Deep Amplicon Sequencing of *POLE* Gene for Effective Endometrial Tumour Diagnostics Using fastGEN Technology.

Rastislav Slavkovský[1], Jana Stránská[1], Nikola Ptáková[2], Kořínková Gabriela[3] , Barbora Blumová[1]
Jiří Drábek[1], Veronika Seidlová[4], Kateřina Pehlíková[4], Petr Brož[5], Marián Hajdúch[1]
[1]Institute of Molecular and Translational Medicine, Faculty of Medicine, Palacký University, Olomouc, Czech Republic
[2]Bioptická laboratoř s.r.o., Plzeň, Czech Republic [3]Department of Clinical and Molecular Pathology, Faculty of Medicine, Palacký University in Olomouc,
[4]Biovendor LM/MDx a.s., Brno, Czech Republic, [4]Bioxsys s.r.o., Ústí nad Labem, Czech Republic

INSTITUTE OF MOLECULAR AND TRANSLATIONAL MEDICINE

Introduction

Tumor DNA testing of *POLE* gene, which is component of DNA polymerase epsilon, is a prerequisite for tumor risk-group assessment and personalized treatment in endometrial carcinoma. *POLE* mutated tumors are low risk and adjuvant chemotherapy should be deescalated. *POLE* mutations occur in 7 - 12% of endometrial cancers and have been associated with high tumor mutation burden, neoantigen load. Retrospective analysis showed that pathogenic *POLE* mutations are associated with clinical benefit to immune checkpoint inhibitor therapy, thus further thorough studies are warranted to validate *POLE* mutation as a predictive biomarker.

Deep amplicon next-generation sequencing (NGS) has a potential to be a suitable method for simultaneous direct detection of all somatic mutation within tested regions.

Aim of the study was to develop and verify a fast NGS library preparation of tumor DNA samples for the detection of **mutations in clinically relevant codons of *POLE* gene** (Tab. 1)

Protein Change	Nucleotid Change	Exon no.	Frequency
P286R	c.857C>G	9	+++
V411L	c.1231G>T/C	13	+++
S297F	c.890C>T	9	++
S459F	c.1376C>T	14	+
A456P	c.1366G>C	14	+
F367S	c.1100T>C	11	+
L424I	c.1270C>A	13	+
M295R	c.884T>G	9	.
P436R	c.1307C>G	13	.
M444K	c.1331T>A	13	.
D368Y	c.1102G>T	11	.

Tab. 1: Clinically relevant pathogenic variants according to Dundr 2021, Cesk Patol; 57(3) and Castillo 2020, J Pathol Mar;250(3). + : semi-quantitative representation of variant frequency in endometrial tumors.

Materials and methods

Samples: DNA isolated from formalin fixed paraffin embedded (FFPE) block of endomerial adenocarcinoma or samples provided in laboratory quality assessments.

NGS library preparation: **NGS method based on proprietary amplicon fastGEN technology (a.k.a. fastGEN Endometrial Cancer Kit, Biovendor)** were introduced into laboratory as described at Fig.1. Usually 4 to 16 samples per run were pooled and run with other fastGEN libraries.

Sequencing: **Illumina MiSeq** using pair-end 2x150 bp reads.

Data analysis: MiSeq Reporter using BWA algorithm for alignment and Somatic Variant Caller for variants detection. Output .VCF data processed by MS-Excel VBA macro or **GENOVESA fastGEN POLE** module.

Results interpretation: **≥ 5 %** mutated allele fraction (MAF) were concluded as **mutation detected**, 1 - 5% were confirmed in duplicates.

Results

In validation study of 12 samples we observed 100 % concordance between fastGEN and alternative method (Tab.2, QIAseq Tumor Mutational Burden Panel - Qiagen, The Cell3™ Target: Pan-Cancer Panel – Nonacus, TruSight Oncology 500 Panel - Illumina or VariantPlex custom GYNcore - Archer)

result	*POLE genotyping*
True Positive	6
True Negative	6
False Positive	0
False Negitive	0
Specificity	100%
Sensitivity	100%

Tab.2: Summary of intra and interlaboratory comparison of results using alternative method.

