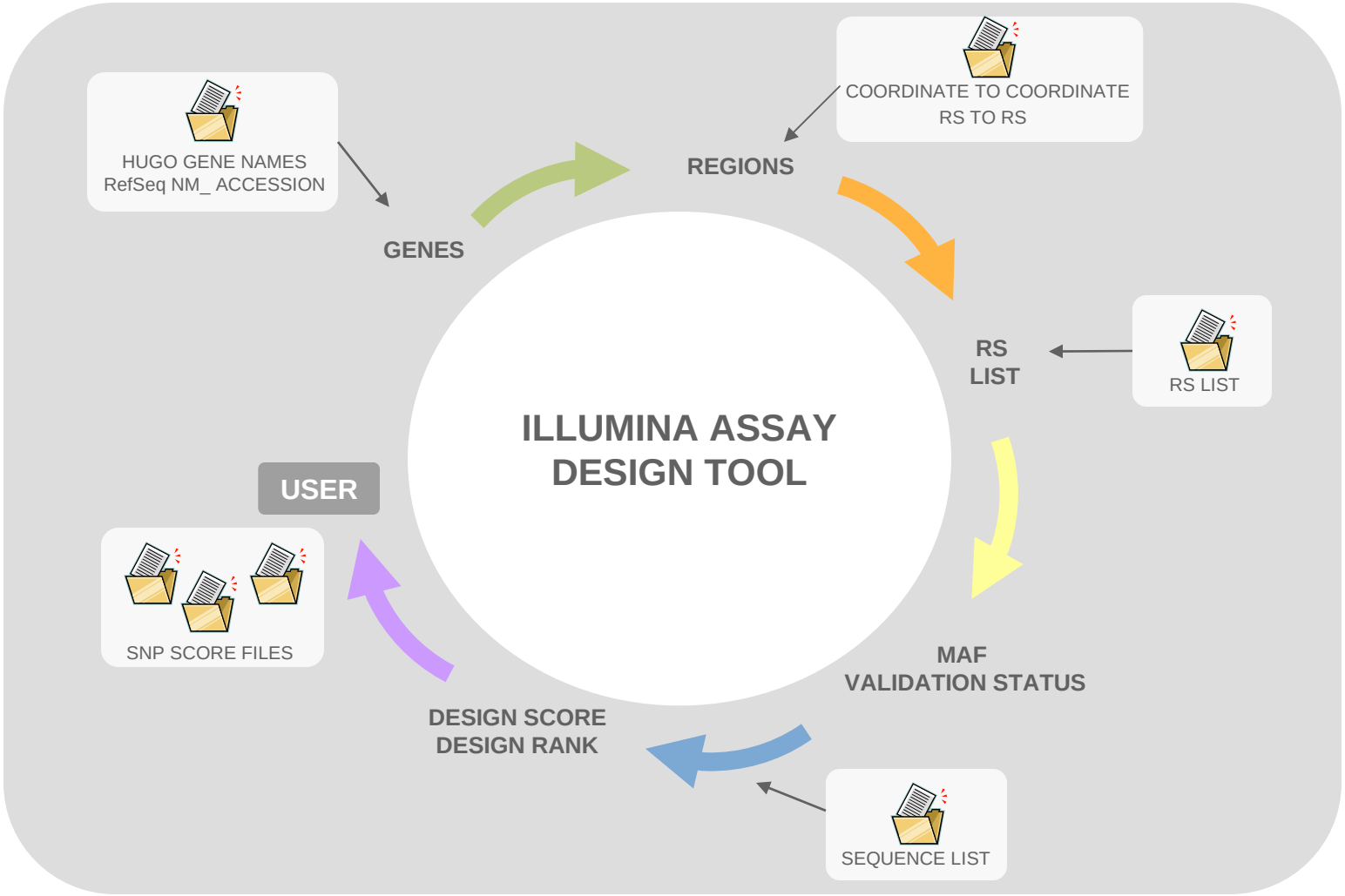
Preliminary Design Phase



** See next page for text explanation*

Preliminary Design Phase

- Files can enter ADT as a GeneList*, RegionList*, RSList*, or SequenceList
- GeneLists get mapped to the human genome using Build 36 to produce regions
- RegionLists use coordinates from Build 36 to identify RS SNPs within those regions
- Internal version of dbSNP version 128 is used to get sequence data
- MAF and Validation Status is then added to each SNP
- Design Score and Design Rank are added for each SNP
- File is returned as a SNPScore file
- SequenceList files only do not have MAF and Validation Status

*Available only for human studies

Preliminary Design

HEADER OF FILE

- Same for all preliminary submission types
- Text and numerical values only
- Exceptions: hyphen in phone/fax, and '@' in e-mail
- Number of SNPs should be '0' if Region or Gene list
- Lowercase Weighting (next slide)
- **Company_Address2 and Order_Comments fields are optional**
- **Lowercase_Weighting and File_Type fields are new, relative to old scoring tool**

City	San Diego
State/Province	CA
Postal_Code	92121
Country	USA
Phone_Number	858-202-4566
Fax_Number	858-202-1234
Email_Address	TechSupport@illumina.com
Order_Description	SNPs for demonstration
Order_Comments	
Assay_Type	GGGT
Number_of_SNPs	1003
Lowercase_Weighting	**0**
File_Type	**RSList**

Preliminary Design

LOWERCASE_WEIGHTING	
0	All nucleotides considered for oligo design (i.e. no ‘masking’). Will rely on Illumina algorithm to identify repetitive/duplicated regions.
1	Lowercase nucleotides not considered for oligo design (i.e. are ‘masked’). Will rely on masked regions and Illumina algorithm to identify repetitive/duplicated regions.

