Human Sample ID Kit

cat. n° PXL-SID-001

February 2020
version 1.0

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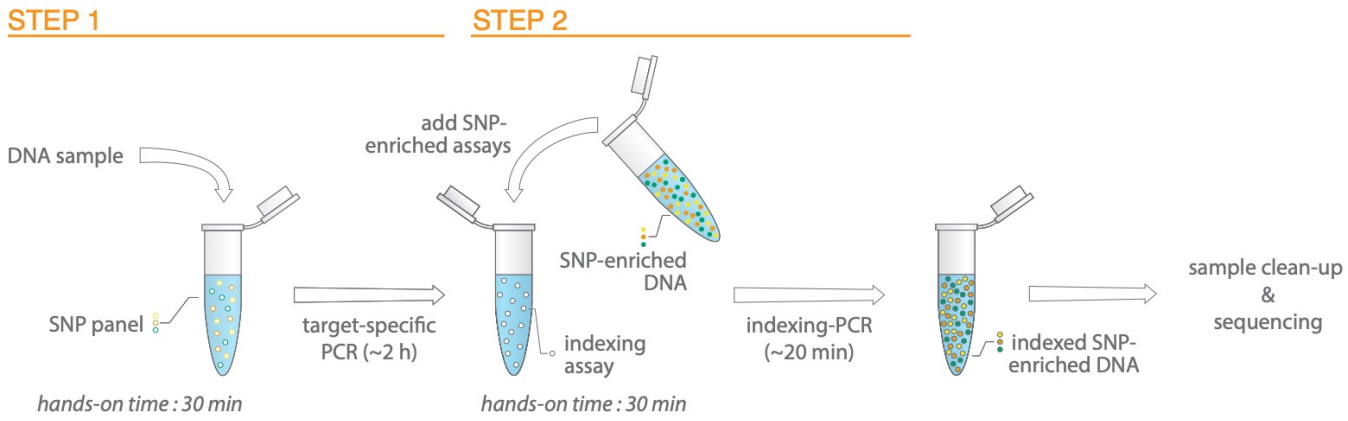
Human Sample ID Kit overview

In molecular diagnostics and research, exome, genome and gene panel sequencing are used routinely. Consequent to the custody transfers and complex workflows, DNA samples are prone to sample mix-ups. It has been estimated that such sample mix-ups occur in up to 1% of the cases, underscoring the need for an independent method for sample identity confirmation.

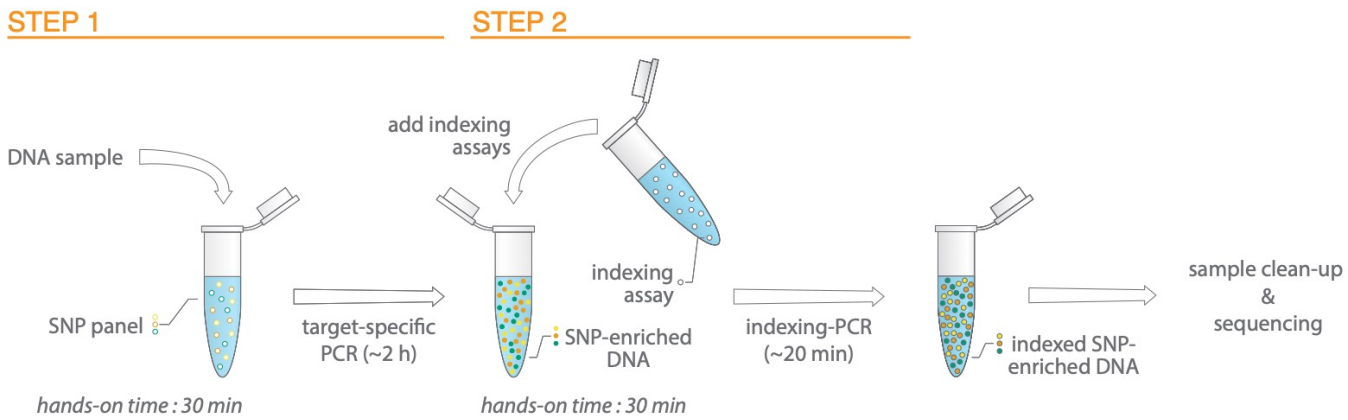
pxlence's Human Sample ID Kit is an easy and flexible resequencing assay that targets 44 polymorphic SNPs and 6 gender markers, creating a highly specific intrinsic genetic label for each sample. Using two simple PCR steps, sequencing-ready libraries are generated that are compatible with Illumina sequencing platforms. The Human Sample ID multiplex panel is designed to be compatible with established target enrichment platforms and has been validated for both high-quality genomic, low-quality formalin-fixed paraffin-embedded (FFPE) and cell-free DNA templates.

