

► Precision ID library preparation protocol (0.5x)

① Target amplification

Component	Volume
5X Ion AmpliSeq HiFi Mix (Red cap)	2.0 ul
Precision ID Identity panel	5.0 ul
Nuclease-free Water	2.0 ul
Reference gDNA (1ng/ul)	1.0 ul
Total	10.0 ul

Thermal Cycling		
99°C	2 min	
99°C	15 sec	X 21
60°C	4 min	
10°C	Hold	

- Sample : 1ng of 2800M, ...

② Partially digest amplicons

Reaction Mix	Volume
Amplified mixture	10.0 ul
FuPa reagent (Brown cap)	1.0 ul
Total	11.0 ul

Thermal Cycling		
50°C	10 min	
55°C	10 min	
60°C	20 min	
10°C	Hold (for up to 1 hr)	

③ Adapters ligation

Reaction Mix	Volume
Digested amplicon mixture	11.0 ul
Switch Solution (Yellow cap)	2.0 ul
TS adapter mix (1 pmole/ul)	1.0 ul
DNA Ligase (Blue cap): LAST	1.0 ul
Total	15.0 ul

* Add the followings in the order listed

Thermal Cycling		
22°C	30 min	
68°C	5 min	
72°C	5 min	
10°C	Hold (for up to 1 hr)	

④ Library amplification

Reaction Mix	Volume
Nuclease-free Water	3.0 ul
2x SuperFi II Master Mix	10.0 ul
Index TS5 (10 pmole/ul)	1.0 ul
Index TS7 (10 pmole/ul)	1.0 ul
Ligated DNA (non-purified)	5.0 ul
Total	20.0 ul

Thermal cycling		
98°C	30 sec	
98°C	10 sec	X 11
61°C	10 sec	
72°C	20 sec	
72°C	5 min	
4°C	Hold	