**Thank you for your interest to present your work at xMAP® Connect 2017!**

* Prepare a presentation title, the title included on the submission should be suitable for published material.
* Abstract text is limited to 3000 characters (approx 500 words).
* We ask to use 4 core elements: 1) background and aim, 2) methods, 3) results, 4) conclusions. We kindly ask you to use the format below.
* Please ensure the submission has been approved by all authors.
* By submitting an abstract, you agree to be present the 8th and 9th of November at the congress, should your abstract be selected.
* Please indicate if you would like to be a speaker or present a poster.

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| **Title:** Differential expression of cancer-related amplifications in metastatic lesions, using bead-based RNA assays. |
| 1) Background and aim:  Multiplex microsphere-based panels are robust and cost-effective molecular assays, but their use to quantify RNA expression in low input samples is limited to few studies. Our group optimised the Invitrogen™ QuantiGene™ Plex Assay (Thermo Fisher Scientific) to classify tumours using low input samples, including microdissected formalin-fixed, paraffin embedded (FFPE) archival tissue and lysates from low numbers of spiked cell lines in blood. The aim of this study was to extend the use of the bead based RNA assay to measure gene expression in FFPE cores comparing matched primary and metastatic tissues.  2) Methods:  Using well annotated cell lines, a 18-plex QuantiGene™ Assay was used to quantify RNA expression of a selection of genes known to be amplified (copy number variations, CNVs) in colorectal cancer. In addition, the ERBB2 (HER2) expression was included to use HER2 positive cell lines as a positive control. RNA was extracted from the cell lines and lysed according to Quantigene assay methodology[[1]](#footnote-1). Following specific hybridization, signal was amplified using the bDNA signal amplification protocol (Quantigene assay) and end point measurement using the Luminex platform. The same workflow was used to quantify expression using FFPE cores from matched primary and metastatic lesions in archival colorectal cancer (CRC) patient material.  3) Results:  As expected a high RNA expression of HER2 (ERBB2) was observed in the HER2 positive cell line BT474 and overexpression was absent in all the colorectal cancer cell lines studied. The high expression of MET and EGFR in SW48, and high expression of MYC and FAM84B in COLO320 cell lines concords with the previously reported gene amplifications [[2]](#footnote-2). Differential expression comparing primary to matched metastatic archival tissues, using the 18-plex on lysed FFPE cores, show that CCND3 is overexpressed in lung metastatic lesions, while KIT overexpression is exclusively found in Liver metastasis.  4) Conclusion:  The use of sensitive bead-based RNA assays provided a robust and simple workflow to compare gene expression in low input FFPE archival material and provides novel insights in distinct overexpression of genes in metastatic lesions from different organs. The presence of highly expressed genes that are specific to primary tumours and differentially expressed in metastatic lesions can provide an opportunity to measure RNA expression in exosomes and circulating tumour cells. |

1. Baldacchino, S., Saliba, C., Scerri, J., Scerri, C., Grech, G. Optimization of a Multiplex RNA-based Expression Assay Using Breast Cancer Archival Material. J. Vis. Exp. (138), e57148, doi:10.3791/57148 (2018). [↑](#footnote-ref-1)
2. Briffa R, Um I, Faratian D, Zhou Y, Turnbull AK, Langdon SP, et al. (2015) Multi-Scale Genomic, Transcriptomic and Proteomic Analysis of Colorectal Cancer Cell Lines to Identify Novel Biomarkers. PLoS ONE 10(12): e0144708. https://doi.org/10.1371/journal.pone.0144708 [↑](#footnote-ref-2)