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SureSelect XT HS2 DNA to prepare libraries for single-cell Whole Genome Sequencing (scWGS) after single-cell Whole Genome Amplification (scWGA)

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We use this collection and it's working.

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Disclaimer

Acknowledgements and Funders:

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This research was funded in part by Aligning Science Across Parkinson's [Grant ID: 000430] through the Michael J. Fox Foundation for Parkinson's Research (MJFF).

Note

ALL IMAGES CAN BE ENLARGED BY CLICKING AT THEM

Critical notes!

Please follow Good Laboratory Practices.

- To prevent DNA contamination, clean all surfaces and equipment before use with DNA AWAY Surface Decontaminant, separate the lab in pre- and post-PCR areas, and use filtered sterile pipette tips.
- For each protocol step that requires theremoval of tube cap strips, reseal the tubes with a fresh strip of caps.
- In all master mixes to be used manually, include 5-10% excess volume in the calculations.

Abstract

We adapted the SureSelect XT HS2 DNA protocol to prepare libraries using as input material samples after single-cell Whole Genome Amplification (scWGA), instead of genomic DNA samples, for library preparation for Illumina single-cell Whole Genome Sequencing (scWGS). This protocol can be employed either manually (Section 2: Option A) or in combination with automation using the Bravo Automated Liquid Handling Platform (Section 2: Option B).

Attachments

861-2221.pdf	2022_SureSelect_Auto...	SureSelect Enzymatic...	SureSelect XT HS2 DN...
1.1MB	5MB	393KB	1.1MB

