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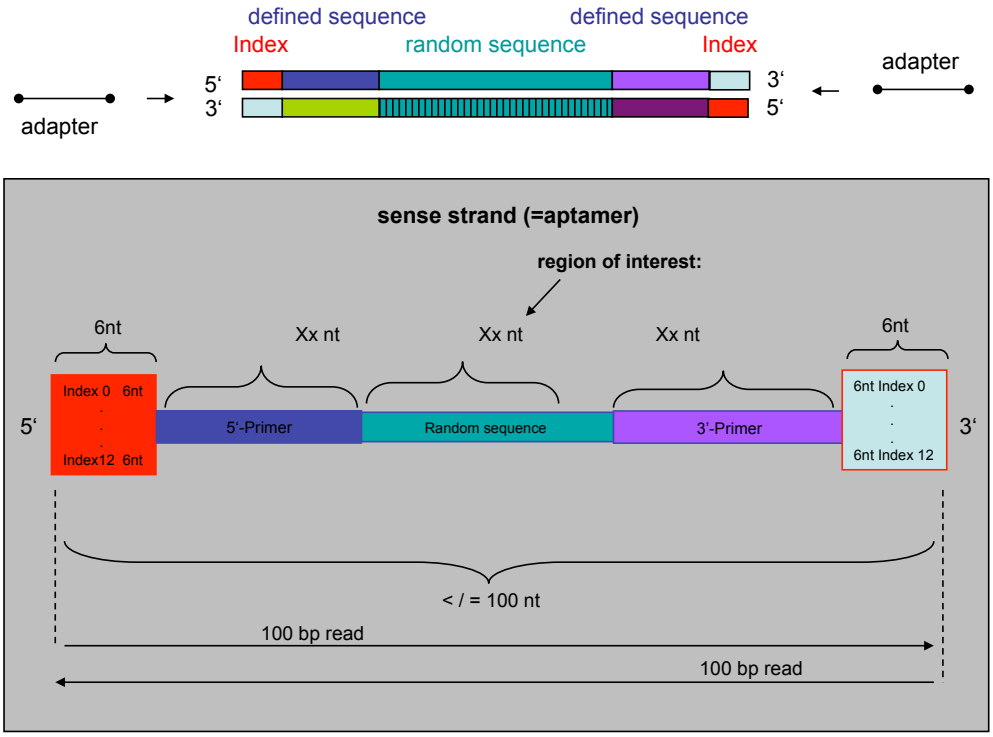
Preparation of Samples for Illumina Next Generation Sequencing

Example:



Design NGS-primers by adding the respective index 1-12 to the 5’-end of your selection primer sequence. Please attach the same index at 5’-end of forward- as well as of reverse primer. It may be necessary to replace 5’-nucleotides from either or both of your original primers in order to keep annealing temperatures of both primers in the same range.

Indexed primers:

Forward-Primer (5’→3’)	
FP-Index 1	ATCACG-FP-Sequence
FP-Index 2	CGATGT FP-Sequence
FP-Index 3	TTAGGC FP-Sequence
FP-Index 4	TGACCA FP-Sequence
FP-Index 5	ACAGTG FP-Sequence
FP-Index 6	GCCAAT FP-Sequence
FP-Index 7	CAGATC FP-Sequence
FP-Index 8	ACTTGA FP-Sequence
FP-Index 9	GATCAG FP-Sequence
FP-Index 10	TAGCTT FP-Sequence
FP-Index 11	GGCTAC FP-Sequence
FP-Index 12	CTTGTA FP-Sequence
FP-Index 13	AGTCAA FP-Sequence

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Reverse-Primer (5'→3')	
RP-Index 1	ATCACG RP-Sequence
RP-Index 2	CGATGT RP-Sequence
RP-Index 3	TTAGGC RP-Sequence
RP-Index 4	TGACCA RP-Sequence
RP-Index 5	ACAGTG RP-Sequence
RP-Index 6	GCCAAT RP-Sequence
RP-Index 7	CAGATC RP-Sequence
RP-Index 8	ACTTGA RP-Sequence
RP-Index 9	GATCAG RP-Sequence
RP-Index 10	TAGCTT RP-Sequence
RP-Index 11	GGCTAC RP-Sequence
RP-Index 12	CTTGTA RP-Sequence
RP-Index 13	AGTCAA RP-Sequence

- PCR using Taq Core Kit from Qiagen:
  - PCR-Mix (for 100 µl-PCR)*

	1x
10x PCR-Puffer*:	10
5'-Primer-Index (100 µM):	1
3'-Primer-Index (100 µM):	1
dNTPs (10 mM)*:	2
Templat (approx: 1µM)	2
H_2O:	83,5
Taq*:	0,5
Total (µl)	100

* Please calculate the number of PCR reactions that are needed to end up with the necessary amount of DNA (depends on number of differently indexed amplicons derived from different experiments that have to be combined to give one sequencing probe. For one sequencing probe minimum of xµg of dsDNA is needed.

  - PCR-program:

1.	94°C	60sec	(denaturation)
2.	xx °C	60sec	(annealing)
3.	72°C	60sec	(elongation)
4.	GOTO 1	rep 5-8	
5.	8°C	endless	

- Check on 2.5% agarose
- Purification with QiaQuick PCR Purification Kit or MiniElute PCR Purification Kit (Qiagen)
- Concentration and purity measurement of each indexed probe (A260/A280)
- Pooling of all indexed probes (sum: minimum 3 µg) (optional: please inquire AptaIT if pooling should be done in your case)
- Shipment of the probes according to the guidelines of the NGS-provider (refrigerated/chilled in EB or H2O).