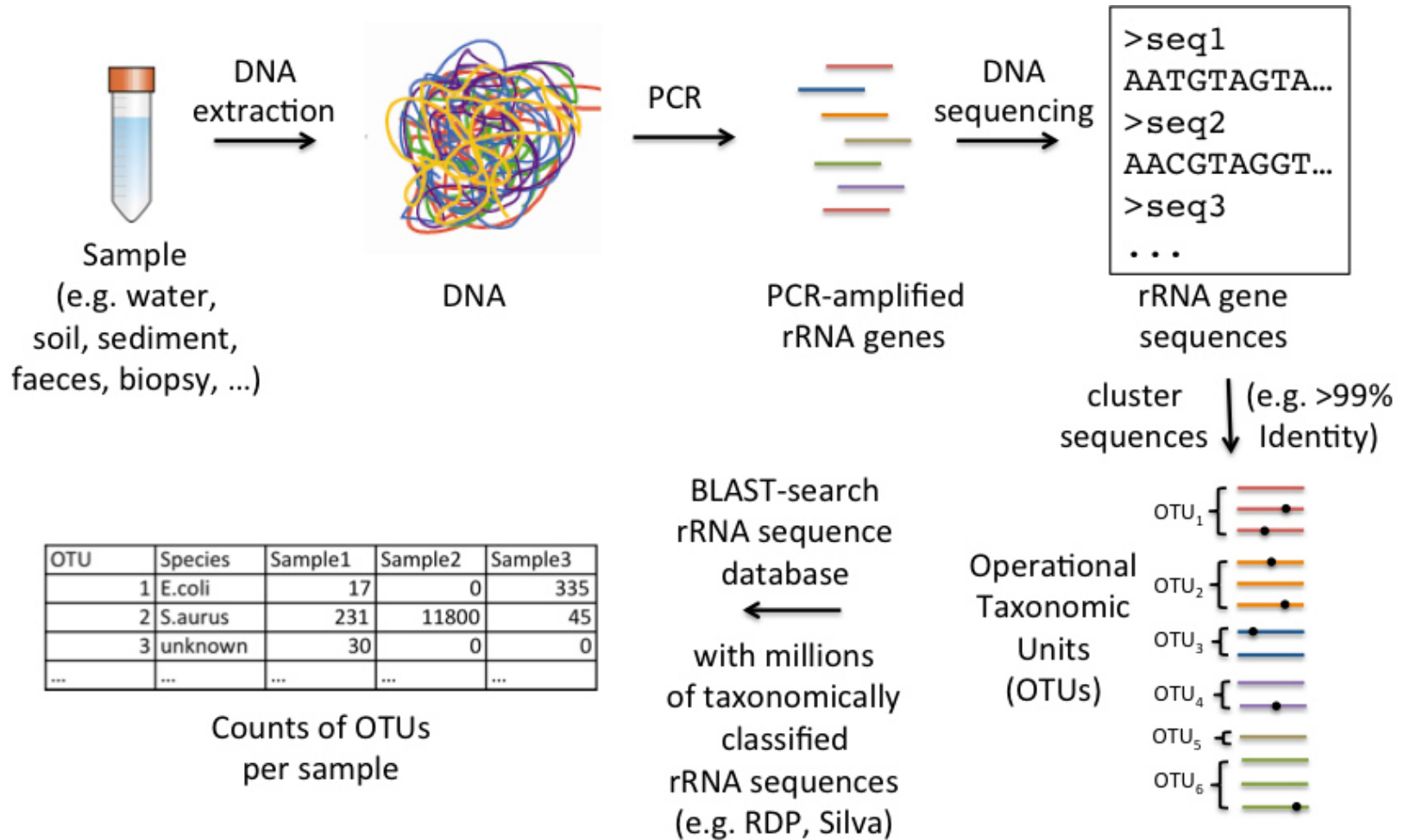


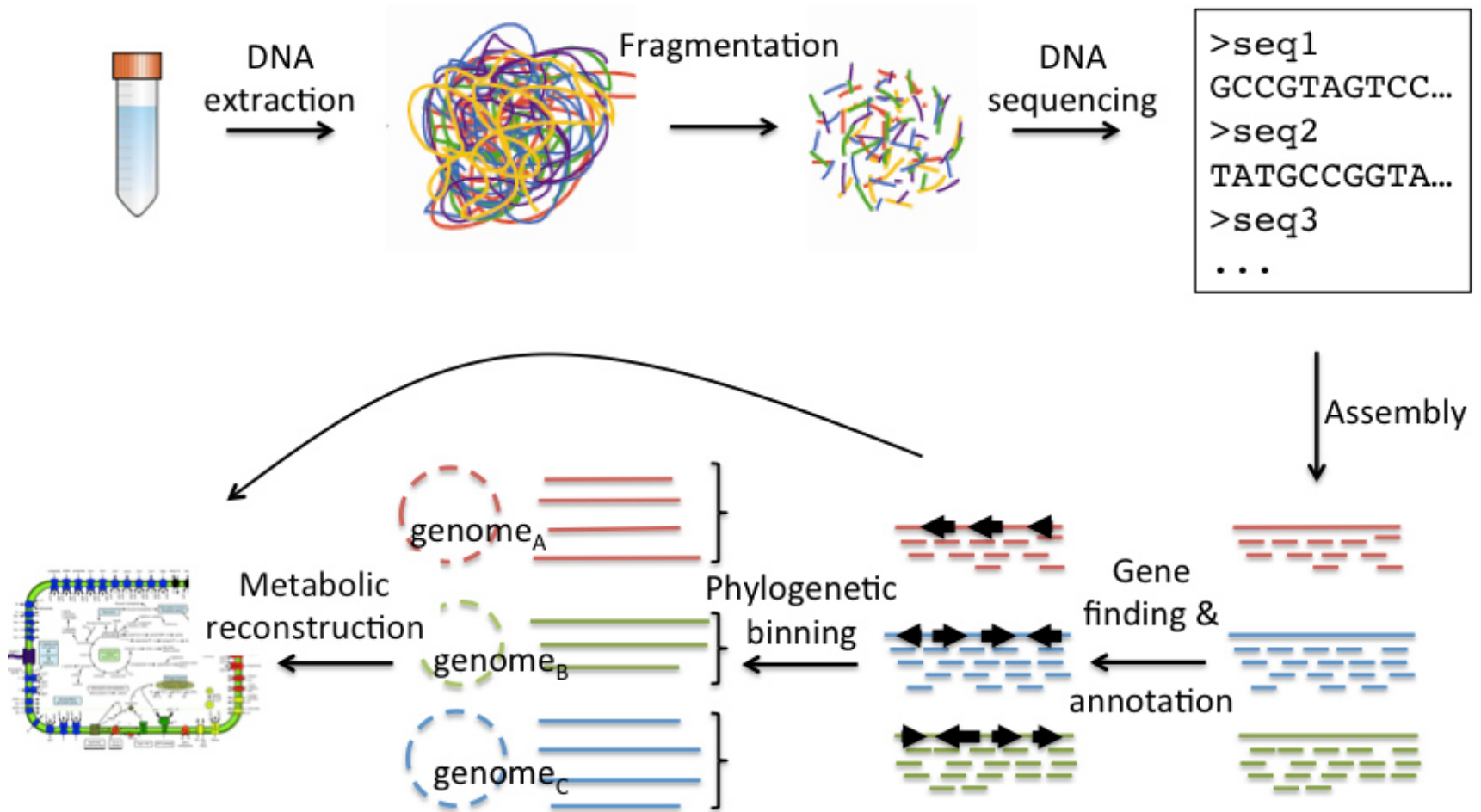
# Příprava celometagenomových knihoven (shotgun)

Petra Vídeňská, Ph.D.

# Sekvenace 16S rRNA

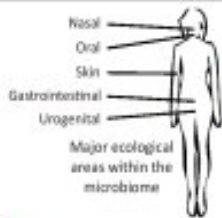


# Celometagenomová sekvenace



# 16S rDNA vs. celometagenom

## Current techniques for human microbiome profiling



Human microbiome sample

Extract DNA

Which organisms are present?

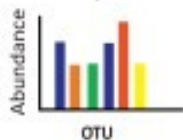
Amplify 16S rRNA gene by PCR and sequence



Bin similar sequences into OTUs



Compare OTUs to databases



Identify OTUs in sample and relative frequencies



Phylogenetic view of community composition

Compare sequences to reference genomes



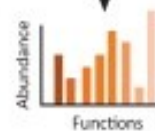
GATTACA  
GATTACA  
GATTCA  
GATTCA

Identify microbial sequences, variants, and polymorphisms in sample

What are the functions of the community?

