TruSeq® Bovine Parentage Sequencing Panel

A high-value solution for parentage testing of cattle breeds with added genotyping of relevant traits in a single assay.

Highlights

- **Enhanced Content Increases Value**
  Sequencing panel includes all International Society of Animal Genetics (ISAG) recommended content plus disease-associated and economically relevant traits
- **Robust Solution Controls Costs**
  Intelligent content design, high accuracy, and reliability eliminate need for costly multiple or repeat testing
- **Intuitive Software Simplifies Data Interpretation**
  Analysis software converts sequencing results to genotype calls automatically without the need for bioinformatics expertise

Introduction

The TruSeq Bovine Parentage Sequencing Panel is a high-value solution for parentage testing of multiple beef and dairy cattle breeds. By offering more information in a single assay than from parentage testing alone, service labs can use the TruSeq Bovine Parentage Sequencing Panel to make more informed herd management recommendations.

A Robust Solution for Parentage Assignment

Though microsatellites or short tandem repeats (STRs) have been the preferred genetic markers for parentage control in cattle, the industry is transitioning to single nucleotide polymorphism (SNP)-based methods.[1] SNPs offer several advantages:[2-4]

- Low mutation rates between generations
- Robust data interpretation
- Compatible with automated, high-throughput analysis
- Suitable for standardized representation of genotyping results

The TruSeq Bovine Parentage Panel uses proven Illumina next-generation sequencing (NGS) technology to deliver accurate genotyping results for SNP-based parentage determination. The streamlined workflow, including library preparation through data analysis, increases efficiency. The high accuracy and reliability reduces the need for repeat testing, helping to control costs. The TruSeq Bovine Parentage Sequencing Panel consolidates parentage assessment and added genotyping of biologically relevant traits into a single test (Table 1).

Table 1: TruSeq Bovine Parentage Sequencing Panel Content

Description	No. of SNPs
SNPs Used for Parentage Assessment	
ISAG Core SNP Targets	100
ISAG Additional SNP Targets	100
Variant Targets Associated with Genetic Disorders/Diseases	
Prenatal/Perinatal Lethal Conditions (HH1, HH3, HH4, MH1, etc)	12
Postnatal/Adult Lethal Conditions (Citrullinemia, Congenital Muscular Dystonia, Cardiomyopathy, Myoclonus, etc)	17
Nonlethal, Adverse Conditions (Marfan Syndrome, Mulefoot, Protoporphyria, Hemophilia A, etc)	19
Variant Targets Associated with Economically Valuable Traits	
Male Fertility	1
Growth and Appearance	4
Meat Quality	4
Milk Quality	6
Total Number of SNP/Variant Targets	263

Content Design Strategy

Developed by Illumina scientists in accordance with recommendations from the ISAG, the TruSeq Bovine Parentage Sequencing Panel contains 263 variant targets. These cover all ISAG-recommended content[1] and additional economically valuable and disease-associated markers (Table 1).

Simple, Integrated Workflow

The TruSeq Bovine Parentage Panel features a streamlined workflow that goes from DNA to report in 1.5 days with minimal hands-on time.

Library Prep

A key element of Illumina NGS is high-quality library preparation. Illumina library prep protocols can accommodate a range of throughput needs, from lower-throughput protocols for small laboratories to fully automated library preparation workstations for large laboratories or genome centers. Different methods are employed to capture regions of interest from input DNA and prepare sequencing libraries. The TruSeq Bovine Parentage Panel uses an amplicon generation approach.

Amplicon generation employs a hybridization followed by extension-ligation approach, creating a single-stranded template from a double-stranded genomic DNA (gDNA) population that is later amplified via PCR (Figure 1). The library preparation protocol can be completed with less than 2.5 hours of hands-on time (Figure 2).

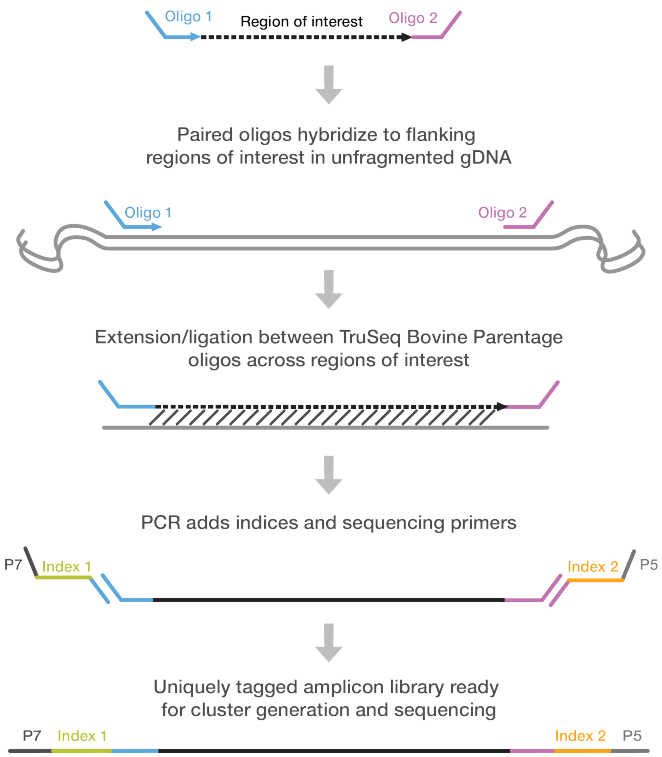

Figure 1: Amplicon Chemistry–The TruSeq Bovine Parentage Sequencing Panel uses an amplicon-based method to prepare sequencing-ready libraries.

