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# **Sekvence Nanopórem**

# Sekvenace nanopórem

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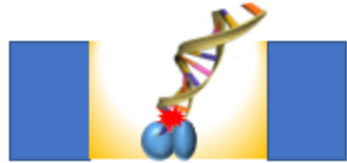


<https://nanoporetech.com/resource-centre/introduction-nanopore-sequencing>

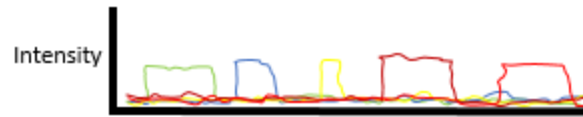
# Třetí generace sekvenátorů

## PacBio SMRT seq

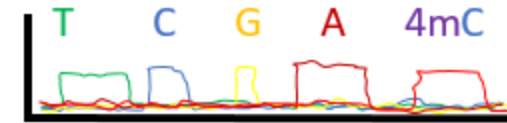
DNA passes thru  
polymerase in an  
illuminated volume



Raw output is fluorescent signal  
of the nucleotide incorporation,  
specific to each nucleotide

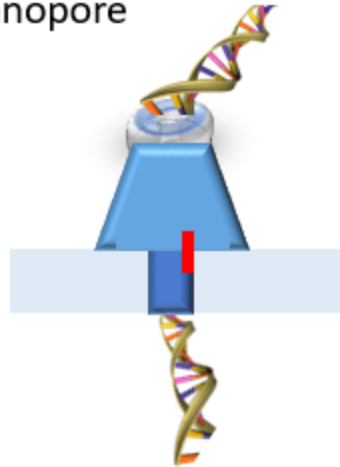


A,C,T,G have known pulse  
durations, which are used to  
infer methylated nucleotides

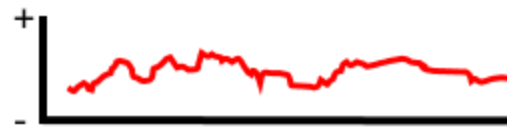


## Oxford Nanopore

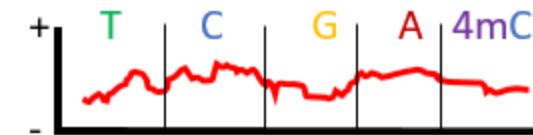
DNA passes thru  
nanopore



Raw output is electrical signal  
caused by nucleotide blocking  
ion flow in nanopore



Each nucleotide has a specific  
electric “signature”

