A QuickStart Guide for the Creation and Ordering of SureSelect Target Enrichment Oligo Sets

Using **eArray**, a free web-based design tool

without the Wizard



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Getting Started Access eArray

- Access eArray at: https://earray.chem.agilent.com/earray/
- Log in to eArray, if this is your first time visiting, click Request for Registration

Agilent Technologies	Guest View Login Page Help
User Login Login Name: demo_user@agilent.com Remember my login name Password: Forgot Password Request for Registration Additional Information Frequently asked questions Information on how to do common tasks Download DNA Analytics 4.0 Download GeneSpring GX	Important Links - Catalog Gene Lists - Published Designs - eArray Product Page - System Requirements The Login Name is the e-mail address used to register - s and Alerts Welcome to eArray 5.4!! Get 15% off your first time eArray design order here



Getting Started Confirm Application Type

• Confirm that the Application Type is TargetEnrichment

Agilent Technologies effrray } Workspace Collaboration Petrilic					Help Logout Welcome Demo User (Aglient, Marketing) Application Type: TargetEnrichment Sector Accession Tree				If necessary, select Switch
			Create Library from Balt Uplo Create Library from Existing B Create Library by Balt Tilling in	alt Group(s) mrg	Sh Varw All Enrichment				
	Search Recet	Unicad			Search Results: 0 matching ren	suits found			Agilent Technologies eArray
Pending Jobs	natching results found			Refresh, <u>View A</u> u	My Libraries	tulte found	Refres	h. View.All	Select View Type : TargetEnrichment Set As Default View CHP CGH DNACapture microRNA
	Job Name Demo_BatTiing micading the desired file, isses pop-up blocking soft		27-Jan- 2009	tion Action	Kame Demo_BatTiingLikeary2 Demo_BatTiingLikeary	Created Date 28-Jan-2009 20-Jan-2009	Action Suste Constant Suste Constant		TargetEnrichment

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Getting Started Select Method

Choose between two methods for creating a SureSelect Target
 Enrichment Set

	Benefits	Caveats	When to Use
Following the Wizard*	 Easy, stream-lined method The Wizard takes the user from initiation of design to submission 	 The user will only be able to download the library details after the library is completed The user will not have access to a "fate" file 	When a simple or straightforward design is planned
Independent of the Wizard	 User can download additional files, including a Fate file that lists the # of baits tiled for each target interval User can fine-tune the design, creating and combining multiple bait groups with a variety of parameter settings User can easily track the success of bait tiling for individual targets during the process of design 	• User will not be guided by a Wizard	When iterations may be desired to get an optimal design for a target set

* This tutorial guides the user on how to create a design independent of the Wizard. If desired, please see the tutorial on how to create a design with the Wizard.



Terms and Definitions

- Bait:
 - A single oligo sequence of pre-determined length (120 bp) that complements a targeted region of the genome

Bait Group:

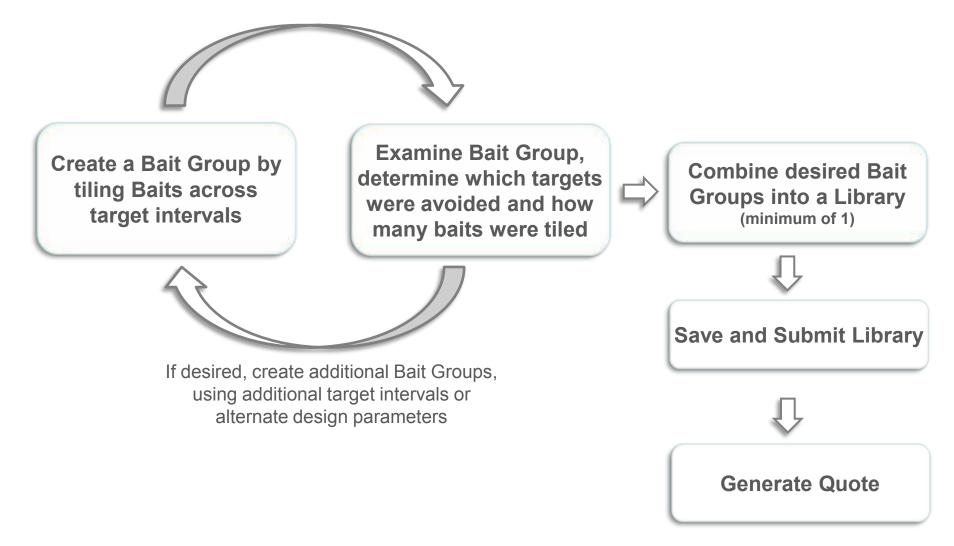
- Consists of a group of Baits designed to complement a single or set of targeted intervals
- May be formed from baits generated within eArray, baits uploaded into eArray, or bait search results within eArray
- Library:
 - Consists of one or more Bait Groups
 - Represents the set of oligos that will be produced for the kit







