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Modified nuclease flush protocol for Nanopore RNA flow cells

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

- This method was developed on PromethION FLO-PRO004RA flow cells and has not been tested in other systems.
- All results shown are for runs sequencing total RNA. PolyA+ runs, or runs experiencing less pore blocking, may see smaller improvements after flowcell flushing.

Abstract

A nuclease flush can be used to recover blocked pores on Oxford Nanopore Technologies (ONT) flow cells to maximise overall sequencing output. The Flow Cell Wash Kit (EXP-WSH004/EXP-WSH004-XL) was developed to remove blockages on DNA flow cells, but does not restore blocked pores on their RNA counterparts. This short protocol describes a simple modification to the Nanopore Flow Cell Flush protocol to enable pore recovery in RNA flow cells.

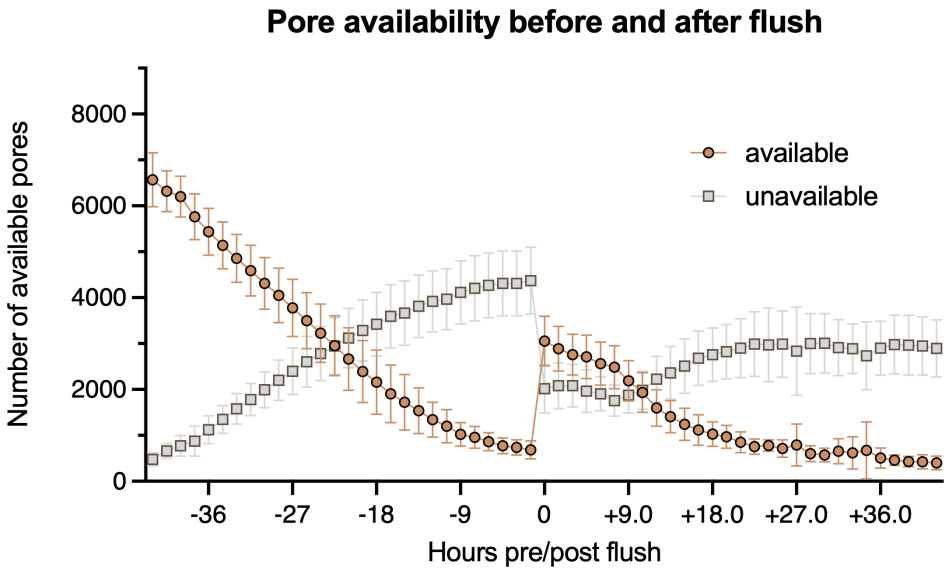

Figure 1. Summary of mean number of available and unavailable pores during RNA sequencing before and after RNase flush (n=9). Progressive blocking leads to increasing unavailable pores, which can be partially returned to their original available state following nuclease flush. Error bars are standard deviations.

This protocol uses two RNases to digest blockage-causing nucleic acids: RNase Cocktail™ Enzyme Mix and RNase H. RNase Cocktail™ contains endoribonucleases RNase A and RNase T1, which cleave at C and U, and G residues, respectively. RNase H, rather than cleaving single-stranded RNA, degrades the RNA strand of DNA-RNA hybrid molecules, allowing it to target the hybrid libraries used for direct RNA sequencing. When used in combination with WMX (a DNase I containing solution provided in the ONT EXP-WSH004 wash kit), blockage-causing DNA, single-stranded RNA or DNA-RNA hybrids can be degraded.

Based on our preliminary results, this protocol can recover up to 50% of available pores present at the start of a run, and can boost the number of sequenced bases by >50%. Additionally, flushing RNA flow cells in accordance with this protocol has a negligible effect on read quality or length.

