# Illumina Sample Preparation Overview



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# Session Objectives

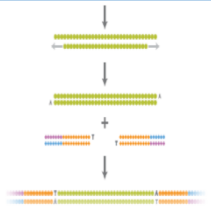
**By the end of this training, you will be able to:**

- Describe the different sample prep workflows
- Discuss sample prep best practices

# Illumina Sequencing Workflow

1

## Library Preparation



2

## Cluster Generation

cBot
MiSeq

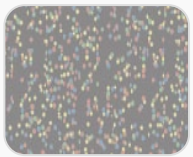
3

## Sequencing

HiSeq
HiScan SQ
GA IIx
MiSeq

4

## Data Analysis



ICS/RTA
CASAVA
MSR
BaseSpace

# Library Preparation Overview

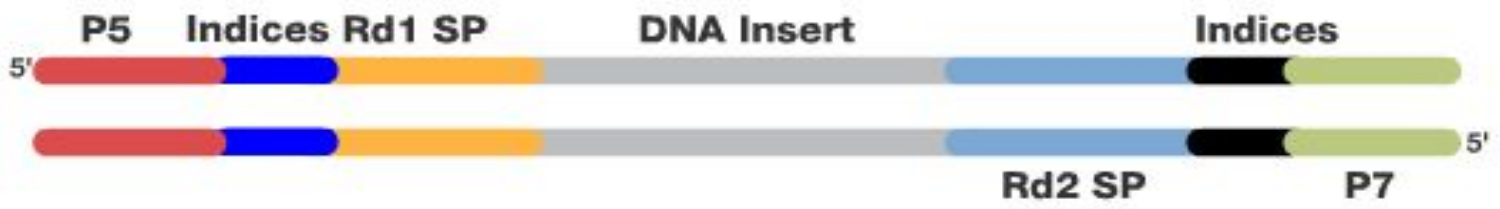
Nucleic Acid

Modify to proper insert size

Add adapters with sites for:

- Flow cell binding
- Sequencing primer binding

# No matter the input, all libraries end up looking similar




Dual Index Library shown

**The aim of the sample prep step is to obtain nucleic acid fragments with adapters attached on both ends**



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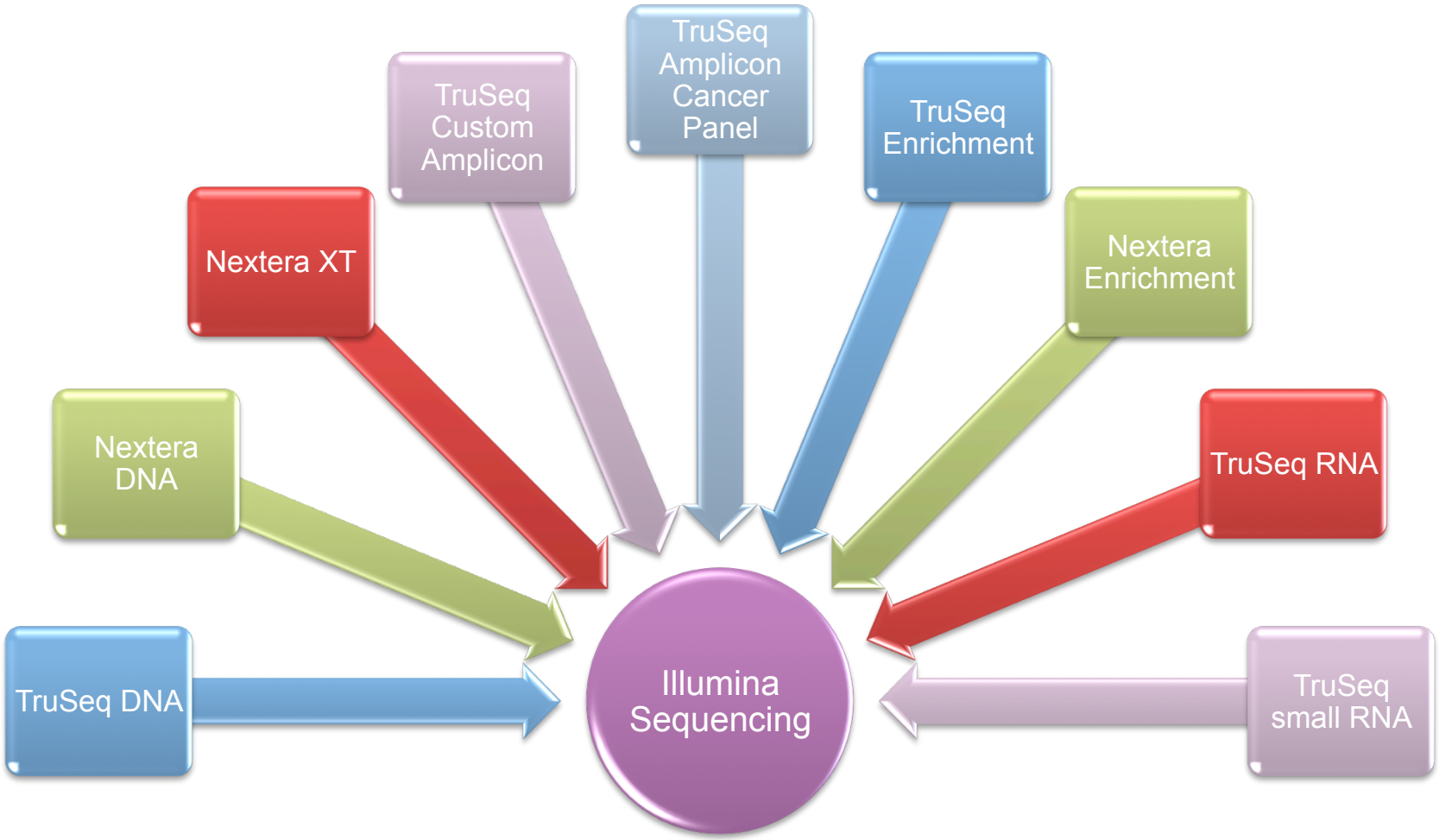
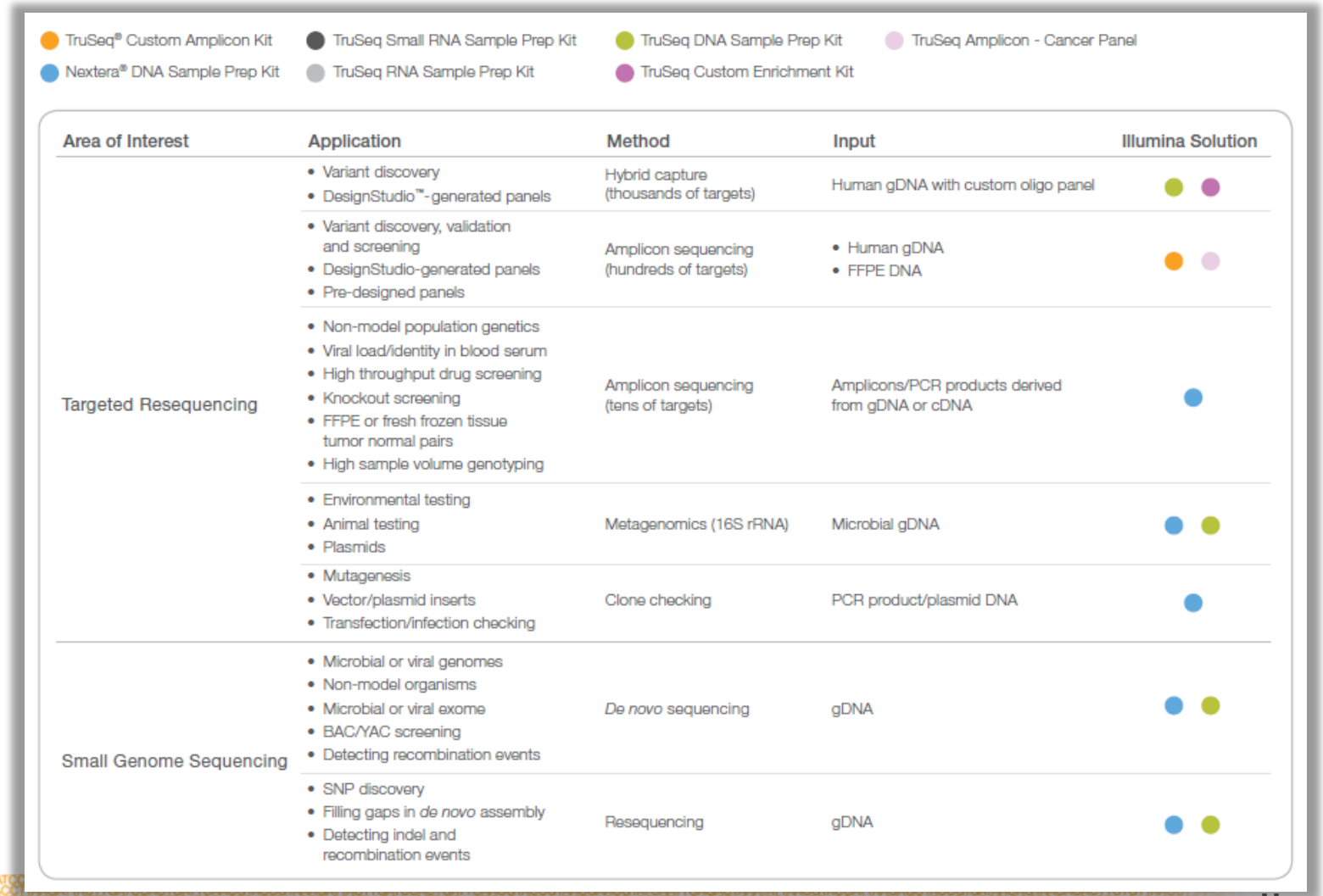
# Selecting the Right Kit



