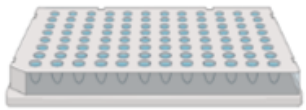
1. & 2. Amplify DNA Targets

DNA

7.5 µL

DNA plate



Safe Stop Point

4°C O/N
-15°C long

+ 5 µL
HiFi Mix

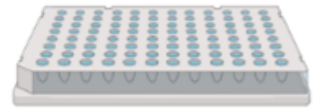
short spin

Mix & transfer

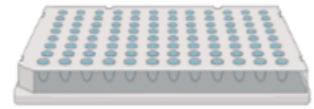
5 µL

5 µL

Pool 1 plate



Pool 2 plate



+ 5 µL
Primer pool 1

+ 5 µL
Primer pool 2

Seal & short spin

AMP_DNA program		
99°C	2min	1X
99°C	15sec	21X
60°C	8min	
10°C	Hold	up to 24h

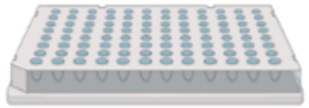
Safe Stop Point

≤10°C O/N
-15°C long

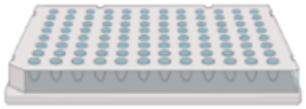
3. Partially digest amplicons

short spin

Amplified Pool 1 plate

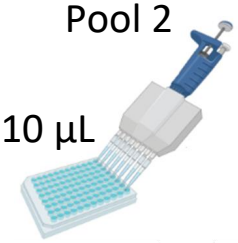


Amplified Pool 2 plate

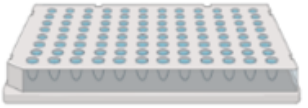


Combine Pool 1 + Pool 2

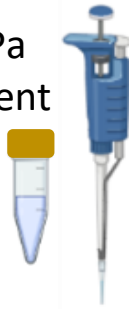
10 µL



Amplified Pool 1 + Pool 2



FuPa reagent



30 µL

+ 2 µL FuPa

Seal & vortex & short spin

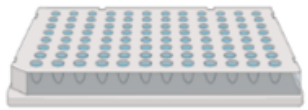
FUPA program	
50°C	10min
55°C	10min
62°C	20min
10°C	Hold (max 1 hr)

Safe Stop Point ≤10°C 1 hr -15°C long

4. Ligate indexes

short spin

Pool 1 + Pool 2
digested amplicons



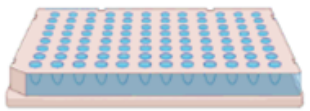
+ 4 µL
Switch solution

Pool 1 + Pool 2
digested amplicons +
Switch solution

---

short spin

index plate



+ 2 µL
Indexes

DO NOT
CHANGE
ORDER OF
REAGENTS

DNA
Ligase

30 µL

+ 2 µL
DNA Ligase

Seal & vortex &
short spin

LIGATE program	
22°C	30 min
68°C	5 min
72°C	5 min
10°C	Pause (up to 24hrs)

≤10°C O/N
-15°C long

5. Clean-up Library

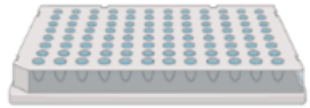
RT 30min

70% EtOH

35mL 100% EtOH
+ 15mL H_2O

short spin

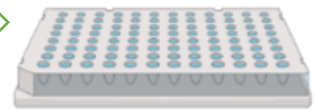
Library plate



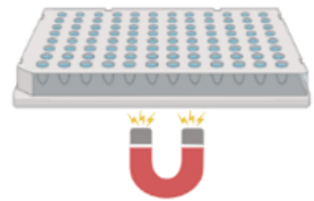
+ 30 µL
AMPureXP

Seal & vortex &
short spin

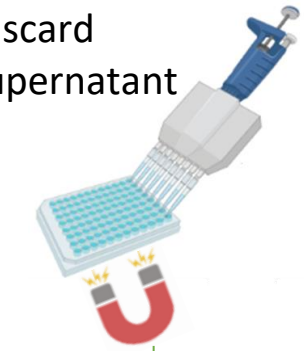
RT 5min



Magnet ≥2 min



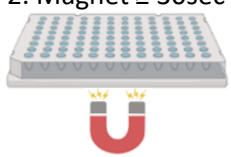
Discard
supernatant



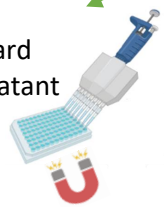
WASH

2X 150µL 70% EtOH

2. Magnet ≥ 30sec

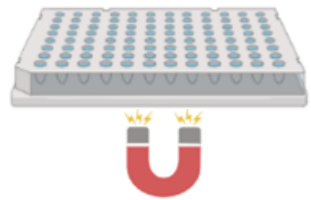


3. Discard
supernatant

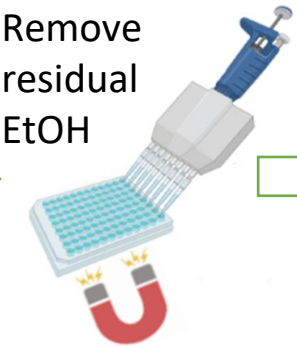


short spin

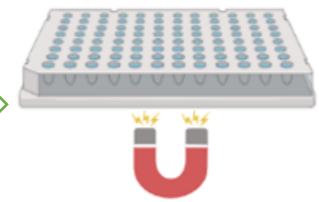
Magnet ≥30 sec



Remove
residual
EtOH



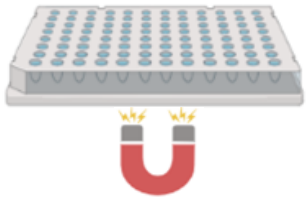
RT ≥10 min



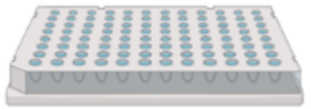
Inspect if all EtOH has evaporated:
NO: remove and RT ≥10 min
YES: Continue amplify library

