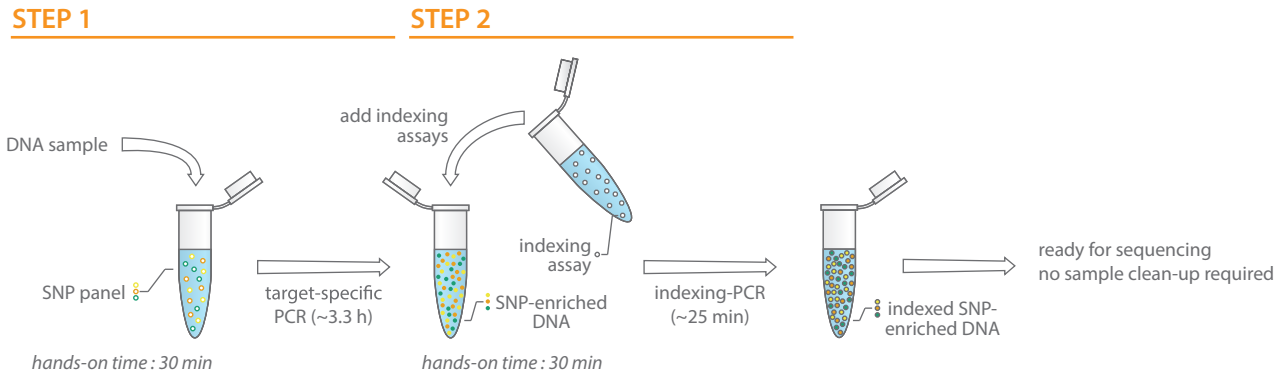
Sample ID panel workflow

This protocol contains a multiplex PCR step for the simultaneous production of 50 amplicon targets in a single tube and an indexing step for the addition of dual indexed adapters,



Required components

Sample ID panel plex (5X)	included in the package
pxlence indexes (17X)	
pxlence PCR mastermix	

Preparations

Create the following cycling conditions in your preferred thermal cycler.

Multiplex PCR

	Temperature (°C)	Time (s)	Number of repetitions
Step 1	98	180	
Step 2	98	15	repeat 40 times
Step 3	60	240	
Step 4	10	∞	

Indexing PCR

	Temperature (°C)	Time (s)	Number of repetitions
Step 1	98	15	
Step 2	60	240	repeat 5 times
Step 3	10	∞	

Protocol

Multiplex PCR

1. Create the multiplex PCR reaction by mixing the components as follows in a 0.2 ml eppendorf :

	Volume (µL)
SsoAdvanced Preamp Supermixv(2X)	7.5
Sample ID panel plex (5X)	3
Template DNA (1-5 ng)	1 to 4.5
Water	0 to 3.5
Total reaction volume :	15

2. Place the eppendorf in a thermocycler and run the Multiplex PCR thermal cycling conditions.

Indexing PCR

3. Take the eppendorf out of the thermal cycler when the Multiplex PCR thermal cycling conditions have been completed.
4. Add 1 µL of the selected 17X i7-index solution and 1 µL of the selected 17X i5-index solution to the eppendorf.
5. Vortex carefully or pipet 10 times up and down.
6. Place the eppendorf in a thermocycler and run the Index PCR thermal cycling conditions.
7. When the Index PCR thermal cycling conditions have been completed, take the eppendorf out of the thermal cycler and store it at -20°C until sequencing.