

1. Introduction

Many cytogenetic laboratories are in the process of adopting microarray screening as the frontline test for a variety of postnatal referrals. Inevitably, high resolution analysis will result in the detection of sub-microscopic chromosomal abnormalities, which must then be confirmed and identified as *de novo* or inherited. In the majority of cases, FISH is likely to be the method of choice for these follow-up investigations. The purchase of commercial FISH probes can however be expensive and those that are currently on the market are of variable quality. Additionally, the extended procurement procedure typical for commercial FISH probes can add considerably to reporting times.

The production of "in-house" FISH probes is an alternative method, with potential advantages both in cost and turnaround times. Here we evaluate production of in-house FISH probes using a 1Mb clone set and examine the various factors which can influence the success of this approach, namely cost, availability of appropriate clones, and validity of results obtained.

2. 1Mb Clone Set

The 1Mb clone set consists of a collection of 96 well plates, containing around 3500 clones. The clones have been selected to provide coverage across the entire genome, including telomeric regions, spaced at approximately 1Mb intervals.

Clones exist as a specific DNA sequence of interest within Bacterial or Plasmid Artificial Chromosomes (BACs/PACs), which are cultured at 37 °C overnight. The DNA sequence of interest is then extracted from the BAC/PAC using a simple Plasmid Extraction kit [Qiagen], and subsequently labeled using a Nick Translation kit [Abbott Molecular]. The labeled FISH probes are then precipitated and purified using a series of washes, before hybridisation using standard laboratory procedures.

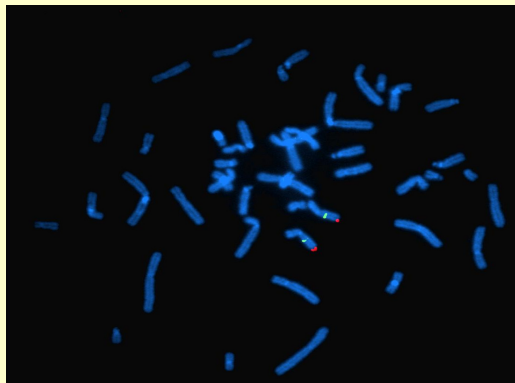


Figure 1. "In House" FISH Probes
Clone RP11-31L22 (11q13.4) labelled using green dUTP and clone RP11-469N6 (11q25) labelled using red dUTP.

3. Comparison of "in-house" & commercial FISH probes

The table below details a comparison of costs, when producing "in house" FISH probes and purchasing commercial FISH probes. This table shows the estimated costs for 250 loci-specific FISH probes, plus 250 telomeric controls.

		TOTAL	
In House	DNA Extraction	£ 385	£7,362
	Labelling	£1,820	
	Purification	£ 60	
	Hybridisation	£ 565	
	Staff*	£4,532	
Commercial	RP11 Probes	£13,475	£19,853
	Telomere Kit	£ 4,000	
	Hybridisation	£ 565	
	Staff*	£ 1,813	

Figure 2. Cost Comparison

This figure clearly highlights the significant reduction in our costs when producing "in-house" FISH probes, compared to that of purchasing commercial probes.

Both commercially purchased and "in-house" probes typically permit 4 tests per vial. The total Commercial cost allows for purchasing one complete telomere kit, however in reality telomeres will be used at differential rates. Therefore additional telomere kits, or individual probes, would have to be purchased to provide the 250 controls as required.

* It would take approximately 7hrs+30mins of staff time (per week) to batch produce 10 probes and perform subsequent FISH procedures, using our "in house" protocols. This would cost around £4532 per annum for a Band 5 Genetic Technologist to carry out this technical work. It would take approximately 3hrs per week, costing around £1813 per annum, to produce the same results with commercial probes.

4. Advantages of an "in-house" FISH Service

- Substantial reduction in consumable costs
- DNA extracted from BACs/PACs can be stored at 4 °C for future labeling
- "In-house" probes can be produced efficiently within 3 days
- FISH results can be reported within 5 working days of probe identification
- An estimated 60% of all abnormal array findings (135K) can be followed up using this 1Mb clone set
- Additional clones can be purchased for as little as \$20 (TCAG, Toronto) and labeled "in-house" for most loci which are not included in the 1Mb set

5. Conclusions

The 1Mb clone set, which has been made available to our laboratory, has proven to be highly resourceful in the production of high quality FISH probes. This method is significantly cost effective and extremely time efficient, both of which are highly beneficial within a diagnostic laboratory.

6. Acknowledgments

Thank you to MRC Human Genetics Unit, Edinburgh, for making the 1Mb clone set available to our laboratory, .

Thank you to Anna Broadwell and Martin Hart who are continuing work within this service, at the South East Scotland Cytogenetics Laboratory .

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References

- <http://www.tcag.ca/>
- <http://www.sanger.ac.uk>
- <http://www.qiagen.com>