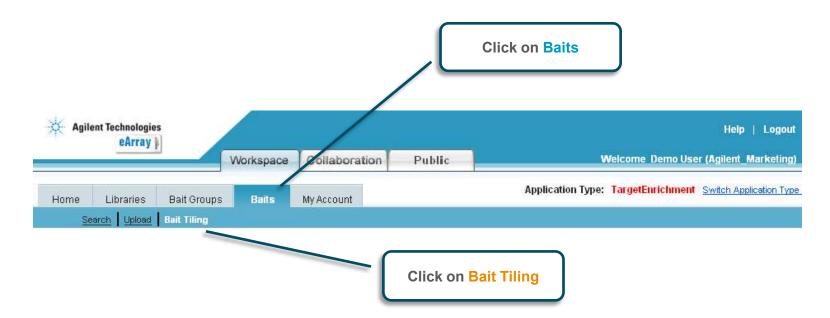
Workflow Summary





Create a Bait Group Design Baits

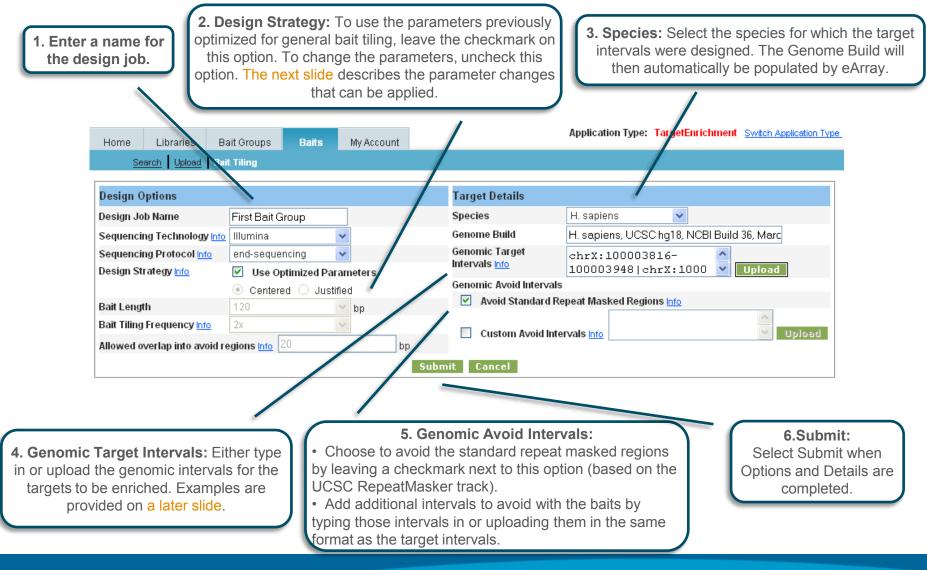
• To create a Bait Group, go to the Baits page, and select Bait Tiling



• This will bring you to the page where you can define design parameters for a Bait Group

