ddPCR™ Genome Edit Detection Assays

ddPCR Genome Detection Assays offer a fast, precise, simple, and cost-effective method for detection of genome editing events. Assays can be obtained for any target using Bio-Rad's easy-to-use, intuitive Digital Assay Site.

ddPCR Genome Edit Detection Assays:

- Enable rapid assessment of HDR and NHEJ edits generated by CRISPR-Cas9 or other genome editing tools
- Detect editing events present at frequencies of ≤0.5%
- Provide absolute quantification of genome editing events from as little as 5 ng of total gDNA (cells from single well of a 96 well plate)
- Distinguish between homozygous and heterozygous edits in clonal populations
- Results are easily analyzed using QuantaSoft™ Analysis Pro Software

Visit **bio-rad.com/web/GenomeEditAssays** for more information.

BIO-RAD

Genome Editing

Genome editing with site-specific, programmable nucleases has revolutionized the ability to edit the genome of any cell. Nucleases, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)–associated Cas nucleases, produce site-specific DNA double-strand breaks (DSBs). DSBs can be repaired by imprecise, error-prone non-homologous end joining (NHEJ) or by homology directed repair (HDR) pathways, leading to targeted mutagenesis (Figure 1).

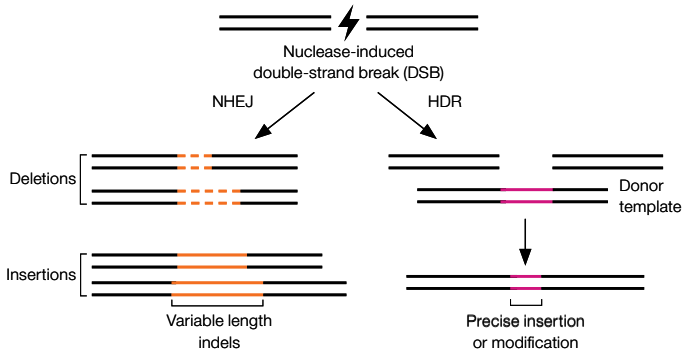

Fig. 1. Targeted genome editing. DSBs introduced by nucleases can be repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ produces insertion and/or deletions (indels) of variable length at the site of the DSB. HDR can introduce precise point mutations or insertions using a DNA donor template.

Creation of Recombinant Cell Lines using CRISPR-Cas9

Introducing CRISPR-Cas9 and guide RNA (gRNA) into cells (with or without a donor template) results in DSBs, which the cellular machinery repairs by NHEJ or HDR. This results in a mixed population of cells with heterogeneous indel errors and varying allelic editing frequencies (Figure 2A). Transfected cells are harvested and analyzed 24–72 hours post-transfection to measure genome editing frequency at the desired locus. Clonal cell lines can then be generated using serial dilutions to isolate single cells followed by an expansion period. Each clonal cell line is then assayed to verify the genome editing event (Figure 2B).

