Here, we present a detailed protocol for easy assembly of One-pot DTECT using off-the-shelf reagents. One-pot DTECT captures targeted genetic signatures using a simple library of adaptors and a unique pair of detection primers.

Only reagents required for One-pot DTECT:
AcuI (NEB #R0641)
T4 ligase (Invitrogen #15224017)
SYBRGreen (ThermoFisher #4367659)
Q5 DNA polymerase (NEB #M0491)

Note: Equivalent reagents can be purchased from other suppliers.

A- Preparation of the competitor DNA fragment (prepare only once):

The competitor DNA fragment requires two complementary oligonucleotides:

Oligo name	Sequence (5'->3')
Competitor DNA fragment#1	AGCCTGTGGTTCCTGAAGATCGCGTCCGAT
Competitor DNA fragment#2	ATCGGACGCGATCTTCAGGAACCACAGGCT

Mixture:
1 µl Competitor DNA fragment #1 (100 µM)
1 µl Competitor DNA fragment #2 (100 µM)
2 µl ligase buffer (5X)
6 µl H_2O

Incubate in a thermocycler at 95°C for 5 minutes followed by a gradual temperature decrease to 15°C at a rate of 0.5°C/s.

Use a nanodrop to measure the concentration of competitor, then dilute it to a concentration of 1 µM using distilled H_2O. The prepared competitor stock (1 µM) is stored at -20°C.

B- Preparation of the library of 16 adaptors (prepare only once):

The library requires the following 17 individual oligonucleotides:

Oligo name	Sequence (5'->3')
Reverse oligonucleotide	CTGGGGCACGGGTAAGAAGCATTCTGTCTCTCTTCTAAgaattcgagctcggtacccg
AA-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**AA**
AC-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**AC**
AG-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**AG**
AT-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**AT**
CA-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**CA**
CC-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**CC**
CG-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**CG**
CT-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**CT**
GA-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**GA**
GC-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**GC**
GG-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**GG**
GT-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**GT**
TA-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**TA**
TC-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**TC**
TG-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**TG**
TT-Adaptor oligonucleotide	cgggtaccgagctcgaattcTTAGAAGAGAGACAGAATGCTTCTTACCCGTGCCCCAG**TT**

Mixture (16 tubes, each tube contains the reverse oligonucleotide + one of the adaptors)**:**
2.5 µl Reverse oligonucleotide (100 µM)
2.5 µl Adaptor oligonucleotide (100 µM)
4 µl ligase buffer (5X)

11 µl H_2O

Incubate the mixtures in a thermocycler at 95°C for 5 minutes followed by a gradual temperature decrease to 15°C at a rate of 0.5°C/s.

Then, add 100 µl H_2O and store the adaptors at -20°C.

C- Preparation of the 2X One-pot DTECT master mix:

Mixture (for 100 µl):
40 µl Ligase buffer (5X)
1 µl AcuI (5 units/µl)
10 µl T4 ligase (1 unit/µl)
20 µl Competitor DNA (1 µM, from A)
29 µl H_2O

D- Preparation of a ready-to-use qPCR master mix:
Given that One-pot DTECT utilizes a unique pair of primers for all detections, a ready-to-use qPCR master mix is prepared:

Oligo name	Sequence (5'->3')
Detection primer #1	gcaattcctcacgagacccgtcctg
Detection primer #2	cgggtaccgagctcgaattcttagaag

Mixture (for 500 µl):
5 µl Detection primer #1 (100 µM)
5 µl Detection primer #2 (100 µM)
250 µl SYBRGreen (2X)
190 µl H_2O

One-pot DTECT protocol:

Example: Quantification of base editing frequency in human primary T-cells
The genomic sequence of the targeted locus is: 5'-…CCCGCTGGCCCTGTAAAGGAAACTGGAACACAAAGCATAGACTGCGGG…3'
gRNA sequence/PAM sequence
Targeted dinucleotide signature

Expected editing: A>G: the nonedited dinucleotide (AC) is expected to become (GC) upon base editing.