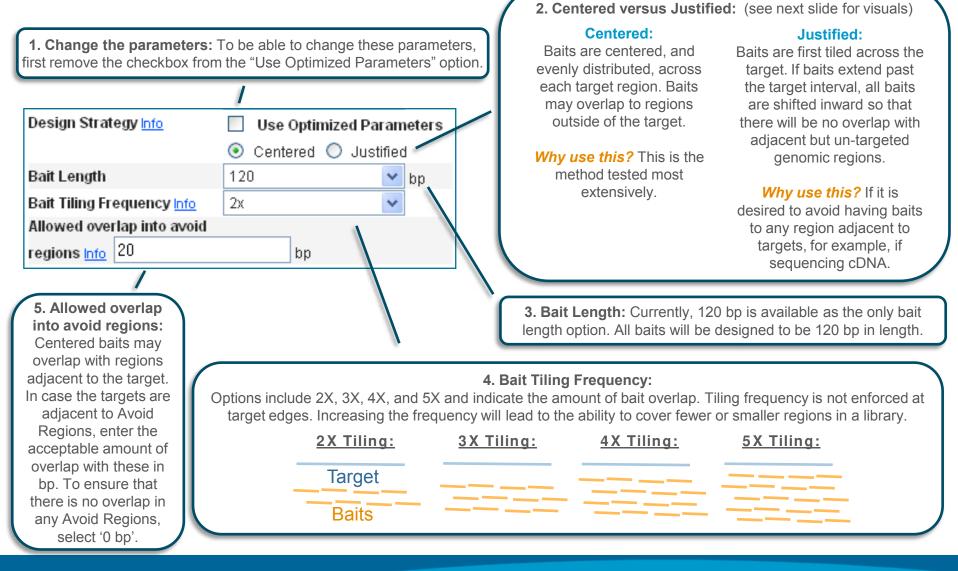


Create a Bait Group Design Options





Create a Bait Group Design Strategy



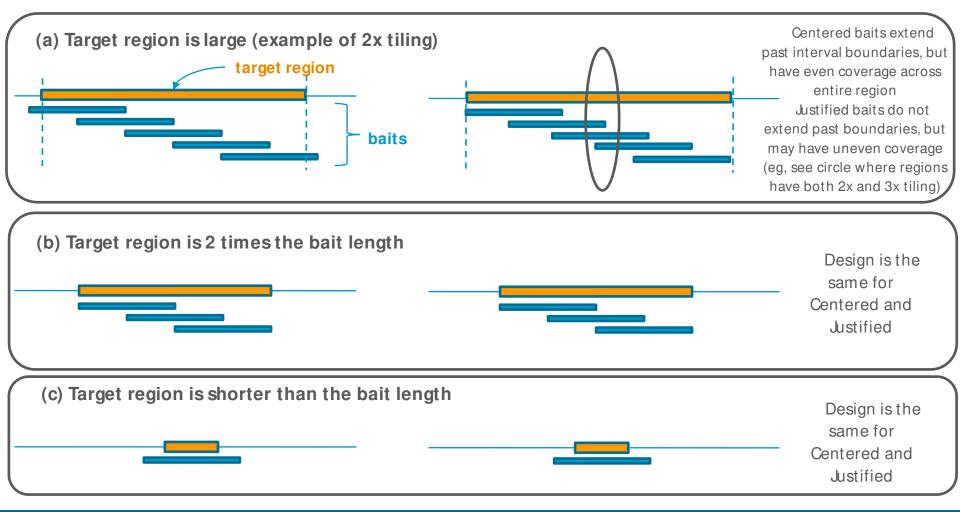


Create a Bait Group

Centered versus Justified

Centered

Justified





Create a Bait Group

Formats for describing Target Intervals

Target Details										
Species	H. sapiens 🗸									
Genome Build	H. sapiens, UCSC hg18, NCBI Build 36, Marc									
Genomic Target Intervals I <u>nfo</u>	chrX:100003816-									
The format for typing	enomic intervals to be targeted. g in the intervals is as follows: 00003948 chrX:100004037-									
10000446	8 chrX:100004314- 5 chrX:100004329- K:100004804-100004888									
there is a long list of int	e separated by the character. If tervals, it would be better to use option 2.									

Option 2: Upload a file that includes the genomic intervals to be targeted: The format for uploading the intervals is as follows:

🗉 exons.txt - WordPad 📃 🗖	×
<u>File E</u> dit <u>V</u> iew Insert F <u>o</u> rmat <u>H</u> elp	
□ ☞ 🖬 ● 🗟 🗰 🐇 階 🏙 ♡	e 9
chrX:10007765-10007844	^
chrX:100111356-100111454	_
chrX:100115321-100115393	
chrX:100115772-100115842	
chrX:100127312-100127561	
chrX:100128441-100128546	
chrX:100129035-100129201	
chrX:100129838-100129928	
chrX:100130023-100130203	
chrX:100132207-100132476	
chrX:100151202-100152352	
chrX:100155272-100155384	
chrX:100160616-100160715	_
chrX:100160929-100161048	×
For Help, press F1	:

Each interval should be presented on a separate line, and the file should be saved as a text file.

When using this option, select Upload, Browse to find the saved file, and then select Upload File.



Create a Bait Group Check Bait Group Status

- The status of your Bait Group can be viewed in the Pending Jobs quadrant on the Home page (see left), or at the bottom of the Baits page (see right)
- To continue, you must be on the Baits page

				_			Application Type: TargetEnn	chement fights an
earch			11 miles	ait Groups Dorn	My Account		appacation type: tai genean	CININA 20101740
) Libraries	O Bait Oroups	O Baits	Search Quose P	at 1 dang				
	11		Design Options			Target Details		
library Nome:			Design Job Name			Species	H. sapiens 💌	
pecies;		Select and Add	Sequencing Technology into	Illumina	*	Genome Build	H. sapiens, UCSC hg18, NCE	Build 36. Marc
130:	[] []	Upload	Sequencing Protocol 1000	end-sequencing	*	Genomic Target	-	~
		an against a	Design Strategy into	Use Optimized	Parameters	Intervals <u>Inte</u>		Upload
			45 V 17704	🕘 Centered 🔅 J	ustified	Genomic Avoid Interv	1011.	
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			Balt Tilling Frequency mits	24	- W.	Custom Avoid I	at a state when	
			Allowed overlap into avoid i	egions into 20	bp	Custom Avera	Intervals (IIIV)	
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			i					
-		No. 10 10 10 10 10 10 10 10 10 10 10 10 10	If you have difficulty downloads	ng the desired file, ho	id down the «Otri» key until a l	File Download dialog box appe	sars. This bypasses pop-up blocking	g software.
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	matching results found		Search Results, + matchin	g results found				
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arch Results: 1					The second	and the second state of th	1.00.000.000	4
earch Results: 1	Job Name Status	Created Job Date Position Action	Job ID Job Na	me Status	Besim Type : Dromonil	Citor Position in Onene	Actions	5

Note:- Your design results will be removed from the system 2 weeks after design completion!



Create a Bait Group View Design Details (I)

• To view the Bait Group design, click on View Design on the Baits page

Agilent Technologies									Help Logout
		Workspace	Collabora	tion Pu	blic		v	Velcome Demo Use	r (Agilent_Marketing)
Home Libraries Ba	ait Groups	Baits	My Account				Application Type:	TargetEnrichment	Switch Application Type
Search Upload Ba	it Tiling								
Design Options					Target Det	tails			
Design Job Name					Species		H. sapiens	*	
Sequencing Technology Info	Illumina	1	1		Genome Bu	ild	H. sapiens, UCSC	hg18, NCBI Build 3	36, Marc
Sequencing Protocol Info	end-sequ	encing 💉 💉	/		Genomic Ta	-		~	
Design Strategy Info	Vse 🗸)ptimized Para	meters		Intervals I <u>nf</u>			× .	Upload
	Cente	red 🔘 Justifi	ed		_	void Intervals			
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Allowed overlap into avoid re	gions <u>Info</u>	20	b	p		om Avoid Interv	ais <u>into</u>		≚ Upload
				Submi	t Cancel				

If you have difficulty downloading the desired file, hold down the <Ctrl> key until a File Download dialog box appears. This bypasses pop-up blocking software.

