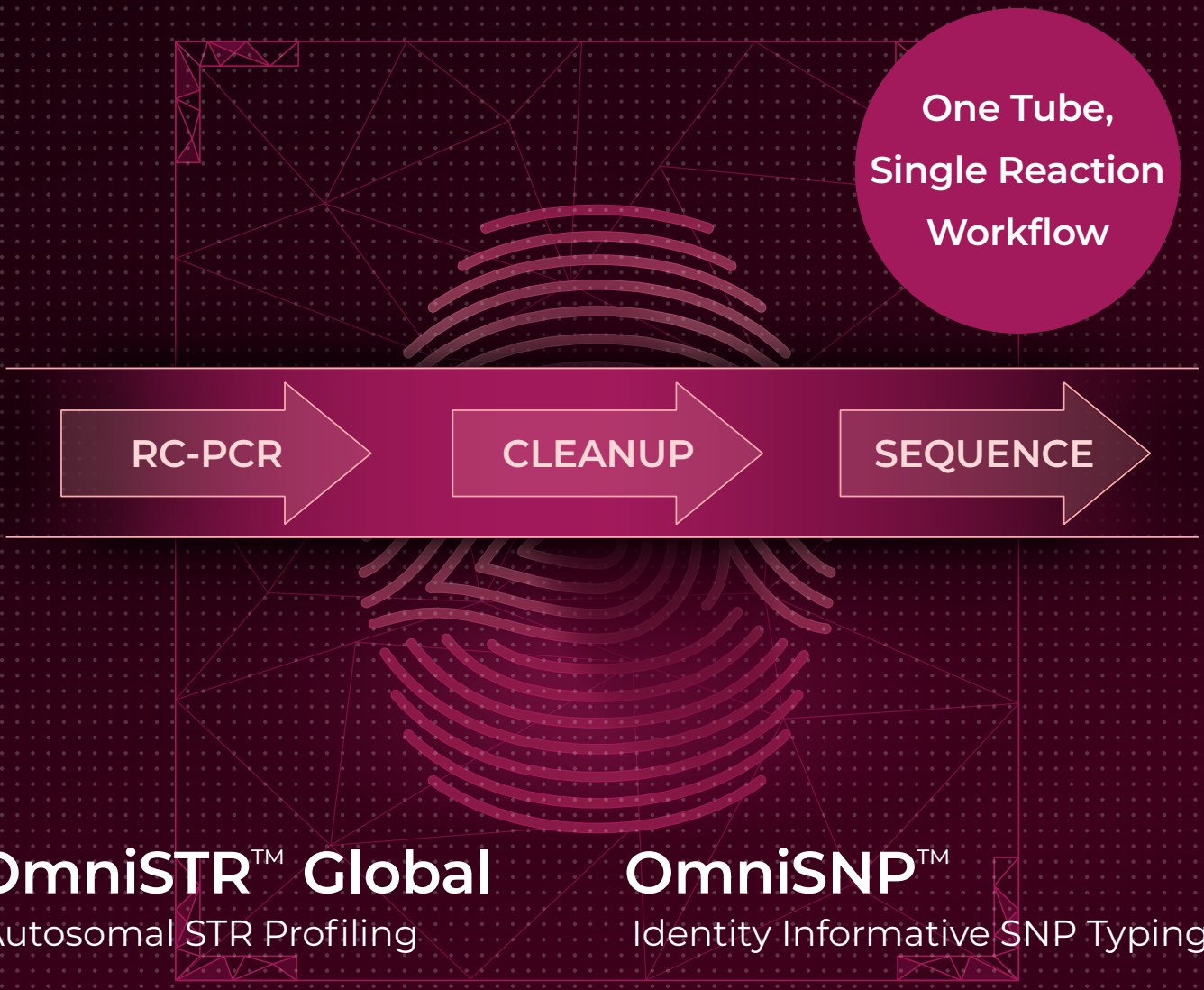
Product Bulletin

IDseek®

The Next Revolution in Forensic MPS

Powered by Reverse Complement PCR



OmniSTR™ Global
Autosomal STR Profiling

OmniSNP™
Identity Informative SNP Typing

mYSTR™
Y-Chromosomal STR Profiling

Mitochondrial DNA
HVR and Full Genome

- **Reliable results, safer and easier**
- **Providing detailed insights that allow for informed decision making**