# Not sure which kit to use?
# Try the "Which Illumina Sample Prep Kit is right for me" document



TruSeq® Custom Amplicon Kit · TruSeq Small RNA Sample Prep Kit · TruSeq DNA Sample Prep Kit · TruSeq Amplicon - Cancer Panel
Nextera® DNA Sample Prep Kit · TruSeq RNA Sample Prep Kit · TruSeq Custom Enrichment Kit

| Area of Interest | Application | Method | Input | Illumina Solution |
|---|---|---|---|---|
| Targeted Resequencing | • Variant discovery<br>• DesignStudio™-generated panels | Hybrid capture (thousands of targets) | Human gDNA with custom oligo panel | |
| | • Variant discovery, validation and screening<br>• DesignStudio-generated panels<br>• Pre-designed panels | Amplicon sequencing (hundreds of targets) | • Human gDNA<br>• FFPE DNA | |
| | • Non-model population genetics<br>• Viral load/identity in blood serum<br>• High throughput drug screening<br>• Knockout screening<br>• FFPE or fresh frozen tissue tumor normal pairs<br>• High sample volume genotyping | Amplicon sequencing (tens of targets) | Amplicons/PCR products derived from gDNA or cDNA | |
| | • Environmental testing<br>• Animal testing<br>• Plasmids | Metagenomics (16S rRNA) | Microbial gDNA | |
| | • Mutagenesis<br>• Vector/plasmid inserts<br>• Transfection/infection checking | Clone checking | PCR product/plasmid DNA | |
| Small Genome Sequencing | • Microbial or viral genomes<br>• Non-model organisms<br>• Microbial or viral exome<br>• BAC/YAC screening<br>• Detecting recombination events | *De novo* sequencing | gDNA | |
| | • SNP discovery<br>• Filling gaps in *de novo* assembly<br>• Detecting indel and recombination events | Resequencing | gDNA | |



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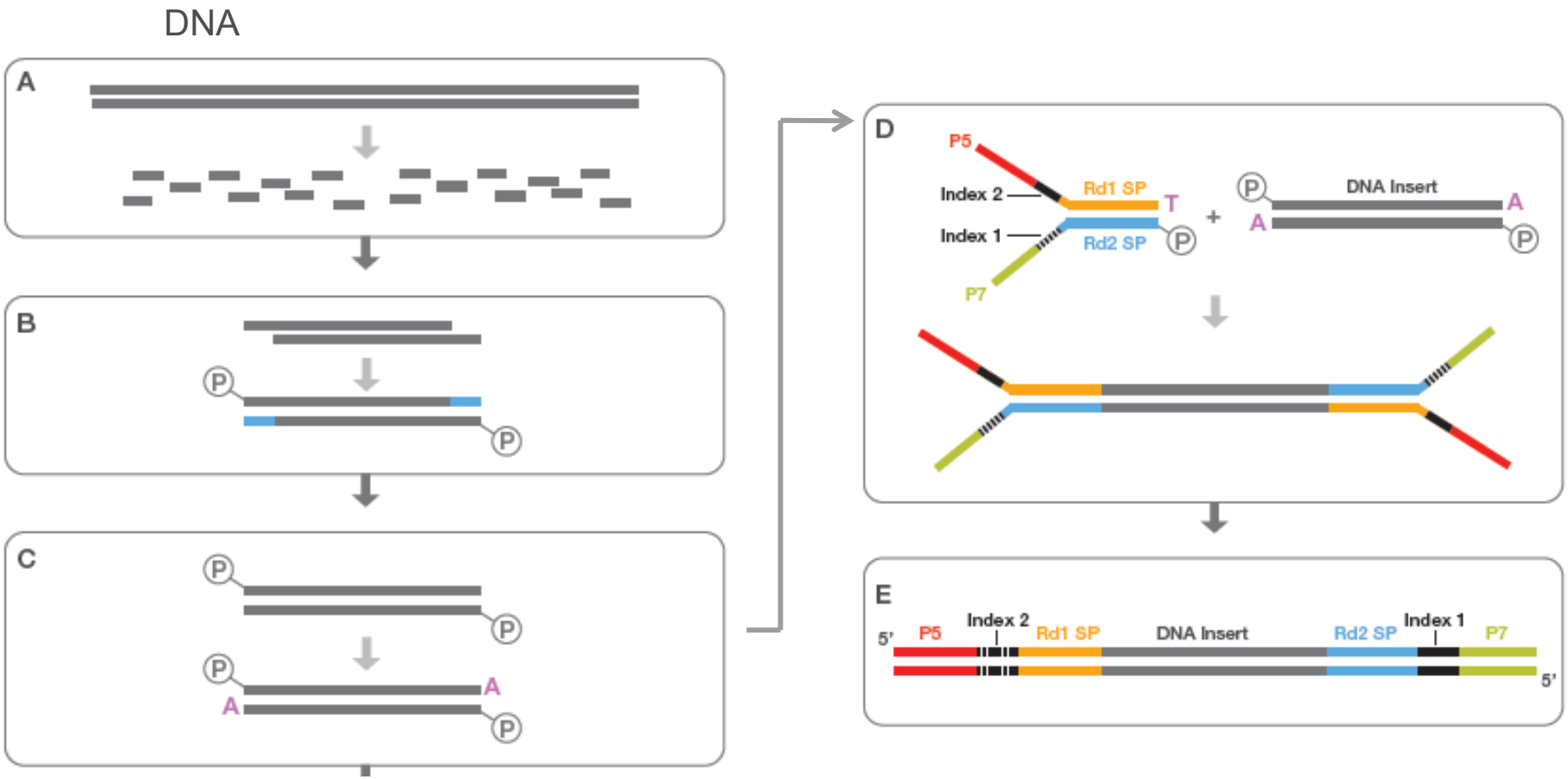
# DNA Sample Prep Options

