

I. Overview of planned Experiments

All experiments are planned as demonstration experiments. For experiments 1, 2, 4, 5, 6 and 8, participants can bring own reagents that they want to test. This should be discussed in advance with the organizers to allow for preparation. Each day will feature a ca. 1/2 hour introduction into the subject

II. Experiments in the course:

1. Transfection of minigenes (Jamal Tazi)

Transfection of two reporter minigene constructs in eukaryotic cells, isolation of the RNA and analysis of the RNA by RT-PCR,

Participants can also bring their own reporter genes for analysis

Reference for the method:

Stoss, O., Stoilov, P., Hartmann, A. M., Nayler, O., and Stamm, S. (1999). The in vivo minigene approach to analyze tissue-specific splicing. *Brain Research Protocols* 4, 383-394.

Tang, Y., Novoyatleva, T., Benderska, N., Kishore, S., Thanaraj, T. A., and Stamm, S. (2005). Analysis of alternative splicing in vivo using minigenes. In *Handbook of RNA Biochemistry*, Westhof, Bindereif, Schön, and Hartmann, eds. (Wiley-VCH), pp. 755-782.

2. Generation of minigenes (Stefan Stamm, Amit Khanna)

Use of a new method to generate minigenes within one week. Participants are encouraged to bring their own Bac clones, if they want to generate their own minigenes

Reference: Kishore, S., Khanna, A., and Stamm, S. (2008). Rapid generation of splicing reporters with pSpliceExpress. *Gene* 427, 104-110.

3. In vitro splicing (Jamal Tazi)

In this demonstration experiment, RNA is in vitro transcribed, spliced in HeLa nuclear extract and the fragments are analyzed by RT-PCR. In a demonstration, the use of radioactively labeled RNA is shown.

Reference: Hicks, M. J., Lam, B. J., and Hertel, K. J. (2005). Analyzing mechanisms of alternative pre-mRNA splicing using in vitro splicing assays. *Methods* 37, 306-313.

4. Bioinformatic analysis (Pierre de la Grange)

The use of databases, especially FAST-DB to analyze splicing events, to predict functions of alternative exons and their regulatory features is demonstrated. Participants can pose own experimental problems prior to the course.

Reference: de la Grange, P., Dutertre, M., Correa, M., and Auboeuf, D. (2007). A new advance in alternative splicing databases: from catalogue to detailed analysis of regulation of expression and function of human alternative splicing variants. *BMC Bioinformatics* 8, 180.

de la Grange, P., Dutertre, M., Martin, N., and Auboeuf, D. (2005). FAST DB: a website resource for the study of the expression regulation of human gene products. *Nucleic Acids Res* 33, 4276-4284.

5. Splice Array Analysis (Pierre de la Grange)

The theory of Affymetrix exon arrays and their analysis will be presented, using the GenoSplice analysis tools that link to the FAST DB database.

6. Screening of drugs that change alternative splicing (Peter Stoilov)

The use of cell lines containing a splicing reporter with dual fluorescence markers is shown. The influence of model substance that change splicing will be demonstrated.

Reference: Stoilov, P., Lin, C. H., Damoiseaux, R., Nikolic, J., and Black, D. L. (2008). A high-throughput screening strategy identifies cardiotoxic steroids as alternative splicing modulators. *Proc Natl Acad Sci U S A* 105, 11218-11223.

7. Cross-link and immunoprecipitation (CLIP) of splicing factors (Branislav Kusenda)

The demonstration experiment explains the CLIP technology and shows the CLIP procedure using prepared experimental steps.

Reference: Ule, J., Jensen, K., Mele, A., and Darnell, R. B. (2005). CLIP: a method for identifying protein-RNA interaction sites in living cells. *Methods* 37, 376-386.

8. Microscopy of splicing proteins (Edouard Bertrand)

The cellular localization of splicing factors and the sub-nuclear localization will be shown using GFP-tagged factors. Background and usage of fluorescent microscopy will be explained.

III. Tentative Timetable:

	mo	tues	wed	thurs	fri	sat
1	Split cells	transfect	RNA	RT-PCR, gel		Final discussion
2	Symposium	PCR, ligate	transform	inoculate	Miniprep, gel	
3		Make template	Splice, PCR	Analyze		
4				Bioinformatic		
5					Array introduction	
6			Treat cells	RT-PCR	gels	
7		Cross link	Imunoprecipitate, gels	Rna isolation	RT-PCR	
8			Microscopy			

