Use of Dispomix homogenization technology for sample disruption in combination with nucleic acid preparation kits from MACHEREY-NAGEL

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Content

Summary

The Dispomix Drive allows optimization of homogenization profiles in terms of mixing speeds, time and intervals to specifically address different sample materials for optimal yield or quality. However, the described experiments are based on a standard profile without any optimization.

Compared with conventional sample disruption methods (mortar, Ultra Turrax, bead mill) the cell disruption with the Dispomix yielded similar to better results (quantity and quality). The Dispomix Drive is easy to operate. Profile optimization may even have a positive influence on some results with sensitive material (e.g. for the RNA-extraction).

1 Isolation of genomic DNA

1.1 Isolation of gDNA from frozen leaf material or lyophilized leaf material with magnetic beads (NucleoMag 96 Plant kit)

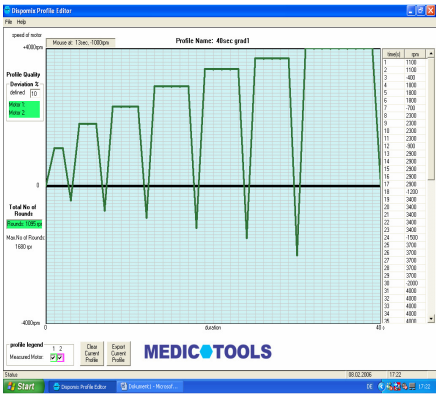
Material and methods:

Dispomix Drive V 1.4 → Profile 09 *) → homogenization in lysis buffer volume on the instrument

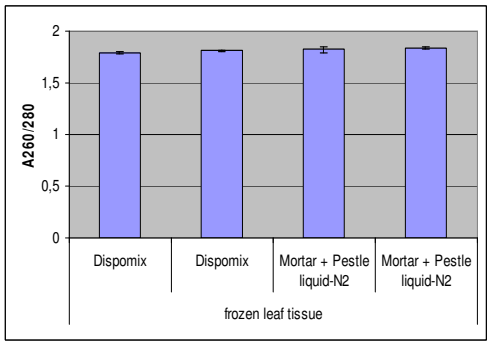
- ⇒ Wheat leaves (storage at – 20°C, not lyophilized)
- ⇒ Mortar / pistil, liquid N_2 (approx. 5 min, until fine powder)
- ⇒ Dispomix tube, master lysate, fresh wheat, (5 x Profile 09)
- ⇒ Purification according to NucleoMag Plant Kit protocol
- ⇒ 50 mg /sample in 400 µl MC1/RNase
- ⇒ Clearing of supernatant (10 min, centrifugation 4600 x g + filtration)
- ⇒ Work-up of sample in NM-Plant-Prep.: 400 µl MC1-lysat
- ⇒ Elution volume: 150 µl
- ⇒ UV-measurement

***) Dispomix Drive, Profile 09**: 40 sec. profile, gradient of intervals with increasing homogenization speeds (up to 4000 rpm) with short backward spins.

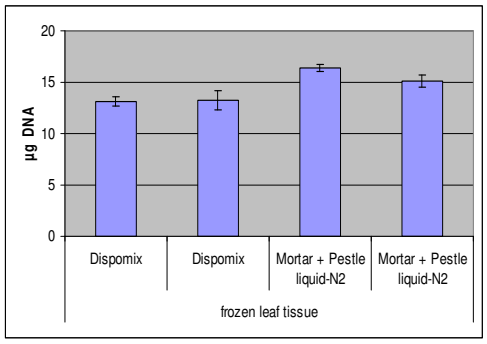
No profile optimization has been done for these tests.



Results:
16 samples have been processed with each method. The graphs show the mean value per micro plate column (8 samples each).



DNA purity: Mean values and SD of n=8



DNA yield: Mean values and SD of n=8

Conclusion:
Both methods show a very similar yield and good gDNA quality.

