Comparison of hybridisation chain reaction and RNAscope® for mRNA detection

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Introduction

RNA *in situ* hybridisation (ISH) methods allow detection of single or multiple targets, with preservation of spatial context. With the demand increasing for mRNA analysis and detection of multiple targets, it is imperative to develop, test and optimise new techniques that can improve not only signal detection, but also fit into busy laboratory workflows and maximise budgets.
In situ hybridisation chain reaction (HCR) is an ISH technique, first described in 2004, consisting of a one-step detection and one-step amplification. It's an enzyme-free method that uses probe pairs and hairpin DNAs to detect single or multiple mRNA targets in various samples, including formalin-fixed, paraffin-embedded (FFPE) samples. RNAscope is another ISH technique, that uses a cocktail of Z probe pairs to detect RNA targets, including in FFPE samples and it has been used in Cancer Research UK – Cambridge Institute (CRUK-CI) for over 10 years successfully.
The main goal in this study was to assess whether HCR was a suitable mRNA detection method and to compare its performance against RNAscope, within the histopathology core facility.

Material and Methods

A 3 Phase study was designed to analyse both single and multiple target HCR performance using different fluorophores and compare it with RNAscope. A total of 72 paraffin sections of mouse small intestine tissue was used for phases 1 and 2, and 23 paraffin sections of mouse lymph node tissue for phase 3.

Workflow	Permeabilization	Hybridisation Probe pairs to RNA	Pre-amplification	Amplification ISH signal	Visualisation
HCR	Heat retrieval with Tris-EDTA, followed by protease	Probe sets hybridise in tandem forming specific initiator sequence	Pair of labelled hairpins hybridise to initiator and a chain reaction is triggered	Chain reaction continues until exhaustion of hairpins	Scanned on PhenoImager™ HT and analysed with HALO software
RNAscope	Heat retrieval with Tris-EDTA, followed by protease	Z probe pairs hybridise in tandem forming specific preamplifier binding site	Pre-amplifier hybridises to Z probe pairs	Multiple amplifiers per pre-amplifier, and label probes linked to HRP	Scanned on PhenoImager™ HT and analysed with HALO software

1. Test singleplex HCR using PPIB, POLR2A and DAPB probes, for each fluorophore (488, 546, 594, 647 and 750)
2. Test singleplex RNAscope using PPIB, POLR2A and DAPB probes, for each OPAL fluorophore (520, 570, 620, 690 and 780)
3. Test multiplex HCR (CD8a - 488; CD3e - 546; CD4 - 594; Ihh - 647; Gli1 – 750; Positive control: POLR2A; Negative control: DAPB)

Results

Phase 1: It was possible to observe specific signal for both positive control probes (PPIB and POLR2A) with all fluorophores, Figure 1 shows the images obtained for POLR2A with each fluorophore.

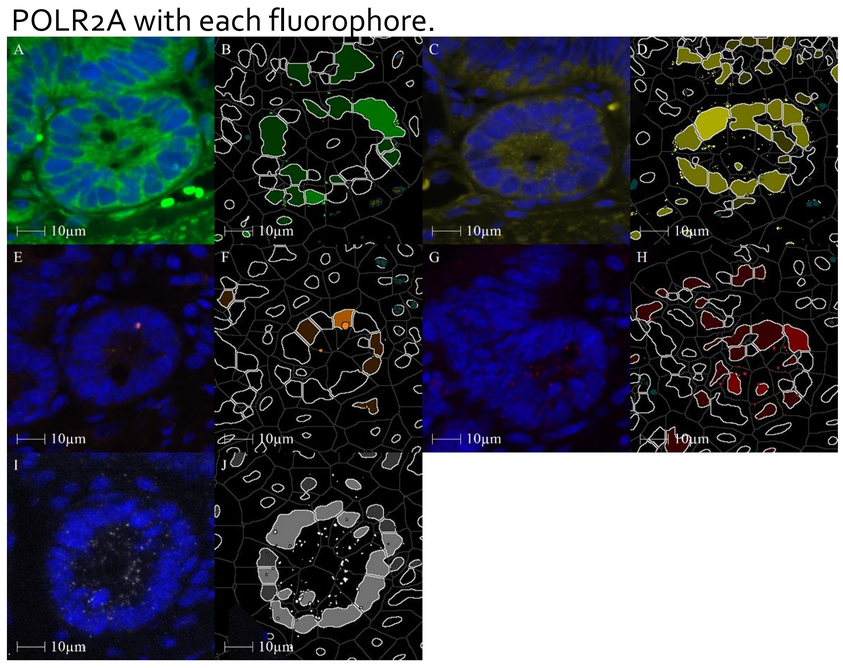

Figure 1 - POLR2A HCR detection, with each fluorophore. A-B) 488 Fluorophore (C-D) 546 Fluorophore (E-F) 594 Fluorophore (G-H) 647 Fluorophore (I-J) 750 Fluorophore; (A; C; E; G; I) Raw images; (B; D; F; H; J) Analysis mark-up.