Since lowercasing in public databases has not been standardized to demonstrate masking, we recommend using ‘0’ for lowercase.

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Preliminary Design

HEADER + BODY OF FILE

Customer_Name	Jim Acierno		
Company_Name	Illumina		
Company_Address1	9885 Towne Centre Drive		
Company_Address2			
City	San Diego		
State/Province	CA		
Postal_Code	92121		
Country	USA		
Phone_Number	858-202-4566		
Fax_Number	858-202-1234		
Email_Address	TechSupport@illumina.com		
Order_Description	Genes for demonstration		
Order_Comments			
Assay_Type	GGGT		
Number_of_SNPs	0		
Lowercase_Weighting	0		
File_Type	GeneList		
Gene_Name	Bases_Upstream	Bases_Downstream	Species
NM_000342	100	50	Homo sapiens
NM_021936	5000	2500	Homo sapiens
NM_015386	0	0	Homo sapiens
NM_000445	0	1000	Homo sapiens
NM_130393	5000	0	Homo sapiens
NM_147174	5000	5000	Homo sapiens
NM_152246	5000	5000	Homo sapiens
NM_019554	5000	5000	Homo sapiens
NM_018647	5000	5000	Homo sapiens
HS6ST2	5000	5000	Homo sapiens
CPT1B	5000	5000	Homo sapiens
B4GALT2	5000	5000	Homo sapiens
SLC22A17	5000	5000	Homo sapiens
SMAP-1	5000	5000	Homo sapiens
S100A4	5000	5000	Homo sapiens
TNFRSF19	5000	5000	Homo sapiens
KCNAB1	5000	5000	Homo sapiens

Standard .csv (comma-delimited) file format

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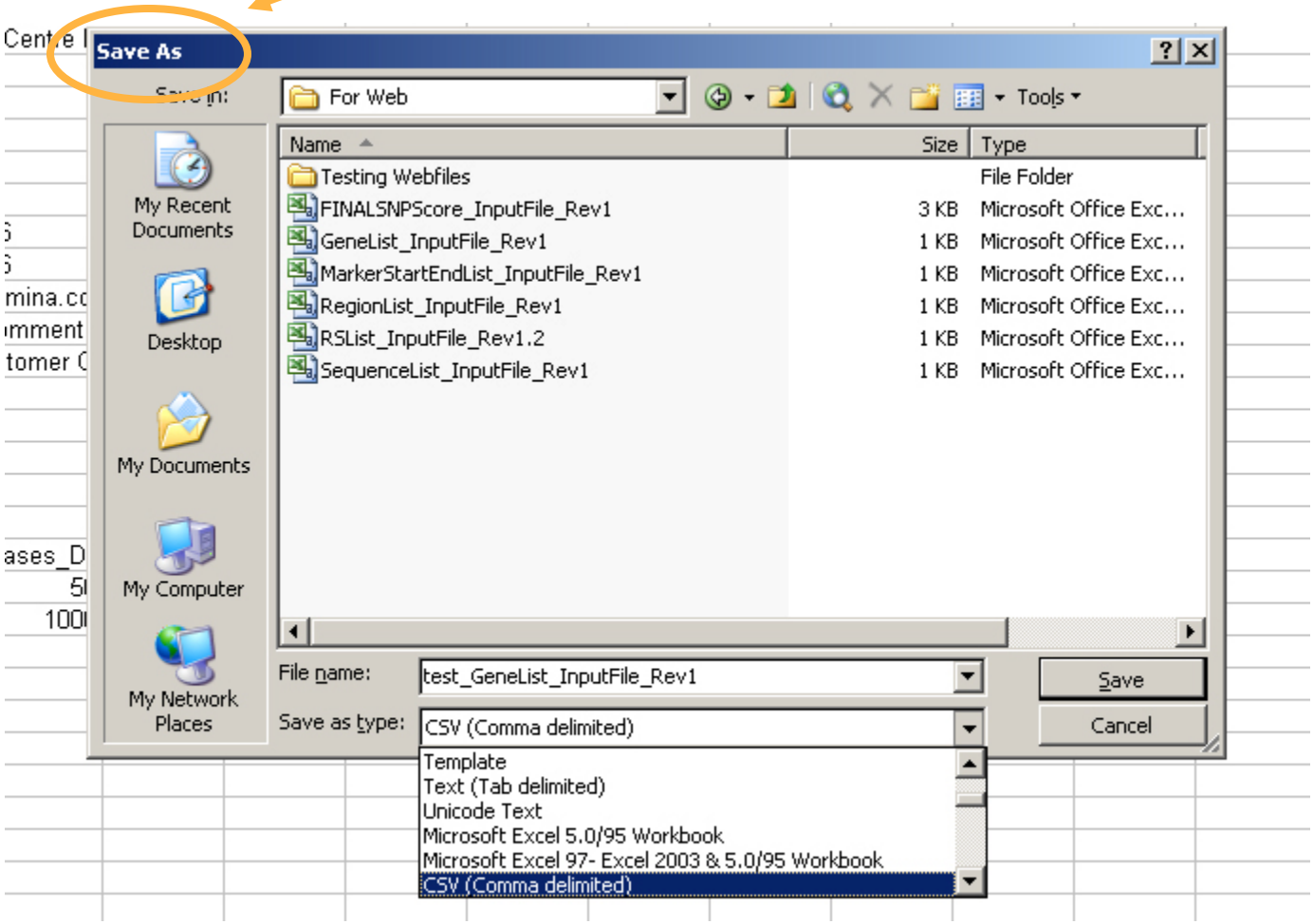
Preliminary Design