Sequence total DNA

Compare sequences to databases



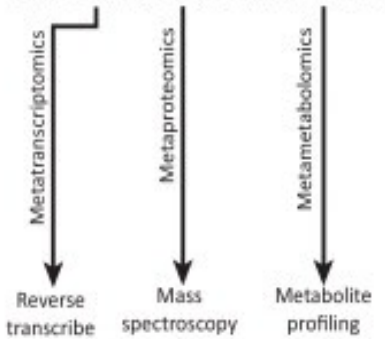
Identify genes, pathways, and relative frequencies in sample

## Emerging methods

Human microbiome sample

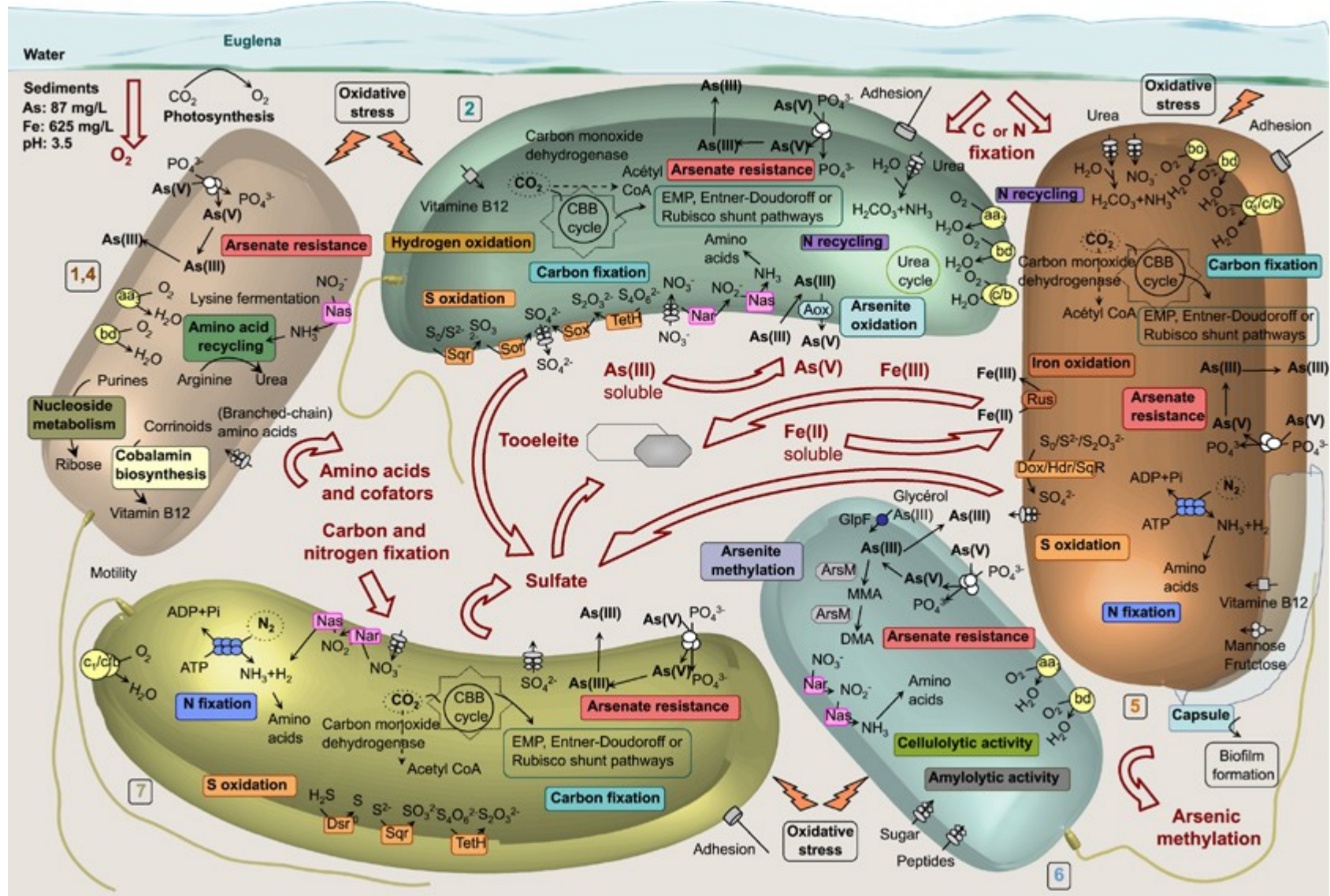
How does the community respond to its environment?

Extract RNA, protein, and small molecules

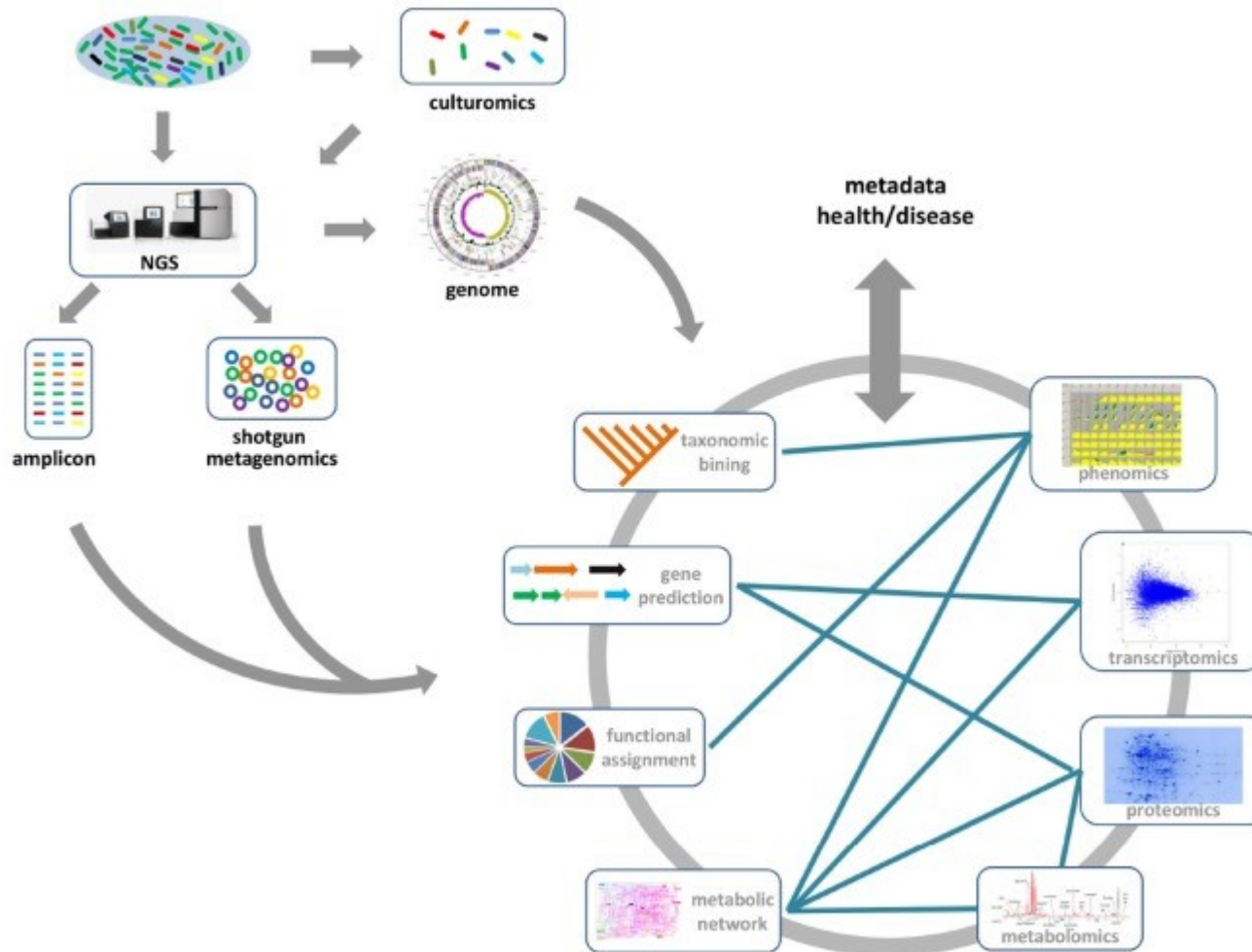


Identify interactions between expressed genes, proteins, and metabolic products in sample

