Overview of tailed amplicon sequencing approach with MiSeq

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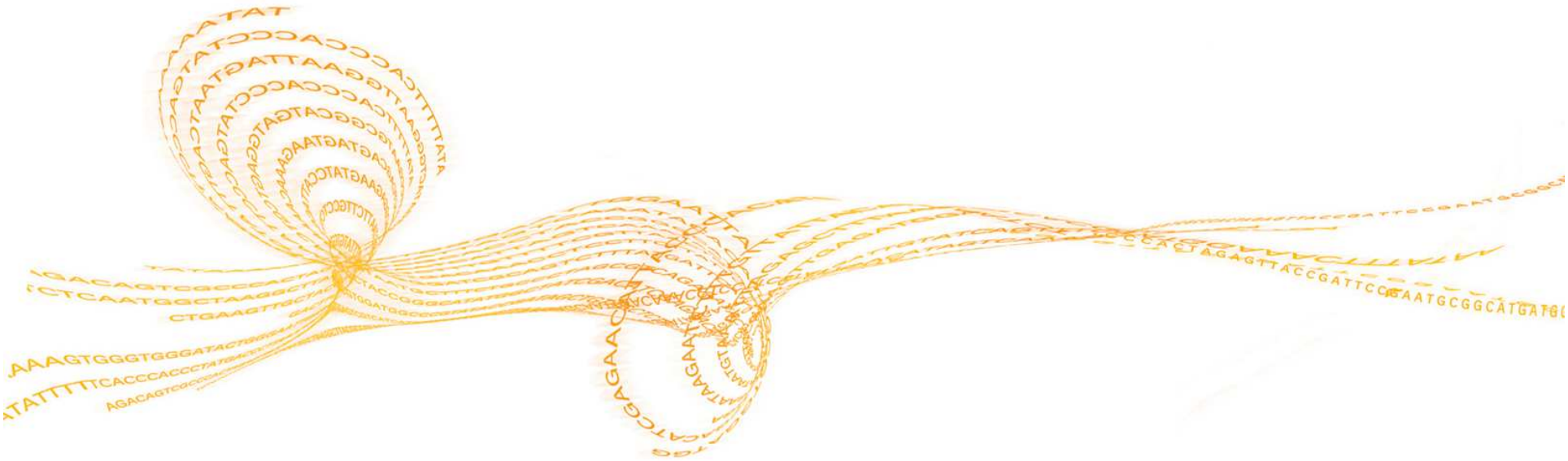

Overview Example Workflow

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Three main steps

PCR#1

- Amplify genomic regions of interest
- Pool all amplicons from the same sample into a single pool (e.g. 5 single plex PCR reactions pooled into one sample)

PCR #2

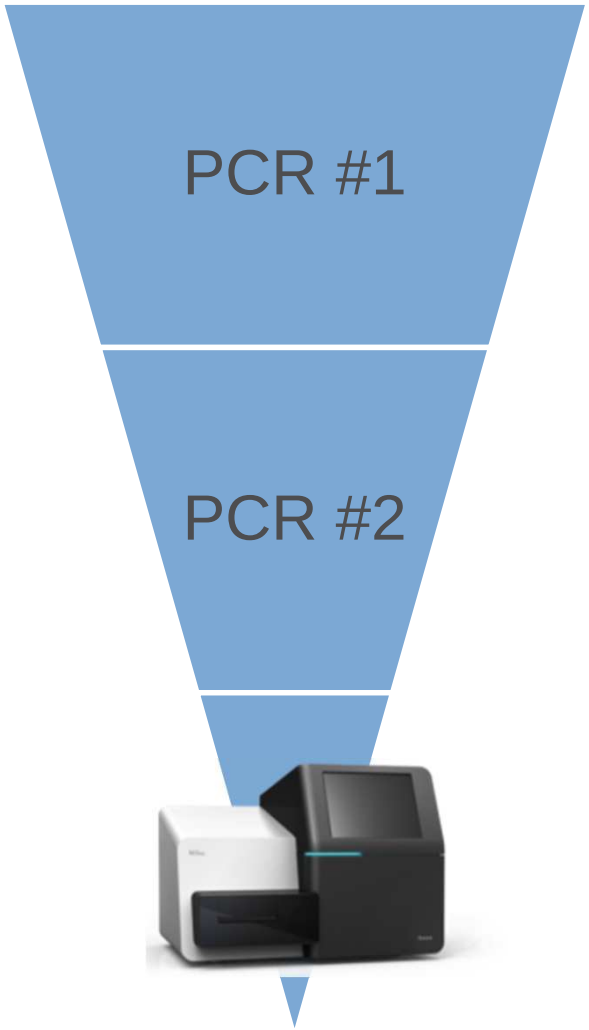
- Amplify pooled amplicons from Step 1 using indexed adapter oligos from ILMN
- Produces barcoded amplicons ready for MiSeq
- Pool up to 96 samples into a single library