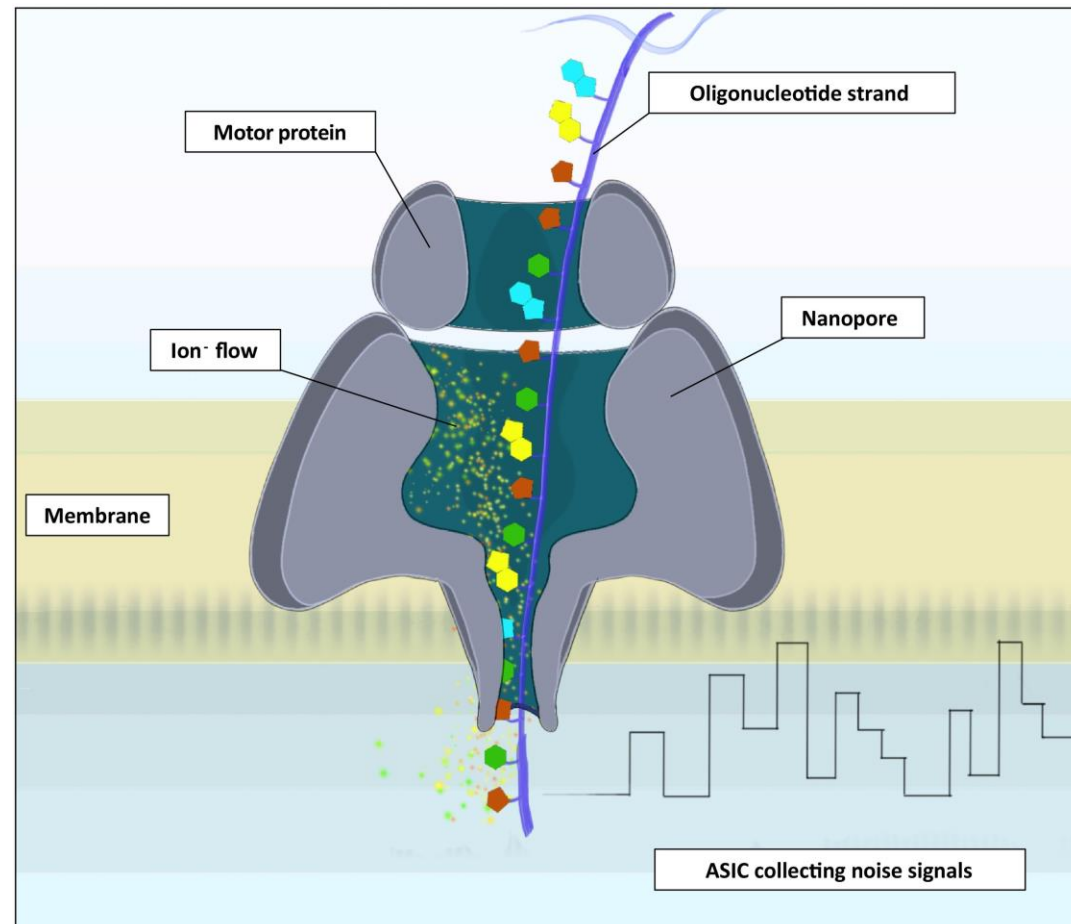


# Třetí generace sekvenátorů

- Dlouhé čtení
- Single-molecule sequencing
- Epigenetika (methylace, změny histonů, ...)
- Snadnější assembly
- Rychlost