Search Results Refresh	: 1 matching res	ults found					
Job ID	Job Name	<u>Status</u>	<u>Design Type</u>	Creation Date	Position in Queue		Actions
BI407162395	FirstBaitGroup	Completed	BaitTiling	05-Feb-2009	NA	Delete View Design	Download Create Bait Group
Refresh						\bigcirc	

If you have difficulty downloading the desired file, hold down the <Ctrl> key until a File Download dialog box appears. This bypasses pop-up blocking software.

Note:- Your design results will be removed from the system 2 weeks after design completion!



Create a Bait Group View Design Details (II)

 Toggle between Design Summary, Design Details, Target Fate, and Bed File to view the details of the bait design (only the first 100 lines will be presented).

Design Summary	Design Details	Target Fate	Bed File						
Number of Input Target	s : 8423								
Number of Valid Targets : 8423									
Number of Baits per Ta	rgets : 4.86								
Number of Targets with	n Baits Designed : 8081								
Number of Baits : 4093	1								
Number of Baits remov	ed due to avoids : 2311								
Standard Deviation of E	9ait Length : 0.0								
Mean of Bait Length : 1	20.0								
Minimum Bait Length : 1	20								
Maximum Bait Length :	120								

First 100 lines of Balt Tiling Design Details for "Bi407149254"									
Design Surmary Design Details Target Fale	Sout File								
Target ID	Genomic Location	Sequence	1 3	Batt Length	0				
ctvX:140476046-140476911	chrit 140476019-140476938	CTCCTCACAGAGACCTCOGOCAGCTTACCTCCACCOGTATCAAGCCOCCCCCTCAGAAGGAGGCCTCTCCCCTTCTCTCTGACCCCCACGTGACGTGACGTGACGTGACGTCACATCCGGCCCCCCAA	120						
ctvX.46989025-48989267	CTVX 469890226-46989145	ADATOACOADOAGOATAAAGATOACOTCCCTOGOCCCTCAACTOGOGOCAGOCTCCOAGACCCTGAGCCAGAGCAGGCTGGGGCCCAGGCTCTDGAGTCACGAACAGGTGCCGGTCCCTGCTCCTCCTCCT	120						
Phys 46585025-46569367	INVERTIGISTIC	CONTRACT AGAIN ASSOCIDED OF TASCET PRANTICATIANT ASSOCIDE CONTRACT AND AN ANTION CONTRACT ASSOCIDE CARCEAN CONTRACT	120						

	First 100 lines of Balt Ti	ing Design Details for "Bl407149254"	
Design Summary Design Details Target Fale Bert File			
Farget ID	Status	Farget Length	Bat Generated
TARGET.ctvix:140470846-140470911	Pass	66	(L)
TARGET chrX 46865025-46865267	Pacs	243	5
TARGET::HVX:24001129-24001301	Pace	173	3

					First 190 lines of Bak	Tiling Design Details for "BH07149254"				
Design Summary	Design Details	Tacanth	iller Bed Film	1						
5	hromesome	A Ch	remosene Start	Chromosome Stop		Batt Name		Score	Strand	
chirX.		1404	176019	140476938	64407149254_0		1000		243	_
chrX		0460	189026	046989145	EH407149254_2		1000			
chrX.		0466	89006	046909205	EH07149254_0		1000		848	
chrX.		0465	69206	046989325	BI407149254_5		1000		1.00	



Create a Bait Group Download the Design (I)

- To download the design results files, click on Download on the Baits page.
- It may be necessary to hold down the Ctrl key until the File Download dialog box appears

Agilent Technologies									Help Logout
		Workspace	Collabora	tion Pub	lic		v	Velcome Demo Use	r (Agilent_Marketing)
Home Libraries B	ait Groups	Baits	My Account				Application Type:	TargetEnrichment	Switch Application Type
Search Upload Ba	iit Tiling								
Design Options					Targe	t Details			
Design Job Name					Specie	ès -	H. sapiens	*	
Sequencing Technology Info	Illumina	•	v		Genon	ne Build	H. sapiens, UCSC	Chg18, NCBI Build 3	36, Marc
Sequencing Protocol Info	end-sequ	iencing 💽	-			nic Target			
Design Strategy Info	Use	Optimized Para	meters		Interva			×	Upload
	Cente	ered 🔘 Justifi	ed		_	nic Avoid Intervals			
Bait Length	120	1	/ bp		~	Avoid Standard Re	peat Masked Regior	is <u>Info</u>	
Bait Tiling Frequency Info	2x		1 and the second se			Custom Avoid Inter			▲ Upload
Allowed overlap into avoid re	egions <u>Info</u>	20	bp)		Custom Avoid Inter			
				Submit	Can	cel			

If you have difficulty downloading the desired file, hold down the <Ctrl> key until a File Download dialog box appears. This bypasses pop-up blocking software.