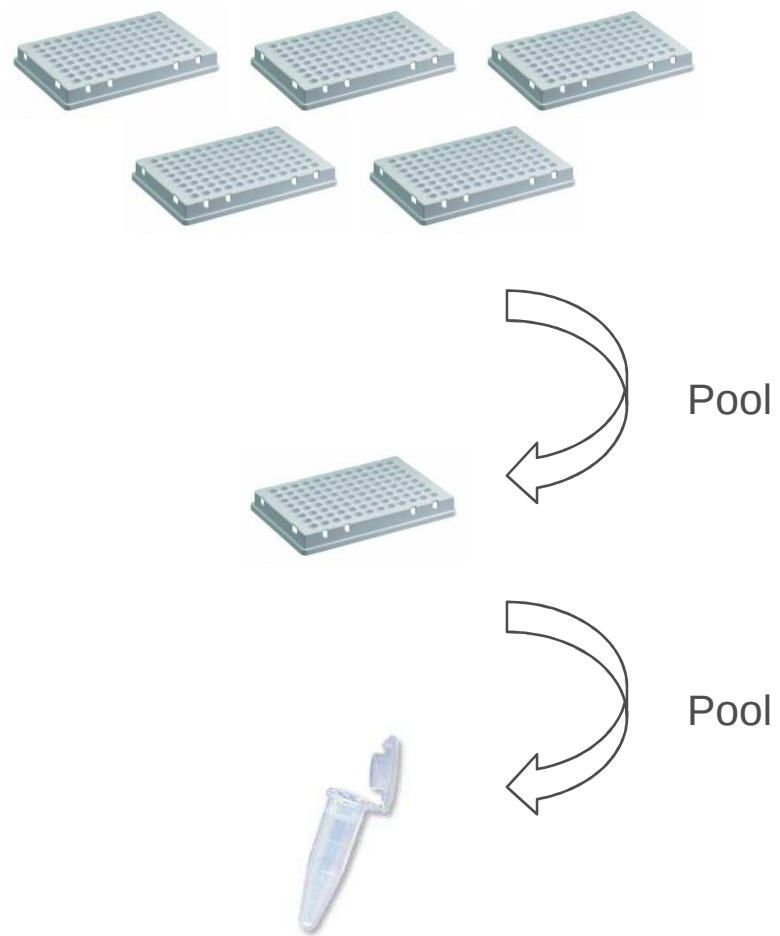
MiSeq

- Sequence pooled library of up to 96 samples in a single MiSeq run
- MiSeq analysis software automatically demultiplexes data to uniquely assign reads to samples

