The OncotypeDX score made cheaper, faster, better

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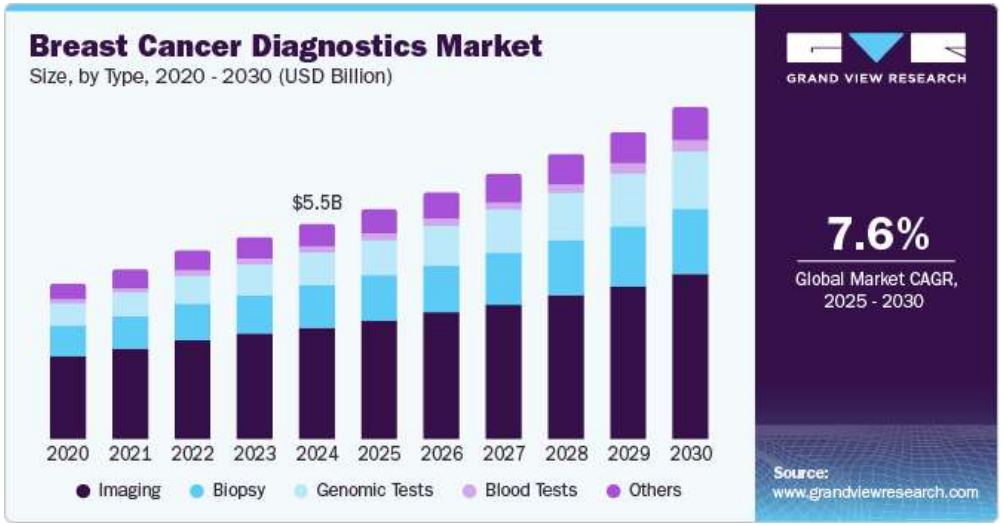
Breast Cancer diagnostics market

- Global breast-cancer incidence 2022: 2.3 M cases (WHO).
- ≈60 % are HR-positive/HER2-negative.
- Global cancer-genetic-testing market: US$ 7.5B (2025) → US$ 18B by 2033 (11.6 % CAGR)
- Oncotype DX: 156.000 tests in 2019 (14% increase from 2018).



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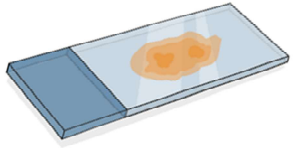
Breast Cancer diagnostics market

Most important Breast cancer commercial tests

Parameter	Oncotype DX	Mammaprint	Prosigna	EndoPredict
Company	Exact Sciences Corp.	Agendia N.V.	Veracyte Inc. (kits from NanoString)	Myriad Genetics Inc.
Price	£2580	£2675	£1686 + Labor and Instrument	£1500
Input Requirement	15 x 5um serial unstained slides	10 x 5um serial unstained slides	1.0mm3 (1-6 10um sections)	5- or 10-µm
Platform	RT-PCR	Microarray	NanoString	qRT-PCR
Service or Kit	Service	Service	Kit	Both
RNA Extraction Required	Yes	Yes	Yes	Yes
cDNA Synthesis	Yes	Yes	Yes	Yes
Number of Genes	21	70	50	12
Eligible Population	HR+, Stage 1 & 2, Node negative and positive (1-3) in Pre-and Post-menopausal	lymph node-negative, ER+ breast cancer and ER- disease (≤3 node positive outside of US)	HR+, Stage 1 & 2, Node negative and positive (1-3 Nodes) in Post-menopausal	(ER)–positive, HER2-negative
Turnaround Time	Within 14 days 90% of the time	14 days	7-14 days	>2 days

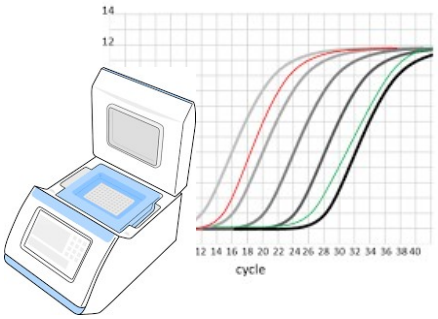
OncotypeDX Breast Cancer Assay

How does it work ?