1.2 Isolation of gDNA from lyophilized leaf material with magnetic beads (NucleoMag 96 Plant kit)

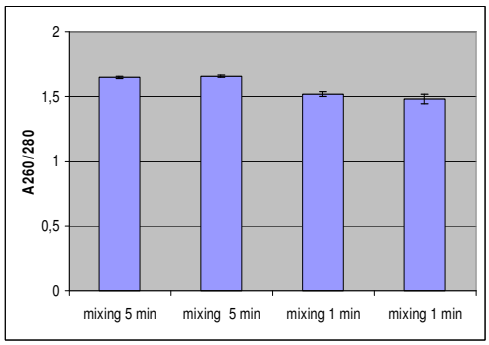
Material and methods:

- ⇒ Lyophilized wheat leaves
- ⇒ 12.5 mg sample (in analogy to the 50 mg moist weight, assuming a dry weight of ¼ to the moist mass) → Lysis buffer: 500 µl C1 + 20 µl RNAse [25 mg/ml] : 65°C ½ h lysis
- ⇒ Dispomix Drive → Profile 09
- ⇒ Sample in lysis buffer homogenized on instrument
- ⇒ 3 x Profile 09 – total volume of master lysate = 20 ml of 500 mg lyophilized wheat leaves
- ⇒ Centrifugation to clear lysate 5500 x g / 5 min
- ⇒ Isolation with NucleoMag Plant Kit Protocol, additional buffer MC5 with 0.05 % Tween20
- ⇒ Elution in 150 µl MC6 buffer

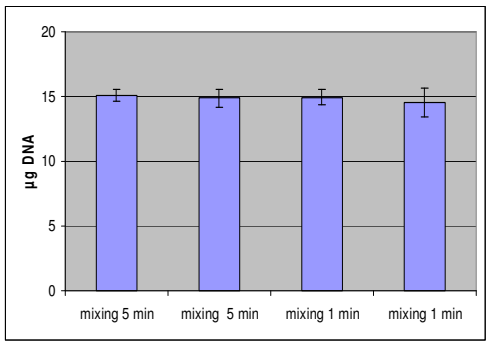
Comparison of different mixing speeds and times for the washing steps of the magnetic beads during the purification procedure. With this experiment a mixing time of 5 min with a moderate speed was compared with a shorter mixing time (1 min) with high speed setting.

Results

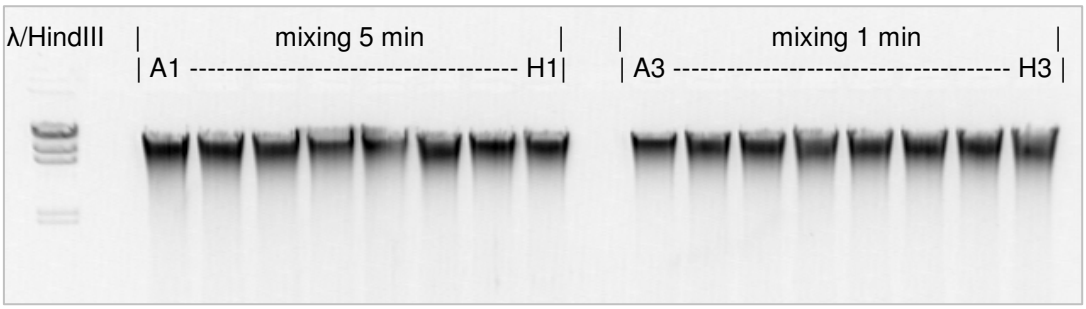
16 samples have been processed with each method. The graphs show the mean value per micro plate column (8 samples each).



DNA purity: Mean values and SD of n=8



DNA yield: Mean values and SD of n=8



1 % Agarose gel / 12 µl each well

Conclusion:

Both methods show equally good yield and quality of the gDNA. Different workup protocols have no effect on the structural integrity of the DNA as shown by electrophoresis. No significant sheering of the genomic DNA can be detected.

1.3 Isolation of gDNA from tissue (pig liver) with silica filter method (NucleoSpin 96 Tissue kit)

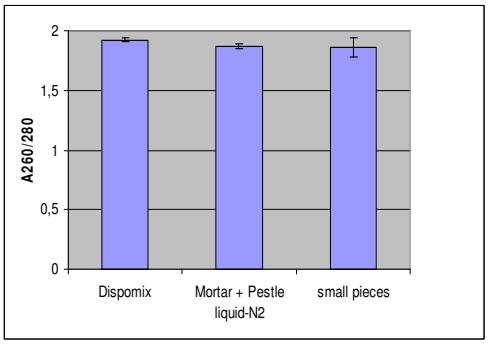
Material and methods:

DNA isolation from pig liver

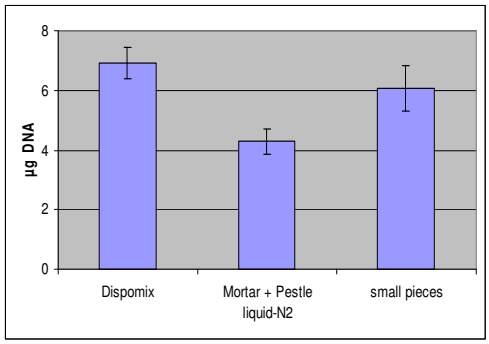
- ⇒ Preparation of master lysates according to 20 mg tissue, lysis in 180 µl T1, proteinase K solution 25 µl (28 mg/ml) per preparation.
- ⇒ Tissue has been homogenized by a) Dispomix b) mortar in liquid N_2 c) cut into small pieces and incubated in the buffer mentioned above (GFL shaker, 300 rpm, 56°C, lysis for 3 h)
- ⇒ RNA digestion 10 µl (20 mg/ml water) for 10 min
- ⇒ Further purification according to NucleoSpin 96 Tissue protocol
- ⇒ Elution with 150 µl buffer BE

Results:

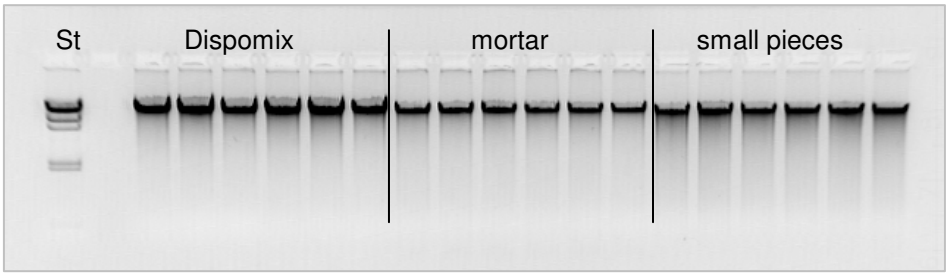
24 samples have been processed with each technology and analyzed individually. As a summary, the mean values are shown in the graphs below.



DNA purity: Mean values and SD of n=24



DNA yield: Mean values and SD of n=24



0,7% Agarose/ 12µl of 150µl eluat/ Standard Lambda Hind III

Conclusion:

The yield of the homogenization with the Dispomix is equivalent to the chemical lysis of small pieces. The mortar resulted in slightly smaller yields (potentially longer processing required). All methods result in equivalent purity. The quality of the DNA is very good with all methods (with no significant sheered DNA).

2 Isolation of RNA

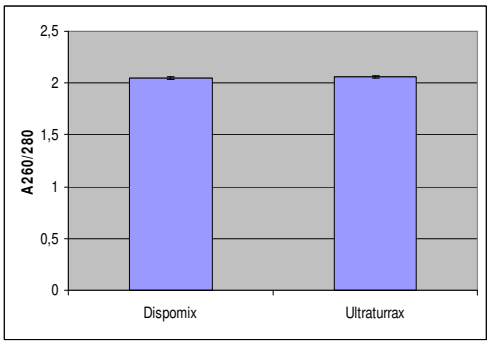
2.1 Isolation of RNA from tissue (mouse liver) with magnetic beads (NucleoMag 96 RNA kit)

Material and methods:

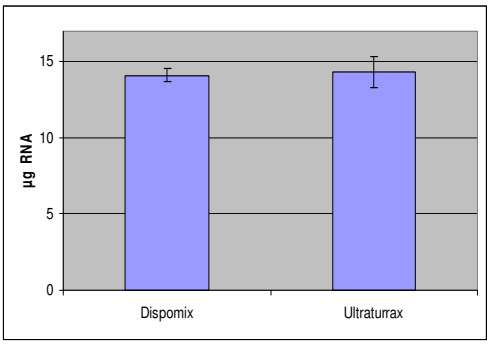
- ⇒ Mouse liver stored in RNALater → 10 mg / preparation
- ⇒ Ultra Turrax (IKA Ultraturrax T25, approx. 5 min at 20'500 rpm)
- ⇒ Dispomix Drive → 5 x Profile 09
- ⇒ Processing according to NucleoMag 96 RNA Kit protocol
- ⇒ Elution volume 150 µl

Results:

24 samples have been processed with each technology and analyzed individually. As a summary, the mean values are shown in the graphs below.