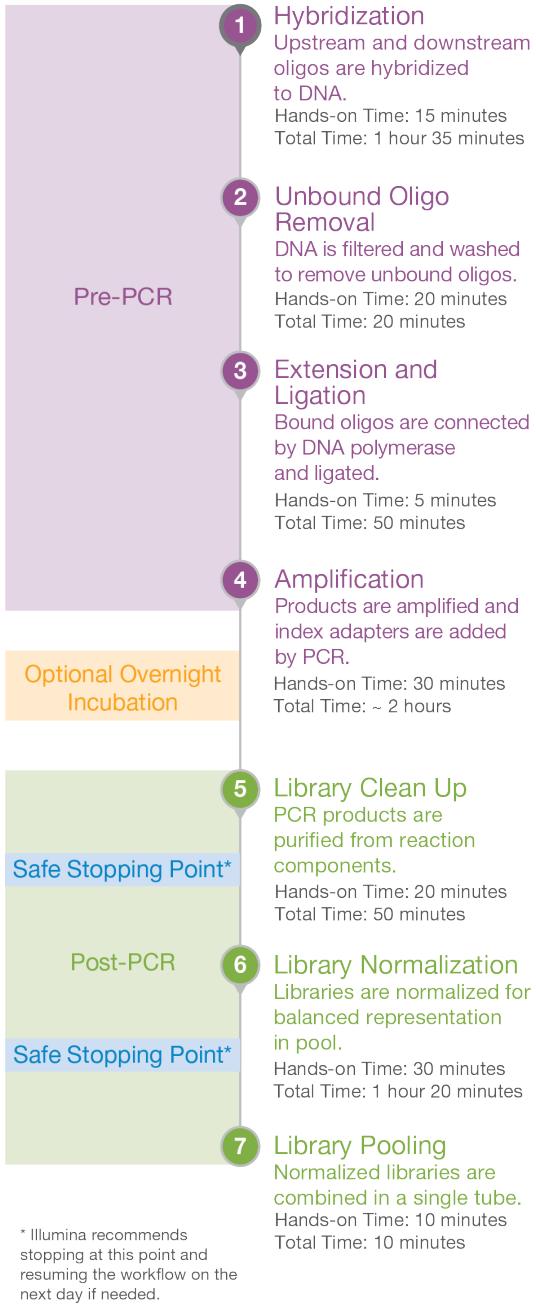

Figure 2: Library Prep Workflow–The TruSeq Bovine Parentage Kit enables simplified library prep with less than 2.5 hours of hands-on time.

Sequencing

After libraries are prepared, they are loaded into a flow cell where they undergo cluster generation. This involves the capture of DNA templates by surface-bound oligos complimentary to adapters added during library prep. Each library fragment is then amplified into distinct, clonal clusters through bridge amplification. The templates are now ready for sequencing on a MiSeq® System (Figure 3), which offers a user-friendly, intuitive interface for easy run setup and operation. The MiSeq System features the high data quality and reliability of Illumina sequencing by synthesis (SBS) chemistry (Table 2), responsible for generating greater than 90% of the world's sequencing data.*

Figure 3: MiSeq System–The MiSeq System integrates cluster generation, amplification, sequencing, and data analysis into a single instrument, enabling rapid, cost-effective NGS.

Data Analysis

After a sequencing run on the MiSeq System is completed, Sequence Genotyper Analysis Software performs genotype interpretations automatically. The software converts variant call files (VCF) generated on the MiSeq System into genotype call files using predesignated target markers for bovine parentage. Sequence Genotyper Software can organize source data and target files, and generate reports for comparison across samples. The Sequence Genotyper Software is available for download from the Illumina website at support.illumina.com/. A MyIllumina account is required.

Table 2: MiSeq System Performance Metrics

MiSeq v3 (150-cycle) Sequencing Reagent Kit	
Read Length (bp)	1 × 121
Output (Gb)	3.8
Samples per Run	96
Run Time (hours)	15
Coverage of ISAG Targets	100%
Reproducibility	100%
Genotype Accuracy	99%

*Data calculations on file. Illumina, Inc. 2015.

Summary

The TruSeq Bovine Parentage Sequencing Panel eases the transition to SNP-based genotyping for parentage determination in cattle. Including all ISAG-recommended content, disease-associated markers, and economically relevant traits, the TruSeq Bovine Parentage Sequencing Panel maximizes the information gained from a single test. Additional economically valuable and disease-associated content enables service labs to make more informed herd management recommendations. The high accuracy and reliability of Illumina sequencing reduces the amount of required testing and shortens the time to answer, leading to efficiency gains and cost savings.

Learn More

To learn more about the TruSeq Bovine Parentage Sequencing Panel and other animal sequencing and genotyping options, visit www.illumina.com/agrigenomics

Ordering Information

Product	No. of Samples	Catalog No.
TruSeq Bovine Parentage Sequencing Panel (includes all necessary reagents)	96	20004795

References

1. ISAG cattle core and additional SNP panel 2013. International Society for Animal Genetics (ISAG) Web site. www.isag.us/committees.asp?autotry=true&ULnotkn=true. Accessed June 13, 2016.
2. Fernández ME, Goszczynski DE, Lirón JP, et al. Comparison of the effectiveness of microsatellites and SNP panels for genetic identification, traceability and assessment of parentage in an inbred Angus herd. *Genet and Mol Biol.* 2013;36(2):185–191.
3. Schütz E and Brenig B. Analytical and statistical consideration on the use of the ISAG-ICAR-SNP bovine panel for parentage control, using the Illumina BeadChip technology: example on the German Holstein population. *Genet Sel Evol.* 2015;47(1):3.
4. Allen AR, Taylor M, McKeown B, et al. Compilation of a panel of informative single nucleotide polymorphisms for bovine identification in the Northern Irish cattle population. *BMC Genet.* 2010;11:5.

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