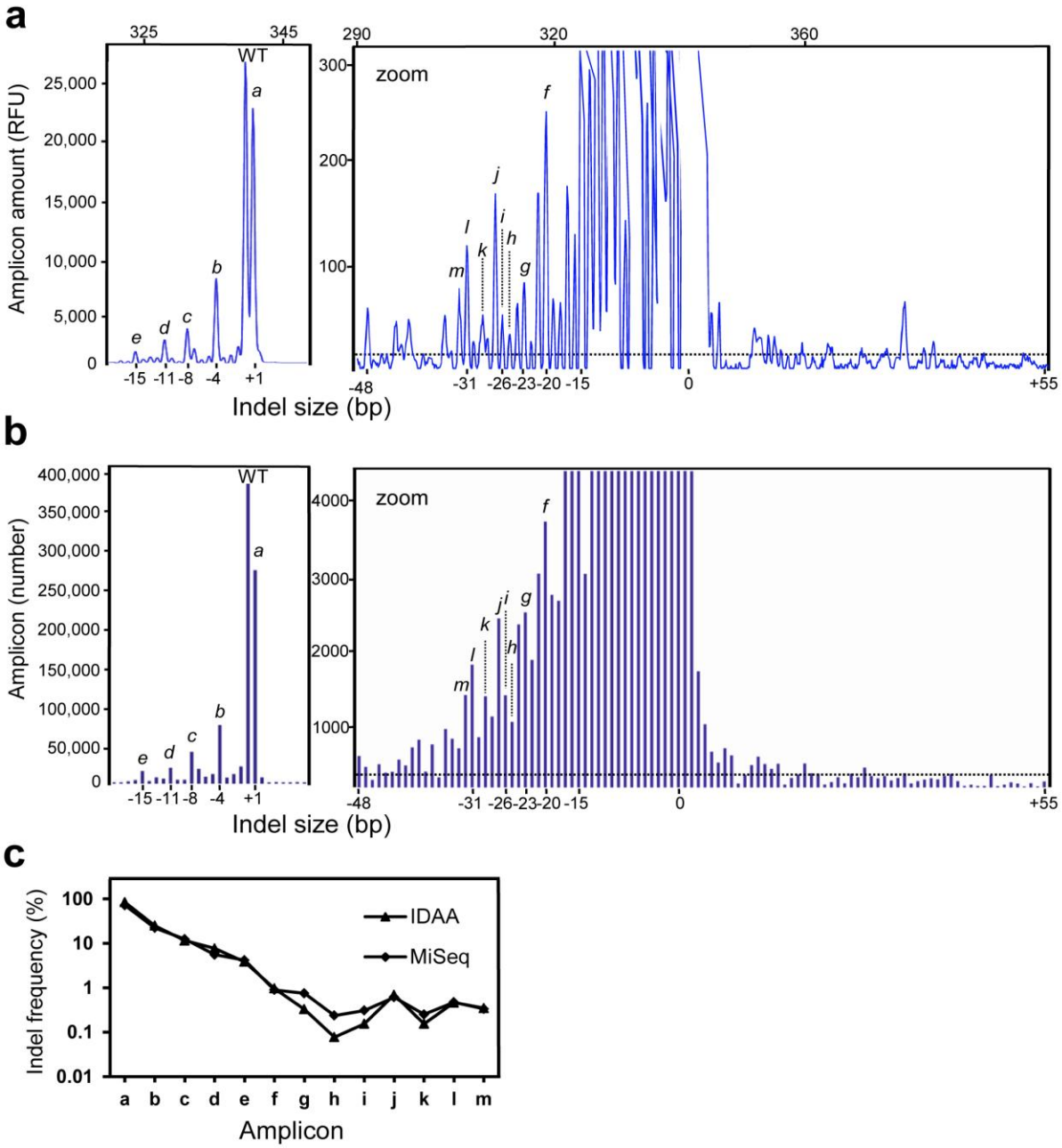
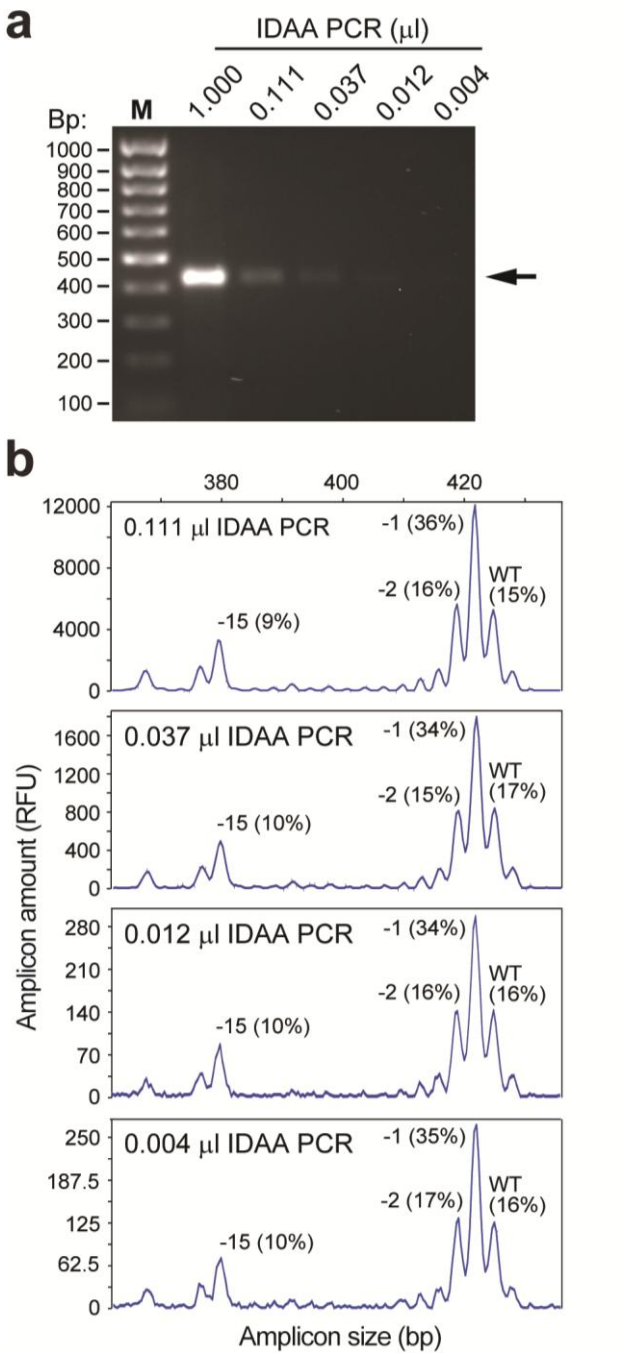


Supplementary Figure 1

Sensitivity and quantitative performance of IDAA performed in an ABI 3500 instrument is comparable to Next-Generation Sequencing (MiSeq).

Indel analysis by **(a)** IDAA or **(b)** MiSeq of PCR amplicons from the *Cosmc* locus edited by CRISPR/Cas9 in a CHO cell pool. On the x-axis, indel sizes (bp) are indicated for some of the amplicons. In the zoom-in panels, a stippled line indicates the background signal level, defined based on the signals from 45-55 bp insertions that were considered background, since CRISPR/Cas9 rarely elicits insertions of such sizes. **(c)** Graphs of frequencies of indels *a-m* from the IDAA and MiSeq analyses. Note the high degree of agreement between the two analyses.

