WILDseq Library Prep v2 Protocol

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Contents

Reverse Transcription

Overview

- cDNA synthesis from RNA template
- Include a -RT control if possible
  - Mix several samples and include extra H20 instead of Reverse Transcriptase
  - PCR this sample as well, checks for contamination of genomic DNA, mastermix, ect.
  - Can also use as negative control to determine product size and yield on tapestation

Materials

Component	Expected Volume/experiment	Check
5x SSIV Buffer	4 μL	
SSIV RT	1 μL	
100 mM DTT	1 μL	
RNAse Out	1 μL	

RT Protocol

1. In PCR strip tubes, prepare 1-5 μg of RNA in a total volume of 10 μl of RNAse/DNAase-free water.
2. Add 1 μl of WS_RT_UMI_NexteraR2 primer (2μM stock)
3. Add 1 μl dNTPs (10 mM each).
4. Denature at 65 C for 5 mins in the PCR machine, then straight onto ice for at least 2 mins, spin briefly to get liquid to the bottom of the tube.
5. Prepare mastermix of RT enzyme and buffers. 1.1x the number of samples.

Component	Volume	MM volume
5x SSIV Buffer	4 μL	
SSIV RT	1 μL	
100 mM DTT	1 μL	
RNAse Out	1 μL	

6. Add 7 μl of RT MM prepared above to each sample and mix, spin briefly to get liquid to the bottom of the tube.
    - Spin briefly to get liquid to bottom of the tube
7. In the PCR machine incubate at 53 C for 10 mins followed by 80 C for 10 mins.
8. Add 1 μl Themolabile Exonuclease I (NEB M0568) to remove excess RT primer.

Component	Volume	MM volume
Thermolabile Exonuclease I	1 μL	
NEBuffer r3.1*	2 μL	

Most PCR buffers are compatible

9. In PCR Machine: heat at 37C for 4 mins followed by 80C for 1 min
    - Spin briefly to get liquid to bottom of the tube
10. Add 1 μl of RNAse H and incubate at 37 C for 20 mins.
11. Dilute final cDNA 1:2 with DNAse/RNAse-free water.

PCR Step 1

Overview

- Aim to keep number of cycles at a minimum.
- Forward primer is an equal mix of primers with different length spacers to generate diversity required for sequencing.

Materials

Component	Expected Volume/experiment	Check
5x SSIV Buffer	4 μL	
SSIV RT	1 μL	
100 mM DTT	1 μL	
RNAse Out	1 μL	

PCR1 Protocol

- Set up PCR reactions on ice
- One reaction per sample plus -RT control

1. Prepare mastermix of below reagents at 1.1x the number of samples

Component	Volume
10uM WS_RT-PCR1_Fwd	1.5 μL
10uM WS_RT-PCR1_Rev	1.5 μL
DNAse/RNAse H20	12 μL

Component	Volume
Kapa Hifi HotStart Ready Mix (2X)	25 μL
Total Master Mix volume	**40 μL**
cDNA from above	10 μL

2. Perform PCR using the following parameters:

Step Name	Steps	Time
Initial Denaturation	Step 1: 95C	3mins
Denaturation	**Step 2: 98C**	**20s**
Annealing	**Step 3: 60C**	**15s**
Extension	**Step 4: 72C**	**15s**
Final Extension	Step 5: 72C	1 min
Hold Step	Step 6: 12C	Hold

10-25 cycles of steps 2-4