In Phase 2 it was also possible to detect signal for PPIB and POLR2A with all fluorophores. Figure 2 compares the results obtained with HCR and RNAscope, demonstrating that RNAscope detected a higher percentage of positive cells, for PPIB and POLR2A, and a lower percentage of positive cells for DAPB, as well as lower variability between fluorophores.

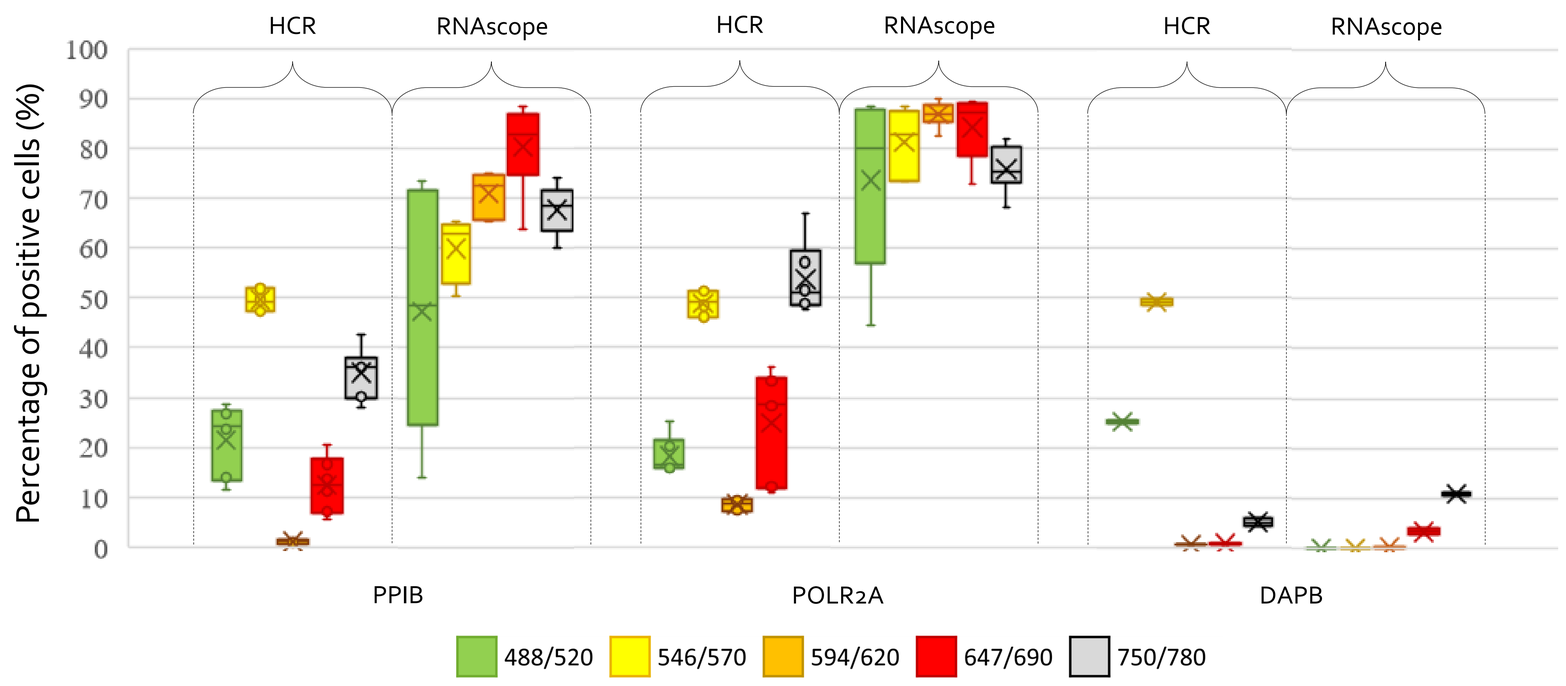

Figure 2- Percentage of positive cells detected with *in situ* HCR vs RNAscope for each probe.
Presented values are the average obtained in the analysis of the whole tissue and ROI on each tissue type. Error bars correspond to data standard deviation, X – Average value, n=6 per bar for PPIB and POLR2A and n=2 for DAPB.

