





# 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

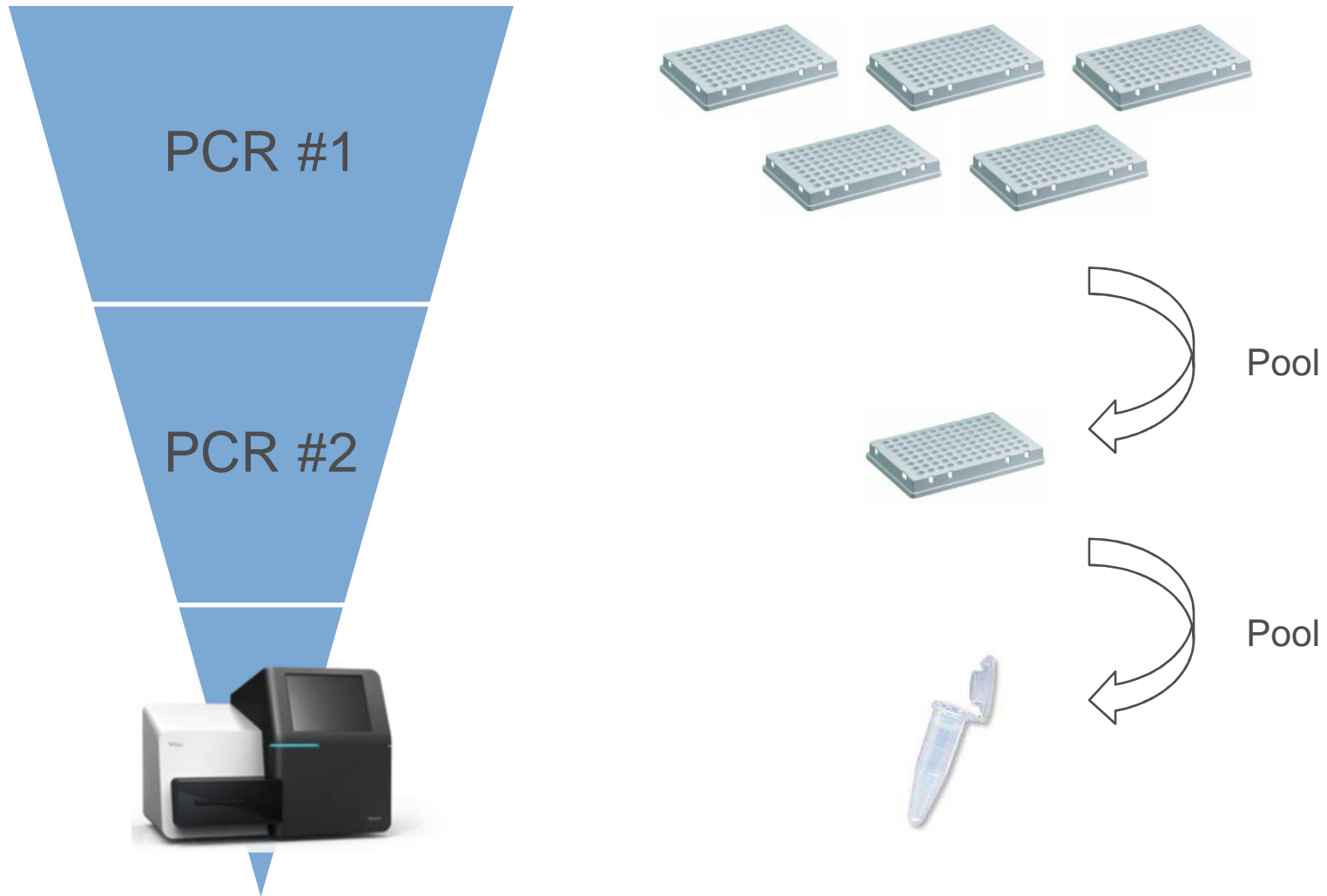
- Sequence pooled library of up to 96 samples in a single MiSeq run
- MiSeq analysis software automatically demultiplexes data to uniquely assign reads to samples



GTATCATTAAAGATTACTTGTATCCACTGATTCACGTTACCGTACCGAACQTATCAATTGAGACTAAATATAACQTACCATTAAAGACTACCGTGCACCGAACGAAAGAAATGATAACAGTAAACACACTTCTGTAAACCTTAAAGGAAACGTATCATTAAAGATTACT  
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# Throughput advantage of NGS

