CRISPincette™ LUNG Quick Guide

Preparation for Assay

- Before starting, spin down all reagents briefly to collect components clinging under the lid and keep them on ice until ready for use.
- In the case of frozen reagents, they are fully thawed and mixed using a vortex mixer. Briefly spin down and keep on ice until ready for use.
- SPRI beads must be sufficiently vortexed before use. It should appear homogenous and consistent in color.
- The 85% ethanol used for Size Selection is prepared and used immediately before the washing process.
- Using a filter tip during all experimental processes is recommended.

Step 1. 1st PCR (Total volume : 25 µL, each)

1) Add DNA sample and each reagent in 8-strip tube under the conditions shown in the table below.

* Perform PCR by adding the Target primer sets to each separated tube.

Reagent	Volume per Sample
● Target primer set 1 ● Target primer set 2	2.5 µL to each tube
○ 2X PCR mix	12.5 µL
DNA sample	Variable (Maximum 10 µL)
DEPC-Water	Up to 25 µL
	Total Volume 25 µL

2) Vortex and spin down.

3) Place the 8-strip tube in the pre-set thermal cycler under the conditions shown in the table below and run the program. (Lid temperature: 105 °C and ramp rate 0.5 °C/sec)

Step description	Temperature	Time	Cycles
Pre-denaturation	98 °C	1 min	1
Denaturation	98 °C	10 sec	30
Annealing/Extension	65 °C	40 sec	
Hold	4 °C	∞	1

Step 2. IVC (Total volume : 10 µL, each)

1) Add 1st PCR product (Step 1.) and each reagent in 8-strip tube under the conditions shown in the table below.

* Separately perform IVC on set 1 and set 2 of 1st PCR products (Step 1.) by adding the Remov Enzyme mix sets to the corresponding 1st PCR product sets. The remaining 1st PCR products are used as 'Control (Non-IVC)' in Step 4.

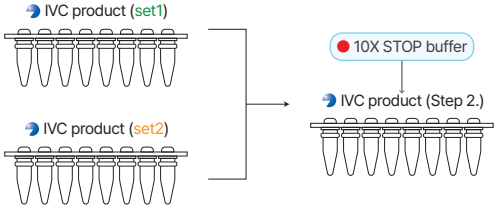
Reagent	Volume per Sample
1st PCR product (set 1 or set 2)	1 µL to each tube
● Remov RXN buffer	4 µL
● Stabilizer	1 µL
● Remov Enzyme mix set 1 ● Remov Enzyme mix set 2	4 µL to each tube
	Total Volume 10 µL

2) Vortex and spin down.

3) Place the 8-strip tube in the pre-set thermal cycler under the conditions shown in the table below and run the program. (Lid temperature: 60 °C)

Step description	Temperature	Time
In vitro Cleavage (IVC)	45 °C	30 min

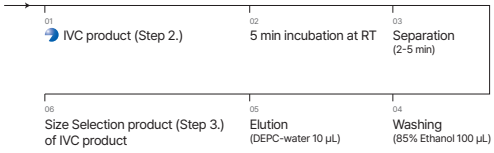
4) After the 30 minutes reaction, collect 10 µL of each IVC product set 1 and set 2 into one well of a new 8-strip tube. And add 2 µL ● 10X STOP buffer to each 8-strip tube.

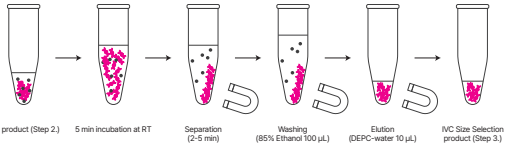


Step 3. Size Selection (with a ratio of 2X)

* Only perform 'Size Selection' on the IVC products (Step 2.).

1) Add 40 µL of SPRI beads (2X ratio) to approximately 20 µL of IVC product (Step 2.). Then, perform 'Size Selection' according to the following steps.





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Step 4. 2nd PCR (Total volume : 25 µL, each)

* Perform PCR on the Control (1st PCR product 1 and 2, Step 1.) and on the IVC Size Selection product (Step 3.), respectively.

1) Add Control and IVC Size Selection product and each reagent in 8-strip tube under the conditions shown in the table below.

Reagent	Volume per Sample
Control (Mix 1st PCR product 1 and 2)	0.5 + 0.5 µL
○ 2X PCR mix	12.5 µL
DEPC water	9 µL
Index primer set index 01-48 (plate)	2.5 µL
	Total Volume 25 µL

Reagent	Volume per Sample
IVC Size Selection product	1 µL
○ 2X PCR mix	12.5 µL
DEPC water	9 µL
Index primer set index 01-48 (plate)	2.5 µL
	Total Volume 25 µL

2) Vortex and spin down.

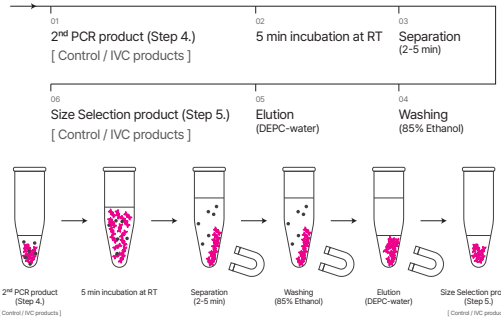
3) Place the 8-strip tube in the pre-set thermal cycler under the conditions shown in the table below and run the program. (Lid temperature: 105 °C and ramp rate 0.5 °C/sec)

Step description	Temperature	Time	Cycles
Pre-denaturation	98 °C	1 min	1
Denaturation	98 °C	10 sec	25
Annealing/Extension	65 °C	40 sec	
Hold	4 °C	∞	1

Step 5. Size Selection (with a ratio of 0.8X)

1) Separately mix the 2nd PCR products (Step 4.) of the Controls and IVC products into each 1.5 mL microtube. Add SPRI beads with a ratio of 0.8X to the 1.5 mL microtube under the conditions shown in the table below.

		Size Selection mixture	
Number of 2nd PCR product	Volume of 2nd PCR product (each)	Volume of 2nd PCR product	Volume of SPRI beads
n	10 µL	(n x 10) µL	(n x 8) µL
12	10 µL	(12 × 10) µL	(12 × 8) µL
24 (Maximum)	10 µL	(24 × 10) µL	(24 × 8) µL



* Final elution volume of DEPC-water

Number of 2nd PCR product	Elution Volume of DEPC-Water
<10	30 µL
10-15	50 µL
15-20	70 µL
20-24	100 µL

Step 6. Library Pooling

1) Qubit® Fluorometer equipment is recommended to measure the concentration (ng/µL) of Size Selection products (Step 5.).

2) Dilute Size Selection products in new 1.5 mL microtubes to 10 ng/µL using DEPC-water.

3) Mix each 10 µL of the diluted Size Selection products in a new tube.

Step 7. Library QC

1) Qubit® Fluorometer equipment is recommended to measure the concentration (ng/µL) of the pooled library.

2) Convert the measured concentration of the pooled library to molar concentration.
(Length of dsDNA : ≒220 bp)

3) Dilute the pooled library to 4 nM with DEPC-water.

Step 8. Sequencing

1) Please refer to the manufacturer's instructions and recommendations.

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