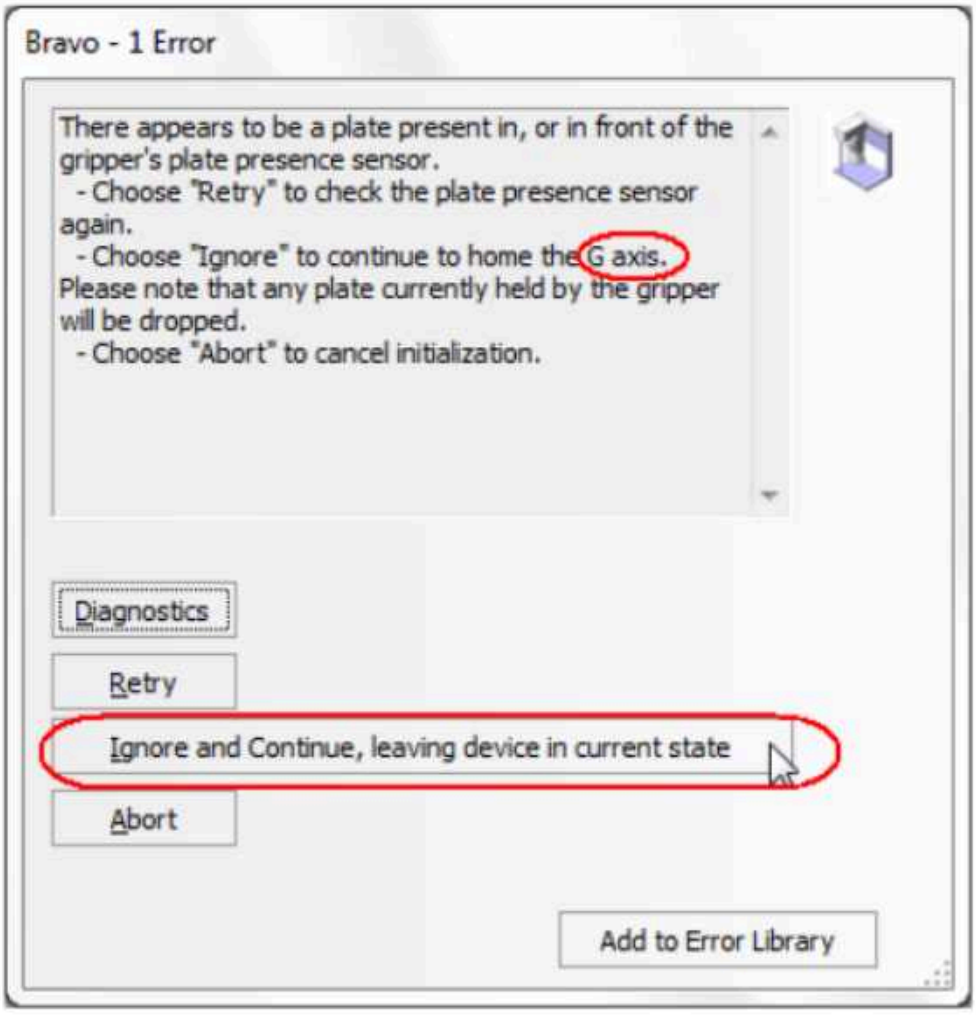
Guidelines

We have used this protocol for DNA products after single-cell Whole Genome Amplification (scWGA) by either PicoPLEX (Takara), ResolveDNA (BioSkryB) and droplet MDA (Samplix). We recommend to bead purify the PicoPLEX and ResolveDNA products prior library preparation. In all cases, the amplicon quantity and size should be assessed using Qubit and TapeStation or other methods prior to SureSelect XT HS2 DNA library preparation.

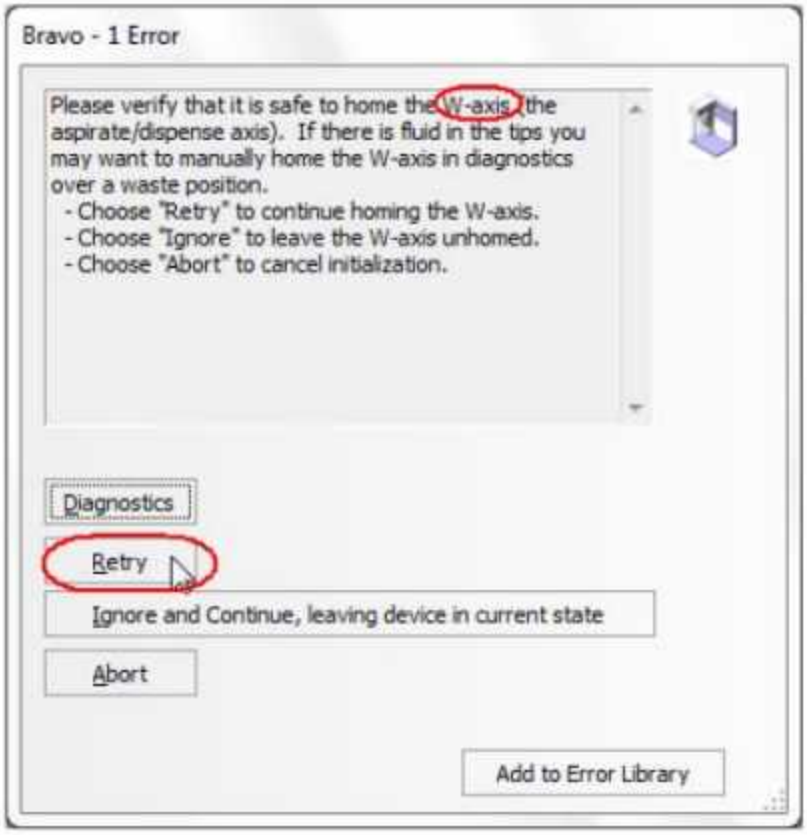
Bravo error/notification messages

Two error/notification messages are encountered when VWorks Bravo software is initializing:

1. If you encounter the G-axis error message shown below, select Ignore and Continue, leaving device in current state.



2. If you encounter the W-axis error message shown below, select Retry.


Bravo - 1 Error
Please verify that it is safe to home the W-axis (the aspirate/dispense axis). If there is fluid in the tips you may want to manually home the W-axis in diagnostics over a waste position.
- Choose "Retry" to continue homing the W-axis.
- Choose "Ignore" to leave the W-axis unhomed.
- Choose "Abort" to cancel initialization.
Diagnostics
Retry
Ignore and Continue, leaving device in current state
Abort
Add to Error Library

