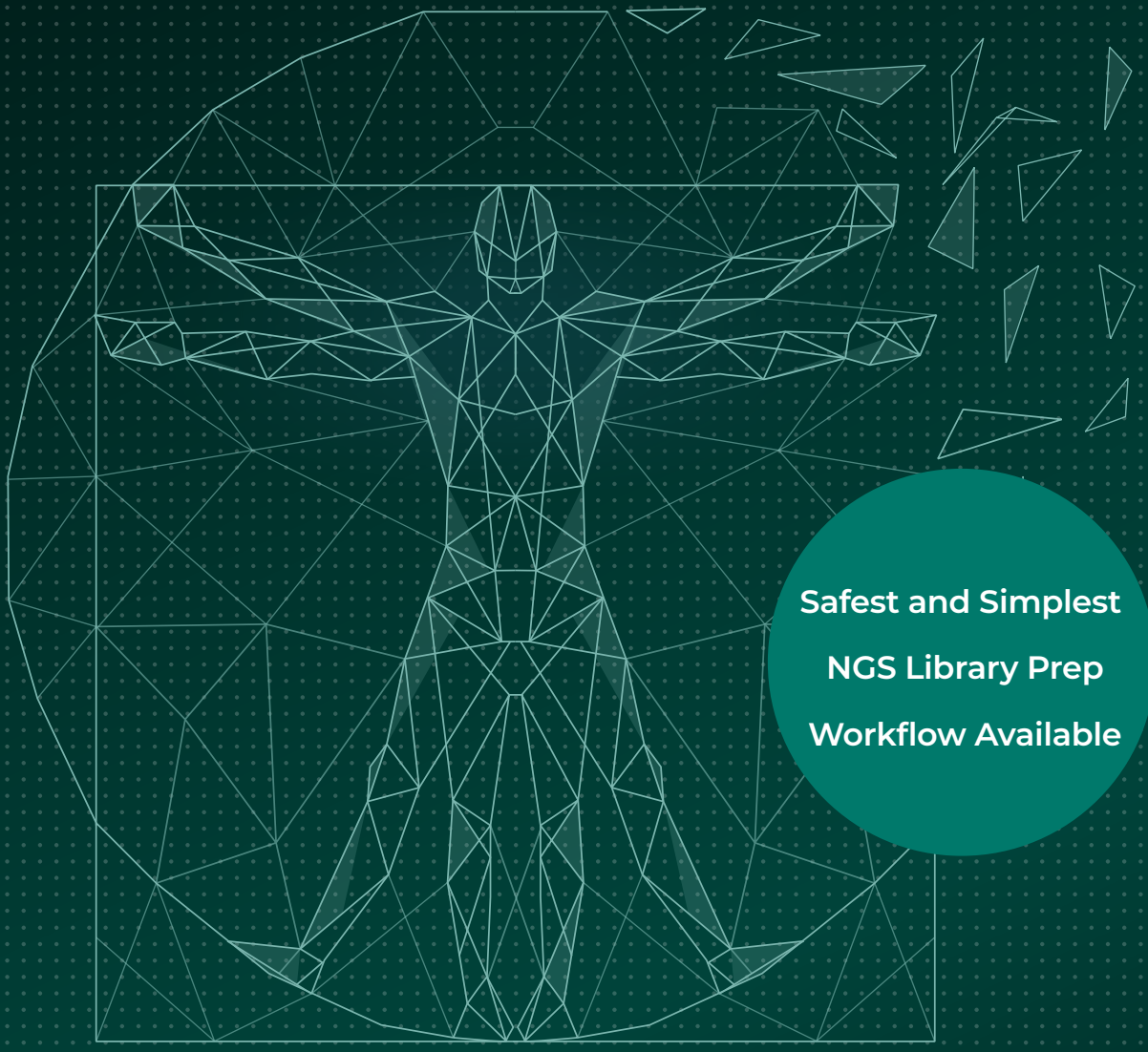
EasySeq™

Human DNA Sample Identification Kit

NGS Library Prep by Reverse Complement PCR



Safest and Simplest NGS Library Prep Workflow Available

- Helping laboratories safeguard sample identity, and associated Whole Exome and Whole Genome Sequencing data integrity and validity

NimaGen.

