

EasySeq™

Human Gene Sequencing *Human WES WGS Sample Tracking 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.

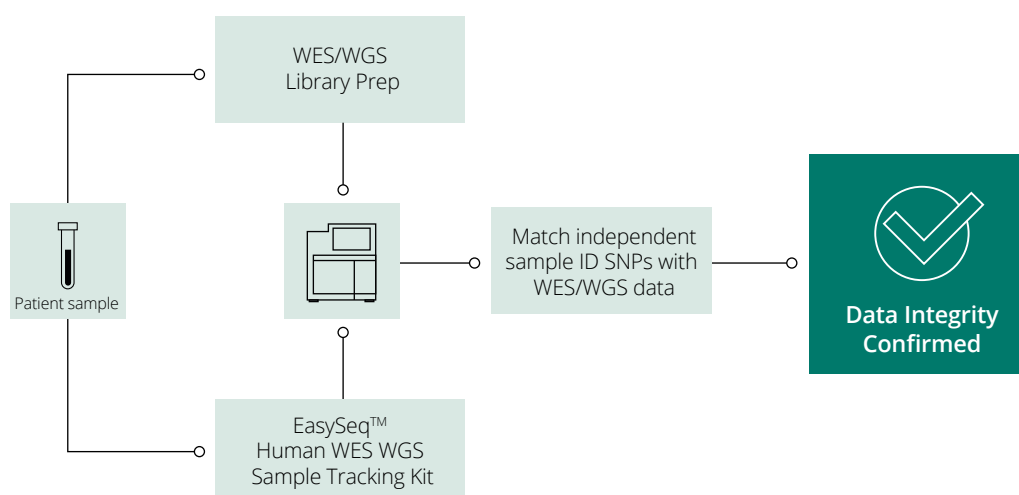
A method for independent confirmation of sample identity is therefore highly desirable, preferably using an identification method that is intrinsic to the WGS/WES data of the biological sample and simple to incorporate into the existing NGS workflow.

EasySeq™ Human WES WGS Sample Tracking Kit, powered by patented Reverse Complement PCR (RC-PCR) technology,

facilitates 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 WES WGS Sample Tracking is complementary to both Whole Exome and Whole Genome Sequencing Sample workflows (Figure 1). Sample IDs are matched with WES/WGS data and, as a result, data integrity and validity can be confirmed.

Figure 1 | Integrated Sample Tracking and Data Confirmation Workflow



EasySeq™ NGS Library Prep by RC-PCR

The Next Revolution in Human Genetics NGS

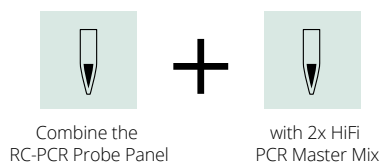
Our unique kits utilize RC-PCR technology to create a simple and safe one-tube, single reaction NGS library prep workflow. Multiplex target amplification, sequencing adaptor addition and sample-specific unique dual indexing all occur simultaneously in a closed-tube workflow, as simple as any normal PCR reaction. Multiplex reactions are then pooled and cleaned-up in a single tube using magnetic beads, thereby eliminating the need to clean-up reactions separately (Figure 2). Therefore, RC-PCR greatly reduces the amount of hands-on steps and the associated risks of pipetting errors, as well as sample swaps and cross-contamination.

RC-PCR kinetics results in high sensitivity and specificity because target-specific primers are synthesized during the reaction. Therefore, concentrations of primers and amplicons are more in line, which reduces potential primer dimerization and off-target primer binding (Figure 3).

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

Figure 2 | EasySeq™ RC-PCR workflow

1 Prepare the RC-PCR Master Mix



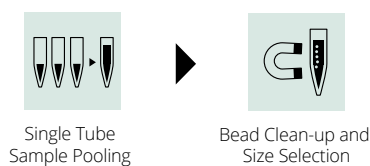
2 Dispense and add DNA



3 RC-PCR



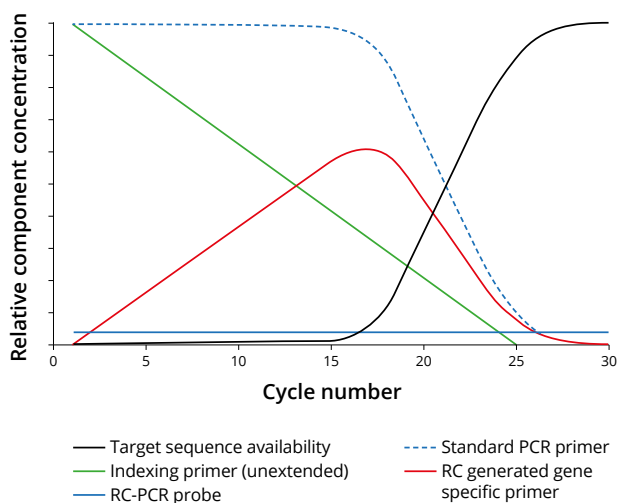
4 NGS Library Clean-up



5 Sequence



Figure 3 | RC-PCR Kinetics



Cost-efficient workflow

- Breakable index plates ensures optimal usage, minimizing waste
- 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 maximizes sequencing instrument flow cell capacity