NimaGen.
Innovators in DNA Sequencing Technologies

IDseek®: The Next Revolution in Forensic DNA Analysis

Introduction:
After more than thirty years of DNA analysis in forensics using classical methods such as PCR followed by Fragment Analysis by capillary elecrophoresis (CE), new technologies based on Massive Parallel Sequencing (MPS) are now changing this field. In addition to unravelling complicated mixed DNA traces, it can also reveal far more information and be used with lower quality and highly degraded samples. Existing MPS library preparation methods are complicated, typically involving multiple hands-on steps, error-prone workflows, increased risk for amplicon contamination and sample swapping.

With the introduction of the novel and innovative Reverse Complement PCR (RC-PCR) technology, NimaGen opens doors for the forensic community to make the transition from classic CE to MPS in the Safest, Fastest and Simplest way possible, without compromise.

Different MPS workflows versus CE

CE	MPS / std library prep	MPS / Nested PCR	MPS / RC-PCR
PCR Amplification	PCR Amplification	PCR Amplification 1	**RC-PCR**
CE Preparation	End-rep. / A-tailing	Purification	**Pooling**
Electrophoresis	Adapter Ligation	PCR Amplification 2	**Purification**
	Purification	Purification	**Quantification**
	Quantification	Normalisation	**Sequencing**
	Pooling	Pooling	
	Quantification	Sequencing	
	Sequencing		

Steps in pink are performed for each sample separately.
Steps in green are performed once per run.

Technology

DNA Sequencing methods typically involve multiple steps, whether it is classical Sanger Sequencing or MPS.

RC-PCR from NimaGen is a breakthrough technology providing MPS library prep in a single, closed-tube workflow, with extremely low handling. Multiplex targeted amplification, adapter addition and Indexing all occur simultaneously in a single closed tube reaction: simply add your DNA to the pre-dispensed index tubes and run the PCR reaction in your thermal cycler.

As a direct consequence of the Unique Dual Indexing in the very first step of the library prep ("Early Indexing"), and the single-step amplification, the risk of sample swapping, PCR contamination and pipetting errors are drastically minimized. Additionally, the reaction kinetics of RC-PCR results in high sensitivity and specificity, due to the fact specific primers are synthesized during the reaction, so concentrations of primers and amplicons are more in line.
MPS targeted library prep will never be easier. See video animation of the working principle at www.rcpcr.com.

The first MPS assay that has the potential to compete with CE for routine forensic casework