Innovators in DNA Sequencing Technologies

Introduction

Complex sample preparation workflows and challenges handling multiple Next-Generation Sequencing (NGS) samples make misidentification of samples at any stage of the analytical process a recognizable concern.

A method for independent confirmation of sample identity is therefore highly desirable, preferably using an identification method that is intrinsic to the WGS/WES data of the biological sample and simple to incorporate into the existing NGS workflow.

EasySeq™ Human DNA Sample Identification Kits, powered by patented Reverse Complement PCR (RC-PCR) technology, facilitate an effective end-to-end identification and tracking of independent samples in a single targeted sequencing assay that is purpose-designed for fast and efficient lab processing.

EasySeq™ Human DNA Sample Identification Kits are complementary to both Whole Exome and Whole Genome Sequencing Sample workflows. Sample IDs are matched with WES/WGS data and, as a result, data integrity and validity can be confirmed.

Another application of the EasySeq™ Human DNA Sample Identification Kit is the conformation of cell line authenticiation in Human Cell-line Culturing.

EasySeq™ Human DNA Sample Identification Kit

EasySeq™ Human DNA Sample Identification Kits comprise an optimized panel of 40 exonic targets, the genotypic profile which can be utilized to extract intrinsic identifiers from the human exome and genome. 37 exonic single nucleotide polymorphisms (SNPs) with high minor allele frequency (MAF), and Amelogenin X - Y and TXLNGY for gender determination (Table 1).

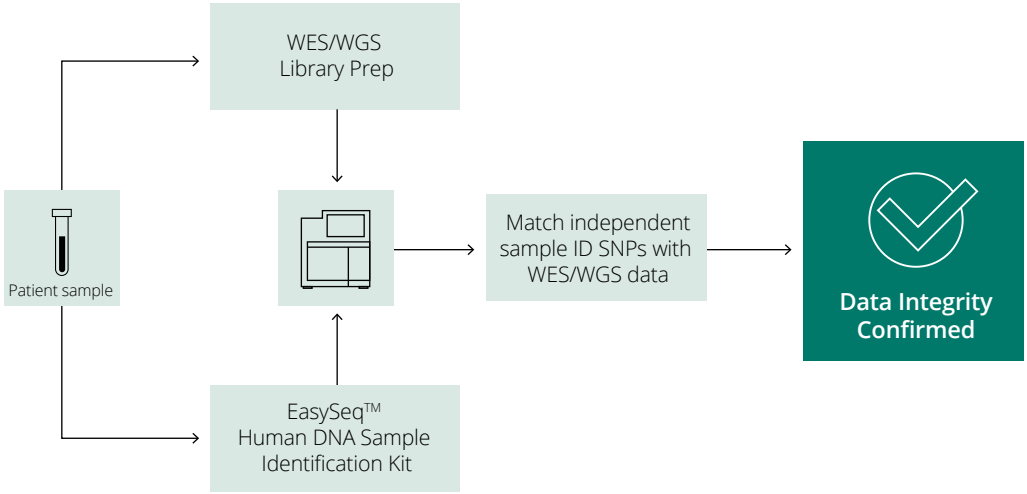
Table 1 | EasySeq™ Human DNA Sample Identification Kit Targets Overview