# Celometagenomové sekvenování



# Vše souvisí se vším



# Příprava celometagenomových knihoven

- [Beckman SPRIworks HT \(for the Illumina platform\)](#)
- [Beckman SPRIworks Fragment Library \(for the Illumina platform\)](#)
- [Illumina TruSeq DNA PCR-Free \(for the Illumina platform\)](#)
- [IntegenX PrepX ILM DNA \(for the Illumina platform\)](#)
- [NEB NEBNext Ultra DNA \(for the Illumina platform\)](#)
- [NuGEN Encore Rapid Library \(for the Illumina platform\)](#)
- [Illumina Nextera DNA \(for the Illumina platform\)](#)
- [Kapa DNA Library \(for the Illumina platform\)](#)
- [Homebrew \(for the 10X Genomics platform\)](#)
- [Life Tech Ion Plus Fragment \(for the Ion platform\)](#)
- [Illumina Nextera XT \(for the Illumina platform\)](#)
- [NEB NEBNext DNA \(for the Illumina platform\)](#)
- [Bioo Scientific NEXTflex Rapid DNA \(for the Illumina platform\)](#)
- [Bioo Scientific NEXTflex PCR-Free \(for the Illumina platform\)](#)
- [Bioo Scientific NEXTflex DNA \(for the Illumina platform\)](#)
- [Illumina TruSeq DNA \(for the Illumina platform\)](#)
- [PacBio DNA Template Prep \(for the PacBio platform\)](#)

<https://genohub.com/ngs-library-preparation-kit-guide/>

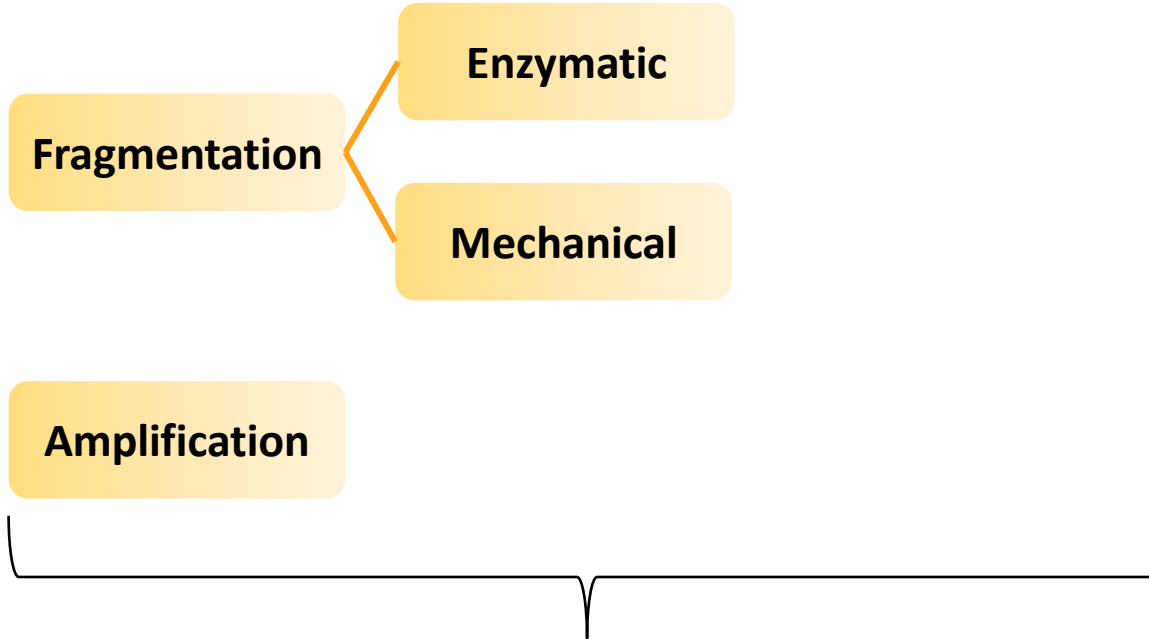
# Kontrola DNA

- Kontrola integrity DNA na gelu
- Kontrola koncentrace vyizolované DNA (NanoDrop, Qubit...)



# Příprava knihoven

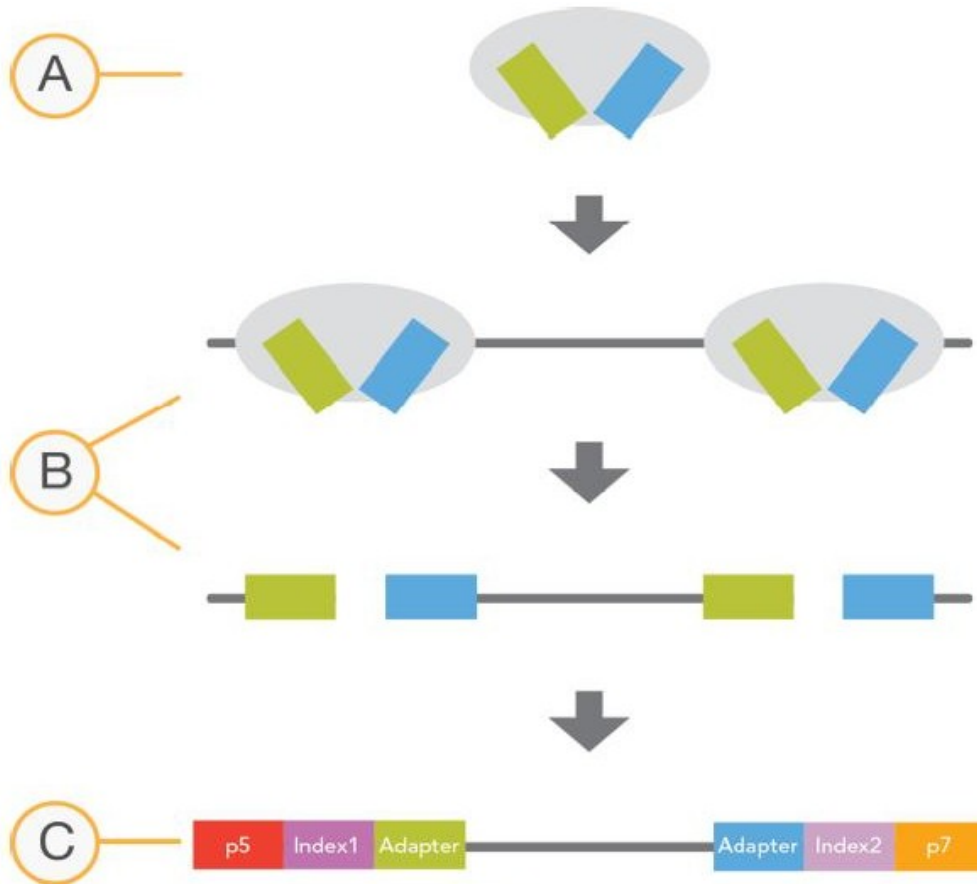
Fragment



Adapter



# Enzymatic fragmentation



Separate fragmentation not required

Tag with enzyme mix

PCR  
Polishes fragment ends and incorporates optional indices

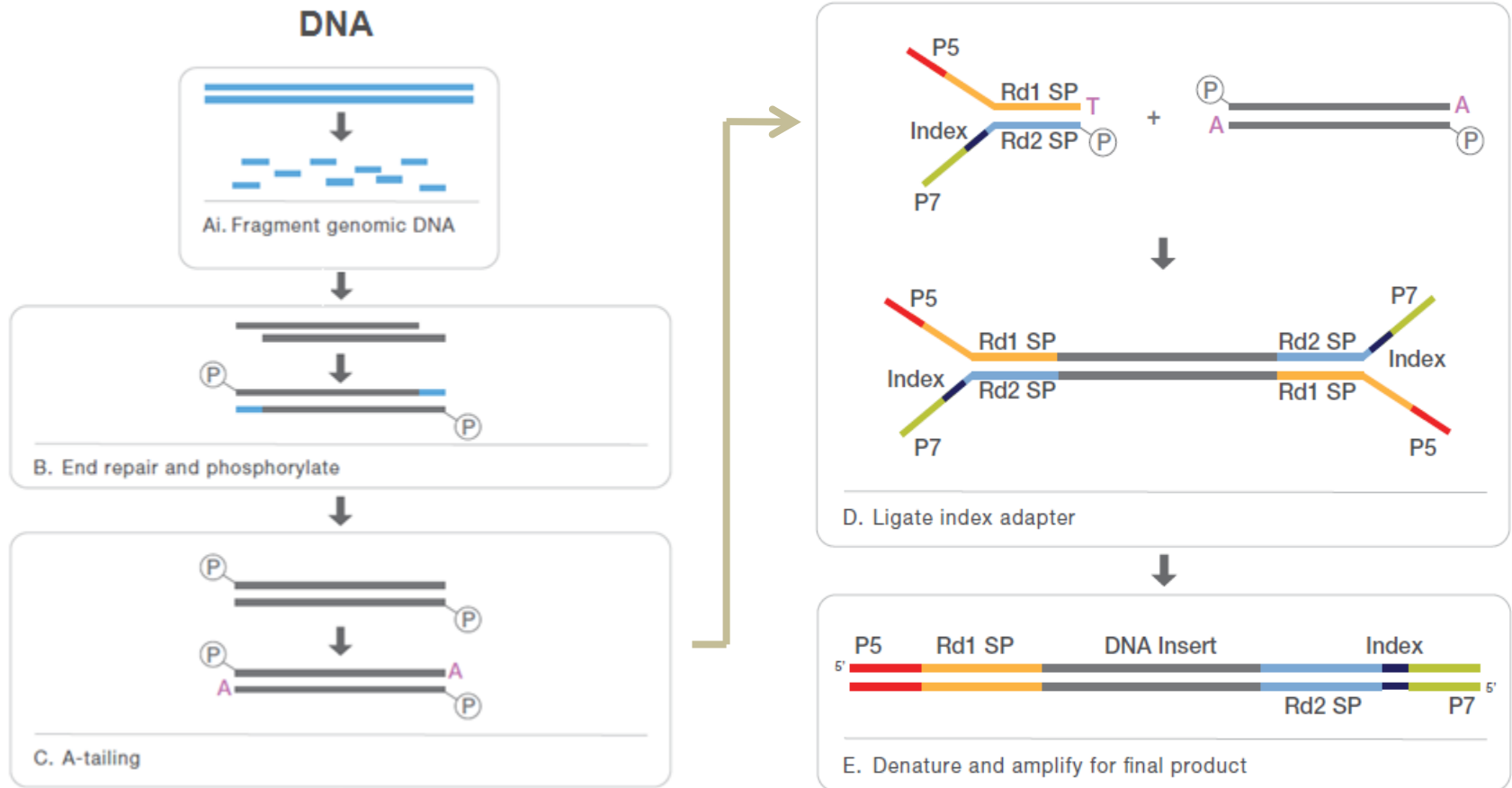
- A Nextera Transposome with Adaptors
- B Tagmentation to Fragment and Add Adaptors
- C Limited Cycle PCR to Add Sequencing Primer Sequences and Indices