Example project of 5 amplicons per sample, 96 sample project



4  
GTATCATTAAAGATTACTTGTCCACTGATTCACGTTACCGTAACCGTATCAATTGAGACTAAATATAACGTACCATTAAAGACTACCGTCCAAACGACGAAAGAAATGATAACAGTAACACACTTCTGTAAACCTTAACCGAACGTATCATTAAAGATTACT  
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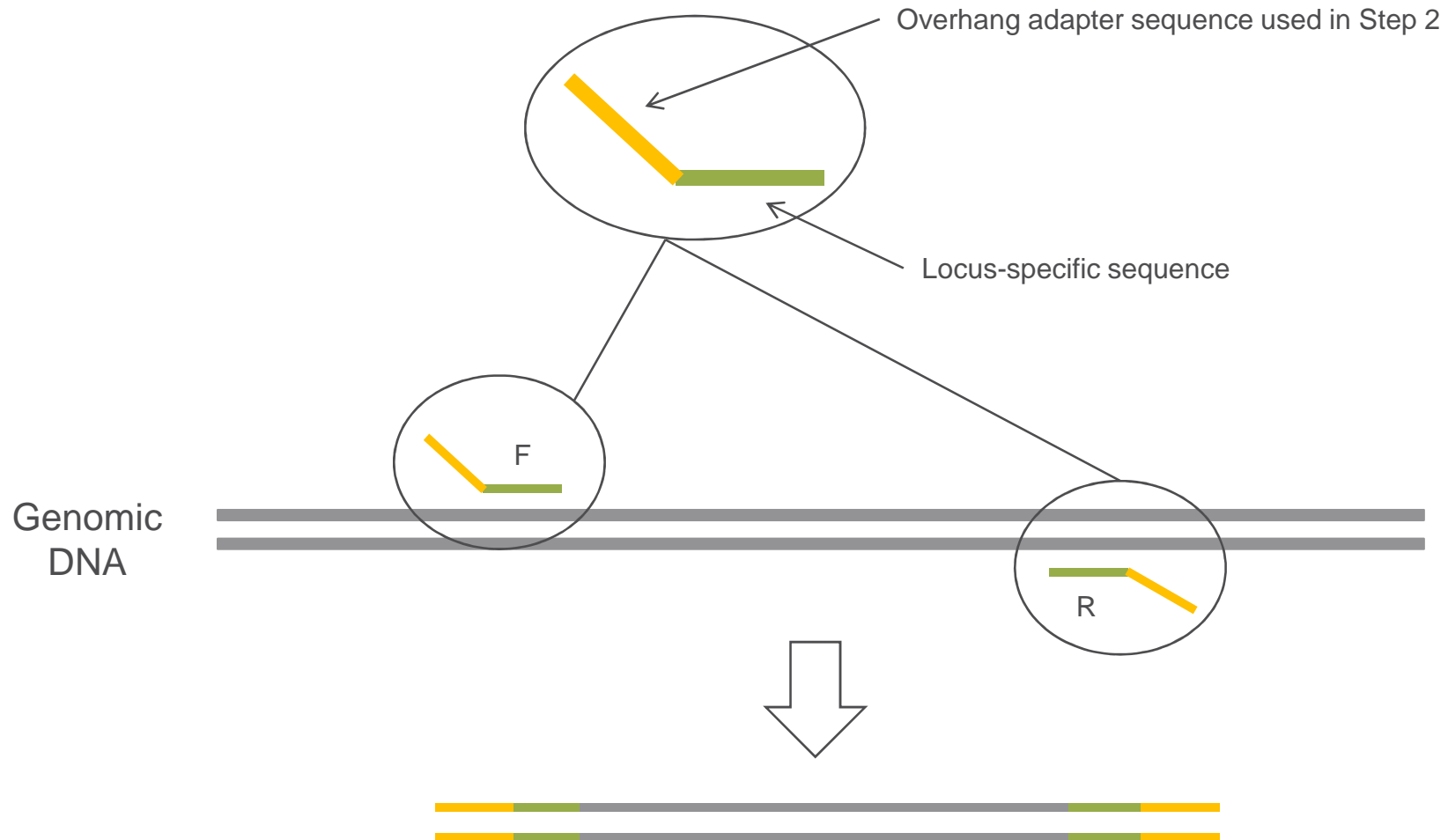


