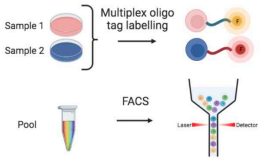
Feb 05, 2024

Optimising sample multiplexing oligos by flow cytometry



DOI
https://dx.doi.org/10.17504/protocols.io.81wgbyjpovpk/v1

Daniel V Brown[1]

[1]Walter and Eliza Hall Institute

Daniel V Brown
Walter and Eliza Hall Institute

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Protocol Citation: Daniel V Brown 2024. Optimising sample multiplexing oligos by flow cytometry. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbyjpovpk/v1

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

Created: May 14, 2023

Last Modified: February 05, 2024

Protocol Integer ID: 81857

Keywords: scRNA-Seq, facs, sample multiplexing, hashtag, sample multiplexing oligos by flow cytometry optmisation, sample multiplexing oligos by scrna, sample multiplexing oligo, optimising sample multiplexing oligo, flow cytometry read, flow cytometry optmisation, fluorescent detection, out with fluorescent detection, labelled oligo, scrna, signal swapping

Disclaimer

Abstract

Optmisation of sample multiplexing oligos by scRNA-Seq is costly and time consuming. A cheaper and faster method is to use a flow cytometry read-out with fluorescent detection oligonucleotides.
This method can also be used to mix samples with different fluorescently labelled oligos and investigate signal swapping.

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Materials

- Phosphate Buffered Saline, without magnesium and calcium
- Sample multiplexing oligos (MULTI-Seq, CellPlex or Hashtag Antibody)
- 10ug/mL DAPI
- 30% BSA stock solution
- Fluorescent detection oligonucleotides

	A	B	C
	Name	**Sequence**	**Modification**
	A647_FB2_detect	/5Alex647N/CCTTAGCCGC TAATAGGTGAGC	5' Alexa 647 modification
	A594_FB2_detect	/5Alex594N/TTGCTAGGAC CGGCCTTAAAGC	5' Alexa 594 modification
	A594_FB1_detect	/5Alex594N/TTGCTAGGAC CGGCCTTAAAGC	5' Alexa 594 modification
	A647_Total-SeqC_detect	/5Alex647N/CTGTCTCTTA TACACATCTCCG	5' Alexa 647 modification
	AF647_oligo_dT_detect	/5Alex647N/TTTTTTTTTTT TTTTTTTTTTTTTTTTTTT	5' Alexa 647 modification

Fluorescent detection oligonucleotides. Order with HPLC modification

MULTI-Seq barcoding oligos. I substituted the poly-A tail for 10x Genomics feature barcode 2 sequence.

	A	B	C
	Name	**Sequence**	**Barcode**
	multiSeq_FB2_BC2	CCTTGGCACCCGAGAATT CCACCACAATGGCTCACC TATTAGCGGCTAAGG	CCAC AATG
	multiSeq_FB2_BC3	CCTTGGCACCCGAGAATT CCATGAGACCTGCTCACCT ATTAGCGGCTAAGG	TGAGA CCT
	multiSeq_FB2_BC4	CCTTGGCACCCGAGAATT CCAGCACACGCGCTCACC TATTAGCGGCTAAGG	GCAC ACGC
	multiSeq_FB2_BC5	CCTTGGCACCCGAGAATT CCAAGAGAGAGGCTCACC TATTAGCGGCTAAGG	AGAG AGAG

