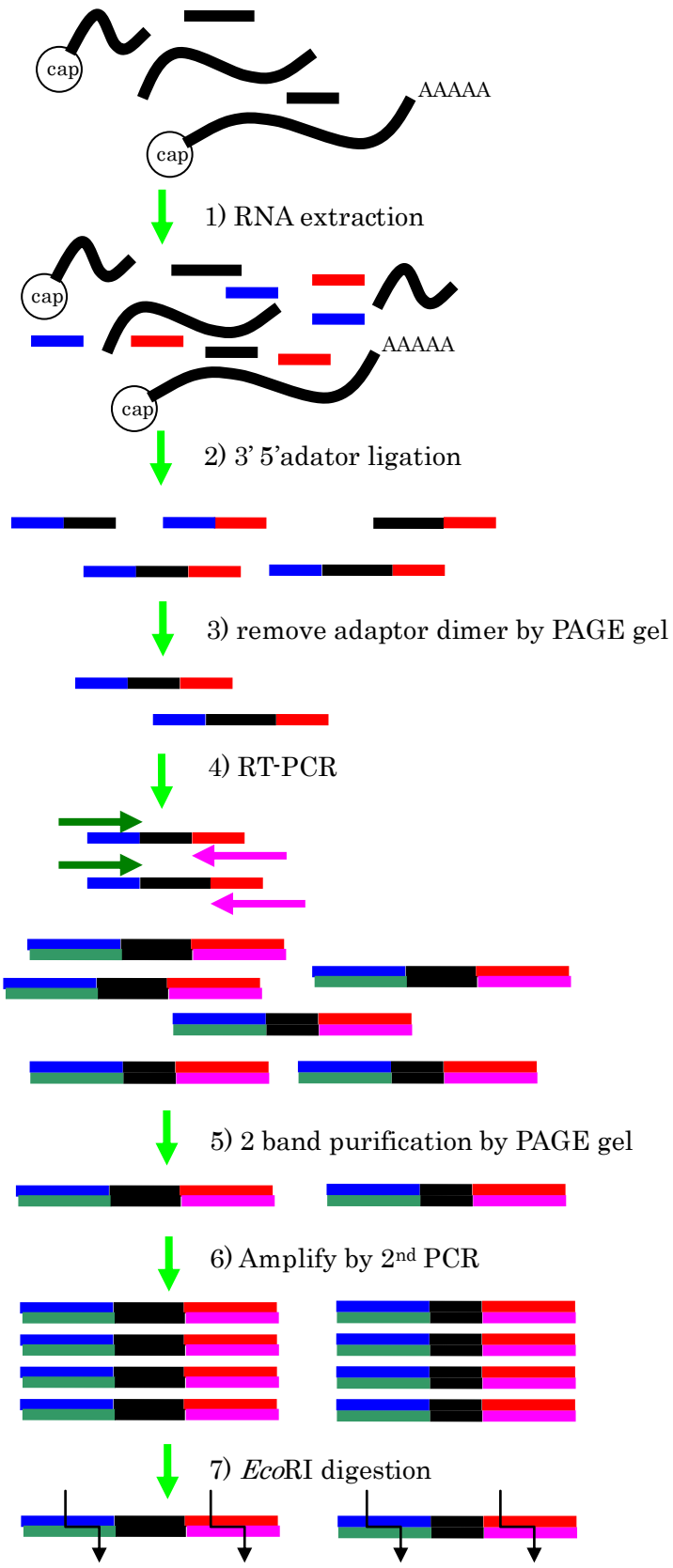
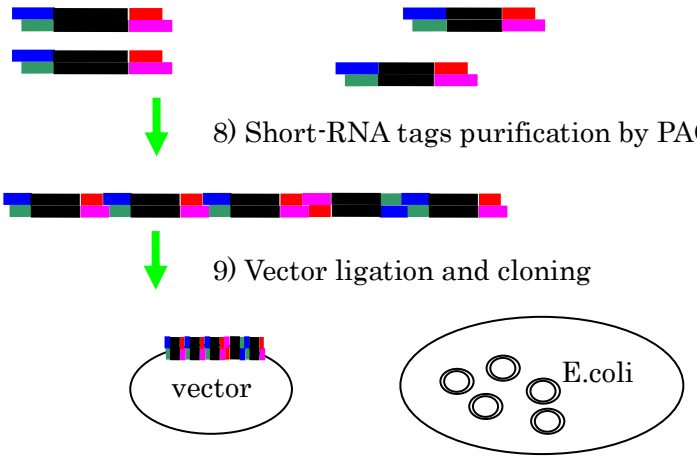

cap
AAAAA
cap
1) RNA extraction
cap
AAAAA
cap
2) 3' 5'adator ligation
3) remove adaptor dimer by PAGE gel
4) RT-PCR
5) 2 band purification by PAGE gel
6) Amplify by 2nd PCR
7) EcoRI digestion



1) HepG2 total RNA are extracted by AGPC method minimizing the time in order to avoid random cleavage of RNA.

2) 3' and 5' RNA-DNA chimeric oligonucleotides are ligated to the short RNAs.

3) Removal of adaptor dimers by PAGE gel electrophoresis

4) Reverse transcriptase and 1^{st} PCR cycle

5) The two main bands are separated by PAGE gel from non-specific products

6) 2^{nd} PCR to amplify of DNA fragment

7) Rhort RNA tags are cut by using the restriction enzyme EcoRI

8) Short RNA tags are purification by PAGE gel and then concatenated

9) Concatemers are cloned into the p-ZeroII vector and transformed

10) sequence by using RISA system (Sanger based).