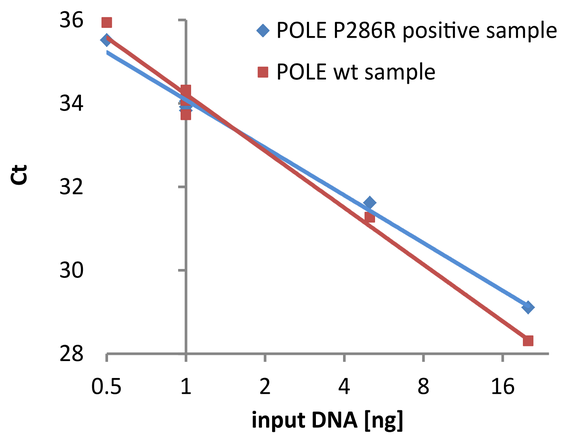

Fig.2: Correlation of Ct values obtained during library preparation with input amount.

Limit of detection for input amount

We have tested the different input amount including limiting amount of **input DNA (1 ng or less).** In all cases (n = 12) we have seen library amplification, with Ct value linearly dependent on logarithm value of input (Fig.2) and correct genotyping result with unbiased MAF(17 – 26%).

Reproducibility of method

Reproducibility was tested using 2 positive samples in 5 repetitions with correct result for each case with st. dev. of MAF below 3% and negative sample were repeated 4 times with negative result in each case (Tab.3).

Frequency of biomarker variants in gynecological tumors

51 gynecological tumor samples including 3 inter-laboratory control samples were tested during 2022. We have detected mutations in 4 different codons (Fig.3). Out of 48 routine samples **6 % were positive for clinically relevant pathogenic *POLE* mutations.** 3 samples (6%) with lower DNA quality were retested to obtain data with sufficient QC values.