# Mechanická fragmentace

- Sonikace
- Nebulizace
- Hydroshear



# Mechanická fragmentace



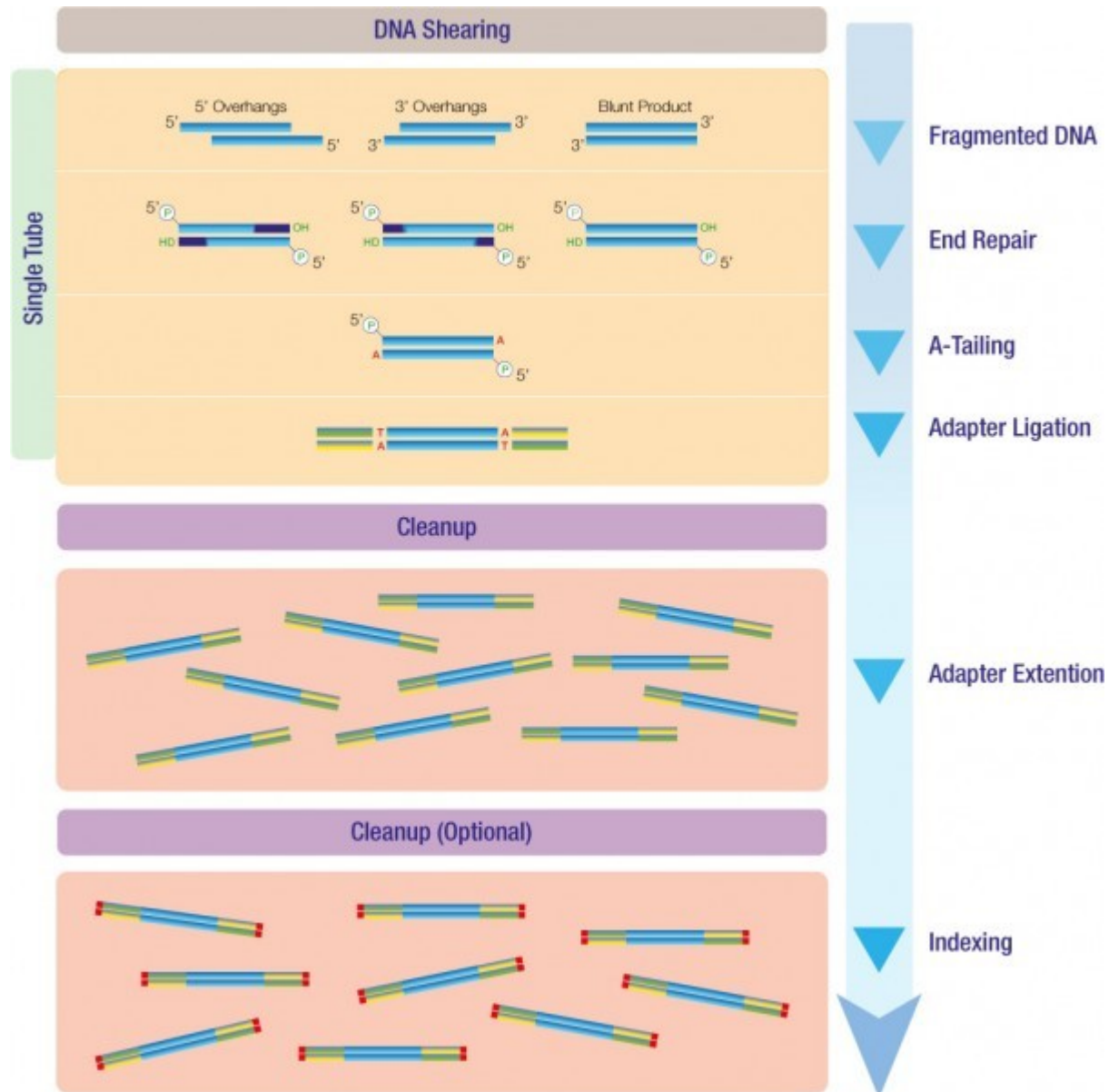
# Obecný postup



A Meridian Life Science® Company

## JetSeq Library Preparation workflow

The fast, one-tube JetSeq Library Preparation protocol offers the fastest turnaround time, with minimal cleanup steps reducing hands-on time and significantly reducing the total time required for preparation of library DNA



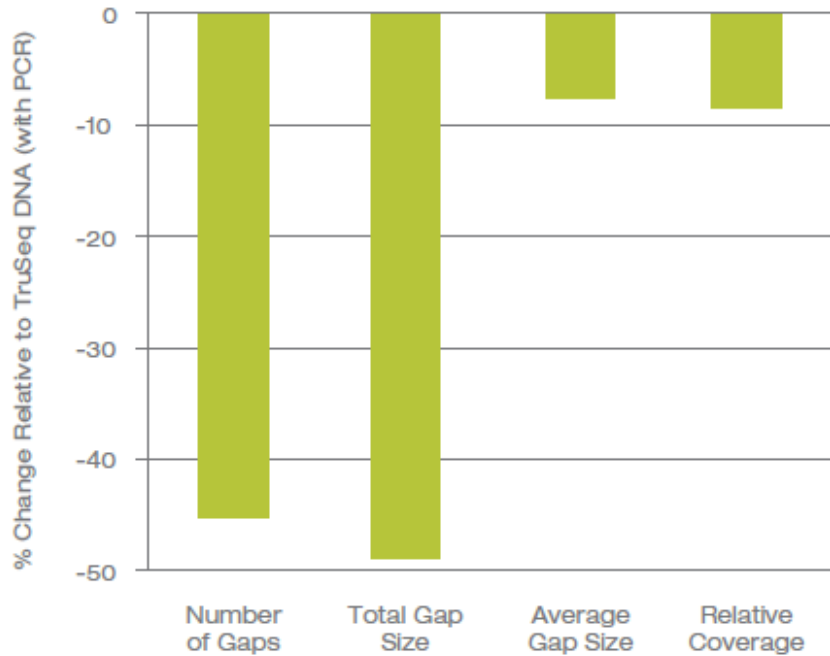
# Nextera XT DNA Sample Prep Kit

- Rapid sample preparation- complete sample prep in 90 minutes
  - Fastest and easiest sample prep workflow
  - Innovative sample normalization
  - Flexible multiplexing
- Optimized for many application
  - Small genomes, plasmids and PCR amplicons
  - Low amount of input DNA (1 ng)
  - High coverage

# TruSeq DNA PCR-Free Prep Kit

- Simple ½ day PCR-free workflow
  - All-inclusive kit w/gel-free size-selection
  - Tunable to a variety of read lengths
  - Low (24spl) and High (96spl) Throughput Options
- Premier genome coverage
  - Lowest & smallest number of gaps
  - No duplicates from PCR
  - Enhanced coverage of high GC/AT rich regions