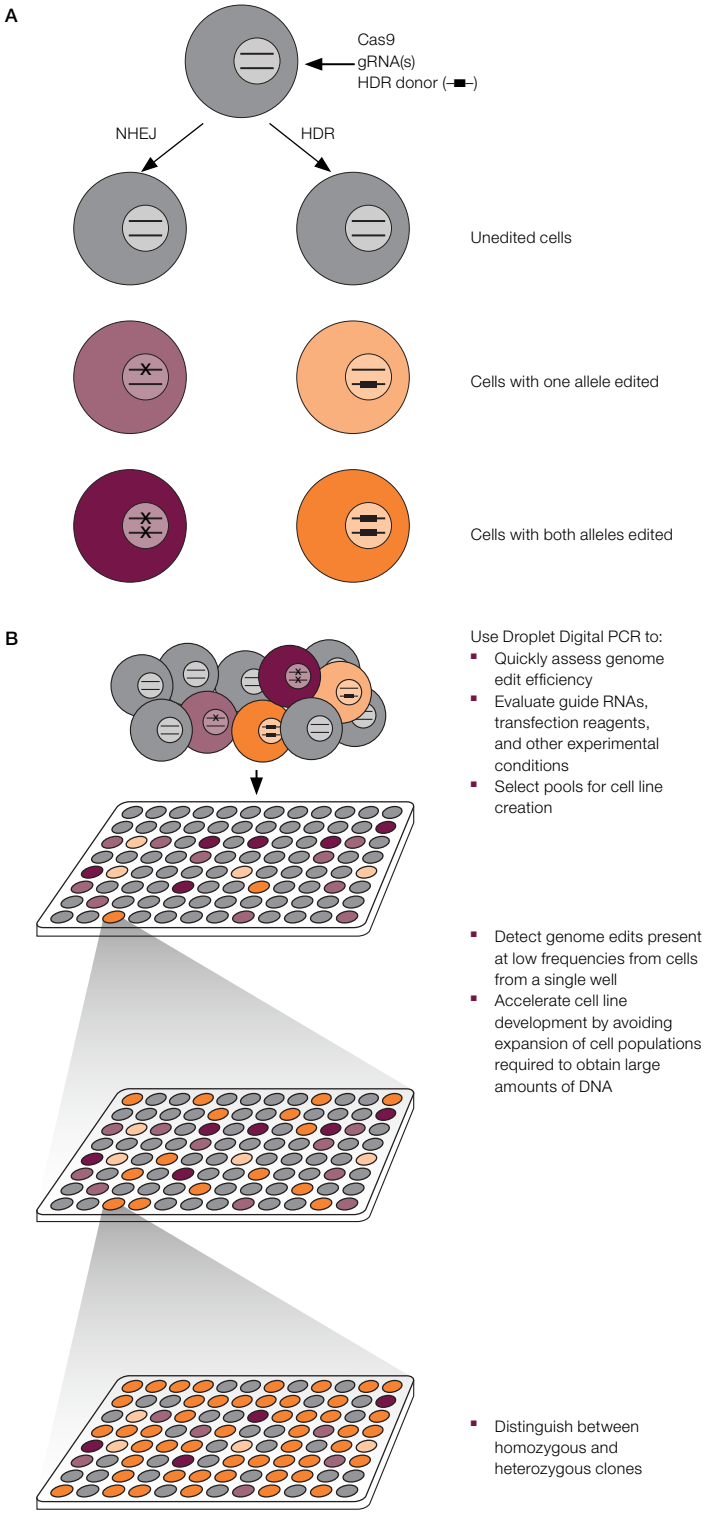

Fig. 2. Creation of clonal cell lines containing desired genome edits. **A**, CRISPR-mediated genome editing can result in unedited cells, cells with one allele edited, and cells with both alleles edited. **B**, To isolate cells with both alleles altered by HDR, a mixed pool of cells is serially diluted. Subpopulations are screened using Droplet Digital PCR (ddPCR), wells with cells of interest are selected and further subdivided until a clonal population containing the desired edit is obtained.

Droplet Digital PCR (ddPCR) for Ultra-Sensitive Quantification of Genome Editing Events

The efficiency and mutation spectrum generated by genome editing varies substantially depending on the type of cells used, transfection method, target sequence, and many other factors. Therefore a simple, quick method is required to accurately characterize and quantify genome-editing events.

The T7 Endonuclease 1 or Surveyor assays are commonly used for detecting genome editing events. However, these methods are semi-quantitative, offer limited sensitivity, are prone to false-positives, and suffer from high background signal when sequence polymorphisms are present. These assays require a large amount of starting material necessitating significant cellular expansion. Further, these methods have limited utility for screening of single-cell derived clones. For a typical diploid target locus, a clone with both alleles successfully altered via genome editing, will be indistinguishable from a clone with one mutated allele and one wild-type allele.

Next generation sequencing is a comprehensive method for assessing genome editing but the turn around time is several weeks, analysis is complex, and costs are high. This makes this method unsuitable for routine screening.

Droplet Digital PCR offers a superior ultrasensitive, rapid, and low-cost method for absolute quantification of genome editing events. ddPCR allows absolute quantification from very small amounts of starting material. Clones harboring homozygous and heterozygous edits can be distinguished and ddPCR Genome Edit Detection Assays can be easily designed for any target of interest. This enables a much more rapid and efficient workflow and provides the ability to quickly identify and focus on desired clones.

ddPCR Genome Edit Detection Assays

Genome edit detection assays, optimized specifically for ddPCR, can be ordered instantly for any target of interest on Bio-Rad's easy-to-use Digital Assay Site (Figure 3). Bio-Rad's assay design algorithms take into consideration everything from primer/probe placement and melting temperature to GC content and even genome specificity to design ddPCR Genome Edit Detection Assays with superior sensitivity, specificity, and accuracy, that accelerate your time to results.

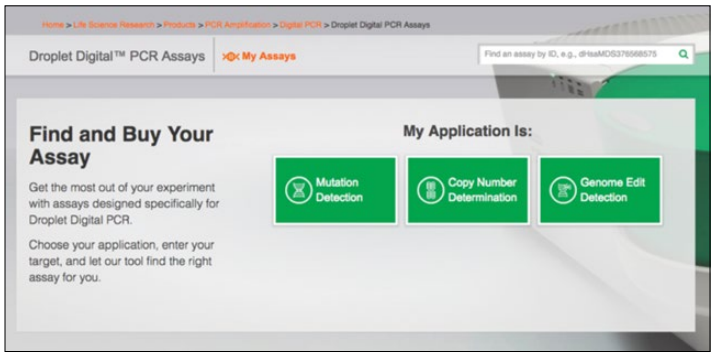

Fig. 3. Order ddPCR Genome Edit Detection Assays for any target on the Digital Assay Site. Easy, step by step workflow allows design of NHEJ and HDR Genome Edit Detection assays in minutes.

