

	Method A	Method S	Method E	Method K	Method N
Description	Native barcoding	PCR barcoding	Rapid barcoding	Rapid PCR barcoding	Native barcoding
Kit name	SQK-LSK109	SQK-PBK004	SQK-RBK004	SQK-RBP004	SQK-LSK109 (Io-Cost)
Step	RT-PCR	RT-PCR	RT-PCR	RT-PCR	RT-PCR
Kit	SuperScript III Platinum	SuperScript III Platinum	SuperScript III Platinum	SuperScript III Platinum	SuperScript III Platinum
Primer sets used	Set 1	Set 2	Set 3	Set 4	Set 4
Cycling conditions	[13]	[13]	[20]	[28]	[13]
Time (min) incl bead purification	268	268	233	278	278
Step	End Prep				End Prep
Kit	NEBNext FFPE & Ultra II				NEBNext Ultra II
Time (min) incl bead purification	23				10
Step	Barcoding	PCR Barcoding	Fragmentation and Barcoding	Rapid PCR Barcoding	Barcoding
Kit	NEB Blunt/TA ligase	NEB LongAmp Hot Start Taq	ONT Fragmentation Mix	NEB LongAmp Hot Start Taq	NEB Blunt/TA ligase
Time (min) incl bead purification	23	223	13	37.5	45
Step	Adaptor Ligation	RAP	RAP	RAP	Adaptor Ligation
Kit	NEB Quick T4 DNA Ligase	ONT Rapid Adaptor	ONT Rapid Adaptor	ONT Rapid Adaptor	NEB Quick T4 DNA Ligase
Time (min) incl bead purification	23	5	5	5	23
# Steps	4	3	3	3	4
# bead purifications	4	2	2	2	3
Total hands-on time (h)	1.2	3.8	0.3	0.7	1.3
Total prep time (h)	5.6	8.3	4.2	5.3	5.9