# Princip sekvenace

- Detekce změny elektrického napětí při průchodu molekuly DNA nanopórem

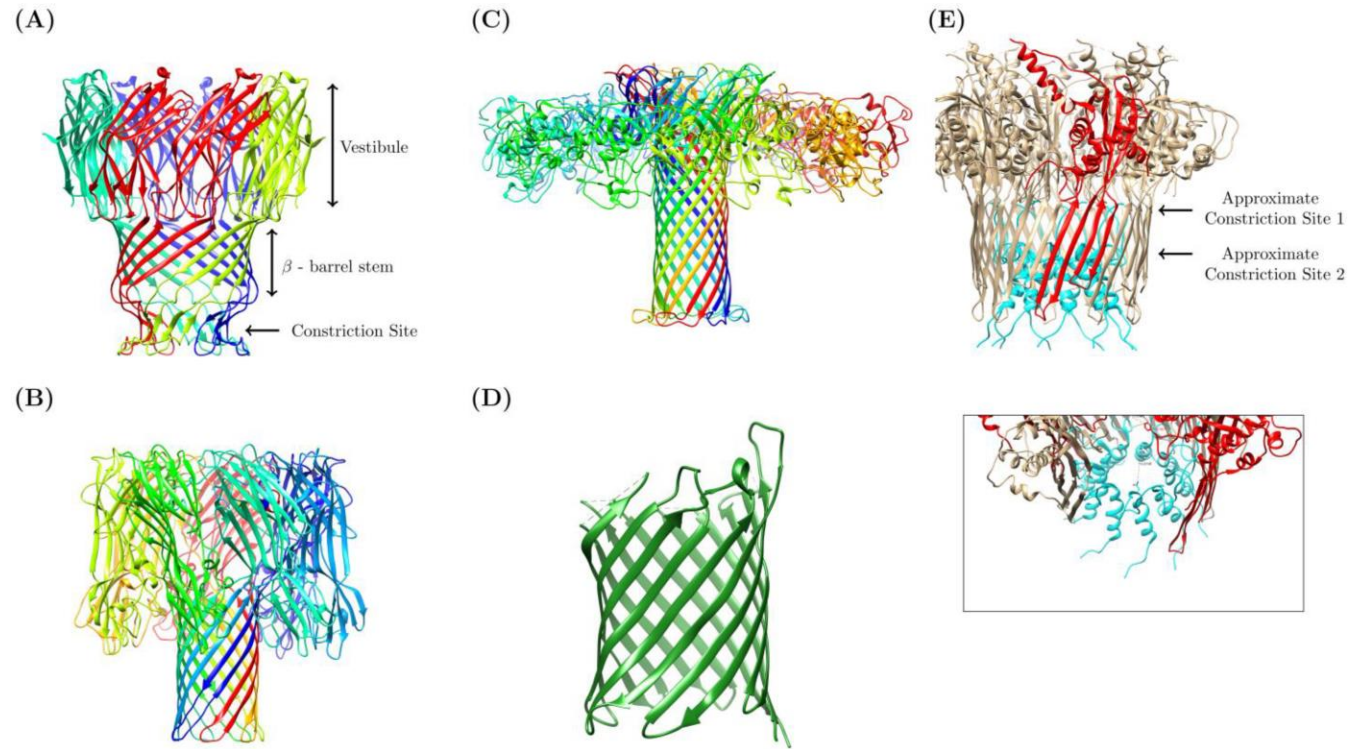


# Nanopór

- Dostatečně malý
- Stabilní
- Editovatelný
  
- Biologický
- Pevný (solid-state)

# Biologický nanopór

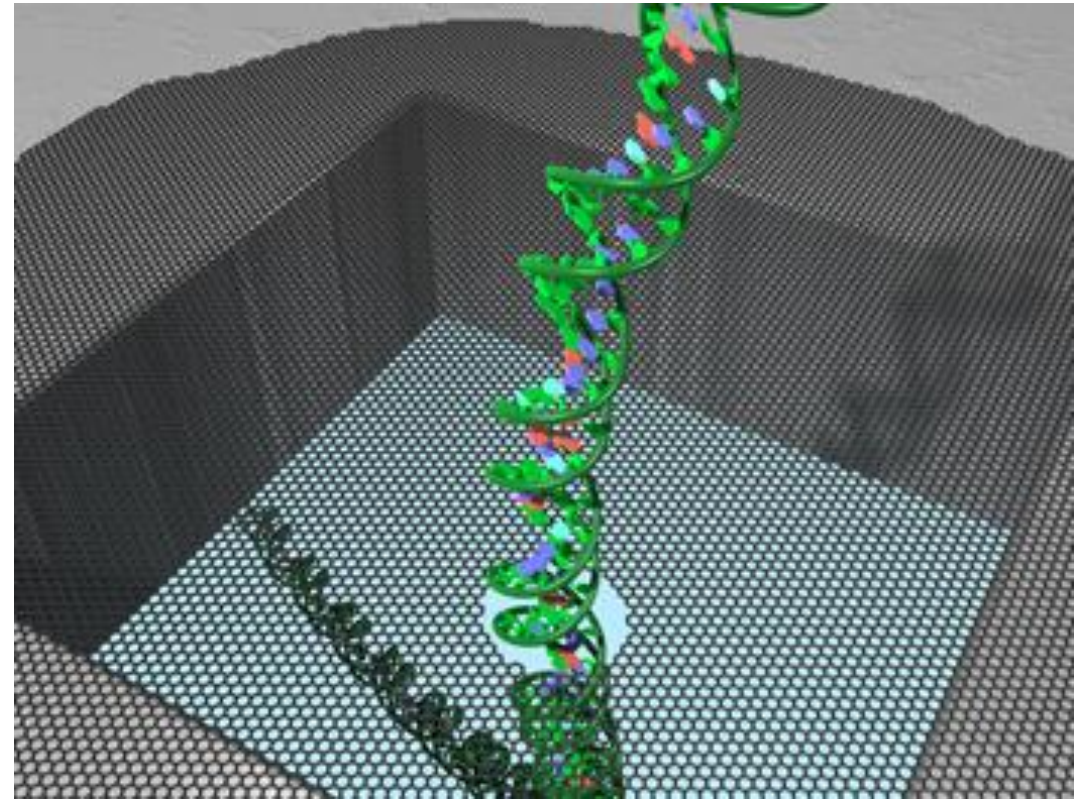
- Alpha hemolysin
- CsgG-CsgF
  
- Průměr ~ 1nm
- Vazebná místa pro modifikaci
  - DNA sonda
  - Molekulární motor
  - Ligandy pro specifické proteiny