Confidence in test results

- Closed-tube RC-PCR workflow significantly reduces hands-on time and pipetting error, minimizing risk of sample contamination
- Sample tracking dye in pre-spotted Unique Dual Indexing plates ensures accuracy
- Unique RC-PCR kinetics promote high-target specificity and coverage uniformity (optimized read-depth balance) from low DNA input)

Choice and flexibility

- 8 variants of 96-well breakable Unique Dual Indexing plates available for matching your sample workload, facilitating up to 768 samples
 - Automation compatibility for low to high-throughput workflows
 - Compatible with various Illumina® platforms
-



EasySeq™ Human WES WGS Sample Identification Kit

The Next Revolution in Human Genetics NGS

EasySeq™ Human WES WGS Sample Tracking Kit comprises an optimized panel of 36 single nucleotide polymorphisms (SNPs), the genotypic profile of which can be utilized to extract intrinsic identifiers from the human exome and genome: 34 exonic SNPs with high minor allele frequency (MAF), and Amelogenin X and Y to determine gender (Table 1).

Table 1 | EasySeq™ Human WES WGS Tracking Kit Exon and Amelogenin Targets

#	dbSNP	Chr#	Location HG38	Gene	MAF 1000 genomes	
1	rs1410592	1	179551371	NPHS2 , AXDND1	G	0.4123
2	rs2229546	1	67395837	IL12RB2	C	0.4449
3	rs10203363	2	227032260	COL4A4	T	0.495
4	rs2819561	3	4362083	SUMF1	A	0.2955
5	rs4688963	4	5748177	EVC	C	0.4714
6	rs309557	5	83538811	VCAN, VCAN-ASI	C	0.4687
7	rs4735258	8	93923709	PDP1	C	0.4952
8	rs4870723	8	120216440	COL14A1	A	0.4099
9	rs7465584	8	123975238	FER1L6	C	0.3962
10	rs1381532	9	97428498	TDRD7	A	0.4551
11	rs1536928	9	122629130	OR1B1	G	0.4101
12	rs1572983	9	101371346	BAAT	C	0.4371
13	rs577993	9	76706955	PRUNE2	C	0.4836
14	rs10883099	10	98459557	HPSE2	G	0.4702
15	rs4617548	11	1611867	SOX6	A	0.4942
16	rs7300444	12	884764	WNK1	T	0.399
17	rs495680	13	33129519	STARD13	T	0.4844
18	rs9532292	13	38859469	FREM2	G	0.4367
19	rs11158685	14	67575857	GPHN, PLEKHH1	G	0.4786
20	rs4577050	15	34236747	SLC12A6	G	0.4279
21	rs1026128	17	73200670	COG1	A	0.479
22	rs1037256	17	73201609	COG1	G	0.4746
23	rs1292053	17	59886176	TUBD1	G	0.4812
24	rs2159132	17	14102122	COX10	A	0.4918
25	rs1805034	18	62360008	TNFRSF11A	C	0.4058
26	rs3826616	18	63987229	SERPINB8	A	0.4515
27	rs9962023	18	23833905	LAMA3	T	0.3756
28	rs10373	20	6119441	FERMT1	A	0.4754
29	rs2296241	20	54169680	CYP24A1	A	0.4581
30	rs4148973	21	42903480	NDUFV3	T	0.4698
31	rs760482	22	38782696	DNAL4	G	0.4125
32	rs2073787	X	110451457	RTL9	A	0.374
33	rs5930933	X	136349199	ADGRG4	T	0.4734
34	rs6568050	X	112454808	RTL4	T	0.4856
35	AMELX	X				
36	AMELY	Y				

Confidence in Data Integrity and Validity

EasySeq™ Human WES WGS Sample Tracking Kit offers laboratories the simplest and safest workflow for NGS sample tracking 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:

- MAF SNPs selected in combination with RC-PCR kinetics provide high discrimination power
- Minimizing pipetting error and preventing sample swaps ensures that correct data interpretation and the right critical samples are matched
- Costly sample re-runs are prevented by capturing misidentified samples
- Complementary to both Whole Exome and Whole Genome Sequencing workflows
- Also validated with challenging cfDNA and FFPE DNA samples

Ordering Information

EasySeq™ NGS Library Prep Kit for Human Sample Identification

Part Number	Description
RC-HEST096	EasySeq™ Human WES WGS Sample Tracking Kit 1 pool/sample, includes PCR Master Mix, 96 rxn

Unique Dual Index Plates for use with Easy-Seq™ Human WES WGS Sample Tracking Kit

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 Name

EasySeq™ Human WES WGS Sample
Tracking Kit

Product Use

For Research Use Only

Version 2.3 – June 2023

Legal Notice

EasySeq and AmpliClean are trademarks of NimaGen.
Illumina is a registered trademark of Illumina, Inc.
AMPure XP is a trademark of Beckman Coulter.

Disclaimer

Although the information in this document is presented in good faith and believed to be correct at the time of printing, NimaGen makes no representations or warranties as to the completeness or accuracy of the information. NimaGen has no liability for any errors or omissions in the materials