Elisabeth®
Pharmacon

NGS for Pathology

EliGene® NGS kits

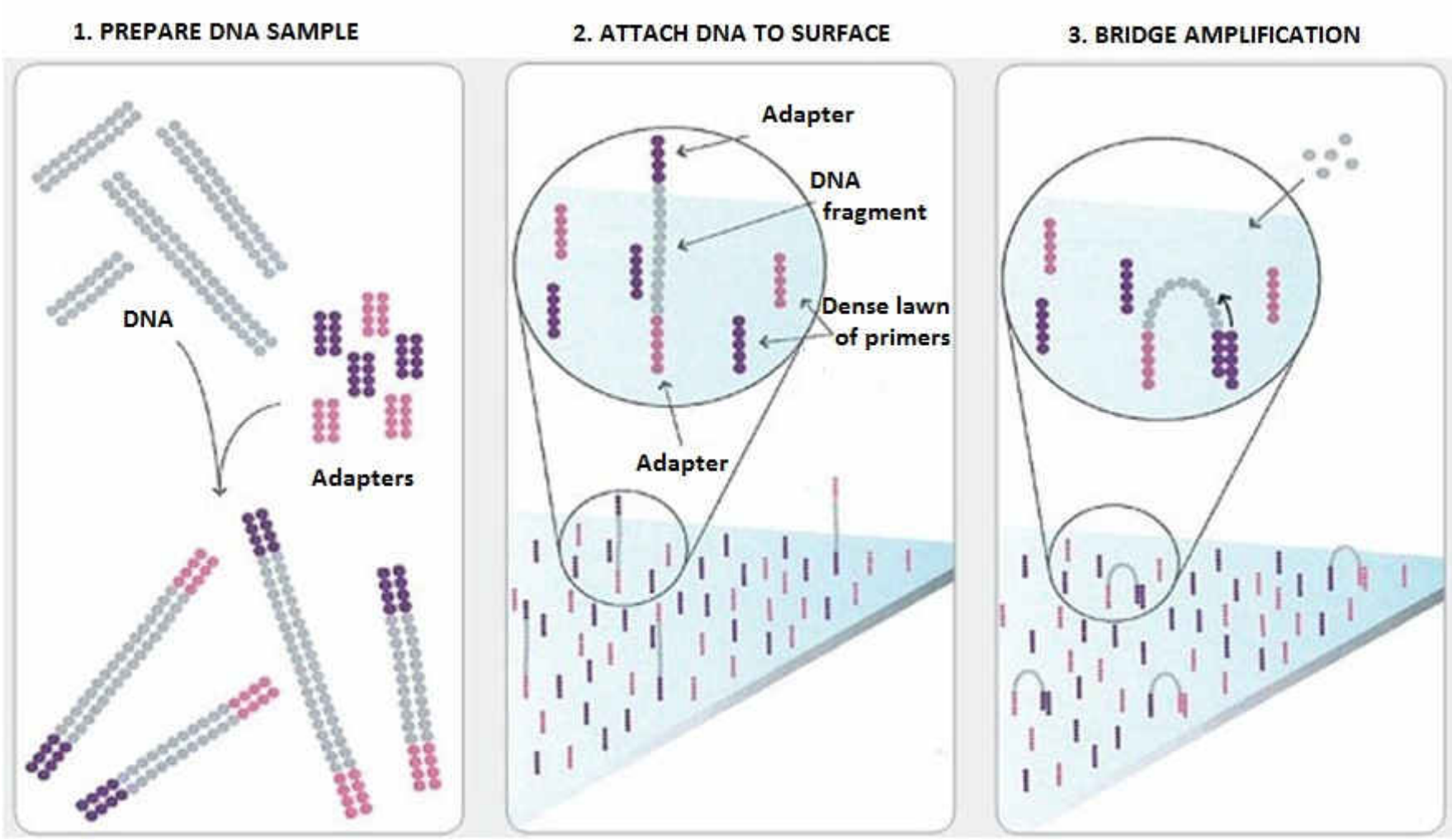
Individual approach

Why NGS sequencing?

