Sourcing Guide for Guide-RNAs and qPCR Primers:
HLS-CATCH BRCA1 Enrichment in Human or Mouse

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This document provides instructions and recommendations for sourcing HLS-CATCH reagents (S. pyogenes Cas9 and guide RNAs) for isolation of ~200kb CATCH fragments containing the human BRCA1 locus and the mouse Brca1 locus.

Supplier	Product	Catalogue #
Guide RNAs		
Integrated DNA Technologies(IDT)	Guide RNAs: Alt-R®CRISPR Cas9 crRNA Alt-R®CRISPR Cas9 tracrRNA Nuclease-Free Duplex Buffer	**Custom** (2 nmol) **1072532** (5 nmole) **11-01-03-01** (10 X 2 ml)
Cas9 Nuclease		
New England Biolabs (NEB)	S.pyogenes Cas9 enzyme, wild type	**M0386T** (20 µM, 400 pmol)
Aldevron	sNLS-SpCas9-sNLS Nuclease	**9212-0.25MG** (60 µM, 250 µg)
Integrated DNA Technologies (IDT)	Alt-R® S.p. Cas9 Nuclease V3, 100 µg	**1081058** (60 µM, 100 µg)
TaqMan™ qPCR Reagents (Recommended)		
ThermoFisher	TaqMan Genotyping Master Mix (all reactions)	4371353
ThermoFisher	Human: BRCA1 TaqMan Assay	Hs00300666_cn
ThermoFisher	Human Ref.: TaqMan RNase P Detection Reagents	4403326
ThermoFisher	Mouse: Brca1 TaqMan Assay	Mm00594285_cn
ThermoFisher	Mouse Ref.: TaqMan Copy Number Assay, Tfrc	4458366
SYBR Green Reagents (Optional Alternative to TaqMan)		
ThermoFisher (or preferred vendor)	PowerUp SYBR Green Master Mix	A25742
ThermoFisher (or preferred vendor)	Target and reference primer sets	custom
DNA Quantification		
ThermoFisher Scientific	Qubit™ Fluorometer/dsDNA HS Assay kit	**Q32851** (100 assays)

Note: If using an electronic pdf version of this document, links in the Supplier column of this table go directly to the referenced product page.

A. Ordering Guide RNAs for Cas9 nuclease (Integrated DNA Technologies [IDT])

About Guide RNAs and HLS-CATCH:

Synthetic two-part guide RNAs (gRNAs) from Integrated DNA Technologies (IDT) should be used with HLS-CATCH. Testing with the SageHLS indicates that gRNAs are more reliable than single-guide RNAs (sgRNAs) produced by in vitro transcription, and slightly more specific than synthetic sgRNAs.

When ordering two-part gRNAs from IDT, the customer must specify the 20 bp protospacer sequence immediately upstream from the PAM site (-NGG-3') in DNA bases. This is illustrated in the figure below (from the IDT website).

The IDT ordering software will convert the entered DNA sequence into RNA base pairs and tag on the 3' portion of the crRNA, which is required for hybridization with the universal tracrRNA to form the active gRNA complex that binds to the Cas9 enzyme.

IDT offers two kinds of crRNA: ALT-R crRNA, and ALT-R crRNA XT which has enhanced RNase resistance. The standard ALT-R crRNA is less expensive and has not been found to provide an advantage for use with HLS-CATCH in Sage Science's internal testing.

Ordering Instructions

1. Synthetic two-part guide RNAs (gRNAs) from Integrated DNA technologies are should be used for HLS-CATCH. **IDT catalogue # Alt-R®CRISPR Cas9 crRNA**

