

## APPLICATION PROTOCOL

### Preparation of Sequencing Cartridge for mixing fastGEN Primers and Primers from Illumina®, Inc.

This protocol is recommended for mixing fastGEN sequencing primers with sequencing primers from Illumina®, Inc.

#### PREPARATION OF SEQUENCING CARTRIDGE

Prepare: 3 x 1.5 ml tube or larger (depending on the sequencer used and the amount of primers)  
3 x **thin** plastic Pasteur pipette  
disposable pipette tips with filter  
thawed sequencing cartridge

1. Mark 1.5 ml tubes as R1SP, ISP a R2SP.
2. Use a tip to puncture (pierce) the position for Read 1. Use a Pasteur pipette to remove the entire volume of primers and transfer them to a new tube labeled R1SP. If required by protocol (see Table 1), add **3 µl** custom sequencing primer R1SP from the fastGEN kit, vortex, centrifuge briefly and return to the custom position in the sequencing cartridge.
3. Use a tip to puncture (pierce) the position for Index. Use a Pasteur pipette to remove the entire volume of primers and transfer them to a new tube labeled ISP. Add custom sequencing primers ISP from the fastGEN kit, vortex, centrifuge briefly and return to custom position in the sequencing cartridge.
4. Use a tip to puncture (pierce) the position for Read 2. Use a Pasteur pipette to remove the entire volume of primers and transfer them to a new tube labeled R2SP. Add custom sequencing primers R2SP from the fastGEN kit, vortex, centrifuge briefly and return to custom position in the sequencing cartridge.
5. If using more than one fastGEN kit, mix the volumes listed (see Table 1). If using only one kit, add only one appropriate primer with the indicated volume.
6. Enter information about custom primers in your Sample sheet.

This protocol is recommended for mixing fastGEN libraries with PhiX or other libraries.

7. Mix the purified DNA pools (sequencing libraries). Denature with NaOH, dilute with chilled HT1 solution.
8. Optional: Add a denatured and diluted PhiX control library.
9. Store in a cool place just before use. Then apply to the sequencing cartridge.

MiSeq				MiniSeq			
fastGEN custom sequencing primers	volume			fastGEN custom sequencing primers	volume		
	R1SP	ISP	R2SP		R1SP	ISP	R2SP
Solid	3 µl	13.5 µl	13.5 µl	Solid			
Solid II	x	13.5 µl	13.5 µl	Solid II	x	11 µl	8.2 µl
Lung	3 µl	6 µl	6 µl	Lung			
Brain	3 µl	3 µl	3 µl	Brain			
POLE	3 µl	6 µl	6 µl	POLE			
POLE/ CTNNB1	x	10.5 µl	10.5 µl	POLE/ CTNNB1	x	8.6 µl	6.4 µl
TP53	x	20 µl	20 µl	TP53	x	24.6 µl	18.3 µl
PIK3CA	x	16.5 µl	16.5 µl	PIK3CA	x	13.5 µl	10.1 µl
CFTR	3 µl	44 µl	44 µl	CFTR			
add Illumina primers to volume	600 µl	600 µl	600 µl	add Illumina primers to volume	550 µl	820 µl	610 µl
Illumina Primer (name)	Read 1 (HP10)	Index 1 (HP12)	Read 2 (HP11)	Illumina Primer (name)	Read 1 (BP10)	Index 1+2 (BP14)	Read 2 (BP11)
Position in sequencing cartridge	12	13	14	Position in sequencing cartridge	24	28	25
application to sequencing cartridge				application to sequencing cartridge			
Position	18	19	20	Position	15	13	14

NextSeq 500/550 Mid Output				NextSeq 500/550 High Output			
fastGEN custom sequencing primers	volume			fastGEN custom sequencing primers	volume		
	R1SP	ISP	R2SP		R1SP	ISP	R2SP
Solid				Solid			
Solid II	x	27 µl	20 µl	Solid II	x	27 µl	27 µl
Lung				Lung			
Brain				Brain			
POLE				POLE			
POLE/ CTNNB1	x	21 µl	16 µl	POLE/ CTNNB1	x	21 µl	21 µl
TP53	x	60 µl	45 µl	TP53	x	60 µl	60 µl
PIK3CA	x	33 µl	25 µl	PIK3CA	x	33 µl	33 µl
CFTR				CFTR			
add Illumina primers to volume	1 330 µl	2 000 µl	1 500 µl	add Illumina primers to volume	1 730 µl	2 000 µl	2 000 µl
Illumina Primer (name)	Read 1 (BP10)	Index 1+2 (BP14)	Read 2 (BP11)	Illumina Primer (name)	Read 1 (BP10)	Index 1+2 (BP14)	Read 2 (BP11)
Position in sequencing cartridge	20	22	21	Position in sequencing cartridge	20	22	21
application to sequencing cartridge				application to sequencing cartridge			
Position	7	9	8	Position	7	9	8

NovaSeq SP; S1; S2				NovaSeq S4			
fastGEN custom sequencing primers	volume			fastGEN custom sequencing primers	volume		
	R1SP	ISP	R2SP		R1SP	ISP	R2SP
<b>Solid</b>				<b>Solid</b>			
<b>Solid II</b>	x	47 µl	27 µl	<b>Solid II</b>	x	67.5 µl	47 µl
<b>Lung</b>				<b>Lung</b>			
<b>Brain</b>				<b>Brain</b>			
<b>POLE</b>				<b>POLE</b>			
<b>POLE/ CTNNB1</b>	x	37 µl	21 µl	<b>POLE/ CTNNB1</b>	x	52.5 µl	37 µl
<b>TP53</b>	x	105 µl	60 µl	<b>TP53</b>	x	150 µl	105 µl
<b>PIK3CA</b>	x	58 µl	33 µl	<b>PIK3CA</b>	x	82.5 µl	58 µl
<b>CFTR</b>				<b>CFTR</b>			
<b>add Illumina primers to volume</b>	<b>2 000 µl</b>	<b>3 500 µl</b>	<b>2 000 µl</b>	<b>add Illumina primers to volume</b>	<b>3 500 µl</b>	<b>5 000 µl</b>	<b>3 500 µl</b>
<b>Illumina Primer (name)</b>	Read 1 (VP10)	Index 1+2 (VP14)	Read 2 (VP11)	<b>Illumina Primer (name)</b>	Read 1 (VP10)	Index 1+2 (VP14)	Read 2 (VP11)
<b>Position in sequencing cartridge</b>	24	23	13	<b>Position in sequencing cartridge</b>	24	23	13
<b>application to sequencing cartridge</b>				<b>application to sequencing cartridge</b>			
<b>Position</b>	5	7	6	<b>Position</b>	5	7	6

In case of any doubts and questions, please contact the application specialist:  
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