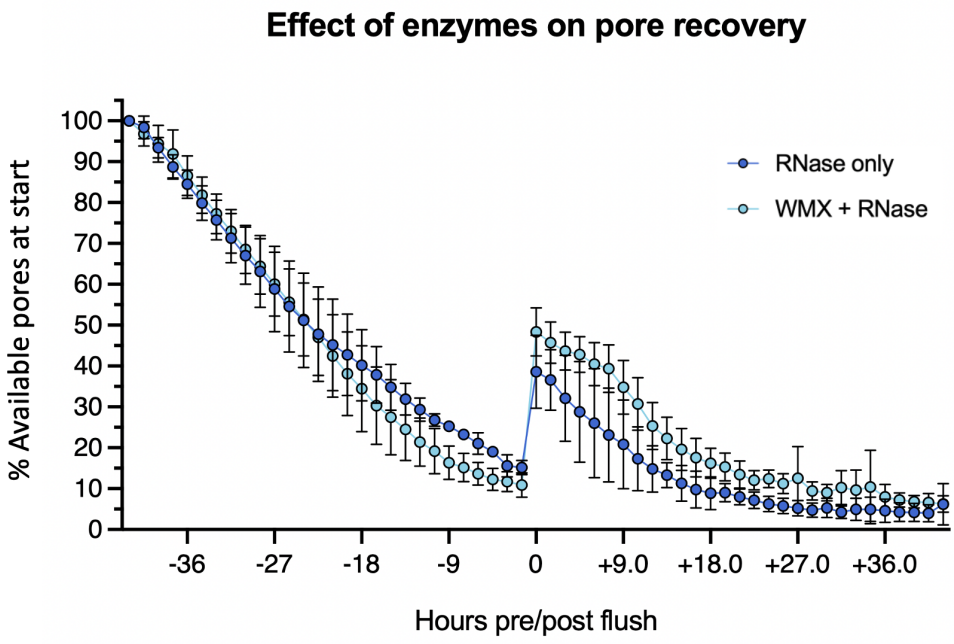

Figure 2. Effect of WMX in RNase Flow Cell Wash Mix on recovery of available pores. Flushing with WMX and RNase (RNase Cocktail™ + RNase H) together (n = 9) increases the % of available pores beyond the effects of flushing with RNase alone (n = 3). Data presented as mean and standard deviations.

Materials

Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL) from Oxford Nanopore Technologies (ONT).
RNase H (NEB, M0297)
RNase Cocktail^{TM} Enzyme Mix (Invitrogen, AM2286)

Protocol

1 Thaw Wash Mix (WMX), RNase H and RNase Cocktail™ on ice. Do not vortex.

2 Thaw one tube of Wash Diluent (DIL) at room temperature.

3 Mix the contents of Wash Diluent (DIL) thoroughly by vortexing, then spin down briefly and place on ice.

4 In a fresh 1.5 ml Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix:

Reagent	Volume per flow cell
Wash Mix (WMX)	2 µl
RNase H	1 µl
RNase Cocktail™	1 µl
Wash Diluent (DIL)	396 µl
Total	**400 µl**

5 Mix well by pipetting, and place on ice. Do not vortex the tube.

6 Follow Oxford Nanopore EXP-WSH004/EXP-WSH004-XL Protocol (accessible from link below) from Step 6 as directed, using the prepared Flow Cell Wash Mix to complete the flow cell flush.

EXP-WSH004/EXP-WSH004-XL Protocol:
https://community.nanoporetech.com/docs/prepare/library_prep_protocols/flow-cell-wash-kit-exp-wsh004/v/wfc_9120_v1_revq_08dec2020/flushing-a-promethion-flow-cell?devices=promethion

7 **Optional: Following the 1 hour incubation and waste removal steps as described in the standard protocol, we recommend the flow cell be loaded with storage buffer as detailed in the "To store the Flow Cell for later use" protocol and a flow cell check be performed to quantify extent of pore recovery.**

Flow Cell storage Protocol:
https://community.nanoporetech.com/docs/prepare/library_prep_protocols/flow-cell-wash-kit-exp-wsh004/v/wfc_9120_v1_revq_08dec2020/to-store-the-promethion-flow-cell-for-later-use?devices=promethion

8 The flow cell may now be stored for later use or used immediately with a fresh RNA library.

Protocol references

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