(A) MspA pore (PDB ID 1UUN) with a constriction site diameter of 1.2 nm, and a stem length of ~3.7 nm (B) Alpha Hemolysin (PDB ID 7AHL) with a constriction site diameter of 2.6 nm and a stem length of 5.2 nm (C) Aerolysin porin (PDB ID 5JZT) with a constriction site diameter of 1 nm, and a stem length ~10 nm. (D) A simplified depiction of OmpG pore (PDB ID 2F1C) with a constriction site of 1.3 nm. (E) CsgG-CsgF mutant (PDB ID 6SI7) with two constriction sites: the original CsgG constriction of 1 nm diameter (chain monomer of the original CsgG pore is depicted in red); and a secondary constriction caused by the insertion of CsgF (Cyan residues) with a 1.5 nm diameter. The insert shows the approximated diameter of the second constriction site, ~1.5 nm. Protein chains are depicted in different colors to help with distinction.

# Pevný nanopór

- Grafenové struktury
- Vyšší napěťové zatížení



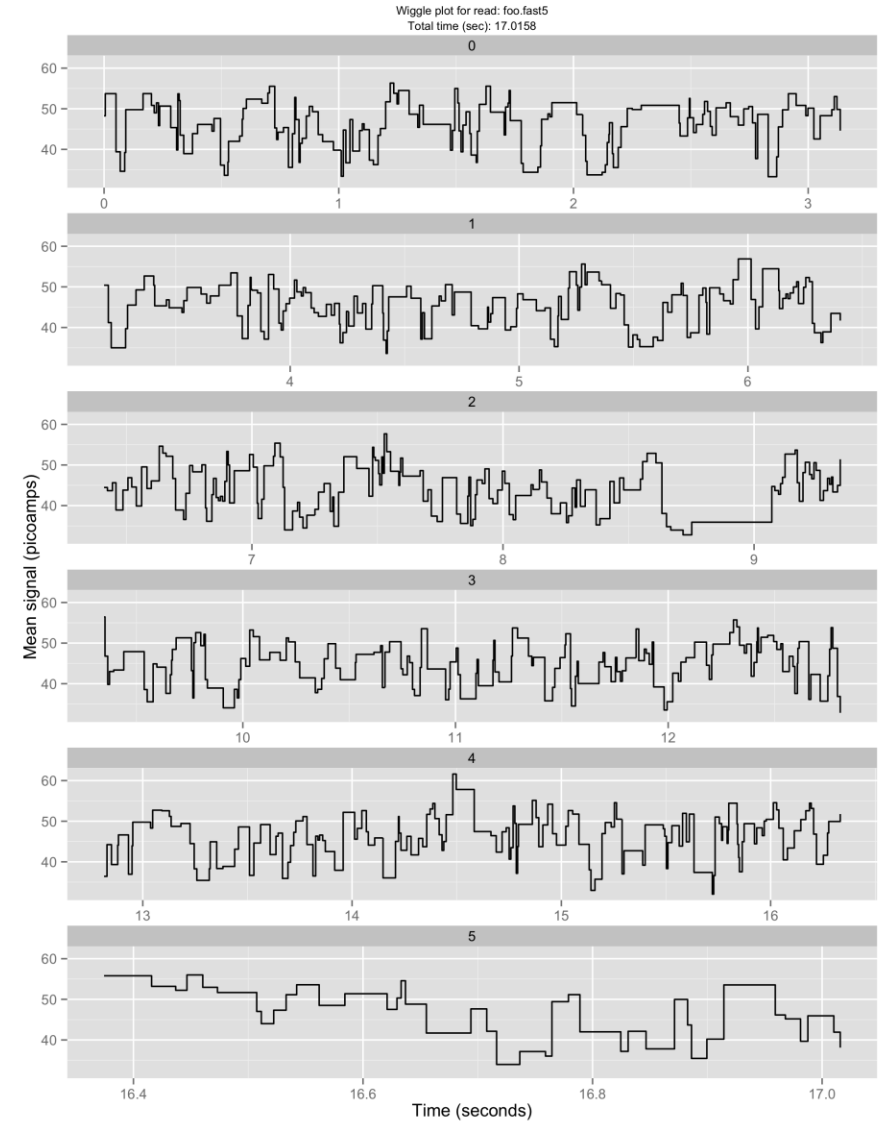


# Laboratorní příprava

- Dostupné kity dle aplikace
- Fragmentace
- Ligace barcodů
- Ligace sekvenační adaptérů

