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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. using a MiSeq sequencer.

PREPARATION OF SEQUENCING CARTRIDGE

Prepare: 3 x 1.5 ml tube
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. Pierce position number 12 (Read 1 SP) with the tip. With Pasteur pipette, remove the entire volume of liquid from position 12 and transfer it to a 1.5 ml tube marked as R1SP. Add **3 µl** fastGEN sequencing primer R1SP from the fastGEN kits to the tube, vortex, centrifuge briefly and return to the position **12** in the sequencing cartridge.
3. Pierce position number 13 (Index SP) with the tip. With Pasteur pipette, remove the entire volume of liquid from position 13 and transfer it to a 1.5 ml tube marked as ISP. Add **13,5 µl** fastGEN sequencing primer ISP Solid Cancer / ***6 µl** ISP Lung Cancer / ***3 µl** ISP Brain Cancer / ***6 µl** ISP POLE Cancer kit, vortex, centrifuge briefly and return to position **13** in the sequencing cartridge.
4. Pierce position number 14 (Read 2 SP) with the tip. With Pasteur pipette, remove the entire volume of liquid from position 14 and transfer it to a 1.5 ml tube marked as R2SP. Add **13,5 µl** fastGEN sequencing primer R2SP Solid Cancer / ***6 µl** R2SP Lung Cancer / ***3 µl** R2SP Brain Cancer / ***6 µl** R2SP POLE Cancer kit vortex, centrifuge briefly and return to position **14** in the sequencing cartridge.

* If more than one type of fastGEN kit is used, mix the indicated volumes. If you are using only one type of kit, add only appropriate primer with the indicated volume.

5. Do not enter information about custom primers in your Sample sheet.

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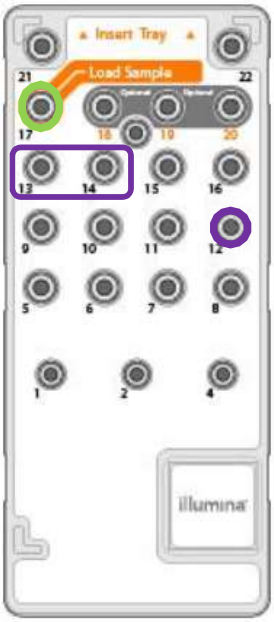
This protocol is recommended for mixing fastGEN libraries with PhiX or other libraries.

6. Mix purified DNA pools (sequencing libraries). Denature with NaOH, dilute with chilled HT1 solution.
7. Optional: Add a denatured and diluted PhiX control library.
8. Store in a cool place just before use. Then apply to the sequencing cassette in position 17.

Kit version	Illumina Primer (name)	Cartridge Position	Volume: fastGEN primers + Illumina primers
v2 and v3	Read 1 (HP10)	12	3 µl + 680 ul Illumina primers
	Index 1 (i7) (HP12)	13	13,5 µl + 680 ul Illumina
	Read 2 (HP11)	14	13,5 µl + 680 ul Illumina

Table 1: Example for mixing Illumina sequencing primers and fastGEN Solid Cancer Kit sequencing primers.



Position	Reagent Name	Description
7	LPM	Linearization Premix
8	LDR	Formamide
9	LMX1	Linearization Mix
10	LMX2	Read 2 Linearization Mix
11	RMF	Resynthesis Mix
12	HP10	Read 1 Primer Mix
13	HP12	Index Primer Mix
14	HP11	Read 2 Primer Mix
15	PW1	Laboratory-grade water
16	PW1	Laboratory-grade water
17	Empty	**Load Samples** (Reserved for sample libraries)
18	Empty	Optional use for custom Read 1 primer
19	Empty	Optional use for custom Index Read primer
20	Empty	Optional use for custom Read 2 primer
21	PW1	Laboratory-grade water
22	Empty	Empty

Picture 1: MiSeq Reagent Cartridge (taken from https://support.illumina.com)

In case of any doubts and questions, please contact the application specialist:
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Links:
https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/miseq-system-custom-primers-guide-15041638-01.pdf