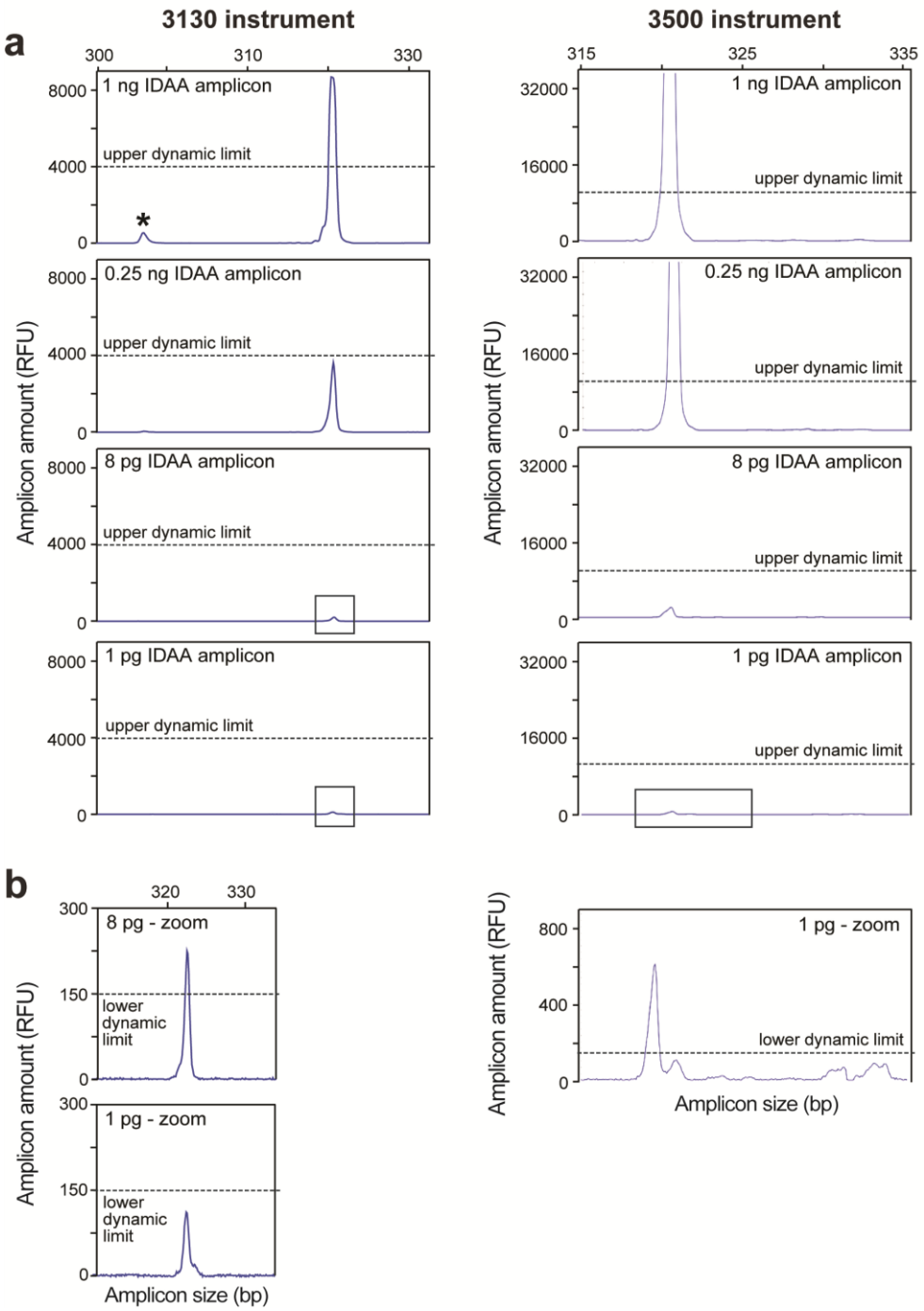


Supplementary Figure 2

IDAA can generate useful indel profiles from minute amounts of IDAA PCR amplicons.

The *Trp53* locus was targeted with CRISPR/Cas9 in a mouse Neuro2A cell pool and the target site was amplified by IDAA PCR. The indicated amounts of the IDAA PCR were analyzed by **(a)** agarose gel electrophoresis (an arrow indicates the IDAA PCR amplicon) or **(b)** IDAA capillary electrophoresis. The size and frequency of selected indels are indicated. IDAA was performed in an ABI 3500 instrument.

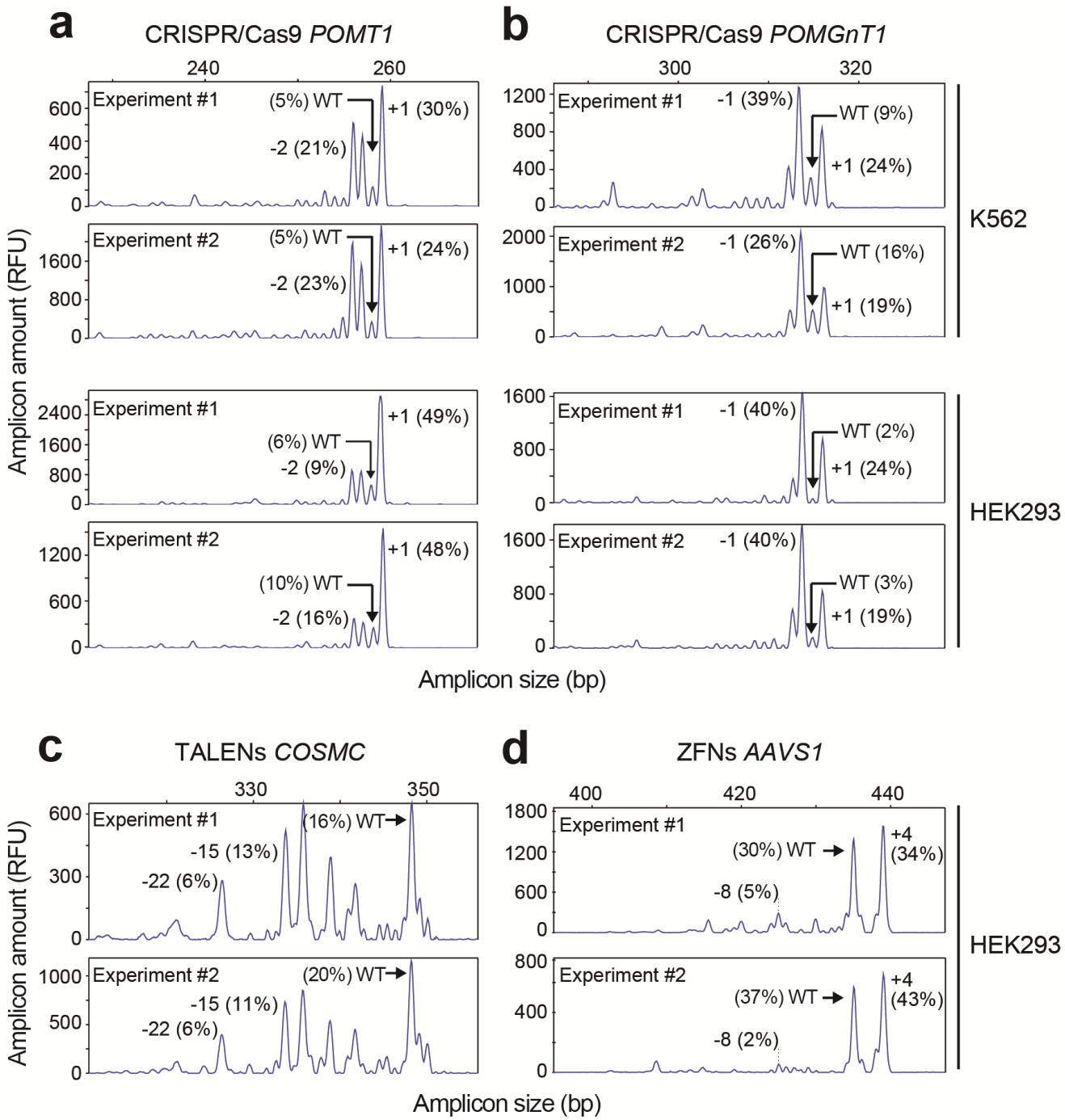
Note that the indel profiles are almost identical across the range of IDAA amplicons analyzed and that a high-quality profile can be generated from amplicon amounts barely visible or undetectable by agarose gel analysis.



Supplementary Figure 3

Upper and lower detection levels for IDAA amplicons in ABI 3130 and 3500 instruments.

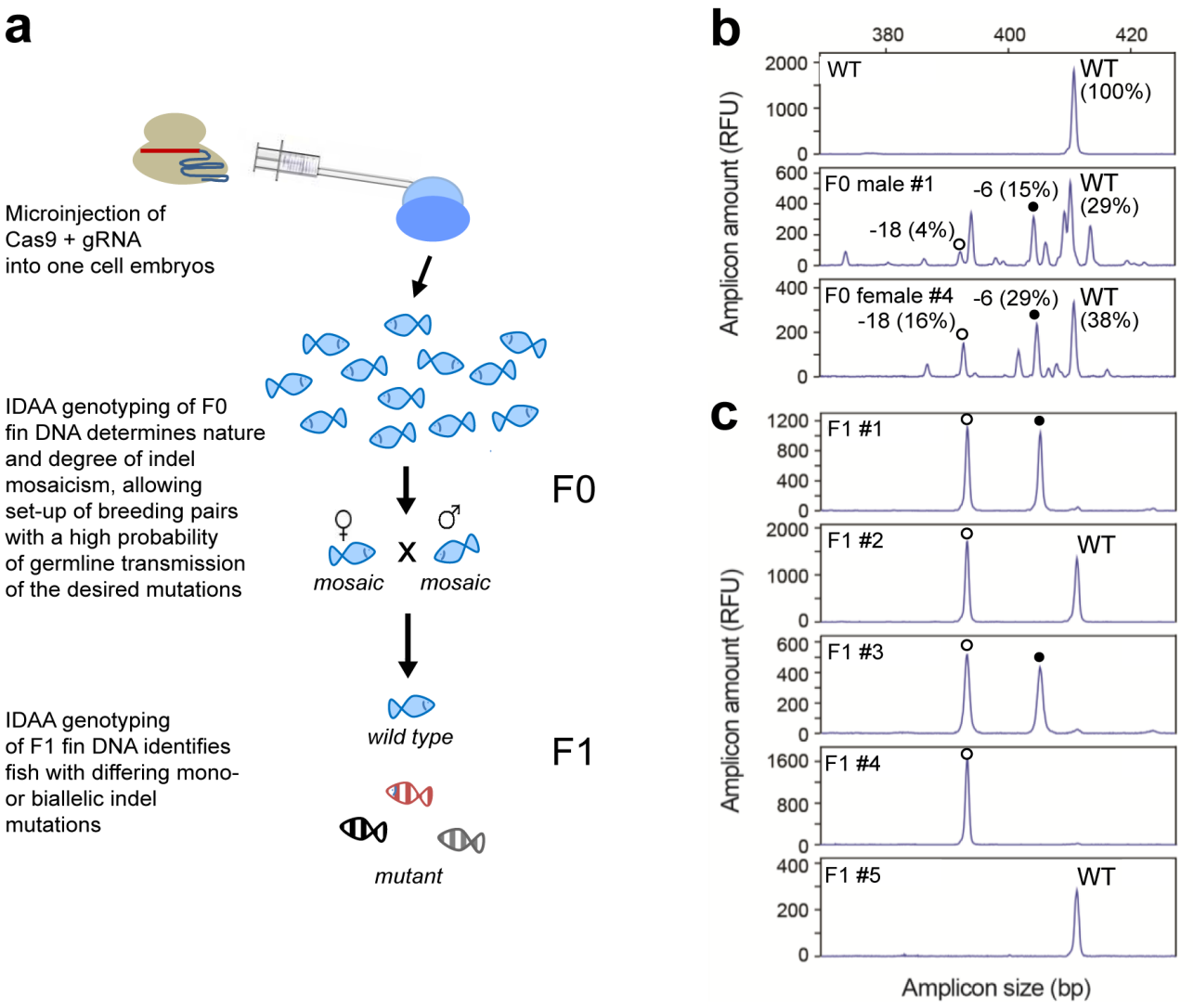
(a) Various amounts of a wt IDAA amplicon from a non-edited control sample were analyzed in ABI 3130 and 3500 instruments. When the loaded amplicons exceed the dynamic range, a smaller peak artifact often appears 20-30 bp ahead of the true signal (indicated by asterisk in the upper 3130 panel). **(b)** Zoom in on the peaks boxed in (a). The lower 3130 panel illustrates that also below the dynamic range, a specific signal can be discriminated from background signals.



