

Figure S10. Recommended protocol and technical aspects for faeces

## Metabolome coverage

ESI	HILIC (Log P < 1)	RP modified (-3 < Log P < 7)	RP (Log P > -1)
+	171 metabolites: aas, heterocyclic comp., carbohydrates, organic oxygen	223 metabolites: aas, acylcarnitines, phospholipids, heterocyclic comp.,organic oxygen, amines	<b>158</b> metabolites: acylcarnitines, phospholipids, heterocyclic comp., aas, organic oxygen
-	44 metabolites: FAs, organic acids, carbohydrates	<b>91</b> metabolites: FAs, BAs, phospholipids, organic acids	90 metabolites: FAs, BAs, phospholipids, organic acids
+/-	242 metabolites: aas, heterocyclic comp., nucleotides, carbohydrates, benzenoids	<b>329</b> metabolites: heterocyclic comp., aas, benzenoids, FAs, sterpoids, BAs	<b>235</b> metabolites: FAs, BAs, heterocyclic comp., organic acids, benzenoids, flavonoids

aas: amino acids; FAs: fatty acids, BAs: bile acids

## Tips

- Filtration highly recommened prior to analysis to avoid rapid deterioration of LC-MS components.

- Normalization is not necesary when using dried feces. Wet fresh samples should be normalized by their water content and the pH be adjusted.

<sup>-</sup> It is preferable to homogenize fresh samples before freezing and lyophilizing to avoid problems derived from the lack of homogeneity.

<sup>-</sup> Dried fecal samples have to be weighted prior to analysis.