# TruSeq DNA Sample Prep Kit Features

| | |
|---|---|
| Highlights | • Includes index sequence by default<br>• Single read or paired-end compatibility<br>• Methylated-Cs for epigenetic applications |
| Sample input and indices | • 1 μg DNA per sample<br>• Index up to 96 samples<br>• Parallel processing of up to 96 samples |
| Specific considerations | • Construct completed at the ligation step<br>• Contains internal quality controls<br>• Optimized for high quality genomic DNA |
| Suitable for: | • MiSeq<br>• GA IIx<br>• HiSeq |

# TruSeq DNA Sample Prep  Workflow



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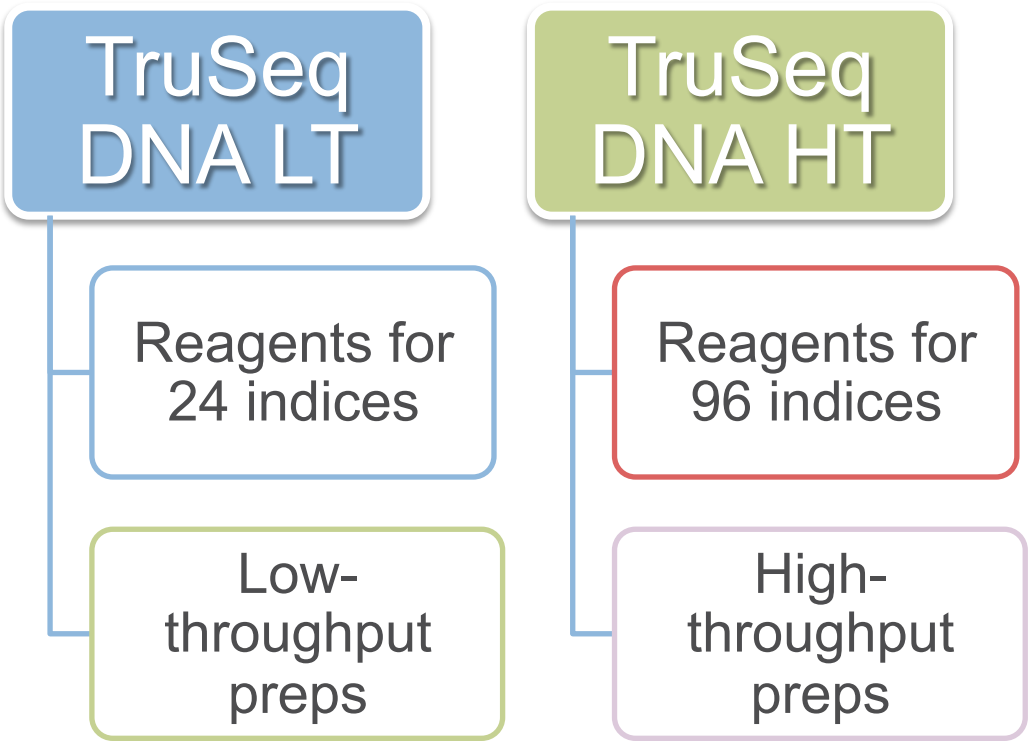
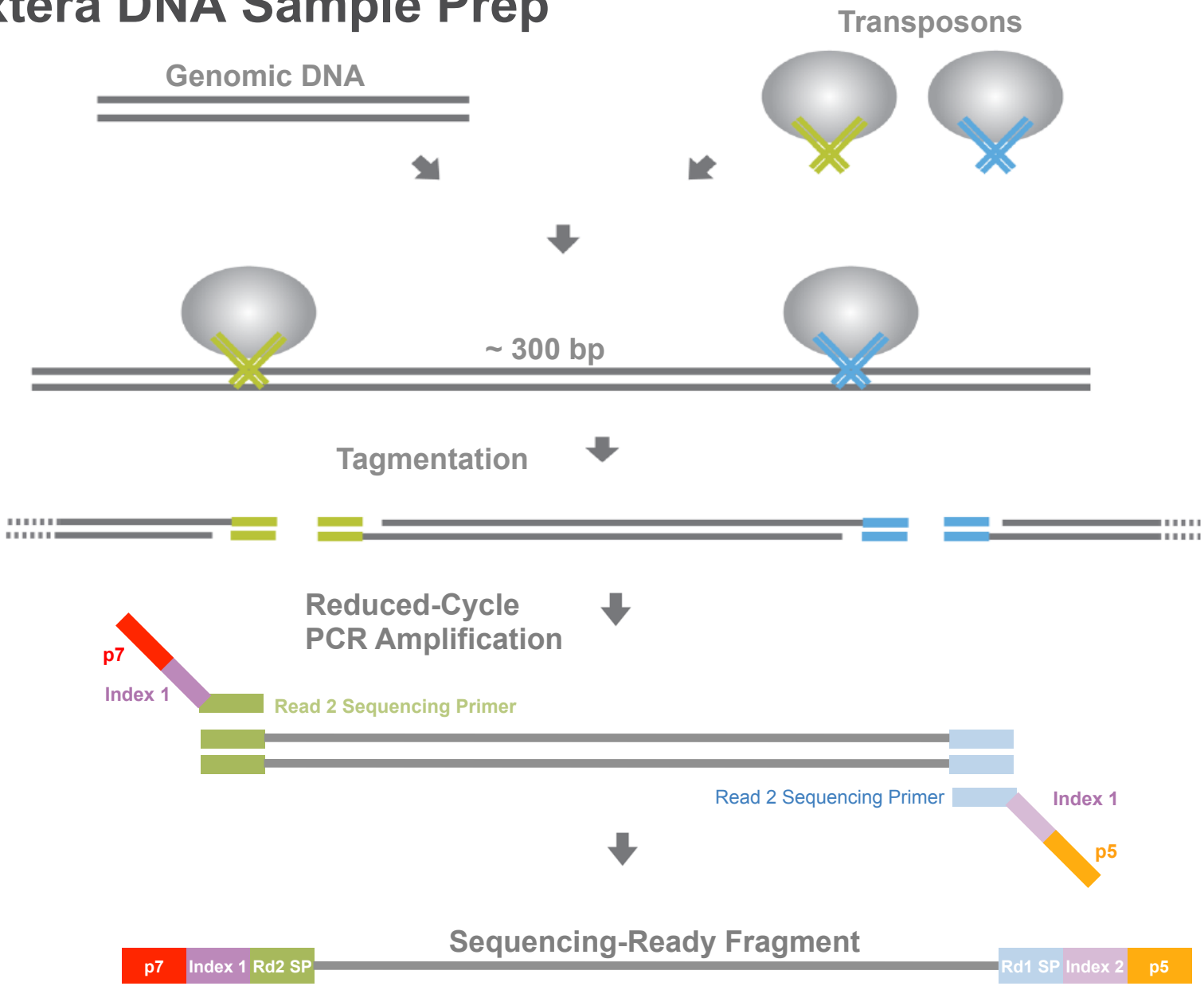
# TruSeq DNA: Two different sample prep options