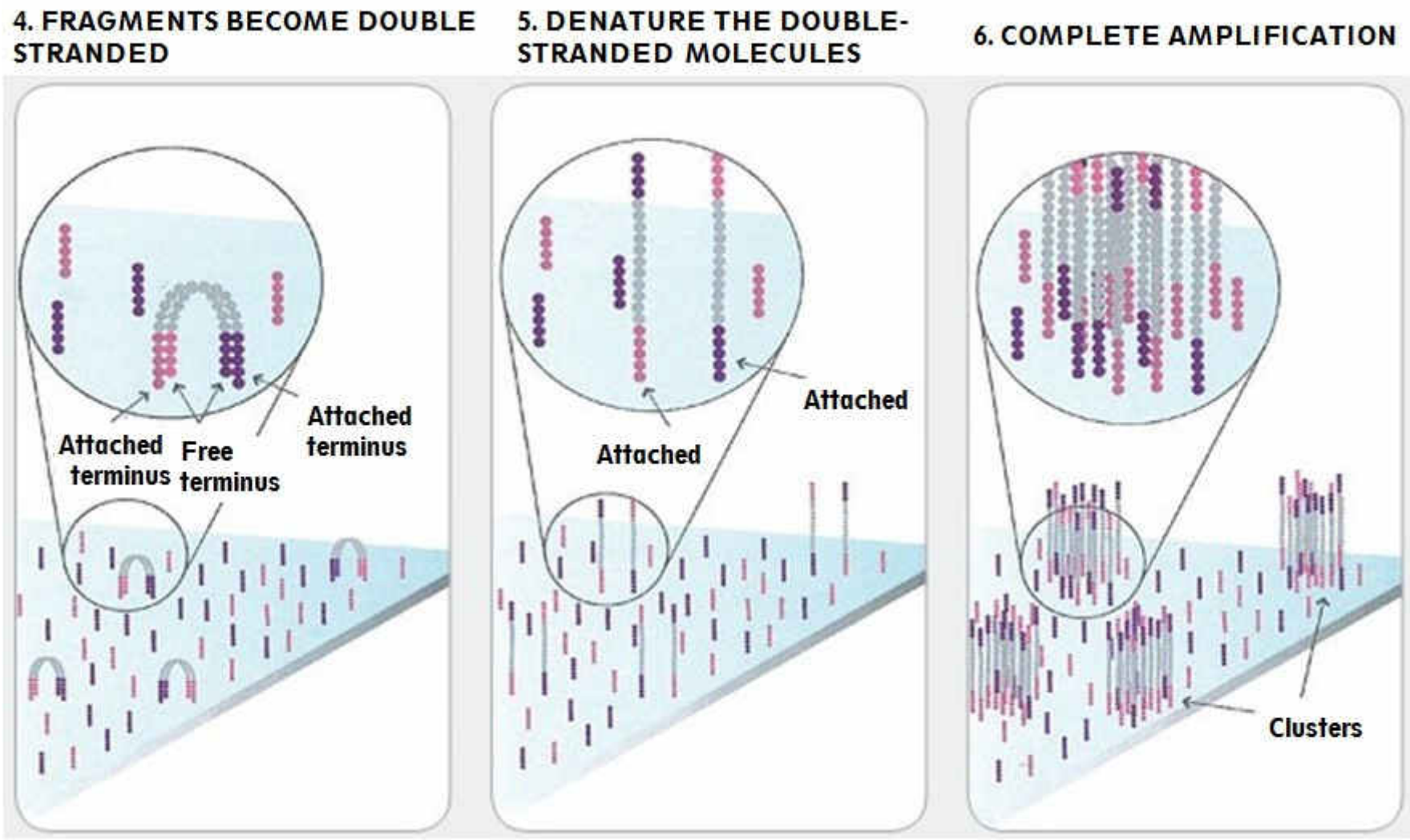
Elisabeth® Pharmacon

- High-throughput
- Determine the base
- Cost-effective
- Analyse many samples = many patients
- Somatic mutations