Search Result: Refresh	s: 1 matching res	ults found				
<u>Job ID</u>	Job Name	<u>Status</u>	<u>Design Type</u>	Creation Date	Position in Queue	Astions
BI407162395	FirstBaitGroup	Completed	BaitTiling	05-Feb-2009	NA	Delete View Design Download greate Bait Group
Refresh						\smile



Create a Bait Group Download the Design (II)

There are four files included in the downloaded zip:

	Example	e of a	-ate file:			
	A	В	С	D	E	
1	TargetID	Status	Target Length	Baits Generated		
2	TARGET:chrX:148476846-148476911	Pass	66	1		
3	TARGET:chrX:46989025-46989267	Pass	243	5		
4	TARGET:chrX:24001129-24001301	Pass	173	3		
5	TARGFT-chrX-152694536-152694863	Pass	328	5		
- H	🔸 🕨 🛛 BaitTiling_fate 🤇 🖏				► I	

second of a Esta file.

Example of a BED file:

🗄 BaitTiling_bed - WordPad
<u>File Edit V</u> iew Insert Format <u>H</u> elp
browser position
track name=BaitTiling description="" visibility=2 color=0,128,0 useScore=1 💳
chrX 148476819 148476938 BI407162395_0 1000 +
chrX 046989026 046989145 BI407162395 2 1000 +
chrX 046989086 046989205 BI4071623953 1000 + 🛛 💌
For Help, press F1 NUM

Example of a TDT (tab delimited table) file:

		A	В	С	D		Ē
	1	TargetID	BaitLocation	Sequence	BaitLength		
1	2	chrX:148476846-148476911	chrX:148476819-148476938	CTCCTCACAGAGCCTC	120		
3	3	chrX:46989025-46989267	chrX:46989026-46989145	AGATGACGAGGAGGAT	120		
4	4	chrX:46989025-46989267	chrX:46989086-46989205	CCCTGAGCCAGAGCAG	120		
	F	-L-V-4000005 4000007	-L-V-40000000 40000000	COTOCACACOCTCAAC	100		1
K	-	🕩 🕂 🔁 BaitTiling_tdt	· · · · · · · · · · · · · · · · · · ·	▲		► I	

Example of a Summary file:

	A	В	С	
1	numInputTargets	8423		Π
2	numValidTargets	8423		
3	numBaitPerTarget	4.86		≡
4	numTargetsWithBait	8081		
5	numBaits	40931		
6	numBaitsRemovedDueToAvoids	2311		
7	lengthSD	0		
8	lengthMean	120		
9	lengthMin	120		
10	lengthMax	120		
11	BaitTiling_sum 🖄 🖬	Ш		



Create Bait Group Create Bait Group

Once satisfied with the Bait Group Design, select Create Bait Group **Agilent Technologies** Help | Logout eArray) Welcome Demo User (Agilent Markeling Collaboration Workspace Public Application Type: TargetEnrichment Switch Application Type Home Libraries Bait Groups-Bails My Account Search Upload Distriction Design Options **Target Details** Design Job Name Species H saplens Genome Build H. sepiens, UCSC hg18, NCBI Build 36, Marc Sequencing Technology http:////uniina Genomic Target Sequencing Protocol Inte end-sequencing Intervals info Upload Design Strategy into Use Optimized Parameters Genomic Avoid Intervals - Cerdered Judified Void Standard Repeat Masked Regions mo Bait Length bp Balt Tilling Frequency Info Custom Avoid Intervals into liploud Allowed overlap into avoid regions info bp Submit Cancel If you have difficulty downloading the desired file, hold down the «Ctrl» key until a File Download dialog box appears. This bypasses pop-up blocking software. Search Results: 1 matching results found

 Andresh
 Job ID
 Job Name
 Status
 Design Type
 Creation Date
 Position in Quesie
 Actions

 BH07162395
 FirstBabGroup
 Completed
 BalTing
 D5-Feb-2009
 NA
 Design Point (Create Balt Group)
 Completed
 Balt Ting
 D5-Feb-2009
 NA
 Design Point (Create Balt Group)
 Completed Balt Ting
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 NA
 Design Point (Create Balt Group)
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 Create Balt Group)
 Completed Balt Group)
 Create Balt Group)<

Check the status of the Bait Group creation in the Pending Jobs quadrant of the Home page

Search				
Libraries	O Bat Groups		0	Gaite
Library Name:	1			
Speciest		Select	and Add	
ELID:		Upload	D: 11	
	serch Reset			
ending Jobs				Refresh Vie
ending Jobs		4		Refresh Vie
ending Jobs	2 matching lesuits found			
ending Jobs	2 matching results found Job Name	Status C	realed Date De	

While waiting for the Bait Group to be created, design additional Bait Groups, if desired