Supplementary Figure 4

IDAA reveals the indel signature of a given gRNA, TALEN or ZFN.

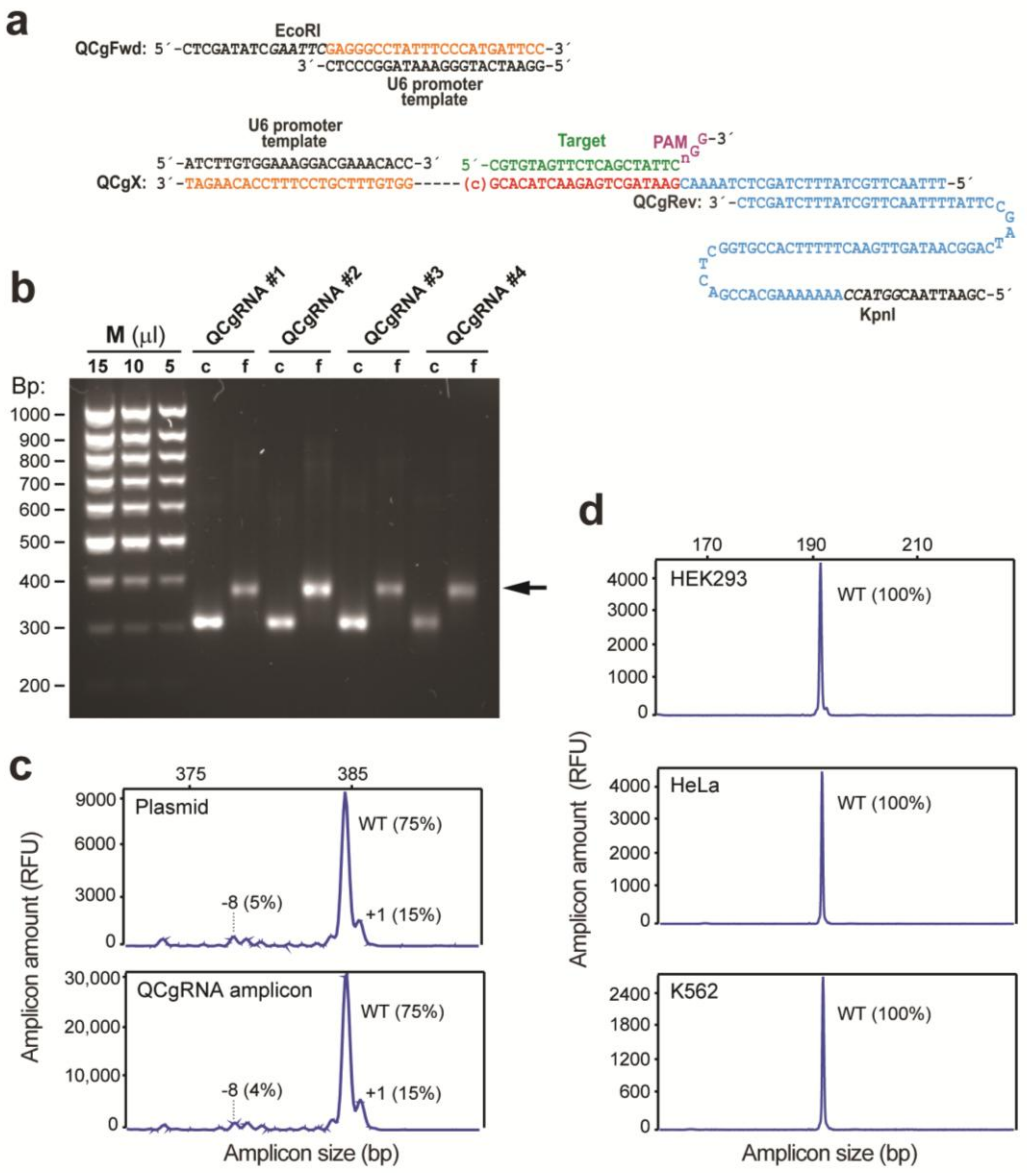
IDAA profiles for two independent experiments using the same construct of FP-linked CRISPR/Cas9 **(a,b)**, TALENs **(c)** or ZFNs **(d)**, targeting various loci in K562 or HEK293 cells, as indicated. Prior to analysis, the nuclease-transfected cells were subjected to bulk FACS for the top 30% most fluorescent cells. The size and frequency of selected indels are indicated. IDAA profiles were generated in an ABI 3130 instrument.



Supplementary Figure 5

Using IDAA to estimate probability of germline transmission of indels from F0 mosaic fish and to identify F1 mutant fish in *Danio rerio* genome editing.

(a) In zebrafish genome editing, a major challenge is determination of the nature and degree of indel mosaicism and hence, probability of germline transmission of indels from F0 fish derived from one-cell embryos injected with Cas9 and gRNA. IDAA enables easy and rapid evaluation of these variables through analysis of F0 fin DNA, allowing optimal set-up of F0 breeding pairs. IDAA also enables easy identification of indels in subsequent generations through fin DNA analysis. (**b**) Somatic IDAA profiles of the *bambi* locus in fin DNA of F0 fish targeted at the one-cell embryo stage via injection of Cas9 and gRNA. F0 male #1 and F0 female #4 were chosen for mating due to the presence of predominant indels indicated by open or closed circles, suggesting high probability of germline transmission of these mutations. **(c)** IDAA profiles of 200 downstream F1 fish, showing that 4 out of 5 fish harbored the predominant indels identified in the F0 breeding pair, of which 3 were in the biallelic state (F1 #1, #3, #4) and one homozygous for an indel (F1 #4). In this example, IDAA was performed on embryos, but IDAA could also have been performed on fin DNA. The size and frequency of selected indels are indicated. IDAA profiles were generated in an ABI 3130 instrument.



Supplementary Figure 6

QCgRNA amplicon expression cassettes.

(a) Schematic showing the various elements of the QCgRNA amplicon primers QCgFwd, QCgX and QCgRev. Sequences annealing to U6 promotor template are shown in orange; gRNA design in red; tracr elements in blue; restriction enzyme sites for sub-cloning to pEPB104 in italics. Note that the gRNA design is incorporated into QCgX as the complementary sequence to the target, which is shown in green. The nucleotide (c) is only included in the QCgX primer, if the gRNA (=target) does not contain a G as the first (5´) nucleotide, which is the case in the present example. **(b)** Agarose gel (2%) electrophoresis check for the formation of full-length (f) products (arrow) by QCgRNA amplicon tri-primer PCRs, as compared to control (c) PCRs containing only QCgFwd and QCgX primers (Step 3 in Procedure). Various amounts of MassRuler Low Range DNA ladder (M) are run alongside to enable quantitation of the QCgRNA amplicons. **(c)** The indel profiles elicited by a gRNA design in a QCgRNA amplicon and a plasmid vector are identical, as illustrated by targeting *GALNT10* in HEK293 cells. The *GALNT10* QCgRNA amplicon was subcloned into pEPB104 plasmid (Addgene #68369; **Supplementary Sequence 1**) using EcoRI and KpnI restriction endonuclease sites present in both constructs. **(d)** A QCgRNA design found non-functional in one cell type typically remains non-functional when tested in other cell types, as illustrated with a *POMT2* QCgRNA. IDAA profiles were generated in an (c) ABI 3500 or and (d) ABI 3130 instrument.