2. For purification of the human BRCA1 gene with HLS-CATCH, the protospacer gRNA sequences of the crRNAs are:

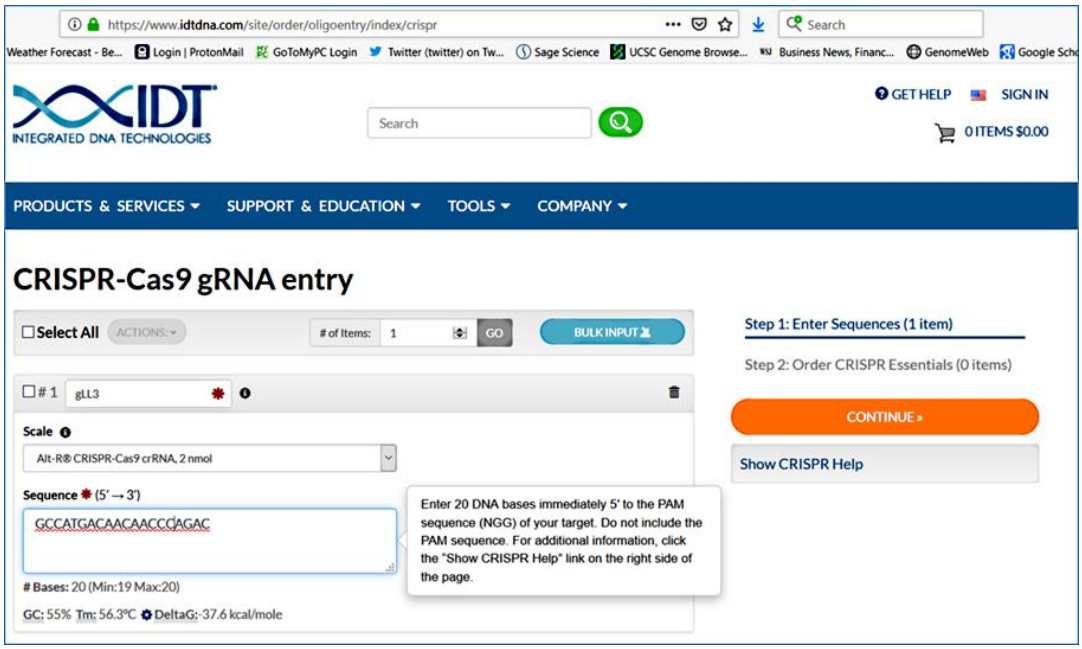
Table 1. Human BRCA1 Guides		
Name*	**crRNA recognition seq (DNA bp)**	**Position in hg38**
gLL3	GCCATGACAACAACCCAGAC	chr17:43,018,501-43,018,520
gR68	CTTATTACATTCTCGGCCAT	chr17:43,216,861-43,216,880

2a. For the mouse Brca1 gene, the protospacer gRNA sequences of the crRNAs are:

Table 2. Mouse Brca1 Guides		
Name*	**crRNA recognition seq (DNA bp)**	**Position in GRCm38**
L-g3	TTCGATGGTAACAACTCGAA	chr11:101,411,140-101,411,159
R-g1	GATAGTATTCCGAACCTGGG	chr11:101,590,993-101,591,012

* Guide names were assigned by Sage Science for internal reference.

3. Go to https://www.idtdna.com/site/order/oligoentry/index/crispr . Refer to the screen images below.



a →
b →
c →

For each guide:

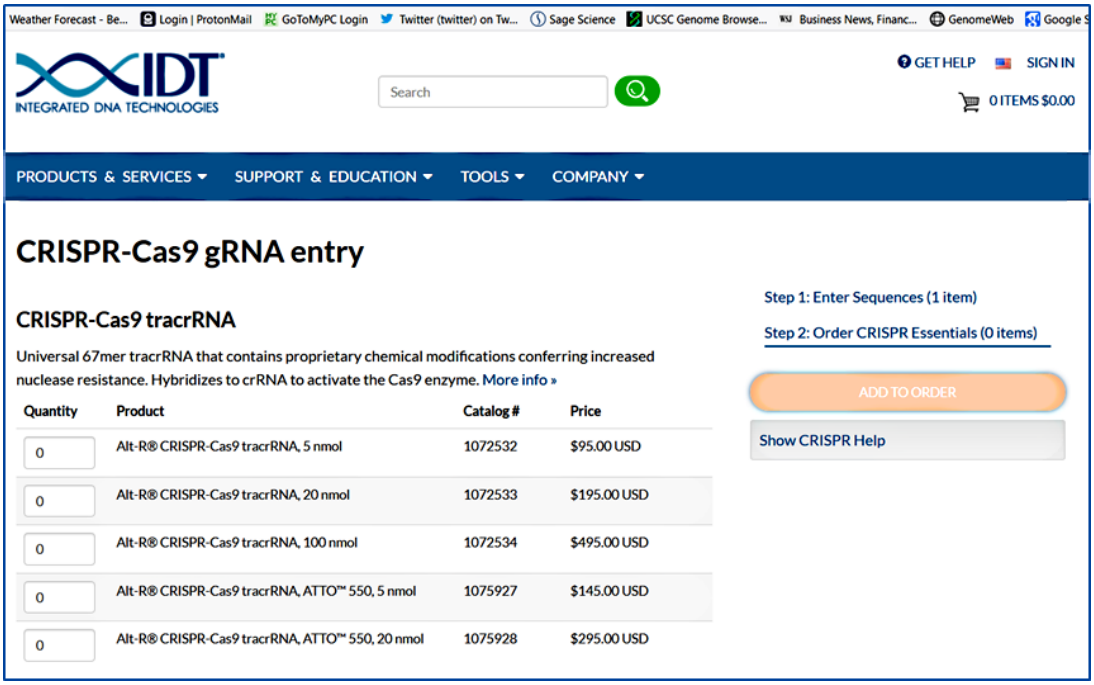
4. Enter the guide name (from Table 1 or 2) (**a**)

