Using CRISPR/Cas9 to generate isogenic cell lines and reference standards for applications in cancer diagnostics

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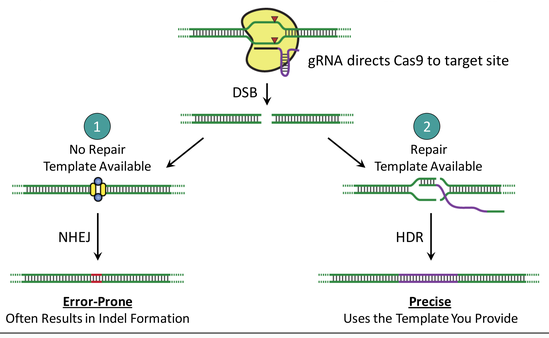
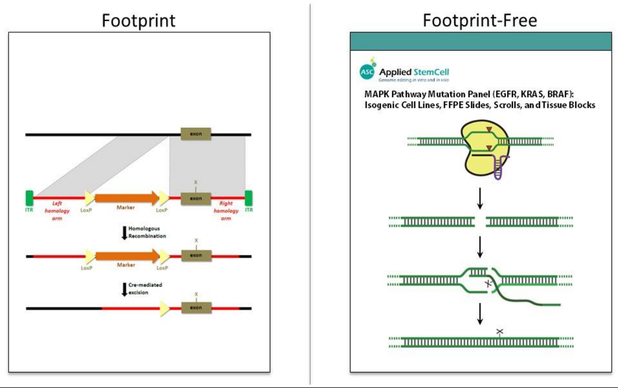
Introduction

- In the era of personalized medicine, precision diagnostics and molecular profiling significantly influence clinical decisions in therapeutic treatment, especially in cancer related treatment.
- Cell line-based reference standards provide a renewable, reproducible, and therefore reliable source of control or reference materials.
- We engineered a series of isogenic cell lines featuring 51 recurrent mutations in the EGFR, KRAS and BRAF genes in the MAPK signaling pathway based on data from the COSMIC database.
- CRISPR/Cas9 was used to engineer precise insertions, deletions, and point mutations in HCT116 and RKO cells.
  - Footprint-free cell line engineering
  - Homozygous mutations
  - Isogenically paired mutant and wild-type cells
- The isogenic cell lines were then used to derive multiple formats of reference materials, including genomic DNA, FFPE slides, and scrolls
- These reference materials are ideal as paired positive and negative controls for multiple applications, such as:
  - Next generation sequencing (NGS)
  - Quantitative PCR (qPCR)
  - *In situ* hybridization (ISH)