SAVING AS A .CSV FILE—USE THE 'SAVE AS' FUNCTION IN EXCEL



Preliminary Design

- Can vary upstream and downstream specifications
- Both RefSeq NM accession and HUGO allowed
- RefSeq NM accession preferred
- Limited to 65,000 SNPs returned. As a guideline, most files with 100 genes with 10kb upstream and downstream will be processed with no problems.

GENE LIST*

Gene_Name	Bases_Upstream	Bases_Downstream	Species
NM_000342	100	50	Homo sapiens
NM_021936	5000	2500	Homo sapiens
NM_015386	0	0	Homo sapiens
NM_000445	0	1000	Homo sapiens
NM_130393	5000	0	Homo sapiens
NM_147174	5000	5000	Homo sapiens
NM_152246	5000	5000	Homo sapiens
NM_019554	5000	5000	Homo sapiens
NM_018647	5000	5000	Homo sapiens
HS6ST2	5000	5000	Homo sapiens
CPT1B	5000	5000	Homo sapiens
B4GALT2	5000	5000	Homo sapiens
SMAP1	5000	5000	Homo sapiens
SMAP-1	5000	5000	Homo sapiens
S100A4	5000	5000	Homo sapiens
TNFRSF19	5000	5000	Homo sapiens
KCNAB1	5000	5000	Homo sapiens
OTOF	5000	5000	Homo sapiens

Conversion to Region List based on most recent Genome Build—currently Build 36

*Available only for human studies

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Preliminary Design

REGION LIST*

Chr	Region_Start	Region_End	dbSNP_Version	Genome_Build_Version	Species	Customer_Annotation
2	519169	657177	128	36	Homo sapiens	Chr2_Linkage_Peak
19	12488990	12963302	128	36	Homo sapiens	Candidate_Gene_1
X	652978	789153	128	36	Homo sapiens	Candidate_Gene_2

Conversion to RS List:

- Currently works from Genome Build Version 36, dbSNP version 128
- Customer_Annotation entries can be at your discretion
- Uses an Illumina-internal version of dbSNP
  - no ins/del, MNPs, SSRs
  - no SNPs with ambiguous or multiple localizations
- Limited to 65,000 SNPs returned, therefore we recommend submitting no more than 10Mb of regions in one file

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Preliminary Design

RS LIST*

- SNP information is obtained from the most up-to-date sources, currently Human Genome Build 36 and dbSNP version 128
- If SNP name has been merged, will return new RS name for future scoring requests
- SNPDome_Must_Keep is a placeholder for future features in development and has no significance at this time

SNP_Name	Ploidy	Species	SNPDome_Must_Keep
rs12345	diploid	Homo sapiens	1
rs11822459	diploid	Homo sapiens	1
rs2634207	diploid	Homo sapiens	1
rs2682117	diploid	Homo sapiens	1
rs12807110	diploid	Homo sapiens	1
rs2682116	diploid	Homo sapiens	1
rs2682115	diploid	Homo sapiens	1
rs2682114	diploid	Homo sapiens	1
rs16912066	diploid	Homo sapiens	1
rs7938608	diploid	Homo sapiens	1
rs7935627	diploid	Homo sapiens	1
rs11040867	diploid	Homo sapiens	1
rs2344348	diploid	Homo sapiens	1
rs4396271	diploid	Homo sapiens	1
rs1077441	diploid	Homo sapiens	1

If you want to use your own RS sequence, you must use Sequence List submission

*Available only for human studies

Preliminary Design

SEQUENCE LIST

SNP_Name	Sequence	Genome_Build_V	Chr	Coordinate	Source	dbSNP_Version	Ploidy	Species	Customer_Strand
OurSNP1	GTGCTGA	0	1	1234	Unknown	Unknown	diploid	Homo sapiens	Forward
Bob-the-SNP	CTCACCT(	99	6	66581982	Internal	Unknown	diploid	Homo sapiens	Forward

CTCACCTGGATGGATGCTCCTGCAGAAATCTATACTAACGATATGTGTGGA
TGCCTGGAACTACAGATGACCGTTGC[A/C]CCGCTCAGCCAGGAGAAGGCA
CTGCATTTAATGTTACTATGGGTTATAAATACCCTTCCTCT