#	SNP	Chr	Gene	Location HG38	Location HG19	MAF ALFA Total	
1	rs1410592	1	NPHS2	179551371	179520506	G	0,374144
2	rs2229546	1	IL12RB2	67395837	67861520	C	0,35182
3	rs10203363	2	COL4A4	227032260	227896976	T	0,44476
4	rs2819561	3	SUMF1	4362083	4403767	A	0,432928
5	rs4688963	4	EVC	5748177	5749904	C	0,375104
6	rs309557	5	VCAN	83538811	82834630	T	0,495618
7	rs7738	7	BLVRA	43807004	43846603	G	0,39911
8	rs4735258	8	PDP1	93923709	94935937	C	0,42266
9	rs4870723	8	COL14A1	120216440	121228679	C	0,492677
10	rs7465584	8	FER1L6	123975238	124987478	C	0,466264
11	rs1381532	9	TDRD7	97428498	100190780	G	0,494669
12	rs1536928	9	OR1B1	122629130	125391409	G	0,48492
13	rs1572983	9	BAAT	101371346	104133628	C	0,315905
14	rs577993	9	PRUNE2	76706955	79321871	C	0,372343
15	rs10883099	10	HPSE2	98459557	100219314	G	0,489745
16	rs4617548	11	SOX6	16111867	16133413	A	0,496018
17	rs7300444	12	WNK1	884764	993930	T	0,426319
18	rs495680	13	STARD13	33129519	33703656	T	0,384773
19	rs9532292	13	FREM2	38859469	39433606	G	0,333352
20	rs11158685	14	PLEKHH1	67575857	68042574	A	0,495745
21	rs4577050	15	SLC12A6	34236747	34528948	G	0,339184
22	rs17715450	16	CDH3	68695882	68729785	C	0,43336
23	rs1026128	17	COG1	73200670	71196809	A	0,474133
24	rs1037256	17	COG1	73201609	71197748	G	0,478103
25	rs1292053	17	TUBD1	59886176	57963537	G	0,450154
26	rs2159132	17	COX10	14102122	14005439	G	0,436677
27	rs1805034	18	TNFRSF11A	62360008	60027241	C	0,47866
28	rs3826616	18	SERPINB8	63987229	61654463	A	0,432558
29	rs9962023	18	LAMA3	23833905	21413869	T	0,30934
30	rs2228611	19	DNMT1	10156401	10267077	C	0,499414
31	rs10373	20	FERMT1	6119441	6100088	A	0,466702
32	rs2296241	20	CYP24A1	54169680	52786219	G	0,469989
33	rs4148973	21	NDUFV3	42903480	44323590	T	0,416006
34	rs760482	22	DNAL4	38782696	39178701	G	0,31297
35	rs2073787	X	RGAG1	110451457	109694685	A	0,44298
36	rs5930933	X	ADGRG4	136349199	135431358	T	0,474134
37	rs6568050	X	ZCCHC16	112454808	111698036	C	0,460272
38	AMELOGENIN-X	X					
39	AMELOGENIN-Y	Y					
40	TXLNGY	Y					

EasySeq™ Human DNA Sample Identification Kit

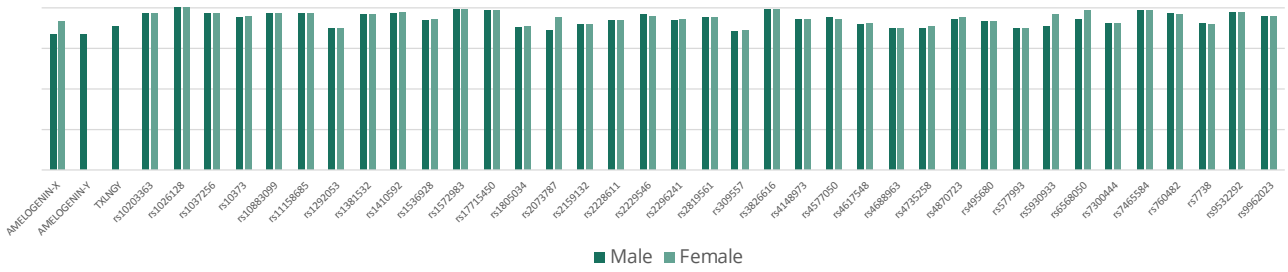
- A set of 40 exonic targets for extensive spread in the exome, to obtain a complete data set
- Additional Y-chromosomal targets for sex determination
- High sensitivity and specificity; extremely low number of off-target reads
- Optimized intra-locus balancing; optimal use of sequencing capacity
- Short amplicons; shorter sequencing time and lower costs

Figure 1 | Integrated Sample Tracking and Data Confirmation Workflow



Read depth distribution = 0.41 – 1.83 of mean read depth. Needed reads for single source samples for minimal 50x coverage: 5000 total.