Throughput advantage of NGS

Example project of 5 amplicons per sample, 96 sample project





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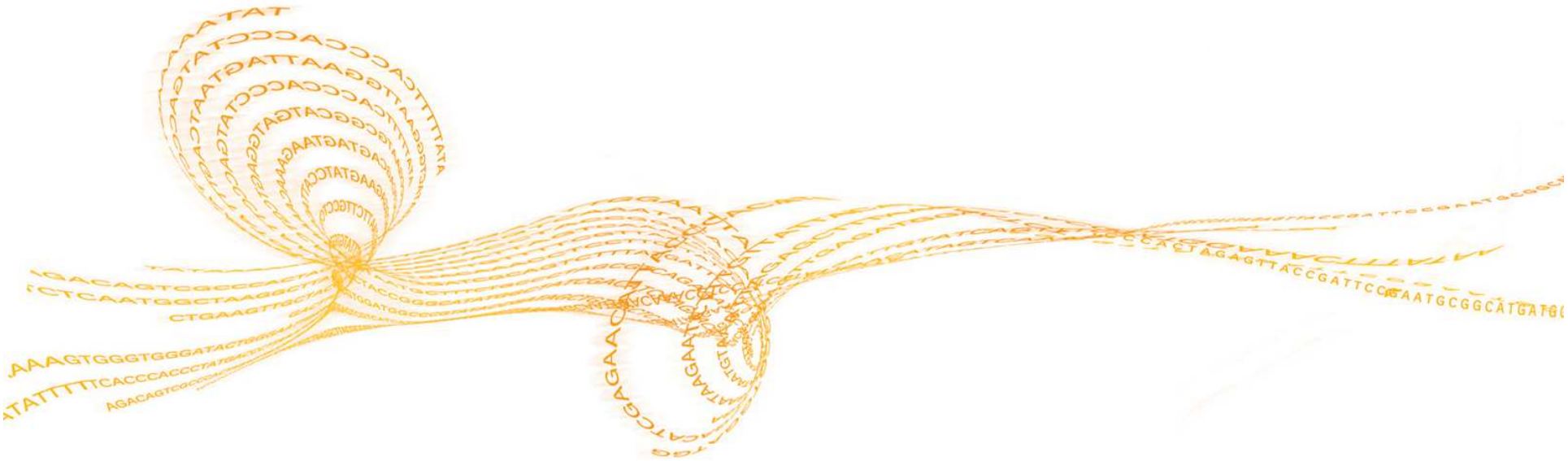

Detailed breakdown

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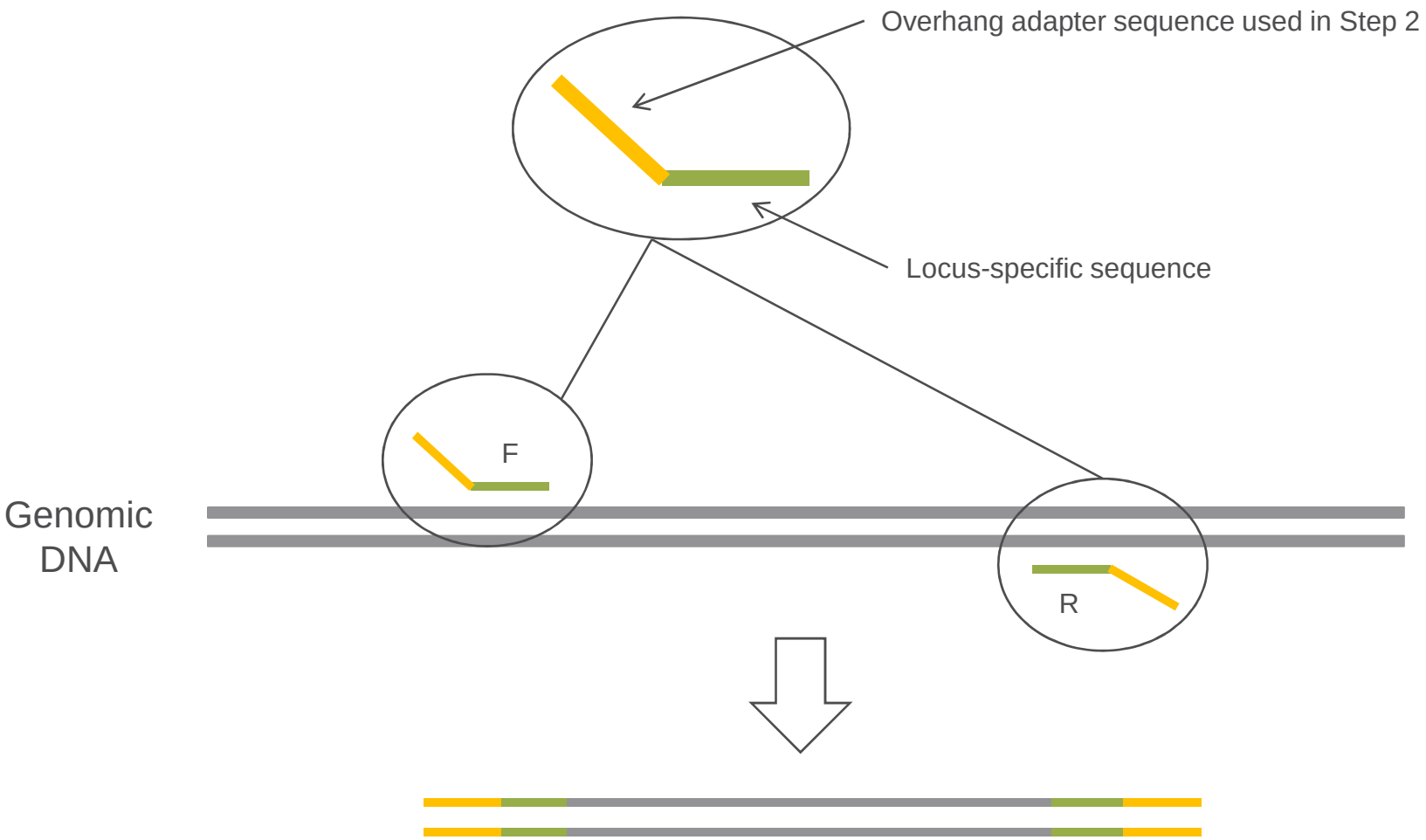
Step 1: PCR to amplify regions of interest



