**TITLE**

*NGS methods for diagnosis and research.*

**Overview of the course**

*The course is aimed at describing the common NGS techniques that are routinely used in diagnostic and research. It will describe the limits, pitfalls, and potentials of those techniques and give an idea of which technique can be chosen for different experimental designs and how results are typically interpreted. Biotechnological, bioinformatics and interpretation aspects are briefly covered. The course mainly focuses on single nucleotide variants from exome-sequencing experiments, however, a brief overview of other NGS-based analyses is provided as well, such as whole-genome sequencing, structural variants identification, and transcriptome analysis. The differences between the analytical pipeline and the biological significance of data coming from constitutive and somatic variants are illustrated. A final exercitation on real exome-sequence data will give a practical idea of how WES data can be provided to researchers and how can be interpreted.*

**References**

* Andolfo I, Rosato BE, Marra R, De Rosa G, Manna F, Gambale A, Iolascon A, Russo R. The BMP-SMAD pathway mediates the impaired hepatic iron metabolism associated with the ERFE-A260S variant. Am J Hematol. 2019 Nov;94(11):1227-1235. doi: 10.1002/ajh.25613. PMID: 31400017.
* Russo R, Andolfo I, Manna F, Gambale A, Marra R, Rosato BE, Caforio P, Pinto V, Pignataro P, Radhakrishnan K, Unal S, Tomaiuolo G, Forni GL, Iolascon A. Multi-gene panel testing improves diagnosis and management of patients with hereditary anemias. Am J Hematol. 2018 May;93(5):672-682. doi: 10.1002/ajh.25058. PMID: 29396846.
* Lasorsa VA, Montella A, Cantalupo S, Tirelli M, de Torres C, Aveic S, Tonini GP, Iolascon A, Capasso M. Somatic mutations enriched in cis-regulatory elements affect genes involved in embryonic development and immune system response in neuroblastoma. Cancer Res. 2022. doi: 10.1158/0008-5472.CAN-20-3788.
* MacArthur, D. G., Manolio, T. A., Dimmock, D. P., Rehm, H. L., Shendure, J., Abecasis, G. R., Adams, D. R., Altman, R. B., Antonarakis, S. E., Ashley, E. A., Barrett, J. C., Biesecker, L. G., Conrad, D. F., Cooper, G. M., Cox, N. J., Daly, M. J., Gerstein, M. B., Goldstein, D. B., Hirschhorn, J. N., … Gunter, C. (2014). Guidelines for investigating causality of sequence variants in human disease. Nature, 508(7497), 469–476. https://doi.org/10.1038/nature13127

**Schedule (~6 hours)**

The course will include three lessons (2 hours each), as it follows.

**Programme**

**Lesson one (Roberta Russo & Immacolata Andolfo, DMMBM): December 6, 3:00 pm**

Seminar room 4th floor Torre Biologica

* NGS approaches: WGS, WES, Targeted-NGS, RNAseq
* NGS-based genetic testing
* NGS-based identification of complex mode of inheritance
* NGS-based identification of genetic modifiers
* Exome Secondary Findings

**Lesson two (Mario Capasso, DMMBM): December 14, 3:00 pm**

Seminar room 4th floor Torre Biologica

* Base calling: Sequencing signal to nucleotides and FASTQ format
* Mapping (BWA, SAM/BAM format, IGV)
* Usage of important NGS toolkits (samtools)
* Mapping statistics
* DNA variant calling
* Variant Call File Format (VCF)
* Filtering DNA variants

**Lesson three (Michele Pinelli, DMMBM): December 20, 3:00 pm**

Seminar room 4th floor Torre Biologica

* Criteria for interpretation for diagnostic and research purpose
* Guidelines
* Online resources
* New disease gene discovery
* Practical exercitation