- Allows for scoring of any custom or proprietary SNPs (non-RS)
- Allows for scoring of SNPs from species other than human
- 100bp of sequence on either side of the polymorphism is recommended
- Will not have full annotation in results such as MAF, Validation Status, etc
- Will still contain Design Score, Design Rank, and Failure Codes
- If submitting your own RS sequence, make sure that SNP_name does not start with ‘rs’ (e.g. change ‘rs14489’ to ‘Smith-rs14489’). Acceptable characters include letters, numbers, periods (.), and dashes (-)

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Preliminary Design

OTHER FILE TYPES AND PLATFORMS:

- If you are interested in obtaining the exact same oligo designs from an OPA that you previously ordered, please contact TechSupport@illumina.com for instructions on using the Existing Designs template.
- Please also contact TechSupport@illumina.com if you will be designing your custom GoldenGate product for use on the BeadXpress platform.

A BRIEF CAUTION ABOUT GENE LIST AND REGION LIST FILES:

- If there are two or more overlapping regions in a file, SNPs that are present in both regions will only be listed as being part of the first region.
- Since GeneLists are first converted into regions, this can be true for GeneLists as well. Even if the genes themselves do not overlap, requesting upstream and downstream SNPs can create overlapping regions in a file. A summary file is provided with the results (SNPScore file) that will give the exact conversion information from gene to region.

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Preliminary Design Summary

- Gene List
- Region List
- RS List
- Sequence List

PRELIMINARY DESIGN

SNP SCORE FILES

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SNP Score File

VERSION IN WHICH SNP WAS LAST MODIFIED

PLACEHOLDER FOR FUTURE FEATURES

SNP_Name	Sequence	Genome_Build_Version	Chr	Coordinate	Source	dbSNP_Version	Ploidy	Species	Customer_Strand	SNPDome_Must_Kee
rs11362	TCCGTCGAC	35	8	6722809	dbSNP;refSNP	100	diploid	HOMO SAPIENS	forward	0

Customer_Annotation	SNP_Score	Designability_Rank	Failure_Codes	Validation_Clas	Validation_Bin
DEFB1$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated

MAF_Caucasian	ChrCount_Caucasian	MAF_African	ChrCount_African	MAF_African_American	ChrCount_African_American	MAF_Asian	ChrCount_Asian	MAF_Unknown	ChrCount_Unknown
0.33	120	-1	0	-1	0	-1	0	-1	0

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SNP Score File

SCORES AND VALIDATION

Customer_Annotation	SNP_Score	Designability_Rank	Failure_Codes	Validation_Class	Validation_Bin
CCL11$RefSeq/34.3$+5000$-5000	0.902	1		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.848	1		2	tw o-hit validated
CCL11$RefSeq/34.3$+5000$-5000	0.495	0.5		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.811	1		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.935	1		2	tw o-hit validated
CCR3$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated
DEFB1$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated
DEFB1$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated

- If starting from gene list, result will be gene plus upstream/downstream notation
- If starting from region list, this result will be notation that you entered on submission

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SNP Score File

SCORES AND VALIDATION

Customer_Annotation	SNP_Score	Designability_Rank	Failure_Codes	Validation_Class	Validation_Bin
CCL11$RefSeq/34.3$+5000$-5000	0.902	1		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.848	1		2	tw o-hit validated
CCL11$RefSeq/34.3$+5000$-5000	0.495	0.5		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.811	1		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.935	1		2	tw o-hit validated
CCR3$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated
DEFB1$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated
DEFB1$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated

- 0.000 – 1.000 scale to reflect ability to design a successful assay
- GoldenGate® validated SNPs that have original assay oligos on file will have SNP_scores of 1.1

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SNP Score File

SCORES AND VALIDATION

Customer_Annotation	SNP_Score	Designability_Rank	Failure_Codes	Validation_Class	Validation_Bin
CCL11$RefSeq/34.3$+5000$-5000	0.902	1		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.848	1		2	tw o-hit validated
CCL11$RefSeq/34.3$+5000$-5000	0.495	0.5		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.811	1		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.935	1		2	tw o-hit validated
CCR3$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated
DEFB1$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated
DEFB1$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated

- Results can be 0, 0.5, or 1
- SNP_Score <0.400 = rank of 0 — low success rate, high risk to OPA
- SNP_Score 0.400 to <0.600 = rank of 0.5 — moderate success rate, moderate risk to OPA
- SNP_Score 0.600 to 1.1 = rank of 1 — high success rate, low risk to OPA
- Addition of Designability Rank simplifies sorting and filtering

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SNP Score File

SCORES AND VALIDATION

Customer_Annotation	SNP_Score	Designability_Rank	Failure_Codes	Validation_Class	Validation_Bin
CCL11$RefSeq/34.3$+5000$-5000	0.902	1		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.848	1		2	tw o-hit validated
CCL11$RefSeq/34.3$+5000$-5000	0.495	0.5		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.811	1		1	non-validated
CCL11$RefSeq/34.3$+5000$-5000	0.935	1		2	tw o-hit validated
CCR3$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated
DEFB1$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated
DEFB1$RefSeq/34.3$+5000$-5000	1.1	1		3	goldengate-validated