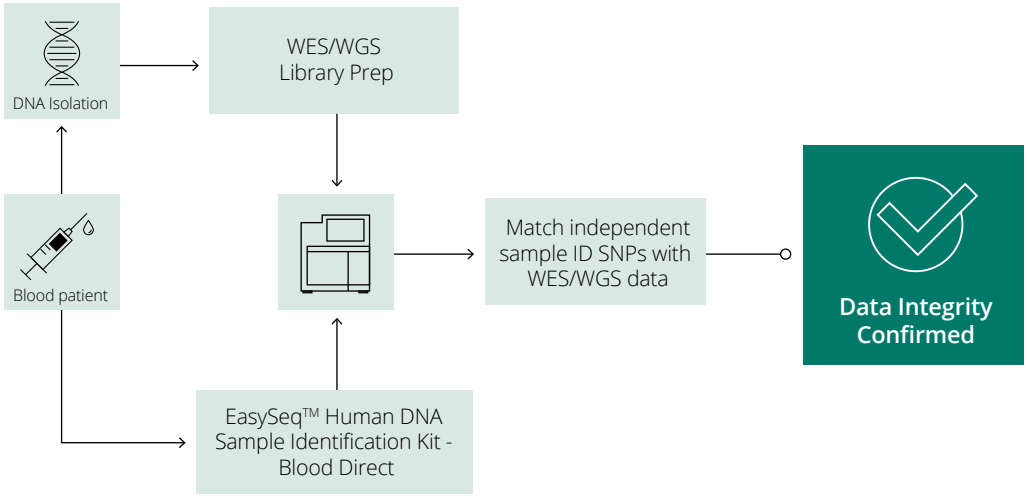
Figure 2 | EasySeq™ Human DNA Sample Identification Target Balance



EasySeq™ Human DNA Sample Identification Kit - Blood Direct

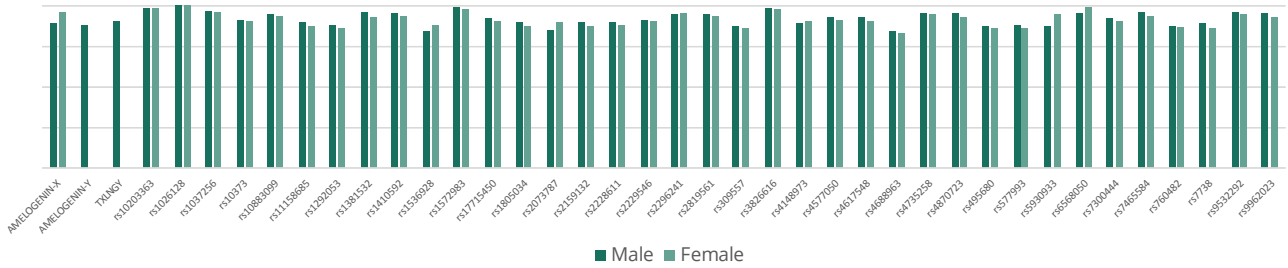
- Directly compatible with fresh or frozen blood and dried bloodspots; validate your sample identity from the very first step of the WEG / WGS workflow, including DNA isolation
- A set of 40 exonic targets for extensive spread in the exome, to obtain a complete data set
- Additional Y-chromosomal targets for sex determination
- High sensitivity and specificity; extremely low number of off-target reads
- Optimized intra-locus balancing; optimal use of sequencing capacity
- Short amplicons; shorter sequencing time and lower costs

Figure 3 | Integrated Sample Identification and Data Confirmation Workflow - Blood Direct



Read depth distribution = 0.48 – 2.14 of mean read depth. Needed reads for single source samples for minimal 50x coverage: 5000 total.

Figure 4 | EasySeq™ Human DNA Sample Identification - Blood Direct Target Balance



EasySeq™ NGS Library Prep by RC-PCR

The Next Revolution in Human Genetics NGS

Our unique kits utilize RC-PCR technology to create a simple and safe one-tube, single reaction NGS library prep workflow. Multiplex target amplification, sequencing adaptor addition and sample-specific unique dual indexing all occur simultaneously in a closed-tube reaction, as simple as any normal PCR. Multiplex reactions are pooled and cleaned-up in a single tube using magnetic beads, thereby eliminating the need to clean-up reactions separately (Figure 5). Therefore, RC-PCR greatly reduces the amount of hands-on steps and the associated risks of pipetting errors, sample swaps and cross-contamination (Figure 6).