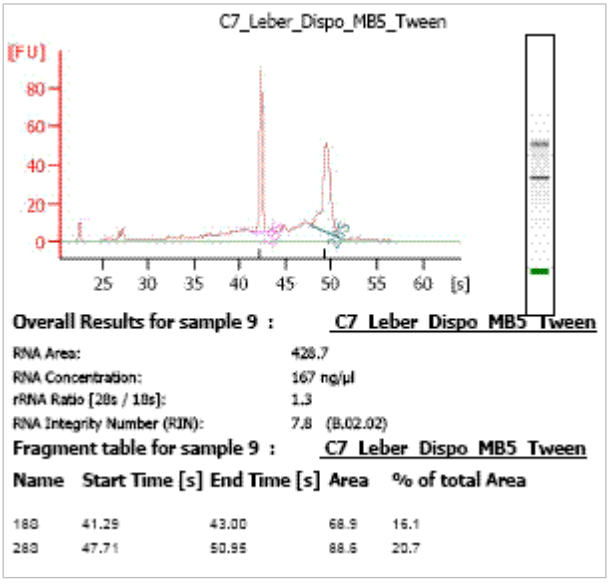


RNA quality: Mean values and SD of n=12

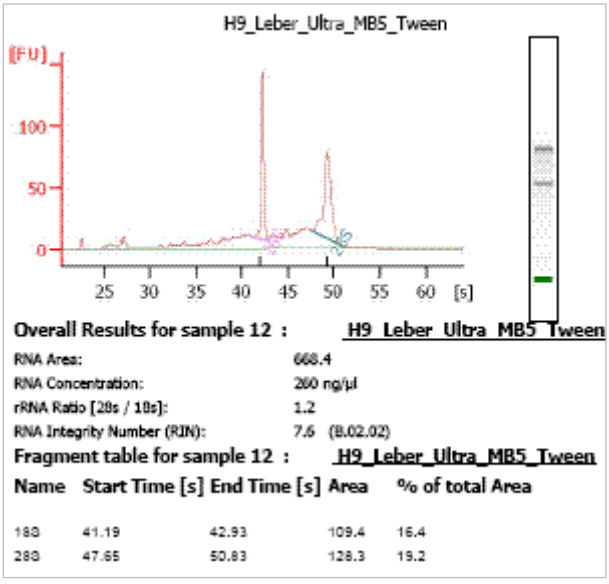


RNA yield: Mean values and SD of n=12

Results from Agilent 2100 Bioanalyzer results (RNA 6000 nano kit)



Sample disruption with Dispomix



Sample disruption with Ultra Turrax

RNA-Integrity: RIN-Number

Sample Name	RIN	mean	
A7_Leber_Dispo	7.4	**7.6**	
B7_Leber_Dispo	7.6		
C7_Leber_Dispo	7.8		
F9_Leber_Ultra	7.6	**7.5**	
G9_Leber_Ultra	7.3		
H9_Leber_Ultra	7.6		

Conclusion:
Both methods show equivalent results. According to the RIN-values no differences in the RNA quality between the different methods can be detected. The preparation with the Dispomix and Ultra Turrax are similar regarding yield and purity.

2.2 Isolation of RNA from tissue (mouse liver) with silica filter kit (NucleoSpin RNA)

Material and methods:

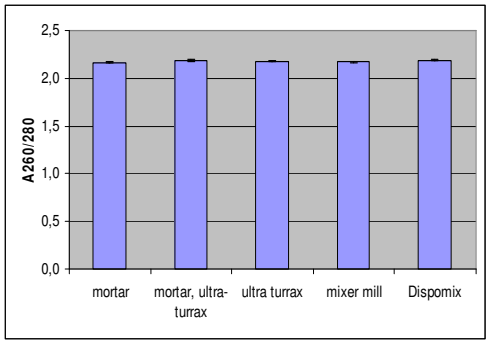
Sample size 10mg mouse liver, triplicates for each process. The tissue was cut into small pieces, mixed and directly used for the processes 3, 4, and 5. The rest of the tissue was frozen in liquid nitrogen, homogenized in a mortar and aliquoted for process 1+2. All samples were treated with ß-Mecaptoethanol.

