MGC course **'The ins and outs of CRISPR-Cas'**

Date: **March 2-3 and 10-11, 2020**

Location: Erasmus MC

Registration

for PhD students with an active interest in genome engineering using this technique AND an interest in understanding the molecular mechanism of its function

The CRISPR-Cas9 system is revolutionizing the field of biomedical science by allowing efficient mammalian genome editing. It is furthermore being repurposed for gene regulation, imaging as well as *in vitro* applications. This course will offer students the latest advances in this technique and allows them interactions with leading scientists in the field. Students will study the underlying biochemical and biophysical principles of Cas9 structure-function relations, through literature study and practical protein visualization using PyMol. Furthermore students will design a genome modification strategy or other CRISPR-Cas application based on their own research project or available case studies. Focus will be on assimilation and application of background and new knowledge in an interactive setting including lectures, seminars, computer assignments, discussion sessions and student presentations.

The course runs for 4 full days within a period of 2 weeks and has assignments that require preparation (2 ECTS)

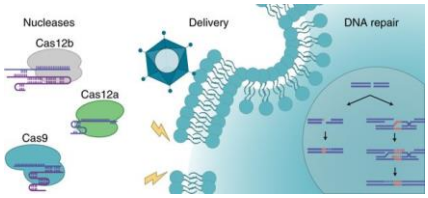
There is a maximum of 24 places.

When registering for this course, please provide a short motivation of max. 100 words, which will be used for selection in case the course is oversubscribed. Students that bring their own CRISPR-Cas project to develop during the course and have an active interest into the molecular mechanism of the system will have a pre during the selection process.

The course is free for all members of the MGC institutes. Course fee for participants from outside these institutes is € 500.

The ins and outs of CRISPR-Cas

May 2019 – Erasmus MC Rotterdam, NL



Wu et al, Nat. Chem. Biol. 14, 642-651, 2018.

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Program from 2019:

Wednesday May 8		**Teacher**
10.00	Introduction to the course and CRISPR-Cas	Joyce Lebbink
10.30	Introduction biochemical principles structure-function	Joyce Lebbink
11.00	PyMol session 1 – DNA, RNA and Cas9 basics	Joyce Lebbink / Niels Galjart
13.00	Lunch	
14.00	PyMol session 1 – continued	Joyce Lebbink / Niels Galjart
15.00	Break	
15.30	Introduction to genome engineering	Sreya Basu
17.00	End of day 1	

Thursday May 9

10.00	Guest seminar	Stan Brouns, TU Delft
11.00	Interactive coffee session	Stan Brouns
12.00	PyMol session Cas9 – 2	Joyce Lebbink / Niels Galjart
13.00	Lunch	
13.30	PyMol session Cas9-2 continued	Joyce Lebbink / Niels Galjart
14.30	Lecture CRISPR-Cas applications – general	Joyce Lebbink
15.00	Break	
15.30	Lecture CRISPR-Cas applications - genome engineering	Sreya Basu
16.30	Paper selection and explanation, group formation	Sreya / Niels / Joyce
17.00	End of day 2 (homework paper preparation for day 3)	

Wednesday May 15 – bring your laptop

10.00	Paper presentation in groups	Sreya / Niels / Joyce
12.00	Seminar	Jurgen Marteijn, Erasmus MC
13.00	Lunch with speaker	Jurgen Marteijn
14.00	Practice problems	Sreya Basu / Niels Galjart
17.00	End of day 3	

Thursday May 16 – bring your laptop

10.00	Design your own project	Sreya Basu / Niels Galjart
12.00	Lunch	
13.00	Present your own project	Sreya / Niels /Joyce
15.00	General discussion	Sreya / Niels / Joyce
16.00	Seminar	Chirlmin Joo, TU Delft
17.00	Discussion and Drinks	Chirlmin/Sreya/Niels/Joyce

Literature, homework to prepare by everyone:

Genome editing by natural and engineered CRISPR-associated nucleases. Wen Y. Wu, Joyce H. G. Lebbink, Roland Kanaar, Niels Geijsen & John van der Oost. *Nature Chemical Biology* 14, pages 642–651 (2018). *Review on genome editing.*

Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V., Scott, D. A., and Zhang, F. (2013) Genome engineering using the CRISPR-Cas9 system. Nat Protoc. 8, 2281–2308. *Protocol for genome engineering.*

Brouns SJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP, Dickman MJ, Makarova KS, Koonin EV, van der Oost J. (2008) Small CRISPR RNAs guide antiviral defense in prokaryotes. Science 321, 960-964. *CRISPR-Cas mediates an antiviral response that counteracts infection.*

Literature, to choose and prepare 1 paper per group for presentation:

Mechanistic papers:

A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. Science. 2012 Aug 17;337(6096):816-21. *Landmark paper on molecular mechanism of CRISPR-Cas9.*

Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. Science. 2016;351:84-8.

Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzales AP, Li Z, Peterson RT, Yeh JR, Aryee MJ, Joung JK. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature. 2015;523:481-5.

Kan, Y., Ruis, B., Takasugi, T. & Hendrickson, E.A. Mechanisms of precise genome editing using oligonucleotide donors. Genome Res (2017).
An elegant mechanistic study unravelling the different homology directed repair pathways that are used during precision gene editing.

Application papers:

Cong, L. et al. Multiplex genome engineering using CRISPR/Cas systems. Science 339, 819-823 (2013).
This report shows successful Cas9 genome editing in mammalian cells.

Genetic screens in human cells using the CRISPR-Cas9 system. Wang T[1], Wei JJ, Sabatini DM, Lander ES. Science. 2014 Jan 3;343(6166):80-4. doi: 10.1126/science.1246981. Epub 2013 Dec 12.

Tabebordbar, M. et al. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. Science 351, 407-411 (2016).
This report demonstrates in vivo AAV-mediated CRISPR/Cas9 delivery for the repair of the Dystrophin gene defect in Mdx-mice

Paquet, D. et al. Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9. Nature 533, 125-125 (2016).
This report describes how the introduction of blocking mutations and optimization of distance effects increase the efficiency of CRISPR-Cas mediated genome editing.

Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A. & Liu, D.R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533, 420-424 (2016).
This report is the first to show efficient and precise programmable base editing.

Wang, H., Yang, H., Shivalila, C. S., Dawlaty, M. M., Cheng, A. W., Zhang, F., & Jaenisch, R. (2013). One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas mediated genome engineering. Cell, 153, 910– 918. doi: 10.1016/j.cell.2013.04.025.

Yang, H., Wang, H., Shivalila, C. S., Cheng, A. W., Shi, L., & Jaenisch, R. (2013). One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas mediated genome engineering. Cell, 154, 1370– 1379. doi: 10.1016/j.cell.2013.08.022.

Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. Cell 159, 647–661, October 23, 2014

Perturb-seq: Dissecting molecular circuits with scalable single cell RNA profiling of pooled genetic screens. Dixit, et al., Cell. 2016 December 15; 167(7): 1853–1866.e17. doi:10.1016/j.cell.2016.11.038

One-step generation of conditional and reversible gene knockouts. Nat Methods. 2017 March ; 14(3): 287–289. doi:10.1038/nmeth.4156

RNA editing with CRISPR-Cas13. *Science* 24 Nov 2017: Vol. 358, Issue 6366, pp. 1019-1027 DOI: 10.1126/science.aaq0180