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Amplicon NGS battles begin in earnest

A short while ago I posted about the [recent exome sequencing comparisons in Genome Research](#). In that post I did ask whether we really need to target the exome at all and if targeted amplicon sequencing might be a better fit for some projects.

In the last few days both Life Technologies and Illumina have released amplicon resequencing products. You can read another good review of Illumina's offering over at [Keith Robinson's blog](#).

I really hope that amplicon NGS is the tool that gets translated into the clinic quickly. Microarrays took over a decade, and only CGH has made it. I am not aware of any gene expression array based clinical tests, either than Mammarray and the upcoming Coloprint from Agendia. Amplicon NGS is similar to the current standard Sanger tests in many ways. Labs will still perform PCR and sequencing, they'll just be doing a different PCR and it will be NGS. This should make adoption seem like less of a hurdle.

The other amplicon competition:

Fluidigm's Access Array, RainDance's new ThunderStorm, Halo Genomics, MIPs, traditional multiplex PCR assays are all competition from the in-house kits of Illumina and Life Technologies. The major differences with all the platforms are the way in which multiple loci are captured and amplified. Microfluidics, emulsion PCR and oligo-probes are the different 'capture' mechanisms. All rely on PCR for the amplification and to add the sequencing platform adapters and barcodes. The cost of the RainDance instrument is very high, AccessArray is medium and the probe based

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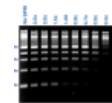


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as Life and Illumina's offerings, all require you to design a panel and order quite a lot to become cost effective.

Ultimately the cost per sample is going to be what makes one of the system here, or one as yet to be released the dominant technology. \$10 rather than \$100 is what we need to get these tests to every cancer patient!

So what have Life Tech and Illumina got to offer?

Life Technologies "AmpliSeq" amplicon sequencing cancer panel for Ion Torrent:

The Ion cancer panel interrogates >700 mutations using 190 amplicons in 46 genes. Using the 314 chip should get 500 fold coverage and allow detection of variants as low as 5%. The AmpliSeq kit can target 480 amplicons (but is scalable from there) in a single tube reaction with just 10ng DNA input from FFZN or FFPE tissue. PCR and sequencing can be completed in a single day, assuming of course you have the one touch system. They have chosen "the most relevant cancer genes" for the initial panel, most probably from COSMIC.

Life Tech are also involved in the CRUK/TSB funded Stratified Medicines Initiative, on which I was worked early on. However I am not sure if they are going to get the ion test out before a full set of Sanger based assays. It will be interesting to see what comes first on this project and could be a good proxy for seeing how much Life Tech still believes in Sanger as a long-term product. Life Tech are aiming to get this into the clinic and are going to seek FDA approval.

There is no pricing on the press release from Life Tech.

I'd agree with the early access Life Tech customers, Christopher Corless, Marjolijn Ligtenberg and Pierre Laurent-Puig at Oregon, Nijmegen and Paris respectively on the likely benefits of amplicon NGS. The simplicity of these methods will hopefully mean clinical genetics labs adopt them quickly.

Illumina's TruSeq custom amplicon (TCSA) sequencing for MiSeq et al:





an integrated normalisation solution so you do not have to quantitate each amplicon set before running on a sequencer. This is going to make some peoples lives much easier as many do still struggle getting this right every time.

TCSA uses the GoldenGate chemistry as I mentioned [at the bottom of a previous post](#). This makes use of an extension:ligation ([see here for one of the original E:L methods](#)) reaction followed by universal PCR to provide better specificity in highly multiplex PCR based reactions. In SNP genotyping GG goes much higher than the 384 plex Illumina are offering on TCSA today. Hopefully this shows the scope for increasing the level of multiplexing.

The benefits of running TCSA on MiSeq are going to be turnaround time and the inbuilt analysis workflows. Of course many users will want to be amplifying 100s-10,000's of samples from FFPE collections and for this purpose Illumina might want to consider modifying their dual-indexing to allow the maximal number of samples to be run in a single HiSeq lane. Right now the limitation of 96 is a pain.

There are no early access customer comments on the TCSA data sheet or Illumina's website. I cant imagine it is going to take too long for the first reports to come out on how well it works though.

Illumina have a pricing calculator on their website so you get to see how much your project is going to cost. Once you have designed an amplicon pool it will let you specify a number of samples and return a project cost inclusive of MiSeq sequencing. I'm not sure who they talked to about the price point for this but it looks like Illumina are aiming at capillary users. The target price is \$0.43 per amplicon or nearly \$200 per sample! Personally I was hoping we would get to under \$50 per sample and as low as \$10 or \$20. I'd also like to see enough indices such that a large project could be run in one lane on HiSeq making the whole project very cost effective and fast.

Imagine 1500 DNA samples from FFPE blocks for lung cancer being screened for the top 50 Cancer genes with just 15 plates of PCR and one PE100 lane on HiSeq. The whole sample prep could be done by one person in a couple of weeks, and the sequencing completed ten days later.



to use the technology.

Hopefully it won't be too long before someone compares the results on all of these as well.

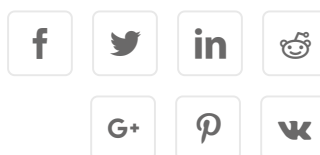
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**Anonymous** 2012-02-10 at 5:27 pm - [Reply](#)

What do you think is the likelihood of being able to dilute the oligo-pool from TSCA projects to enable another plate of samples to be run?

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The new home of the Core Genomics blog, and a site for NGS users to tell people who they are and what they do (on the map), and share knowledge (on the Enseqlopedia NGS methods wiki).

This site is aimed at the whole
NGS community - users, core labs
& services, technology providers.

Thanks for looking - James.

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