Supplementary Information

Genome editing using FACS enrichment of nuclease expressing cells and indel detection by amplicon analysis

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Supplementary Table 1 | Nuclease-2A-FP expression systems

Cas9 + gRNA ("all-in-one") expression vectors							
Fluorescent protein	**Delivery mode**	**Nuclease elements**	**Promoters: †gRNA *Cas9-FP**	**gRNA swapping possible (swapping method) OR custom gRNA**	**Plasmid name**	**Source/ Laboratory**	**Reference/Link**
GFP	Lentiviral transduction	Cas9 + 4 gRNAs	†mU6/hU6 /H1/7SK *hUbC	Yes (Golden Gate)	pLV hUbC-Cas9-T2A-GFP	Addgene/ Charles Gersbach	1/ https://www.addgene.org/53190/
EGFP	Transfection	Cas9 + 1 gRNA	†hU6 *CBh	Yes (BbsI)	pSpCas9(BB)-2A-GFP (PX458)	Addgene/ Feng Zhang	2/ https://www.addgene.org/48138/
GFP	Lentiviral transduction	Cas9 + 1 gRNA	†hU6 *EFS	Yes (BsmBI)	pL-CRISPR.EFS.GFP	Addgene/ Benjamin Ebert	3/ https://www.addgene.org/57818/
tagRFP	Lentiviral transduction	Cas9 + 1 gRNA	†hU6 *EFS	Yes (BsmBI)	pL-CRISPR.EFS.tRFP	Addgene/ Benjamin Ebert	3/ https://www.addgene.org/57819/
tagRFP	Lentiviral transduction	Cas9 + 1 gRNA	†hU6 *SFFV	Yes (BsmBI)	pL-CRISPR.SFFV.tRFP	Addgene/ Benjamin Ebert	3/ https://www.addgene.org/57826/
eGFP	Lentiviral transduction	Cas9 + 1 gRNA	†hU6 *SFFV	Yes (BsmBI)	pL-CRISPR.SFFV.GFP	Addgene/ Benjamin Ebert	3/ https://www.addgene.org/57827/
BFP	Transfection	Cas9 + 1 gRNA	†hU6 *Cbh	Yes (BbsI)	pU6-(BbsI)_CBh-Cas9-T2A-BFP	Addgene/ Ralf Kühn	4/ https://www.addgene.org/64323/
mCherry	Transfection	Cas9 + 1 gRNA	†hU6 *Cbh	Yes (BbsI)	pU6-(BbsI)_CBh-Cas9-T2A-mCherry	Addgene/ Ralf Kühn	4/ https://www.addgene.org/64324/
mCherry	Transfection	Cas9 + 1 gRNA	†hU6 *Cbh	Yes (BbsI)	pU6-(BbsI)_CBh-Cas9-T2A-mcherry-P2A-Ad4E4orf6	Addgene/ Ralf Kühn	4/ https://www.addgene.org/64222/ Note: co-expresses viral protein to suppress NHEJ and thus promote knockin
BFP	Transfection	Cas9 + 1 gRNA	†hU6 *Cbh	Yes (BbsI)	pU6-(BbsI)_CBh-Cas9-T2A-BFP-P2A-Ad4E4orf6	Addgene/ Ralf Kühn	4/ https://www.addgene.org/64220/ Note: co-expresses viral protein to suppress NHEJ and thus promote knockin
mCherry	Transfection	Cas9 + 1 gRNA	†hU6 *Cbh	Yes (BbsI)	pU6-(BbsI)_CBh-Cas9-T2A-mcherry-P2A-Ad4E1B	Addgene/ Ralf Kühn	4/ https://www.addgene.org/64221/ Note: co-expresses viral protein to suppress NHEJ and thus promote knockin
BFP	Transfection	Cas9 + 1 gRNA	†hU6 *Cbh	Yes (BbsI)	pU6-(BbsI)_CBh-Cas9-T2A-BFP-P2A-Ad4E1B	Addgene/ Ralf Kühn	4/ https://www.addgene.org/64218/ Note: co-expresses viral protein to suppress NHEJ and thus promote knockin
mCherry	Transfection	Cas9 + 1 gRNA	†hU6 *Cbh	Yes (BbsI)	pU6-(BbsI)_CBh-Cas9-T2A-mcherry-H1-(BamHI)	Addgene/ Ralf Kühn	4/ https://www.addgene.org/64217/ Note: allows co-expression of a user-specified shRNA
tGFP	Transfection	Cas9 + 1 gRNA	†hU6 *CMV, EF1a	Yes (BamHI and BsmBI)	pCas-Guide-EF1a-GFP	OriGene	http://www.origene.com/CRISPR-CAS9/Detail.aspx?sku=GE100018
GFP	Transfection	Cas9 + 1 gRNA	†hU6 *CBh	No	CRISPR/Cas9 Knockout Plasmid	Santa Cruz Biotechnolog	http://www.scbt.com/crispr-cas9_system.html

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GFP	Transfection	Cas9 + 1 gRNA	†hU6 *CMV	Custom gRNA	CRISPR/Cas-GFP	Sigma-Aldrich	http://www.sigmaaldrich.com/technical-documents/articles/biology/crispr-cas-gfp-vector.html
OFP	Transfection	Cas9 + 1 gRNA	†hU6 *CMV	No	GeneArt CRISPR Nuclease Vector	Thermo Fisher Scientific	https://www.thermofisher.com/order/catalog/product/A21174
ZsGreen	Transfection OR Lentiviral transduction	Cas9 + 1 gRNA	†hU6 *EFS	Yes (BsmBI) OR custom gRNA	pCLIP-All-EFS-ZsGreen	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-gRNA-plus-Cas9-Cloning-Vector-(EFS-ZsGre.aspx
tRFP	Transfection OR Lentiviral transduction	Cas9 + 1 gRNA	†hU6 *EFS	Yes (BsmBI) OR custom gRNA	pCLIP-All-EFS-tRFP	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-gRNA-plus-Cas9-Cloning-Vector-(EFS-tRFP).aspx
GFP	Transfection	Cas9 + 1 gRNA	†hH1 *EF1	No	EF1-T7-hspCas9-T2A-GFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
RFP	Transfection	Cas9 + 1 gRNA	†hH1 *EF1	No	EF1-T7-hspCas9-T2A-RFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
GFP	Transfection	Cas9 + 1 gRNA	†hH1 *CAG (=CBh)	No	CAG-T7-hspCas9-T2A-GFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
RFP	Transfection	Cas9 + 1 gRNA	†hH1 *CAG (=CBh)	No	CAG-T7-hspCas9-T2A-RFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
GFP	Transfection	Cas9 + 1 gRNA	†hH1 *CMV	No	CMV-T7-hspCas9-T2A-GFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
mCherry	Transfection OR Lentiviral transduction	Cas9 + 1 gRNA	†hU6 *CMV	Custom gRNA	pCRISPR-CG01	GeneCopoeia	http://www.genecopoeia.com/product/crispr-cas9/
RFP	Transfection	Cas9 + 1 gRNA	†hH1 *CMV	No	CMV-T7-hspCas9-T2A-RFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
EGFP	Transfection	Cas9 nickase + 1 gRNA	†hU6 *CBh	Yes (BbsI)	pSpCas9n(BB)-2A-GFP (PX461)	Addgene/ Feng Zhang	2/ https://www.addgene.org/61592/
GFP Puromycin on one plasmid of the nickase pair	Transfection	Cas9 nickase + 1 gRNA	†hU6 *CBh	Custom gRNA	Double Nickase Plasmid	Santa Cruz Biotechnology	http://www.scbt.com/crispr-cas9_system.html
GFP/RFP	Transfection	Cas9 nickase + 2 gRNAs	†Dual U6 * Many possible	Custom gRNA	pD14xx-xx NickaseNinja	DNA2.0	https://www.dna20.com/products/crispr?gclid=CLmtw-H9_MMCFUTjcgoddXAAIw
GFP	Transfection	Cas9 nickase + 1 gRNA	†hH1 *EF1	No	EF1-T7-hspCas9-nickase-T2A-GFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
RFP	Transfection	Cas9 nickase + 1 gRNA	†hH1 *EF1	No	EF1-T7-hspCas9-nickase-T2A-RFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
GFP	Transfection	Cas9 nickase + 1 gRNA	†hH1 *CAG (=CBh)	No	CAG-T7-hspCas9-nickase-T2A-GFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
RFP	Transfection	Cas9 nickase + 1 gRNA	†hH1 *CAG (=CBh)	No	CAG-T7-hspCas9-nickase-T2A-RFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
GFP	Transfection	Cas9 nickase + 1 gRNA	†hH1 *CMV	No	CMV-T7-hspCas9-nickase-T2A-GFP-H1-gRNA	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
RFP	Transfection	Cas9 nickase + 1	†hH1 *CMV	No	CMV-T7-hspCas9-nickase-T2A-RFP-	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors

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		gRNA			H1-gRNA		
Cas9 expression vectors							
Fluorescent protein	**Delivery mode**	**Nuclease elements**	**Promoter (Cas9-FP)**	**Cloning method**	**Plasmid name**	**Source/ Laboratory**	**Reference/Link**
EGFP	Lentiviral transduction	Cas9	EFS-NS	N/A	lentiCas9-EGFP	Addgene/ Phil Sharp, Feng Zhang	5/ https://www.addgene.org/63592/
EGFP	Transfection OR Lentiviral transduction	Cas9	CAG (=CBh)	N/A	pCas9_GFP	Addgene/ Kiran Musunuru	6/ https://www.addgene.org/44719/
GFP	Transfection	Cas9 (NB: *Staphylococcus aureus*)	CAG (=CBh)	N/A	pSaCas9_GFP	Addgene/ Kiran Musunuru	https://www.addgene.org/64709/
EGFP	Transfection	Cas9	CBh	N/A	CAS9PBKS	Addgene/ Eric Bennett	This paper/ https://www.addgene.org/68371/
GFP	Transfection	Cas9	CMV	N/A	Cas9-GFP	Sigma-Aldrich	http://www.sigmaaldrich.com/technical-documents/articles/biology/crispr-cas-gfp-vector.html
ZsGreen	Transfection OR Lentiviral transduction	Cas9	EFS	N/A	pCLIP-Cas9-Nuclease-EFS-ZsGreen	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-CRISPR-Cas9-Nuclease-Expression-Vect-(2).aspx
ZsGreen	Transfection OR Lentiviral transduction	Cas9	hCMV	N/A	pCLIP-Cas9-Nuclease-hCMV-ZsGreen	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-CRISPR-Cas9-Nuclease-Expression-Vect-(6).aspx
tRFP	Transfection OR Lentiviral transduction	Cas9	EFS	N/A	pCLIP-Cas9-Nuclease-EFS-tRFP	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-CRISPR-Cas9-Nuclease-Expression-Vect-(3).aspx
tRFP	Transfection OR Lentiviral transduction	Cas9	hCMV	N/A	pCLIP-Cas9-Nuclease-hCMV-tRFP	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-CRISPR-Cas9-Nuclease-Expression-Vect-(7).aspx
copGFP	Transfection OR Lentiviral transduction	hspCas9	CMV or MSCV	N/A	Not available	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
mCherry	Transfection	Cas9	CMV	N/A	CP-C9NU-01	GeneCopoeia	http://www.genecopoeia.com/product/crispr-cas9/
EGFP	Lentiviral transduction	Cas9	CMV	N/A	CP-LvC9NU-02	GeneCopoeia	http://www.genecopoeia.com/product/crispr-cas9/
EGFP	Transfection	Cas9 nickase	CAG (=CBh)	N/A	pCas9D10A_GFP	Addgene/ Kiran Musunuru	6/ http://www.addgene.org/44720/
ZsGreen	Transfection OR Lentiviral transduction	Cas9 nickase	EFS	N/A	pCLIP-Cas9-Nickase-EFS-ZsGreen	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-CRISPR-Cas9-Nickase-Expression-Vecto-(2).aspx
tRFP	Transfection OR Lentiviral	Cas9 nickase	EFS	N/A	pCLIP-Cas9-Nickase-EFS-tRFP	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-CRISPR-Cas9-Nickase-Expression-Vecto-(3).aspx
copGFP	Transfection OR Lentiviral transduction	Cas9 nickase	CMV Or MSCV	N/A	Not available	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
Cas9 nickase	Cas9 nickase	Cas9 nickase	CMV	N/A	CP-C9NI-02	GeneCopoeia	http://www.genecopoeia.com/product/crispr-cas9/

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gRNA expression vectors containing FPs							
Fluorescent protein	**Delivery mode**	**Nuclease elements**	**Promoters: †gRNA *FP**	**gRNA swapping possible (swapping method) OR Custom gRNA**	**Plasmid name**	**Source /Laboratory**	**Reference/Link**
EGFP	Lentiviral, in-vivo mouse targeting	1 gRNA	†hU6 *hSYN1	Yes (SapI)	PX552	Addgene/ Feng Zhang	7/ https://www.addgene.org/60958/
EGFP	Lentiviral transduction	1 gRNA	†hU6 *EFS	Yes (BsmBI)	pLKO5.sgRNA.EFS.GFP	Addgene/ Benjamin Ebert	3/ https://www.addgene.org/57822/
TagRFP	Lentiviral transduction	1 gRNA	†hU6 *EFS	Yes (BsmBI)	pLKO5.sgRNA.EFS.tRFP	Addgene/ Benjamin Ebert	3/ https://www.addgene.org/57823/
EGFP	Transfection	1 gRNA	†hU6 *PGK1	Yes (SacI)	pU6_gRNA_handle_U6t	Addgene/ Timothy Lu	8/ https://www.addgene.org/49016/
TagBFP	Lentiviral transduction	1 gRNA	†hU6 *PGK	Yes (BbsI)	pKLV-U6gRNA(BbsI)-PGKpuro2ABFP	Addgene/ Kosuke Yusa	9/ https://www.addgene.org/50946/
ZsGreen	Transfection OR Lentiviral transduction	1 gRNA	†hU6 *EFS	Yes (BsmBI)	pCLIP-gRNA-EFS-ZsGreen	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-gRNA-Cloning-Vector-(EFS-ZsGreen).aspx
tRFP	Transfection OR Lentiviral transduction	1 gRNA	†hU6 *EFS	Yes (BsmBI)	pCLIP-gRNA-EFS-tRFP	transOMIC	http://www.transomic.com/Catalog-Products/transEDIT-gRNA-Cloning-Vector-(EFS-tRFP).aspx
copGFP	Transfection OR Lentiviral transduction	1 gRNA	†U6 Or H1 *EF1a	No	N/A	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
RFP	Transfection OR Lentiviral transduction	1 gRNA	U6 Or H1 *EF1a	No	N/A	System Biosciences	https://www.systembio.com/crispr-cas9/Vectors
mCherry	Lentiviral transduction	1 gRNA	†hU6	Custom gRNA	pCRISPR-LvSG03	GeneCopoeia	http://www.genecopoeia.com/product/crispr-cas9/
ZFNs							
Fluorescent protein	**Delivery mode**	**Nuclease elements**	**Promoter (FP-ZFN)**	**Cloning method**	**Plasmid name**	**Source/ Laboratory**	**Reference/Link**
GFP	Transfection	ZFN-L (FokI-ELD)	CMV	Custom ZFN	pZFN1-GFP	Sigma-Aldrich	http://www.sigmaaldrich.com/technical-documents/articles/biology/fluorescent-protein-linked-zinc-finger-nucleases.html
RFP	Transfection	ZFN-R (FokI-KKR)	CMV	Custom ZFN	pZFN2-RFP	Sigma-Aldrich	http://www.sigmaaldrich.com/technical-documents/articles/biology/fluorescent-protein-linked-zinc-finger-nucleases.html
GFP	Lentiviral transduction	ZFN-L (FokI-ELD)	CMV	Custom ZFN	GFP-ZFNL-IDLV	Sigma-Aldrich	http://www.sigmaaldrich.com/life-science/zinc-finger-nuclease-technology/integrase-deficient-lentivirus.html
RFP	Lentiviral transduction	ZFN-R (FokI-KKR)	CMV	Custom ZFN	RFP-ZFNR-IDLV	Sigma-Aldrich	http://www.sigmaaldrich.com/life-science/zinc-finger-nuclease-technology/integrase-deficient-lentivirus.html
GFP	Transfection	ZFN-L (FokI-	CMV	Custom ZFN	GFP-2A-ZFNL-2A-	Sigma-Aldrich	http://www.sigmaaldrich.c

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		ELD) + ZFN-R (FokI-KKR) "all-in-one"			ZFNR		om/technical-documents/articles/biology/fluorescent-protein-linked-zinc-finger-nucleases.html
TALENs							
Fluorescent protein	**Delivery**	**Nuclease elements**	**Promoter**	**Cloning method**	**Plasmid name**	**Source/ Laboratory**	**Reference/Link**
EGFP	Transfection	TALEN-L	CMV SV40	Golden Gate	pcDNA3.1(−)-EGFP	Xin Huang	10
DsRed	Transfection	TALEN-R	CMV SV40	Golden Gate	pcDNA3.1(−)-DsRed	Xin Huang	10
EGFP	Transfection	TALEN-L (FokI-ELD)	CAG (=CBh)	BsmBI	pTAL_GFP	Addgene/ Kiran Musunuru, Chad Cowan	11/ http://www.addgene.org/TALEN/Musunuru/
RFP	Transfection	TALEN-R (FokI-KKR)	CAG (=CBh)	BsmBI	pTAL_RFP	Addgene/ Kiran Musunuru, Chad Cowan	11/ http://www.addgene.org/TALEN/Musunuru/
Donor vectors							
RFP	Transfection	Donor for HDR only	EF1a (RFP)		HDR Plasmid	Santa Cruz Biotechnology	http://www.scbt.com/crispr-cas9_system.html

BFP, blue fluorescent protein; CAG, CMV early enhancer/chicken β-actin; CBh, chicken β-actin hybrid; CMV, cytomegalovirus; MSCV, murine stem cell virus; EBFP2, enhanced blue fluorescent protein 2; EFS, elongation factor-1 short; EGFP, enhanced green fluorescent protein; RFP, red fluorescent protein; GFP, green fluorescent protein; hUbC, human ubiquitin C; N/A, not applicable; OFP, orange fluorescent protein; PGK1, phosphoglycerate kinase-1; SFFV, spleen focus-forming virus; SYN1, synapsin-1; tRFP, turbo red fluorescent protein.