Lysis variations:

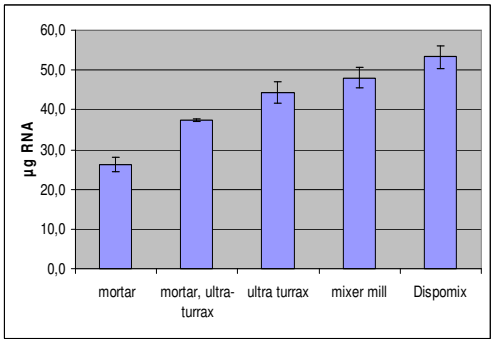
- ⇒ Mortar: homogenize with liquid nitrogen, weigh aliquot into Eppendorf tube, add lysis buffer, vortex, apply 350 µl on NS Filter. (approx. 5 min, until fine powder)
- ⇒ Mortar + Ultra Turrax: homogenize with liquid nitrogen, weigh aliquot into Eppendorf tube, additional Ultra Turrax treatment of tissue, apply 350 ul on NS Filter. (approx. 5 min with mortar until fine powder, afterwards addition of buffer and additional 5 min Ultraturrax, 20'000 rpm at room temperature)
- ⇒ Ultra Turrax: weigh aliquot into Eppendorf tube, Ultra Turrax treatment of tissue, apply 350 µl on NS Filter. (approx. 5 min at 20'000 rpm)
- ⇒ Retsch mill: weigh aliquot into rack with tube strips (2 balls at the bottom, 2 balls on top of the tissue), lysis buffer, close tubes with cap strips, milling (3 min, 30 Hz), apply 350 µl on NS Filter.
- ⇒ Dispomix: weighing of aliquot into Dispomix tube, homogenize on Dispomix Drive with profile 09, apply 350 µl on NS Filter. (5 x Profile Nr 09)

Processing according to NS RNA II protocol. Elution in 100 µl water.

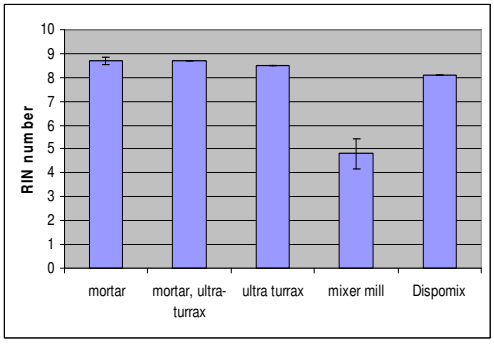
Analytic – Results:



RNA quality. Mean values and SD of n=3

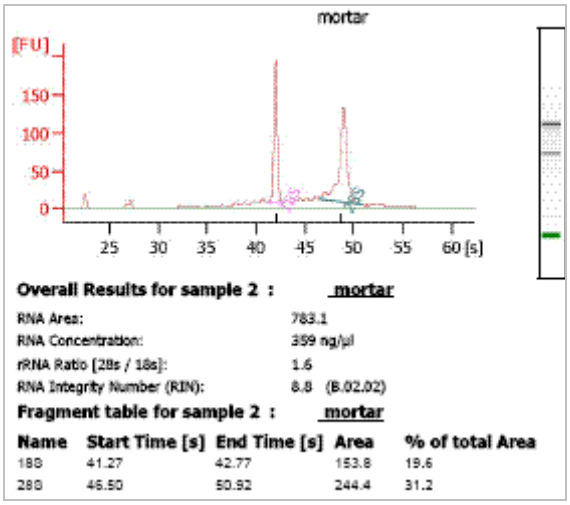


RNA yield. Mean values and SD of n=3

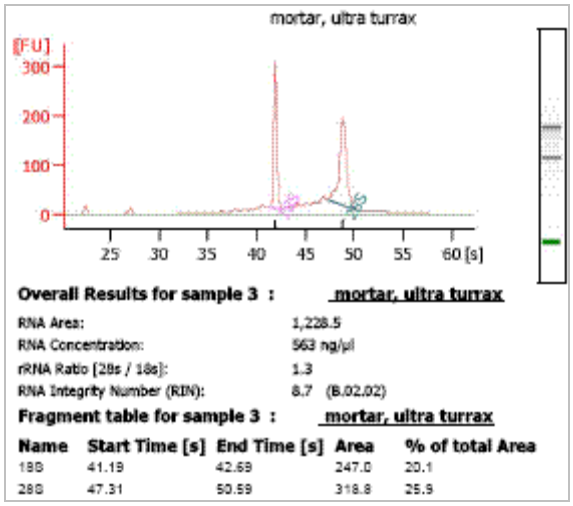


RNA quality: RIN number and SD n=2

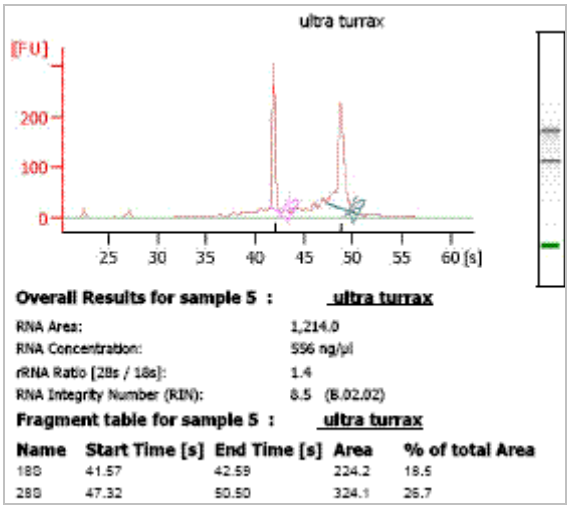
Results from Agilent 2100 Bioanalyzer results (RNA 6000 nano kit)



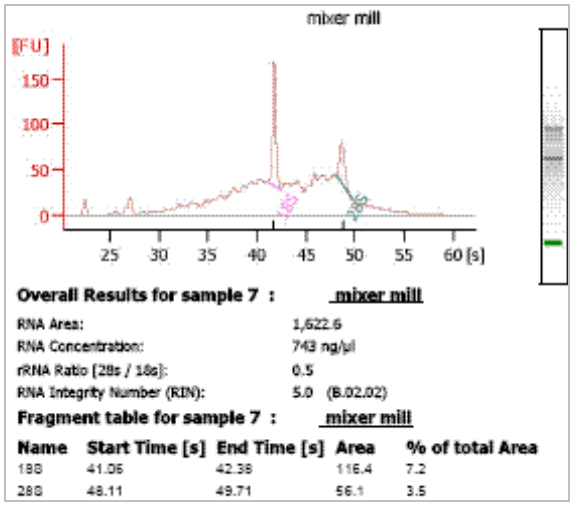
Sample disrupted with mortar



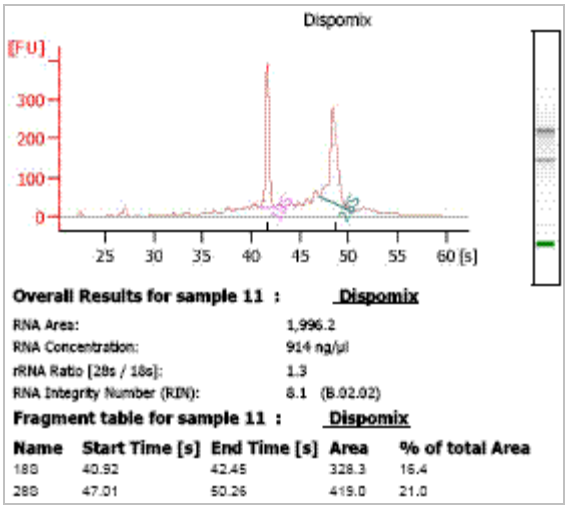
Sample disrupted with mortar and Ultra Turrax



Sample disrupted with Ultra Turrax



Sample disrupted with mixer mill



Sample disrupted with Dispomix

Conclusion:
- The homogenization with Mortal only results in low yield, but with a good RNA quality. The yield can be increased by an additional Ultra Turrax treatment.
- The Ultra Turrax alone also results in a good yield and a good RNA quality.
- The Retsch mill results in good a yield but shows significant degradation of the RNA.
- The Dispomix results in very good yield and a good RNA quality.

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