# TruSeq DNA PCR-Free Data Quality



Access more of the genome with reduced library bias and gaps



# TruSeq Nano DNA Prep Kit

- Simple less than one day workflow
  - All-inclusive kit w/gel-free size-selection
  - Tunable to a variety of read lengths
  - Low (24spl) and High (96spl) Throughput Options
- Premier genome coverage
  - Low sample input (100 ng)
  - Lowest & smallest number of gaps
  - Enhanced coverage of high GC/AT rich regions

# Další možnosti příprav knihoven DNA, RNA - Illumina

# TruSeq Synthetic Long-Read DNA Library Prep Kit

- Designed for two applications: preparing DNA libraries for long-read assembly and phasing analysis from whole genome sequencing data
- Leverages TruSeq and Nextera chemistries with the high accuracy of short sequencing reads to construct long synthetic fragments with high assembly accuracy or efficient phasing
- Enables phasing of *de novo* mutations and the identification of co-inherited alleles in a population

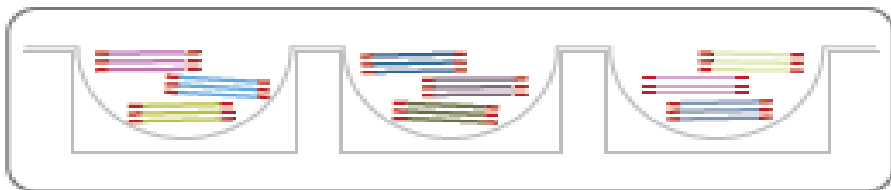
# TruSeq Synthetic Long-Read DNA Library Prep Kit



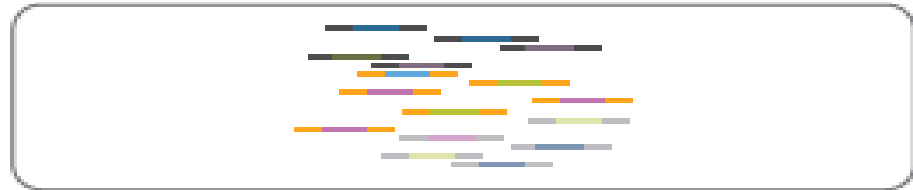
Library construction begins with genomic DNA that is fragmented to lengths of approximately 10 kb. Adapters are ligated to the fragments.



Fragments are tagged and a PCR reaction labels them with unique indexes. The fragments from all 384 wells are pooled, purified, and size selected.



Fragments are clonally amplified across 384 wells.



Fragments are sequenced. The TruSeq Long-Read Assembly App constructs long fragments from the shorter sequencing reads.

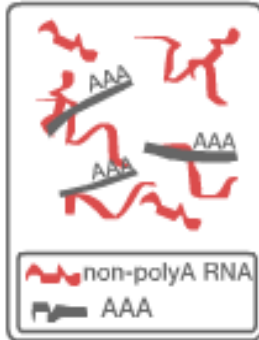
# Příprava RNA knihoven

- TruSeq RNA Sample Prep Kit v2
- TruSeq Stranded mRNA HT Sample Prep Kit
- TruSeq Stranded Total RNA LT Sample Prep Kit
- TruSeq Small RNA Sample Prep Kit

# 1. TruSeq® RNA Sample Prep Kit V2

- Simple 12 hours workflow
  - All-inclusive kit w/gel-free size-selection
  - Tunable to a variety of read lengths
- High-Troughput Gene Expression studies
  - Improvements in the RNA to cDNA conversion
  - polyA selection of mRNA

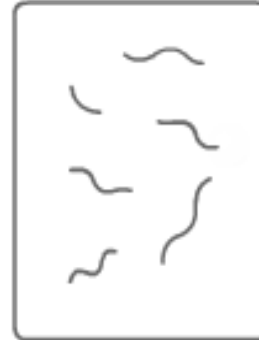
# 1. TruSeq<sup>®</sup> RNA Sample Prep Kit V2



Total RNA



mRNA Purification



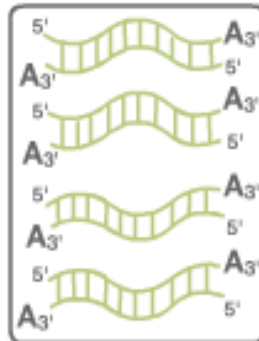
Fragmentation



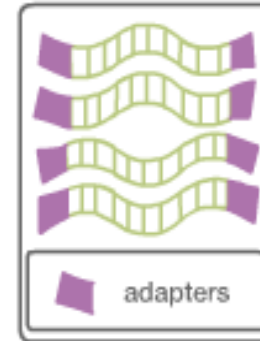
1st Strand cDNA  
Synthesis



2nd Strand  
cDNA Synthesis



3' Adenylate



Adapter Ligation



Enrich DNA  
Fragments

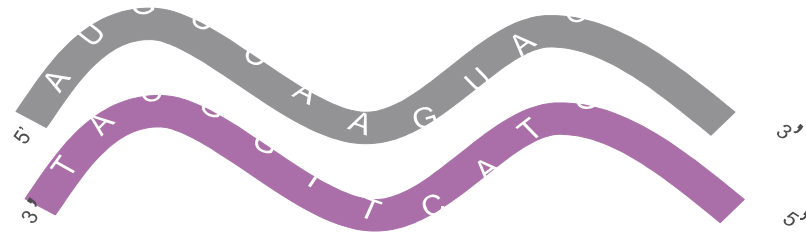
## 2. TruSeq<sup>®</sup> Stranded mRNA Sample Prep Kit

- Simple less than 2 days workflow
  - All-inclusive kit w/gel-free size-selection
  - Tunable to a variety of read lengths
- High-Troughput Gene Expression studies
  - Improvements in the RNA to cDNA conversion
  - polyA selection of mRNA
  - Precise measurement of strand orientation
  - Compatible with many sample types including low-quality and FFPE



## 2. TruSeq<sup>®</sup> Stranded mRNA

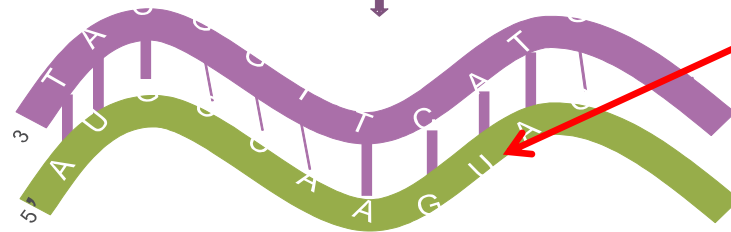
**First strand  
cDNA synthesis  
(w random hex)**



RNA

First  
Strand  
cDNA

**Second strand  
cDNA synthesis  
(w random hex)**

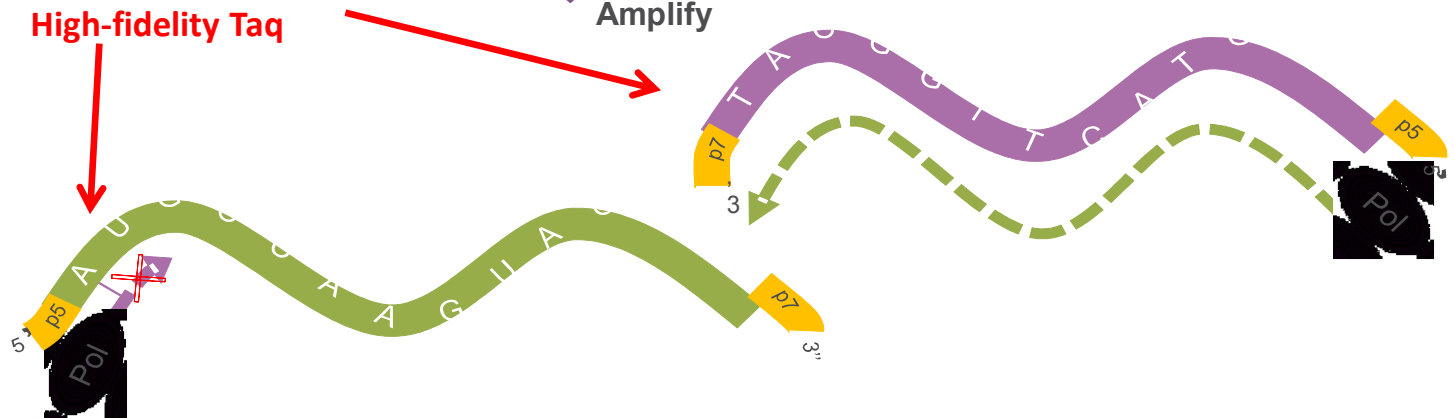


**Incorporates dUTP instead of dTTP**

First  
Strand  
Second  
Strand

Ligate Adapters

Enrich DNA fragments /  
Amplify

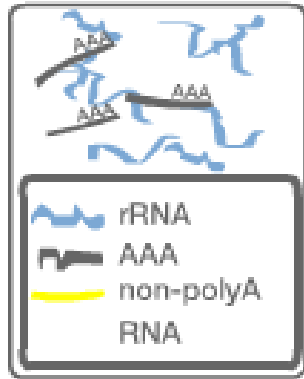


**High-fidelity Taq**

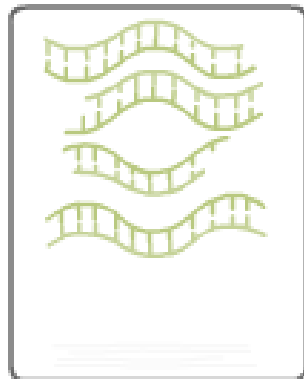
# 3. TruSeq<sup>®</sup> Stranded Total RNA Sample Prep Kit