Unless stated otherwise, Cas9 protein is from *Streptococcus pyogenes*. When known, the species of the U6 and H1 promoters is indicated: h=Homo sapiens, m=Mus musculus.

Supplementary Table 2 | IDAA primers

Primer name	Sequence (5' to 3')
***GALNT10*-Fwd**	*AGCTGACCGGCAGCAAAATTG*GCTTGCTCCCCTCCTACTCT
***GALN10*-Rev**	ACAACAGCCAGGGAAACATC
***KRAS*-Fwd**	*AGCTGACCGGCAGCAAAATTG*AAAAGGTACTGGTGGAGTATTTGA
***KRAS*-Rev**	TCATGAAAATGGTCAGAGAAACC
***B4GALT4*-Fwd**	*AGCTGACCGGCAGCAAAATTG*TCGCCCTCAGGAATGTAAAG
***B4GALT4*-Rev**	TTTCCCAGAACTTGAACCCA
***B4GALT3*-Fwd**	*AGCTGACCGGCAGCAAAATTG*CATAGTCTGGTTCCCCTCCA
***B4GALT3*-Rev**	CGAGTCTTCTGGGGACACAT
***GALNT3*-Fwd**	*AGCTGACCGGCAGCAAAATTG*TCCCTCCAGGTGAGTGTTTC
***GALNT3*-Rev**	AAAGCAAACAGTGTGTACATATTCAA
***Trp53*-Fwd**	*AGCTGACCGGCAGCAAAATTG*GCCCAGCTTTCTTACTGCCT
***Trp53*-Rev**	CATGCGAGAGACAGAGGCAA
***VEGFA*-Fwd**	*AGCTGACCGGCAGCAAAATTG*GTCGAGGAAGAGAGAGACGG
***VEGFA*-Rev**	CGAGAACAGCCCAGAAGTTG
***AAVS1*-Fwd**	*AGCTGACCGGCAGCAAAATTG*CCTTACCTCTCTAGTCTGTGCTAG
***AAVS1*-Rev**	CGTAAGCAAACCTTAGAGGTTCTGG
***COSMC*-Fwd**	*AGCTGACCGGCAGCAAAATTG*AGGGAGGGATGATTTGGAAG
***COSMC*-Rev**	TTGTCAGAACCATTTGGAGGT
***POMGnT1*-Fwd**	*AGCTGACCGGCAGCAAAATTG*TAGTTCGTGCTCTGTGAGGC
***POMGnT1*-Rev**	AATAGGAGCCAGTGGCAGTG

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***POMT1*-Fwd**	*AGCTGACCGGCAGCAAAATTG*TTGGTTTCCTGTGTTCACCTC
***POMT1*-Rev**	CCGGCATCAAATGTAGGTCT
***POMT2*-Fwd**	*AGCTGACCGGCAGCAAAATTG*CCTGGCAGAGTCCGAGCT
***POMT2*-Rev**	GACAGCAGCGTCACCAAG
***Bambi*-Fwd**	*AGCTGACCGGCAGCAAAATTG*TTCGCGATCGGGGATAGTTG
***Bambi*-Rev**	CGATGGCTGTTCTTCTCACG

The common extension of the IDAA-Fwd primers is indicated in italics. For species of the targeted genes, see Supplementary Table 3.

Supplementary Table 3 | Nuclease target sites

Target gene (nuclease)	Target sequence (5' to 3')
GALNT10* **(gRNA)**	ACTCTCTCAGCATCGGTCAT
KRAS* **(gRNA)**	TAGTTGGAGCTGGTGGCGT
***B4GALT4*¤ (ZFN)**	GGCATCTACGTCATCcaccaGGTGAGCGTGGGGGCAGAC
GALNT3* **(QCgRNA #1)**	CGTGTAGTTCTCAGCTATTC
GALNT3* **(QCgRNA #2)**	AGATCTATGGATGCAATATC
GALNT3* **(QCgRNA #3)**	TATGGAAGTAACCATAACCG
GALNT3* **(QCgRNA #4)**	ACTGGAGTCTTTCATTTGGC
***Trp53*§ (gRNA)**	TGTACGGCGGTCTCTCCCA
VEGFA* **(gRNA)**	GACCCCCTCCACCCCGCCTC
AAVS1* **(ZFN)**	ACCCCACAGTGGggccacTAGGGACAGGAT
***COSMC*¤ (TALEN)**	TGACTTATCACCCCAACCAGGTAgtagaaggctgttGTTCAGATATGGCTGTTACTTTTA
POMGnT1* **(gRNA)**	GAGGGACACATGGGCCTTCG
POMT1* **(gRNA)**	ACCAGATAGTGTTGGAGCTC
POMT2* **(gRNA)**	CTTCGAGGCGGTCGGCTGGT
***Bambi* † (gRNA)**	GGTTTCTCTGTGGTTTCAGC

*Homo sapiens; §Mus musculus; ¤Cricetulus griseus; †Danio rerio. For TALEN and ZFN target sites, the nuclease-binding sequence is in uppercase.

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Supplementary Note | Peak Scanner™ 2 Software and GeneMarker® (Demo) Software.

Peak Scanner™ 2 Software. Thermo Fisher Scientific´s free software for fragment analysis performs all the tasks needed for IDAA and is very easy to use (see **Supplementary Manual**). Peak Scanner works with data files from 310, 3100, 3130 and 3730, but not 3500 instruments. To obtain Peak Scanner™ 2, register at http://resource.thermofisher.com/pages/WE28396/, whereafter the software can be downloaded. Installation formally requires a computer with 32-bit operating system, a processor of at least 2.3 GHz and Windows 7.0. Peak Scanner does, however, work on some 64-bit computers. If not, download a "virtual machine application" such as VirtualBox from Oracle for PC or Mac (https://www.virtualbox.org/) or Parallels Desktop for Mac (http://www.parallels.com; licence available through IT in many institutes). A virtual machine is a software computer that allows users to run additional operating systems, such as the 32-bit version of Windows 7.0, and thereby applications for that operating system, such as Peak Scanner, on their desktops. After installing a virtual machine, request IT at your institute to install Windows 7.0 32-bit on your computer or purchase Windows 7.0 and select the 32-bit operating system during installation, then download and install Peak Scanner™ 2 as described above.

GeneMarker® (Demo) Software. A free trial version (access limited to 70 days) of Softgenetics´ commercial software for fragment analysis. It is very easy to use, can be downloaded smoothly on most computers and uses data files from most fragment analyzers. The generated IDAA profiles, however, cannot be printed or saved. To obtain GeneMarker® (Demo), register at http://www.softgenetics.com/gm_demo_form.php, where after the software can be downloaded.

Supplementary Data | Sequences of pEPB104 and CAS9PBKS

pEPB104, Addgene #68369

Plasmid containing the U6 promoter, but no sgRNA or Cas9 elements. Can be used as template for the QCgRNA PCR as well as cloning vector for the resultant QCgRNA amplicon expression cassette. The sequence of the U6 promoter (sequence ID gb|M14486|HUMUG6) and flanking EcoRI restriction endonuclease sites was synthesized by GeneArt (Thermo Fisher Scientific) and inserted into the EcoRV site of pMA-T, which is flanked 3´ by a KpnI site. This allows excision of the U6 promoter by EcoRI/KpnI digestion and insertion of a QCgRNA amplicon expression cassette after its digestion with same enzymes. Below is shown the sequence of the U6 promoter in upper case and the flanking restriction endonuclease sites in lower case letters.

gatatcgaattcGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAA
TTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCT
TGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATT
TCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGgaattcgatatcggtacc

CAS9PBKS, Addgene #68371

Plasmid expressing Cas9-2A-EGFP under control of the CBh promotor. The construct was generated by excising the CBh-Cas9-2A-EGFP-bGH_PA_terminator sequence from PX458[2] using KpnI/NotI restriction

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endonucleases and insertion into the multiple cloning site of pBluescript KS (Stratagene) digested with same enzymes.