# Nextera Sample Prep Kit Features

| | |
|---|---|
| Highlights | • Fastest sample prep, 90 min.<br>• Single read or paired-end compatibility |
| Sample input and indices | • 50 ng DNA per sample<br>• Index up to 96 samples<br>• Parallel processing of up to 96 samples |
| Specific considerations | • Construct completed at the PCR step<br>• Improved performance with GC regions<br>• Gel-free protocol |
| Suitable for: | • MiSeq<br>• GA IIx<br>• HiSeq |

# Nextera DNA Sample Prep



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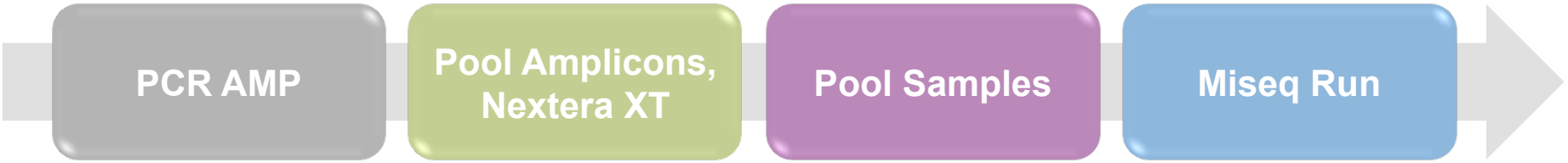
# Nextera XT Sample Prep Kit Features

| | |
|---|---|
| Highlights | • Fastest sample prep, 90 min.<br>• Optimized for small genomes, PCR amplicons and plasmids |
| Sample input and indices | • 1 ng DNA per sample<br>• Index up to 96 samples |
| Specific considerations | • No library quantification needed<br>• Sample normalization is included |
| Suitable for: | • MiSeq<br>• GA IIx<br>• HiSeq |

# Nextera XT for PCR Amplicons

*Quick workflow for long or short range PCR*




**Short Range - *CFTR***

| | | | |
|---|---|---|---|
| 7 Exons<br>255-416 bp range | 90 min total<br>15 min hands on | 12 samples | 2x36 (6.5hrs)<br>95%>Q30 |

**Long Range – *BRCA1/2***

| | | | |
|---|---|---|---|
| 22 Amplicons<br>1-6kb range<br>68.5kb total | 90 min total<br>15 min hands on | 5 samples | 2x76(15hrs)<br>95%>Q30 |

# Which version of Nextera should I use?

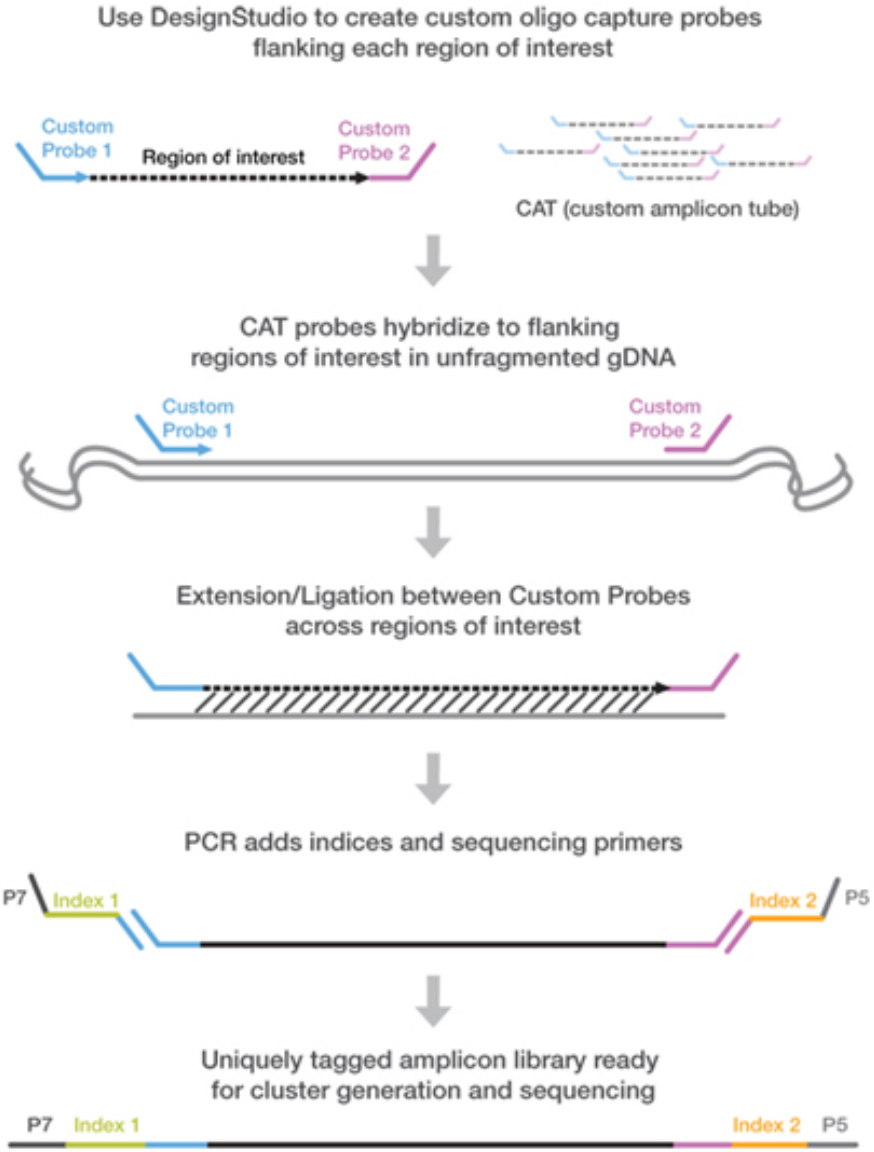
| | Nextera | Nextera XT |
|---|---|---|
| Applications | **Large/complex genomes** (human, mammalian, plants, invertebrates) | **Small genomes, amplicons, plasmids** (bacteria, archea, viruses, PCR amplicons, plasmids) |
| DNA input | 50 ng | 1 ng |
| Post tag cleanup? | Zymo | None |
| Sample normalization | Manual (e.g. bioanalyzer, qPCR) | Bead-based normalization included |
| Indexing | 96 | 96 |

# Amplicon and Enrichment Sequencing Options

# TruSeq Custom Amplicon Features

| | |
|---|---|
| Highlights | • Create projects with DesignStudio<br>• Integrated normalization procedure<br>• Amplicon Viewer software |
| Sample input and indices | • 250 ng DNA per sample<br>• Up to 384 amplicons per sample<br>• Index up to 96 samples |
| Specific considerations | • Construct completed at the PCR step<br>• Contains internal quality controls<br>• Gel-free protocol |
| Suitable for: | • MiSeq only! |