The protocol consists of two PCR steps: a multiplex PCR step for the simultaneous amplification of 50 targets and an indexing step for the addition of sample specific barcodes (dual indexes). Two workflow options are available: 1) the standard protocol consists of a two-step two-tube protocol (page 5/14) and 2) the alternative protocol is a two-step single-tube version (page 9/14). After a simple clean-up, the libraries are ready to be sequenced.

Standard protocol



Single-tube protocol



Required components

Supplied by pxlence

Human Sample ID Primers (7.5X)	included in the package	store at -20 °C
Master Mix 1 (2X)	included in the package	store at -20 °C
Master Mix 2 (2X)	included in the package	store at -20 °C
Human Sample ID Index Set (7.5X)*	PXL-IND-001	store at -20 °C

*For more options see appendix 1 or contact info@pxlence.com.

Other suppliers

KAPA qPCR library Illumina Kit (KAPA Biosciences)	universal	KK4824
	for ABI Prism	KK4835
	for Bio-Rad	KK4844
	for Roche LightCycler 480	KK4854
QIAquick PCR Purification Kit (Qiagen)	50 columns	28104
	250 columns	28106
AMPure XP for PCR purification (Beckman Coulter)	5 mL	A63880
	60 mL	A63881
	450 mL	A63882

Standard protocol

Multiplex PCR

1. Setup the multiplex PCR reaction by mixing the following components in a 0.2 mL PCR tube.

	volume (µL)
Master Mix 1 (2X)	7.5
Human Sample ID primers (7.5X)	2
template DNA (0.2-20 ng)	1 to 5.5
water	0 to 4.5
total reaction volume:	15

2. Seal the tube, vortex briefly and spin down

3. Place the tube in a thermal cycler and run the Multiplex PCR thermal cycling program

Multiplex PCR program

	temperature (°C)	time (s)	number of cycles
step 1	95	180	1
step 2	95	15	25
step 3	60	240	
step 4	12	∞	1

4. Take the PCR tube out of the thermal cycler upon completion of the Multiplex PCR program

Note: You can safely stop at this point and store the Multiplex product at -20 °C before indexing.

Indexing PCR

1. Setup the indexing PCR reaction by mixing the following components in a new 0.2 mL PCR tube. As template, 2 µL of the Multiplex PCR product is used.

	volume (µL)
Master Mix 2 (2X)	7.5
i7 index primer (7.5X)	2
i5 index primer (7.5X)	2
Multiplex PCR product	2
water	1.5
total reaction volume:	15

2. Seal the tube, vortex briefly and spin down

3. Place the tube in a thermal cycler and run the Indexing PCR program

Indexing PCR program

	temperature (°C)	time (s)	number of cycles
step 1	95	180	1
step 2	95	15	5
step 3	62	120	
step 4	12	∞	1

4. Take the PCR tube out of the thermal cycler upon completion of the Indexing PCR thermal cycling program.

   Note: You can safely stop at this point and store the indexed PCR product at -20 °C before pooling of the libraries.

Library pooling

Option A: volumetric pooling

While this method provides a time saving and cost-effective approach for pooling libraries, it may introduce more variance in the coverage distribution of the reads across your samples during sequencing (compared to option B).

1. Add equal volumes of each Indexed reaction (e.g. 2-5 µL) to a clean microcentrifuge tube and mix by vortexing.

Option B: equimolar pooling

This method will ensure the most even distribution of the reads across your samples during sequencing.

1. Follow the recommendations of your preferred qPCR library quantification method (e.g. KAPA qPCR library Illumina kit) and quantify triplicate samples using 1:80 000 and 1:400 000 dilutions.

   Important: For calculation of the library concentrations, use an average library size of 217 bp.

2. Pool the individual libraries in a clean microcentrifuge tube to a proper concentration (e.g. 10-30 nM) and mix by vortexing.

Library pool clean-up

Clean-up of the library is required to remove residual oligonucleotides and products that could impair the sequencing performance. It is recommended to use either the QIAquick PCR Purification kit of Qiagen (see manufacturer for further details) or AMPure XP beads of Beckman Coulter (see below).