ATGGACTATAAGGACCACGACGGAGACTACAAGGATCATGATATTGATTACAAAGACGATGACGATAAGATGGCCCCAAAGAAGAA
GCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGGGC
CGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGA
TCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAG
AACCGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCC
TGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCA
TCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGGCCCACATGATCAAGT
TCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACA
ACCAGCTGTTCGAGGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGC
TGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCA
ACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGG
CCCAGATCGGCGACCAGTACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAA
CACCGAGATCACCAAGGCCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCT
CGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGC
CAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGA
GGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCG
GCAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCT
CTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGA
CAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAG
CCTGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGAG
CGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCA
AGAAAATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAA
AATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGA
CAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACA
CCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCG
ACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCG
GCCAGGGCGATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGTGAAGGTGG
TGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAG
GGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGT
GGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAACTGGACAT
CAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCAGA
AGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAA
CGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCAT
CAAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGTACGACGAGA
ATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGT
GCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCT
GGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTA
CCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTCT
GATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCC
AAGTGAATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTG
ATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAA
GTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAA
TCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCGAGCTG
GAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACTTC
CTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCAC
TACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCT
ACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCG
CCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCACCAGAGCA
TCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGGGAGGCGACAAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCA
AAAAAGAAAAAGGAATTCGGCAGTGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAGAATCCTGGCCCAGTGAG
CAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGG
CGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCT
CGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCC
GAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACC
CTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGC
CACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTG
CAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCC
GCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGAC
GAGCTGTACAAGGAATTCTAA

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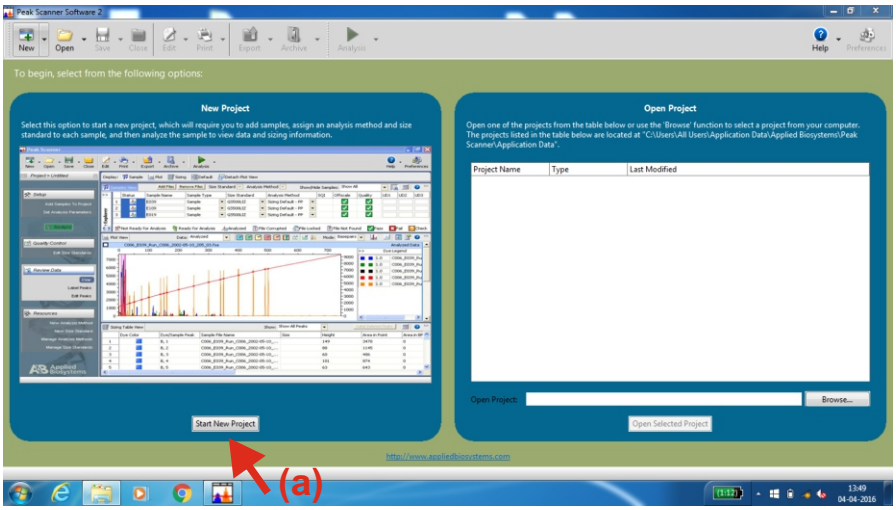
Peak Scanner™ 2 Software step-by-step guide (page 1/3)

This quick-guide shows the few, essential steps needed from import of data files from ABI Genetic Analyzers 310, 3100, 3130 or 3730 instruments to determination of amplicon abundance and size. Peak Scanner offers several additional analysis tools than shown here, but these are not essential for determining the indel pattern of an edited sample.

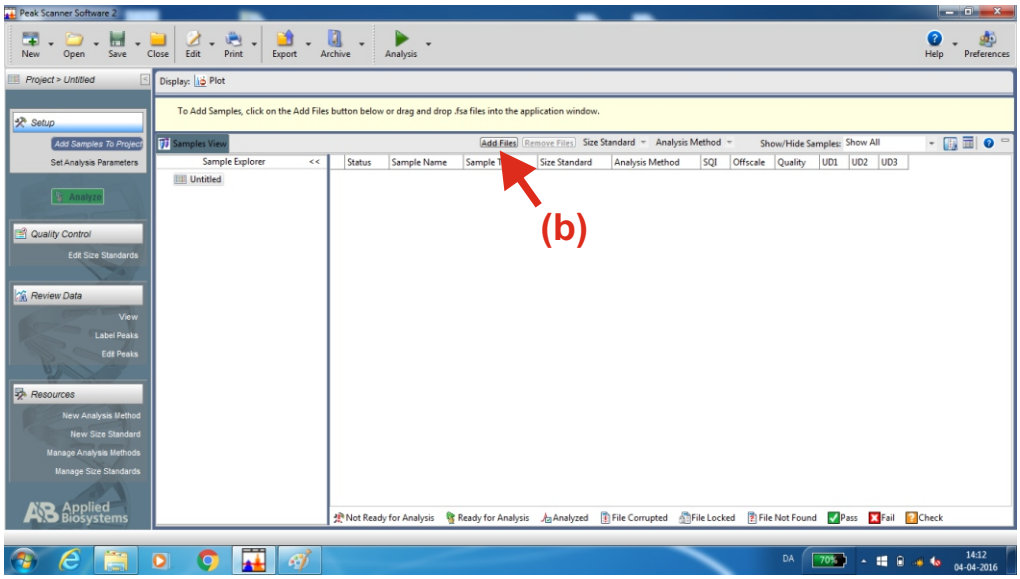
1. Open Peak Scanner™ 2.

Upon opening Peak Scanner, this screen image will appear. Click "Start New Project" button (a).



2. Import sample files.

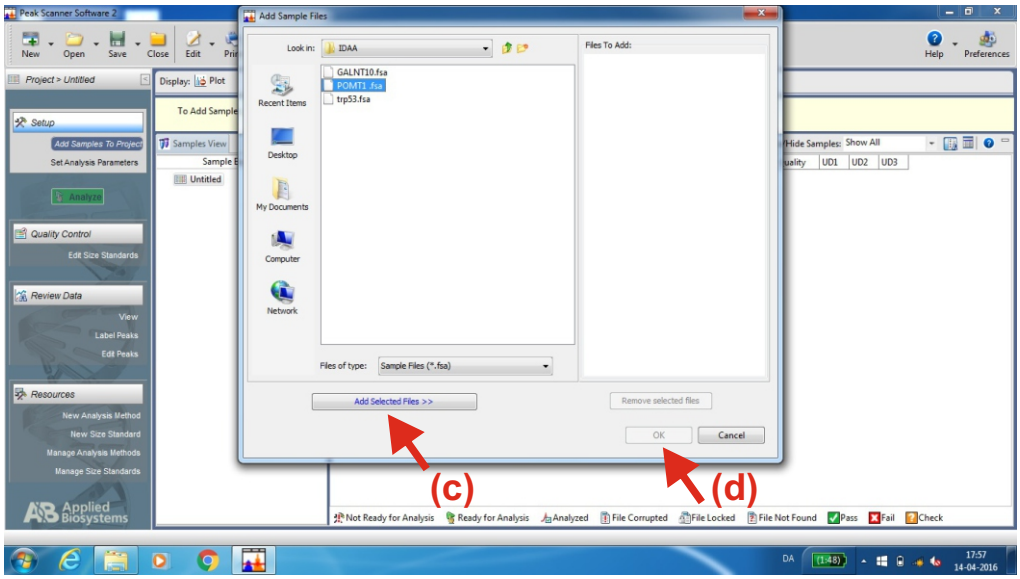
Click the "Add Files" button (b) in the window that has opened.



In the next window that opens, go to the folder where you have your ".fsa" data files from the ABI Genetic Analyzer. Click on the file to be analyzed (if more than one, press the shift key and click on several .fsa files). The clicked files will be highlighted like the "POMT1.fsa" file in this example.

Then, click "Add Selected Files>>" button (c) and finally the "OK" button (d).

(Alternatively, the .fsa files may be dragged-and-dropped into the window)



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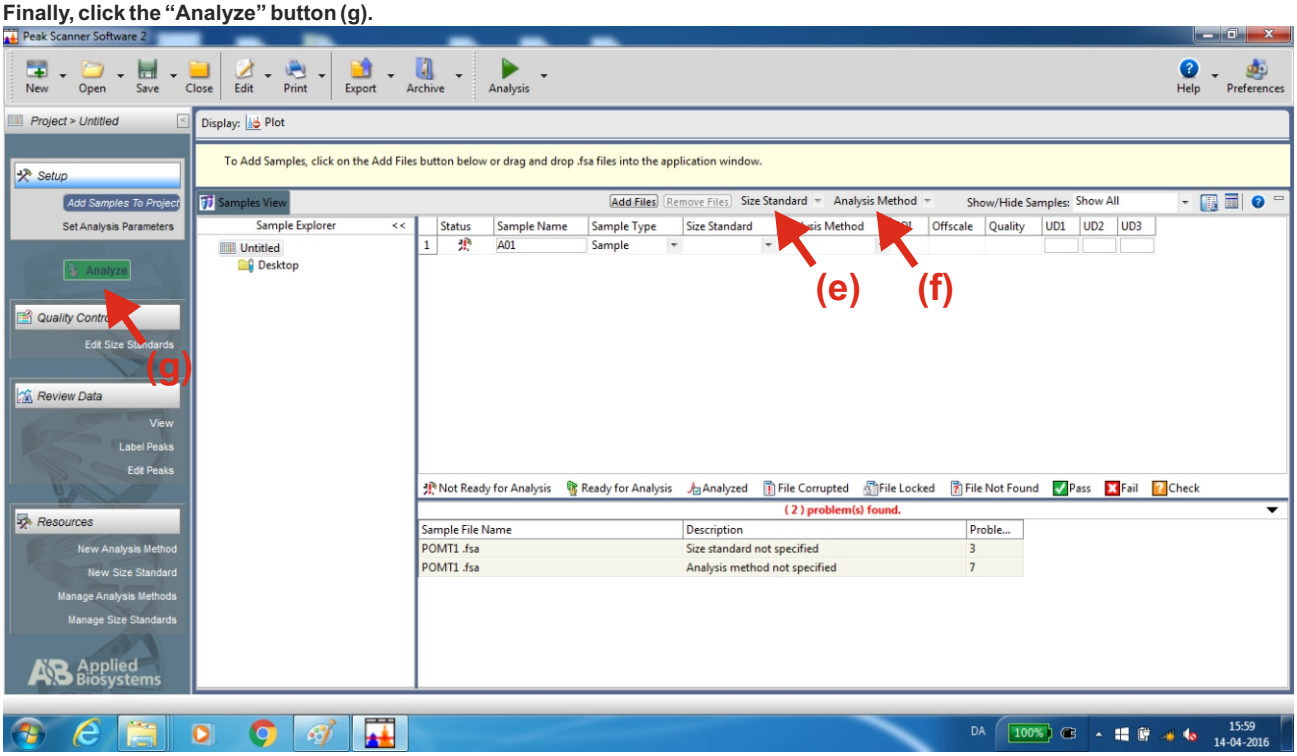
Peak Scanner™ 2 step-by-step guide (page 2/3)

3. Select size standard and analysis method.

First, click the "Size Standard" button (e), and from the pull-down menu click the size standard used (for IDAA, typically GS500LIZ).