Cas9-Mediated Genome Engineering



Cas9 for Footprint-Free Genome Engineering



Engineering Clinically Relevant Mutations

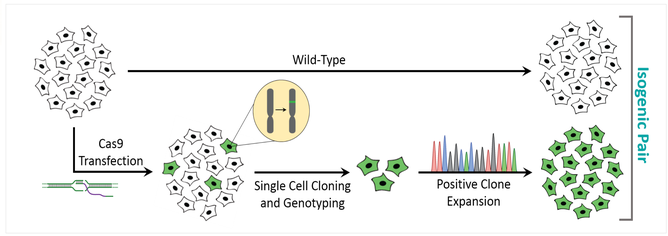
Control Every Aspect Of Your Workflow

ONCOREF™

Homozygous mutations

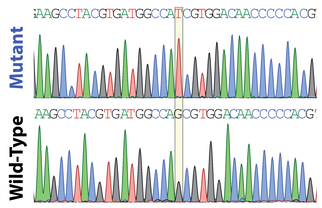
Footprint-free

Paired Isogenic Lines



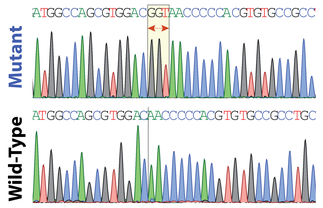
Example 1: Point Mutation

EGFR c.2303G>T p.S768I



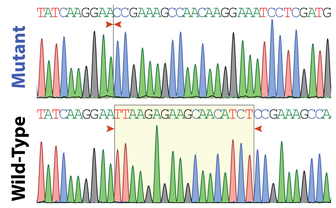
Example 2: Small Insertion

EGFR c.2310_2311insGGT p.D770_N771insG



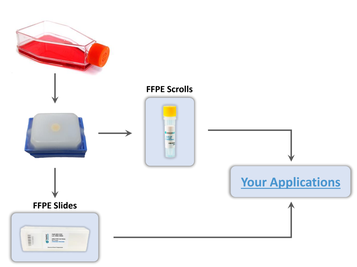
Example 3: Deletion

EGFR c.2239_2256del18 p.L747_S752del

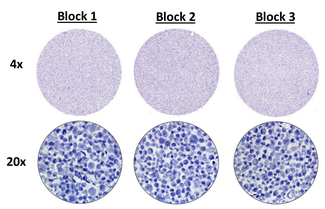


Consistent Source of Biorelevant Specimens

Generation of FFPE-Mimetic Specimens Using FFPE Cell Line Blocks

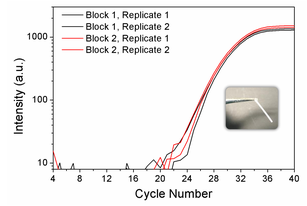


✓ Consistent and reproducible block generation



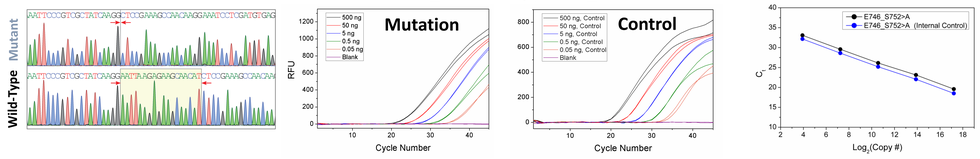
5 µm Sections, Hematoxylin Stained

BRAF V600E TaqMan Assay



Genomic DNA for qPCR Assay Development and Validation

EGFR c.2237_2254del18, p.E746_S752>A



Genomic DNA reference standards perform efficiently as reference material for qPCR applications:

- Ability to detect mutations using qPCR platforms
- Amplification efficiency close to 1

MAPK Mutation Panel: Engineered Variants

Mutation Information				
Gene	**Exon**	**Amino Acid**	**Nucleic Acid**	**COSMIC ID**
EGFR	18	G719S	2155G>A	6252
EGFR	18	*G719C	2155G>T	6253
EGFR	18	G719A	2156G>C	6239
EGFR	19	*L747_S752delLREATS	2239_2256del18	6255
EGFR	19	*L747_P753>S	2240_2257del18	12370
EGFR	19	*E746_S752>D	2238_2255del18	6220
EGFR	19	*L747_P753>Q	2239_2258>CA	12387
EGFR	19	*E746_S752>A	2237_2254del18	12367
EGFR	19	*E746_S752>V	2237_2255>T	12384
EGFR	19	*E746_T751delELREAT	2236_2253del18	12728
EGFR	19	*E746_T751>A	2237_2251del15	12678
EGFR	19	*L747_T751delLREAT	2239_2253del15	6254
EGFR	19	*L747_T751delLREAT	2240_2254del15	12369
EGFR	19	*E746_T751>V	2237_2252>T	12386
EGFR	19	*E746_T751>I	2235_2252>AAT	13551
EGFR	19	*K745_E749delKELRE	2233_2247del15	26038
EGFR	19	E746_A750delELREA	2235_2249del15	6223
EGFR	19	*E746_A750delELREA	2236_2250del15	6225
EGFR	19	*L747_T751>P	2239_2251>C	12383
EGFR	19	*L747_T751>S	2240_2251del12	6210
EGFR	19	*L747_T751>Q	2238_2252>GCA	12419
EGFR	19	*L747_A750>P	2239_2248>C	12382
EGFR	19	*L747_A750>P	2238_2248>GC	12422
EGFR	19	*L747_E749delLRE	2239_2247del9	6218
EGFR	19	*E746_A750>IP	2235_2248>AATTC	13550
EGFR	20	S768I	2303G>T	6241
EGFR	20	V769_D770insASV	2307_2308insGCCAGCGTG	12376
EGFR	20	*D770_N771insG	2310_2311insGGT	12378
EGFR	20	*H773_V774insH	2319_2320insCAC	12377
EGFR	20	T790M	2369C>T	6240
EGFR	21	L858R	2573T>G	6224
EGFR	21	L861Q	2582T>A	6213
KRAS	2	G12C	34G>T	516
KRAS	2	G12S	34G>A	517
KRAS	2	G12R	34G>C	518
KRAS	2	G12V	35G>T	520
KRAS	2	G12D	35G>A	521
KRAS	2	G12A	35G>C	522
KRAS	2	*G13C	37G>T	527
KRAS	2	*G13S	37G>A	528
KRAS	2	*G13R	37G>C	529
KRAS	2	G13D	38G>A	532
KRAS	2	*G13A	38G>C	533
KRAS	2	*G13V	38G>T	534
BRAF	15	V600K	1798_1799GT>AA	473
BRAF	15	V600R	1798_1799GT>AG	474
BRAF	15	*V600E	1799_1800TG>AA	475
BRAF	15	V600E	1799T>A	476
BRAF	15	*V600D	1799_1800TG>AT	477
BRAF	15	V600M	1798G>A	1130
BRAF	15	V600G	1799T>G	6137

Summary

- CRISPR/Cas9 is a powerful tool for isogenic cell line engineering
- This approach can be used to introduce diverse genetic variants, including precise point mutations, insertions, deletions, and complex gene mutations.
- Cell lines can be used as a starting material for derivative products including genomic DNA, FFPE blocks, matched frozen cell pellets, and others.
- Molecular reference standards derived from these cell lines provide a renewable source of consistent and biorelevant specimens
- The CRISPR/Cas9 platform enables rapid development of cell line based reference materials, and ASC plans to expand our panel by 200 mutations in 2017

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