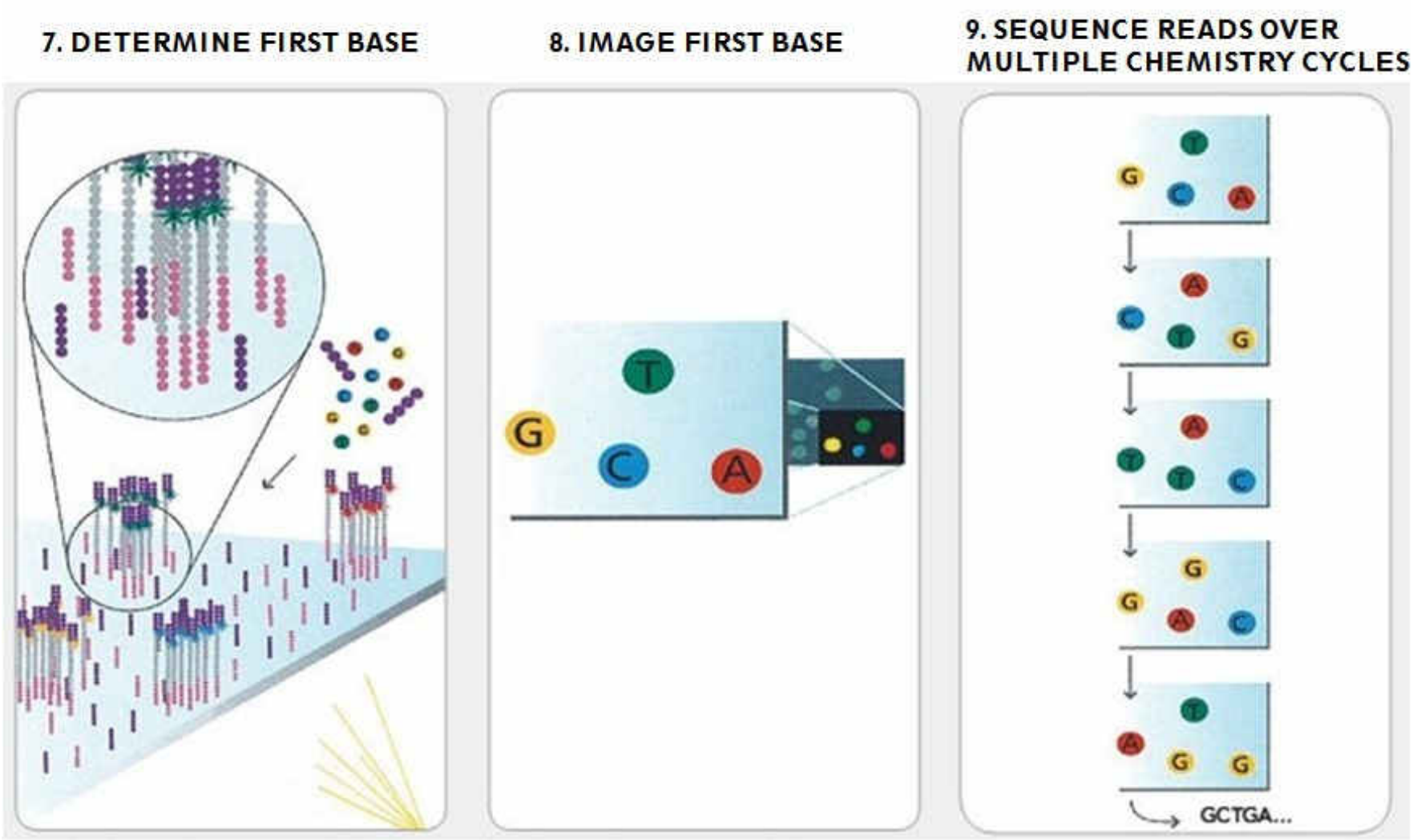
Illumina sequencing technology



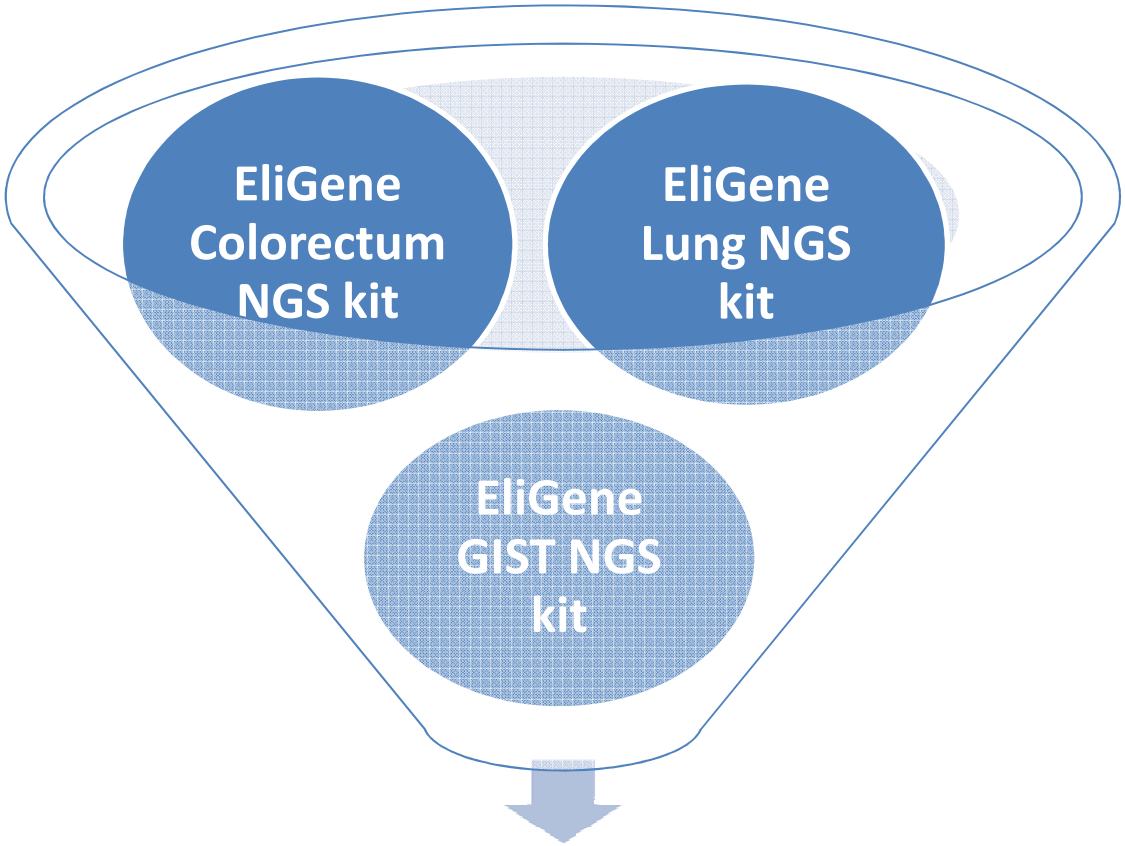
Illumina sequencing technology



Illumina sequencing technology



