SeCore™ Workflow Quick Reference Card-CE-IVD

Amplification

1. Add DNA (15–30 ng/µL) to the bottom of each tube/well in the amount indicated to the right.
2. Create a mastermix, for N+1 samples, of Amp Mix and *Taq* using the volumes indicated to the right. Pulse vortex 2-3 times.
3. Add mastermix to the wells containing DNA (20 µL for Class I reactions and 23 µL for Class II reactions).
4. Cover and centrifuge briefly. Place plate in thermal cycler.

Class I (n=1)	
Amp Mix:	19.8 µL
Taq:	0.2 µL
DNA:	5 µL

Class II (n=1)	
Amp Mix:	22.8 µL
Taq:	0.2 µL
DNA:	2 µL

Cycles	Temperature	Time
1	95°C	4 min
35	95°C	20 sec
	63°C	20 sec
	72°C	40 sec
1	72°C	5 min
1	4°C	∞

ExoSAP-IT® Reagent Cleanup

5. Remove 5 µL of PCR product and combine with loading dye. Load onto a 2% agarose gel to check for amplification.
6. Add 4 µL of ExoSAP-IT reagent to the bottom of each well. Centrifuge ~5 seconds.
7. Vortex thoroughly for ~10 seconds. Centrifuge briefly. Place plate in thermal cycler.

Cycles	Temperature	Time
1	37°C	20 min
	80°C	20 min
1	4°C	∞

Sequencing Reactions

8. Add 40 µL of ultra pure water to **Class II** reactions only. Vortex and centrifuge briefly.
9. Add 2 µL of ExoSAP-IT reagent–treated amplicon to a 96-well optical plate.
10. Add 8 µL of the appropriate sequencing primer to these same wells. Vortex and centrifuge briefly. Place plate in thermal cycler.

Cycles	Temperature	Time
25	95°C	20 sec
	50°C	15 sec
	60°C	60 sec
1	4°C	∞

Ethanol Precipitation

11. Add 2 µL of PPT buffer to each well. Centrifuge briefly.
12. Add 40 µL of 100% (absolute) ethanol to each well. Vortex for 1 min.
13. Centrifuge for 30 min at 2,000 x g.
14. Invert on a paper towel and centrifuge inverted for 10–60 seconds at 500 x g.
15. Add 100 µL of 70%–80% ethanol to each well. DO NOT vortex.
16. Centrifuge for 5 min at 2,000 x g.
17. Invert on paper towel and centrifuge inverted for 1 min at 500 x g.
18. Add 15 µL of Hi-Di™ Formamide to each pellet.
19. Denature the samples at 95°C in a thermal cycler for 2 min.

Instrument	Parameters	POP -6™ polymer	POP -7™ polymer
3730/3730*xl*	Run Module	StdSeq36	FastSeq50
	Injection Time	5 sec	5 sec
	Run Time	1800 sec	1900 sec
3500/3500xL	Run Module	StdSeq50	FastSeq50
	Injection Time	Default	Default
	Run Time	3780 sec	1400 sec
3130/3130*xl*	Run Module	RapidSeq36	NA
	Injection Time	10 sec	NA
	Run Time	1800 sec	NA

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CE IVD (CE/IVD in European Union Only)