It was possible to detect all 5 targets with multiplex HCR, that being said the CD4 values were lower than expected. Furthermore, co-localisation of targets was possible, where positive signal for CD3e, Ihh and Gli1 was observed in the same cell, as shown in Figure 3.

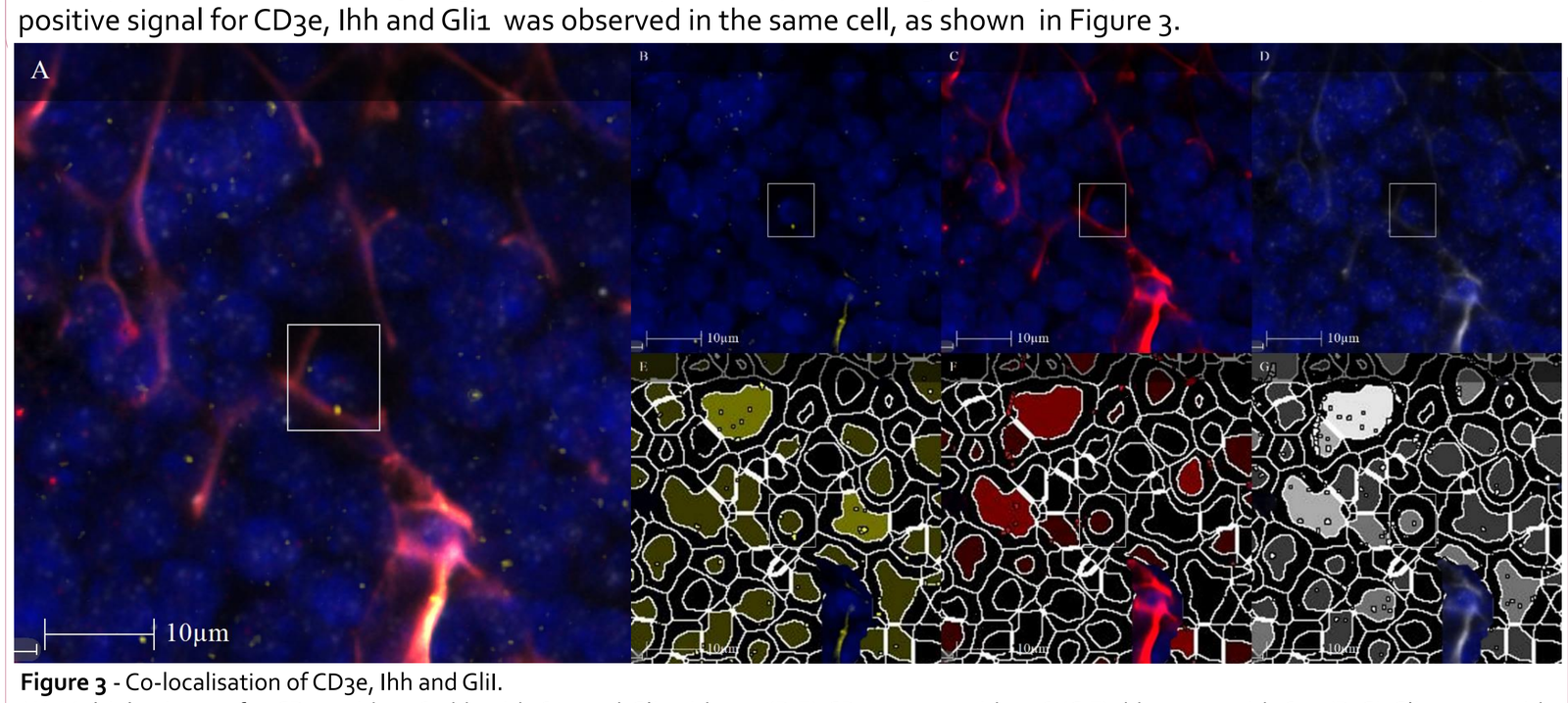

Figure 3 - Co-localisation of CD3e, Ihh and GliI.
(A) Multiplex image for CD3e with 546, Ihh with 647 and GliI with 750 (B;E) CD3e target with 546 (C;F) Ihh target with 647 (D;G) GliI target with 750.CD3e positive cells in yellow, Ihh positive cells in red and GliI positive cells in white.

Conclusion

- HCR detects both medium and low abundance mRNA targets, independent of the fluorophore used.
- RNAscope was a more robust technique, showing consistency between fluorophores, unlike HCR where variability can be observe between fluorophores.
- Multiplex detection of 5 targets using HCR was possible, including co-localised targets.
- HCR can be used to detect mRNA targets, but further optimisation and studies should be performed for optimal results.

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References

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