# TruSeq Custom Amplicon Workflow



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# TruSeq Amplicon – Cancer Panel Features

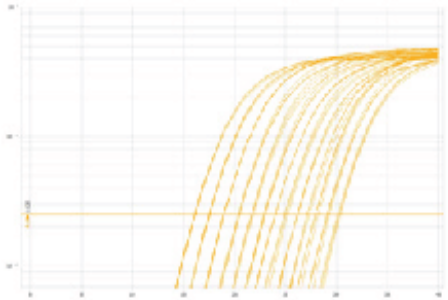
| | |
|---|---|
| Highlights | • Most relevant cancer loci in a single panel<br>• Suited for FFPE samples<br>• Integrated normalization procedure<br>• Amplicon Viewer software |
| Sample input and indices | • 250 ng DNA per sample<br>• 48 genes are targeted with 212 amplicons<br>• Index up to 96 samples |
| Specific considerations | • Construct completed at the PCR step<br>• Contains internal quality controls<br>• Gel-free protocol |
| Suitable for: | • MiSeq only! |

# TruSeq Amplicon – Cancer Panel

Pre-screen Samples

Assay Biochemistry

Sequencing and Analysis



FFPE QC Kit

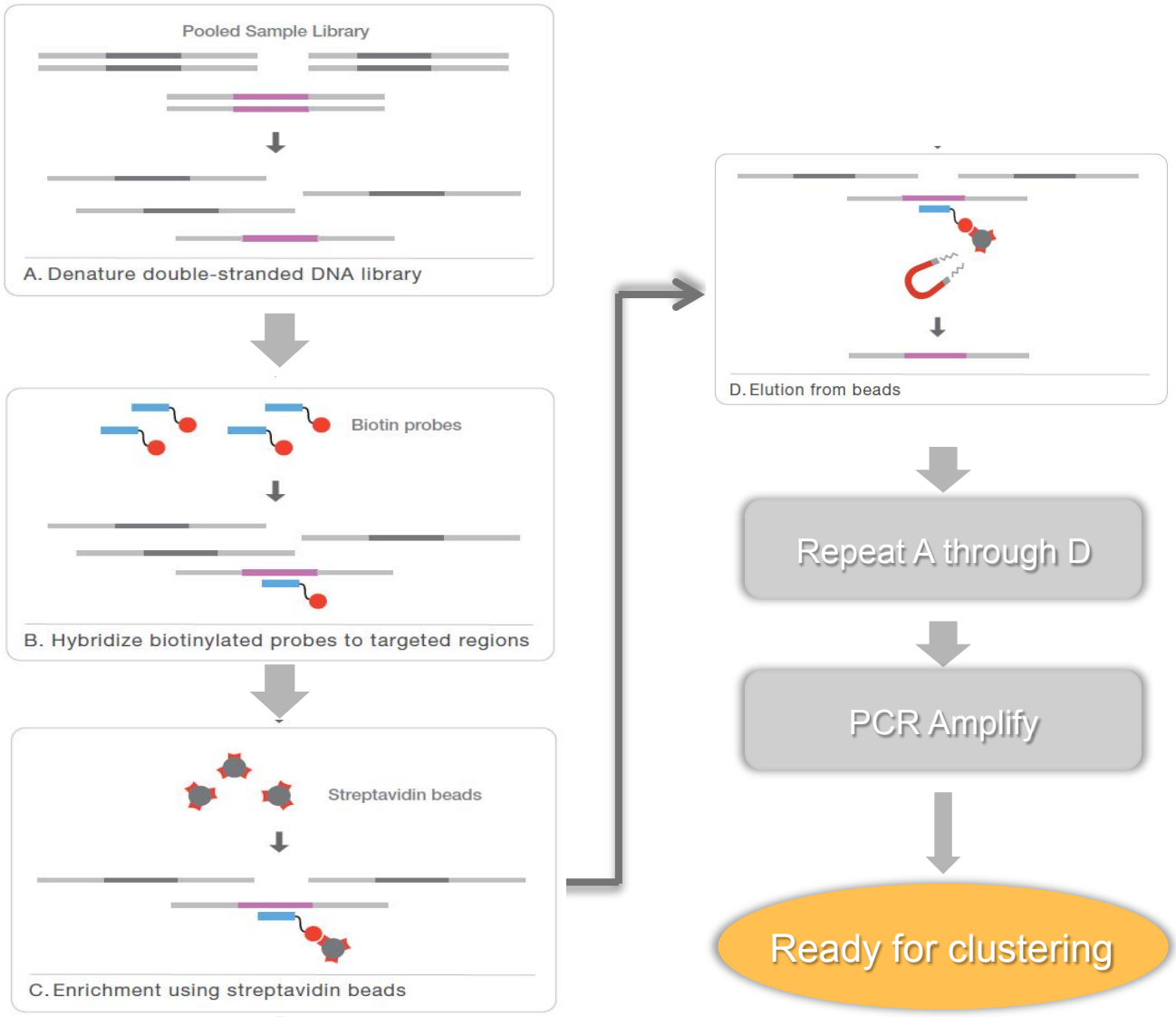
TruSeq Amplicon - Cancer Panel

Automated Sequencing and Variant Calling

# TruSeq Enrichment Sample Prep Kit Features

| | |
|---|---|
| Highlights | • Targeted resequencing of human exome and associated regulatory regions<br>• Create Custom Enrichment with DesignStudio |
| Sample input and indices | • 500ng TruSeq DNA library per enrichment<br>• Index up to 6 samples per Exome Enrichment<br>• Index up to 12 samples per Custom Enrichment |
| Specific considerations | • Contains internal quality controls<br>• Gel-free protocol |
| Suitable for: | • GA IIx<br>• HiSeq<br>• MiSeq (for certain Custom Enrichments) |