Figure 6 | Workflow Comparison

Workflow Competitors	Workflow NimaGen
PCR amplification	Reverse Complement - PCR amplification
End repair / dA-tailing	Pooling and Purification
Adapter Ligation	Quantification / Quality Control
PCR amplification with specific UDI primers	Sequencing
Pooling and Purification	
Quantification / Quality Control	
Sequencing	

RC-PCR kinetics results in high sensitivity and specificity, as target specific primers are synthesized during the reaction, so concentrations of primers and amplicons are more in line, reducing potential primer dimerization and off-target primer binding (Figure 7).

Every EasySeq™ NGS Library Prep Kit consists of two parts: an assay-specific part, that includes the target specific Probe Panel and the RC-PCR Master Mix, and a universal part, the index (IDX) plate(s) containing pre-spotted and dehydrated Unique Dual Index primers.

Figure 5 | EasySeq™ RC-PCR workflow

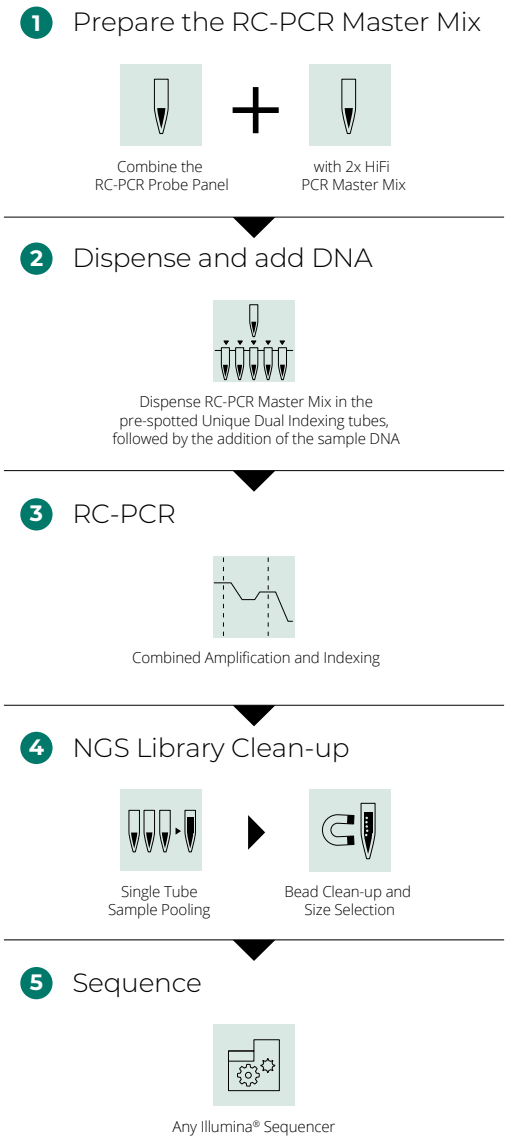
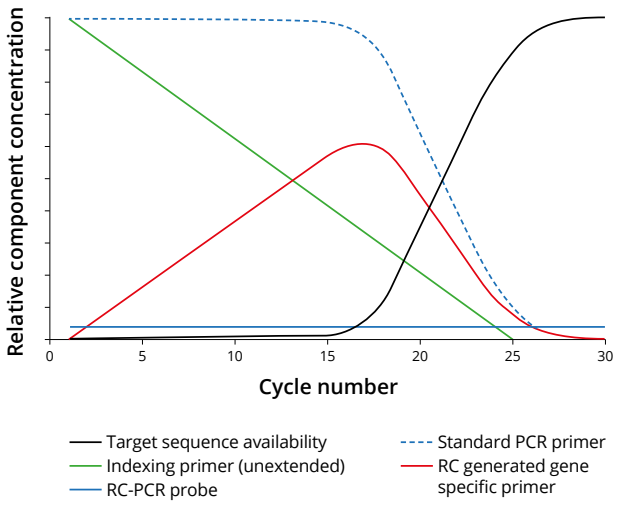


Figure 7 | RC-PCR Kinetics



NimaGen.