Materials

Commercial Reagents:

1. Equipment and consumables for DNA quality control:

DNA quantification. Here we used Qubit (Thermo Fisher Scientific):

Equipment	
Qubit Fluorometer	NAME
Fluorometer	TYPE
Invitrogen	BRAND
Q33238	SKU
https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238	LINK

- Qubit™ dsDNA BR Assay Kit **Thermo Fisher Scientific Catalog #**Q32853
- Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #**Q32854
- Qubit™ Assay Tubes **Invitrogen - Thermo Fisher Catalog #**Q32856

2. Nucleic acid analysis platforms. Here we used TapeStation (Agilent):

Equipment	
4150	NAME
TapeStation System	TYPE
Agilent	BRAND
G2992AA	SKU
https://www.agilent.com/store/en_US/Prod-G2992AA/G2992AA	LINK

- Optical tube strips (8x Strip) **Agilent Technologies Catalog #**401428
- Optical tube strip caps (8x strip) **Agilent Technologies Catalog #**401425
- D1000 ScreenTape **Agilent Technologies Catalog #**5067-5582
- D1000 Reagents **Agilent Technologies Catalog #**5067-5583
- High Sensitivity D1000 ScreenTape **Agilent Technologies Catalog #**5067-5584
- High Sensitivity D1000 Reagents **Agilent Technologies Catalog #**5067-5585

SureSelect XT HS2 performed manually:

- SureSelect XT HS enzymatic fragmentation kit, 16 reactions **Agilent Technologies Catalog #**5191-4079

  or
- SureSelect Enzymatic Fragmentation Kit, 96 reactions **Agilent Technologies Catalog #**5191-4080
- SureSelect XT HS2 DNA Library Preparation Kit with Index Primer Pairs (Choose one of the following: Agilent G9981A (index 1-16), G9985A (index 1-96), G9985B (index 97-192), G9985C (index 193-288) or G9985D (index 289-384))

SureSelect XT HS2 with automation (Bravo):

- SureSelect Enzymatic Fragmentation Kit, 96 Reactions, Auto **Agilent Technologies Catalog #**5191-6764
- SureSelect XT HS2 DNA Library Preparation Kit with Index Primer Pairs (Choose one of the following: Agilent G9985A (index 1-96), G9985B (index 97-192), G9985C (index 193-288) or G9985D (index 289- 384))

Consumables:

- UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Catalog #**10977049
- Low TE Buffer **Invitrogen - Thermo Fisher Catalog #**12090-015
- Ethanol (molecular biology grade, ≥99.8%) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**51976-500ML-F
- Plasticware compatible with the selected thermal cycler. Here we used PCR 8-tube strips w/o caps
  8-tube strips for qPCR/PCR, without caps **VWR International (Avantor) Catalog #**732-1517
- Low binding filtered tips (sterile)
- Low-binding 1.5ml DNase/RNase-free (sterile). Here we used DNA LoBind Tubes,
  DNA LoBind® Tubes **Eppendorf Catalog #**0030108418
- Powder-free gloves
- DNA AWAY™ Surface Decontaminant, Surface decontaminant; 8.5 oz. (250mL) **Thermo Fisher Catalog #**7010PK
- Cleaning wipes for surface cleaning. Here we used Conti Washcloth Dry Brosch Direct PH5959

Consumables for SureSelect XT HS2 with automation:

- Processing plate:
  Armadillo PCR Plate, 96-well, clear, clear wells, barcoded **Thermo Fisher Scientific Catalog #**BC2396
- PCR plate:
  PCR plates, 96-well, Armadillo™ Standard Semi-skirted **VWR International (Avantor) Catalog #**731-0649
- Deep well plate for AMPure beads and master mixes:
  Nunc™ DeepWell™ Plates with Shared-Wall Technology, 96U, 1mL, sterile **Thermo Fisher Catalog #**260251
- Waste Plate: Axygen™ Storage Microplates **Fisher Scientific Catalog #**P-2ML-SQ-C
- VersiCap Mat, 96-well, domed cap strips **Thermo Fisher Scientific Catalog #**AB1810
- Agilent Bravo 250uL Pipette Tips, Sterile, Filtered, for 96LT head **Catalog #**19477-022
- Reservoir, single cavity, polypropylene, 300 mL, 96 pyramids base geometry, 44 mm height, 25/pk **Agilent Technologies Catalog #**201244-100