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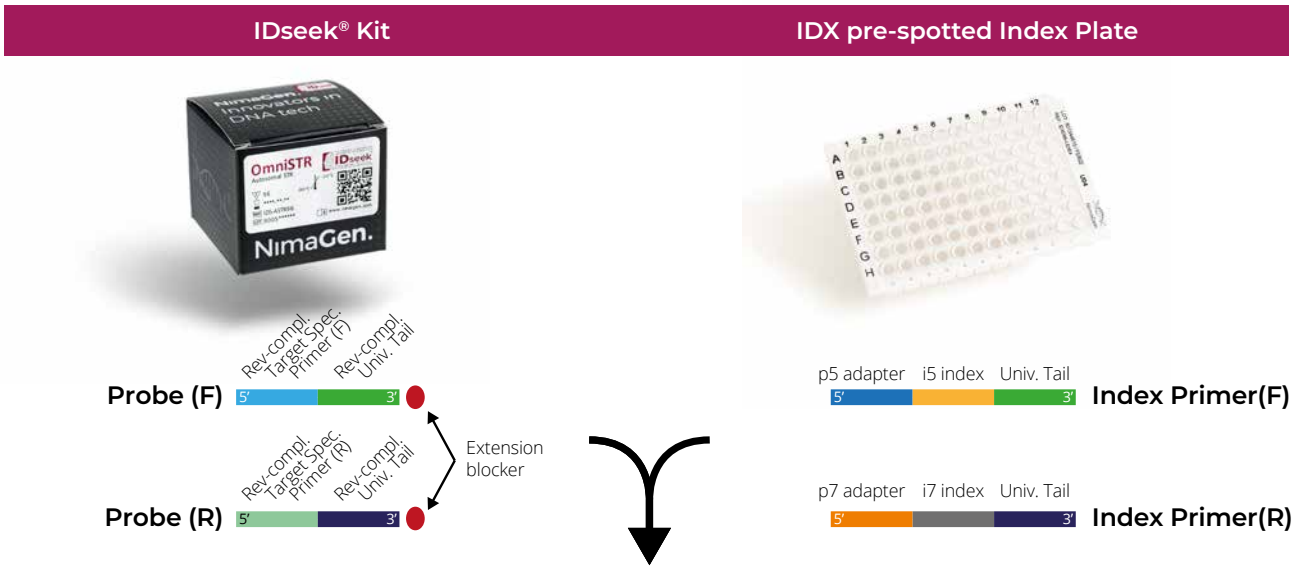
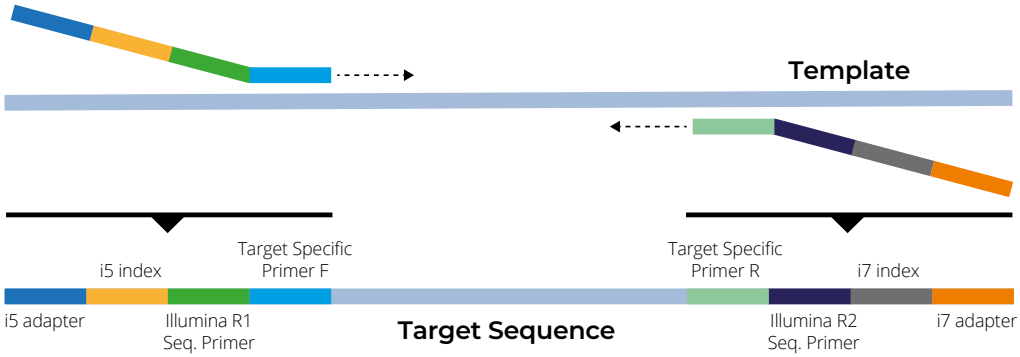
IDseek® MPS library prep: How does it work?

NimaGen offers a range of forensic MPS library prep kits that all work with the same principle. Libraries from different kits can be combined in a single Illumina® run, to make efficient use of your sequencing capacity. Every kit consist of two parts. One assay specific part, that includes the target specific probe mixes and the RC-PCR Master Mix, and one universal part: The IDX plate(s), containing pre-spotted and dehydrated Unique Dual Index primers. All IDX plates are breakable per 8 wells, to maximize efficiency.



Combine the probe Panel with the mastermix, dispense in the IDX plate, add sample DNA and start the RC-PCR program.

At the first annealing step, RC-PCR probe tails hybridize to the IDX primer tails, followed by extension of the IDX primers with gene specific primer sequences. This step synthesizes functional Indexed primers including Illumina® adapters. In the following cycles, target regions are amplified, while creating more primers.



This results in a ready-to-sequence, Illumina comaptible MPS library, in a single step. IDseek® assays are highly specific and sensitive due to the synthesis of primers during the reaction, keeping primer concentration more in line with target availability.

IDseek® Multiplex STR Kits for MPS

Powered by Reverse-Complement PCR

Short Tandem Repeat (STR) analysis is the far most used tool in forensic DNA analysis for generating unique genetic profiles. Forensic science uses the variability in the human population in STR lengths, enabling scientists to distinguish DNA samples, and match with databases to identify crime scene samples. In the classic analysing method, DNA fragments are separated and detected using CE in order to determine allele length.