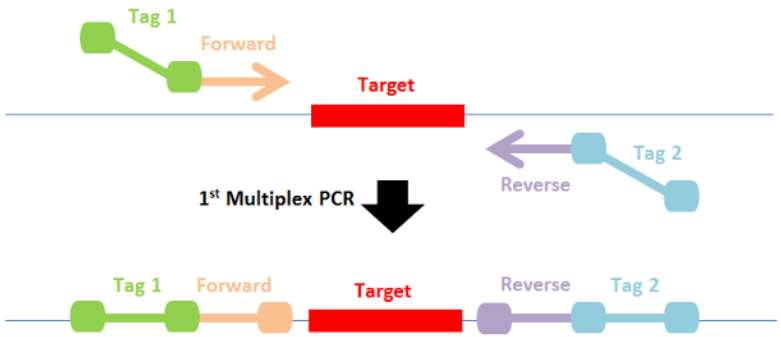
Working scheme



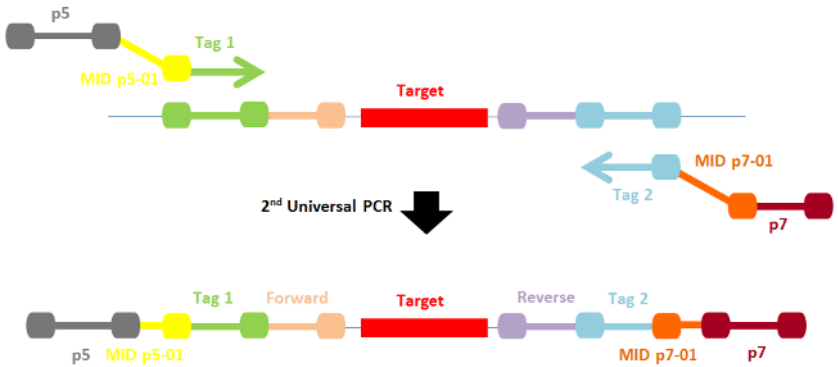
EliGene Adaptor-IL NGS kit

MiSeq Reagent Nano Kit, v2 (500 cycles)