1. Before use, allow the AMPure XP beads to reach room temperature and homogenize by vortexing thoroughly.
2. Prepare a fresh 500 µL molecular grade 70% ethanol solution.
3. Transfer 100 µL of the Human Sample-ID library pool to a new 1.5 mL microcentrifuge tube.

   Note: If the total volume of your library is below 100 µL, adjust the volume to 100 µL using nuclease-free water or Tris-HCl (pH 8) buffer.

4. Add 180 µL (ratio 1.8:1) of homogenous AMPure XP beads and mix by pipetting up and down.
5. Incubate at room temperature for 5 min.
6. Briefly pulse-centrifuge and place the tube on a magnetic stand until the solution becomes clear. Approximately 5 min.

7. Keep the tube on the magnetic stand and carefully remove and discard the cleared solution. Do not disturb the beads while removing the supernatants.

8. Add 200 µL of 70% ethanol while keeping the tube on the magnetic stand

9. Incubated at room temperature for 1 min and remove the ethanol

10. Repeat the ethanol washing steps 8 and 9

11. Briefly spin down the tube and let the beads resettle on the magnetic stand for 30 s. Pipet any residual ethanol from the tube.

12. Allow the samples to air dry until the residual ethanol has completely evaporated.

13. Add 25 µL of nuclease-free water or Tris-HCl (pH 8) to the tube and vortex thoroughly to resuspend the beads.

14. Incubate at room temperature for 2 min.

15. Place the tube back on the magnetic stand until the solution becomes clear. Approximately 2 min.

16. Transfer the cleared solution (approximately 25 µL) to a new microcentrifuge tube. Do not disturb the beads while removing the supernatants.

Library pool quantification

1. Follow the recommendations of your preferred qPCR library quantification method (e.g. KAPA qPCR library Illumina kit) and quantify triplicate samples using 1:80 000 and 1:400 000 dilutions.

2. Dilute the library pool to your preferred loading concentration for your Illumina instrument.

   Important: For calculation of the library concentrations, use an average library size of 217 bp.

Single-tube protocol

Multiplex PCR

1. Setup the multiplex PCR reaction by mixing the following components in a 0.2 mL PCR tube

	volume (µL)
Master Mix 1	7.5
Human Sample ID primers (7.5X)	2
template DNA (0.2-20 ng)	1 to 5.5
water	0 to 4.5
total reaction volume:	15

2. Seal the tube, vortex briefly and spin down.

3. Place the tube in a thermal cycler and run the Multiplex PCR thermal cycling program.

Multiplex PCR program

	temperature (°C)	time (s)	number of cycles
step 1	95	180	1
step 2	95	15	25
step 3	60	240	
step 4	12	∞	1

Indexing PCR

4. Take the PCR tube out of the thermal cycler upon completion of the Multiplex PCR thermal cycling program

5. Create the indexing PCR reaction by adding 2 µL of i7 index primer (7.5X) and 2 µL of i5 index primer (7.5X) to the Multiplex PCR product.

6. Seal the tube, vortex briefly and spin down

7. Place the tube in a thermal cycler and run the Indexing PCR thermal cycling program without the enzyme activation step.

Indexing PCR program

	temperature (°C)	time (s)	number of cycles
step 1	95	15	5
step 2	62	120	
step 3	12	∞	1

8. Take the PCR tube out of the thermal cycler upon completion of the Indexing PCR thermal cycling program.

   Note: You can safely stop at this point and store the Indexed product at -20 °C before pooling of the libraries.

Library pooling, clean-up and quantification

The following steps are identical to the standard protocol. Continue the library pooling, clean-up and quantification as described at page 6 to 7.

Appendix 1: Indexes

Human Sample ID Index Set uses a combinatorial indexing system. The twelve i7 indexes can be combined with any of the eight i5 indexes, forming in total 96 dual index combinations.

Adapter sequence

The following sequence is used for Read 1 and Read 2 adapter trimming.