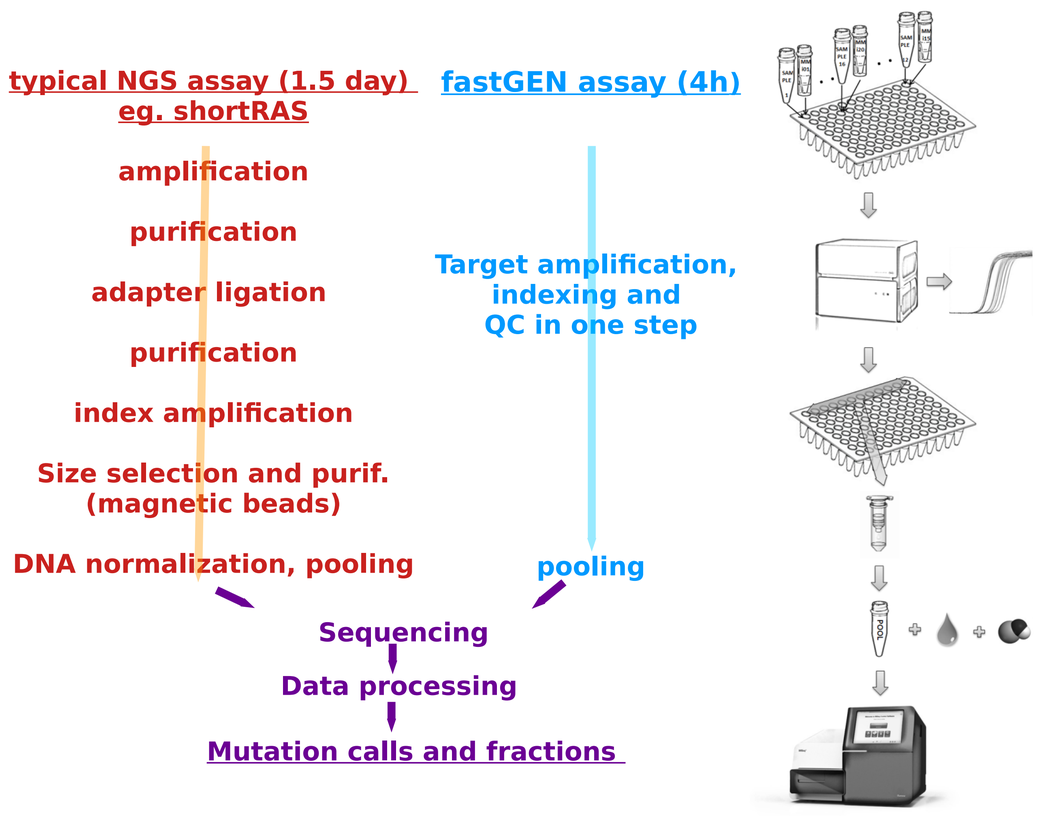

Fig.1: Scheme of procedures in **typical** and **fastGEN** NGS assays. (typical assay is described for example in Slavkovsky 2022, Neoplasma Vol.69)

Sample ID	Expected status	rep. ID	Result status	MAF	Sequencing depth (X)
13947	S459F 31 %	A	S459F	28%	56 150
		B	S459F	31%	14 448
		C	S459F	30%	37 602
		D	S459F	25%	9 245
		E	S459F	28%	18 606
		average		28%±2.5%	27 210
13752	P286R 19%	A	P286R	21%	33 346
		B	P286R	20%	9 218
		C	P286R	22%	14 348
		D	P286R	19%	10 652
		E	P286R	21%	13 759
		average		21%±1.2%	16 265
14069	wt	A	wt	ND	>1000*
		B	wt	ND	>1000*
		C	wt	ND	>1000*
		D	wt	ND	>1000*

Tab.3: Results of reproducibility of 3 samples. For each repetition new library was prepared and sample was sequenced and analysed independently. * values for sequencing depth for all the studied regions, wt – wild-type genotype (negative)

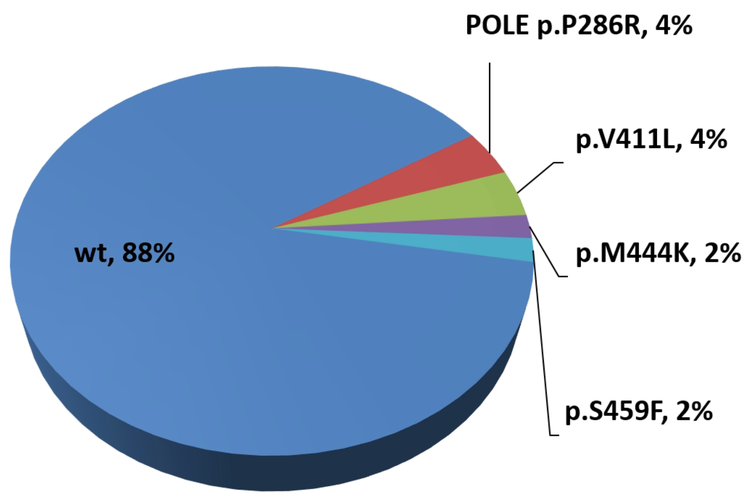

Fig.3: Proportion of cancer samples with *POLE* mutations detected during 2022 at DNA laboratory of IMTM. Totally 51 samples were analyzed including 3 positive interlaboratory control samples (V411L, P286R, S459F).

Fig.4: fastGEN kits contain 16 ready-to-use master mixes with sample indexes and sequencing primers.

Conclusion

We showed that **deep fastGEN NGS assay is a suitable method for routine diagnostic of *POLE* as biomarker** where several possible mutations need to be analyzed and enough number of samples is available. Especially in case of small FFPE derived samples, direct single-step library preparation could be beneficial for improving the quality of the results. FastGEN kits are nowadays commercially available from Biovendor Group (Fig.4)

Acknowledgment : The work was supported Supported by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and Ministry of Education, Youth and Sport of the Czech Republic grant LM2018132.