# TruSeq Enrichment Workflow Overview




Targeted resequencing to focus on subset of genome

**Exome**
Gene encoding sequences

**Custom**
Researcher/user-defined regions



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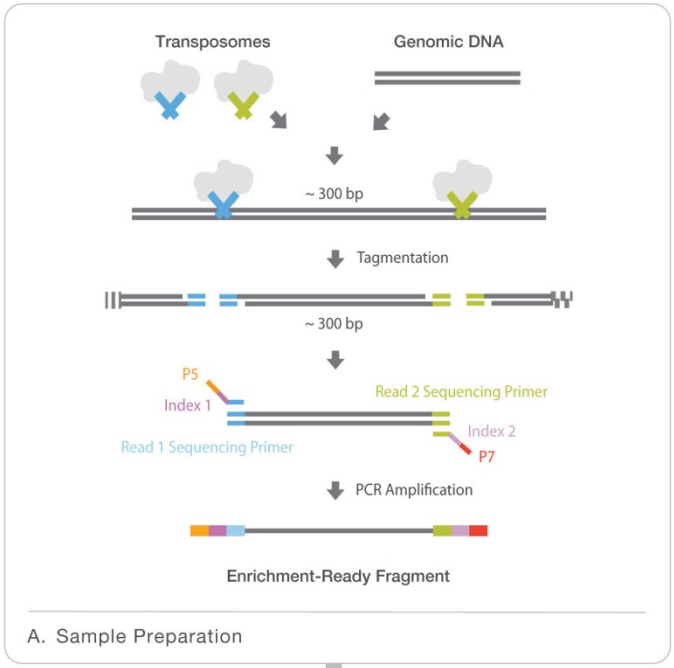
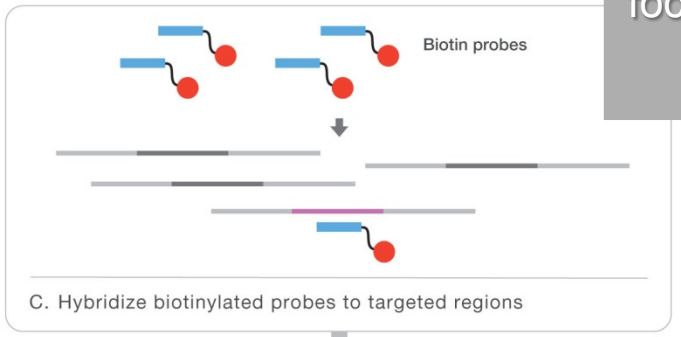
# Nextera Enrichment Sample Prep Kit Features

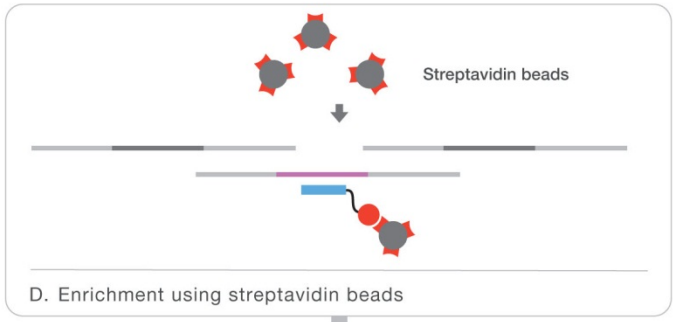
| | |
|---|---|
| Highlights | • Targeted resequencing of human exome and associated regulatory regions<br>• Create Custom Enrichment with DesignStudio |
| Sample input and indices | • 50 ng DNA input<br>• Index up to 12 samples per Enrichment |
| Specific considerations | • Contains internal quality controls<br>• Gel-free protocol |
| Suitable for: | • GA IIx<br>• HiSeq<br>• MiSeq (for certain Custom Enrichments) |

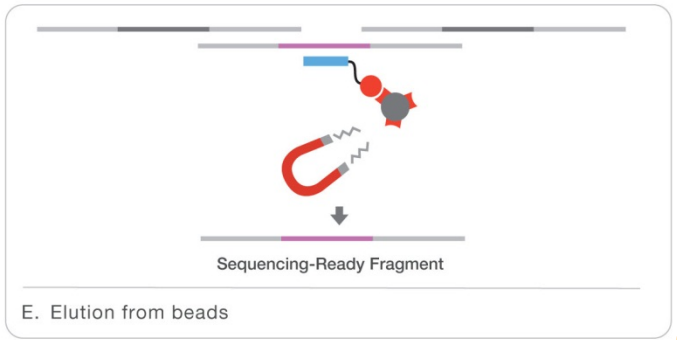
# Nextera Enrichment Workflow








Targeted resequencing to focus on subset of genome

**Exome**
Gene encoding sequences

**Custom**
Researcher/user-defined regions

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# TruSeq Enrichment or Nextera Enrichment?

| | TruSeq Enrichment | Nextera Enrichment |
|---|---|---|
| Library prep input | 1ug | 50ng |
| Library prep time (hrs) | 12 | 3 |
| Prep & Enrich kits | Separate | Bundled |
| Total workflow (days) | 3.5 | 2.5 |
| Pre-enrich pooling (E/C) | 6/12 | 12/12 |
| Dual-indexing support | No (Up to 24 single-indexes) | Yes (Up to 96 dual-indexes) |
| % Enrichment (aligned/+padded) | 55%/65% | 55%/65% (60%/70% typical) |
| % Region Dropout (E/C) | <1%/<5% | <1%/<5% |
| Cov Uniformity (0.2X Mean) | ≥80% | ≥80% |
| Cov Uniformity (0.5X Mean) | ≥60% | ≥60% |

E = exome; C = custom

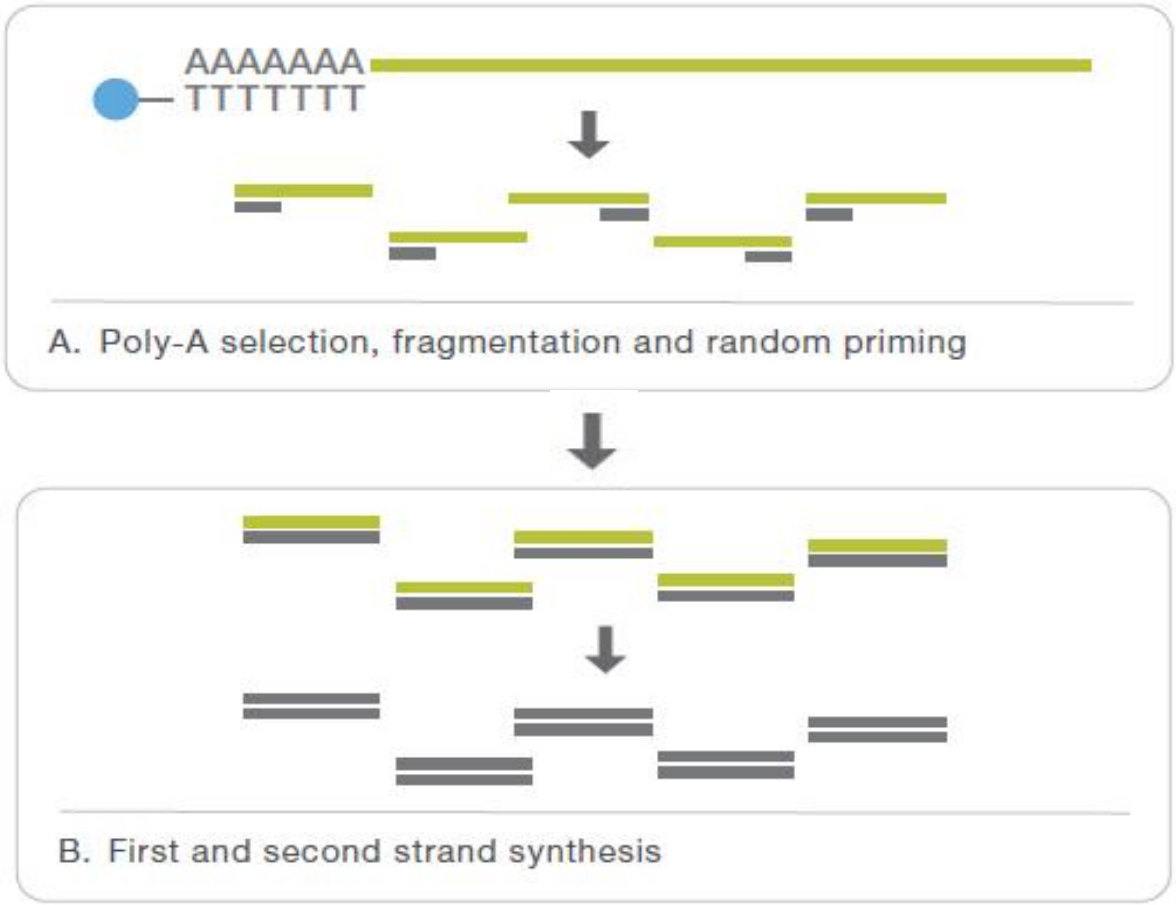
# RNA Sample Prep Options

# TruSeq RNA Sample Prep Kit Features

| | |
|---|---|
| Highlights | • Includes index sequence by default |
| Sample Input and indices | • 0.1-4ug of total RNA or 10-400ng of mRNA<br>• Up to 24 indices<br>• Parallel processing of up to 96 samples |
| Specific considerations | • Construct completed at the ligation step<br>• Contains internal quality controls<br>• Gel-free protocol |
| Suitable for: | • MiSeq<br>• GA IIx<br>• HiSeq |