Examine Bait Group Look for Missed Targets

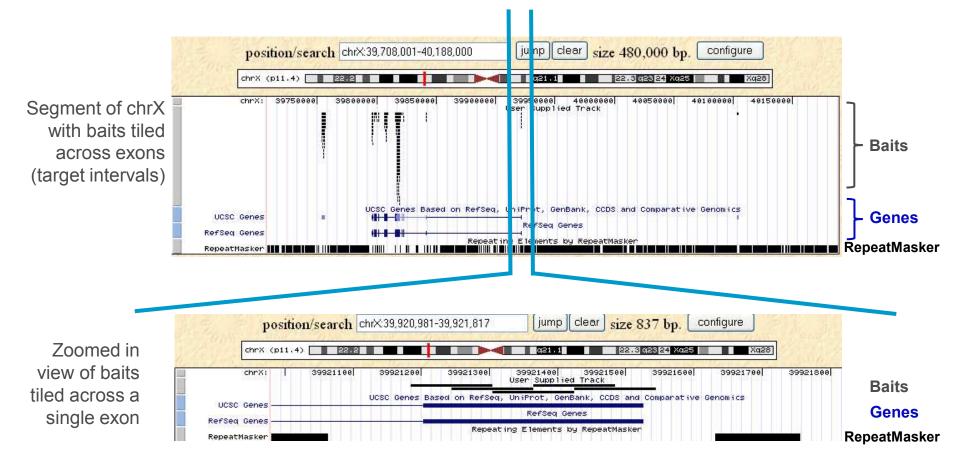
- Open the BaitTiling_fate file in Excel
- Sort the file for Status to identify invalid (failed) genomic coordinates
 - These are targets for which the genomic coordinates could not be correctly assigned
- Sort the file for Baits Generated to identify targets for which no baits were assigned
 - In this example, according to the BaitTiling_sum file (left): 8423 8081 = 342 targets were not assigned baits; these can be viewed on the right in the BaitTiling_fate file

						_				
	4	А	В	C 🔺		A	В	С		D
4	1	numInputTargets	8423	$>$ \square	1	TargetID	Status	Target Lengt	Baits G	ienerated
	2	numValidTargets	8423		2	TARGET:chrX:114947632-114949510	Pass	187		0
	3	numBaitPerTarget	4.86		3	TARGET:chrX:101598384-101598491	Pass	108	3	0
4	4	numTargetsWithBait	8081		4	TARGET:chrX:103060664-103060787	Pass	124		0
		numBaits	40931		5	TARGET:chrX:52260569-52260678	Pass	110		0
	6	numBaitsRemovedDueToAvoids	2311		6			1852		0
	7	lengthSD	0		7	TARGET:chrX:134674851-134675089	Pass	239		0
	8	lengthMean	120		8		Pass	11	,	0
	9	lengthMin	120		0			11	·	0
	10	lengthMax	120		9	TARGET:chrX:135213958-135214032	Pass	/9		0
		\mapsto 🕨 BaitTiling_sum 🖉 🚺			10	TARGET:chrX:52397077-52397187	Pass	111	· \	/



Examine Bait Group View Design in UCSC Browser

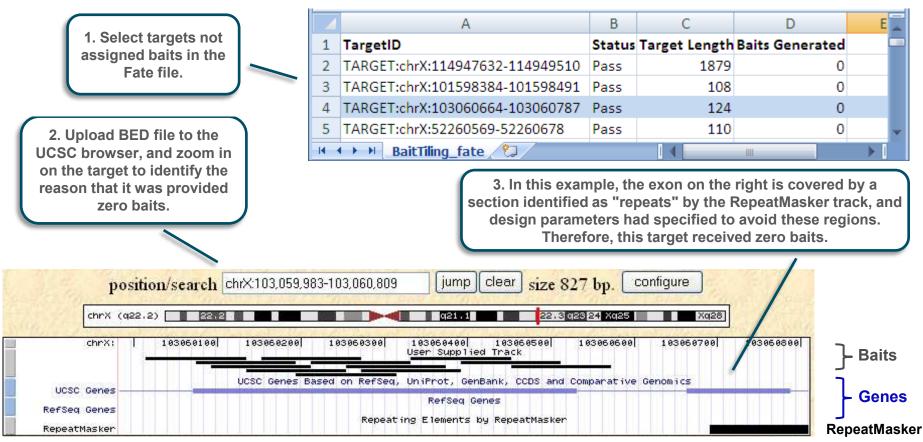
• The BED file can be uploaded into the UCSC browser as an easy method to visualize the probes tiled across targets of interest





Examine Bait Group

Identify Targets for Potential Re-Tiling



If the target is still desired, it is possible to create a new, additional Bait Group for this target with new design parameters that allow repeat regions. Both Bait Groups can later be included in the final library.