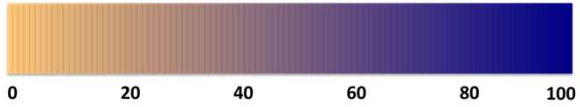
15 x 5µm serial unstained slides of formalin fixed paraffin embedded (FFPE) tissue

RNA extraction



Quantification of gene expression by RT-qPCR

Calculation of the Recurrence Score (RS)



- Low Risk (RS 0–17)
- Intermediate Risk (RS 18–30)
- High Risk (RS ≥31)

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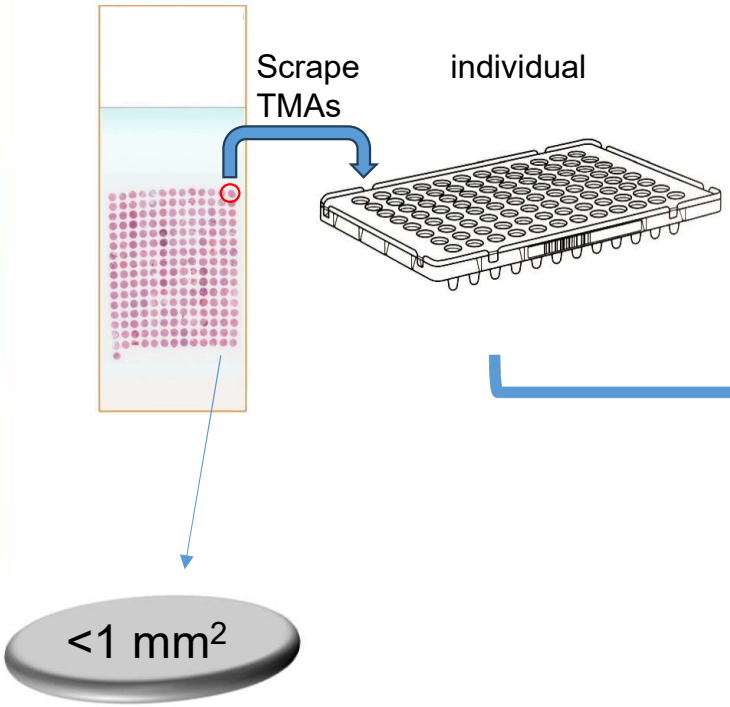
BioClavis Breast Cancer TempO-seq Assay

How does it work ?



Deparaffinization and lysis → TempO-Seq

No purification
No RNA extraction
No retro-transcription
One single test quantifies 21 genes