MPS technologies adds significant benefits over CE analysis. It is not limited by available fluorescent channels, nor does it suffer from primer design to amplicon size / length constraints which allows it to overcome a number of complicating factors:

- **Allowing the recovery of fuller profiles from challenging samples due to reduced amplicon sizes**
- **Providing the full base sequence in and around the repeat motifs, further increasing the discriminative power of STR markers.**
- **Higher multiplexes for more STRs to be detected simultaneously avoiding the need for separate secondary workflows.**
- **Differentiation of genetic profiles from complex mixtures, commonly associated with crime scenes.**

All amplicons in NimaGen's STR multiplex kits are designed to meet the shortest possible fragment lengths and are compatible with Illumina® MiSeq™ systems, with 2 x 10 bp Unique Dual Index reads. This combination of state-of-the-art features with the possibility for automated data analysis without human intervention makes this kit the ideal solution for the transition from CE to MPS.

Features:

- Sensitive: 100% of markers called, down to 60 pg of DNA input
- Marker balance optimized for 1 ng input DNA, with limited impact on low inputs down to 60 pg
- High on-target read percentage, even with low input
- Robust and inhibitor tolerant, tested for Humic Acid, Tannic Acid, Bacterial DNA

FDSTools graph for marker D5S818 on 2800M reference DNA

FDSTools Table (D5S818 / 2800M)

CE Allele	MPS Allele	Reads	PctOfHighest	PctOfMarker	Correction
12	CE12_ TCTA[12]	1651 → 1751	100.00%	49.49%	(+6.08%)
12	CE12_ TCTA[12]_+16A>G	1608 → 1708	97.55%	48.28%	(+6.24%)

Example of D5S818 sequence variation in a flanking region of a homozygous CE sample (Promega®, 2800M). Analysis performed using FDSTools software (Netherlands Forensic Institute, The Hague, NL, doi.org/10.1016/j.fsigen.2016.11.007)

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IDseek® OmniSTR™ Global

Autosomal STR Profiling

The OmniSTR™ Kit is a single reaction, multiplex library prep kit for autosomal STR profiling, including all the US and European expanded core loci, plus SE33, D4S2408, D6S1043, D9S1122, D17S1301, D20S482, PentaD, PentaE and DYS391. All targets have been designed for short amplicons, while maintaining the most informative sites in the flanking regions.

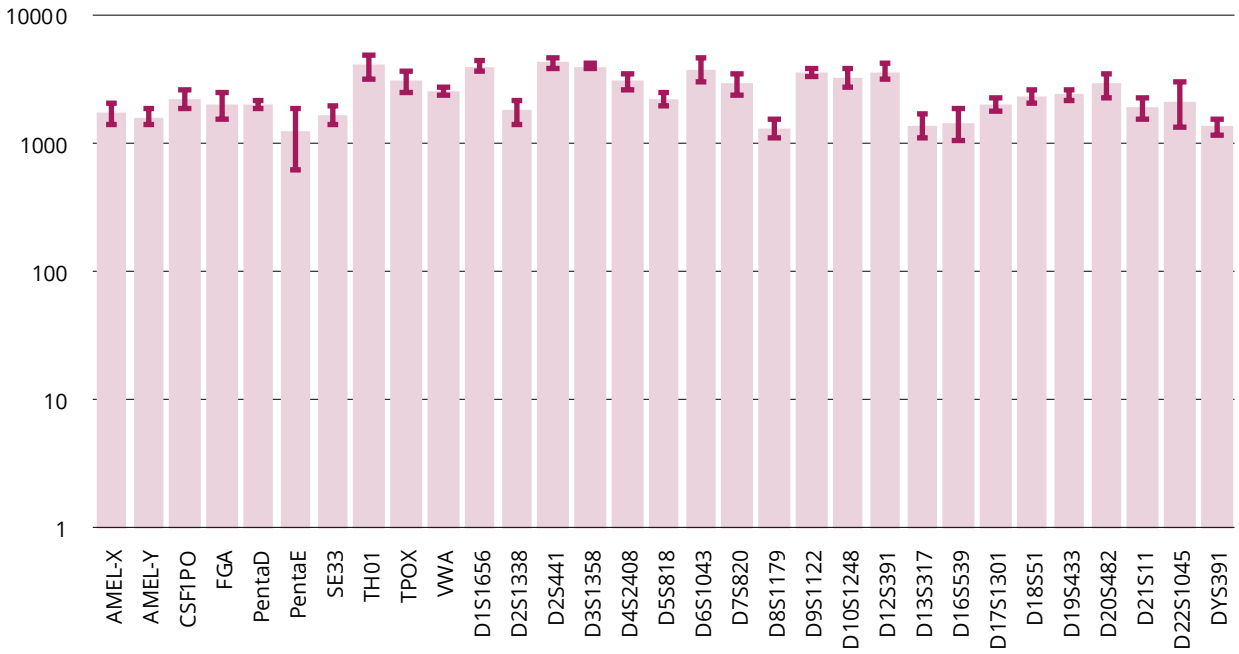
Forensic STR profiling by MPS tend to be applied in specialty cases due to high hands-on time investments. The unmatched simplicity, robustness, sensitivity and safety of IDseek now facilitates a routine MPS application, hardly exceeding hands-on time of CE.