5. Select "Alt-R®CRISPR Cas9 crRNA, 2 nmol" (**b**). The 2 nmol scale is enough for 8-10 HLS-CATCH samples.

6. Enter or copy and paste crRNA recognition sequence (from Table 1 or 2) (**c**)

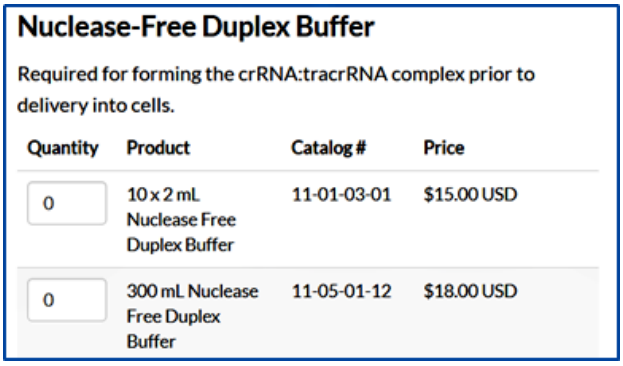
7. Press "CONTINUE". The screen shown below will load.

8. Order the universal tracrRNA from IDT (**d**). The smallest synthesis scale of tracrRNA (5 nmol) offered by IDT is enough for 18 HLS-CATCH samples (cat#1072532)



d →

9. Use IDT's Nuclease-Free Duplex Buffer to resuspend and anneal the crRNA and tracrRNA. Ordering information is found by scrolling to the bottom of the web page shown above.



Nuclease-Free Duplex Buffer

Required for forming the crRNA:tracrRNA complex prior to delivery into cells.

Quantity	Product	Catalog #	Price
0	10 x 2 mL Nuclease Free Duplex Buffer	11-01-03-01	$15.00 USD
0	300 mL Nuclease Free Duplex Buffer	11-05-01-12	$18.00 USD

B. Ordering Cas9 *S. pyogenes* nuclease

About S. pyogenes Cas9 enzyme and HLS-CATCH:

Three different Cas9 enzymes have been tested with equally good results in HLS-CATCH:

IDT and **Aldevron** sell SpCas9 enzymes modified with Nuclear Localization Signals (NLS) for genome editing. **New England Biolabs** sells wild-type SpCas9 enzyme without NLS modifications. We find that these three enzymes perform equally well in HLS-CATCH.

IDT and Aldevron also sell a "HiFi" version of SpCas9. In HLS-CATCH procedures, we have not found that the HiFi Cas9 enzyme performs better than the "standard" Cas9 versions.

Cas9 products validated with HLS-CATCH

Cas9 Enzymes that have been tested with HLS-CATCH are shown below. Note that the Aldevron and IDT Cas9 enzymes are sold at 61 µM concentration, while the NEB enzyme is sold at 20 µM concentration. The volume of Cas9 need per CATCH reaction, and the number samples that can be run (with a minimum order) are also shown below:

Validated Enzymes for HLS-CATCH

Supplier	Product	Catalogue #	Vol. per CATCH Rx	# CATCH samples
NEB	S.pyogenes Cas9 enzyme, wild type	**M0386T** (20 µM, 400 pmol)	**8 µl**	**2**
Aldevron	sNLS-SpCas9-sNLS Nuclease	**9212-0.25MG** (60 µM, 250 µg)	**2.6 µl**	**9**
IDT	Alt-R® S.p. Cas9 Nuclease V3	**1081058** (60 µM, 100 µg)	**2.6 µl**	**3**

C. Biological samples for HLS-CATCH demonstration:

One of the following cell preparations should be used as a starting material for HLS-CATCH demonstration of BRCA1/Brca1 enrichment :

- White blood cells isolated from prescreened human whole blood, or mouse whole blood using the SageHLS Cell Preparation kit (CEL-MWB1).
- Lymphoblastoid cultured cell lines.

If using WBCs from whole blood, ACD (acid citrate dextrose) preserved blood should be used. When stored at 4°C, ACD whole blood samples can be used for WBC preparation for up to 5 days.

CAUTION!: Human cells and cell lines should be handled only by trained users under all applicable biosafety regulations (typically BL-2 biohazard conditions).

D. Quantitative PCR reagents for detection of BRCA1 and mouse Brca1 CATCH targets (Recommended)

About ThermoFisher Taqman™ qPCR Assays

ThermoFisher TaqMan qPCR reagents for detecting CATCH targets are recommended. When ordering TaqMan assay, it is critical to use "copy number" assays for the CATCH targets. Copy number assays are designed to determine the genomic DNA copy number of the gene target. The product numbers for these kits end in "_cn". Similarly, the reference gene kits are named "…Copy Number Reference Assay, …".