Detection of HDR Edits using ddPCR HDR Genome Edit Detection Assays

ddPCR Homology Directed Repair Genome Edit Detection (HDR GED) Assays use a modified mutation detection assay strategy. The ddPCR HDR GED Assay contains primers, a FAM probe that is designed to bind the HDR edit, and a non-fluorescent probe that prevents binding of the FAM probe to unedited alleles. ddPCR HDR GED Assays must be run duplexed with a reference assay containing a HEX probe. Thus, two amplicons are generated (Figure 4). FAM positive droplets represent the population of alleles with the desired HDR edit and the HEX positive droplets represent the total number of alleles. Ratio of FAM positive droplets to HEX positive droplets provides the fractional abundance of HDR edited alleles (Figure 5).

ddPCR HDR Genome Edit Detection Assays:

- Can be designed to detect single base pair substitutions, insertions, and deletions up to 25 bp
- Can be designed for donor homology arms ≤450 bp
- Are available with amplicon length-matched reference assays for human, mouse, and rat
- Quantify precise edits with sensitivity ≤0.5%
- Are quantifiable using QuantaSoft Analysis Pro Software

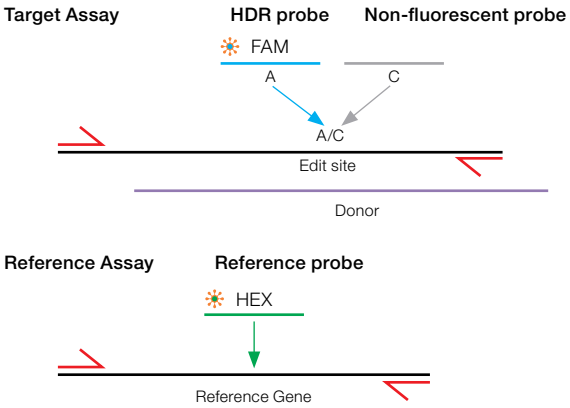

Fig. 4. HDR assay design.

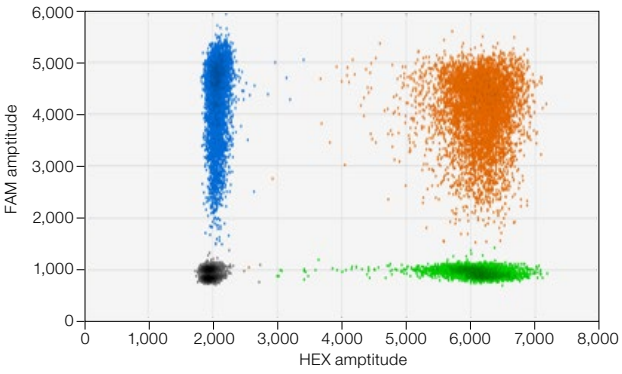

Fig. 5. HDR assay readout. HDR mutation positive control (WT + 20% gblocks gene fragment containing a single base pair substitution) with HDR GED + HDR REF Assay. The HDR edits are FAM+HEX-, while the HEX+ and FAM+HEX+ positive clusters represent the total number of copies, including WT, NHEJ, and HDR edits.

Detection of NHEJ Edits Using ddPCR NHEJ Genome Edit Detection Assays

NHEJ assays are duplexed primer probe-based ddPCR assays (Figure 6). A FAM-labeled probe is designed to bind a reference sequence distant from the nuclease target site but still within the amplicon. A second HEX NHEJ/drop-off probe binds WT sequence at the nuclease target site, such that NHEJ-induced indels block the probe from binding. In a 2-dimensional view of the ddPCR analysis, droplets with signal from both the FAM and HEX probe contain wild-type amplicons while droplets that are FAM-positive but HEX-negative contain amplicons with mutations at the target site (Figure 7). The ratio of FAM positive to FAM/HEX double positive droplets provides the fractional abundance of NHEJ mutations.

ddPCR Genome Edit Assays for NHEJ:

- Detect indels up to 40 bp in length symmetrically on either side of the cut-site, or 80 bp asymmetrically
- Quantify precise edits with sensitivity ≤0.5%
- Are quantifiable using the “drop-off assay” analysis option in QuantaSoft Analysis Pro

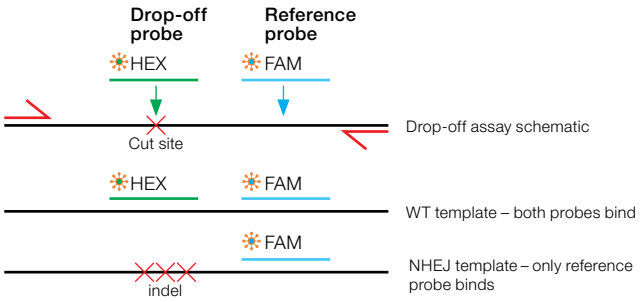

Fig. 6. NHEJ assay design.