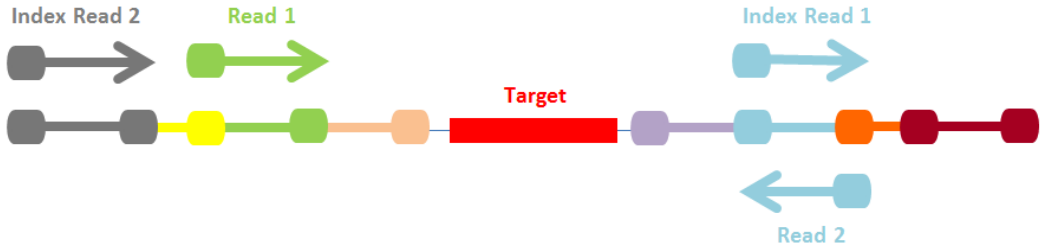
Scheme of sequencing process



Multiplex PCR of target regions



Incorporation of MIDs and p7, p5 adaptors



Structure of final product

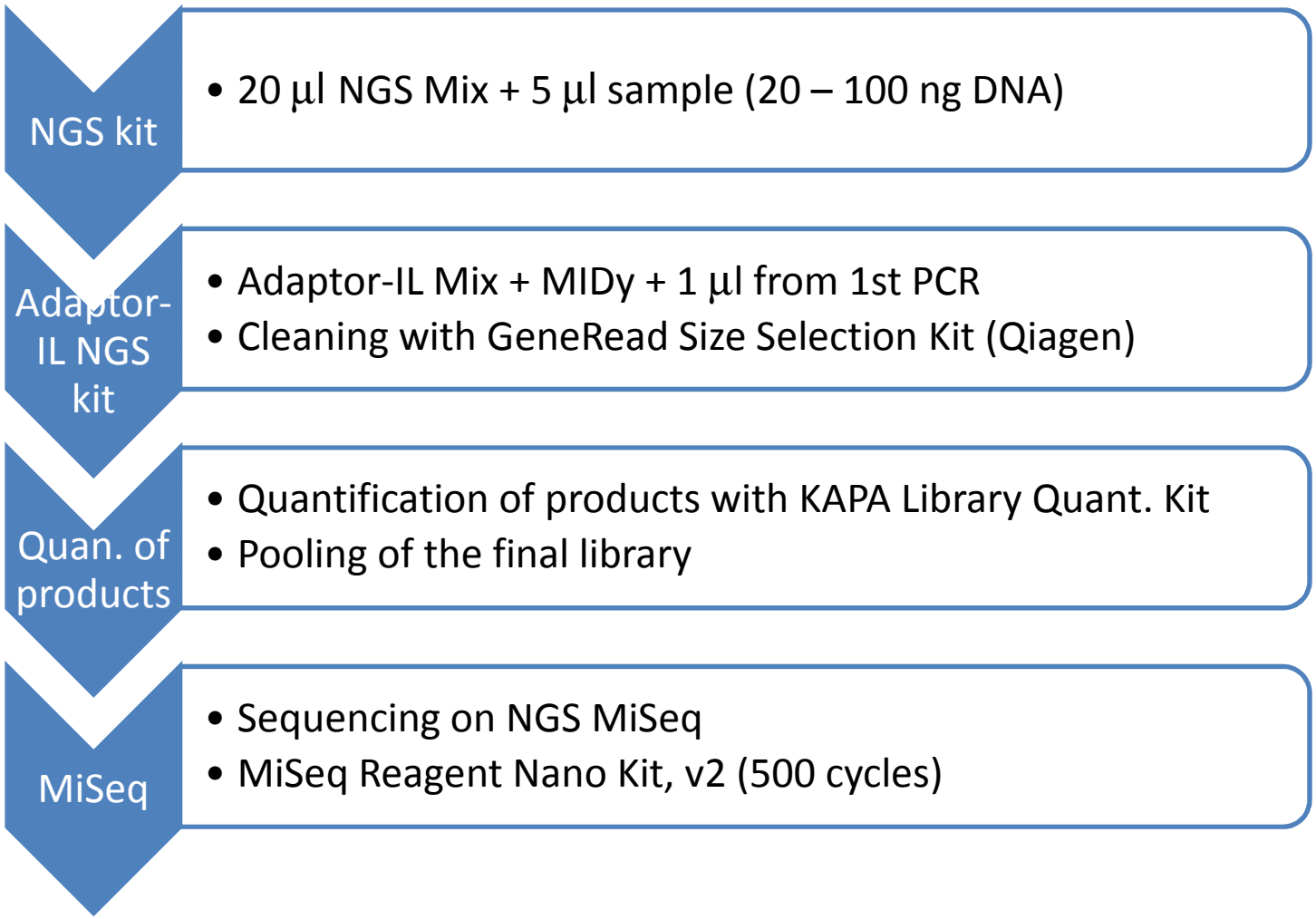
Sequenced regions

EliGene Colorectum NGS kit:
KRAS, NRAS – exon 2, exon 3, exon 4
BRAF – exon 15

EliGene Lung NGS kit:
EGFR – exons 18, 19, 20, 21

EliGene GIST NGS kit:
ckit – exons 9, 11, 13, 14, 15, 16, 17
PDGFRa – exons 8, 10, 12, 14, 18

Workflow


NGS kit
• 20 µl NGS Mix + 5 µl sample (20 – 100 ng DNA)
Adaptor-IL NGS kit
• Adaptor-IL Mix + MIDy + 1 µl from 1st PCR
• Cleaning with GeneRead Size Selection Kit (Qiagen)
Quan. of products
• Quantification of products with KAPA Library Quant. Kit
• Pooling of the final library
MiSeq
• Sequencing on NGS MiSeq
• MiSeq Reagent Nano Kit, v2 (500 cycles)

Data analysis

- evaluation with Illumina Variant Studio software
- as filter should be used min. 100x coverage of particular variant + consider the allele frequency according to % TTC
- minimal % TTC in sample at least 10%

Why EliGene® NGS kits?

- ready-to-use mastermix with hot-start polymerase (mastermix + sample)
- standartized workflow with quantification with KAPA Library Quantification Kit (Kapa Biosystems)
- positive control in kits for verification of workflow
- high robustness of the whole workflow

EliGene®

Elisabeth® Pharmacon

Chlamyc
Legior
Mycopl
Influe

C

Ur

Anaplasr

Adenovirus RT (CE)
Enterovirus LC (CE)
Norovirus LC (CE)

Coeliac 3.0 RT (DQ2.2, DQ2.5, DQ8 | CE from 2017)
Coeliac RT (DQ2.5, DQ8, DR4 | CE)
Coeliac RT28 (DQ2.5, DQ8 | CE)
Lactose Intolerance C-13910T RT (CE)
Lactose Intolerance G-2201A RT (CE)
Spondylitis HLA-B27 RT (CE)