# TruSeq RNA Workflow Overview

**RNA to cDNA**



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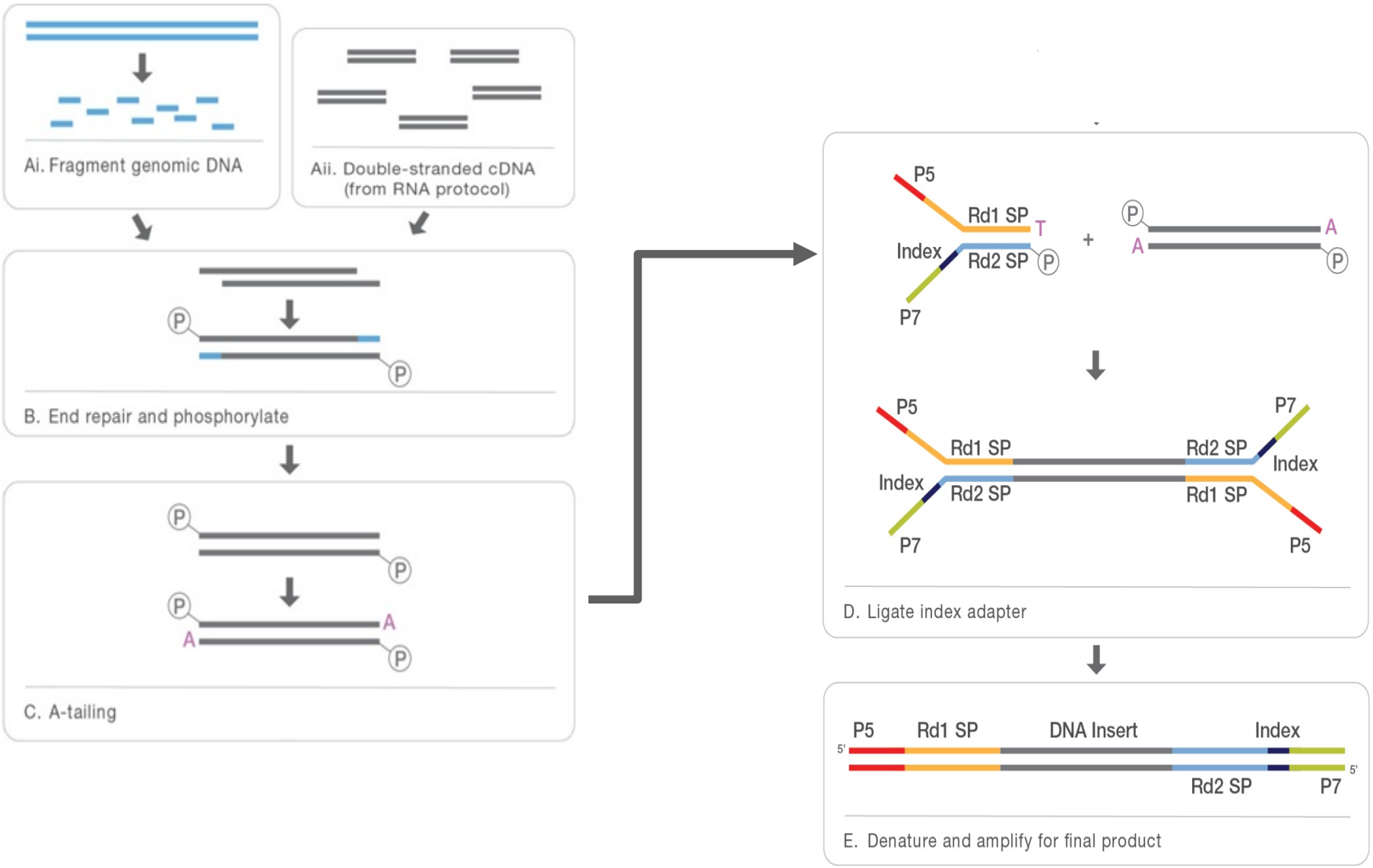
# TruSeq DNA and RNA Workflow Overview



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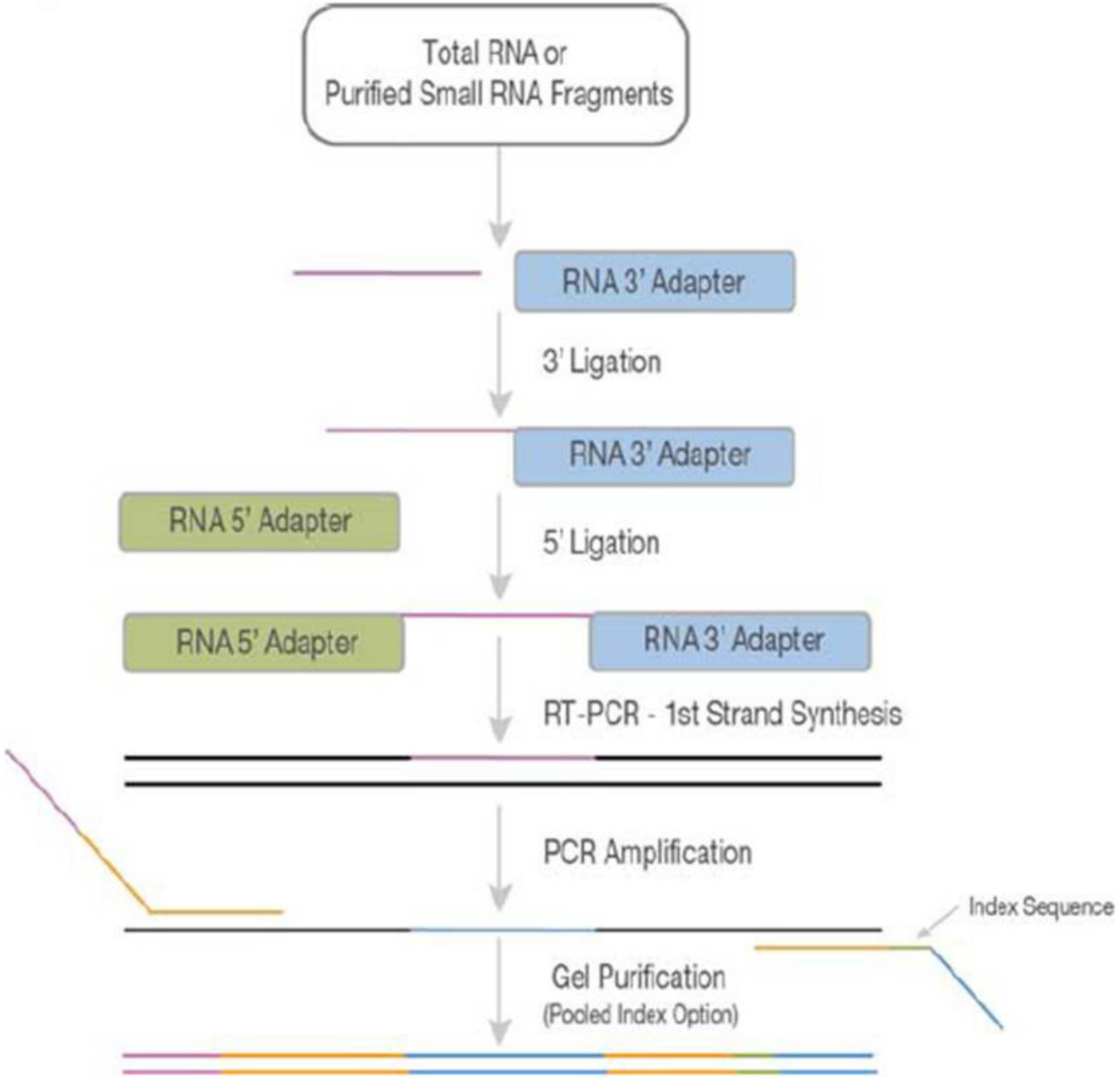
# TruSeq small RNA Sample Prep Kit Features