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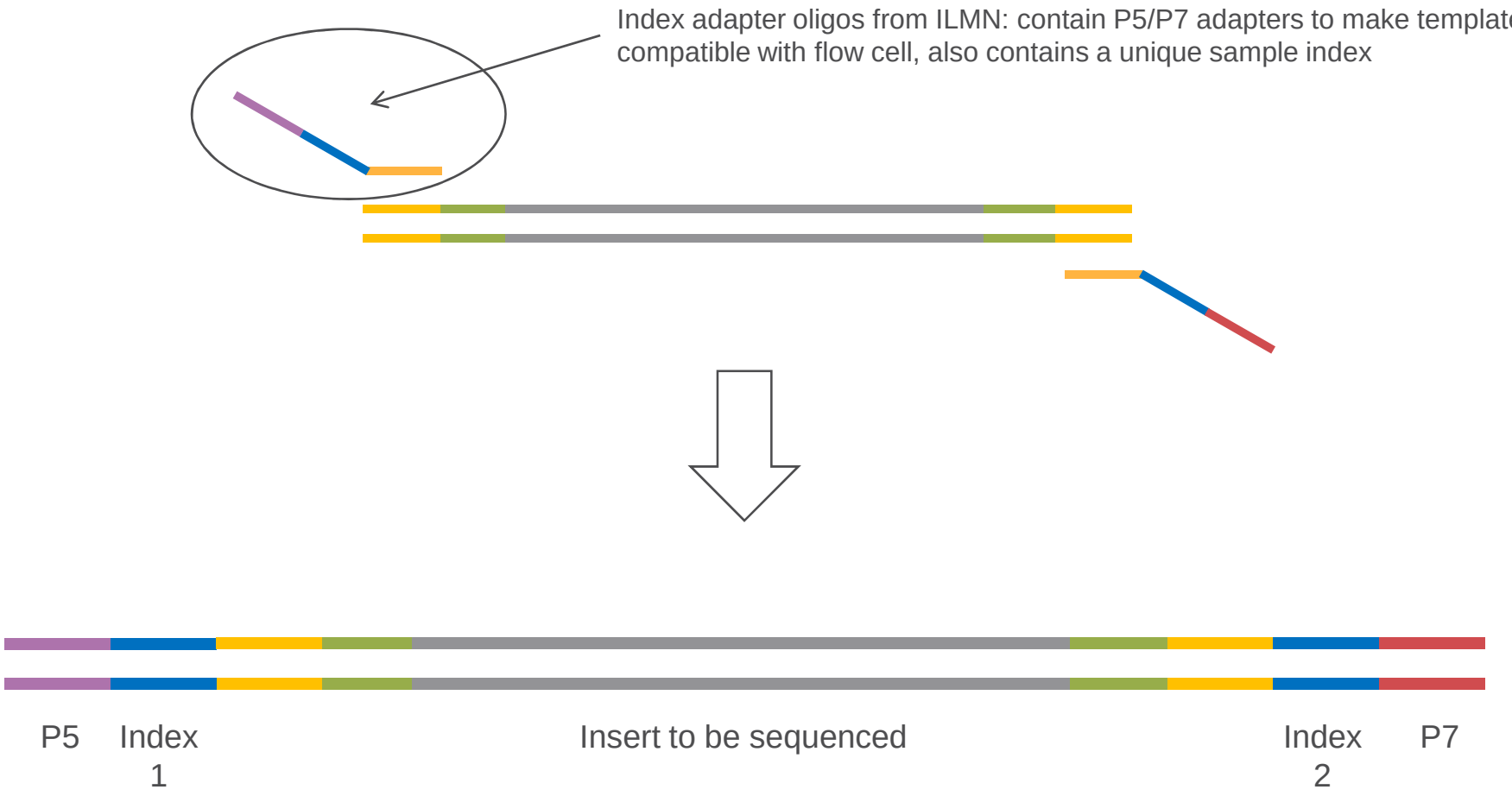
Step 2: 2nd round of PCR to add ILMN indices and sequencing adapters