Table Amplicon IDseek® OmniSTR

Marker	Length (HG38)	US - EU	Marker	Length (HG38)	US - EU
AMEL-X	105	• •	D3S1358	124	• •
AMEL-Y	111	• •	D4S2408	124	
CSF1PO	147	• •	D5S818	144	•
D10S1248	119	• •	D6S1043	117	
D12S391	139	• •	D7S820	185	•
D13S317	144	•	D8S1179	130	• •
D16S539	226	• •	D9S1122	116	
D17S1301	134		DYS391	158	
D18S51	167	• •	FGA	165	• •
D19S433	128	• •	PentaD	175	
D1S1656	157	• •	PentaE	81	
D20S482	119		SE33	287	•
D21S11	209	• •	TH01	83	• •
D22S1045	124	• •	TPOX	88	•
D2S1338	200	• •	VWA	165	•
D2S441	134	• •			

- Recommended Input: 1 ng
- Sensitivity: Down to 60 pg
- Inhibition: 1 ng input DNA Tested with Tannic Acid (15 µM), Humic Acid (400 ng), E.coli DNA (10 ng)

IDseek® OmniSTR™ Target Balance (1 ng)



IDseek® mYSTR™

Y-Chromosomal STR Profiling

The mYSTR™ kit is a single reaction, multiplex library prep kit for Y-Chromosomal STR profiling, ampiflying 27 Y-chromosomal STR markers plus Amelogenin in a single multiplex. All targets have been designed for short amplicons, while maintaining the most informative sites in the flanking regions.

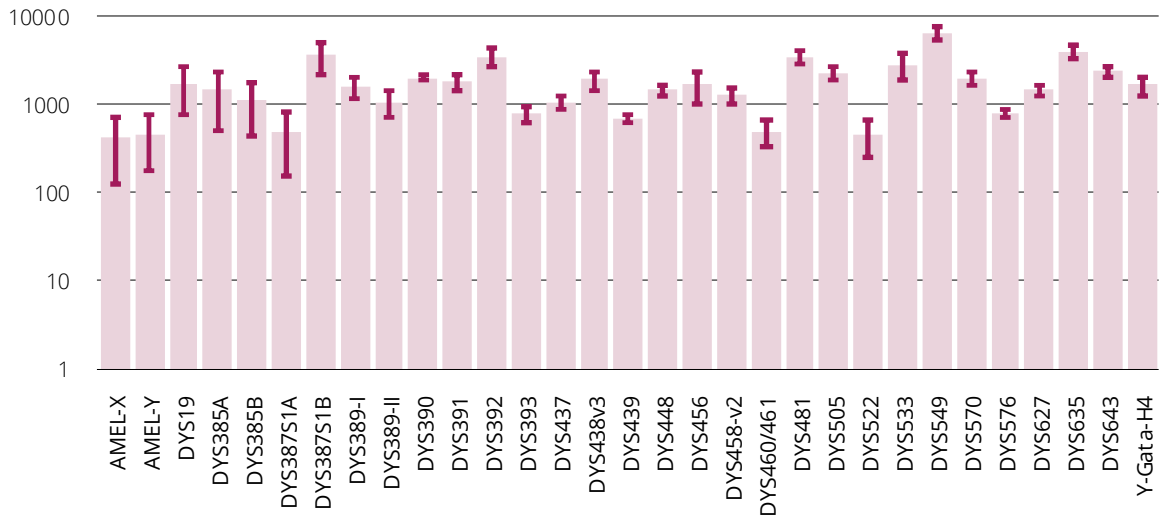
Forensic STR profiling by MPS tend to be applied in specialty cases due to high hands-on time investments. The unmatched simplicity, robustness, sensitivity and safety of IDseek® now facilitates a routine MPS application, hardly exceeding hands-on time of CE.