Next, click the "Analysis Method" button (f), and select "Sizing Default - PP" if the IDAA primers were present in the analyzed samples (the typical scenario, since IDAA capillary electrophoresis is normally performed on crude PCRs). Otherwise, select "Sizing Default - NPP" if IDAA amplicons were purified prior to capillary electrophoresis.

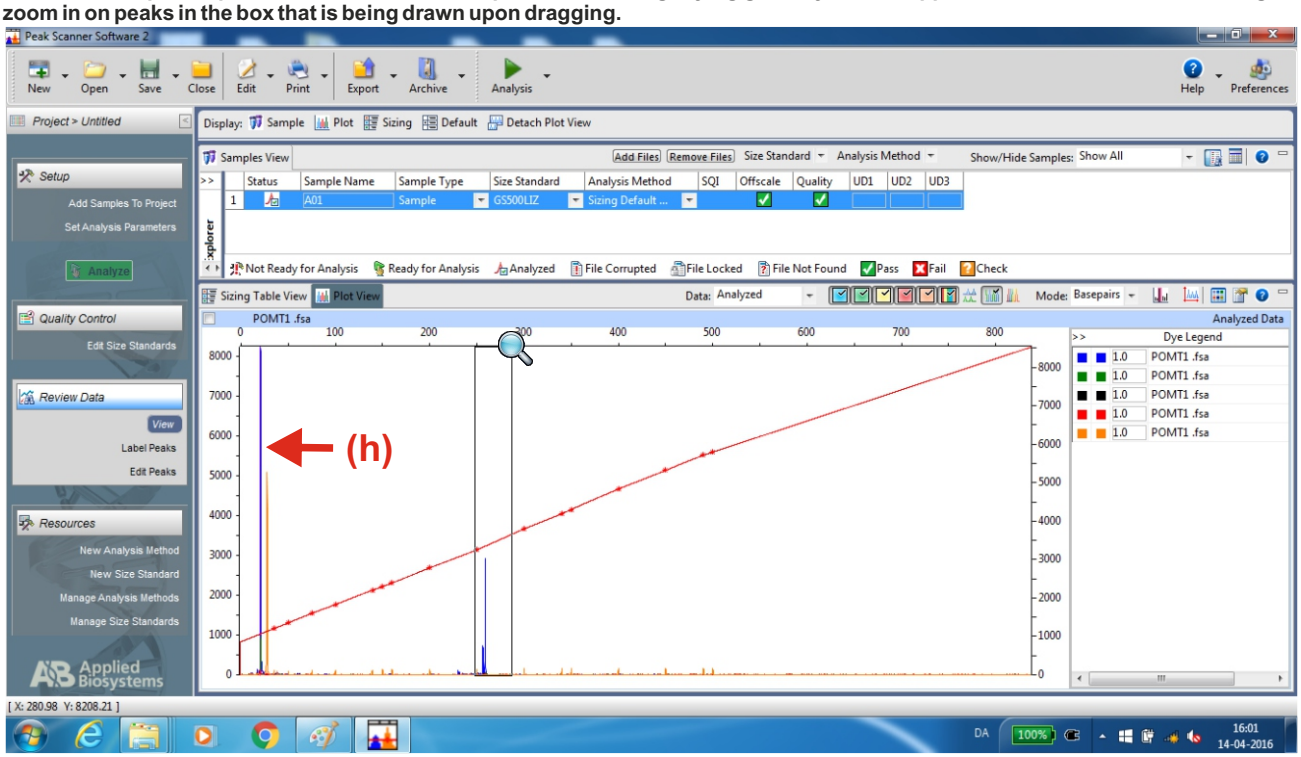
Finally, click the "Analyze" button (g).



4. Zoom in on the amplicon peaks.

The window that now opens shows the IDAA profile as a blue trace with peaks for all the different amplicons present in the sample as well as a FamFwd primer peak around 20 nt (h). Size standard peaks are orange.

To zoom in on peaks, place the cursor over the top x-axis for a magnifying glass symbol to appear as the cursor. Click and drag to zoom in on peaks in the box that is being drawn upon dragging.

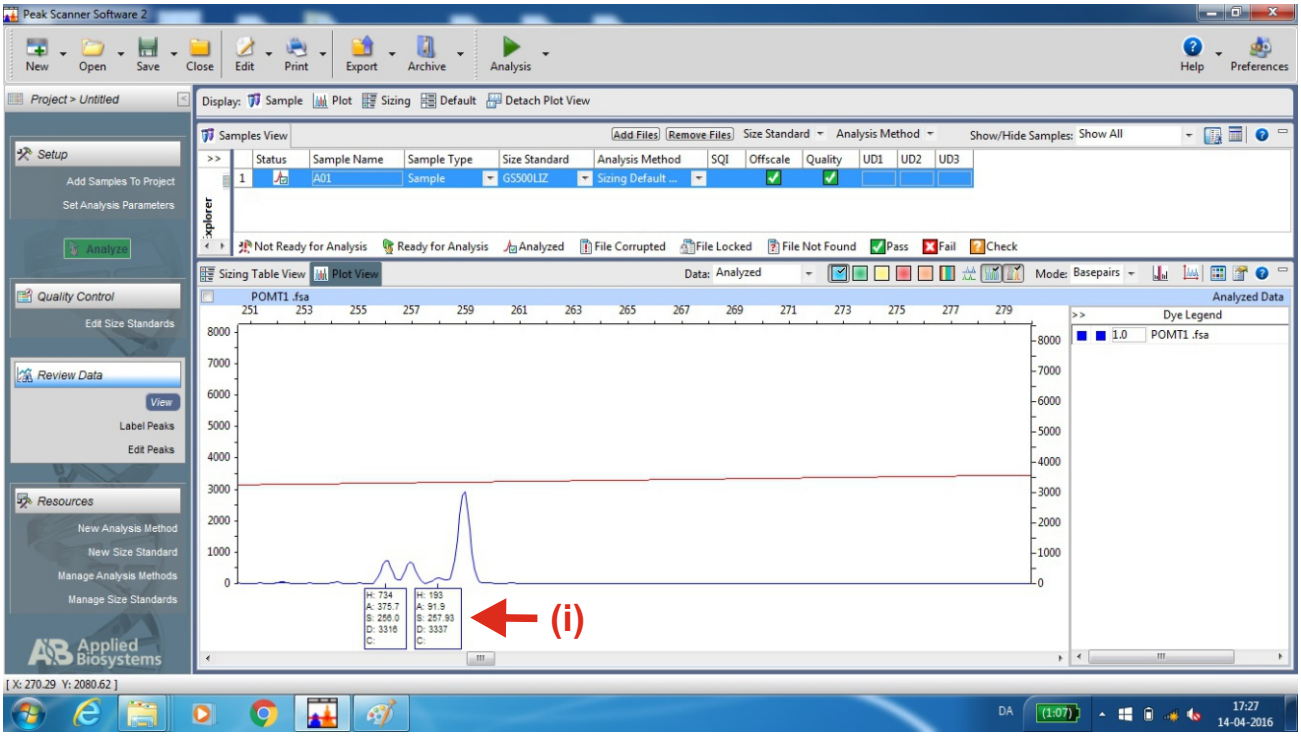


Peak Scanner™ 2 step-by-step guide (page 3/3)

5. Analyze high-abundance peaks.

Click on a peak and in the box that opens (i), view peak height (H), peak area (A) and amplicon size (S). In below example, two peaks were clicked.

To zoom in on low-abundance peaks, place the cursor on the y-axis for a magnifying glass to appear. Click and box to zoom in.



6. Analyze low-abundance peaks.

Click on peaks to analyze as described in step 5. The determined sizes of the high-lighted amplicons are 252.12 (i.e. 252), 256.0 and 257.93 (i.e. 258) bp. Thus, amplicon sizes are determined with small deviations from the actual size.