- Simple less than 2 days workflow
  - All-inclusive kit w/gel-free size-selection
  - Tunable to a variety of read lengths
- Sequencing of mRNA and broad range of non-coding RNA (lincRNA, snRNA, snoRNA and other RNA)
  - Improvements in the RNA to cDNA conversion
  - RiboZero kits remove rRNA – rRNA depleted RNA
  - Precise measurement of strand orientation
  - Compatible with many sample types including low-quality and FFPE

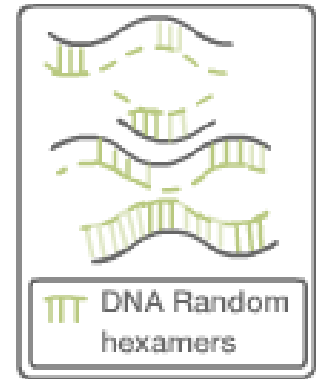
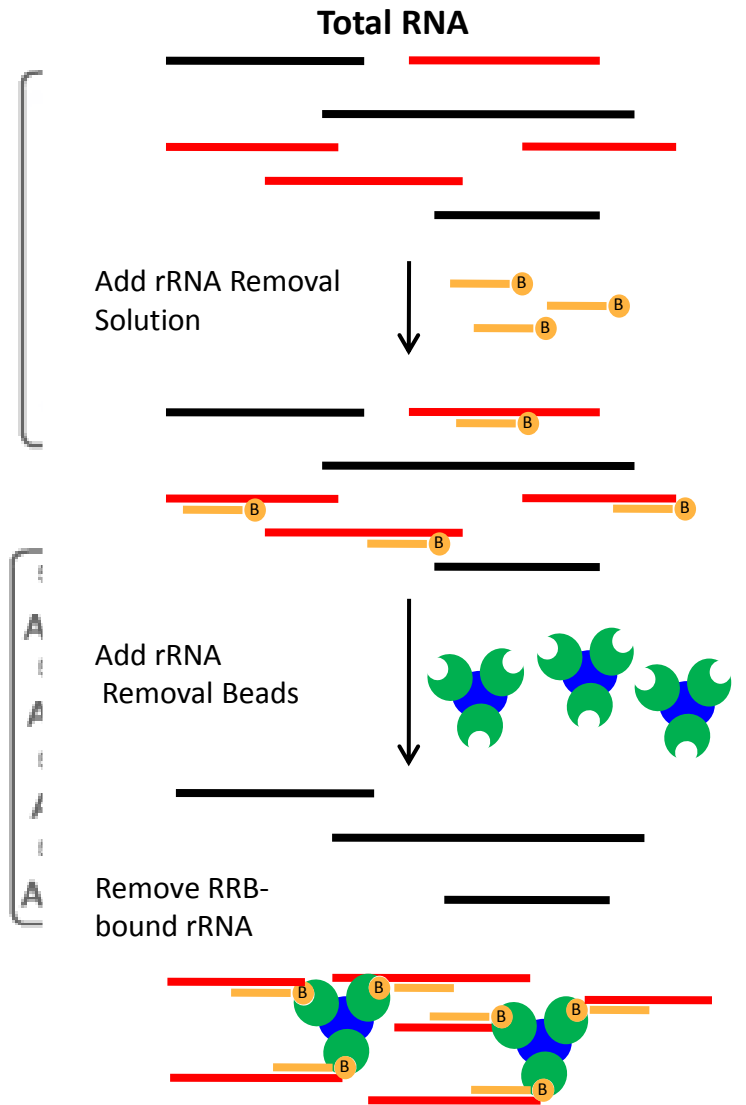
# 3. TruSeq<sup>®</sup> Stranded Total RNA



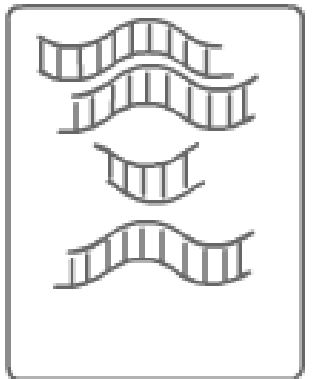
Total RNA



2nd Strand cDNA Synthesis



1st Strand cDNA Synthesis

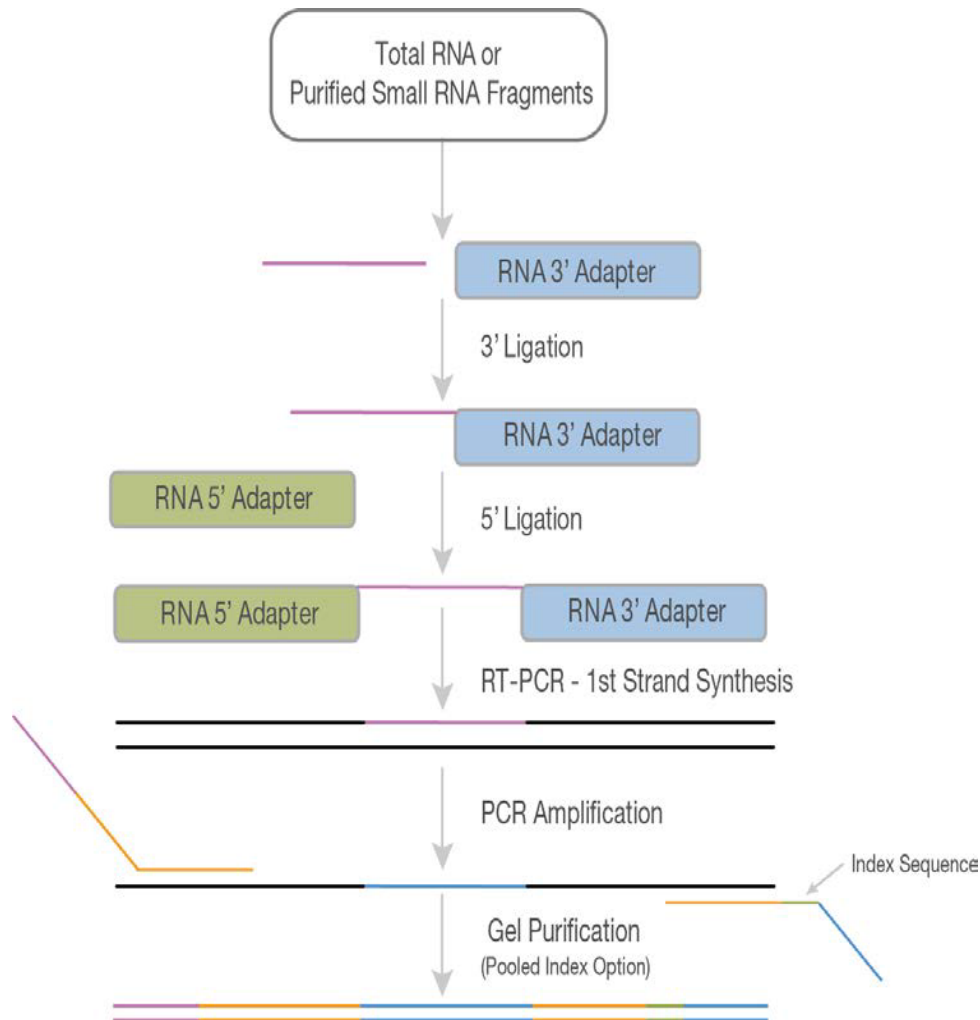


Enrich DNA Fragments

# TruSeq Small RNA Sample Prep Kit

- Simple less than 2 days workflow
- Sequencing of small mRNA – for small RNA discovery and profiling applications
  - Enables preparation of a variety of RNA species, using total RNA or purified small RNA as input.
  - Takes advantage of the natural structure common to most known microRNA molecules
  - Characterize variations such as isomiRs with single-base resolution