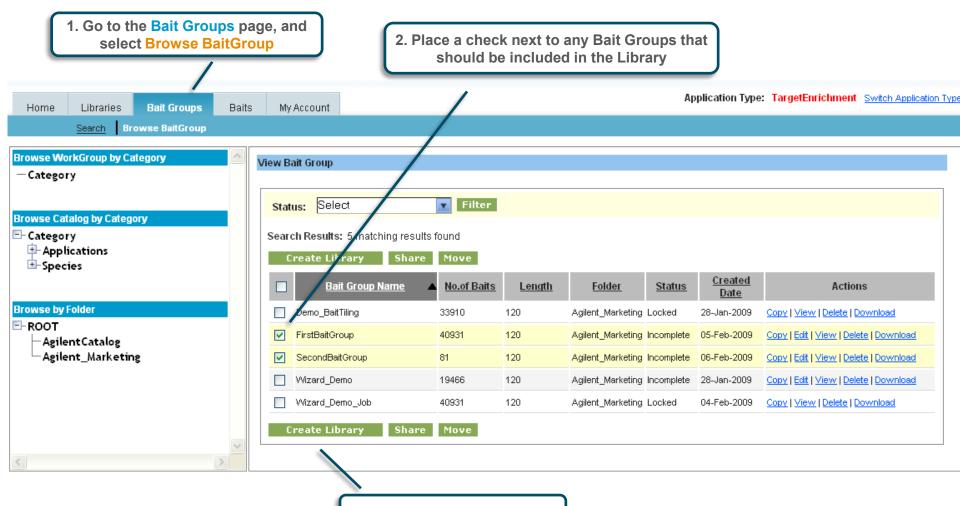
Complete Design for all Bait Groups

- Before proceeding to create the Library, complete the design for all Bait Groups that might be included in that Library
- Reasons for using multiple Bait Groups include:
 - To use different design options for different targets
 - To include Bait Groups from previous projects
 - To maintain separate Bait Groups based on target types; for example, a Bait Group of chrX exons, a Bait Group for Kinases, and so on

	matching results found					
Refresh Job ID	Job Name	<u>Status</u>	Design Type	Creation Date	Position in Queue	Actions
BI407194606	SecondBaitGroup	In Queue	BaitTiling	06-Feb-2009	Job 1 of 1	Delete
BI407162395	FirstBaitGroup	Completed	BaitTiling	05-Feb-2009	NA	Delete <u>View Design Download</u>
Refresh						



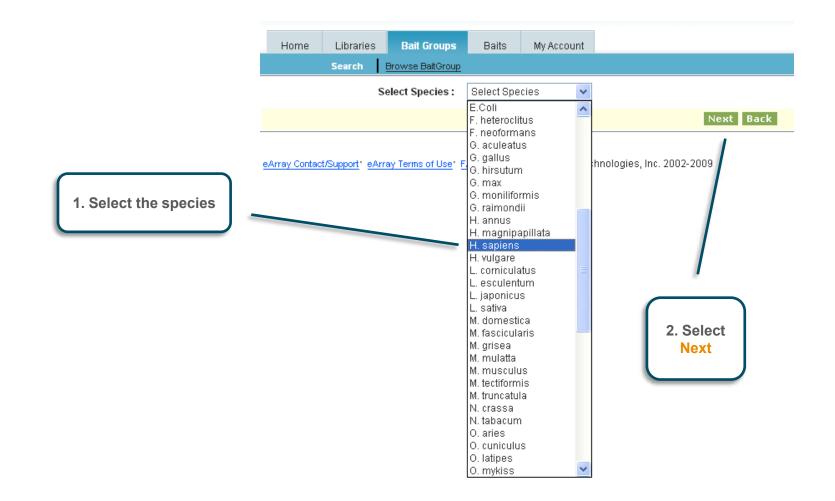
Create the Library Select Bait Groups to Include in the Library