- Numeric and text representation of the same parameter
- Simplifies sorting and filtering

SNP Score File

FAILURE CODES

Critical Failures (Undesignable):		Warnings (Designable):	
101	Flanking sequence is too short.	301	SNP in duplicated/repetitive region.
102	SNP or sequence formatting error. SNP must match the format: [X/Y] Possible causes: • Spaces found in submitted sequence. • More than one set of brackets in sequence • Missing brackets around SNP • SNP alleles not separated by a "/"	302	Tm outside assay limits
103	Top/Bot strand cannot be determined. • Low sequence complexity	340	Another SNP in this list is equal to or less than 60 nucleotides away.
104	SNP is not appropriate for Illumina platform. Possible causes: • Tri- or quad-allelic SNP • Insertion or deletion polymorphism • SNP contains characters other than A,G,C, or T	399	Multiple contributing issues
105	SNP is located in the Mitochondrial genome • not recommended for GoldenGate OPAs due to high copy number of target Mitochondrial DNA		
106	Degenerate nucleotide(s) in assay design region. • e.g. W, R, S, N, etc.		

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Validation Status

GoldenGate validation status:	SNP has been previously designed and successfully generated polymorphic results on the Illumina platform. Designed oligonucleotides have 100% sequence match to those previously designed.
Two-hit validation status:	Both alleles of the SNP have been seen in two independent methods and populations
Non-validated:	SNP seen in only one method or population. Even if it has a high design score, there is still an increased chance that it is monomorphic

SNP Score File

MINOR ALLELE FREQUENCIES

Results for Caucasian, Yoruban, African-American, Han Chinese, Japanese, and Unknown

MAF_Caucasian	ChrCount_Caucasian	Study_Caucasian
-1	0	
-1	0	
-1	0	
-1	0	
0.25	118	HapMap-CEU
0.4	120	HapMap-CEU
0.15	120	HapMap-CEU
0.33	120	HapMap-CEU

- Queries HapMap and reviewed studies in dbSNP version 128
- Frequencies from largest studies are returned
- Reviewed studies list compiled by Illumina scientists and qualify based peer-reviewed publication, study design and size, and verified results
- List of reviewed studies is available by contacting Illumina's Scientific and Technical Support team

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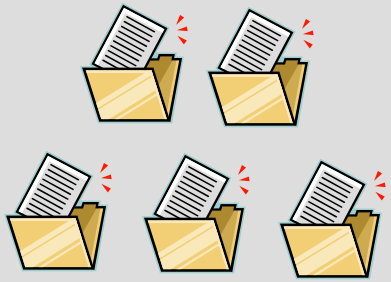
SNP SCORE FILES



Results from
Preliminary Design

FILTERED BY USER TO MEET THEIR PERSONALIZED RESEARCH CRITERIA

FINAL SNP SCORE FILE

- 96-plex, or 384-1536 plex (in multiples of 96)
- Ready to be placed as an Order

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Recommendations for Designing Your OPA

- In addition to your research requirements (e.g. spacing, MAF, etc), use GoldenGate validated SNPs first, as they have the highest chance of converting into functional assays.
- Even GoldenGate validated SNPs with scores <1.1 should be used preferrentially, as they have a >90% chance of having the same original assay oligos designed as were used to validated the SNP.
- Next, use two-hit validated SNPs with scores >0.60 (Design Rank 1).
- Also, remember that assays for SNPs that are less than or equal to 60 base pairs apart from each other (failure code 340) should not be included in the same OPA.
- It is recommended that you not use SNPs with failure codes 301, 302, 340, or 399 in your final OPA design.
- SNPs with failure codes 101 through 106 cannot be included in your final OPA order.

Final SNP Score File

ORDERING

Customer_Name	Jim Acierno										
Company_Name	Illumina										
Company_Address1	9885 Towne Centre Drive										
Company_Address2											
City	San Diego										
State/Province	CA										
Postal_Code	92121										
Country	USA										
Phone_Number	858-202-4566										
Fax_Number	858-202-1234										
Email_Address	TechSupport@illumina.com										
Order_Description	SNPs for demonstration										
Order_Comments											
Assay_Type	GGGT										
Number_of_SNPs	1536										
Lowercase_Weightir	0										
Design_Iteration	**Final**										
Scale(Number_of_	**5**										
Purchase_Order_N	**1234567**										
File_Type	SNPScore										
SNP_Name	Sequence	Genome_Build	Chr	Coordinate	Source	dbSNP_Versi	Ploidy	Species	Customer_Strand	Customer_Annotatio	SNP_Score
BobTheSNP	GTGCTGAGCTCCT(	35	19	567001	private	124	diploid	human	forward	Chr19Peak	0.85
rs1019107	TTCCAGAATAATTA	35	4	32761348	dbSNP	86	diploid	human	forward		0.902
rs10438808	GCCATTCAAATCCA	35	4	32763571	dbSNP	119	diploid	human	forward		0.848
rs1129844	CACTTCTGTGGCTG	35	6	112584998	dbSNP	86	diploid	human	forward		0.495
rs1860183	TTCCCTCTACTCCC	35	6	15985111	dbSNP	92	diploid	human	forward		0.811
JSA-alpha012	TAACATACTGAGCT	35	2	33265985	lab	92	diploid	human	forward		0.935
BK-link3-lod2	TCAAGGACTTGGC	35	3	46265198	dbSNP	119	diploid	human	forward		1.1
rs1047031	GATCTGATCATTAC	35	8	6715608	dbSNP	100	diploid	human	forward		1.1
rs11362	TCCGTCGACGAGG	35	1	6722809	dbSNP	100	diploid	human	forward	Chr1Peak	1.1