Table Amplicon IDseek® mYSTR™

Marker	Length (HG38)	Marker	Length (HG38)
AMEL-X	106	DYS448	243
AMEL-Y	112	DYS456	149
DYS19	212	DYS458	166
DYS385A	261	DYS460/461	272
DYS385B	249	DYS481	140
DYS387S1A	231	DYS505	173
DYS387S1B	244	DYS522	303
DYS389-I	153	DYS533	220
DYS389-II	127	DYS549	135
DYS390	174	DYS570	125
DYS391	159	DYS576	129
DYS392	121	DYS627	262
DYS393	183	DYS635	154
DYS437	205	DYS643	144
DYS438	136	Y-Gata-H4	200
DYS439	220		

- Recommended Input: 1 ng
- Sensitivity: Down to 60 pg
- Inhibition: 1 ng input DNA Tested with Tannic Acid (15 µM), Humic Acid (400 ng), E.coli DNA (10 ng)

IDseek® mYSTR™ Target Balance (1 ng)



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IDseek® Mitochondrial DNA

HVR and Full Genome

The use of MPS for the analysis of Mitochondrial DNA (mtDNA) adds great benefits for Forensic Scientists, especially for samples like hairs, teeth and bones, coming from crime scenes or disasters, or ancient DNA samples. Also, for the analysis of any samples that contain very low quantities or highly degraded nuclear DNA, mtDNA analysis can add extra power in creating genetic profiles.

Since every human cell contains up to 2000 mitochondria, and the mtDNA is more stable than nuclear DNA, the higher sensitivity of mtDNA analysis can add additional tools to the standard autosomal or Y-chromosomal genetic profiling.

With the introduction of the IDseek® mtDNA sequencing assays, the High Variable Region (HVR) or the entire mtDNA genomes can be easily sequenced, providing additional information, like haplotypes and heteroplasmy.

Like every IDseek® assay, the workflow is extremely straightforward and safe. In contrast to the STR and SNP assays, the mtDNA kits have two probe pools per sample, separating overlapping fragments in order to obtain full length sequences after alignments.

The IDseek® Mitochondrial DNA HVR Sequencing kit is based on two (2) multiplex reactions of 5 amplicons each. The amplicon lenghts are 150 – 230 bp.
The IDseek® Mitochondrial DNA Full Genome Sequencing is based on two (2) multiplex reactions of 50 amplicons each. The amplicon lenghts are 150 – 250 bp.