# Detailed breakdown

5 GTATCATTAAAGATTACTTGATCCACTGATTCACGGTACCGTAACGAAACQTATCAATTGAGACTAAATATTAACQTACCATTAAAGACTACCGTCCAAACGAAAGAAATGATAACAGTAACACACTTCTGTTAACCTTAACGAAACGTATCATTAAAGATTACT  
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# Step 1: PCR to amplify regions of interest

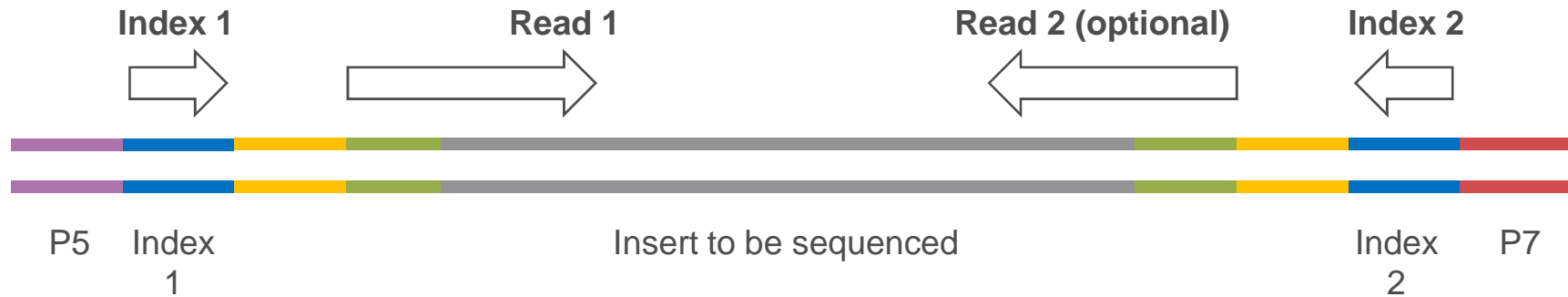


6  
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## Step 3: Sequence on MiSeq



### ► 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

8 GTATCATTAAAGATTACTTGTCCACTGATTCACGTTACCGTAACCGTATCAATTGAGACTAAATATAACGTACCATTAAAGACTACCGTCCAAACGAAAGAAATGATAACAGTAACACACTTCTGTTAACCTTAACCGAACGTATCATTAAAGATTACT  
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# What materials are necessary?

	Description	Step used	Customer Provided	ILMN Provided
<b>Locus-specific PCR primers</b>	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	✓	
<b>Index Adapter Primers for second PCR</b>	Used to add sequencing adapters and sample-specific indices to samples  <b><i>Nextera Index Kit (96 Indices, 384 Samples) FC-121-1012</i></b>	Step 2		✓
<b>PCR Reagents</b>	Mastermix (includes nucleotides and polymerase) for PCR reactions	Steps 1 & 2	✓	
<b>Sequencing Reagents</b>	TruSeq SBS kits for sequencing with MiSeq System  <b><i>MiSeq Reagent Kit (300-cycles – PE) MS-102-1001</i></b>	Step 3		✓

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# 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**AGATGTGTATAAGAGACAG**N**-[locus specific sequence]
- ▶ 5' **TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAG**NN**-[locus specific sequence]
- ▶ 5' **TCGTCGGCAGCGTC**AGATGTGTATAAGAGACAG**NNN**-[locus specific sequence]
  
- ▶ Append to 5' end of reverse PCR primers:
- ▶ 5' **GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAG-[locus specific sequence]
- ▶ 5' **GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAG**N**-[locus specific sequence]
- ▶ 5' **GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAG**NN**-[locus specific sequence]
- ▶ 5' **GTCTCGTGGGCTCGG**AGATGTGTATAAGAGACAG**NNN**-[locus specific sequence]
  
- ▶ **14 or 15 nt PCR Overlap Sequences**
- ▶ **N, NN, and NNN are mixed sequence bases added to introduce sequence complexity**

GTATCATTAAAGATTACTTGTCCACTGATTCACGGTACCGTAACCGTATCAATTGAGACTAAATATAACGTACCATTAAAGACTACCGTCCAAACGGAAGAAATGATAACAGTAACACACTTCTGTAAACCTTAACCGAAGCTATCATTAAAGATTACT  
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