*** fields in orange are new fields, relative to the Preliminary Files**

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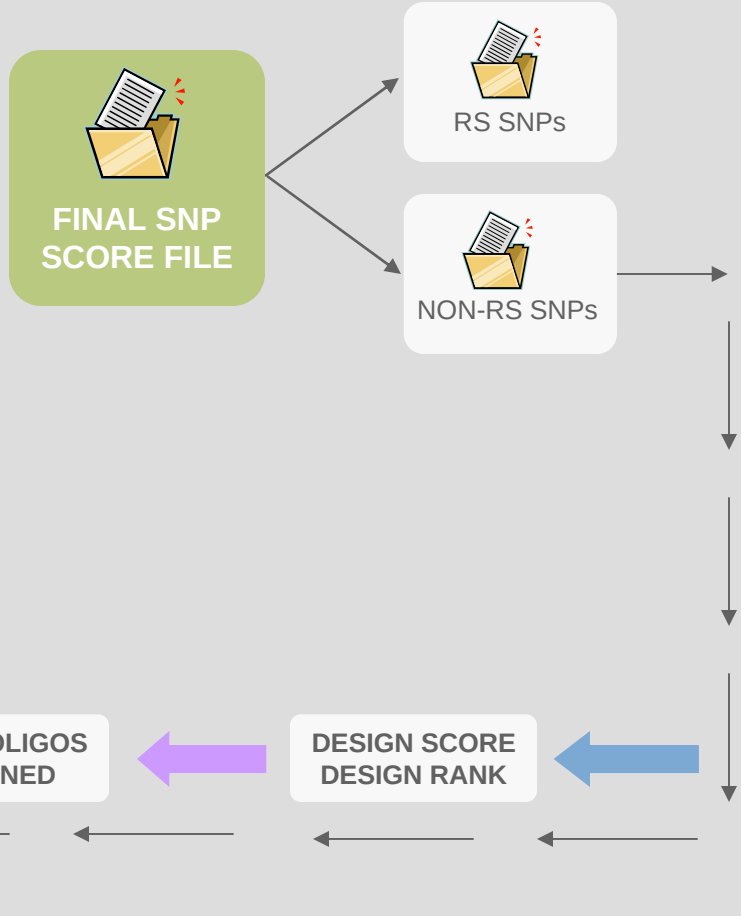
Final SNP Score File

ORDERING A CUSTOM OPA

ILLUMINA ASSAY DESIGN TOOL

- Final SNP Score file is first split into RS and non-RS SNPs
- Non-RS SNPs get Design Score, Design Rank, and have Assay Oligos designed

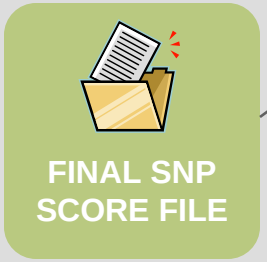


Final SNP Score File

ORDERING A CUSTOM OPA

ILLUMINA ASSAY DESIGN TOOL

- RS SNPs have updated dbSNP information retrieved
- List is then split into GoldenGate validated and non-GoldenGate validated SNPs
- Non-GoldenGate validated SNPs get Design scores and ranks, and have Assay Oligos Designed