Confidence in Data Integrity and Validity

EasySeq™ Human Sample Identification Kits offer laboratories the simplest and safest workflow for NGS sample identification available in the market. RC-PCR in combination with the optimized panel provides a number of distinctive features and benefits that help safeguarding sample identity and data integrity:

Confidence in test results

- MAF SNPs selected in combination with RC-PCR kinetics provide high discrimination power
- Minimizing pipetting error and preventing sample swaps ensures correct data interpretation and the right critical samples are matched
- Sample tracking dye in pre-spotted Unique Dual Indexing plates ensures accuracy
- Also validated with challenging cfDNA and FFPE DNA samples
- Blood Direct Kit enables to include the DNA isolation step in the validation process, to exclude possible sample swabs or contamination

Cost-efficient workflow

- One closed-tube, single reaction workflow with simultaneous indexing and target amplification reduces labor time
- Single tube sample pooling for library clean-up significantly reduces usage of magnetic beads and consumables
- Well-balanced read distributions maximize sequencing instrument flow cell capacity
- Costly sample re-runs are prevented by capturing misidentified samples

Choice and Flexibility

- Complementary to both Whole Exome and Whole Genome Sequencing workflows
- Compatible with various Illumina® platforms
- 8 variants of 96-well breakable Unique Dual Indexing plates available for matching your sample workload, facilitating up to 768 samples per run

Ordering Information

EasySeq™ Human DNA Sample Identification Kit

Part Number	Description
RC-SID096	EasySeq™ Human DNA Sample Identification Kit *1 pool/sample, includes PCR Master Mix, 96 rxn*

EasySeq™ Human DNA Sample Identification Kit - Blood Direct

Part Number	Description
RC-BDSID096	EasySeq™ Human DNA Sample Identification Kit - Blood Direct *1 pool/sample, includes PCR Master Mix, 96 rxn*

Magnetic Beads for NGS Library Clean-up

Part Number	Description
AP-005	AmpliClean™ Cleanup Kit, Magnetic Beads (AMPure XP alternative), 5 mL

Note: AmpliClean™ Magnetic Beads are ordered separately to complete the workflow from input DNA to sequencing-ready NGS libraries.

Unique Dual Index Plates for use with EasySeq™ Human DNA Sample Identification Kits

Part Number	Description
IDX096-U01	96 Dehydrated, Colored Unique Dual Indexes *Pre-spotted in 96-well plate - UDI #0001-0096*
IDX096-U02	96 Dehydrated, Colored Unique Dual Indexes *Pre-spotted in 96-well plate - UDI #0097-0192*
IDX096-U03	96 Dehydrated, Colored Unique Dual Indexes *Pre-spotted in 96-well plate - UDI #0193-0288*
IDX096-U04	96 Dehydrated, Colored Unique Dual Indexes *Pre-spotted in 96-well plate - UDI #0289-0384*
IDX096-U05	96 Dehydrated, Colored Unique Dual Indexes *Pre-spotted in 96-well plate - UDI #0385-0480*
IDX096-U06	96 Dehydrated, Colored Unique Dual Indexes *Pre-spotted in 96-well plate - UDI #0481-0576*
IDX096-U07	96 Dehydrated, Colored Unique Dual Indexes *Pre-spotted in 96-well plate - UDI #0577-0672*
IDX096-U08	96 Dehydrated, Colored Unique Dual Indexes *Pre-spotted in 96-well plate - UDI #0673-0768*

NimaGen.

Product and Company Information

NimaGen B.V.
Hogelandseweg 88
6545 AB Nijmegen
The Netherlands

T +31 (0)24 820 0241
E info@nimagen.com
www.nimagen.com

Product Names

- EasySeq™ Human DNA Sample Identification Kit
- EasySeq™ Human DNA Sample Identification Kit - Blood Direct

Product Use

For Research Use Only

Version 1.1 – October 2023