Equipment:

- Thermal Cycler with 96-well, 0.2 ml block. Here we used Multigene Optimax Gradient Thermal cycler with 96 well block (Labnet MB0520)
- Manual version: Magnetic separator compatible with0.2 ml tubes, strips of plates. Here we used either Magnetic Separator - PCR Strip (Takara 635011) or ResolveDNA Dual Volume Strip Tube Magnet (BioSkryB PN100226)
- Plate or strip tube centrifuge. Here we used SciSpin Mini microfuge, blue, 7000rpm (Sciquip SS-6050) and plate centrifuge (Starlab N2631-0008)
- 1.5 ml tube centrifuge. Here we used Mini-Centrifuge (Fisherbrand 16617645)
- PCR cooler. Here we used PCR-Cooler, Blue, Capacity: 0.2 mL (Eppendorf 1019228)
- General lab pipettes (single and multi-channel)
- Nuclease-free filtered pipette tips sterile, if possible, use low binding tips
- 96-well plate mixer. Here we used Vortex mixer. Here we used WhirliMixer (Fisons Scientific 1993-520) and iSwix VT Digital Vortex Mixer (Appleton Woods ST6000)
- Ice bucket
- For automated version: Bravo NGS Option A robot (Agilent G5573A)

SMARTer-Seq™ Magnetic Separator - PCR Strip **Takara Bio Inc. Catalog #**635011

Equipment

SciSpin MINI Microfuge BLUE, inc 2 rotors of SS-6058 & SS-6059
NAME

SciQuip
BRAND

SS-6050
SKU

https://sciquip.co.uk/scispin-mini-microfuge.html
LINK

Equipment

Plate Centrifuge
NAME

StarLab
BRAND

N2631-0008
SKU

https://www.starlabgroup.com/en/product/plate-centrifuge-n2631-0008.html
LINK

Equipment

Mini-Centrifuge 100-240V, 50/60Hz Universal Plug, Grey
NAME

minicentrifuge
TYPE

Fisherbrand™
BRAND

16617645
SKU

https://www.fishersci.co.uk/shop/products/fisherbrand-standard-mini-centrifuge/16617645
LINK

Equipment

iSwix VT Digital Vortex Mixer	NAME
Appleton	BRAND
ST6000	SKU
https://www.appletonwoods.co.uk/product/iswix-vt-digital-vortex-mixer-parent/	LINK

Safety warnings

Safety Warnings:
Please follow the Safety Data Sheets (SDS) for all reagents for safe handling and safety hazards.

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SureSelect Enzymatic... 393KB

SureSelect XT HS2 DN... 1.1MB

Files

SEARCH

Protocol

NAME

Section 1: Enzymatic DNA Fragmentation (Manually)

VERSION 1

CREATED BY

Ester Kalef-Ezra
University College London

OPEN →

Protocol

NAME

Section 2: NGS library preparation for sequencing

VERSION 1

CREATED BY

Ester Kalef-Ezra
University College London

OPEN →

Protocol

NAME

Section 3: Libraries quality control (QC)

VERSION 1

CREATED BY

Ester Kalef-Ezra
University College London

OPEN →

Protocol references

This protocol was adapted with minor modifications from the following Agilent protocols:

SureSelect Enzymatic Fragmentation Kit
https://www.agilent.com/cs/library/usermanuals/public/G9702-90050.pdf

Manual version: SureSelect XT HS2 DNA System
https://www.agilent.com/cs/library/usermanuals/public/G9985-90000.pdf

Automated version: SureSelect XT HS2 DNA System Automated using Agilent NGS Bravo Option A https://www.agilent.com/cs/library/usermanuals/public/G9985-90020.pdf