RS SNPs

RETRIEVE UP-TO-DATE dbSNP SEQUENCE AND INFORMATION

MAF VALIDATION STATUS

RS SNPs GOLDENGATE VALIDATED

RS SNPs NOT GOLDENGATE VALIDATED

DESIGN SCORE DESIGN RANK

ASSAY OLIGOS DESIGNED

NON-RS SNPs

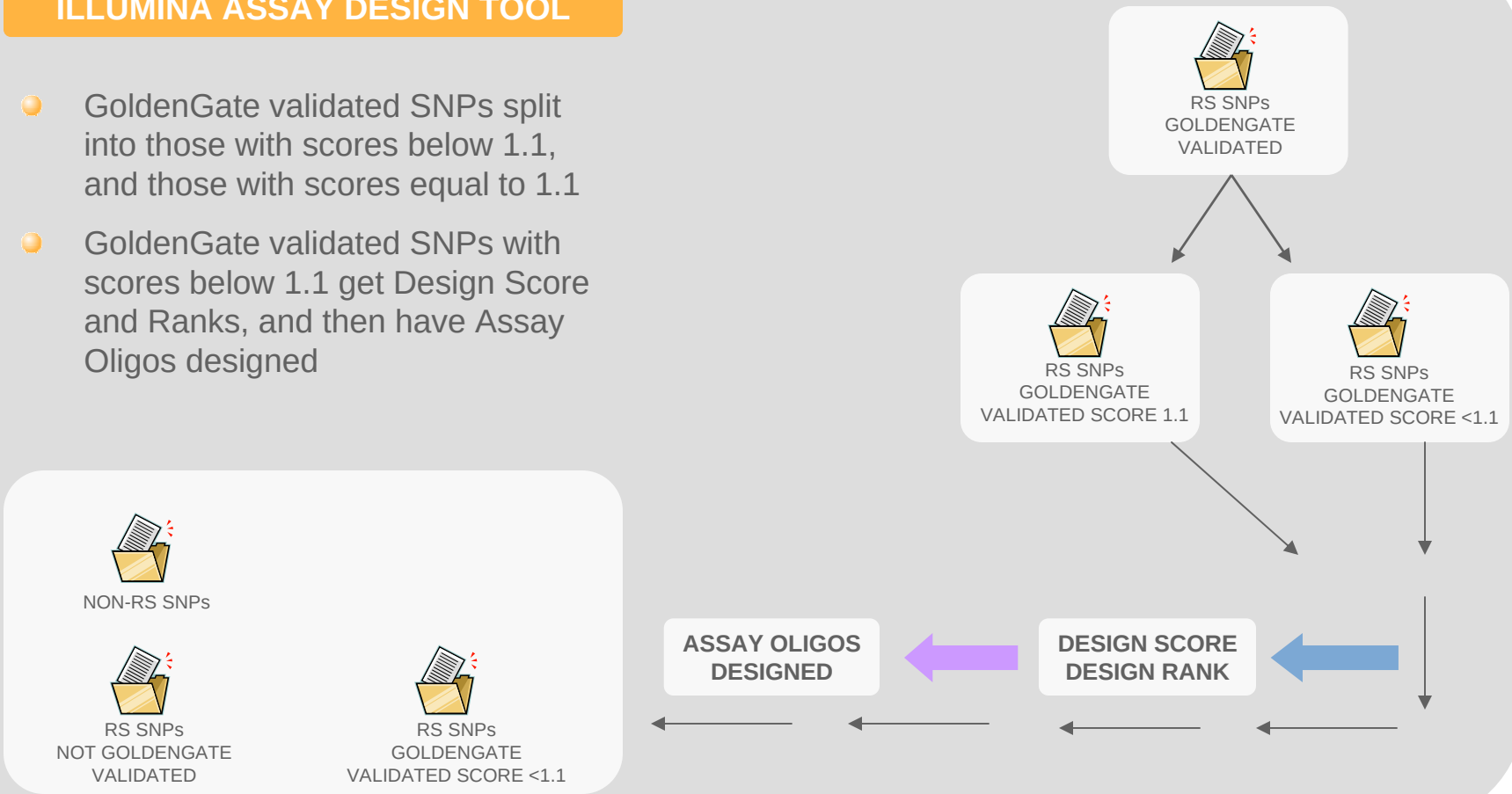
RS SNPs NOT GOLDENGATE VALIDATED

Final SNP Score File

ORDERING A CUSTOM OPA

ILLUMINA ASSAY DESIGN TOOL

- GoldenGate validated SNPs split into those with scores below 1.1, and those with scores equal to 1.1
- GoldenGate validated SNPs with scores below 1.1 get Design Score and Ranks, and then have Assay Oligos designed