	A	B	C
	multiSeq_FB2_BC6	CCTTGGCACCCGAGAATTCCATCACAGCAGCTCACCTATTAGCGGCTAAGG	TCACAGCA
	multiSeq_FB2_BC7	CCTTGGCACCCGAGAATTCCAGAAAAGGGGCTCACCTATTAGCGGCTAAGG	GAAAAGGG
	multiSeq_FB2_BC8	CCTTGGCACCCGAGAATTCCACGAGATTCGCTCACCTATTAGCGGCTAAGG	CGAGATTC
	multiSeq_FB2_BC9	CCTTGGCACCCGAGAATTCCAGTAGCACTGCTCACCTATTAGCGGCTAAGG	GTAGCACT
	multiSeq_FB2_BC10	CCTTGGCACCCGAGAATTCCACGACCAGCGCTCACCTATTAGCGGCTAAGG	CGACCAGC
	multiSeq_FB2_BC11	CCTTGGCACCCGAGAATTCCATTAGCCAGGCTCACCTATTAGCGGCTAAGG	TTAGCCAG
	multiSeq_FB2_BC12	CCTTGGCACCCGAGAATTCCAGGACCCCAGCTCACCTATTAGCGGCTAAGG	GGACCCCA
	multiSeq_FB2_BC13	CCTTGGCACCCGAGAATTCCACCAACCGGGCTCACCTATTAGCGGCTAAGG	CCAACCGG

Safety warnings

Please follow all Manufacturer safety warnings and recommendations.

Prepare multiplexing reagent

1 For CellPlex and Hashtag antibody the reagent comes ready to use.

1.1 Prepare a dilution series for titration if desired

MULTI-Seq oligo preparation

2 Mix anchor and barcode strands in 1:1 molar ratio in PBS (without FBS or BSA at 2 µM concentration (10X stock).

2.1 This is 6uL 50uM anchor LMO, and 15uL 10uM barcode oligo to 129uL plain PBS.

2.2 Make one unique barcode solution per sample.

2.3 Total 25 µL per sample.

3 Make a 10X solution of the Co-Anchor in PBS.

3.1 Add 3uL 50uM co-anchor to 141uL plain PBS.

3.2 Add 6uL 100uM fluorescent detection oligo. For example:
Alexa 647 feature barcode 2 detection oligo.

3.3 It is highly recomended to label in at least duplicate. On these occasions I label the other replicate with a Alexa 594 detection oligo and mix immediately before FACS analysis.

Sample preparation

4 I use suspension cell lines to titrate cell multiplexing oligos so the sample preparation is easy.

4.1 Prepare a single-cell suspension of the sample to be tested.

4.2 Wash cells once in plain PBS without additives. I centrifuge suspension cell lines at 400xg.
Resuspend in PBS.

4.3 Count cells and transfer 100k to 1M cells (preferably 500k) into a 1.5mL tube for labelling

Labelling samples with multiplexing oligos

5 This is largely based on original protocols but with the addition of a fluorescent secondary oligo.

5.1 Resuspend cells in 180uL of plain PBS.

5.2 Add 20 µL 10X Anchor:Barcode solution and pipette gently to mix 10 – 15 times.

5.3 Incubate on ice for 5 minutes.

5.4 Add 20 µL Co-Anchor solution and pipette gently to mix.

5.5 Incubate 5 minutes longer on ice.

5.6 Add 1.5mL of 1% BSA in PBS (ice cold) to quench.

5.7 Add 1.5mL of 1% BSA in PBS (ice cold) to quench.

5.8 Add 1mL of 1% BSA in PBS (ice cold) to quench.

5.9 Centrifuge cells at 4°C 400xg 5min.

5.10 Resuspend cells in the remaining 100uL of supernatant then transfer to a new 1.5mL tube.

5.11 Add 1.9mL 1% BSA in PBS and spin 5 minutes 400g at 4°C.

5.12 Repeat wash 2 more times for a total of 3 washes.

5.13 Resuspend cells in 500 µL of PBS + 1% BSA and transfer to 5mL polystyrene FACS tube

Flow cytometry

6 Use a FACS analyser to compare the signal of labelled samples to a control sample where only the fluorescent detection oligo has been added.
If you have replicate labelled samples with different fluorescent detection oligos, you may combine them at this step.

6.1 Add DAPI to a final concentration of 0.1ug/mL (1/100 stock tube).

6.2 Gate for debris, single cells and viable cells.

6.3 Aquire at least 10,000 viable cells per test.

6.4 Analyse with relevant software.

Protocol references

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