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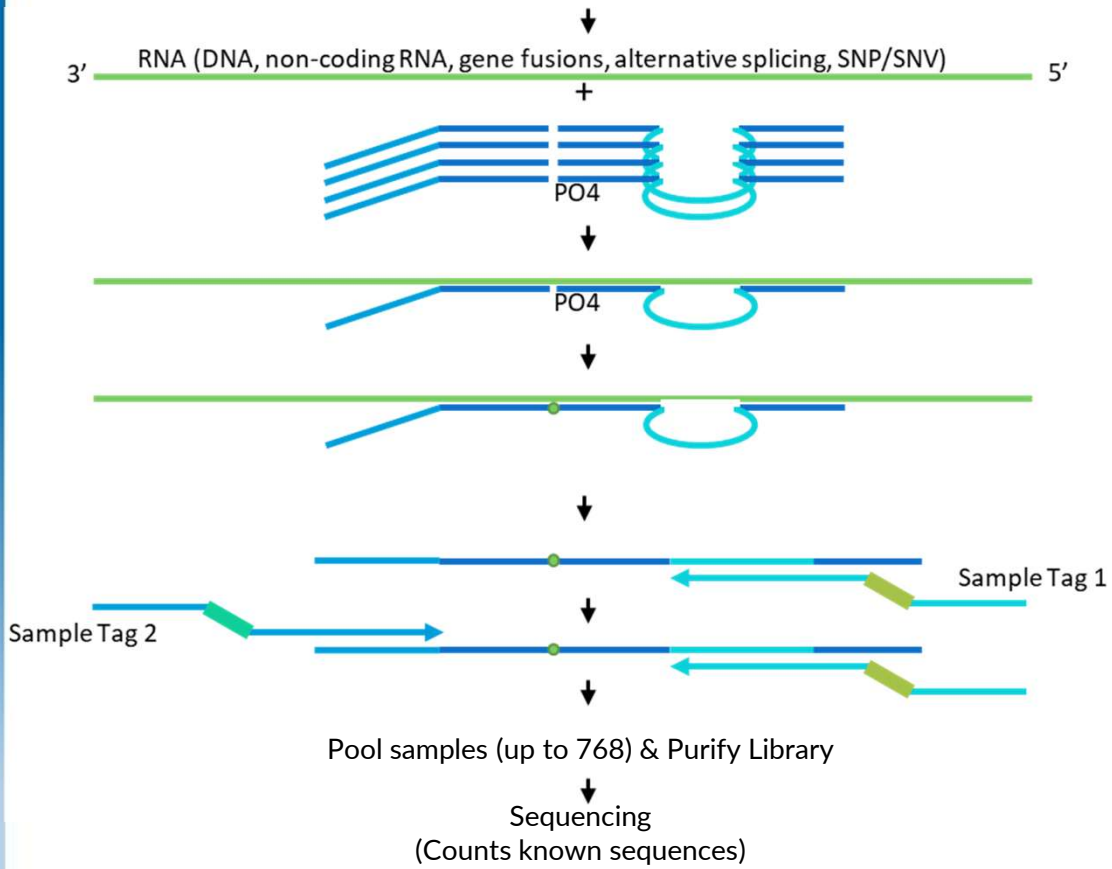
The TempO-seq Assay



Step 1: Hybridization step
Detector Oligos anneal to target, adjacent to each other

Step 2: Exonuclease step
Removes excess and mis-hybridized Detector Oligos

Step 3: Ligation step
Creates an amplifiable probe.
Single-base specificity

Step 4: PCR Amplification (Single-Plex)
Addition of sample-specific tags
Addition of Illumina sequencing adaptors