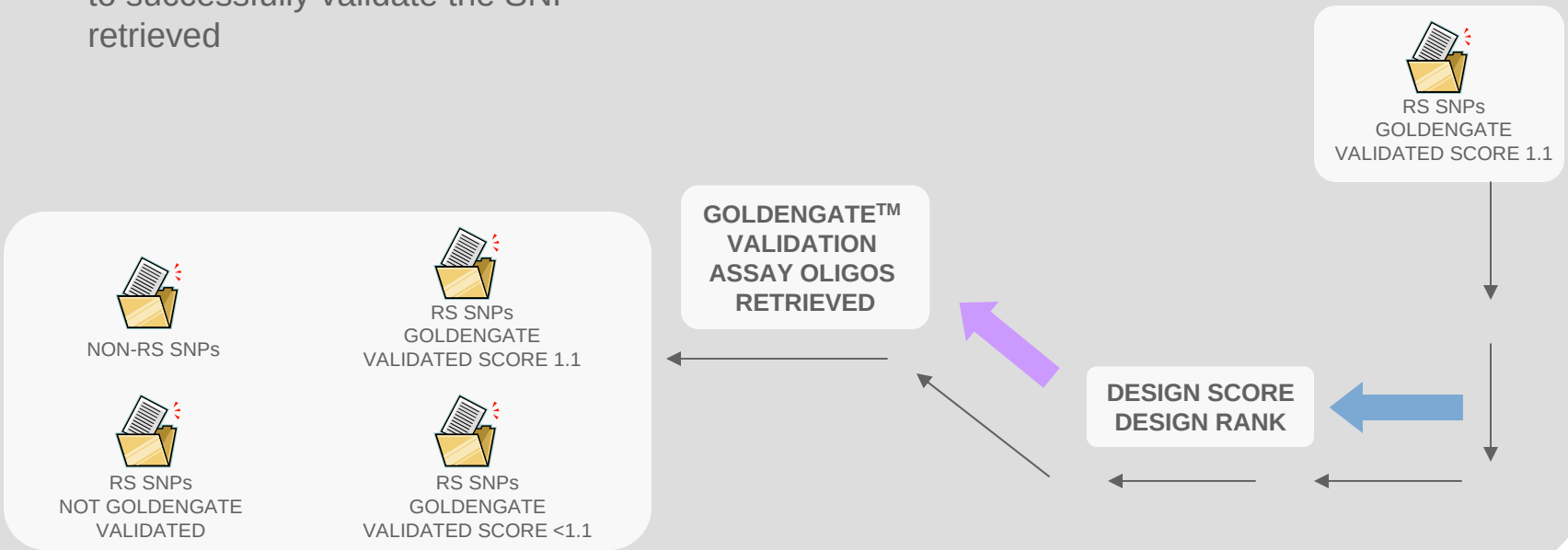


Final SNP Score File

ORDERING A CUSTOM OPA

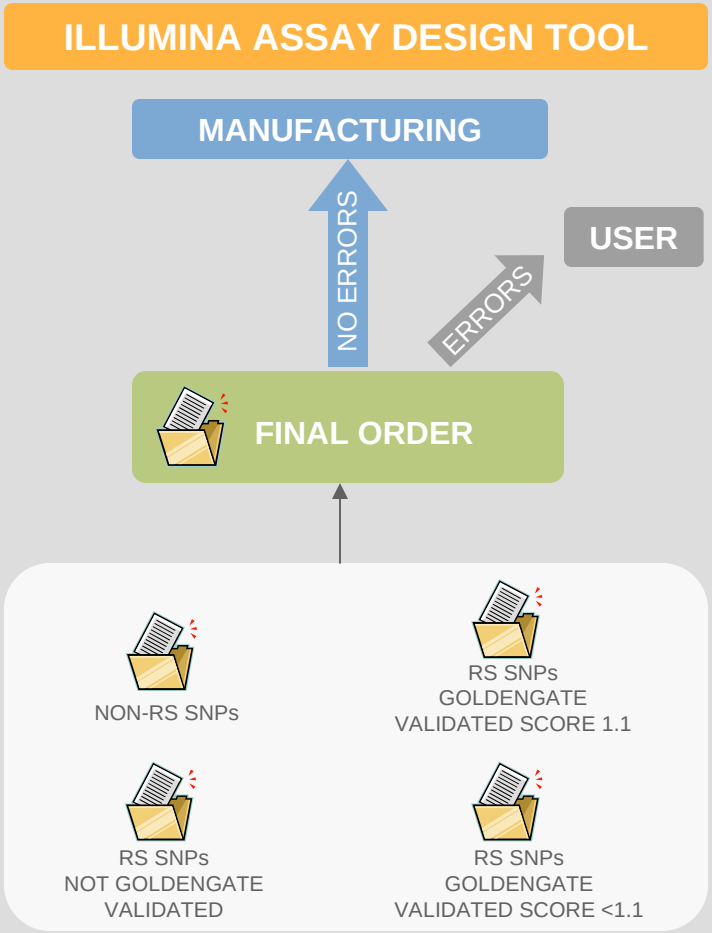
ILLUMINA ASSAY DESIGN TOOL

- GoldenGate validated SNPs with scores equal to 1.1 get Design Score and Ranks, and then have the original Assay Oligos that were used to successfully validate the SNP retrieved



Final SNP Score File

ORDERING A CUSTOM OPA



- The four files are then merged back into the Final Order
- If there are errors, the file is returned to you
- If there are no errors, the file is then sent to Manufacturing, and an order confirmation is sent

Final SNP Score File

ORDERING SPECIFICATIONS

- Any SNP_Name that begins with ‘rs’ will have all entries updated with the most current information from dbSNP
- Similar to Preliminary Design phase, if you wish to use your own RS sequences, you must add a prefix to the SNP_Name so that it does not begin with ‘rs’
- Announcements of when Illumina will migrate to a new Genome Build or dbSNP version will be made well in advance
- Validation Status is updated by both dbSNP version and internal Illumina results as they become available

OPA Design and Ordering Summary

1. Send Preliminary Files to **TechSupport@illumina.com** (Region, Gene, RS, Sequence).
2. You will receive your results (SNPScore Files) within 1-2 business days via e-mail.
3. Repeat steps 1 & 2 if necessary.

4. Arrive at final list of SNPs for custom OPA (96-plex, or 384-1536-plex).
5. Send Final SNPScoreFile to **TechSupport@illumina.com** (optional).
6. Receive and review results, if no errors proceed to number 7. If errors, make corrections and return to number 5.

7. Complete BeadStation or BeadLab order form. If working through a distributor, submit Final SNPScore file to distributor for them to complete and submit the order form. Orders can also be placed through our iCom site. Please visit **https://icom.illumina.com** for more details.
8. E-mail completed order form to **Orders@illumina.com** (or submit to iCom).
9. Receive Order Confirmation.

Illumina Scientific and Technical Support

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