6. Amplify Library

Cleaned library plate
Library on beads



Remove from magnet



4410 µL 1x Lib Amp mix

+

490 µL 10X Library Amp primers

Amplification master mix

Vortex briefly

+ 50 µL Amplification master mix

Seal & vortex & short spin

Beads in mix during amplification

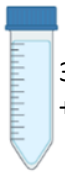
AMP_7 program		
Cycles	**Temp.**	**Time**
1	98°C	2 min
7	98°C	15 sec
	64°C	1 min
Hold	10°C	up to 24 hrs

Safe Stop Point

≤10°C O/N
-15°C long

7. 2nd Clean-up

RT 30 min



70% EtOH

35mL 100% EtOH
+ 15mL H_2O

FIRST ROUND

short spin

Library plate

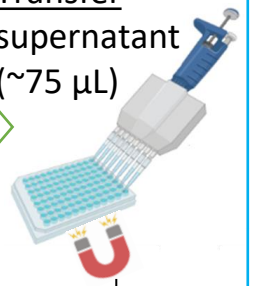
+ 25 µL AMPureXP

Seal & vortex & short spin

RT 5 min

Magnet ≥5 min

Transfer supernatant (~75 µL)



SECOND ROUND

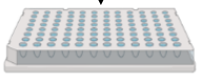


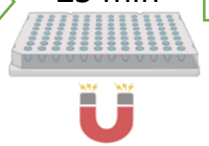
Plate with library in supernatant

+ 60 µL AMPureXP

Seal & vortex & short spin

RT 5 min

Magnet ≥5 min



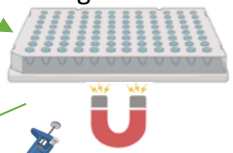
Discard supernatant (~135 µL)

Plate with library on beads

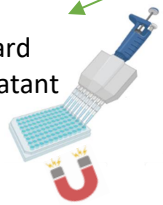
WASH

2X 150µL 70% EtOH

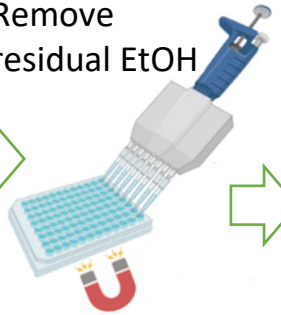
2. Magnet ≥ 30sec



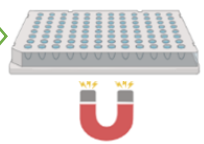
3. Discard supernatant



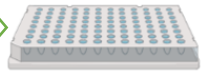
Remove residual EtOH



RT ≥10 min



Remove from magnet

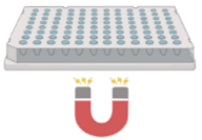


+ 30 µL Low TE

Seal & vortex & short spin

resuspend

Magnet ≥5 min



Transfer 27 µL supernatant to new plate & seal

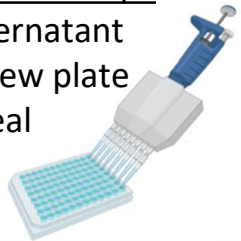
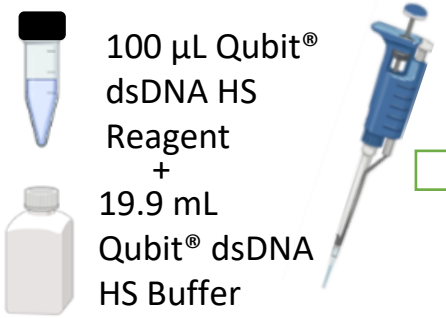


Plate with 27 µL final library

Safe Stop Point

-15°C ≤30 days

8. Qubit

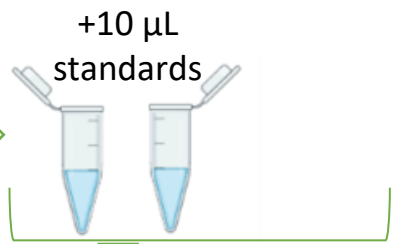


Qubit working solution

Vortex briefly

96 x 197 µL working solution to individual tubes

2 x 190 µL working solution to individual tubes



Excel template

short spin

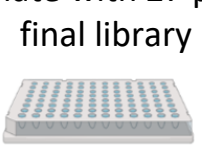
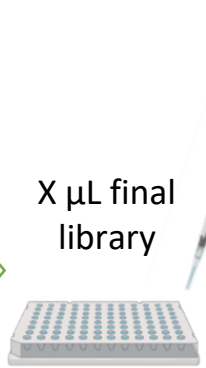


Plate with 27 µL final library

9. Pooling

Plate with 27 µL final libraries

X µL final library



Y µL Low TE

Plate with 2 nM libraries

Transfer 5 µL

Combine all 8 wells in 1 tube