3. Select Create Library

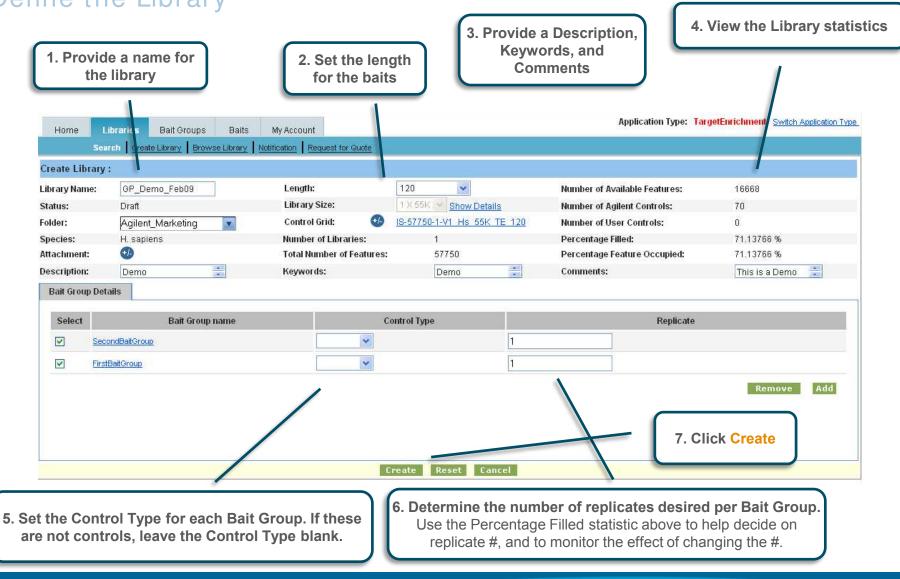


Create the Library Select the Species





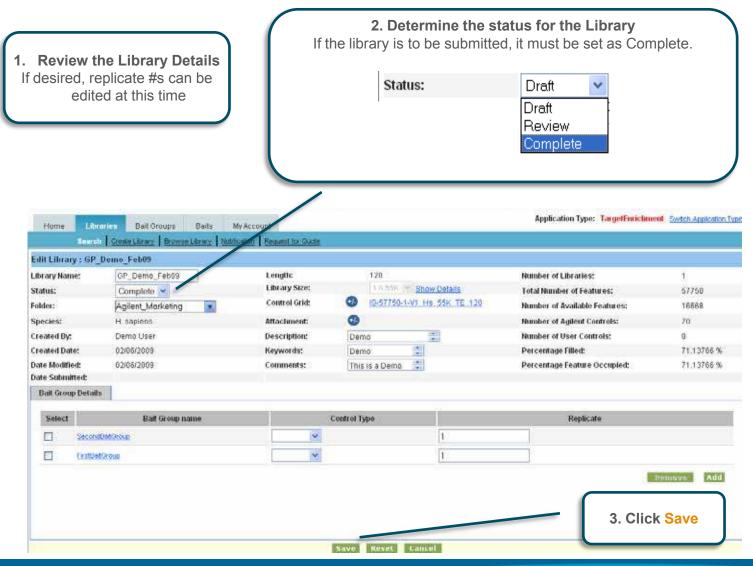
Create the Library Define the Library





Create the Library

Review Library Details and Submit as Complete





Create the Library Finalize Submission

1. Provide a comment and click Yes	2. Provide a comment in the next window and click on Design check list
Agilent Technologies eArray	Home Libraries Bait Groups Baits My Account Search Create Library Browse Library Notification Request for Guote Warning : You are about to submit this Library. Once saved no changes can be made. Image: Search
Home Libraries Bait Groups Baits My Account Search Create Library Browse Library Notification Request for Quote Warning : You are about to complete this Library. Once saved no changes can be made. Mathematical State Mathematical State	Please type comments here::
Please type comments here: This is a Demo Are you sure you want to continue ?	S. Read and check off the Design check list, and click Done File Folder for contracted by any other deside of the second lateral Folder for contracted by any other deside of the second lateral Folder for any other deside in the second lateral for any other deside by any other deside any other desi
	If and the sense of extreme is the program of and the sense mean of a spin-table is the block here is equive the characterization of a spin-table sense of a spin-table sense of the spin-table sense of a spin-table sense of a spin-table sense of a spin-table sense of the spin-table sen

4. Click Yes to finalize the submission of the Library, making it available for getting a quote. This is not a kit order, this is only a submission so that the kit can be ordered.



Generate a Quote Select Library

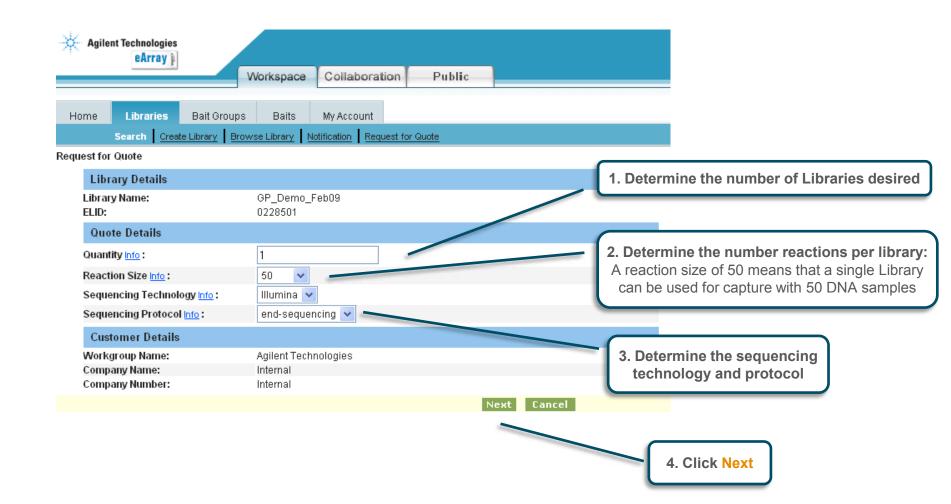
1. Select Browse Library on the Libraries page to find the Library

W	lorkspace Collaboration Pub	lic				Welcome Demo User (Aplient Mark
ome Libraries and Groups Search Grane Library Brown	Baits My Account es Ebreay Notecalon Request for Guide				Аррінса	tion Type: TargetEnrichment Switch Austonia
wse WorkGroup by Category ategory	View Library List					
vae Catalog by Category ategory - Applications - Species	Design Format: Select Search Results: 4 matching tes Share Hove	Section 1994	Select		Filler	•2
	Literary Norse	 Eolder, Name 	Status	CreatedDate	Leouth	Actions
vse by Folder	Demo BatTikigLikrary	Aglent_Marketing	Submitted	28-Jan-2009	120	Menter I Guestie I Decembro II Charonae, Conferret Taran
тоот	Ceno_BatTling_Library2	Agilent_Marketing	Submitted	20-Jan-2009	120	View I Swate Download Change Control Type
- AgilentCatalog - Agilent_Marketing	Detto_Wizard_Library	Aglent_Marketing	Submitted	05-Feb-2009	120	View Guste Downtowil Chanse Control Type -
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