SEQUENOM™

***Fact Sheet**: Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood*

On Monday October 6, 2008 the PNAS published a study entitled, "Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood" with key author Stephen R. Quake, cofounder and Chair of the Scientific Advisory Board of Fluidigm Corp.

The study involved high-throughput shotgun sequencing of cell-free DNA from 18 samples of maternal plasma, correctly identifying all the normal cases, the nine Trisomy 21 cases, two cases of Trisomy 18, and one case of Trisomy 13. All of the aneuploidy cases had gestational ages greater than 14 weeks (second trimester).

The sequencing approach is a variant of digital PCR, which has been already well characterized in a number of experiments and publications. The ability of Solexa/Illumina (and other next generation sequencing technologies) sequencing to detect aneuploidies in maternal plasma is, therefore, not surprising, but certainly all research in this field is welcome. Sequenom scientists were pleased to see that the results described in the Quake manuscript support findings from their own experiments. It should be noted that there are some trade-offs between sequencing and digital PCR; sequencing has more sensitivity than a number of non-sequencing variants of digital PCR for aneuploidies, like Trisomy 21, but not for point mutations. This advantage was explicitly anticipated and described in a paper that Dennis Lo, of The Chinese University of Hong Kong, published in PNAS in August 2007(Lo YMD et al (2007) PNAS USA 104: 13116-13121.). Dr. Dennis Lo is an exclusive consultant with Sequenom.

Although the Quake manuscript provides yet further elaboration of one derivative of Dr. Dennis Lo's breakthrough work in non-invasive prenatal genetic testing, the following should be kept in mind when assessing the implications of this paper:

1. Sequenom has already licensed early patents or patent applications, from Oxford University, CUHK, Cytonix, and others, that cover the screening method described in the Quake paper. Hence the results in the new Quake PNAS paper are unlikely to result in any significant new intellectual property.
2. The results reported in the Quake manuscript reported feasibility only in the 2nd trimester or 3rd trimester. Maternal blood was drawn shortly after invasive chorionic villus sampling (CVS) or amniocentesis, which is contrary to the current best practice (in other studies) where samples are preferably collected immediately prior to an invasive procedure or less preferably collected *several days* after an invasive procedure. The current study by Quake is unusual in the extremely short time (15 to 30 minutes) after the invasive procedures when blood was taken. Indeed, at least two previous studies have reported that there is a significant increase in circulating fetal DNA concentration at 10 minutes following amniocentesis, similar to the timeframe described in this study (Samura O et al (2003) Clin Chem 49: 1193-1195; and Miura K et al (2006) Clin Chem 52:

2121-2123). We note in particular that the paper by Samura, et al, has appeared to have been misquoted by the authors of the Quake study as not showing a significant increase post-invasive procedure. In Samura, et al, the authors are quoted as "*The present study showed that amniocentesis is associated with a significant increase in the fetal DNA concentrations* [in 79% of the samples], *representing a transfer of either fetal cells or fetal DNA to the maternal circulation*." The Samura manuscript clearly shows the contrary. It thus remains to be seen whether the sequencing approach would work satisfactorily in real-world samples collected from pregnant women prior to an invasive procedure such as amniocentesis.

3. As currently structured the test described in the Quake manuscript has a Cost of Goods (COGS) of $700 just for sequencing. This likely implies COGS around $1,000 for the entire sampling and testing process, not counting royalty obligations described below.
4. To practice Solexa sequencing on prenatal samples may require, at a minimum, licensing from the following organizations which add significantly to the already prohibitive COGS:
    a. Solexa/Illumina for the sequencing platform;
    b. Sequenom's exclusive sublicense of the Cytonix intellectual property relating to whole genome sequencing, and
    c. Sequenom's exclusively licensed patents from Oxford University and the Chinese University of Hong Kong on detecting fetal nucleic acid in serum or plasma.
5. Any test based on fetal nucleic-acids derived from maternal blood requires a means of quantifying the amount of fetal material in a sample to avoid errors from insufficiency of the analyzed genetic material (i.e. sampling error). To this end, the Methods section of Quake manuscript describes PCR amplification of the DYS14 locus on Y chromosome inherited by the fetus from the father, which infringes the Lo "540" patent that Sequenom has exclusively licensed. It is noteworthy that the study team also obtained multiple sequence reads from the Y chromosomes. And, as mentioned above, there are other relevant patents to contend with that Sequenom has also exclusively licensed.
6. The Quake study appears to require about 2 weeks of sample preparation, library preparation, sequencing and bioinformatic analysis time; this process will need to become far more efficient if it is to meet the needs of pregnant women. For example, the SEQUENOM T21 test can be completed in two days.

For all these reasons, the testing methodology described in the Quake manuscript is still only of academic interest and is far from being commercially feasible. We are confident that, as DNA sequencing costs continue to drop, there will eventually be a cost-effective sequencing platform that Sequenom will add to its non-invasive prenatal testing franchise if it offers advantages over other detection platforms. Sequenom is aggressively investigating all these approaches and has strategically in-licensed several platform technologies including opti-nanopore sequencing and Digital PCR, among others. Also, Sequenom continues to actively search for other technology licensing opportunities, as well. With a dominant and growing IP estate, we expect that Sequenom, to the exclusion of others, may have the freedom to decide which of many technologies to employ in the commercialization of non-invasive prenatal genetic testing.