1 hour hands-on time

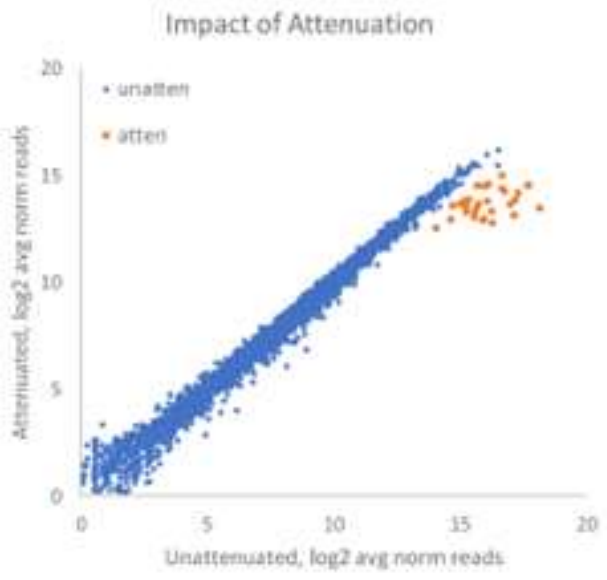
No proprietary equipment needed

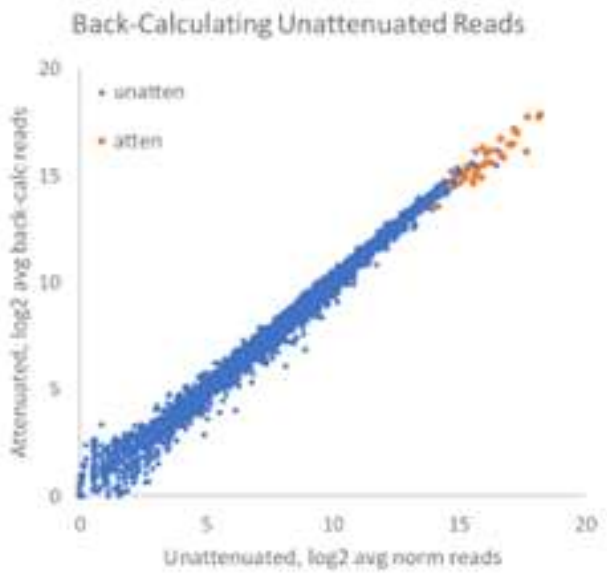
Easily automated

The TempO-seq Assay

Attenuation

- Attenuation of highly abundant transcripts improves total counts from low expressed genes
- Attenuation is gene specific and customizable
- No impact on Differential Expression
- Attenuated gene counts can be corrected to obtain their unattenuated read counts





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TempO-Seq vs Oncotype DX

Methods

A TempO-Seq custom panel was designed, which included probes targeting the same 21 genes that are used in the OncotypeDX test (additional genes can be easily targeted).

After testing the panel on commercial breast cancer TMA, the probes targeting the high expressors have been **attenutated**.

Additional probes were designed and added to the panel for the low expressors **(probe overdesign).**

The panel was tested on our OncotypeDX cohort, after optimizing the **extraction free assay**

Performance was assessed by developing testing several machine learning models

Reproducibility was assessed by comparing technical and biological replicates.

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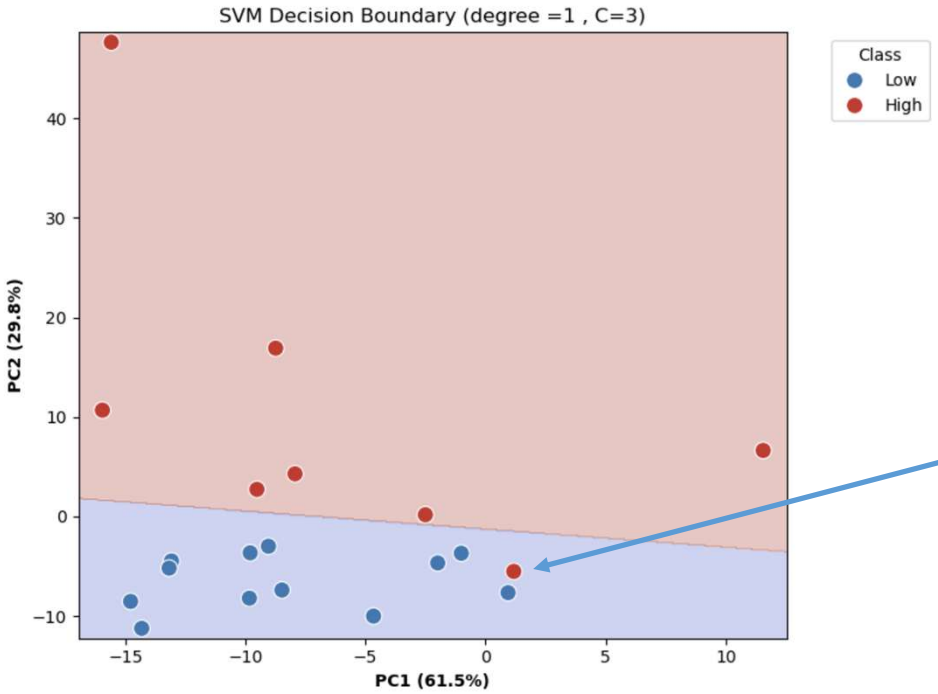
Strong correlation with OncotypeDx Score

- The OncotypeDX gene signature was used to develop a Support Vector Machine (SVM) model that was able to differentiate patients according to their OncotypeDX score (e.g., low vs high).