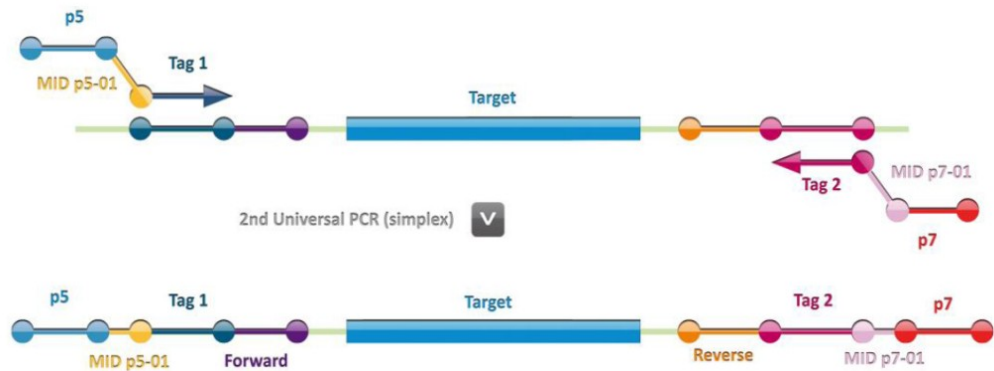
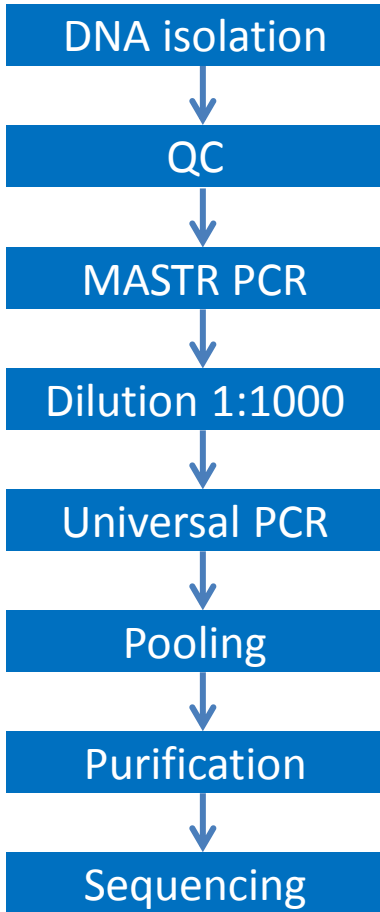
# TruSeq Small RNA Sample Prep Kit



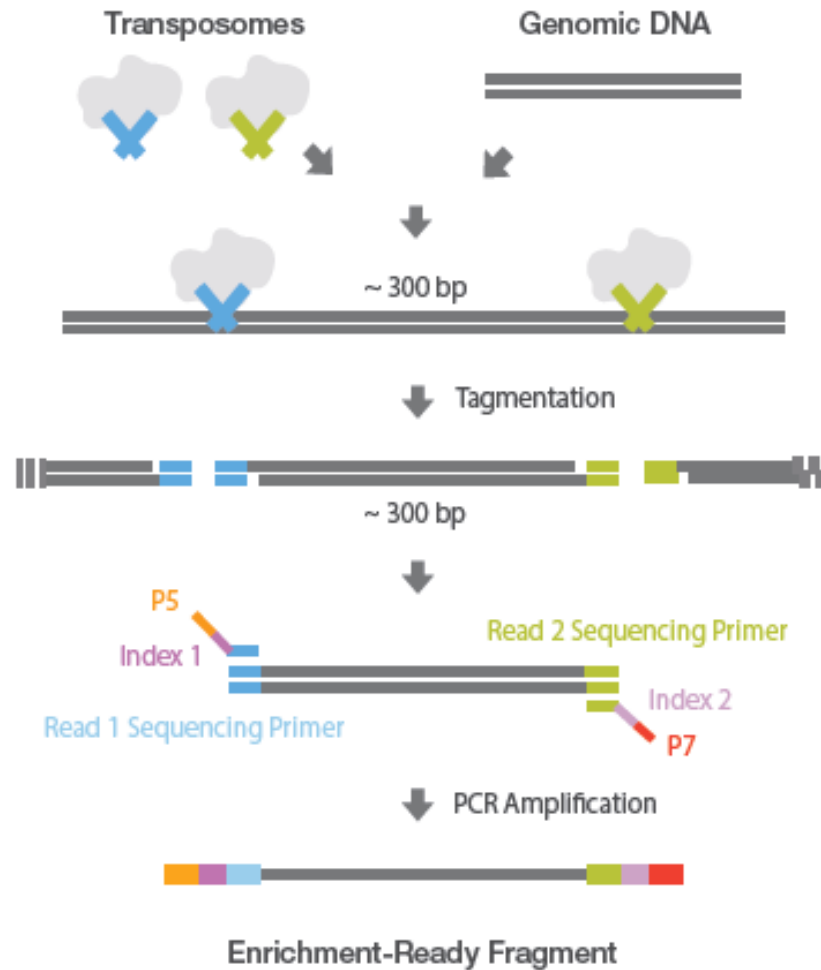
# Cíleně obohacené knihovny

- 1. Multiplexové PCR
- 2. Obohacení pomocí sond

# Ukázka multiplexové PCR (Multiplicom)

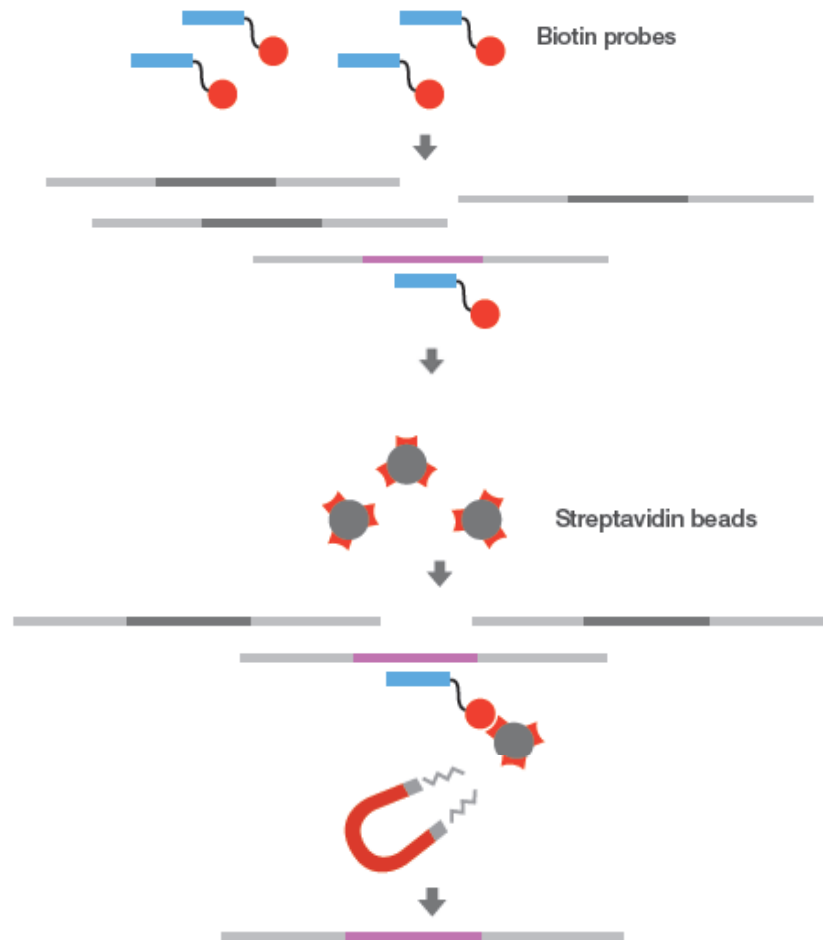


# Ukázka obohacení pomocí sond





# Ukázka obohacení pomocí sond

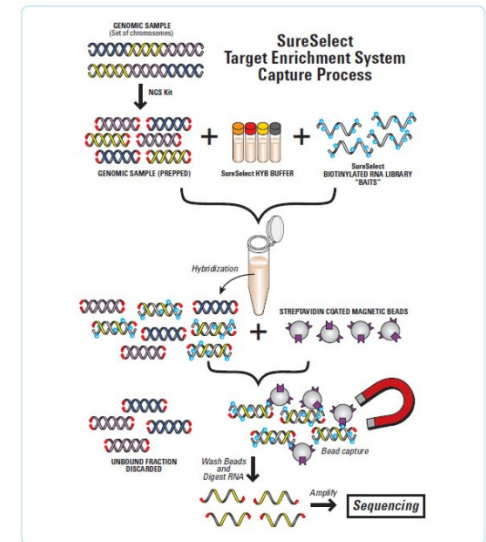
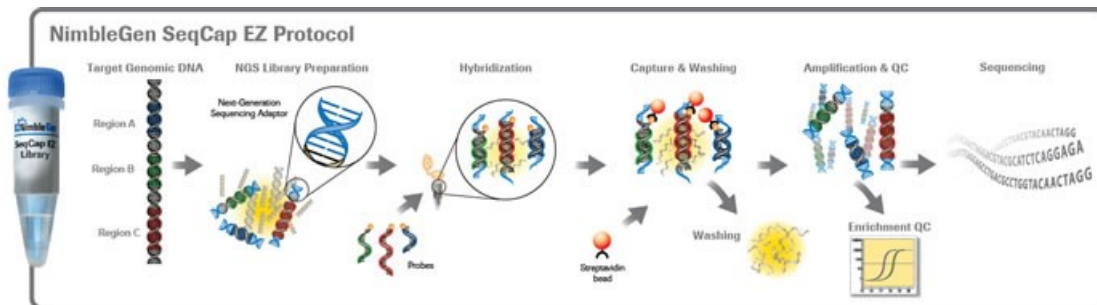