NOTE: Most TaqMan BRCA1/Brca1 mRNA expression assays cannot be used to quantify genomic DNA, because the amplicons are designed to cross mRNA splice boundaries, in order to minimize possible interference from contaminating genomic DNA.

The following reagents from ThermoFisher should be used:

Human BRCA1 TaqMan Assay	**Reference: TaqMan RNase P Detection**
Cat# Hs00300666_cn	Cat# 4403326
Mouse Brca1 TaqMan Assay	**Reference: TaqMan Copy Number Reference Assay, mouse, Tfrc**
Cat# Mm00594285_cn	Cat# 4458366
TaqMan Genotyping Master Mix (all reactions): Cat# 4371353	

Reaction conditions for TaqMan assays:

Volume	Reagent
10 µl	TaqMan Genotyping Master Mix
1 µl	TaqMan probe mix (BRCA1 or Brca1)
1 µl	TaqMan Copy Number Reference Assay probe mix (RNase P or Tfrc)
6 µl	*0.33% beta-cyclodextrin (bCD) reagent , from the Sage Science (HIT-XXXX) kit
2 µl	DNA sample from SageHLS elution well, or genomic DNA standard dilution
20 µl	**Total Volume**

**Beta-cyclodextrin is important for avoiding PCR inhibition by trace amounts of SDS in CATCH products.*

qPCR conditions:

Almost all qPCR cyclers have preset cycling programs for TaqMan assays. Run all qPCR assays in standard curve mode, using a serially diluted human (or mouse) genomic DNA standard. Standard curve mode allows an estimation of both yield and enrichment factor. Use a 10-fold dilution series of genomic DNA samples ranging from 10^5 down to 10^1 haploid genome copies per reaction (assuming 3.3 pg per haploid human genome).

E. SYBR Green qPCR reagents for detection of BRCA1 / Brca1 CATCH targets (Optional)

TaqMan assays are somewhat easier to setup (only 1 tube for each target+reference replicate) and have lower CV's, they are significantly more expensive than SYBR Green qPCR assays. Below are some primers that have validated for qPCR for the BRCA1/Brca1 CATCH targets. These primers were designed using the Primer-BLAST web application at NCBI, using a Tm of 55C and an amplicon length range of 70-110bp.

Human BRCA1 and reference primers for SYBR qPCR	
Human BRCA1 primers (within BRCA1 exon 18, hg38, chr17:43,063,190-43,063,782)	
BRCA1-1F TTTCACGGAGATAGAGAGGT	BRCA1-1R ATTAAAGGGCTGTGGCTTTA
Human RNase P RNA gene (RPPH1) primers (chr14:20,343,077-20,343,148)	
Reference: RNaseP-3F CGGAGGAGAGTAGTCTGAAT	RNaseP-3R AAGTGAGTTCAATGGCTGAG
Mouse Brca1 and reference primers for SYBR qPCR	
Mouse Brca1 primers(GRCm38/mm10, Brca1 intron 21/22, chr11:101,492,016-101,492,102)	
375-F GACCTCCCTCGATACATTCA	374-R GCTTTCTCTACC CTTGACAC
Reference: Mouse randomly-selected single-copy sequence (chr2:52,313,255-52,313,348)	
457-F GCTGTGGAGACAGACTTCTATG	458-R TGAAACCTGACTGGCCTAAAC

Reaction conditions for SYBR Green qPCR assays:

Volume	Reagent
10 µl	ThermoFisher PowerUp SYBR Green Master Mix Cat# A25742 (or preferred vendor)
2 µl	Primer mix, each primer at 10uM concentration (preferred vendor)
6 µl	*0.33% beta-cyclodextrin (bCD) reagent , from the Sage Science (HIT-XXXX) kit
2 µl	DNA sample from SageHLS elution well, or genomic DNA standard dilution
20 µl	**Total Volume**

**Beta-cyclodextrin is important for avoiding PCR inhibition by trace amounts of SDS in CATCH products.*

SYBR Green Cycling conditions

1x 50C, 2min
1x 95C, 10min
40x 95C, 15sec; 60C, 1min
melt curve 60C > 95C at 0.15C/sec ramp

(These are the default conditions if using ThermoFisher QuantStudio qPCR cyclers.)

F. Qubit HS assay for total DNA in eluted HLS-CATCH products

Qubit HS assays on all elution fractions are carried out mainly to assess the health of the input cells and overall performance of the assay. 2-5 ul aliquots of each elution fraction should be used. Typical results for BRCA1/Brca1 CATCH elution produced in the Qubit HS and qPCR assays are illustrated on the final page of the HLS-CATCH training manual.