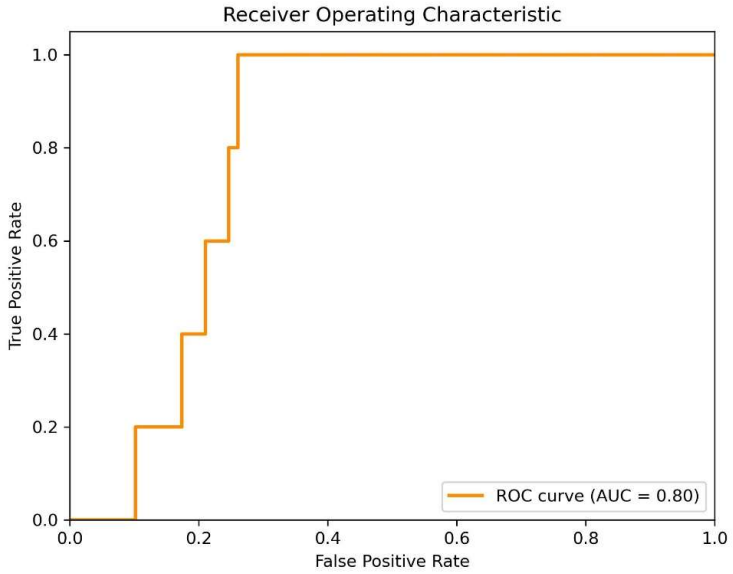


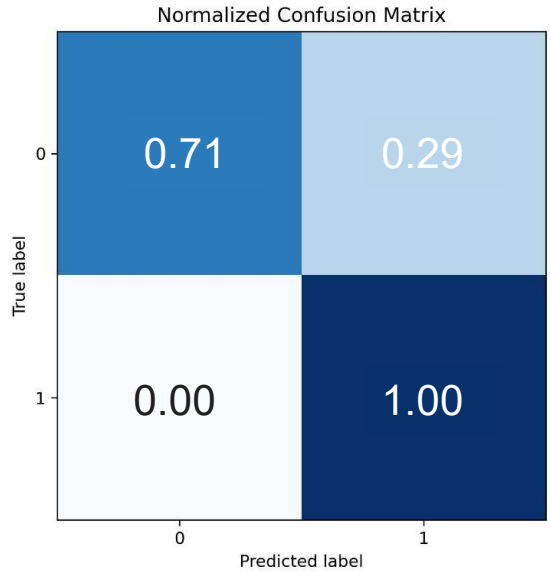
- Our model classified correctly 100% of patients with low OncotypeDX score (<18)
- Only one patient was misclassified among those featuring moderate to high OncotypeDX risk score. Such patient had a border line score of 18.

TempO-Seq vs Oncotype DX

We investigated whether the TempO-Seq assay data, based on the OncotypeDX signature, could be used to develop more accurate models.

For this reason, we tested different machine learning algorithms to classify the patients to their actual recurrence status rather than to the OncotypeDX score they received.





A logistic regression model resulted in AUC = 0.8

100% of the patients with recurrence were classified correctly

71% of the patients without recurrence were classified correctly

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TempO-Seq assay accuracy

The developed model was used to classify a different core from the tumor of the same patients. The model performed well in identifying the majority of the patients with recurrence. Classification accuracy on non-recurrence patients was in line with the OncotypeDX model.

		TempO-Seq	
Real	0	46%	54%
	1	18%	82%
		0	1
		Predicted	

		OncotypeDX*	
Real	0	52%	48%
	1	37%	63%
		0	1
		Predicted	

* OncotypeDX moderate and high-risk scores were considered together in the confusion matrix.

Summary

A TempO-Seq custom panel which included the OncotypeDX signature was tested resulted in good classification accuracy, low required input and good reproducibility

The panel can be easily optimized by adding probes targeting genes included in other commercially available panels

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