# Srovnání obou přístupů

<b>Amplicon Sequencing</b> <i>Amplification-based selection</i>	<b>Targeted Enrichment</b> <i>Hybridization-based selection</i>
<ul style="list-style-type: none"><li>• Discover common and rare mutations<ul style="list-style-type: none"><li>• Point mutations</li><li>• Insertions/deletions (indels)</li><li>• Copy number variations (CNVs)</li></ul></li></ul>	<ul style="list-style-type: none"><li>• Discover common and rare mutations<ul style="list-style-type: none"><li>• Point mutations</li><li>• Insertions/deletions (indels)</li><li>• Copy number variations (CNVs)</li><li>• Gene rearrangements</li></ul></li></ul>
<ul style="list-style-type: none"><li>• Ideal for characterizing up to 0.5 Mb</li></ul>	<ul style="list-style-type: none"><li>• Ideal for assaying &gt;0.5 Mb</li></ul>
<ul style="list-style-type: none"><li>• Very cost-effective</li></ul>	<ul style="list-style-type: none"><li>• Cost-effective</li></ul>
<ul style="list-style-type: none"><li>• Fast turnaround time</li></ul>	<ul style="list-style-type: none"><li>• Moderate turnaround time</li></ul>

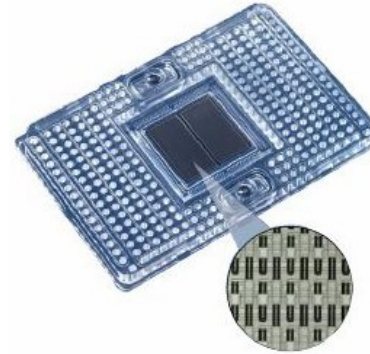
# Firmy na trhu

- Illumina – TruSeq Custom Amplicon, Nextera Rapid Capture Custom Enrichment
- Roche – NimbleGen SeqCap
- Agilent – SureSelect

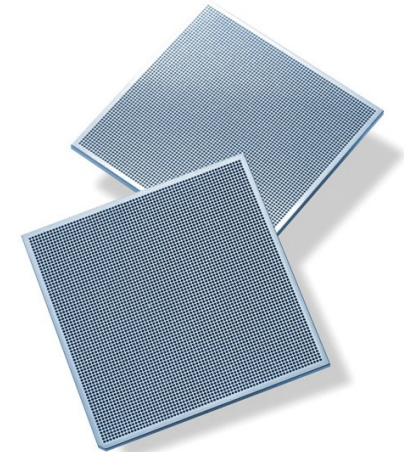
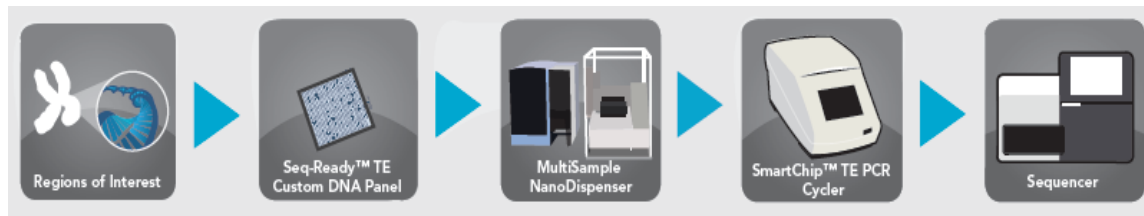


# Další možnosti

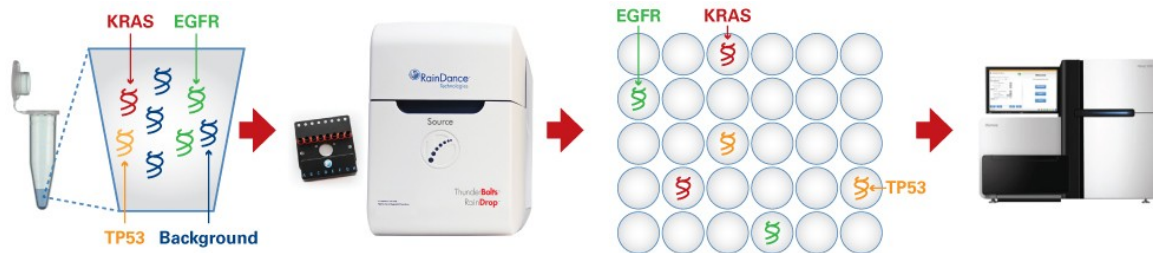
- Fluidigm



- Wafergen SmartChip



- RainDance technology



Prepare Samples,  
Primers and PCR Reagents

Enrich Gene Targets

Analyze with  
Illumina NGS

# Automatizace přípravy knihoven

## Manual

Options	Benefits	Potential Challenges
<p><b>Capital Cost:</b> Low.</p> <p><b>Number of Samples:</b> Low.</p>	<ul style="list-style-type: none"><li>• No major capital expenditure, as it does not require a dedicated system (although it does require the use of ancillary equipment and tools that labs may not already own).</li><li>• Is more flexible to incorporate changes to a protocol or method.</li></ul>	<ul style="list-style-type: none"><li>• Large quantity of consumables to purchase and manage.</li><li>• Requires high level of hands-on attention.</li><li>• Results are subject to variability, depending on who is running the protocol. Requires skilled technicians.</li><li>• More prone to errors than other methods.</li></ul>

## Microfluidics

Options	Benefits	Potential Challenges
<p><b>Capital Cost:</b> Low to high range.</p> <p><b>Number of Samples:</b> Microfluidics instruments cater to different ranges of throughput, from low to high (depending on the application and platform). Multiple microfluidics instruments can work together to allow for higher-volume processing.</p>	<ul style="list-style-type: none"><li>• Reduced hands-on time.</li><li>• High reproducibility, with reduced chance of human error.</li><li>• Some systems are dedicated to one type of application or have a narrow focus; others are more flexible and offer a broader set of applications.</li><li>• May enable lower sample input; favorable for situations where sample material is scarce.</li></ul>	<ul style="list-style-type: none"><li>• Normally requires use of assays supported by the equipment vendor.</li><li>• Ability to customize assays is more limited than with robotic liquid handling solutions.</li></ul>

## Robotic liquid handlers

Options	Benefits	Potential Challenges
<p><b>Capital Cost:</b> Mid-range to high, with some lower-range options.</p> <p><b>Number of Samples:</b> Most are geared for high throughput. Some mid-range and lower-throughput options are available, but require more user intervention.</p>	<ul style="list-style-type: none"><li>• Increases reproducibility with reduced hands-on time.</li><li>• Can be programmed for various lab needs, making it ideal for labs that require high flexibility.</li><li>• Allows researchers to adjust experimental conditions.</li><li>• Open robotic liquid handlers do not require reagents from specific vendors.</li></ul>	<ul style="list-style-type: none"><li>• Can use a large amount of consumables.</li><li>• Less capable systems may require substantial user intervention, reducing walk-away time.</li><li>• While being highly flexible, users do need to have programming expertise to develop or tweak assays.</li><li>• Ensuring that new programs work consistently and accurately demands time for validation and optimization. Changes to protocol may also require validation.</li><li>• The most highly automated walk-away options are expensive; can cost upwards of \$150,000 (USD).</li></ul>

# Automatizace přípravy knihoven



NeoPrep™  
Library prep reimagined

<http://www.illumina.com/systems/neoprep-library-system.html>



[www.illumina.com/neoprep](http://www.illumina.com/neoprep)