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Sample Identification with the AmpliSeq™ for Illumina Sample ID Panel

Optional product provides sample identification for use with any AmpliSeq for Illumina panel.

Introduction

The AmpliSeq for Illumina Sample ID Panel is an optional component in the AmpliSeq for Illumina Sequencing Solution. Compatible with any AmpliSeq for Illumina human DNA panel, the Sample ID Panel enables quick and accurate sample identification. The AmpliSeq for Illumina Sample ID Panel is included as part of the panel gene content in certain AmpliSeq for Illumina fixed panels. For all other panels, including community and custom panels, it is available as an accessory product (Table 1).

Panel content

The AmpliSeq for Illumina Sample ID Panel consists of eight single nucleotide polymorphism (SNP)-targeting primer pairs and one gender discriminating primer pair (Table 2). Sample ID Panel primer pairs can be added before template amplification to generate a unique fingerprint or signature for each sample.

Sample discrimination power

The AmpliSeq for Illumina Sample ID Panel can achieve a sample discrimination power of ~1:5000. This discrimination power assumes complete independence between SNPs, full conformance with Hardy-Weinberg assumptions, and no missing or incorrect sample ID genotyping calls. Actual discrimination power may vary. Provided study sizes are < 5000 independent, unrelated samples, the Sample ID Panel can accurately identify and discriminate every sample in the study.

Integrated, streamlined workflow

The AmpliSeq for Illumina Sample ID Panel requires only two added steps to any AmpliSeq for Illumina panel workflow: spike-in of prepared Sample ID Panel primers to the target amplification reaction and making the selection to include the Sample ID Panel in the data analysis. Aside from these two additional steps, library preparation, sequencing, and data analysis can proceed without further changes to the workflow for AmpliSeq for Illumina Panel being used. Reference Guides for all panels include instructions if the Sample ID Panel is being used.

Table 1: Overview of Sample ID Panel availability

Sample ID Panel included	Sample ID Panel available as accessory
BRCA Panel	Cancer Hotspot Panel v2
Comprehensive Panel v3	Comprehensive Cancer Panel
Myeloid Panel	Focus Panel
	Community DNA Panels
	On-Demand Panels
	Custom DNA Panels (human)

Table 2: Sample ID Panel content

Code no.	Chromosome	Start	End	ID	Ref allele	Alt allele
1	X	113114957	11315075	AMELX		
1	Y	6737913	6737999	AMELY		
2	3	193207380	193207380	rs6444724	T	C
3	4	169663615	169663615	rs6811238	T	G
4	5	178690725	178690725	rs338882	G	A
5	7	137029838	137029838	rs321198	T	C
6	10	17193346	17193346	rs3780962	A	G
7	12	6945914	6945914	rs2269355	C	G
8	18	9749879	9749879	rs9951171	G	A
9	22	33559508	33559508	rs987640	T	A

Data interpretation

The AmpliSeq for Illumina Sample ID Panel assigns every sample a signature, represented by a nine-letter code. The first letter in the code denotes the gender call for the sample. The remaining letters in the code denote the genotypes called at the eight SNPs in the panel ct (Table 3). For example the DNA sample NA12878 would be assigned FYGACRCRW, and the DNA sample NA12877 would be assigned MCTGYRSAW.

Output from Sample ID Panel analysis can be viewed in three formats: a .txt file with just the nine-letter codes, a .vcf file with more detailed information for additional downstream analysis, and a .csv (Excel-compatible) file with the signature codes and detailed information for ease of viewing.

Table 3: Sample ID Panel signature code

Genotype call	Code (IUPAC)	Genotype call	Code (IUPAC)
AC	M	GT	K
AG	R	AA	A
AT	W	CC	C
CG	S	GG	G
CT	Y	TT	T
No call	N		

Gender call	Code
Male	M
Female	F
No call	N

Accurate sample identification

To demonstrate the accuracy of the AmpliSeq for Illumina Sample ID Panel, libraries were prepared from 12 biological samples (four replicates per sample) using the AmpliSeq for Illumina Comprehensive Cancer Panel with Sample ID Panel spike-in during library prep. Sample ID Panel data output showed that all replicates for each sample have the same signature, as expectedct (Figure 1).

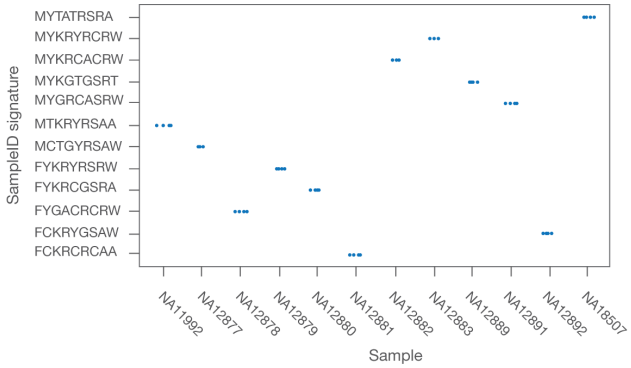

Figure 1: Accurate sample identification with theSample ID Panel — The Sample ID Panel was included as part of library prep and sequencing with the AmpliSeq for Illumina Comprehensive Cancer Panel. Analysis shows that replicates for each of 12 biological samples all have the same Sample ID signature.

Sample ID Panel applications

The AmpliSeq for Illumina Sample ID Panel provides sample tracking for various sequencing applications, including:

- **Multiple samples from the same individual:** The Sample ID Panel enables sample tracking for multi-tissue samples collected from the same individual, or multiple samples collected over time for longitudinal studies.
- **Samples from closely related individuals:** The Sample ID Panel can be used to identify and track samples from closely related individuals (eg, siblings), as the signatures will be highly similar.
- **Paired tumor/normal samples:** The Sample ID Panel can be used for verification and tracking of tumor and normal samples from the same individual. Mutations in the tumor sample may result in an inconsistent signature.

Sample ID Panel troubleshooting

Certain potential challenges with interpretation of Sample ID Panel results have been anticipated and possible solutions are provided (Table 4).

Table 4: Sample ID Panel troubleshooting

Observation	Possible cause	Solution
Numerous "no calls" (Ns) in signature	Sample ID Panel was not spiked in correctly to the primer pool	Repeat library prep with proper spike-in of Sample ID Panel
	SampleID amplicons had low coverage	Check coverage in SampleID .csv file
Inconsistent signature of samples from the same individual	Sample contamination	Avoid cross-contamination when adding or transferring samples and during PCR setup
	Low coverage for SampleID amplicons	Check coverage in SampleID .csv file
	Mutations in tumor sample	Additional sequencing to confirm

Summary

The AmpliSeq for Illumina Sample ID Panel is an optional component in the AmpliSeq for Illumina Sequencing Solution. Compatible with any AmpliSeq for Illumina panel, the Sample ID Panel features an integrated, streamlined workflow with only one added step during library prep and automated data analysis. With a discrimination power of ~1:5000, the AmpliSeq for Illumina Sample ID Panel provides added value for sample identification and tracking for various sequencing applications.

Ordering Information

Order AmpliSeq for Illumina products online at www.illumina.com

Product	Catalog No.
AmpliSeq for Illumina Sample ID Panel (96 reactions)	20019162

Learn More

To learn more about the AmpliSeq for Illumina Sequencing Solution, visit www.illumina.com/products/by-brand/ampliseq.html

References

1. Pakstis AJ, Speed WC, Fang R, et al. SNPs for a universal individual identification panel. *Hum Genet*. 2010;127(3):315–324.
2. Coriell Institute for Medical Research. www.coriell.org. Accessed September, 2018.

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