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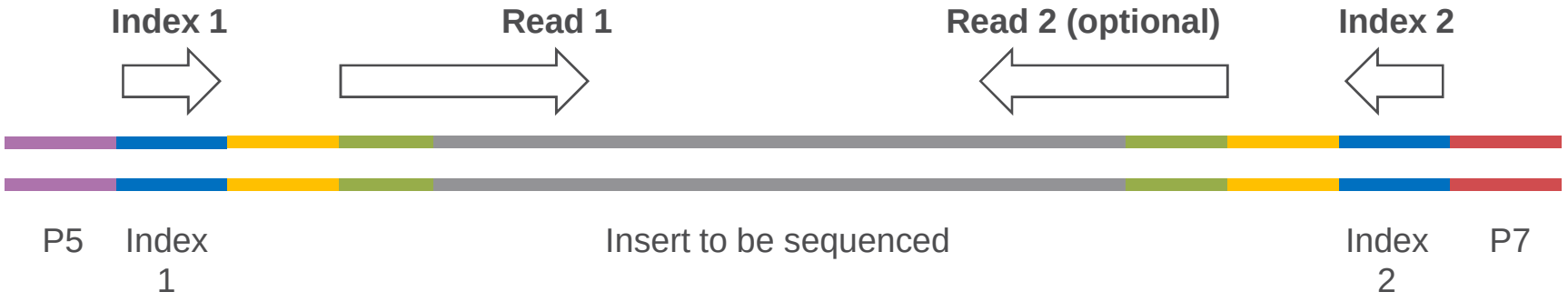
Step 3: Sequence on MiSeq



- **Sequencing order on MiSeq system**
  - Read 1 – sequence amplicons in Forward direction up to 150 bp
  - Index 1 – read first barcode
  - Index 2 – read second barcode (software can now uniquely identify the sample)
  - Read 2 – sequence amplicons in Reverse direction up to 150 bp