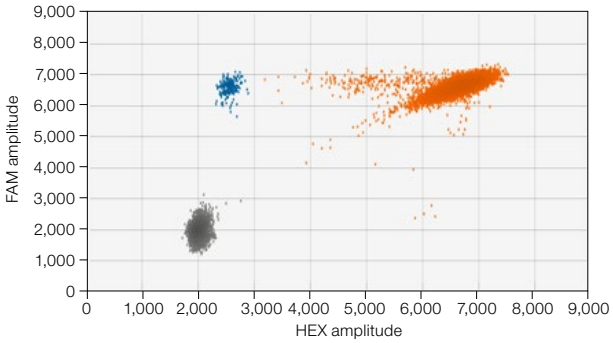

Fig. 7. NHEJ assay readout. NHEJ mutation-positive control (WT + 1% gblocks gene fragment containing a one base pair deletion at the predicted cut site). The WT cluster is positive for both FAM and HEX, while the NHEJ mutant single-positive droplets are positive for FAM only.

Ordering Information

Catalog #	Description
12002312	**ddPCR HDR Gene Edit Assay**, 100 rxns
12002313	**ddPCR HDR Gene Edit Assay**, 500 rxns
12003796	**ddPCR HDR Gene Edit Pkg***, 1,000 rxns
12003805	**ddPCR HDR Ref Assay**, predesigned, 100 rxns
12003806	**ddPCR HDR Ref Assay**, predesigned, 500 rxns
12003793	**ddPCR HDR Ref Pkg**, predesigned, 1,000 rxns
12002314	**ddPCR NHEJ Gene Edit Assay**, 100 rxns
12002315	**ddPCR NHEJ Gene Edit Assay**, 500 rxns
12003794	**ddPCR NHEJ Gene Edit Pkg**, 1,000 rxns

* Sequences of primers and probes of genome edit detection assays are provided

Available HDR Reference Assays

Use Unique Assay ID to find desired reference assay at **bio-rad.com/web/GenomeEditAssays**

Unique Assay ID	Gene Name
dHsaRFS19383242	*Rpp30*, human
dHsaRFS83653015	*Rpp30*, human
dHsaRFS57922788	*Rpp30*, human
dHsaRFS32192561	*Rpp30*, human
dHsaRFS96462334	*Rpp30*, human
dHsaRFS70732107	*Rpp30*, human
dHsaRFS45001880	*EIF2C1*, human
dHsaRFS19271653	*EIF2C1*, human
dHsaRFS83541426	*EIF2C1*, human
dHsaRFS57811199	*EIF2C1*, human
dHsaRFS32080972	*EIF2C1*, human
dHsaRFS96350745	*EIF2C1*, human
dRnoRFS70620518	*Rpp30*, rat
dRnoRFS44890291	*Rpp30*, rat
dRnoRFS19160064	*Rpp30*, rat
dRnoRFS83429837	*Rpp30*, rat
dRnoRFS57699610	*Rpp30*, rat
dRnoRFS31969383	*Rpp30*, rat
dRnoRFS96239156	*ApoB*, rat
dRnoRFS70508929	*ApoB*, rat
dRnoRFS44778702	*ApoB*, rat
dRnoRFS19048475	*ApoB*, rat
dRnoRFS83318248	*ApoB*, rat
dRnoRFS57588021	*ApoB*, rat
dMmuRFS31857794	*Rpp30*, mouse
dMmuRFS96127567	*Rpp30*, mouse
dMmuRFS70397340	*Rpp30*, mouse
dMmuRFS44667113	*Rpp30*, mouse
dMmuRFS18936886	*Rpp30*, mouse
dMmuRFS83206659	*Rpp30*, mouse
dMmuRFS57476432	*ApoB*, mouse
dMmuRFS31746205	*ApoB*, mouse
dMmuRFS96015978	*ApoB*, mouse
dMmuRFS70285751	*ApoB*, mouse
dMmuRFS44555524	*ApoB*, mouse
dMmuRFS18825297	*ApoB*, mouse

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