CTGTCTCTTATACACATCT

i7 Indexes

index name	i7 bases for sample sheet
i7-01	TAAGGCGA
i7-02	CGTACTAG
i7-03	AGGCAGAA
i7-04	GGACTCCT
i7-05	TAGGCATG
i7-06	CTCTCTAC
i7-07	CGAGGCTG
i7-08	AAGAGGCA
i7-09	GTAGAGGA
i7-10	GCTCATGA
i7-11	CGGAGCCT
i7-12	TAGCGCTC

i5 indexes

	i5 bases for sample sheet	
index name	NovaSeq, MiSeq, HiSeq 2000/2500	iSeq, MiniSeq, NextSeq, HiSeq 3000/4000
i5-01	TATCCTCT	AGAGGATA
i5-02	GTAAGGAG	CTCCTTAC
i5-03	ACTGCATA	TATGCAGT
i5-04	CGTCTAAT	ATTAGACG
i5-05	TCGACTAG	CTAGTCGA
i5-06	CCTAGAGT	ACTCTAGG
i5-07	GCGTAAGA	TCTTACGC
i5-08	TTATGCGA	TCGCATAA

More indexing options, including unique dual indexes, are available using Nextera™-compatible indexing primers from Illumina or IDT. For more information contact info@pxlence.com.

Appendix 2: Human Sample ID targets

chr	reference SNP ID	position	REF	ALT	MAF 1000genomes
chr1	rs1410592	179551371	A	G	0.412
chr1	rs2076356	209638541	T	G	0.433
chr1	rs2013162	209795339	C	A	0.404
chr2	rs2229267	169235885	A	G	0.461
chr2	rs1560221	178589667	A	A	0.488
chr2	rs2163009	178590480	T	T	0.488
chr2	rs10498027	214955289	G	A	0.350
chr2	rs3738985	44275649	A	A	0.297
chr4	rs4688963	5748177	T	C	0.471
chr4	rs2736982	87613083	A	A	0.326
chr5	rs4669	136056737	T	C	0.420
chr5	rs3088052	139121126	T	C	0.427
chr5	rs7823	54456158	T	T	0.422
chr7	rs10265207	33970334	C	T	0.428
chr7	rs7738	43807004	A	G	0.475
chr8	rs3808554	103324868	A	G	0.449
chr9	rs3124768	133439376	G	A	0.484
chr9	rs639225	27202872	A	G	0.414
chr9	rs7859201	74800368	A	C	0.461
chr10	rs6163	102837167	C	A	0.410
chr10	rs2673794	68166340	T	C	0.483
chr10	rs1131824	77184832	G	A	0.406
chr10	rs17109674	94032006	G	A	0.378
chr11	rs1043388	6608435	C	T	0.315
chr12	rs60637	1806958	C	A	0.489
chr12	rs7300444	884764	C	T	0.399
chr13	rs3742165	24892817	T	C	0.484
chr13	rs9532292	38859469	A	G	0.437
chr14	rs7161192	64170429	C	A	0.366
chr16	rs2296409	68679827	G	G	0.392
chr16	rs2296408	68679920	C	C	0.392
chr16	rs17715450	68695882	C	A	0.467
chr16	rs3762171	70512331	G	A	0.470
chr17	rs2285479	10632701	G	A	0.470
chr17	rs2285475	10639154	T	G	0.466
chr17	rs5910	44372421	G	A	0.397
chr17	rs1052706	73196524	G	A	0.449
chr18	rs9962023	23833905	T	T	0.376
chr18	rs2298628	49929553	C	T	0.411
chr19	rs2228611	10156401	T	T	0.466
chr19	rs11084673	32862558	G	A	0.280
chr20	rs10373	6119441	A	A	0.475
chr21	rs2249057	46353189	C	A	0.290
chr22	rs4820268	37073551	G	G	0.456
chrX	AMELX				
chrY	AMELY				
chrY	KDM5D				
chrY	SRY				
chrY	TXLNGY				
chrY	USP9Y				
chrY	UTY				

Appendix 3: Compatible enrichment platforms

All 44 included polymorphic SNPs in the Human Sample ID kit are carefully selected to be fully compatible with the following commonly used enrichment platforms.

manufacturer	enrichment kit
Agilent	SureSelect Human All Exon V5 [+UTR]
	SureSelect Human All Exon V6 [+UTR]
	SureSelect Human All Exon V7
	SureSelect Clinical Research Exome [+ v2]
	SureSelectXT Human All Exon V6+COSMIC
	SureSelect Focused Exome
	ClearSeq Inherited Disease Panel
Roche	SeqCap EZ Exome + UTR
	SeqCap EZ MedExome
	SeqCap EZ HGSC VCRome
	SeqCap EZ Prime Exome
	SeqCap EZ Inherited Disease Panel
Illumina	TruSeq DNA Exome
	AmpliSeq Exome Panel
	TruSight One Sequencing Panel [+ expanded]
IDT	xGen Exome Research Panel v1
	xGen Exome Research Panel v2

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