IDseek® OmniSNP™

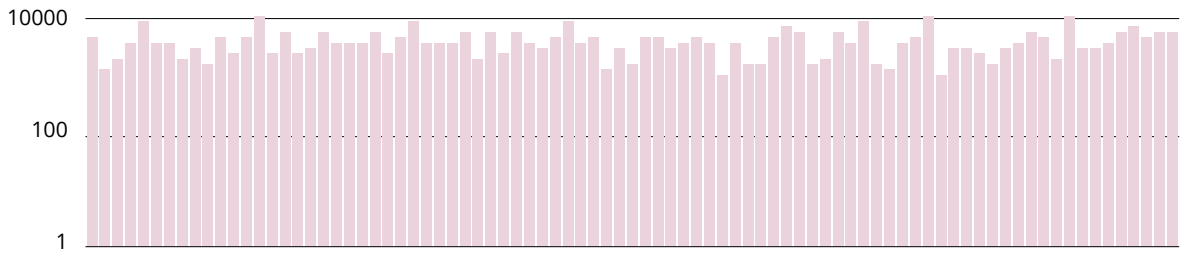
Identity Informative SNP Typing

New technologies have increased the spectrum of sample types that can be subjected to DNA analysis. While several human ID markers are offering amplification of short amplicons, several forensic samples (e.g., human remains and touch evidence) do not always contain sufficient quantity and quality of DNA to be typed with current methodologies.

As a result, challenging samples with high DNA fragmentation may not contain sufficient intact templates for successful PCR amplification. Several studies have reported that DNA fragments in highly degraded samples are very short down to approximately 50 bases in length. Only a limited number of DNA markers, if any, may be detected with such short fragments. Several methods to improve DNA recovery from compromised samples have been developed but had limited or no success.

Two advances now may make it possible to analyze highly degraded DNA: Reverse Complement PCR (RC-PCR) and MPS. With the design of the OmniSNP™ panel, fragments as short as approximately 50–100 nucleotides in length are targeted, increasing the success of typing targets from highly degraded DNA samples.

IDseek® OmniSNP™ Coverage and SNP content



The RC-PCR system may be an effective alternative to the current forensic genetic methods used to analyze highly degraded DNA.

Magdalena M Bus et.al., BioTechniques 71: 00–00 10.2144/btn-2021-0031

Ordering Information

IDseek® Kits for Forensic MPS

Library Preparation kits for Illumina® MPS sequencing, including RC-PCR HiFi Master Mix

Part Number	Description	
IDS-ASTR96	IDseek® OmniSTR™ Global, *Autosomal STR Profiling kit*	96 rxn
IDS-YSTR96	IDseek® mYSTR™, *Y-Chromosomal STR Profiling kit*	96 rxn
IDS-YSTR96	IDseek® OmniSNP™, *Identity Informative SNP Typing kit*	96 rxn
IDS-HVR96	IDseek® Mitochondrial DNA HVR, *Sequencing by MPS kit*	96 rxn
IDS-MTC96	IDseek® Mitochondrial DNA Full, *Sequencing by MPS kit*	96 rxn

IDX Pre-spotted, Dried Down, Colored Index plates for RC-PCR Kits

1 x 96-well Index Plate compatible with RC-PCR kits with 1 probe panel/sample

Part Number	Description	
IDX096-U01	96 Dried Unique Dual Indexes. *Pre-spotted in 96 well plate. UDI# 0001 - 0096*	96 rxn
IIDX096-U02	96 Dried Unique Dual Indexes. *Pre-spotted in 96 well plate. UDI# 0097 - 0192*	96 rxn
IDX096-U03	96 Dried Unique Dual Indexes. *Pre-spotted in 96 well plate. UDI# 0193 - 0288*	96 rxn
IDX096-U04	96 Dried Unique Dual Indexes. *Pre-spotted in 96 well plate. UDI# 0289 - 0384*	96 rxn

2 x 96-well Index Plates compatible with RC-PCR kits with 2 probe panels/sample

IDX096-U01D	2 x 96 Dried Unique Dual Indexes. *Pre-spotted in two identical 96 well plates. UDI# 0001 - 0096*	96 rxn
IDX096-U02D	2 x 96 Dried Unique Dual Indexes. *Pre-spotted in two identical 96 well plates. UDI# 0097 - 0192*	96 rxn
IDX096-U03D	2 x 96 Dried Unique Dual Indexes. *Pre-spotted in two identical 96 well plates. UDI# 0193 - 0288*	96 rxn
IDX096-U04D	2 x 96 Dried Unique Dual Indexes. *Pre-spotted in two identical 96 well plates. UDI# 0289 - 0384*	96 rxn

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Product and Company Information

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Product use
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