# Squiggle

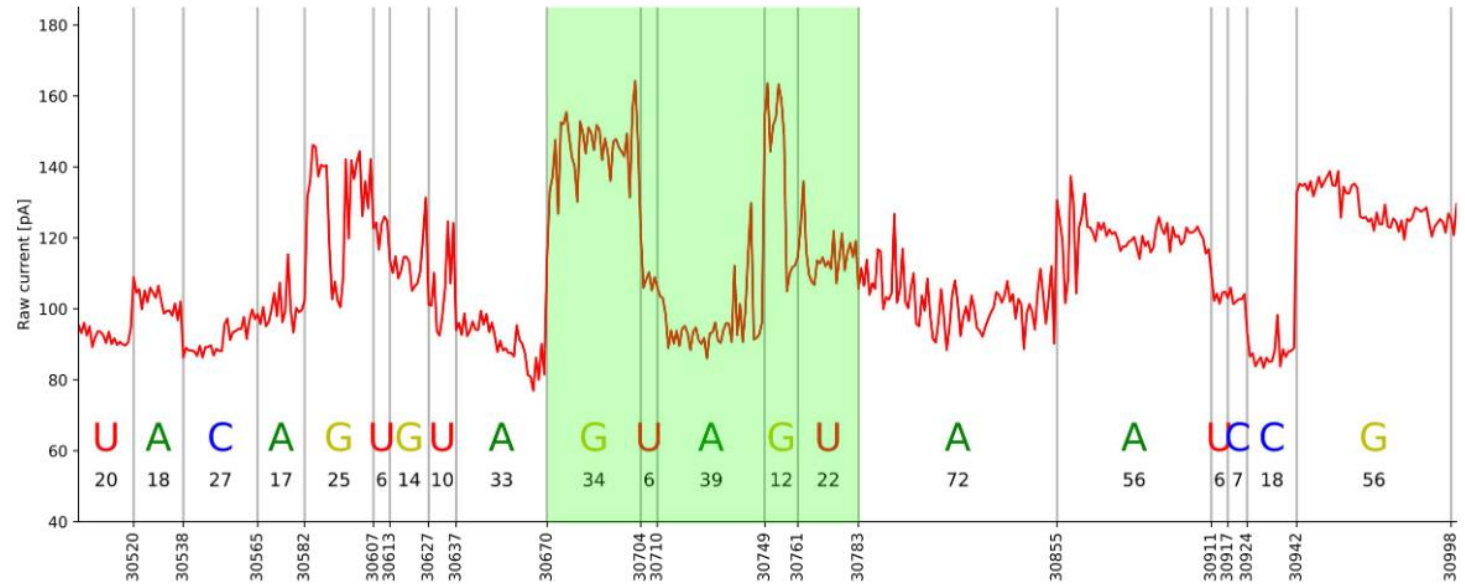
- Signálový výstup
- Čím je ovlivněn?



# Basecalling

- Guppy
- Open-source software

Basecalling: squiggle to basecalls



# Zpracování dat

- Výstup ve formátu \*.fast5 (hdf)
- Obsahuje metadata
  
- Export do \*.fastq

# Porovnání s Illumina

	Oxford Nanopore	Illumina
Délka readů	< 2 Mbp	< 600 bp
Přesnost	87-98 %	99 %
Cena za Gbp	\$ 50-200 (minION) \$ 20-40 (PromethION)	\$ 40-60 (NextSeq) \$ 10-35 (NovaSeq)
Real time	Ano	Ne
DNA modifikace (methylace)	Ano	Ne