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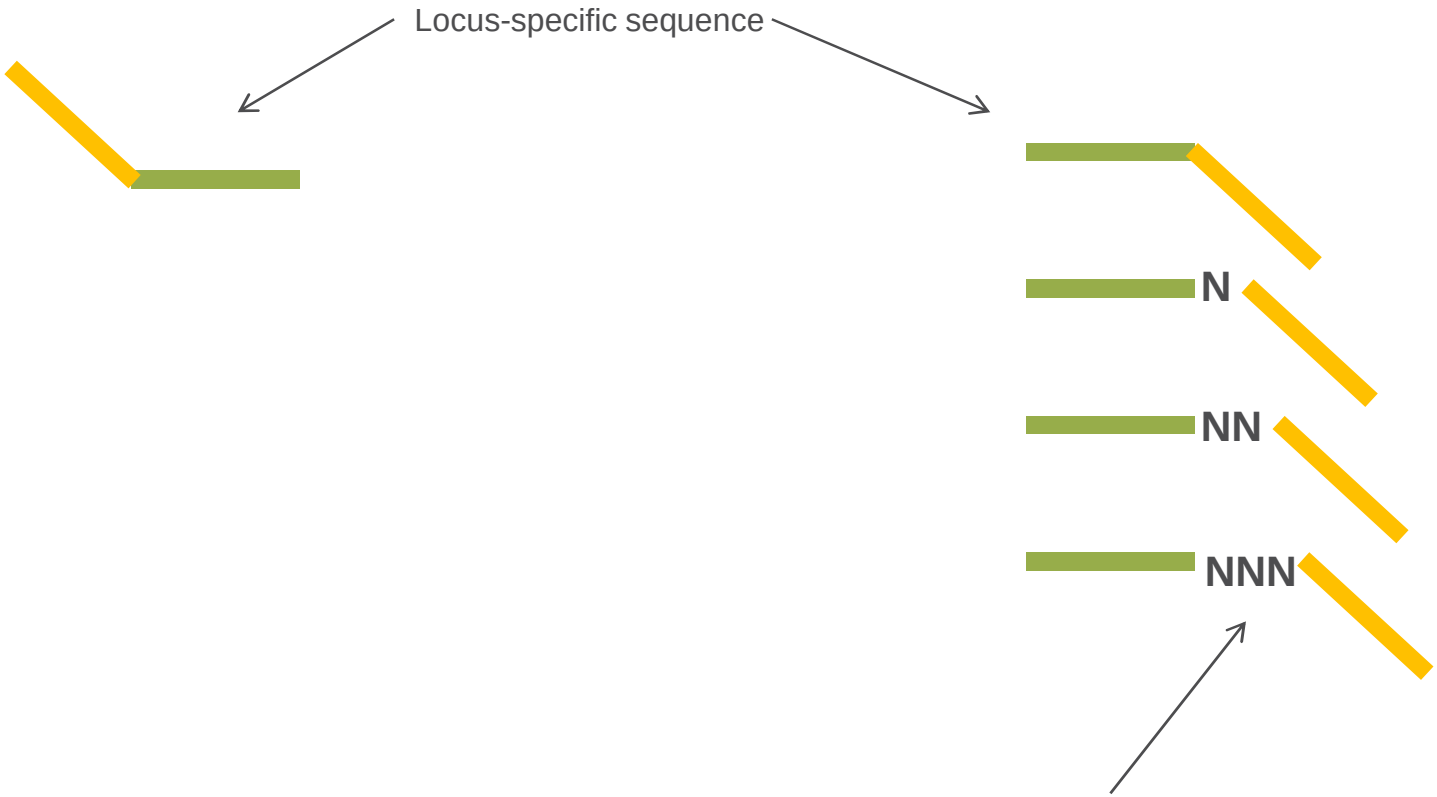
What materials are necessary?

	Description	Step used	Customer Provided	ILMN Provided
Locus-specific PCR primers	Used to amplify genomic regions of interest to be sequenced on MiSeq (e.g. 5 exons from a sample). Oligo primers include overhang adapter sequence	Step 1	✓	
Index Adapter Primers for second PCR	Used to add sequencing adapters and sample-specific indices to samples ***Nextera Index Kit (96 Indices, 384 Samples) FC-121-1012***	Step 2		✓
PCR Reagents	Mastermix (includes nucleotides and polymerase) for PCR reactions	Steps 1 & 2	✓	
Sequencing Reagents	TruSeq SBS kits for sequencing with MiSeq System ***MiSeq Reagent Kit (300-cycles – PE) MS-102-1001***	Step 3		✓

Primer considerations for PCR #1



1 Forward PCR primer

4 Reverse PCR primers

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Sequences of Round 1 PCR Primers: Assuming complex, or mixed, types of amplicons

- Append to 5’ end of forward PCR primer:
- 5’ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus specific sequence]

- Append to 5’ end of reverse PCR primers:
- 5’ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus specific sequence]

Sequences of Round 1 PCR Primers: For simple amplicons with very low complexity

- **Append to 5’ end of forward PCR primer:**
- 5’ **TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAG-[locus specific sequence]
- 5’ **TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAGN-[locus specific sequence]
- 5’ **TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAGNN-[locus specific sequence]
- 5’ **TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAGNNN-[locus specific sequence]

- **Append to 5’ end of reverse PCR primers:**
- 5’ **GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAG-[locus specific sequence]
- 5’ **GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAGN-[locus specific sequence]
- 5’ **GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAGNN-[locus specific sequence]
- 5’ **GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAGNNN-[locus specific sequence]

- **14 or 15 nt PCR Overlap Sequences**
- N, NN, and NNN are mixed sequence bases added to introduce sequence complexity

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