| | |
|---|---|
| Highlights | • Includes index sequence by default |
| Sample Input and indices | • 1 ug (total RNA) or 10-50 ng (purified miRNA)<br>• Up to 48 indices<br>• Parallel processing of up to 96 samples |
| Specific considerations | • Construct completed at the PCR step |
| Suitable for: | • MiSeq<br>• GA IIx<br>• HiSeq |

# TruSeq Small RNA Workflow Overview



# Sample Prep Best Practices

# Quantification Methods of Sequencing Libraries

**UV- spectrophotometer**
*Nanodrop*

- Detects all nucleic acids nonspecifically
- Contaminants elevate values

***Bioanalyzer 2100***

- Accuracy highly dependent on dilution and sample handling
- Can narrow in on library peak

**Fluorescence-based ds-DNA assay**
*Qubit or PicoGreen*

- Detects all double-stranded DNA
- Does not discriminate incomplete libraries

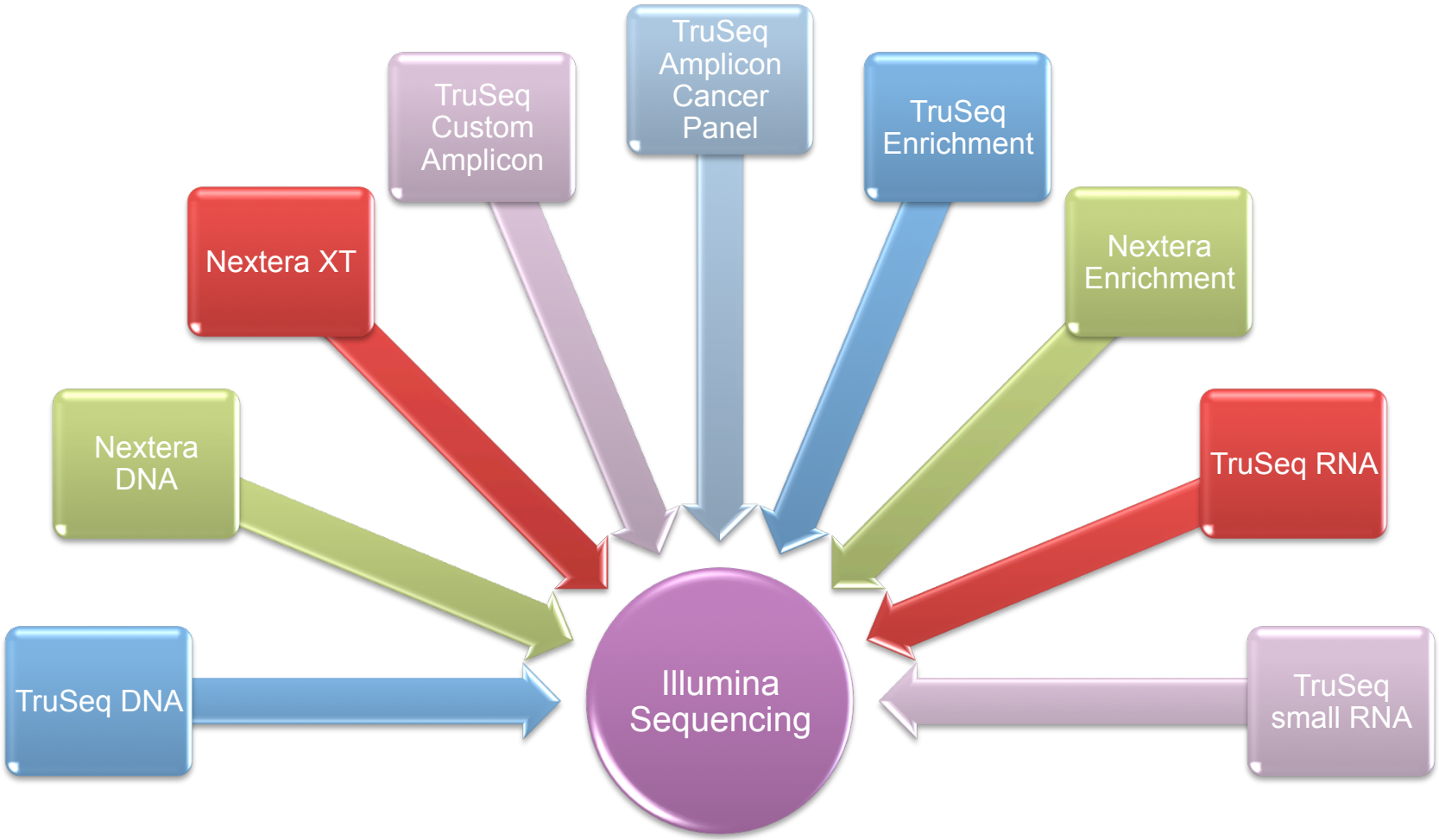
**qPCR**

- Specifically measures full-length libraries
- Detection very sensitive

# Recommended Library QC methods

| | | Quality Check on Bioanalyzer 2100 | Recommended Quant Method | Comments |
|---|---|---|---|---|
| Sample Prep Method | **TruSeq DNA (WGS)** | ✓ | qPCR | |
| | **TruSeq DNA (enrichment)** | ✓ | Qubit | Qubit is sufficient when used after DNA sample prep and before enrichment |
| | **TruSeq RNA** | ✓ | qPCR | |
| | **TruSeq Enrichment** | ✓ | qPCR | |
| | **TruSeq small RNA** | ✓ | Bioanalyzer | Bioanalyzer is sufficient because reduced precision needed due to:<br>• low concentration libraries<br>• short read lengths |
| | **Nextera/XT DNA** | ✓ | Qubit | |
| | **TruSeq Custom Amplicon** | No | None | Built-in Normalization step<br>Final library is single stranded |

# Summary



# Questions?