1) **Step 1 – Amplification of the HEK2 locus and AcuI-tagging:**

Design of AcuI-tagging primers:
Overall structure of an AcuI-tagging primer: (5') GCAATTCCTCACGAGACCCGTCCTG – 15nt targeted DNA sequence – CTGAAG – 14 nt targeted DNA sequence juxtaposed to the targeted dinucleotide of interest (3')

In the above example, a possible AcuI-tagging oligonucleotide to capture AC or GC signatures is:

Oligo name	Sequence (5'->3')
AcuI-tagging primer	GCAATTCCTCACGAGACCCGTCCTGCCCGCTGGCCCTGTACTGAAGAAGGAAACTGGAAC
Reverse primer	GATGTCTGTTTCCCTGATGTAATGA

Mixture:
0.25 µl AcuI-tagging primer (100 µM)
0.25 µl Reverse primer (100 µM)
0.25 µl dNTP (10 mM)
5 µl Q5 polymerase buffer (5X)
0.2 µl Q5 polymerase (2 units/µl)
x µl gDNA (1-200 ng)
y µl H_2O (up to 25 µl reaction)

PCR protocol:
95°C 1 min.
40 cycles of 95°C 10 s, 58°C 15 s, 72°C 30 s.
72°C 1 min.

(Optional but recommended) The AcuI-tagging PCR amplicon can be purified on a 2% agarose/TAE gel. Gel purification can decrease background signals.

2) Step 2 – Capture of genetic signatures:

Mixture:
2.5 µl One-pot DTECT master mix (2X, from C)
1 µl Diluted (1/100th) AcuI-tagging PCR (or 2.5 fmol PCR if purified on gel)
0.25 µl Selected adaptor (from B)
1.25 µl H_2O

In this example, two captures are conducted with the GT or GC adaptors, for their complementarity to AC and GC, respectively. Signature capture in control (unedited) samples are critical as they provide positive and negative controls.

Capture protocol:
25°C 10-60 min
65°C 10 s

3) Step 3 – Detection by qPCR (quantification):

Mixture:
9 µl ready-to-use qPCR master mix (from D)
1 µl One-pot DTECT capture

Load technical qPCR duplicates (lines 2-3, 4-5, 6-7 and 8-9 in table below) for each sample.

qPCR protocol:
50°C 2 min.
95°C 10 min.
40 cycles of 95°C 10 s, 60°C 30 s.

Analysis of the results and quantification of editing:

Retrieve Ct measured by the qPCR to directly obtain editing %:

	G	H	I
1	Equation standard curve	slope a	-3.3245
2		b	7.5504

qPCR duplicates ↓ (column C); Mean Ct = (C2+C3)/2; Capture efficiency = 10^((D2-I2)/I1)

	A: Target	B: Adaptor	C: Ct values	D: Mean Ct	E: Capture efficiency	F: Editing (%)
2	HEK2 (duplicate 1)	GT (WT)	16.185	15.953	0.002969307	99.47
3			15.720			
4		GC (edit)	23.441	23.504	1.58885E-05	0.53
5			23.568			
6	HEK2 (duplicate 1)	GT (WT)	15.817	15.936	0.003003594	80.01
7			16.055			
8		GC (edit)	18.065	17.939	0.000750318	19.99
9			17.812			
10	HEK2 (duplicate 2)	GT (WT)	15.914	15.876	0.003131606	99.17
11			15.838			
12		GC (edit)	22.791	22.782	2.621E-05	0.83
13			22.772			
14	HEK2 (duplicate 2)	GT (WT)	16.013	15.922	0.003032462	77.45
15			15.832			
16		GC (edit)	17.769	17.704	0.000882782	22.55
17			17.639			

F2: =(**F2**/(F2+F4))*100 — **Positive control (confirms experiment worked)**
F4: =(**F4**/(F2+F4))*100 — **Negative control (capture background)**
F6: =(**F6**/(F6+F8))*100 — **Capture WT signature in edited sample**
F8: =(**F6**/(F6+F8))*100 Editing frequency (duplicate 1) — **Capture edited signature in edited sample (duplicate 1)**
F10: **Positive control duplicate 2 (confirms experiment worked)**
F12: **Negative control duplicate 2 (capture background)**
F16: =(**F16**/(F14+F16))*100 Editing frequency (duplicate 2) — **Capture edited signature in edited sample (duplicate 2)**

Comments or questions can be addressed directly to Dr. Pierre Billon (pierre.billon@ucalgary.ca).

Citing One-pot DTECT: Please cite our publication on the development and application of One-pot DTECT: Baudrier, Benamozig et al., *Under review*, 2023

Other versions:
One-pot DTECT protocol-v1: July 2023 (pre-peer-review version)
One-pot DTECT protocol-v2: December 2023 (peer-reviewed version)